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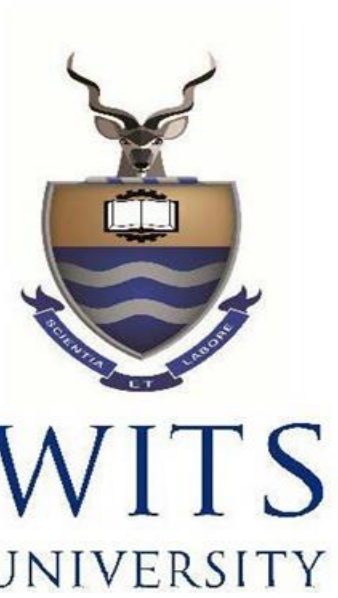
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LYCOPENE: PROTECTIVE POTENTIAL AGAINST DIET-INDUCED METABOLIC DERANGEMENTS IN WISTAR RATS



Mercy Omoye Shafe

A thesis submitted to the Faculty of Health Sciences, School of Physiology, University of the Witwatersrand, in fulfilment of the requirements for the degree of Doctor of Philosophy.

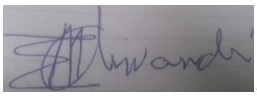
Johannesburg, 2025

DECLARATION

I, **Mercy Omoye Shafe**, student number **2404174**, declare that this thesis, titled **"Lycopene: Protective Potential Against Diet-Induced Metabolic Derangements in Wistar Rats"** is my original work. It is being submitted for the degree of **Doctor of Philosophy (Ph.D.)** at the **University of the Witwatersrand, Johannesburg, Republic of South Africa**. It has not been submitted, in whole or in part, for any degree or examination at any other university or institution. All sources used or cited have been appropriately acknowledged in the references.

Signed…..  …… Date ……10/06/2025…….

Name of Primary Supervisor: ……..Professor Eliton Chivandi……...

Signature of Primary Supervisor: … …….Date …...…10/06/2025…

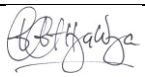
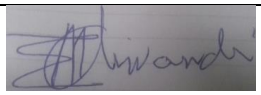
Agreement by Co-Authors

By signing this declaration, the co-authors listed below, including the student's supervisors, confirm their consent to the inclusion of the collaboratively authored review paper on lycopene, primarily written by the student, as part of this thesis.

Article 1

Title: Lycopene: A potent Antioxidant with Multiple Health Benefits

Journal of Nutrition and Metabolism Volume 2024, Article ID 6252426, 17 pages
https://doi.org/10.1155/2024/6252426

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Comments by the primary supervisor: As the PhD candidate, Mercy Shafe actively participated in the study design and data collection. It is from the study concept that she came up with the frame/structure of the review article. She wrote the first draft of the review article, made corrections following input from the supervisors. She submitted the article to the journal, made corrections following reviewer inputs, and published the article as first and corresponding author.

DEDICATION

I dedicate this thesis to **Almighty God**, whose guidance, wisdom, and grace have been my constant source of strength and inspiration throughout this journey. Without His blessings, this achievement would not have been possible.

CONFERENCE PRESENTATIONS ARISING FROM THIS RESEARCH

Oral presentations

1. **M.O. Shafe,** N.M. Gumede, T.T. Nyakudya and E. Chivandi. Lycopene: effects on body and liver mass, haematology, and metabolic substrate content of female Wistar Rats fed a high-fructose diet. **14th cross-Faculty Postgraduate Symposium**, 6th-8th September 2023, University of Witwatersrand, Johannesburg, Republic of South Africa.

2. **M.O. Shafe,** N.M. Gumede, T.T. Nyakudya and E. Chivandi. Lycopene: supplementation mitigated dietary fructose-induced increase liver lipid content in male Wistar Rats. **School of Physiology Research Day**, 13th September 2023, University of Witwatersrand, Johannesburg. Republic of South Africa.

Postal presentation

1. **M.O. Shafe**, N.M. Gumede, T.T. Nyakudya and E. Chivandi. Supplemental lycopene promotes bone and gastrointestinal tract growth, prevent bone loss, in growing male Wistar Rats fed a high-fructose diet. **Faculty of Health Sciences Research Day and Postgraduate Expo**, 5th September 2024, University of Witwatersrand, Johannesburg. Republic of South Africa.

PUBLICATIONS BASED ON THIS RESEARCH

Published review article

1. **M.O. Shafe,** N.M. Gumede, T.T. Nyakudya and E. Chivandi. (2024). Lycopene a potent antioxidant with multiple health benefits. *Journal of Nutrition and Metabolism,* pp. 1-17. https://doi.org/10.1155/2024/6252426

Research article submitted for publication

1. **M.O. Shafe**, N.M. Gumede, T.T. Nyakudya and E. Chivandi (2025). Supplemental lycopene reduces feed intake, increases fluid consumption, and enhances bone and stomach growth in growing Wistar Rats fed a high-fructose diet. Submitted to *Journal of Medicinal Food.*

ABSTRACT

Early-life nutrition and dietary supplements significantly impact long-term health by either increasing the risk of metabolic disorders or enhancing well-being in adulthood. Lycopene has potent antioxidant activity hence this study evaluated its potential to protect against dietary fructose-induced metabolic derangements in Wistar rats mimicking adolescents fed an obesogenic diet. Ninety-six 21-day-old Wistar rats (48 females and 48 males) were assigned to different groups at random and received treatment regimens for 12 weeks as follows: (1) standard rat chow (SRC) with plain drinking water (PDW) and plain gelatine cubes (PG), (2) SRC with a 20% fructose solution (FS) and PG, (3) SRC with FS and 100 mg/kg/day fenofibrate in gelatine cubes. Groups 4, 5, and 6 had SRC, FS, and lycopene in gelatine cube at 30, 60, and 100 mg/kg/day, respectively. Feed and fluid consumption, and body mass, were measured. Terminally, the fasted rats were weighed and euthanised. Collected blood was used to assess treatment effects on general health and oxidant and antioxidant status. Gastrointestinal viscera macro-morphometry, visceral and epididymal fat, liver fat, and kidney and liver macro- and micro-morphometry and femora and tibiae indices were determined.

Female and male rats fed the control diet had higher ($p < 0.05$) feed intake compared to high fructose diet fed, fenofibrate treated and lycopene supplemented counterparts. Compared to control diet fed and fenofibrate-treated, lycopene-supplemented rats had lower feed intake but higher fluid intake ($p < 0.05$). Both female and male rats showed significant growth during the trial ($p < 0.05$). Lycopene increased the small intestine and stomach masses in male rats. Treatment regimens did not affect visceral fat in either male or female rats, nor epididymal fat mass in males ($p > 0.05$). Fenofibrate increased the females' high-density lipoprotein (HDL), glycated haemoglobin (HbA1c), and fasting blood glucose (FBG) concentrations, and the males' FBG concentration ($p < 0.05$). The rats' haemoglobin, haematocrit, triglycerides, serum total cholesterol, low-density lipoprotein (LDL), leptin, creatinine, blood urea nitrogen (BUN), neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), and thiobarbituric acid reactive substances (TBARS) concentrations, BUN/creatinine ratio, alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), and glutathione peroxidase 1 (GPX-1) activities, glomerular tuft area, proximal convoluted tubule outer and epithelial areas showed no significant changes across treatment groups ($p > 0.05$). Lycopene prevented fructose-induced increase in urinary space observed in male rats. In females, treatments did not affect serum

globulin, total protein, albumin, bile acids and uric acid concentrations ($p > 0.05$). Supplemental lycopene attenuated dietary fructose-induced serum albumin, total protein, globulin, total bile acids, and uric acid increases concentrations in males. High-dose supplemental lycopene increased hepatic GPX-1 and catalase (CAT) activities in male rats. In both sexes fenofibrate caused hepatocyte hypertrophy. Lycopene mitigated dietary fructose-induced increases in liver lipid content and steatosis.

Dietary fortification with lycopene showed significant prophylactic potential against metabolic disturbances caused by dietary fructose in Wistar rats. It improved bone, kidney, liver, and gastrointestinal health and enhanced antioxidant activity, supporting better metabolic outcomes and overall well-being in both sexes.

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First and foremost, I offer my deepest gratitude to my Father, 'The Eleburuike' (God whose care for us overflows), for His abundant grace, unfailing love, and constant guidance throughout this academic pursuit. His presence has been my foundation and source of strength in overcoming every challenge along the way.

To my supervisors, **Professors Eliton Chivandi, Trevor T, Nyakudya and Dr. Nontobeko M. Gumede**, I sincerely thank you for your invaluable mentorship. Your guidance, patience, and support have not only shaped my academic journey but also helped me grow as a person. The lessons learnt will continue to guide me throughout my life and career.

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To my **family, Mum, my siblings**, and the memory of my late father, **Pa Raymond Akhiwu Ohuh,** whose love, guidance, and prayers continue to inspire me. I also express my deepest gratitude to my loving, caring, and understanding husband, **Engr. Dirisu Shafe**, for your support and encouragement, and for allowing me to come to South Africa to pursue my PhD.

TABLE OF CONTENTS

TITLE PAGE.....i

DECLARATION.....ii

DEDICATION.....iv

CONFERENCE PRESENTATIONS ARISING FROM THIS RESEARCH.....v

PUBLICATIONS BASED ON THIS RESEARCH.....vi

ABSTRACT.....vii

ACKNOWLEDGMENTS.....ix

CHAPTER ONE: INTRODUCTION AND JUSTIFICATION.....1

1.0 Introduction and justification.....2

1.1 Aims and objectives.....5

1.2 Hypothesis.....6

1.3 References.....7

CHAPTER TWO: LITERATURE REVIEW.....15

2.0 Introduction.....16

2.1 Metabolic syndrome: a historical perspective.....16

2.2 Metabolic syndrome trends.....17

2.3 Components of metabolic syndrome.....18

2.3.1 Central obesity.....18

2.3.2 Insulin resistance.....19

2.3.3 Dyslipidaemia.....20

2.4 Complications of metabolic syndrome.....21

2.4.1 Metabolic dysfunction-associated steatotic liver disease.....21

2.4.2 Type 2 diabetes mellitus.....22

2.4.3 Chronic kidney disease.....23

2.4.4 Cardiovascular diseases.....23

2.4.5 Bone disorders and osteoporosis.....24

2.4.6 Haematological changes.....24

2.5 Metabolic programming.....24

2.6 Experimental models of metabolic programming.....25

2.6.1 Chemical intervention models.....25

2.6.2 Genetic models 26
2.6.3 Diet-based models 27
2.7 High fructose diets and their role in metabolic syndrome pathogenesis 27
2.8 Management strategies for metabolic disorders 29
2.8.1 Lifestyle interventions 29
2.8.2 Conventional pharmacological treatment 30
2.8.3 Phytotherapy 30
2.9 References 32
2.10 Lycopene: A potent antioxidant with multiple health benefits – Published review article 58
2.10.1 Summary 59
2.10.2 Introduction 59
2.10.3 Lycopene: Biochemistry and physical properties 61
2.10.4 Lycopene: Absorption, transportation, and distribution 62
2.10.5 Lycopene autoxidation 65
2.10.6 Biological activities of lycopene 66
2.10.6.1 Antiobesity effects 68
2.10.6.2 Antioxidant effects 69
2.10.6.3 Hypocholesterolaemic effects 70
2.10.6.4 Hepatoprotection 71
2.10.5.6 Osteoprotection 74
2.10.6.7 Anti-inflammatory effects 75
2.10.6.8 Antidiabetic effects 76
2.10.6.9 Anticancer effects 77
2.10.6.10 Gastroprotection 77
2.10.6.11 Neuroprotection 78
2.10.6.12 Cardioprotection 79
2.10.6.13 Lung protection 79
2.10.6.14 Sperm quality enhancement and fertility promotion 80
2.10.6.15 Protection of skin health 81
2.10.6.16 Protective effect on vision 81
2.10.7 Lycopene: Protective effects against toxins 82
2.10.8 Lycopene: Safety and potential toxicity 82
2.10.9 Conclusion 82

2.10.10 References....84
CHAPTER THREE: EFFECT OF LYCOPENE ON GROWTH PERFORMANCE AND GASTROINTESTINAL VISCERA MACROMORPHOMETRY OF WISTAR RATS FED A HIGH-FRUCTOSE DIET....112
3.0 Introduction....113
3.1 Materials and methods....114
3.1.1 Ethical approval....114
3.1.2 Chemicals and reagents....114
3.1.3 Animals, housing, and general care....114
3.1.4 Experimental design....115
3.1.5 Measurements: body mass, feed, fluid, and calorie intake....118
3.1.6 Terminal procedures and sample collection....118
3.1.7 Measurement of bone morphometry....118
3.1.8 Statistical analysis....119
3.2 Results....119
3.2.1 Body mass....119
3.2.2 Feed, fluid, and calorie intake....121
3.2.3 Long bone indices....127
3.2.4 Visceral organ macro-morphometry....130
3.3 Discussion....135
3.3.1 Growth performance: body mass, feed and fluid intake, and calorie consumption....135
3.3.2 Long Bone Growth....139
3.3.3 Morphometry of Visceral Organs....140
3.4 Conclusion....142
3.5 References....143
CHAPTER FOUR: EFFECT OF LYCOPENE ON BLOOD GLUCOSE, GLYCATED HAEMOGLOBIN, HAEMOGLOBIN, HAEMATOCRIT, SERUM LEPTIN CONCENTRATION AND LIPID PROFILE IN GROWING WISTAR RATS FED A HIGH FRUCTOSE DIET....151
4.0 Introduction....152
4.1 Materials and methods....154
4.1.1 Ethical approval....154
4.1.2 Chemicals and reagents....154
4.1.3 Animals, housing, and general care....154
4.1.4 Experimental design....154
4.1.5 Terminal procedures, sample collection and measurements....154

4.1.6 Determination of serum lipoproteins, and total cholesterol 155
4.1.7 Determination of glycated haemoglobin concentrations 155
4.1.8 Determination of serum leptin concentration 155
4.1.9 Statistical analysis 156
4.2 Results 156
4.2.1 Haemoglobin and haematocrit concentrations 156
4.2.2 Fasting blood glucose and glycated haemoglobin concentrations 158
4.2.3 Lipid profile 161
4.2.4 Serum leptin concentration 163
4.3 Discussion 165
4.3.1 Haemoglobin and Haematocrit Concentrations 165
4.3.2 Glucose and Lipid Profile 165
4.3.3 Leptin concentration 168
4.4 Conclusion 169
4.5 References 171
CHAPTER FIVE: EFFECT OF LYCOPENE ON KIDNEY HEALTH IN GROWING WISTAR RATS FED A HIGH FRUCTOSE DIET 178
5.0 Introduction 179
5.1 Materials and methods 181
5.1.1 Ethical approval 181
5.1.2 Chemicals and reagents 181
5.1.3 Animals, housing, and general care 181
5.1.4 Experimental design 181
5.1.5 Terminal procedures, sample collection, and measurements 181
5.1.6 Determination of kidney function and injury biomarkers 182
5.1.7 Determination of kidney micromorphometry 182
5.1.8 Statistical analysis 183
5.2 Results 183
5.2.1 Kidney mass 183
5.2.2 Serum biomarker concentrations 185
5.2.3 Kidney histomorphology 188
5.3 Discussion 198
5.3.1 Kidney masses 198

5.3.2 Renal function and injury biomarkers 199
5.3.3 Kidney histomorphometry 202
5.4 Conclusion 205
5.5 References 206
CHAPTER SIX: EFFECT OF LYCOPENE ON LIVER HEALTH IN GROWING WISTAR RATS FED A HIGH FRUCTOSE DIET 217
6.0 Introduction 218
6.1 Materials and methods 219
6.1.1 Ethical approval 219
6.1.2 Chemicals and reagents 220
6.1.3 Animals, housing, and general care 220
6.1.4 Experimental design 220
6.1.5 Terminal procedures, sample collection and measurements 220
6.1.6 Determination of hepatic lipid content 221
6.1.7 Determination of serum and hepatic oxidative stress and antioxidant status 221
6.1.8 Determination of serum surrogate markers for liver function 222
6.1.9 Liver histology assessment 222
6.1.10 Statistical analysis 222
6.2 Results 223
6.2.1 Liver, visceral fat pad, and epididymal fat pad masses 223
6.2.2 Liver lipid content 226
6.2.3 Systemic and hepatic oxidative stress and antioxidant status 228
6.3 Discussion 244
6.3.1 Abdominal fat and liver mass 244
6.3.2 Hepatic lipid yield 245
6.3.3 Oxidative stress and systemic antioxidant response 246
6.3.4 Serum biomarkers of hepatic function 247
6.3.5 Hepatic microarchitecture 251
6.4 Conclusion 252
6.5 References 254
CHAPTER SEVEN: CONCLUSIONS, LIMITATIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH 266
7.0 Introduction 267
7.1 Summary of key findings, limitations, and recommendations 267

7.2 Overall conclusion 270
7.3 References 271
APPENDICES 273
Appendix 1: Animal ethics approval certificate 274
Appendix 2: First amendment to ethics clearance 275
Appendix 3: Second amendment to ethics clearance 276
Appendix 4: Published review article (Chapter 2) 277

LIST OF TABLES

CHAPTER TWO

Table 2. 1: Diagnostic criteria for metabolic syndrome across different organisation 17
Table 2. 2: Lycopene concentration in fresh fruits and processed food products 60
Table 2. 3: Physical properties of lycopene 64
Table 2. 4: Lycopene concentration in some human tissues 65
Table 2. 5: Meta-Analyses and Clinical Trials of Lycopene in Human Studies 66

CHAPTER THREE

Table 3. 1: Effect of lycopene on the lengths, masses and seedor ratio of tibiae and femora in growing female Wistar rats fed a high-fructose diet 128
Table 3. 2: Effect of lycopene on the lengths, masses and seedor ratio of tibiae and femora in growing male Wistar rats fed a high-fructose diet 129
Table 3. 3: Effect of lycopene on absolute and relative masses and length of the visceral organs in growing female rats fed a high-fructose diet 131
Table 3. 4 Effect of lycopene on absolute and relative masses and length of the visceral organs in growing male rats fed a high-fructose diet. 133

CHAPTER FOUR

Table 4. 1: Effect of lycopene on haemoglobin and haematocrit concentration in growing female and male Wistar rats fed a high-fructose diet..... 157
Table 4. 2: Effect of lycopene on lipid profile in growing female and male Wistar rats fed a high-fructose diet..... 162

CHAPTER FIVE

Table 5. 1: Effect of lycopene on absolute and relative kidney masses in growing female and male Wistar rats fed a high-fructose diet 184

Table 5. 2: Effect of lycopene on serum concentrations of creatinine, BUN, BUN/creatinine ratio, KIM-1 and NGAL in growing female and male Wistar rats fed a high-fructose diet 186
Table 5. 3: Effect of lycopene on kidney morphometry in growing female Wistar rats fed a high-fructose diet..... 189
Table 5. 4: Effect of lycopene on kidney morphometry in growing male Wistar rats fed a high-fructose diet..... 192

CHAPTER SIX

Table 6. 1: Effect of lycopene on absolute and relative masses of the liver and visceral fat pads of growing female Wistar rats fed a high-fructose diet..... 224
Table 6. 2: Effect of lycopene on the absolute and relative masses of the liver, visceral fat pads, and epididymal fat pads of growing male Wistar rats fed a high-fructose diet 225
Table 6. 3: Effect of lycopene on serum markers of hepatic function in growing female Wistar rats fed a high fructose diet..... 234
Table 6. 4: Effect of lycopene on serum markers of hepatic function in growing male Wistar rats fed a high fructose diet..... 235
Table 6. 5: Effect of lycopene on liver steatosis, lobular inflammation, and hepatocyte hypertrophy scores in growing female Wistar rats fed a high-fructose diet 242
Table 6. 6: Effect of lycopene on liver steatosis, lobular inflammation, and hepatocyte hypertrophy scores in growing male Wistar rats fed a high-fructose diet 243

LIST OF FIGURES

CHAPTER TWO

Figure 2. 1 : Molecular structure of lycopene 63

CHAPTER THREE

Figure 3. 1: Flowchart of study design 117
Figure 3. 2: Weekly body masses (A) and induction and terminal body masses (B) of female Wistar rats 120
Figure 3. 3: Weekly body masses (A) and induction and terminal body masses (B) of male Wistar rats..... 121
Figure 3. 4: Effect of lycopene on weekly feed (A), total feed (B), weekly fluid (C), total fluid (D), weekly calorie (E), and total calorie intake (F) of female Wistar rats fed a high-fructose diet. 125
Figure 3. 5: Effect of lycopene on weekly feed (A), total feed (B), weekly fluid (C), total fluid (D), weekly calorie (E), and total calorie intake (F) of male Wistar rats fed a high-fructose diet. 127

CHAPTER FOUR

Figure 4. 1: Effect of lycopene on fasting blood glucose (A) and glycated haemoglobin (B) concentration in growing female Wistar rats fed a high-fructose diet. 159

Figure 4. 2: Effect of lycopene on fasting blood glucose (A) and glycated haemoglobin (B) concentration in growing male Wistar rats fed a high-fructose diet. 160

Figure 4. 3A: Effect of lycopene on serum leptin concentration in growing female Wistar rats fed a high-fructose diet. 163

Figure 4. 3B: Effect of lycopene on serum leptin concentration in growing male Wistar rats fed a high-fructose diet……. …….164

CHAPTER FIVE

Figure 5. 1 Representative photomicrographs illustrating the effect of lycopene on kidney histology (H&E staining, 40x magnification, scale bar = 50 µm) in growing female Wistar rats fed a high-fructose diet. 194

Figure 5. 2: Representative photomicrographs illustrating the effect of lycopene on kidney histology (H&E staining, 40x magnification, scale bar = 50 μm) in growing male Wistar rats fed a high-fructose diet. 196

CHAPTER SIX

Figure 6.1A: Effect of lycopene on liver lipid content in growing female Wistar rats fed a high-fructose diet…….226

Figure 6.1B: Effect of lycopene on liver lipid content in growing male Wistar rats fed a high-fructose diet…….227

Figure 6. 2: Effect of lycopene on serum TBARS (A) concentration and GPX-1 (B) activity and hepatic TBARS (C) concentration, GPX-1 (D) and CAT (E) activities in growing female Wistar rats fed a high-fructose diet..... 230

Figure 6. 3: Effect of lycopene on serum TBARS (A) concentration and GPX-1 (B) activity and hepatic TBARS (C) concentration, GPX-1 (D) and CAT (E) activities in growing male Wistar rats fed a high-fructose diet..... 232

Figure 6.4: Representative photomicrographs showing the effects of lycopene on liver histology in growing female Wistar rats fed a high-fructose diet (H&E staining, 40x magnification, scale bar = 50 μm). 237

Figure 6.5: Representative photomicrographs showing the effects of lycopene on liver histology in growing male Wistar rats fed a high-fructose diet (H&E staining, 40x magnification, scale bar = 50 μm)..... 239

LIST OF ABBREVIATION

ADP:	Adenosine diphosphate
AESC:	Animal Ethics Screening Committee
ALB:	Albumin
ALP:	Alkaline phosphatase
ALT:	Alanine aminotransferase
AMP:	Adenosine monophosphate
ARRIVE:	Animal Research: Reporting of In Vivo Experiment
AST:	Aspartate aminotransferase
AOAC:	Association of Official Analytical Chemists
ATP:	Adenosine triphosphate
BM:	Body mass
BMI:	Body mass index
BUN:	Blood urea nitrogen
CA:	Corpuscular area
CAT:	Catalase
CKD:	Chronic kidney disease
CVD:	Cardiovascular disease
CYP7A1:	Cholesterol 7α-hydroxylase
DCTEA:	Distal convoluted tubules epithelial area
DCTIA:	Distal convoluted tubules inner area
DCTOA:	Distal convoluted tubules outer area
DNA:	Deoxyribonucleic acid
ELISA:	Enzyme-Linked Immuno-Sorbent Assay
F:	Females
FBG:	Fasting blood glucose
FENO:	Fenofibrate
FS:	Fructose solution
FXR:	Farnesoid X receptor
GFR:	Glomerular filtration

GGT:	Gamma-glutamyl transferase
GIT:	Gastrointestinal tract
GLOB:	Globulin
GLUT:	Glucose transporter
GLP-1:	Glucagon-like peptide-1
GPX-1:	Glutathione peroxidase-1
GSH:	Glutathione
GTA:	Glomerular tuft area
H_0:	Null hypothesis
H_1:	Alternative hypothesis
H & E:	Haematoxylin and eosin
HbA1c:	Glycated haemoglobin
Hb:	Haemoglobin
HCT:	Haematocrit
HDL:	High-density lipoprotein
HDL-C:	High-density lipoprotein Cholesterol
HDLY:	High dose lycopene
HepG2:	Human liver cancer cell line
HFCS:	High fructose corn syrup
IDF:	International Diabetes Federation
IL-6:	Interleukin 6
JNK:	c-Jun N-terminal kinase
KIM-1:	Kidney injury molecule-1
L:	Large
LDL:	Low-density lipoprotein
LDLY:	Low dose lycopene
M:	Males
MAFL:	Metabolic dysfunction-associated fatty liver
MAPK:	Mitogen-activated protein kinase
MASH:	Metabolic dysfunction-associated steatohepatitis
MASLD:	Metabolic dysfunction-associated steatotic liver disease

MCP-1:	Monocyte chemoattractant
MDA:	Malondialdehyde
MDLY:	Medium dose lycopene
MetS:	Metabolic syndrome
mRNA:	Messenger ribonucleic acid
n:	Number
NCEP ATP:	National Cholesterol Education Program Adult Treatment Panel
NF-kB:	Nuclear factor-kappa B
NGAL:	Neutrophil gelatinase-associated lipocalin
OPG:	Osteoprotegerin
PDW:	Plain drinking water
PCTEA:	Proximal convoluted tubules epithelial area
PCTIA:	Proximal convoluted tubules inner area
PCTOA:	Proximal convoluted tubules outer area
PG:	Plain gelatine cube
PND:	Postnatal day
PPAR:	Peroxisome proliferator-activated receptor
RANKL:	Receptor activator of nuclear factor kappa B ligand
ROS:	Reactive oxygen species
S:	Small
SD:	Standard deviation
SGLT2:	Sodium-glucose cotransporter 2
SOD:	Superoxide dismutase
SRC:	Standard rat chow
SST:	Serum separator tube
TBA:	Total bile acids
TBARS: `	Thiobarbituric acid reactive substances
TBIL:	Total bilirubin
TC:	Total cholesterol
TCI:	Total calorie intake
TFI:	Total feed intake

TG:	Triglycerides
TNF:	Tumour necrosis factor
TP:	Total protein
T2DM:	Type 2 diabetes mellitus
UA:	Uric acid
USA:	Urinary space area
VAT:	Visceral adipose tissue
VLDL:	Very low-density lipoprotein
w/v:	Weight/volume
WC:	Waist circumference
WHO:	World Health Organisation
WHR:	Waist-to-hip ratio
WRAF:	Wits Research Animal Facility
%BM:	Percentage body mass
α:	Alpha
β:	Beta

CHAPTER ONE: INTRODUCTION AND JUSTIFICATION

1.0 Introduction and justification

Obesity, a growing global concern affecting both children and adults (Koliaki et al., 2023), significantly elevates susceptibility to metabolic disorders and related diseases (Rhee, 2022)**.** The condition develops when dietary energy intake surpasses energy expenditure (Lean et al., 2018). This imbalance in energy metabolism is exacerbated by excessive intake of high-calorie diets and sedentary lifestyles; the major contributors to the rapid increase in obesity worldwide (Heindel et al., 2024). Notably, the global rise in childhood obesity (Pulungan et al., 2024), serves as significant predictor of adult obesity, with affected children facing a higher risk than their non-obese peers (Hampl et al., 2023). As obesity persists into adulthood, it becomes closely linked to metabolic syndrome (MetS), a combination of metabolic abnormalities that heighten the risk of cardiovascular diseases, type 2 diabetes mellitus (T2DM), and stroke (Sharif et al., 2024). MetS is characterised by high blood pressure, elevated lipid levels, gynoid obesity, and increased fasting blood glucose levels (Pluta et al., 2022; Ma et al., 2023). Metabolic syndrome impacts between 25.3% and 34.2% of the adult human global population (Saklayen, 2018) and is projected to increase to 53% by 2035 (Engin, 2017). It affects 22%, 24%, 3%, and 5% of women, men, children and adolescents, respectively (Lovre & Mauvais-Jarvis, 2015; Noubiap et al., 2022). Children in low-income countries experience a higher prevalence of MetS (Noubiap et al., 2022), as people age, the prevalence increases affecting nearly 40% of individuals in their sixties (Swarup et al., 2024). While men generally have a higher prevalence, certain ethnic groups of women show slightly higher rates (Jahangiry et al., 2019; Rus et al., 2023).

This widespread prevalence underscores the role of diet-induced metabolic disorders, which drive the overproduction of reactive oxygen species (ROS) during the metabolism of fuel biomolecule, ultimately resulting in oxidative stress (Jiang et al., 2021). Such oxidative stress is mainly attributed to the excessive intake of corn-fructose-sweetened foods and beverages (Ma et al., 2022), whose metabolism creates a highly oxidative environment that leads to these metabolic disorders (Vona et al., 2021). Fructose is naturally found in fruits, beetroots, sugar cane, and honey and is also industrially produced for use in various food and beverage products (Carvalho et al., 2018; Dholariya & Orrick, 2022). Excessive fructose consumption, particularly during prenatal life has been associated with the onset of obesity, dyslipidaemia, insulin resistance, T2DM, metabolic dysfunction-associated steatotic liver disease (MASLD), and Mets in adulthood (Pereira et al., 2017; Zhou et al., 2020; Tamimi et al., 2021). These conditions can further be complicated

by cardiovascular (Busnatu et al., 2022), renal (Nakagawa & Kang, 2021) and haematologic diseases (Wang et al., 2021). Upon absorption from the small intestine, fructose is largely metabolised in the liver to generate energy, fat and or glucose (Merino et al., 2020) as determined by the energy balance and metabolic requirements of the body. While the liver is the key organ where fructose metabolism takes place, the kidneys also contribute to its metabolism (Dholariya & Orrick, 2022). Excessive fructose consumption has been linked to increased body mass and abnormal enlargement of gastrointestinal organs, including the stomach and intestines (Guney et al., 2023). This abnormal growth can impact the digestive system's overall function. Additionally, fructose disrupts the delicate balance of gut microbiota, the ecosystem of beneficial bacteria residing in the intestines (Beisner et al., 2020). These disruptions can alter digestion, nutrient absorption, and overall gut health (Dong et al., 2023; Sánchez-Terrón et al., 2023). Excessive fructose intake contributes to reduced bone mineral density resulting in a weakened skeletal system (Han et al., 2022). The dietary fructose induced metabolic imbalance leads to oxidative stress which exacerbates dyslipidaemia (Tangvarasittichai, 2015). Specifically, oxidative stress from excessive dietary fructose intake promotes the buildup of triglycerides and total cholesterol, elevates the levels of low-density lipoprotein (LDL) cholesterol and reduces high-density lipoprotein (HDL) cholesterol (Zhang et al., 2017; Baumann et al., 2022). This disruption in lipid metabolism contribute to metabolic dysfunction and increasing the risk of cardiovascular and other metabolic diseases (Chakraborty et al., 2023).

The global burden of metabolic disorders is exacerbated by the high cost of synthetic medications and inadequate healthcare systems, particularly in developing countries, making these treatments largely inaccessible (Mwanza et al., 2023). This has sparked a growing interest in medicinal plants and natural phytochemicals which are known for their diverse biological effects (Theodoridis et al., 2023).

Synthetic pharmaceuticals like fenofibrate and metformin, are commonly prescribed to treat MetS and its associated complications (Dutta et al., 2023; Deerochanawong et al., 2024). However, these widely used medications can be cost-prohibitive in low-income population and are associated with notable side effects (He et al., 2021; Nguyen et al., 2022). This has led to a growing interest in more affordable, plant-based ethnomedicines (Hiben et al., 2019). Despite their benefits, some medicinal plants can be inherently toxic potentially harming vital organs like the liver, heart and

kidneys (Mensah et al., 2019; Philips & Theruvath, 2024). However, many of the medical plants contain bioactive phytochemicals, such as carotenoids, which provide numerous health benefits without the associated toxicity (Nyirenda et al., 2023).

Among these carotenoids, lycopene, a liposoluble compound predominantly found in fruits and vegetables (Pathak & Sagar, 2023), has potent antioxidant properties (Ge et al., 2023). It also exhibits anti-inflammatory (Divyadharsini et al., 2023), hepatoprotective (Abdel-Rahman et al., 2018), cardioprotective (Hsieh et al., 2022), neuroprotective (Paul et al., 2020), and anticancer effects (Sahin et al., 2019). Lycopene also shows benefits against obesity and T2DM (Figueiredo et al., 2024), by reducing oxidative stress and inflammation (Chen et al., 2019) via mechanisms that target critical pathways like c-Jun N-terminal kinase (JNK), mitogen-activated protein kinase (MAPK), and nuclear factor-kappa B (NF-kB) (Long et al., 2024; Song et al., 2024). Furthermore, lycopene prevents bone mineral loss associated with osteoporosis by promoting an increase in the osteoprotegerin (OPG) to receptor activator of nuclear factor kappa B ligand (RANKL) ratio, thereby inhibiting bone resorption (Qi et al., 2021; Xia et al., 2024).

Despite these therapeutic benefits, the disease-preventing potential of lycopene has not been fully explored. Most research has focused on therapeutic interventions, including plant-based ethnomedicines, to combat metabolic disorders (Korivi & Liu, 2021). However, these studies often utilise chemically induced models, like streptozotocin and alloxan-induced diabetes mellitus models (Athmuri & Shiekh, 2023), which do not effectively replicate the metabolic derangements typically caused by diet. Additionally, these studies predominantly involve male animals (Queiroz et al., 2021; Sasongko et al., 2022; Rehman et al., 2023), despite the known differences in metabolic responses between sexes.

Considering the growing prevalence of diet-induced metabolic disorders in children and adolescents, coupled with the extensive health benefits of lycopene, it is crucial to explore its potential for prevention. This highlights the urgent need to evaluate the prophylactic effects of orally administered lycopene in mitigating dietary fructose-induced metabolic derangements in growing female and male Wistar rats.

1.1 Aims and objectives

The primary aim of the study was to evaluate the potential protective effect of orally administered lycopene against metabolic derangements induced by a high-fructose diet, from weaning to adulthood in Wistar rats. The study was structured to explore the effect of lycopene on four specific sub-study areas:

1. **Growth performance**, assessed by determining effects on:
    a) body mass
    b) feed, fluid, and calorie consumption
    c) long bone growth
    d) morphometry of gastrointestinal tract visceral organs
2. **Metabolic and haematological markers,** assessed by determining effects on:
    a) fasting blood haematocrit, haemoglobin, and glycated haemoglobin
    b) fasting blood glucose concentrations
    c) fasting blood triglycerides (TG) and serum total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL)
    d) fasting serum leptin concentration
3. **Kidney health,** evaluated by determining effects on:
    a) kidney mass (absolute and relative)
    b) serum kidney function biomarkers [blood urea nitrogen (BUN), creatinine, and BUN/creatinine ratio]
    c) serum markers indicating kidney tubular damage, including neutrophil gelatinase-associated lipocalin **(**NGAL) and kidney injury molecule 1 (KIM-1)
    d) kidney histomorphology
4. **Liver health,** assessed by:
    a) liver mass (absolute and relative)
    b) absolute and relative mass of the visceral fat pad (in both sexes) and epididymal fat pad (in males) as indicators of obesity
    c) hepatic lipid content
    d) antioxidant and oxidant markers [Fasting serum and hepatic glutathione peroxidase-1 (GPX-1), hepatic catalase (CAT), and serum and hepatic thiobarbituric acid reactive substances (TBARS)]

e) hepatic steatosis, inflammation, and hypertrophy scores
f) serum markers of hepatic function [total bilirubin, globulin, albumin, total protein, total bile acids, uric acid, alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST)]
g) liver histomorphology

1.2 Hypothesis

H_0: Orally administered lycopene has no protective effect against metabolic derangements induced by a high fructose diet in growing Wistar rats.

H_1: Orally administered lycopene has a protective effect against metabolic derangements induced by a high fructose diet in growing Wistar rats.

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CHAPTER TWO: LITERATURE REVIEW

Preview

This chapter is divided into two sections. The first section serves as an introduction to the literature review, focusing on metabolic syndrome (MetS). This section specifically addresses the description, key components, diagnostic criteria, underlying pathophysiology, treatment strategies and associated health risks of MetS. The second section is made up of my published review article, titled "*Lycopene: A Potent Antioxidant with Multiple Health Benefits*." This published article has been incorporated into this chapter since it contains a detailed review of the nature, sources, biological activities, therapeutic and prophylactic potential and pertinent *in vitro* and *in vivo* research on lycopene. It must be noted that lycopene is the phytochemical used as the experimental intervention to access its protective effects against dietary fructose-induced metabolic ill-health in growing Wistar rats fed a fructose-infused drinking solution in a model mimicking growing children fed an obesogenic diet.

2.0 Introduction

Metabolic syndrome (MetS) refers to a cluster of related metabolic disturbances that notably increase the likelihood of developing cardiovascular disease, T2DM, and other metabolic health complications (Rus et al., 2023). It is diagnosed when individuals present with at least three out of five specific conditions: hypertension, central obesity, insulin resistance, hypertriglyceridemia, and low levels of high-density lipoprotein (HDL) cholesterol (Kao & Huang, 2021). Genetic predispositions, environmental conditions, , and lifestyle choices, such as unhealthy diets and lack of physical activity, play a crucial role in the onset of MetS (Kang et al., 2021; Chomiuk et al., 2024). Insulin resistance, which impair cellular responsiveness to insulin, serves as a central feature of MetS and a key factor in its progression (Nolan & Prentki, 2019). Additionally, chronic inflammation and oxidative stress actively contribute to both the development and worsening of MetS (Weinberg Sibony et al., 2024). MetS is a growing public health concern, with its prevalence rising globally due to increasing prevalence of obesity and sedentary lifestyles (Jemal et al., 2023). Early detection and lifestyle interventions, such as dietary modifications and regular exercise, are essential to mitigate MetS' associated health risks (Oppert et al., 2021).

2.1 Metabolic syndrome: a historical perspective

The concept of metabolic syndrome has evolved over several decades (Mendrick et al., 2018). In 1923, Swedish physician Eskil Kylin first documented the co-occurrence of hypertension, hyperglycaemia and gout (Kylin, 1923). Later, in 1947, Jean Vague identified central obesity as a key factor associated with metabolic disturbances, noting its link to cardiovascular diseases (Vague, 1947). Gerald Reaven coined the term "Syndrome X," in 1988, emphasising insulin resistance as the syndrome's core characteristic and its contribution to a heightened risk of cardiovascular disease (CVD) and T2DM (Reaven, 1988).

Over time, MetS' description was expanded to include dyslipidaemia, in the form of elevated plasma triglycerides, reduced HDL cholesterol levels and hypertension (Alberti et al., 2009). This broader definition provided a more comprehensive understanding of MetS enabled and enables clinicians and researchers to better identify individuals at risk and implement targeted interventions (Agabiti et al., 2023). As the criteria evolved, the World Health Organisation (WHO), the International Diabetes Federation (IDF), and the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III), proposed diagnostic guidelines to standardise

the identification (Jha et al., 2023; Russell et al., 2024). These guidelines underscored the syndrome's multifactorial nature and its increasing prevalence, driven by the global rise in obesity and sedentary lifestyles (Koliaki et al., 2023). Table 2.1 outlines the diagnostic criteria for MetS as defined by various organisations.

Table 2. 1: Diagnostic criteria for metabolic syndrome across different organisation

Parameter/criteria	**Harmonized criteria-2009**	**IDF-2005**	**NCEP ATP III-2001**
Criteria for diagnosing MeTs	Any 3 of the following:	Abdominal obesity along with any other 2	Any 3 of the following:
Waist circumference (cm) Men Women	 ≥ 90 cm ≥ 80 cm	 ≥ 90 cm ≥ 80 cm	 ≥ 102 cm ≥ 88 cm
HDL-C Men < 40 Women < 50	 < 1.03 mmol/L < 1.30 mml/L	 < 1.03 mmol/L < 1.30 mml/L	 < 1.03 mmol/L < 1.30 mmol/L
Blood pressure (mmHg)	130/85 mmHg	130/85 mmHg	130/85 mm/Hg
Fasting blood glucose	5.6 mmol/L	5.6 mmol/L	6.1 mmol/L
Triglyceride	≥ 1.70 mmol/L (150 mg/dL)	≥ 1.70 mmol/L (150 mg/dL)	≥ 1.70 mmol/L (150 mg/dL)

Adapted from Russell et al. (2024); IDF: International Diabetes Federation, NCEP ATP: National Cholesterol Education Program Adult Treatment Panel, HDL-C: High-density lipoprotein cholesterol.

2.2 Metabolic syndrome trends

MetS represents a growing global health issue. Its increasing incidence is primarily linked to the rising rates of obesity, physical inactivity, and the persistent consumption of unhealthy diets

(Castro-Barquero et al., 2020). It affects approximately 20 to 25% of adults worldwide, with notable regional variations (Bhalwar, 2020). MetS prevalence is 16.1% in Africa, 21.3% in Asia, 37.1% in the United States of America, 10.5% in Europe, and exceeds 21.5% in mainland China (Ye et al., 2023). Urbanised and industrialised regions report higher prevalence rates due to lifestyle shifts and dietary changes (Nsabimana et al., 2024). Age and gender are key factors, as the prevalence of MetS generally increases with age and differs between sexes, influenced by population demographics and lifestyle factors (Jiang et al., 2018). Women often experience higher prevalence rates than men, particularly after menopause, due to hormonal changes that promote central obesity and increase the risk of metabolic complications (Opoku et al., 2023; Raman et al., 2023). Older adults exhibit higher MetS prevalence compared to younger individuals, as age-related changes, including increased insulin resistance, central adiposity, and declining physical activity contribute to its development (Chang & Halter, 2003; Palmer & Jensen, 2022). Alarmingly, MetS is emerging among children and adolescents, posing long-term health risks (Kelishadi et al., 2016). Recent reports show that approximately 3% of children aged 6 to 12 years and 5% of adolescents aged 13 to 18 years globally are affected by MetS (Noubiap et al., 2022). This early onset of MetS highlights the need for proactive measures to target the lifestyle factors driving the increasing prevalence of MetS in younger populations (Pacheco et al., 2019).

2.3 Components of metabolic syndrome

2.3.1 Central obesity

Central obesity, marked by excess abdominal and visceral fat, is a critical feature of MetS and a significant indicator of metabolic health risks (Paley & Johnson, 2018). The increasing global prevalence of obesity highlights its growing impact on public health (Tiwari & Balasundaram, 2023). Globally, 1.9 billion adults are overweight, with 650 million are classified as having obesity (Haase et al., 2020). Projections indicate that by 2025, 2.7 billion adults will be overweight, with more than 1 billion classified as obese (Boutari & Mantzoros, 2022).

Between 1980 and 2019, global obesity incidence rose significantly, with the prevalence of obesity in men growing from 3.2% to 12.2%, and in women from 6% to 15.7% (Boutari & Mantzoros, 2022). Projections suggest that by 2030, 1 billion individuals globally, with one in five women and one in seven men, will be affected by obesity (Ahmed & Konje, 2023). Additionally, it is projected

that by 2025, 206 million children and adolescents aged 5 to 19 will be affected, with this number expected to increase to 254 million by 2030 (Jebeile et al., 2022).

Understanding the distinction between general and central obesity, as well as their measurement, is crucial in addressing the growing MetS epidemic (Safaei et al., 2021). General obesity refers to the overall accumulation of body fat and is commonly assessed through body mass index (BMI) (Freisling et al., 2017). In contrast, central obesity is measured using waist circumference (WC) or waist-to-hip ratio (WHR), which specifically reflect fat distribution in the abdominal region (Molla et al., 2020). Central obesity is particularly harmful to metabolic health, as it is closely linked to insulin resistance, dyslipidaemia, hypertension, and inflammation, all hallmark features of MetS (Fahed et al., 2022; Molla et al., 2020). Additionally, central obesity is a key risk factor for metabolic dysfunction-associated steatotic liver disease (MASLD), a condition characterised by fat accumulation in the liver, which often coexists with MetS and exacerbates metabolic dysfunction (Ding et al., 2023). Central obesity develops from an imbalance between caloric intake and energy expenditure and is further influenced by genetic predisposition, hormonal regulation, and environmental factors (Romieu et al., 2017; Mazza et al., 2024). This condition is primarily driven by accumulation of visceral adipose tissue (VAT), which surrounds internal organs in the abdominal cavity (Yamamoto et al., 2020). Unlike subcutaneous fat, VAT is metabolically active and secretes a variety of bioactive as adipokines (Clemente-Suárez et al., 2023). These adipokines include leptin, adiponectin, resistin, and the pro-inflammatory cytokines tumour necrosis factor alpha (TNF-α), interleukin 6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), and visfatin (Clemente-Suárez et al., 2023). Impaired adipokine secretion from VAT is a key factor in the development of MetS, as it promotes systemic inflammation, insulin resistance, and atherogenesis (Lau et al., 2017).

2.3.2 Insulin resistance

Insulin resistance develops when the body's cells exhibit reduced responsiveness to insulin, the hormone responsible for regulating glucose metabolism (Ormazabal et al., 2018). Normally, insulin facilitates glucose uptake into myocytes, adipocytes and hepatocyte, maintaining healthy blood glucose concentration (Chadt & Al-Hasani, 2020). In insulin resistance this process falters as cells fails to effectively absorb glucose, while the liver produces excess glucose, resulting in hyperglycaemia, a hallmark of T2DM (Dandona et al., 2017). Insulin resistance also reduces

glycogenesis, limiting the ability of hepatocytes and muscle cells to store glucose as glycogen; further contributing to hyperglycaemia (Rahman et al., 2021). Concurrently, altered fat metabolism increases the release of free fatty acids from adipose tissue and promotes lipid buildup in the liver and muscles, contributing to obesity and dyslipidaemia (Giudetti, 2023). This imbalance stimulates excess lipogenesis and disrupts cellular homeostasis, which triggers oxidative stress (Yoon et al., 2021). The process generates an excessive amount of ROS that damage cellular structures and worsen insulin signalling (Bhatti et al., 2022). Chronic low-grade inflammation, often triggered by adipose tissue in obesity exacerbates these effects by releasing pro-inflammatory cytokines, TNF-α and IL-6 that disrupt insulin function and amplify metabolic dysfunction (Rehman & Akash, 2016).

2.3.3 Dyslipidaemia

Dyslipidaemia occurs in two forms: primary and secondary. Primary dyslipidaemia results from inherited genetic mutations that interfere with lipid metabolism, while secondary dyslipidaemia arises from lifestyle choices or underlying medical conditions that impact lipid concentrations (Jacobsen et al., 2024). Elevated blood cholesterol affects 39% of the global adult population, with women experiencing a slightly higher prevalence at 40% compared to 37% in men (Ghazwani et al., 2023). Over the past 30 years, the global prevalence of dyslipidaemia has risen rapidly (Pirillo et al., 2021), significantly contributing to disease burden in both developed and developing countries (Nandasena et al., 2023).

Dyslipidaemia results from abnormal lipid concentration in the blood, marked by increased LDL-cholesterol and triglycerides, along with reduced HDL-cholesterol concentrations (Eslami & Shidfar, 2019). Dyslipidaemia contributes significantly to the onset of CVD, such as atherosclerosis and coronary artery disease (Jacobsen et al., 2024). This abnormal lipid concentration in the blood is closely linked to insulin resistance and central obesity, both of which worsen the lipid profile (Malik et al., 2019). In patients with dyslipidaemia, the accumulation of LDL-cholesterol in blood vessel walls leads to plaque formation, narrowing the arteries and heightening the risk of heart attacks, strokes, and other metabolic complications (Das & Ingole, 2023). Additionally, dyslipidaemia leads to lower-than-normal plasma HDL-cholesterol levels, which impairs the transport of excess cholesterol from periphery tissues to the liver for excretion, thereby reducing protection against cardiovascular diseases (Ahmed et al., 2016). Elevated plasma

triglyceride concentrations further aggravate cardiovascular risk (Lee et al., 2017) because the liver's impaired ability to process and clear these fats in insulin-resistant individuals contributes to high lipid concentrations (Heeren & Scheja, 2021).

2.4 Complications of metabolic syndrome

2.4.1 Metabolic dysfunction-associated steatotic liver disease

Metabolic dysfunction-associated steatotic liver disease (MASLD) is a chronic liver condition characterised by the accumulation of fat in the liver, exceeding 5% of its weight, in the absence of substantial alcohol intake (Gerges et al., 2021). It (MASLD) is increasingly recognised as hepatic manifestation of MetS and a global public health concern (Mitrovic et al., 2022). The prevalence of MASLD has increased considerably, reflecting the growing rates of obesity, insulin resistance and T2DM (Vetrano et al., 2023). Approximately 38% of people worldwide are affected by MASLD, with regional variations influenced by genetic, lifestyle, and metabolic factors (Wong et al., 2023). MASLD includes a range of conditions, from simple metabolic dysfunction-associated fatty liver, (MAFL), which is simple steatosis, to metabolic dysfunction-associated steatohepatitis (MASH), which manifests with hepatic inflammation and damage (Zhang et al., 2019). Over time, MASH can progress to advanced fibrosis, cirrhosis, and hepatocellular carcinoma, significantly increasing morbidity and mortality (Boldys & Buldak, 2024). The progression from benign steatosis to MASH is influenced by "second hit" mechanism, such as increased oxidative stress, the breakdown of lipid, and the activation of inflammatory cytokine, all of which contribute to the worsening of liver damage (Myint et al., 2023).

2.4.1.1 MASLD pathophysiology in metabolic syndrome

Components of MetS, especially insulin resistance and central obesity, are key contributors to the development of MASLD (Reccia et al., 2017). Insulin resistance disrupts normal lipid metabolism, leading to increased hepatic free fatty acids uptake and upregulation of *de novo* hepatic lipogenesis (Smith et al., 2020). These processes contribute to triglycerides accumulation in hepatocytes, a hallmark of MASLD (Smith et al., 2020). Concurrently, impaired VLDL secretion and reduced fatty acid β-oxidation exacerbate hepatic lipid overload (Bhatt & Smith, 2015). Adipose tissue dysfunction in MetS further aggravates MASLD by releasing the pro-inflammatory cytokines TNF-α and IL-6, which mediate hepatic inflammation and fibrosis (Basil et al., 2024).

Additionally, increased lipid metabolism generates excessive ROS causing oxidative stress-mediated cell organelles damage and mitochondrial dysfunction resulting in hepatocyte injury and liver disease progression (Shi et al., 2021).

2.4.1.2 Clinical implications

MASLD is strongly associated with an increased CVD risk, a leading cause of death in patients with this condition (Targher et al., 2020). Furthermore, MASLD exacerbates insulin resistance, creating a bidirectional relationship with T2DM (Caussy et al., 2021). Patients with MASLD are also at higher risk for chronic kidney disease (CKD), emphasising the clinical implications of this condition (Heda et al., 2021). MASLD diagnosis involves a combination of clinical evaluation, imaging techniques (ultrasonography or transient elastography), and the exclusion of other diseases (Ajmera & Loomba, 2021; Selvaraj et al., 2021). Liver biopsy is considered the gold standard for differentiating MASH from simple steatosis and evaluating fibrosis severity, but due to its invasive nature, it is not typically performed on a routine basis (Berger et al., 2019).

2.4.1.3 Management strategies

A healthy, low-calorie diet combined with regular physical activity forms the cornerstone foundation of lifestyle modifications designed to reduce hepatic fat content and improve insulin sensitivity (Ali et al., 2024). These foundational strategies are pivotal in the management of MASLD (Keating et al., 2024). A 7 to 10% reduction in body weight has been demonstrated to markedly decrease liver fat content, alleviate MASH, and lessen fibrosis and inflammation (Brunner et al., 2019). Pharmacological options, though limited, offer additional support. The insulin-sensitising agents; pioglitazone and GLP-1 receptor agonists, have shown promise in enhancing liver outcomes (Gastaldelli et al., 2021; Liu, 2024). Fenofibrate, a PPAR-α agonist, is extensively used to treat dyslipidaemia and lowers plasma LDL-cholesterol and triglyceride concentrations (Kumar Dan et al., 2022) and it has also shown potential to attenuate hepatic inflammation thus improve liver health in patients with MASLD (Mahmoudi et al., 2021).

2.4.2 Type 2 diabetes mellitus

T2DM is a long-term metabolic condition characterised by insulin resistance and impaired glucose homeostasis (Goyal et al., 2023). It is strongly associated with central obesity, as visceral fat secretes pro-inflammatory cytokines that exacerbate insulin resistance (Wondmkun, 2020). Dyslipidaemia which is marked by higher plasma triglycerides and lower HDL cholesterol levels,

commonly coexists with the condition thus heightening cardiovascular diseases risk (Strikić et al., 2023). T2DM significantly contributes to MASLD development by promoting hepatic fat accumulation through hyperglycaemia (Targher et al., 2021). The disease's progression increases the risk of the associate complications: neuropathy, retinopathy and kidney disease (Alweendo et al., 2023). Lifestyle interventions, such as adopting a healthy balanced diet and staying physically active through regular exercise, are essential for managing this metabolic disease (Galaviz et al., 2015). Pharmacological agents including metformin, GLP-1 receptor agonists, and SGLT2 inhibitors used to treat T2DM enhance insulin sensitivity and regulate blood glucose concentration (Gastaldelli et al., 2021; Liu, 2024). Early diagnosis and proactive management remain crucial to preventing disease progression and associated complications (Antar et al., 2023).

2.4.3 Chronic kidney disease

Kidney disease, a growing global health concern, is characterised by the gradual decline in kidney function (Géza Pethő et al., 2024). It often results from risk factors such as high blood pressure, diabetes, and excessive body weight, which are hallmarks of metabolic dysfunction that contributes to renal vasculature and nephron damage (Zoccali et al., 2023). Chronic kidney disease progresses silently, with patients frequently unaware of declining function until later stages (Wolpert et al., 2023). Early intervention and management, including controlling blood pressure and blood glucose concentration, can slow disease progression, especially in individuals with MetS (Shubrook et al., 2022). In advanced stages, dialysis or kidney transplantation may be necessary. Preventive measures, which include lifestyle modifications, are essential for reducing the global burden of kidney disease (Okpechi et al., 2024).

2.4.4 Cardiovascular diseases

Cardiovascular diseases remain the leading cause of death worldwide, creating substantial health and economic challenges (Amini et al., 2021). Atherosclerosis, driven by lipid accumulation and chronic inflammation, is the primary mechanism in CVD development (Malekmohammad et al., 2021). Insulin resistance and hypertension, which co-exist with MetS, intensify endothelial dysfunction, accelerating disease progression (Petrie et al., 2018). Obesity, poor dietary habits, and physical inactivity significantly contribute to increased CVD prevalence (Wang et al., 2024). Dyslipidaemia, characterised by elevated plasma LDL-cholesterol and triglycerides concentrations, further promotes atherogenesis (Xiao et al., 2016). Lifestyle modifications,

including consumption of a balanced diet and regular exercise in combination with targeted pharmacological therapies, slow disease progression (Kaminsky et al., 2022). Early diagnosis and proactive management are vital to reducing CVD-related morbidity and mortality (Almansouri et al., 2024).

2.4.5 Bone disorders and osteoporosis

Bone health is vital for overall well-being. The skeletal system provides structural support and protects vital organs (Wilson-Barnes et al., 2022). Osteoporosis, characterised by weakened bones and increased fracture risk, often results from an imbalance between bone resorption and formation (Pouresmaeili et al., 2018). Advancing age, hormonal changes, physical inactivity, and inadequate intake of calcium and vitamin D contribute to the development of osteoporosis (Voulgaridou et al., 2023; Smit et al., 2024). Metabolic dysfunction, insulin resistance and chronic inflammation associated with MetS, have been linked to impaired bone remodelling and increased osteoporosis risk (Martiniakova et al., 2024). The condition is more prevalent in postmenopausal women, due to reduced estrogen levels (Charde et al., 2023). Early detection through bone mineral density tests and lifestyle changes, including weight-bearing exercise and proper nutrition are essential for maintaining bone health (Rondanelli et al., 2022).

2.4.6 Haematological changes

Haematological changes reflect the systemic impacts of metabolic dysfunction on metabolic health (Williams et al., 2023). Insulin resistance and inflammation have been reported to alter red and white blood cell indices, increasing oxidative stress and vascular damage (Szalai et al., 2023; Bambo et al., 2024). Metabolic syndrome has been shown to heighten platelets activation, increasing the risk of thrombosis (Barale & Russo, 2020). Monitoring haematological parameters provides early insight into metabolic derangements. Addressing metabolic risk factors through lifestyle modification can improve haematological profiles and reduce associated health risks (Essawi et al., 2023).

2.5 Metabolic programming

Metabolic programming describes how early-life environmental factors, including nutrition, hormonal signals, and stressors, shape long-term metabolic health and disease risk (Brasiel et al., 2020). Prenatal and early postnatal periods are critical windows during which metabolic pathways

are established, and disruptions during this time of developmental plasticity can have lasting effects (Daoust et al., 2021). Poor maternal nutrition, overnutrition, or exposure to stress during pregnancy can alter fetal development, leading to changes in insulin sensitivity, adiposity, and energy balance (Şanlı & Kabaran, 2019). These changes are mediated through epigenetic processes like DNA methylation and histone modification, which influence gene expression without altering the genetic code (Aboud et al., 2023).

Maternal obesity and gestational diabetes have been shown to increase the offspring's susceptibility to metabolic disorders and diseases including obesity, T2DM, and cardiovascular diseases (Ormazabal et al., 2022). Similarly, early-life exposure to high-fat or high-fructose diets can program pathways that predispose individuals to metabolic derangements in adulthood (Zheng et al., 2016; Block & El-Osta, 2017). Interventions during this critical developmental period, for instance through balanced maternal diets, breastfeeding, and appropriate post-weaning nutrition can mitigate adverse outcomes and promote healthier metabolic profiles (Martín-Rodríguez et al., 2022). Understanding metabolic programming underscores the need for preventive strategies targeting early developmental stages to curb the intergenerational transmission of metabolic dysfunction (Hsu et al., 2021).

2.6 Experimental models of metabolic programming

Various experimental models, among many chemical, genetic, and diet-induced approaches, are employed to investigate metabolic programming (Wong et al., 2016). These models offer crucial insights into how early-life environmental factors shape long-term metabolic health and contribute to disease susceptibility (Zhou et al., 2020) and or resistance**.**

2.6.1 Chemical intervention models

Chemically induced models of metabolic programming use chemical agents to alter physiological processes, mimicking the impact of environmental exposures on metabolism (Vatashchuk et al., 2022; La Merrill et al., 2024). These models allow researchers to investigate how early-life chemical exposures influence long-term metabolic outcomes and are widely used to study disorders like obesity, diabetes, and cardiovascular diseases (Sargis et al., 2019). Chemical agents, including toxins and endocrine disruptors, are applied to induce metabolic changes, enabling the assessment of their impact on gene expression and organ function (Hamid et al., 2021). For

instance, streptozotocin and alloxan are frequently used to induce diabetes in animal models by damaging pancreatic β-cells (Fajarwati et al., 2023; Ghasemi & Jeddi, 2023). While chemically induced models can replicate specific aspects of the pathophysiology of diet-induced metabolic disorders, their mechanisms differ. Diet-induced models rely on prolonged exposure (intake and or administration) to dietary insult(s) to trigger metabolic changes (Zhao et al., 2022), whereas chemically induced models rapidly induce specific conditions including impaired insulin sensitivity, diabetes mellitus, or liver dysfunction (Athmuri & Shiekh, 2023). However, chemically induced models do not fully capture the multifactorial nature of diet-induced pathophysiology, which involves the complex interplay of genetics, diet, and the environment (Flessa et al., 2022). Despite these limitations, chemically induced models remain valuable for exploring specific metabolic pathways and disease mechanisms (Wu & Yan, 2015; Murakami et al., 2022), even though they may not comprehensively reflect the broader spectrum of diet-induced metabolic diseases (Wong et al., 2016).

2.6.2 Genetic models

Genetically induced metabolic programming involves modifying the genome to examine its impact on metabolic pathways and long-term health outcomes (Abraham et al., 2023). Genetic modifications, for example, gene knockouts or overexpression, to induce specific metabolic alterations are used (Lee et al., 2020; Eisenhut et al., 2024). An interesting example is the ob/ob mouse model, which is leptin deficient, is widely used to study obesity and metabolic disorders, while the db/db mouse model, which lacks leptin receptors, is employed to explore diabetes-related pathologies (Frühbeck et al., 2017; Fuchs et al., 2018). These models allow researchers to explore how genetic factors contribute to metabolic diseases like obesity, diabetes, and cardiovascular disorders (Górczyńska-Kosiorz et al., 2024). By altering specific genes, scientists can observe changes in metabolic pathways, helping to pinpoint genetic predispositions to these conditions (Li et al., 2020). Genetically induced models provide insights into the molecular mechanisms underlying metabolic diseases and offer potential targets for therapeutic interventions (Sulaiman, 2024).

2.6.3 Diet-based models

Dietary interventions during critical developmental periods alter metabolism through diet-induced metabolic programming (Bonet et al., 2024). These models highlight the impact of early-life nutrition on future health, particularly in increasing the risk of obesity, diabetes, and heart disease (Rajamoorthi et al., 2022). Adjusting macronutrient composition or calorie intake replicates the effects of poor or excessive diets in humans and animal research models (Crean et al., 2024). High fructose significantly impacts metabolism, leading to insulin resistance, obesity and liver dysfunction, which mirror metabolic disturbances observed in human conditions (Dornas et al., 2015). Diet high in fat, cholesterol, and carbohydrates further disrupt metabolic pathways contributing to similar metabolic alterations (Franco-Juárez et al., 2021; Sacks & Andraski, 2021). This programming highlights how excessive intake, particularly of fructose, drives long-term health complication such as obesity and T2DM (Taskinen et al., 2019).

2.7 High fructose diets and their role in metabolic syndrome pathogenesis

Fructose, a monosaccharides present in various fruits, vegetables, and honey, has become a significant focus in metabolic health research (Agarwal et al., 2024). Over the past few decades, its consumption has risen dramatically, largely due to its inclusion in processed foods and sweetened beverages in form of high-fructose corn syrup (Malik & Hu, 2015). This increased dietary intake has raised concern about its impact on health, particularly in contributing to the development of MetS (Khitan & Kim, 2013). Different mechanisms have been suggested to elucidate the pathogenesis of fructose-induced metabolic derangements, all linked to the unique way fructose is metabolised in the liver and other tissues (Herman & Birnbaum, 2021).

The liver primarily metabolises fructose, following a unique pathway that bypasses key regulatory steps of glycolysis (Feinman & Fine, 2013). Upon entering hepatocytes, fructose undergoes rapid phosphorylation by fructokinase, converting it into fructose-1-phosphate, a process that requires ATP (Dholariya & Orrick, 2022). This unregulated pathway drives an excess flow of substrates into lipogenesis, leading to increased triglyceride synthesis and promotes hepatic fat accumulation (Geidl-Flueck & Gerber, 2023). Fructose metabolism, in contrast to glucose, does not trigger substantial insulin release and does not depend on insulin for cellular absorption (Softic et al., 2017), as it is transported into cells via glucose transporter 5 (GLUT5), whereas glucose uptake is

regulated by insulin through GLUT4 (Chadt & Al-Hasani, 2020). This allows fructose to bypass the usual energy regulation mechanisms, contributing to metabolic dysregulation (Lodge et al., 2024). Additionally, excessive fructose intake promotes the production of uric acid as a byproduct, which induces oxidative stress and inflammation (Baharuddin, 2024). Furthermore, fructose disrupts lipid profiles by stimulating the secretion of VLDLs, exacerbating systemic insulin resistance (Zhang et al., 2017).

Although the liver serves as the main organ for fructose metabolism, other tissues also contribute to its metabolic effects (Tesz & Bence, 2020). In the kidneys, fructose metabolism generates uric acid locally, exacerbating oxidative stress and potentially causing kidney damage (Jung et al., 2020). In adipose tissue, it stimulates lipogenesis, enhancing fat storage (Azevedo-Martins et al., 2024). In the small intestine, fructose is partially metabolised to glucose and lactate before releasing it into the bloodstream, where it contributes to systemic metabolic effects (Jang et al., 2018). Beyond its metabolic effects on various tissues, fructose disrupts satiety and energy regulation by reducing leptin production and impairing appetite control (Johnson et al., 2023). This disruption interferes with normal weight regulation, promoting overeating and increased caloric intake (Johnson et al., 2023). Excessive fructose intake also activates the brain's reward pathways, further encouraging overeating (Luo et al., 2015). These combined effects significantly contribute to the development of obesity, MASLD, and other components of MetS (Lara-Romero & Romero-Gómez, 2024). Recent studies underscore fructose's role in promoting inflammation and oxidative stress, which amplify metabolic dysfunction (Cheng et al., 2022; Baharuddin, 2024). Upon metabolism, fructose triggers the production of pro-inflammatory mediators and increases oxidative stress, both of which disrupt normal cellular functions (Gomez-Pinilla et al., 2021). This creates a vicious cycle, where inflammation and oxidative stress perpetuate metabolic disturbances particularly insulin resistance and hepatic fat accumulation (Onyango, 2022). These mechanisms feed into one another, amplifying their negative impact and accelerating the progression of metabolic syndrome (Masenga et al., 2023). The severity of these effects varies, depending on the dose and duration of fructose exposure, as well as factors such as age, sex, genetic predisposition and baseline metabolic health (Ismaiel & Dumitrascu, 2020; Aoun et al., 2022; Mcvicker et al., 2024; Fadhul et al., 2025). Individuals with pre-existing metabolic conditions, for example, obesity

or glucose intolerance, display a more pronounced and accelerated development of metabolic dysfunction (Piché et al., 2020).

Recognising fructose as a major contributor to metabolic disturbances is crucial for addressing MetS (Hannou et al., 2018). While naturally occurring fructose in whole fruits poses minimal risk due to the presence of fibre and other nutrients (Dreher, 2018), excessive fructose intake from processed foods remains a significant concern (Barreto et al., 2022). Therefore, identifying therapeutic and prophylactic interventions with minimal side effects is essential to address diet-induced metabolic disorders, including MetS. A deeper understanding of fructose's role can lead to targeted strategies that prevent or reverse MetS and its associated comorbidities.

2.8 Management strategies for metabolic disorders

Management strategies for metabolic disorders focus on addressing the underlying causes, improving lifestyle factors like diet and exercise, and incorporating pharmacological interventions to prevent complications and promote overall health (Martemucci et al., 2024).

2.8.1 Lifestyle interventions

The high prevalence of metabolic disorders, observed in both adults, children and adolescents, is strongly linked to the overconsumption of calorie-dense foods combined with a sedentary lifestyle (Ruiz et al., 2019; Almoraie et al., 2021). Lifestyle interventions address these challenges by focusing on diet, physical activity, and behavioural changes (Katangwe-Chigamba et al., 2024). Adopting a balanced, nutrient-rich diet that limits processed foods and added sugars, promotes a healthy metabolism and supports weight management across all age groups (Cena & Calder, 2020). Regular physical activity improves insulin sensitivity, lowers blood pressure, and enhances cardiovascular health (Nystoriak & Bhatnagar, 2018). Behavioural modification including stress reduction techniques and adequate sleep are essential for optimal metabolic function (Portell et al., 2023; Mukherjee et al., 2024). These interventions not only prevent the onset of metabolic disorders but also reduce complications in individuals already affected (Kim et al., 2021). Incorporating lifestyle changes into daily routines promotes sustainable improvement by enhancing metabolic health and preventing the progression of associated diseases over time (Dyer, 2023).

2.8.2 Conventional pharmacological treatment

Pharmacological treatments for MetS target various components of the condition: insulin resistance, dyslipidaemia, hypertension, and inflammation (Swarup et al., 2024). Commonly used conventional pharmacological agents, include statins, which lower cholesterol concentrations and reduce cardiovascular risk (Sizar et al., 2024), and metformin, which enhances insulin sensitivity and regulate blood glucose concentrations (Horakova et al., 2019). Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers are frequently recommended for managing hypertension and provide extra protective to the kidneys (Chen et al., 2024). In this context, fenofibrate emerge as a targeted treatment for managing dyslipidaemia, a hallmark of MetS (Deerochanawong et al., 2024).

Fenofibrate stimulates peroxisome proliferator-activated receptor alpha (PPAR-α), which boosts lipid metabolism and leads to a significant decrease in plasma triglycerides concentrations (Zhang et al., 2022). It also increases HDL-cholesterol and lowers LDL-cholesterol concentrations, thereby improving the overall lipid profile (Kim et al., 2019). Additionally, fenofibrate mitigates hepatic fat accretion, alleviating MASLD, a common comorbidity of MetS (Esler & Bence, 2019). Beyond lipid regulation, it demonstrates anti-inflammatory effects by influencing cytokine production and reducing oxidative stress, which together help mitigate cardiovascular risks and improve metabolic health (Jin et al., 2023). However, its use is associated with adverse effects, including liver cell damage, gastrointestinal discomfort, and muscle-related complications, including myopathy (Duan et al., 2018; Zhou et al., 2020; Mahmoudi et al., 2022). Pharmacological agents, including fenofibrate, are effective in managing metabolic syndrome; however, they are often costly, associated with side effects, and may be inaccessible to low-income populations (Kim et al., 2024; Li et al., 2024).These limitations underscore the importance of exploring alternative, long-term prophylactic interventions to prevent diet-induced metabolic derangements effectively.

2.8.3 Phytotherapy

Phytotherapy, the use of plant-derived medicines, has become an increasingly popular approach to managing MetS (Faris Abdulghani et al., 2024). Many medicinal plants contain bioactive compounds that can regulate lipid metabolism, reduce oxidative stress, and enhance insulin

sensitivity (Wang et al., 2020; Ansari et al., 2023; Huang et al., 2024). These plant-based interventions often come with fewer side effects compared to synthetic pharmacological agents, making them an attractive alternative for long-term disease management (Rahman et al., 2022). Additionally, they are generally more affordable and widely accessible, further contributing to their appeal in diverse populations (Eshete & Molla, 2021). One such plant-derived compound is lycopene, a potent antioxidant found in tomatoes and other red fruits (Ramírez et al., 2022). Lycopene has shown promise in improving metabolic health by reducing inflammation, regulating lipids, and enhancing insulin sensitivity (Saeed et al., 2020; Kulawik et al., 2024). As a result, it represents a valuable phytotherapeutic option in the prevention and management of MetS.

The next section, following the references contains the published review article on lycopene, which was the compound used in this study.

2.9 References

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2.10 Lycopene: A potent antioxidant with multiple health benefits – Published review article

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2.10.1 Summary

Lycopene is a naturally occurring carotenoid predominantly found in tomatoes and tomato-based products. Like other phytochemicals, it exhibits health beneficial biological activities that can be exploited when it is used as a dietary supplement. *In vitro* and *in vivo*, lycopene has been demonstrated to mitigate oxidative stress-induced metabolic dysfunctions and diseases including inflammation, obesity, and diabetes mellitus. Lycopene has been shown to alleviate metabolic diseases that affect the bone, eye, kidney, liver, lungs, heart, and nervous system. This review presents the state of the art regarding lycopene's health benefits and its potential applications in health system delivery. Furthermore, lycopene's protective effects against toxins, safety in its use, and possible toxicity are explored.

2.10.2 Introduction

The use of medicinal plants has deep historical roots, ingrained in the traditional healing practices of diverse cultures worldwide (Petrovska, 2012). Throughout centuries, indigenous communities and ancient civilizations have harnessed the therapeutic properties of plants, passing down invaluable knowledge through generations (Rasool Hassan, 2012). Ethnomedicine, a field dedicated to studying traditional medicinal practices, has played a crucial role in documenting this wealth of wisdom. The effectiveness of ethnomedicinal plants in disease management is attributed to their constituent bioactive phytochemicals, such as carotenoids, which are known to have multiple health benefits (Upadhyay & Dixit, 2015). Lycopene, a fat-soluble carotenoid, is one of the most abundant and important carotenoids (Arballo et al., 2021). It has potent antioxidant activity (Przybylska, 2020). This carotenoid, a bioactive organic pigment, is found in pink grapefruit, papaya, guava, apricot, watermelon, and vegetables but is highly concentrated in tomatoes and tomato-derived products (Bin-Jumah et al., 2022). It has been reported to be one of the strongest antioxidants among carotenoids (Imran et al., 2020). As one of the most potent antioxidants, its capacity to neutralise singlet oxygen is double that of β-carotene, ten times greater than that of α-tocopherol, and one hundred and twenty-five times more effective than glutathione (Przybylska, 2020). Lycopene, isolated from *Lycopersicum esculentum* (tomato) in 1903, was named after the fruit from which it was isolated (Joshi et al., 2020). More than 85% of the lycopene in the diet is derived from tomatoes and tomato-based products (Joshi et al., 2020). In addition to fruits and vegetables, lycopene is also found in some food ingredients, as shown in Table 2.2

(Bramley, 2000; Shi & Le Maguer, 2000). While overall tomatoes are a good source of lycopene, research has demonstrated that different tomato and other fruit varieties have different lycopene content (Imran et al., 2020). In addition to varietal differences, the microenvironment in which the tomato and or other lycopene-containing fruit are grown, for example,

Table 2. 2: Lycopene concentration in fresh fruits and processed food products

Fruit/processed food product	Lycopene content (mg/100g)
Apricot Fresh tomatoes	0.11-5.3
Carrot	0.65-0.78
Cooked tomatoes	3.70
Fresh tomatoes	0.72-4.2
Ketchup	9.90-13.44
Papaya	0.11-5.3
Pink grapefruit	0.35-3.36
Pink guava	5.23-5.5
Pumpkin	0.38-0.46
Rosehip	0.68-0.71
Sweet potato	0.02-0.11
Tomato paste	5.40-150
Tomato sauce	6.20
Watermelon	2.30-7.20

Source: Bramley, 2000; Shi & Le Maguer, 2000

temperature, humidity, edaphic conditions, and fruit maturity status at harvest also influence lycopene content (Grabowska et al., 2019). Where the soil microbiome has favourable microbes, a 36% increase in lycopene has been reported (Grabowska et al., 2019).

Several studies have investigated the potential of lycopene to mitigate risk factors for obesity, type 2 diabetes mellitus, and cardiovascular diseases; conditions characterised by dyslipidaemia, oxidative stress and inflammation (Świątkiewicz et al., 2023). These studies have shown that lycopene improved outcomes of these metabolic diseases (Abir et al., 2023a). Lycopene, known for its antioxidant properties, has been found to reduce oxidative stress, a significant contributor to the development of metabolic diseases (Guo et al., 2023). Additionally, it has been shown to mitigate inflammation and dyslipidaemia, thereby reducing the risk of cardiovascular diseases and insulin resistance (Rane et al., 2019; Tierney et al., 2020). Research suggests that regular consumption of lycopene as a dietary supplement can potentially remediate insensitivity to insulin, hypertension, and obesity-related metabolic complications (Mounien et al., 2019; Rejali et al., 2022).

2.10.3 Lycopene: Biochemistry and physical properties

In nature, over 750 carotenoids have been identified (Arunkumar et al., 2020). About 40 to 50 are found in the human diet, and lycopene is the sixth most common carotene in food products (Doyle, 2020; Meléndez-Martínez, 2020). Two main categories of carotenoids exist: hydrocarbon carotenoids and xanthophylls. Hydrocarbon carotenoids such as α, β-, and γ-carotene lycopene are made up of hydrogen and carbon, while xanthophylls, for example, lutein, β-cryptoxanthin, and zeaxanthin, contain oxygen along with carbon and hydrogen (Holzapfel et al., 2013; Arballo et al., 2021). Lycopene, as an aliphatic straight-chain hydrocarbon, contains two unconjugated double bonds and 11 conjugated bonds (Kumar et al., 2017). Its conjugated double bonds are subject to isomerization through heat, light, and chemical reactions (Doyle, 2020). Lycopene is found in *trans*- and *cis*-isomers, but the *cis*-isomers are better absorbed and have greater bioavailability than *trans*-lycopene (Zhu et al., 2020; Caseiro et al., 2020). All-*trans*, 5-*cis*, 9-*cis,* 13-*cis*, and 15-*cis* are the most common forms of lycopene isomers, and the 5-*cis* isomer is the most stable isomer (Rao & Rao, 2007; Honda et al., 2017). The molecular structure and physical properties of lycopene are shown in Figure 2.1 (Gupta et al., 2018) and Table 2.2, respectively (Amjad et al., 2020; Joshi et al., 2020).

2.10.4 Lycopene: Absorption, transportation, and distribution

Following ingestion, lycopene released from the food matrix combines with micelles containing bile salts, cholesterol, and fatty acids (Story et al., 2010) and is then absorbed. Due to its hydrophobicity, the dissolution of lycopene within micelles in the small intestines facilitates its absorption (Przybylska, 2020) through the passive diffusion of lipids across the unstirred water layer in the enterocytes (Yeap et al., 2013). Inside the absorptive enterocyte, lycopene, together with free fatty acids, monoglycerides, and fat-soluble vitamins, is packaged into chylomicrons and released into the lymphatic system for transportation into the bloodstream and liver (Kumar et al., 2017). A fibre-rich diet has been proven to decrease the absorption of lycopene. Such fibrous diets also mediate the adsorption of lycopene, resulting in an over 40% reduction in plasma lycopene (Failla et al., 2008). Several factors, among them alcohol, smocking, gender, age, hormonal status, and other dietary elements, affect the absorption of lycopene (Caseiro et al., 2020). As healthy individuals grow older, the bioavailability of lycopene tends to decrease, possibly due to age-related structural changes in the gastrointestinal tract that result in reduced absorptive efficiency (Durairajanayagam et al., 2014). Humans absorb about 10% to 30% of dietary lycopene; the rest is excreted through faeces (Durairajanayagam et al., 2014; Joshi et al., 2020). The lycopene in heated and processed tomato products is better absorbed compared to that from fresh, unprocessed tomatoes (Doyle, 2020). Thermal exposure during cooking and processing of lycopene-containing foods breaks the food matrix and converts the natural (all-trans) lycopene structure to its *cis* geometric isomer, which is 2.5 times better absorbed from the gastrointestinal tract (Chauhan et al., 2011; Petyaev, 2022). Following its absorption from the small intestines, lycopene is distributed to the various body tissues (Durairajanayagam et al., 2014). The assimilation of lycopene by the tissues from lipoproteins is mediated by certain membrane receptors known as scavenger receptor class B type 1 (SR-B1) and cluster of differentiation 36 (CD36) (Arballo et al., 2021). In humans, the concentration of lycopene in the testes is ten times greater than that found in other tissues (Joshi et al., 2020). This high concentration in the testes is followed by its concentration in the adrenal gland, liver, prostate, breast, pancreas, skin, colon, ovary, lung, stomach, kidney, adipose tissue, and cervix (Joshi et al., 2020). However, cis-lycopene is mainly distributed in the liver and adipose tissue (Zhu et al., 2020). Table 2.4 illustrates the concentration of lycopene in various human tissues (Rao & Agarwal, 2000; Kaur et al., 2017). Lycopene, the primary carotenoid found in human plasma, exhibits a half-life of approximately 2 to 3 days. Its

concentration in plasma and tissues ranges between 0.2-21.4 nmol/g and 0.15-21.36 nmol/g, respectively (Rao & Agarwal, 2000; Joshi et al., 2020). In their study, Zaripheh et al. (2003) reported that in rats, lycopene was most concentrated in the liver, adipose tissue, adrenal tissue, and spleen.

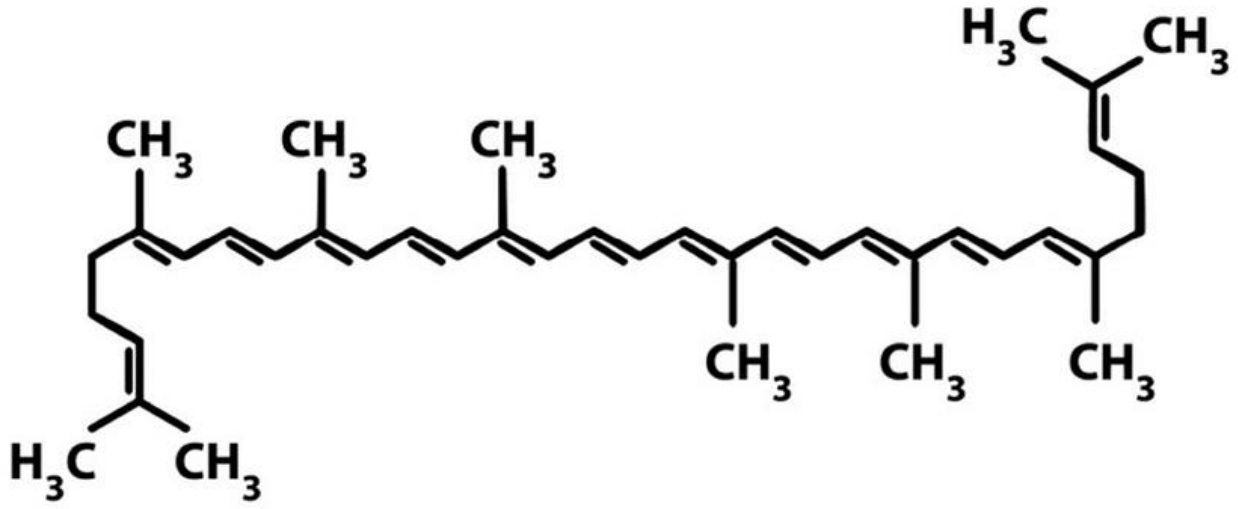


Figure 2. 1 : Molecular structure of lycopene

Table 2. 3: Physical properties of lycopene

Property	Value/Normal range
Boiling point	660.9°C at 760mmHg
Crystal form	Long red needles separate from a mixture of carbon disulfide and Ethanol
Density	0.889 gm. /cm3
Flash point	350.7°C
Main hazards	Combustible
Melting Point	172-175 ℃
Molecular Weight	536.85 Da
Powder form	Dark reddish-brown
Refractive index	1.531
Solubility	Soluble in chloroform, hexane, benzene, carbon disulfide, acetone, petroleum, tetrahydrofuran, carbon disulphide, ether, and oil; Insoluble in water, ethanol, and methanol
Stability	Sensitive to light, oxygen, high temperature, acids, catalyst, and metal ions
Vapour pressure	1.33·10−16 mmHg (25°C)

Source: Amjad et al., 2020; Joshi et al., 2020.

Table 2. 4: Lycopene concentration in some human tissues

Tissue	Lycopene (nmol/g wet weight)
Adipose	0.2-1.3
Adrenal	1.9-21.6
Brainstem	Not detectable
Breast	0.8
Colon	0.3
Liver	1.3-5.7
Lung	0.2-0.6
Ovary	0.3
Prostate	0.8
Skin	0.4
Stomach	0.2
Testis	4.4-21.4

Source: Rao & Agarwal, 2000; Kaur et al., 2017

2.10.5 Lycopene autoxidation

Known to be thermolabile, lycopene undergoes auto-oxidation when exposed to both light and oxygen (Kumar et al., 2017). The heat, light, and oxygen-induced lycopene degradation gives rise to acetone, methyl-heptenone, laevulinic aldehyde, and glycoxal, a colourless compound that produces a grass-like smell (Kumar et al., 2017). In addition to the attractive colour of the final lycopene degradation products, their biodegradation also affects their flavour and nutritive value (Tahmasebi & Emam-Djomeh, 2021).

2.10.6 Biological activities of lycopene

The meta-analyses and clinical trials of lycopene in human studies are shown in Table 2.5 below.

Table 2. 5: Meta-Analyses and Clinical Trials of Lycopene in Human Studies

Biological effects	Mechanisms of action	References
Anticancer	Suppressed cell proliferation, induced cell cycle arrest and increased apoptosis in breast cancer cell lines.	(Gloria et al.,2014)
	Decreased insulin-like growth factor-1 (IGF-1) and increased apoptosis in prostate cancer cell.	(Tjahjodjati et al., 2020)
Cardioprotection	Enhanced endothelial function and decreased triglyceride levels in patients with ischemic heart failure.	(Karimian et al., 2022)
	Increased flow-mediated dilation and total oxidative status decreased	(Xaplanteris et al., 2012)
	Increased HDL, Paraoxonase-1 (PON-1), Lecithin cholesterol acyltransferase (LCAT), decreased serum amyloid A (SAA) and cholesteryl ester transfer protein (CETP) activities	(McEneny et al., 2022)
Antidiabetic	Reduced levels of fasting blood glucose in patients with type 2 diabetes mellitus	(Inoue et al., 2023)
	Decreased glycated haemoglobin (HbA1c) levels and fasting blood glucose concentration	(Leh et al., 2021)
Anti-inflammatory	Inhibited NF-kB and c-Jun N-terminal kinase (JNK) activation. Suppressed the expression of COX-2, iNOS, TNF-α, IL-1β and IL-6	(Cha et al., 2017)
Antioxidant	Increased bone mineral density	(Sahni et al., 2009)
	Increased SOD, GSH-px and decreased MDA	(Xu et al., 2019)

Sperm quality enhancement and fertility promotion	Decreased lipid peroxidation and fragmentation of sperm DNA	(Ghyasvand et al., 2015)
	increased arachidonic acid to docosahexaenoic acid ratio	(Filipcikova et al., 2015)
	Reduced oxidative stress and enhanced sperm quality	(Nouri et al., 2019)
Hepatoprotection	Protection against steatosis and liver damage	(Donghia et al., 2024)
	Regulated oxidative stress and liver enzyme levels in individuals with metabolic syndrome.	(Mirahmadi et al., 2023)
Antiobesity	Decreased body weight, BMI, waist circumference, total cholesterol, and increased HDL levels	(Yao et al., 2021)
Reno-protection	Elevated levels of serum lycopene reduce the risk of mortality in individuals with CKD	(Zhong et al., 2022)
	Increased consumption of lycopene decreased the occurrence of CKD in women	(Shi et al., 2024)
Lung protection	Decreased airway neutrophil influx and decreased activity of neutrophil elastase in sputum.	(Wood et al., 2008)
	Increased SOD, CAT and decreased MDA, TNF-α, IL-1β and IL-6 levels in chronic obstructive pulmonary disease (COPD)	(Kırkıl et al., 2012)
Neuroprotection	Elevated serum levels of lycopene are associated with a decreased risk of Alzheimer's disease (AD) mortality in adults.	(Min & Min, 2014)
	Enhanced cognitive function in middle age	(Kesse-Guyot et al., 2014)
Gastroprotection	Decreased bleeding index and reduction in the percentage of gingivitis.	(Chandra et al., 2007)
	Increased consumption of lycopene improved bowel	(Wang et al., 2023)

	function and helped prevent chronic constipation	
Osteoprotection	Stimulated WNT/β-catenin and ERK1/2 pathways, increased the expression of RUNX2, alkaline phosphatase, and COL1A and decreased RANKL in Saos-2 cells.	(Russo et al., 2020)
	Increased total antioxidant capacity, decreased lipid peroxidation, protein oxidation and N-telopeptide of type 1 collogen	(MacKinnon et al., 2011)

2.10.6.1 Antiobesity effects

Obesity results from an excessive buildup of body fat. It has a detrimental effect on a person's metabolic health and overall well-being (Albrahim & Robert, 2022). The development of obesity is influenced by a variety of factors with complicated origins that involve psychological, environmental, socioeconomic status, and biological components (Sommer et al., 2015; Franks and McCarthy, 2016; Zenebe & Caroline, 2018). The risk of cardiovascular diseases, cancer, depression, dyslipidaemia, type 2 diabetes mellitus, non-alcoholic fatty liver diseases (NAFLD), and hypertension is heightened in obese individuals (Brahimaj et al., 2019; Lavie et al., 2021; Lazarus & Edward, 2022; Overby & Ferguson, 2022). Obesity elevates the prevalence of oxidative stress by disrupting the balance between oxidants and antioxidant activity (Masschelin et al., 2020), which leads to the presence of "unpaired mitochondria" (individual mitochondria within a cell that have not fused or aligned with others to form interconnected networks) and an upsurge in reactive oxygen species (Israel et al., 2021). Consequently, the normal functioning of adipose tissue is disrupted, resulting in an increased production of adipocytokines and a reduction in adiponectin levels, which contribute to the occurrence of metabolic syndrome (Hasan et al., 2018; Naomi et al., 2023). Numerous studies have reported on the health-beneficial antioxidant activity of lycopene. In male Wistar rats exposed to a high-fat diet for 12 weeks, supplementation with lycopene at 25 mg/kg body weight for a period of 4 weeks was shown to reduce plasma interleukin 6 (IL-6), tumour necrosis factor alpha (TNF-α), leptin, very low-density lipoprotein (VLDL), low-

density lipoprotein (LDL), and total cholesterol (TC), but it elevated plasma high-density lipoprotein (HDL) levels (Baz et al., 2022). The supplemental lycopene also reduced malondialdehyde (MDA) concentration but increased hepatic superoxide dismutase (SOD) and catalase (CAT) activities in the liver tissue, demonstrating that it (lycopene) potentially is a potent antioxidant that decreases hepatic oxidative stress by increasing systemic antioxidant and enzymes activities (Baz et al., 2022). Pre- and/or post-weaning supplementing Sprague-Dawley rat pups whose dams were fed a high-fat diet with lycopene at 1% improved the offspring's brown adipose tissue (BAT) development, reduced accumulation of white adipose tissue (WAT), and enhanced serum antioxidant capacity and blood glucose homeostasis (Senkus et al., 2021). In mice fed a high-fat diet, lycopene was shown to improve glucose and lipid metabolism and decrease body weight gain by stimulating WAT browning and activating BAT through modulation of peroxisome proliferator-activated receptor gamma (PPARG) (Zhu et al., 2020). In another study, where lycopene was administered at 25 and 50 mg/kg body weight for 3 months to male Wistar rats, results showed increased HDL, improved antioxidant, and oxidant biomarkers, decreased triglycerides (TG), LDL, apolipoprotein-B (Apo-B), and β-hydroxybutyrate, but boosted hepatic PPARG levels (Albrahim & Alonazi, 2021). Furthermore, tomato oleoresin, which contains 10 mg/kg body weight of lycopene, when orally administered to male Wistar rats for 6 weeks, mediated a significant increase in the expression of messenger RNA (mRNA) of adiponectin, forkhead box 01 (Fox01), fatty acid translocase/cluster of differentiation 36 (FAT/CD36), and sirtuin 1 (SIRTI), but downregulated PPARG expression in the adipose tissue of obese rats (Luvizotto et al., 2015).

2.10.6.2 Antioxidant effects

Oxidative stress is recognised as a significant contributing factor to an increased risk of cancer, the onset and progression of various metabolic and chronic disorders (Sharifi-Rad et al., 2020). The concept of oxygen radicals has been established for the past 50 years; however, its role in the advancement of diseases was discovered in the past two decades (Liu, 2020). In several biological processes that are vital for life, free radicals play an important role, such as the destruction of intracellular bacteria by phagocytes like macrophages and granulocytes (Sharifi-Rad et al., 2020). Excessive production of reactive oxygen species (ROS) causes protein, deoxyribonucleic acid (DNA), and lipid damage (Andrés et al., 2021).Damage to these cellular molecules leads to tissue

injury and interruption of vital cellular processes (Klran et al., 2023). Consuming diets rich in antioxidants or supplementing with bioactive molecules such as vitamins, tannins, and carotenoids may offer protection against oxidative damage (Gu et al., 2008). Carotenoids like lycopene are potent antioxidants that inhibit or hinder the advancement of diverse disorders triggered by ROS (Przybylska, 2020). Carotenoid antioxidant activity has been investigated in multilamellar liposomes by measuring the inhibition of the formation of thiobarbituric acid-reactive substances. Lycopene was shown to be the most potent antioxidant in the sequence: lycopene, ϒ-tocopherol, astaxanthin, canthaxanthin, α-carotene, β-carotene, bixin, zeaxanthin, lutein, α-tocopherol, glutathione, cryptoxanthin, crocin, and lipoic acid (Joshi et al., 2020; Leh & Lee, 2022). Lycopene attenuates ROS effects through radical addition or adduct formation, electron transfer to the radical, and allylic hydrogen abstraction (Bin-Jumah et al., 2022), and radical addition and allylic hydrogen abstraction contribute to its antioxidant effects (Santos-Sánchez et al., 2019). Lycopene has been reported to enhance the status of enzymatic (catalase, superoxide dismutase, and peroxidase) and non-enzymatic antioxidants like vitamins C and E from their radicals by increasing the cellular antioxidant defence system (Durairajanayagam et al., 2014). In addition, lycopene acts as an antioxidant in systems that produce singlet oxygen but behaves as a pro-oxidant in systems that create peroxide (Black et al., 2020). In low doses, it acts as an antioxidant, but at high doses, it acts as a pro-oxidant (Elvira-Torales et al., 2019). Factors such as lycopene concentration, tissue oxygen tension, and interaction with other antioxidants have been reported to influence the pro-oxidant potency of lycopene (Bin-Jumah et al., 2022). In situation where there is an imbalance between antioxidant defences and ROS production, such as during inflammation or exposure to environmental toxins (Poljšak & Fink, 2014), lycopene may switch from its antioxidant role to a pro-oxidant role (Black et al., 2020). Under these conditions, lycopene radicals may contribute to oxidative stress by reacting with cellular components and promoting further ROS generation (Lucas et al., 2022). Studies have suggested that under conditions of low oxygen levels, its antioxidant properties predominate (Saini et al., 2020; Varela et al., 2022).

2.10.6.3 Hypocholesterolaemic effects

An imbalance in the level of cholesterol in the body results in a lipid disorder known as hypercholesterolemia, a notable risk factor for atherosclerosis and related conditions such as coronary and cerebrovascular diseases (Gidding & Allen, 2019; Vaduganathan et al., 2022).

Several animal and human trials have investigated the association between lycopene and cholesterol. Male broiler chickens fed a standard grower diet supplemented with lycopene at 100 mg/kg body weight for three weeks had significantly reduced serum total cholesterol, triglyceride, very low-density lipoprotein, and increased high-density lipoprotein content compared with counterparts fed the control diet (Mezbani et al., 2019). In apolipoprotein E knockout mice fed a high-fat diet and lycopene supplementation at 60 mg/kg body weight daily for fourteen weeks, the administered lycopene significantly decreased both total cholesterol and triglycerides, beginning from the sixth week to the end of the experiment (Kim et al., 2022). Similarly, male Wistar rats given a high-fat diet and 50 mg/kg body weight of lycopene daily for three months had significant reductions in plasma total cholesterol, triglycerides, and low-density lipoprotein levels but increased high-density lipoprotein cholesterol compared to the group given a high-cholesterol diet (Albrahim, 2022). The reported cholesterol-lowering effects of lycopene are attributed to reduced cholesterol synthesis through the inhibition of the expression and activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and the modulation of LDL receptor activity (Palozza et al., 2012). The findings obtained from human studies have been inconsistent. In a systematic review and meta-analysis of 12 and 11 trial arms consisting of 781 and 854 participants, respectively, supplementation of lycopene significantly increased HDL-cholesterol levels when compared to the control group; however, no significant difference was observed in the triglyceride levels (Inoue et al., 2021). The conflicting findings observed from human studies could be attributed to the differences in study design, characteristics of the populations under investigation, and the source and dose of lycopene utilised (Tierney et al., 2020; Rattanavipanon et al., 2021).

2.10.6.4 Hepatoprotection

In a healthy human adult, the liver weighs approximately 1.5kg and is the largest gland and visceral organ (Beyoğlu & Idle, 2020). It plays a vital role in metabolic processes such as bile production, digestion, detoxification of xenobiotics, metabolism of lipids, proteins, carbohydrates, immune regulation, and storage of vitamins (Kalra et al., 2022; Mohammed, 2022). Among the major causes of global mortality is liver disease (Seto & Mandell, 2021). Liver diseases may be caused by several factors; viral infections, ischemia, alcohol-induced damage, autoimmune diseases, and genetic defects such as alpha-1 antitrypsin deficiency, hereditary hemochromatosis, citrin deficiency, hereditary fructose intolerance, cystic fibrosis, cholesteryl ester storage disease, type

IV glycogen storage disease, and Wilson disease (Scorza et al., 2014; Kong et al., 2019; Bouchecareilh, 2020). Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disease (Pouwels et al., 2022). Globally, the prevalence of NAFLD is about 25%, 13%, 23%, and 32% in Africa, Europe, and the Middle East, respectively (Maurice & Manousou, 2018). This disease is characterised by the accumulation of macrovesicular steatosis in ≥ 5% of hepatocytes without secondary causes such as alcohol intake, drugs, or liver diseases (Maurice & Manousou, 2018; Spiers et al., 2022). Patients with type 2 diabetes, dyslipidaemia and obesity are at increased risk of developing NAFLD (Han et al., 2023). Recent studies have shown that consumption of carotenoids such as lycopene can remarkably reduce the chances of developing liver diseases such as NAFLD (Elvira-Torales et al., 2019). In their study, Li et al. (2018), using beta-carotene-15,15'-oxygenase and beta-carotene-9',10'-oxygenase double knockout mice, the oral administration of lycopene at 2.3 mg/g for 24 weeks resulted in significantly decreased severity of hepatic steatosis and triglyceride levels but significantly increased sirtuin 1 and fatty acid oxidation compared to control counterparts fed a high-fat diet. Furthermore, lycopene mediated a decrease in inflammation. In a tramadol-induced hepatotoxicity rat model, supplemental lycopene at 15 mg/kg body weight for 15 days mitigated the hepatotoxicity by increasing antioxidant activity, reducing fatty acid breakdown and necrosis, lipid peroxidation, inhibiting DNA fragmentation, and apoptosis (Sadek et al., 2018) (Kadry et al., 2018). Lycopene administered at 5, 10, and 20 mg/kg body weight for 6 weeks in a rat model of NAFLD was shown to mediate hepatoprotective effects, as seen with reduced activities of aspartate transaminase and alanine transaminase and concomitant reductions in malondialdehyde, free fatty acids, and LDL cholesterol concentrations (Jiang et al., 2016). These findings were associated with elevated hepatic superoxide dismutase and glutathione concentrations, but with reduced cytochrome P450 2E1 and tumour necrosis factor-alpha expression and decreased hepatic fat (Jiang et al., 2016). The above-mentioned experimental studies provide a clear insight that the administration of lycopene not only inhibits ROS but also improves the activity of antioxidant enzymes, thereby providing beneficial effects against NAFLD.

2.10.6.5 Renoprotection

Chronic kidney diseases (CKD) have become a global public health issue, affecting more than 200 million people worldwide (Gori et al., 2021). Chronic kidney disease is a common term used to describe different disorders that permanently affect the structure and function of the kidneys for

over a period of three months (Santos-Araújo et al., 2023). This can be diagnosed when the abnormalities in kidney or glomerular filtration rate are lower than 60 ml/min/1.73m^2 and albuminuria is characterised by an albumin to creatinine ratio above 30 mg/g (Jankowski et al., 2021). Patients with CKD are more prone to developing end-stage renal disease, a condition that requires expensive management by either dialysis or kidney transplantation (Hasan et al., 2018). Patients suffering from CKD commonly display a high incidence of arrhythmias, venous thromboembolism, heart failure, and ischemic heart disease, which significantly increase mortality (Virani et al., 2020; Warrens et al., 2022). The increase in the prevalence of cardiovascular disease (CVD) in CKD patients is associated with oxidative stress, chronic inflammation, and vascular endothelial dysfunction (Ravarotto et al., 2018). These three factors create an intricate cycle, resulting in pathological variations and playing a crucial role in the initiation and progression of CVD in CKD patients (Akchurin, 2015; Rapa et al., 2019). Among these factors, oxidative stress is a key mediator in the intricate pathways linked to the progression of CKD (Rapa et al., 2019). As a result, the utilisation of antioxidant therapy is one of the significant approaches to averting and mitigating the advancement of CKD (Zhong et al., 2022). Lycopene is a potent antioxidant and an efficient free radical scavenger that has been investigated and shown to protect the kidney against chemically induced damage (El-Karm, 2019; Bedir et al., 2021). In female Wistar rats fed a high-fat diet, the supplementation of 200 ml of lycopene extract twice a week for 8 weeks significantly reduced plasma creatinine, urea, serum angiotensin-converting enzymes, renal tissue malondialdehyde, and C-reactive protein levels but increased total protein and tissue antioxidant enzyme levels (Khan et al., 2016). Shams et al. (2015) observed that lycopene protected against the advancement of diabetic nephropathy and improved renal function by inhibiting the advanced glycation product and its receptors (AGE-RAGE) pathway. Lycopene has been shown to inhibit LDL-cholesterol peroxidation, which can damage the kidneys (Zhong et al., 2022). Furthermore, supplemental lycopene was shown to decrease MDA, RAGE, and TNF-α levels in the kidneys of male Wistar rats fed a high fat diet for 6 weeks (Pierine et al., 2014), and similarly, lycopene orally administered at 25 and 50 mg/kg body weight daily for 3 months protected the kidneys of male Wistar rats fed a high fat diet by inhibiting the expression of nuclear factor kappa B, interleukin 1 beta, tumour necrosis factor alpha, decreasing oxidative stress, increasing nuclear factor erythroid 2-related factor 2, and stimulating B-cell lymphoma 2, hence shielding kidney tissue against damages (Albrahim & Robert, 2022).

2.10.5.6 Osteoprotection

Oxidative stress caused by reactive oxygen species influences the activity of both osteoclasts and osteoblasts (Antioksidan et al., 2017). This is thought to impact the pathogenesis of skeletal system disorders, including osteoporosis, the most common skeletal metabolic disease (Mehta et al., 2018). Osteoporosis often develops in older adults and is characterised by an alteration of the bone microarchitecture, typified by a decline in bone mineral density, which contributes to an elevated risk of fractures (Erdayanti et al., 2022). Such bone fractures notably occur at the distal forearm, vertebral column, and proximal femur (Cauley et al., 2014). Complications associated with osteoporosis, particularly hip fractures, result in a mortality rate that is four times higher in the global adult population (Erdayanti et al., 2022). Despite its preponderance in the elderly, osteoporosis has been shown to impact individuals of various age groups, but postmenopausal women are at high risk (Shaki et al., 2021; Bhatnagar & Kekatpure, 2022), due to a decrease in estrogen production which results in increased oxidative stress and osteoclast-induced bone resorption (Shihab et al., 2018). Studies have shown that children born to parents with a history of osteoporosis and fractures are more prone to the development of osteoporosis (Walallawita et al., 2020). In addition to genetic predisposition, poor nutrition, excessive alcohol consumption, smocking, caffeine intake, and medication side effects, for example, glucocorticoids can cause the development of osteoporosis (Tu et al., 2018; Godos et al., 2022; Muñoz-garach & Garc, 2020; Wang et al., 2022). Lycopene has been shown to have an advantageous effect on skeletal health (Xu et al., 2017). It has been shown to play a vital role in protecting postmenopausal women from experiencing bone loss by upregulating alkaline phosphatase, type 1A collagen, runt-related transcription factor 2, triggering the activation of the wingless-related integration site/beta-catenin and extracellular signal-regulated kinase 1/2 pathways, and downregulating receptor activator of nuclear factor kappa-B ligand (Transl et al., 2020). In mice fed a high-fat diet, supplemental lycopene at 15 mg/kg body weight for 10 weeks increased serum levels of total antioxidant capacity (T-AOC), SOD, and reduced the levels of MDA and AGEs, RAGE, and NF-kB expressions in the tibias and femurs (Xia et al., 2022). In male albino rats, orally administered lycopene at 30 mg/kg body weight once daily over an 8-week period mitigated glucocorticoid-induced osteoporosis (Mityas et al., 2019), and in diabetic male rats, lycopene suppressed bone resorption, enhanced osteopreotegerin and RANKL expression ratios by preventing oxidative

damage and reducing inflammation (Shan et al., 2021). These research findings demonstrate that lycopene has osteoprotective properties.

2.10.6.7 Anti-inflammatory effects

Inflammation is an immune response mechanism that is triggered when exposed to various harmful stimuli, such as damaged cells, microorganisms, poisonous and allergenic substances (Chen et al., 2018). It serves as a crucial stage in the process of tissue regeneration, repair, and remodelling, as well as the restoration of tissue haemostasis in impaired areas (Greten et al., 2020). Inflammatory mediators include the cytokines interleukin (IL)-1, IL-5, IL-6, IL-12, IL-1β, TNF-α, and interferon γ (Molnar et al., 2021), and chemokines like IL-8, monocyte chemoattractant protein 1, cyclooxygenase, vascular cell adhesion molecule 1, matrix metalloproteinase, free radicals, growth factors, and prostaglandins serve as regulatory mediators in the process of inflammation (Fernandes et al., 2015). On stimulation, these mediators activate endothelial cells, causing increased vascular permeability and the deployment of neutrophils, eosinophils, monocytes, and mask cells to the injury site, which helps eliminate the harmful agents and facilitate the healing process (Khan et al., 2021). However, inflammation is known to contribute to the development and progression of various diseases, including but not limited to CKD, cancer, diabetes mellitus, cardiovascular disease, NAFLD, obesity, asthma, rheumatoid arthritis, osteoporosis, autoimmune, and neurodegenerative disorders (Zhong & Shi, 2019; Franceschi et al., 2020; Agca et al., 2022). The consumption of natural antioxidants for maintaining human health has become popular, especially in developed nations (Karaköy et al., 2022). In a study using female Wistar rats, lycopene was shown to alleviate palmitic acid-induced neuro-inflammation by reducing oxidative stress and inhibiting the toll-like receptor 4 (TLR4) and nuclear factor kappa-B p65 (NF-kB p65) signalling pathways (Ugbaja et al., 2021). Lycopene supplementation mitigated metalaxyl-induced liver damage in male albino rats by restoring antioxidant status, improving liver function, and alleviating liver injury-associated complications (Hassan et al., 2018). In lycopene-treated endothelial cells, lycopene inhibited the activation of TNF-α but enhanced the expression of heme oxygenase-1 (HO-1) through the upregulation of nuclear factor erythroid 2-related factor 2 signalling pathways (Yang et al., 2017). Another experimental study reported that in male albino rats, orally administered lycopene at 10 mg/kg body weight for 21 days effectively protected the colon epithelial mucosa against acetic acid-induced colitis and oxidative injury (Hussein et al.,

2020). In C57BL/6 mice chronically exposed to cigarette smoke for 60 days, lycopene was shown to restore redox status and mitigate hepatic inflammation (Fonseca et al., 2021). In addition, Li et al. (2023) reported that lycopene mitigated the dysregulation of lipid metabolism and the inflammatory response induced by lipopolysaccharide in the rat testes. Thus, evidence is plentiful demonstrating the anti-inflammatory effects of lycopene both *in vitro* and *in vivo*.

2.10.6.8 Antidiabetic effects

Diabetes mellitus (DM) causes hyperglycaemia and, if inadequately managed, can result in damage to the heart, eyes, and kidneys (Saeedi et al., 2019). The global prevalence of diabetes is approximately 9.3%, which corresponds to about 463 million individuals. However, it is predicted to rise by 25% in 2030 and 51% in 2045 (Arroo & Brunelle, 2023). Diabetes mellitus is classified into three major types: type 1 (insulin-dependent), type 2 (non-insulin-dependent), and gestational diabetes mellitus (Sakran et al., 2022). Among these, type 2 diabetes mellitus predominates and accounts for about 90% of all cases worldwide (Saeedi et al., 2019).

Scientific evidence shows that lycopene can potentially be used to prevent and treat diabetes mellitus (Zhu et al., 2020). In streptozotocin-induced diabetes model, dietary fortification with lycopene mediated increased serum insulin concentrations, decreased urine and blood sugar concentrations, and reduced diabetes-induced pancreatic injury (Ozmen et al., 2016). In diabetic Wistar rats, orally administered lycopene at 40 mg/kg body weight significantly decreased serum MDA, cortisol, and blood glucose concentration but increased SOD, CAT, and glutathione peroxidase (GSH-Px) activities at 10, 20, and 40 mg/kg body weight (Eze et al., 2018). Furthermore, supplemental lycopene was shown to attenuate renal damage in diabetic rats (Xie et al., 2022). In STZ-induced diabetic rats, at 4 mg/kg body weight, lycopene ameliorated B-cell lymphoma-extra-large and B-cell lymphoma 2 (Bcl-2) concentrations and reduced the expression of Bcl-2-associated X protein (BAX) in the hippocampus (Soleymaninejad et al., 2017). Interestingly, orally administered lycopene was shown to increase SOD and GSH-Px activities and lower MDA concentrations in rat pancreatic tissue (Yin et al., 2019), but it mediated increased plasma insulin concentrations and reduced blood and liver lipid content, fasting blood glucose and glycosylated haemoglobin concentration, and homeostatic model assessment for insulin resistance in diabetic rats (Yin et al., 2019).

2.10.6.9 Anticancer effects

Cancer is a major global health challenge and is the second primary reason for mortality in the United States (Siegel et al., 2022)**.** The ingestion of tomatoes and tomato-based products has been associated with a reduced occurrence of different types of cancer (Xu et al., 2021)**.** *In vivo* and *in vitro* research has demonstrated that lycopene hinders the growth and multiplication of prostate cancer cells, inhibits the cell cycle, and induces apoptosis (Mirahmadi et al., 2020). Dietary supplementation with lycopene mitigated the growth of breast cancer cells by suppressing the activity of the insulin-like growth factor 1 receptor (IGF-1R) signalling pathway (Khan et al., 2021). While research shows that the consumption of a lycopene-rich diet could be beneficial in reducing the risk of pancreatic cancer (Mehta et al., 2018). In a rat model, the consumption of lycopene was shown to reduce the progression and proliferation of ovarian cancer (Holzapfel et al., 2017), and in human studies, cisplatin-based chemotherapy in combination with lycopene supplementation enhanced cervical cancer treatment (Aktepe et al., 2021). Furthermore, in animal models of hepatocellular carcinoma, administered lycopene suppressed the onset and development of cancer (Mekuria et al., 2020). In human colorectal adenocarcinoma cell line, treatment with lycopene was shown to exhibit genotoxicity, anti-proliferative, and apoptotic effects (Ataseven & Joha, 2023), a demonstration of its anticancer effects.

2.10.6.10 Gastroprotection

The incidence of peptic ulcer disease (PUD) has substantially increased, affecting approximately 5 to 10 percent of the general population (Abbasi-Kangevari et al., 2022). The corrosive effects of acid and pepsin on the gastro-duodenal mucosa cause peptic ulceration through exposure of the mucosa's lining to gastric acid and digestive enzyme actions (Tarasconi et al., 2020). Peptic ulcer disease is primarily caused by the extensive use of nonsteroidal anti-inflammatory drugs (NSAIDS) and *Helicobacter pylori* infection (Irshad et al., 2023). Other contributing factors include surgery, severe illness, burns, Zollinger-Ellison syndrome, excessive alcohol intake, smoking, psychological and physical stress (Lee et al., 2017; Asali et al., 2018; Yim et al., 2021). The excessive production of ROS is the major factor in stress-induced ulcers (Kumar et al., 2021). Thus, the utilisation of strong antioxidants may be beneficial in the management of ulcers (Zhang et al., 2020). In male Albino rats, lycopene administered at 200 mg/kg body weight for 10 days was shown to protect against ethanol-induced mucosa injury (Al-Razzuqi et al., 2018). In their

study, Chen et al. (2021) found that supplemental lycopene at 10, 50, 100, and 150 mg/kg body weight reduced gastric juice secretion in adult male Kunming mice when compared to the gastric injury control group. However, at high doses (150 mg/kg body weight), lycopene exacerbated absolute ethanol-induced acute gastric mucosal injury. In addition to mediating for protection against alcohol-induced gastrointestinal tract mucosal injury, lycopene has been shown to suppress gastric acid secretion and combat infection by *Heliobacter pylori* (Antioksidan et al., 2017).

2.10.6.11 Neuroprotection

Neurodegenerative diseases (NDs) are characterised by gradual loss of neurons and are associated with the formation of protein aggregates (Lamptey et al., 2022). These diseases are considered a major medical challenge as it affects millions of patients globally (Cho et al., 2018)**.** Alzheimer's, Parkinson's, Huntington's, prion and motor-neuro diseases, amyotrophic lateral sclerosis, spinocerebellar ataxia, and spinal muscular atrophy are common NDs (Lamptey et al., 2022; Duggirala et al., 2023; Dutta et al., 2023). Despite age being the leading factor in the onset of all neurodegenerative disorders, recent discoveries indicate that the combination of a person's genetic makeup and environmental influences can contribute to an elevated risk of developing NDs (Liu et al., 2022). Regardless of the various factors causing these NDs, a key feature common to all is the onset and development of neuronal cell death (Fricker et al., 2018). The progression of NDs is characterised by increased ROS production, which causes oxidative stress (Olufunmilayo et al., 2023). Administered lycopene has been shown to attenuate memory loss due to age, cognitive impairments, neuronal damage, and synaptic dysfunctions in the brain (Zhao et al., 2018). Additionally, lycopene was observed to mitigate age-related oxidative stress by suppressing lipid peroxidation and enhancing GSH, SOD, and CAT activities (Zhao et al., 2018). Dietary fortification with lycopene was demonstrated to decrease age-related neuroinflammation by attenuating microgliosis and combating inflammation (Zhao et al., 2018). Furthermore, lycopene mediated the reduction in the accumulation of amyloid beta 1-42 in the brains of aged CD-1 mice (Zhao et al., 2018) and when used as a supplement, it upregulated the mitogen-activated protein kinase (MARK)/extracellular signal-regulated kinase (ERK) signalling pathway, inhibited oxidative stress and neuronal apoptosis, and protected against bisphenol-induced neurotoxicity in the hippocampi of adult male rats (EL Morsy & Ahmed, 2020). It was also shown to decrease palmitic acid-induced brain oxidative stress and neuroinflammation and to inhibit the Toll-like

receptor 4 (TLR4)/nuclear factor kappa-light chain enhancer of activated B cells p65 (NF-kB-p65) pathway in female rats (Ugbaja et al., 2021). In mice with Alzheimer's disease induced by β amyloid, lycopene reduced oxidative stress, decreased neuronal loss, improved synaptic plasticity, and inhibited neuroinflammation (Guo et al., 2023).

2.10.6.12 Cardioprotection

Globally, cardiovascular diseases (CVDs) stand at the forefront as the leading cause of human mortality (Tierney et al., 2020). Studies have shown that in 2019, CVDs caused 17.8 million fatalities, and this trend is projected to increase by 2030 to 23 million (Przybylska & Tokarczyk, 2022). Several epidemiological studies have confirmed the significance of lycopene in preventing CVDs (Cheng et al., 2019). For instance, lycopene supplementation has been shown to reduce C-reactive protein levels, interleukin-6, pulse wave velocity, blood pressure, intercellular adhesion molecule 1 and enhance vascular health through flow mediated dilation of the endothelium (Cheng et al., 2017)**.** Lycopene supplementation at a dosage of 5 mg/kg body weight for 21 days was shown to confer protection against atrazine-induced cardiotoxicity in mice (Lin et al., 2016)**.** In Brown-Norway/Lewis rat model, lycopene treatment was demonstrated to have the potential to mitigate vascular arteriosclerosis in allograft transplantation by inhibiting Rho-associated kinases and by regulating the expression of nitric oxide/cyclic guanosine monophosphate signalling pathways (He et al., 2016), which indicates that lycopene has the potential to alleviate vascular arteriosclerosis. In another study**,** lycopene administered for 4 weeks at 10 mg/kg body weight reduced inflammation and apoptosis during post-myocardial infarction remodelling by suppressing the NF-KB signalling pathway in mice (He et al., 2015). Additionally, supplemental lycopene improves endothelial function in individuals suffering from CVDs (Gajendragadkar et al., 2014).

2.10.6.13 Lung protection

In male C57BL/6 mice, dietary lycopene supplementation at 25 or 50 mg/kg body weight mitigated cigarette smoke-induced pulmonary emphysema (Campos et al., 2019)**.** Literature shows that lycopene or matrine treatment alone offered minimal protection against lipopolysaccharide-induced acute lung injury in mice, but when co-administered, significant mitigatory effects were observed (Li et al., 2019). These results indicate that lycopene and matrine in combination may

function as an alternative to glucocorticoid therapy in treating acute lung injury (Li et al., 2019). In a study conducted by Mustra et al. (2021), supplemental lycopene at 90 mg/kg body weight for 22 weeks effectively suppressed tobacco carcinogen/cigarette smoke (NNK/CS)-induced emphysema, chronic bronchitis, and preneoplastic lesions. Furthermore, dietary lycopene significantly decreased NNK/CS-induced buildup of total cholesterol and upregulated mRNA expression of peroxisome proliferator-activated receptor alpha (PPARα), ATP-binding cassette (ABC) transporters ABCA1 and ABCG1, and liver X receptor alpha (LXRα) in the lungs of ferret model. These findings suggest that lycopene could act as a preventative agent against the adverse effects of tobacco smoke on lung health and lipid metabolism.

2.10.6.14 Sperm quality enhancement and fertility promotion

Infertility is a prevalent health problem that affects roughly 48 million couples and 186 million individuals globally (Ombelet, 2020). ROS-induced oxidative stress is a primary contributor to various reproductive complications (Ojo et al., 2023). In varicocele-induced rats, supplemental lycopene was shown to protect sperm against DNA damage by mediating upregulation of antioxidant responses that quenched ROS, which manifested with improved sperm viability, Johnson's score, membrane integrity, and the expression of B-cell lymphoma 2-associated X-protein (BAX) (Babaei et al., 2022). Similarly, in men with oligozoospermia, supplemental lycopene for 12 weeks at 25 mg/kg body weight attenuated oxidative stress and improved sperm quality (Nouri et al., 2019). In their study, Yamamoto et al. (2017) observed that the consumption of tomato juice with 30 mg of lycopene for a duration of 12 weeks increased plasma lycopene concentration and sperm motility and decreased the white blood cell count in the seminal plasma of the tomato juice group compared to the control group of infertile men. Dietary supplementation with lycopene at 20 mg per day for 3 months prior to the scheduled *in vitro* fertilization (IVF) treatment increased the arachidonic acid to docosahexaenoic acid ratio in the seminal fluid and resulted in 7 natural pregnancies in addition to 15 pregnancies following the IVF procedure (Filipcikova et al., 2015). In methotrexate-induced ovarian damage, pretreatment with lycopene at 5 mg/kg body weight for 5 days prevented infertility and was shown to mediate increased GSH activity as well as decreased MDA and myeloperoxidase concentrations (Turkler et al., 2020). These findings suggest that lycopene alleviates imbalances in polyunsaturated fatty acids and can serve as a preventive agent against infertility.

2.10.6.15 Protection of skin health

The skin, constituting approximately 15% of the total body weight (Doyle, 2020), plays a vital role in preventing excessive water loss from the body and maintaining the body temperature within an optimal range (Osilla et al., 2022). It provides protection against toxic substances, free radicals, physical damage, and ultraviolet radiation (Balić & Mokos, 2019). The latter causes the development of skin conditions and diseases through sunburn, photoaging, and excessive ROS production within the skin, which damages DNA and causes skin cancer (Brand et al., 2018; Balić & Mokos, 2019; Mogavero et al., 2021). Lycopene is extensively used as an ingredient in cosmetic products due to its demonstrated ability to protect the skin from aging and photodamage (Mogavero et al., 2021). Anbualakan et al. (2023) showed that lycopene can prevent and/or treat sunburn and photoaging and that it could potentially be effective against UV-induced skin cancers. As a dietary supplement, lycopene has been demonstrated to improve skin appearance and pigmentation and mitigate erythema (Zhang et al., 2023).

2.10.6.16 Protective effect on vision

Age-related ophthalmic conditions, inclusive of macular degeneration, glaucoma, cataracts, and diabetic retinopathy, are key contributors to gradual and permanent vision loss (Abu-Amero et al., 2016). In diabetic patients, serum lycopene concentration has been observed to be lower than normal (Li et al., 2018). Importantly, due to its consistently lower levels in diabetics, it has been suggested that serum lycopene concentration might serve as a diagnostic tool for diabetic retinopathy (Li et al., 2018). Using ARPE-19 cells derived from human retinal pigment epithelium, Goug et al. (2017) demonstrated that lycopene suppressed growth of human RPE cells against oxidative stress-induced cell loss findings which suggests that it (lycopene) may protect against RPE proliferative disease and old-age related macular degeneration. Oxidative stress and inflammation have been shown to be associated with the pathogenesis of eye-related conditions (Dammak et al., 2023). As a dietary supplement, lycopene has been proven to mitigate the risk of developing eye diseases associated with old age (Jiang et al., 2019). This could be due to its demonstrated ability to prevent cataract formation both *in vitro* and *in vivo* (Mehta et al., 2018).

2.10.7 Lycopene: Protective effects against toxins

Toxins are natural and harmful chemical substances that adversely impact health (Rameshrad et al., 2017). They cause specific organ toxicity, for example, skin, eye, kidney, liver, blood, cardiovascular, respiratory, reproductive, endocrine, immune, and nervous system damage (Rameshrad et al., 2017; Briffa et al., 2020). Through their actions, toxins disrupt homeostasis, alter gene expression, and cancer-related metabolic signalling pathways (Hedayati et al., 2019). Research has demonstrated that lycopene as a dietary supplement effectively mitigates the deleterious effects of myco-, bacterial and chemical toxins (Liu & Chen, 2016; El-karim, 2019; Karaca et al., 2019; Wan et al., 2022), fungicides (Macar et al., 2023), pesticides (Ahmed, 2015), herbicides (Zhu et al., 2022), and fluoride (Li et al., 2017). It is hypothesised that lycopene mediates protection against toxins through its potent antioxidant, chelating, and antiapoptotic properties (Hedayati et al., 2019).

2.10.8 Lycopene: Safety and potential toxicity

There is no specified daily prescription for dietary lycopene intake, but epidemiological studies have recommended an intake of 2 to 20 mg daily of lycopene (Saini et al., 2020). It has been shown that consumption of up to 100 mg of lycopene daily does not elicit adverse outcomes (Przybylska, 2020). In a toxicological study conducted on rats, feeding a diet fortified with lycopene at 1% (w/w) did not elicit any side effects (Jonker et al., 2003). Similarly, using lycopene at 200 mg/kg body weight per day as a dietary supplement has also been shown not to negatively impact animals (Gupta et al., 2003). Generally, it is asserted that lycopene can be used as a safe dietary supplement during pregnancy and lactation (Hanson et al., 2018). Although in pregnant women, high dietary intake of lycopene has been shown to mitigate the risk of developing preeclampsia (Kang et al., 2022). Imran et al. (2020) reported that excessive chronic consumption of tomato juice, a rich source of lycopene, caused lycopenemia. Findings from both animal and human studies suggest that although lycopene could generally be used as a safe dietary supplement, some caution must be exercised against excessive intake.

2.10.9 Conclusion

The extensive studies carried out on lycopene highlight its exceptional potential to promote overall health and well-being. Its varied spectrum of benefits places it as a potent natural compound which

can contribute to the promotion of health either as a prophylactic and ore therapeutic agent against metabolic diseases. In order to fully exploit its potential and increase its utility in health delivery, it is crucial to undertake additional research to comprehensively elucidate the health beneficial mechanisms underlying lycopene's medicinal properties. Furthermore, in order to enjoy optimal utility from the use of lycopene, there is need to evaluate and recommend effective dosages for efficacy and prevention of possible side effects of abnormally high doses.

2.10.10 References

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CHAPTER THREE: EFFECT OF LYCOPENE ON GROWTH PERFORMANCE AND GASTROINTESTINAL VISCERA MACROMORPHOMETRY OF WISTAR RATS FED A HIGH-FRUCTOSE DIET

3.0 Introduction

Dietary alteration during early life can increase susceptibility to metabolic disorders or promote better health outcomes in adulthood (Reynolds et al., 2015). Metabolic syndrome, a worldwide epidemic impacting individuals of various age groups (Bitew et al., 2021), is projected to affect approximately 3.3% of children and adolescents globally (Al-Hamad & Raman, 2017). Its prevalence ranges from 0.2% to 38.9% (Al-Hamad & Raman, 2017). The heightened prevalence of metabolic disorders is linked to an upsurge in fructose consumption, among various other factors (Aoun et al., 2022). Fructose is absorbed in the small intestine and metabolised majorly by the liver, through fructolysis, glycolysis, lipogenesis and gluconeogenesis (Hannou et al., 2018).

The gastrointestinal tract (GIT) plays a vital role in the digestion and absorption of nutrients (Basile et al., 2023). Excessive dietary fructose intake has been reported to disrupt energy balance, regulation of body mass, growth, and induce changes in organ morphology, particularly in the GIT system (Guney et al., 2023). Consuming high fructose impacts gut microbiota composition, causing an increase in lipopolysaccharides production from Gram-negative bacteria (Beisner et al., 2020). This shift in microbiota alters the morphometry of the GIT viscera and accessory organs (Zhang et al., 2017). This alteration may increase the likelihood of developing metabolic disorders (Taskinen et al., 2019). High fructose intake affects bone metabolism by disrupting the absorption, reabsorption and elimination of crucial vitamins and minerals essential for promoting healthy bone growth (Kuan et al., 2018). In growing children and adolescents, a balanced diet is a necessity for proper bone growth and development and helps mitigate the risk of osteoporotic fractures in later life (Proia et al., 2021). Osteoporosis, a skeletal disorder, is marked by the reduction in bone mass, diminished bone mineral density, deterioration of microarchitectural integrity and weakened bone strength (Barnsley et al., 2021). This leads to increased fragility of bones and higher risk of fractures, particularly in the spine, hip, and humerus (Morin et al., 2014). Over 200 million individuals globally are affected by osteoporosis (Akkawi & Zmerly, 2018). Statistics indicate that vertebral or femoral fractures occur approximately every 200 seconds (De Martinis et al., 2021). It has been demonstrated that individuals with spinal cord injuries exhibit increased susceptibility to fractures in the tibia and femur (Zheng et al., 2021). Osteoporosis affects individuals across all ethnic groups and both sexes (Askari et al., 2019). Approximately 33% of women and 20% of men, usually age 50 and above will experience a fragility fracture at some point in their lifetime (Shen et al., 2022).

There is a dire need to mitigate the high fructose diet-induced alterations in growth, GIT viscera morphometry and bone development. Functional foods, which are rich in secondary plant metabolites, can be used to mitigate such diet-induced changes. Secondary plant metabolites play a key role in defending plants from herbivory, as well as from bacterial, fungal, protozoal, and viral threats (Al-Khayri et al., 2023), while also demonstrating health-promoting biological activities (Divekar et al., 2022), which can be tapped into to mitigate diet-induced metabolic derangements. Lycopene, a liposoluble carotenoid prevalent in tomatoes and watermelons, has antioxidant and anti-inflammatory activities (Rasmus & Kozłowska, 2023). In the GIT it has been shown to protect the GIT mucosae integrity leading to improved nutrient absorption (Rajput et al., 2021), bone health (Costa-Rodrigues et al., 2018), and promotion of growth (Wu et al., 2024). This study therefore evaluated the prophylactic potential of orally administered lycopene to protect against dietary fructose-induced metabolic derangements, focusing on the growth performance and gastrointestinal visceral macromorphometry in growing Wistar rats.

3.1 Materials and methods

3.1.1 Ethical approval

Approval for the study's ethical clearance was given by the Animal Ethics Screening Committee at the University of Witwatersrand (AESC 2022/03/02/C). The research protocols and procedures were thoroughly reviewed to ensure compliance with ethical standards. The research was conducted at the Wits Research Animal Facility (WRAF) in Johannesburg, South Africa and it subscribed to the ARRIVE protocol.

3.1.2 Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade. Fenofibrate was obtained from Sigma-Aldrich, based in St. Louis, Missouri, USA. Fructose was purchased from Nature's Choice in Randvaal, South Africa, while lycopene was supplied by Changsha Staherb Natural Ingredient Co., Ltd (Hunan, China).

3.1.3 Animals, housing, and general care

The study involved ninety-six 21-day-old male and female Wistar rat pups. These weanling rats were selected from litters of first-time dams, with each litter containing between 8 and 12 pups. The rats were individually housed in standard Perspex cages at the Wits Research Animal Facility

(WRAF), University of the Witwatersrand, with wood shavings used as bedding, which was changed twice a week. They were kept on a 12-hour light/dark cycle, with the lights on from 7 am to 7 pm, and the room temperature was controlled at 24±2°C. The rats had *ad libitum* access to either a standard rat chow or a 20% fructose solution, depending on their treatment group. The commercial rat feed (Pet Food Industry of Southern Africa) had the following nutritional composition: 40 g/kg fibre, 70 g/kg ash, 100 g/kg moisture, 220 g/kg protein, 50 g/kg oils and fats, 12 g/kg linoleic acid, 7.5 g/kg phosphorus, 12 g/kg calcium, 16,000 IU/kg vitamin A, 2,000 IU/kg vitamin D, and 100 g/kg vitamin E.

3.1.4 Experimental design

On postnatal day 23 (PND), after a two-day habituation period, the rats were randomly assigned to one of the following treatment groups:

group 1 (control): were given standard rat chow (SRC), plain drinking water (PDW), and a plain gelatine cube (PG).

group 2 (negative control): SRC, a 20% w/v fructose solution (FS) as drinking fluid, and PG.

group 3 (positive control): SRC, FS, and 100 mg/kg body mass per day of fenofibrate in gelatine cube (FENO).

group 4 (low dose): SRC, FS, and Lycopene at 30 mg/kg body mass per day in gelatine cube (LDLY).

group 5 (medium dose): SRC, FS, and Lycopene at 60 mg/kg body mass per day in gelatine cube (MDLY).

group 6 (high dose): SRC, FS, and Lycopene at 100 mg/kg body mass per day in gelatine cube (HDLY).

Groups 4, 5, and 6 were designed to assess the potential preventive effects of lycopene against metabolic dysfunction induced by a high-fructose diet. Each group consisted of 16 rats, with an equal number of 8 males and 8 females per group. The rats were administered to their respective treatments over a 12-week period. The fenofibrate dose (Kopf et al., 2014) and lycopene doses

(Gustin et al., 2004; Zhang et al., 2020) were selected based on previously documented studies. The gelatine cubes used to deliver fenofibrate and lycopene were prepared according to the method described by Kamerman et al. (2004), with a slight adjustment. Instead of using 16 g of brown sugar, 8 g of Selati sugar (RCL Foods and Sugar Milling Ltd, South Africa) were used, as a higher sugar concentration led to decreased intake.

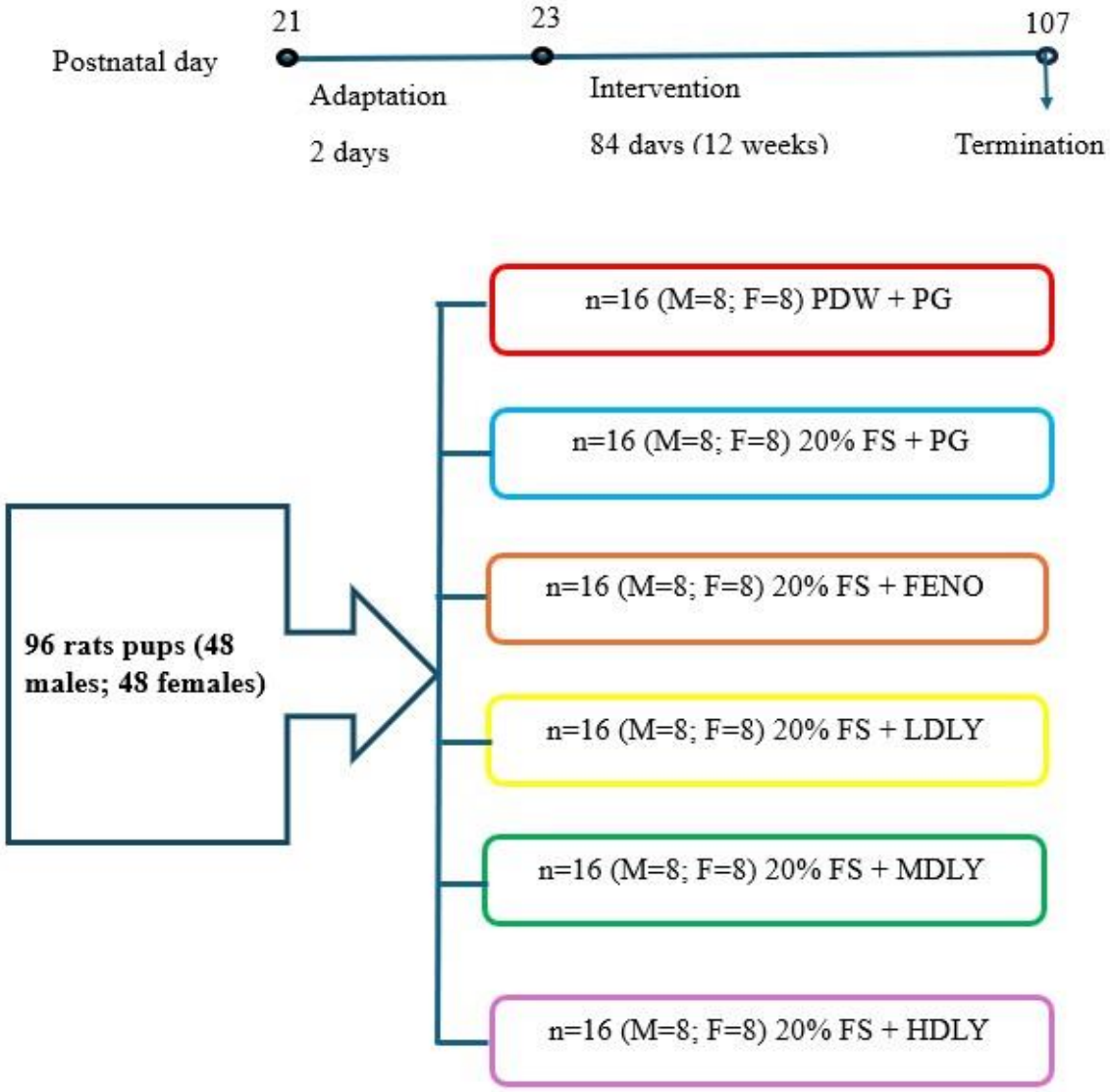


Figure 3. 1: Flowchart of study design

KEY: PDW= plain drinking water; FS= fructose solution (20%); FENO= fenofibrate 100 mg.kg^{-1}BM day^{-1}; LDLY= low dose lycopene 30 mg.kg^{-1}BM day^{-1}; MDLY= medium dose lycopene 60 mg.kg^{-1}BM day^{-1}; HDLY= high dose lycopene 100 mg.kg^{-1}BM day^{-1}; PG= plain gelatine cube

3.1.5 Measurements: body mass, feed, fluid, and calorie intake

At the start of the experiment, the rats were weighed, and their weights were recorded twice weekly using a Snowrex Electronic Scale from Clover Scale (Pty) Ltd, Johannesburg. This allowed for tracking their growth performance, general health, and ensuring accurate doses of fenofibrate and lycopene during the 12-week treatment. Throughout the intervention, feed and fluid intake were monitored weekly. At the end of each week, the remaining feed and fluid were subtracted from the total amounts provided to each rat. Weekly feed and fluid consumption were then calculated as percentages of body mass, expressed as g/100g and ml/100g, respectively. Total weekly calorie intake was determined by multiplying the consumption of feed and fructose by their respective calorie values, and summing the results. The cumulative intake of feed, fluid, and calories for each experimental group was then calculated by adding up the weekly totals over the 12-week period.

3.1.6 Terminal procedures and sample collection

On postnatal day 106, the rats underwent an 11 hours overnight fast, although plain drinking water was made available to prevent dehydration. In the morning of termination (postnatal day 107), the body mass of each rat was measured using a Snowrex Electronic Scale from Clover Scale (Pty) Ltd, Johannesburg. The rats were then euthanised using an intraperitoneal injection of sodium pentobarbitone (Euthapent; Kyron Laboratories, Johannesburg) at a dose of 200 mg/kg body mass. A midline abdominal incision was performed to extract and weigh the pancreas, liver, kidneys, stomach, caecum, small and large intestines, and visceral and epididymal fat pads (in male rats) using an electronic balance (Presica 310M, Instruments, Johannesburg, South Africa). The lengths of the small and large intestines were recorded using a ruler placed on a chilled dissection board. The left hind leg femoral attachment to the pelvis was also excised, sealed, and stored in Ziplock bags at -20°C for subsequent measurement of the long bones.

3.1.7 Measurement of bone morphometry

The femora and tibiae, which had been frozen, were removed from the freezer and left to thaw at room temperature for approximately 45 minutes. Once thawed, the bones were carefully defleshed and separated using scissors and scalpels. The bones were then placed in an oven (LABOTEC (Pty) Ltd., South Africa) set at 50°C for five days to ensure they reached constant mass. The bone masses were measured using an electronic scale (Presica 310M, Instruments, Johannesburg, South Africa). Tibia length was measured from the tibial head to the medial malleolus, while the femur

length was measured from the distal femoral articular surface to the greater trochanter, using a digital Vernier calliper (Major Tech (Pty) Ltd., KTV 150 digital calliper, Elandsfontein, South Africa).

The bone mass to length ratio was calculated using the formula: bone mass to length ratio = mass of bone (mg) / length of bone (mm), as outlined by Seedor et al. (1991).

3.1.8 Statistical analysis

The data was analysed using GraphPad Prism version 9.0 (Graph-Pad Software Inc., San Diego, USA) and presented as mean ± standard deviation. Parametric data from multiple groups were analysed using a one-way ANOVA, while a repeated measures ANOVA was used to analyse weekly body mass, feed, fluid, and calorie intake data. Tukey's *post hoc* test was used for the mean comparison. Statistical significance was defined as $p < 0.05$.

3.2 Results

3.2.1 Body mass

Figures 3.2 and 3.3 below present the mean weekly, induction, and terminal body masses of the female and male rats, respectively. In the female rats, there were no significant differences ($p > 0.05$) observed in the mean induction, weekly body, and terminal body masses across treatment groups (Figure 3.2). Similarly, there were no significant differences ($p > 0.05$) observed in the induction body mass of the male rats (Figure 3.3). In both female and male rats, significant growth ($p < 0.00001$) was evident across the treatment groups from induction through termination (Figure 3.2 and 3.3). The mean weekly body mass of male rats fed the control diet was significantly higher ($p < 0.05$) compared to their counterparts administered medium doses of lycopene at weeks 3 and 4 and fenofibrate at weeks 10, 11, and 12 as interventions (Figure 3.3). Similarly, the terminal body mass of male rats fed the control diet was significantly higher ($p < 0.05$) compared to those fed a high fructose diet and fenofibrate as an intervention. However, the terminal body mass of male rats fed control, high fructose, and high fructose diets with low, medium, and high doses of lycopene as an intervention were similar ($p > 0.05$) (Figure 3.3).

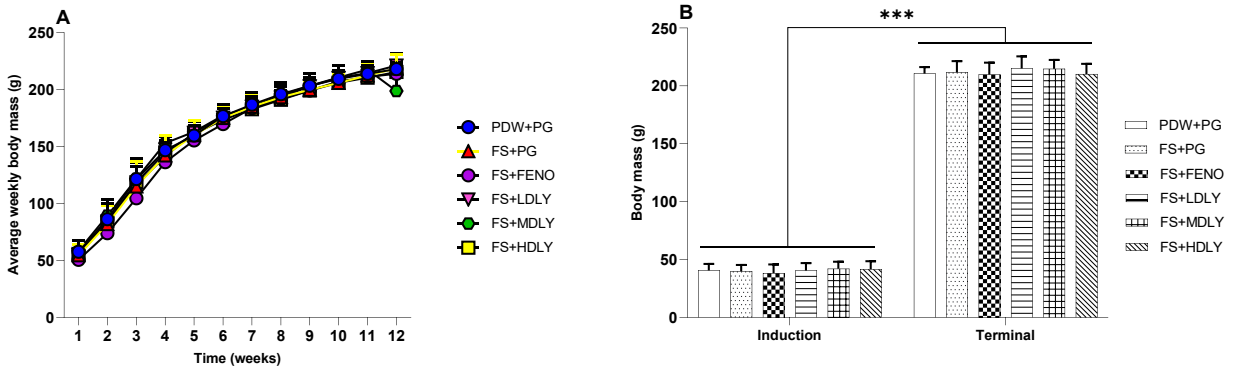


Figure 3. 2: Weekly body masses (A) and induction and terminal body masses (B) of female Wistar rats

No significant difference ($p > 0.05$) in induction, mean weekly and terminal body mass, across treatment groups. *** $p < 0.0001$ induction compared to terminal body mass. PDW + PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 mg/kg body mass per day (mg. kg^{-1}BM day^{-1}) of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 mg.kg^{-1}BM day^{-1} in gelatine cube. Data are expressed as mean ± SD, n = 8 per treatment group.

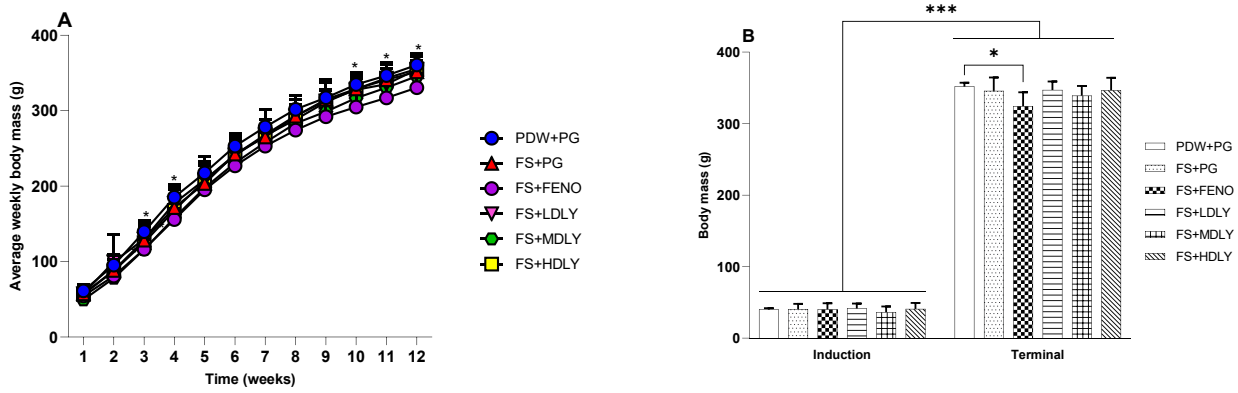


Figure 3. 3: Weekly body masses (A) and induction and terminal body masses (B) of male Wistar rats

***** $p < 0.05$ mean weekly body masses at weeks 3 (FS + LDLY), 4 (FS + MDLY), 10, 11, and 12 (FS + FENO) compared to control (PDW + PG); terminal body masses (FS + FENO) compared to control (PDW + PG) ******* $p < 0.0001$ induction vs terminal. PDW + PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 $mg.kg^{-1}BM\ day^{-1}$ of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube. Data are expressed as mean ± SD, n = 8 per treatment group.

3.2.2 Feed, fluid, and calorie intake

Figures 3.4 and 3.5 below show the feed, fluid, and calories intake in female and male rats respectively. In the females, there was no significant difference ($p > 0.05$) across treatment groups in week 1, but a significant difference ($p < 0.05$) from week 2 to week 12. From week 3 through week 12, rats fed a high fructose diet, and high fructose diet with low, medium, and high doses of lycopene as an intervention had significantly ($p < 0.05$) lower weekly feed intake compared to control. In weeks 2, 3, 4, 6, 7, 8, 9, and 11 rats that had fenofibrate as an intervention had significantly ($p < 0.05$) lower weekly feed intake compared to control counterparts. From week 5 to week 11, rats administered fenofibrate had significantly ($p < 0.05$) higher weekly feed intake compared to counterparts fed the high fructose diet, and or the high fructose diet supplemented with low, medium, or high doses of lycopene (Figure 3.4A). The total feed intake (TFI) of fructose-fed rats and their counterparts fed the high fructose diet with low and medium lycopene doses as supplements was similar ($p > 0.05$). Additionally, there was no difference in TFI between rats supplemented with the low and high lycopene doses ($p > 0.05$; Figure 3.4B). There was no significant difference ($p > 0.05$) in the weekly fluid intake across treatment groups from week 1 to week 5 and week 11. However, at weeks 6 and 10, orally administered fenofibrate had significantly ($p < 0.05$) lower weekly fluid intake compared to control and low-dose lycopene, respectively. At week 12, low-dose lycopene had significantly ($p < 0.05$) higher weekly fluid intake compared to

control counterparts (Figure 3.4C). The total fluid intake of rats fed the control diet, high fructose diet and high fructose diet supplement with medium dose lycopene was similar ($p > 0.05$; Figure 3.4D). Likewise, the total fluid intake of rats fed a high fructose diet supplemented with medium and high lycopene doses were similar ($p > 0.05$; Figure 3.4D). There was no significant difference ($p > 0.05$) in the rats' total weekly calorie intake from weeks 2 to 6, weeks 8 and 11, and weeks 12. In week 1, compared to control counterparts, rats fed the high fructose diet and those fed the high fructose diet with the medium dose lycopene supplement had significantly ($p < 0.05$; Figure 3.4E) higher total weekly calories intake. In week 7, rats fed the high fructose diet with fenofibrate as an intervention had significantly higher ($p < 0.05$) total calorie intake compared to their counterparts fed the control, high fructose diet, and high fructose diet with a medium dose of lycopene, respectively. Similarly, in weeks 9 and 10, rats administered fenofibrate had significantly ($p < 0.05$) higher total weekly calorie intake compared to their counterparts fed high fructose diet, and high fructose diet supplemented with medium and high doses of lycopene (Figure 3.4E). The total calorie intake of rats fed the control diet, high fructose diet, and high fructose diet supplemented with high-dose lycopene was similar ($p > 0.05$). Similarly, fructose-fed rats and counterparts fed the high fructose diet with low, medium, and high lycopene doses as supplements were similar ($p > 0.05$). There was no difference in total calorie intake between rats supplemented with low, medium, and high lycopene doses ($p > 0.05$; Figure 3.4F).

In the male Wistar rats, there was a significant difference ($p < 0.05$) across treatment groups from week 1 to week 12. From week 1 through week 12, rats fed a high fructose diet, high fructose diet with fenofibrate, high fructose diet with low, medium, and high doses of lycopene as an intervention had significantly ($p < 0.05$) lower weekly feed intake compared to control. However, during week 2, rats fed the high fructose diet supplemented with medium lycopene doses showed a significant reduction ($p < 0.05$) in weekly feed intake compared to their control counterparts. From weeks 7 to 12, rats fed the high fructose diet with fenofibrate as an intervention had significantly higher ($p < 0.05$) weekly feed intake compared to their counterparts fed the high fructose diet with low and high doses of lycopene, respectively. However, during week 10, rats administered fenofibrate had significantly ($p < 0.05$) higher weekly feed intake compared to their counterparts fed the high fructose diet with low, medium, and high lycopene doses as supplements. In weeks 9-12, rats administered fenofibrate had significantly ($p < 0.05$) higher weekly feed intake compared to their counterparts fed a high fructose diet (Figure 3.5A). There was no difference in

the TFI between rats supplemented with the low, medium, and high lycopene doses ($p > 0.05$). Similarly, the TFI of rats fed the high fructose diet with medium and high lycopene doses was similar ($p > 0.05$; Figure 3.5B). There was no significant difference ($p > 0.05$) in the male rats' weekly fluid intake across treatment groups from week 1 to weeks 3, 5 to 7, 9, and 12. In week 4, compared to their control counterparts, rats fed the high fructose diet with medium lycopene doses as supplement had significantly ($p < 0.05$) lower weekly fluid intake. However, at weeks 10 and 11, rats fed the high fructose diet, high fructose diet with low, medium, and high lycopene doses as supplements had significantly ($p < 0.05$) higher weekly fluid intake compared to their counterparts fed the control diet. In weeks 8 and 10, rats administered fenofibrate had significantly ($p < 0.05$) lower fluid intake compared to high-dose lycopene in week 8 and compared to low- and high-dose lycopene in week 10 (Figure 3.5C). The total fluid intake of rats fed the control diet, high fructose diet and high fructose diet supplement with low dose lycopene was similar ($p > 0.05$). Similarly, the total fluid intake between rats fed high fructose diet with fenofibrate as intervention and high fructose diet supplemented with medium dose lycopene was similar (Figure 3.5D). There was no significant difference ($p > 0.05$) in the total weekly calorie intake from weeks 1 to 6, 8, 10 and 12 across treatment groups. In weeks 7, 9 and 11, rats administered fenofibrate had significantly ($p < 0.05$) higher total weekly calories intake compared to low-dose lycopene in week 7, compared to low- and medium dose lycopene in week 9 and compared to low- and high dose lycopene and high fructose diet in week 11 (Figure 3.5E). In the male Wistar rats, the total calorie intake of fructose fed rats, and their counterparts fed the high fructose diet with low and high lycopene doses as supplements was similar ($p > 0.05$). Similarly, rats administered fenofibrate were similar to the control ($p > 0.05$). Additionally, there was no difference in the total calorie intake between rats supplemented with low and high lycopene doses ($p > 0.05$; Figure 3.5F).

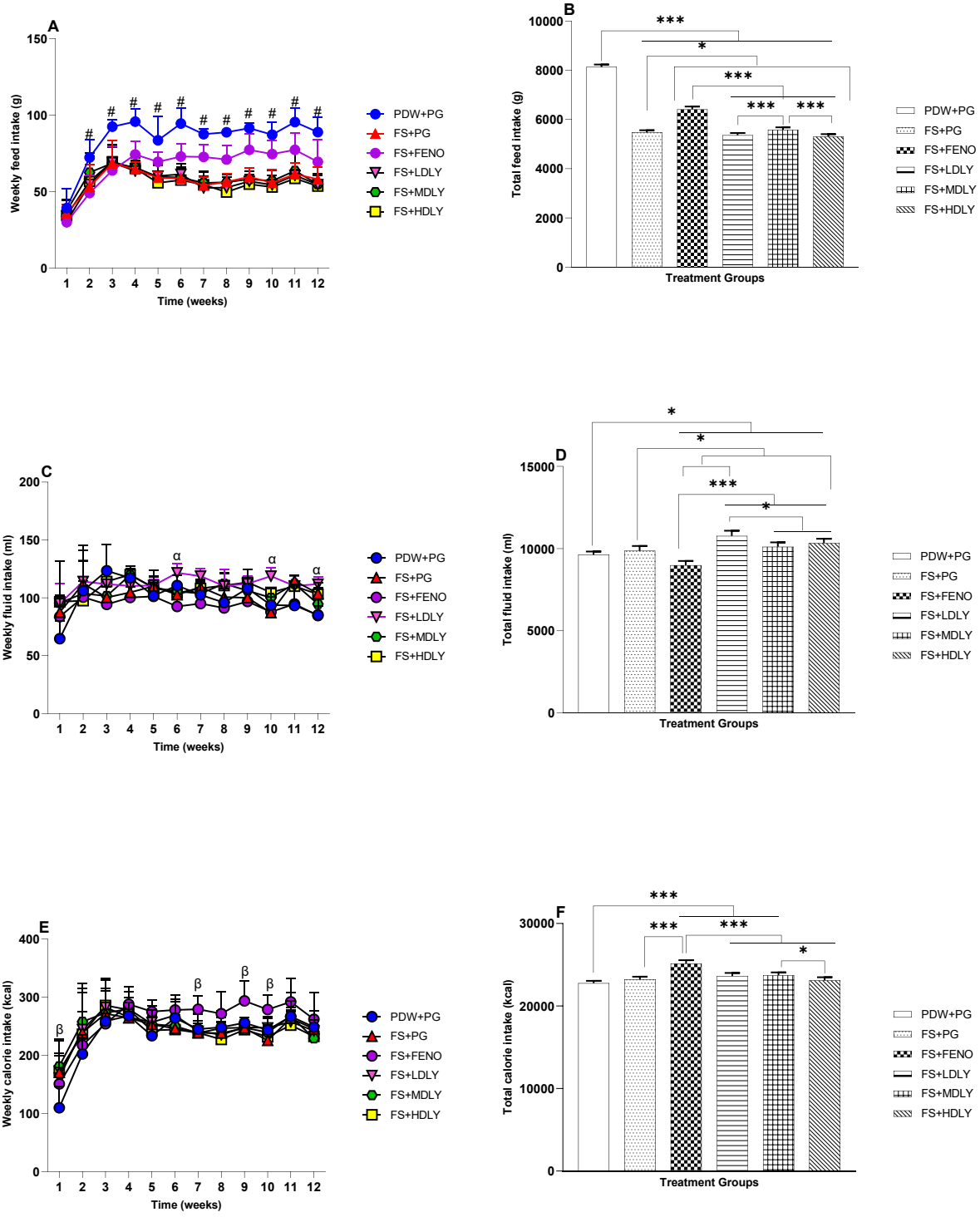

A
Weekly feed intake (g)
Time (weeks)
PDW+PG
FS+PG
FS+FENO
FS+LDLY
FS+MDLY
FS+HDLY
B
Total feed intake (g)
Treatment Groups
C
Weekly fluid intake (ml)
D
Total fluid intake (ml)
E
Weekly calorie intake (kcal)
F
Total calorie intake (kcal)

Figure 3. 4: Effect of lycopene on weekly feed (A), total feed (B), weekly fluid (C), total fluid (D), weekly calorie (E), and total calorie intake (F) of female Wistar rats fed a high-fructose diet.

$^{\#, \alpha, \beta}$ $p < 0.05$, *= significantly different at $p < 0.05$, ***= significantly different at $p < 0.0001$. PDW + PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 $mg.kg^{-1}$BM day^{-1} of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 $mg.kg^{-1}$BM day^{-1} in gelatine cube; FS + MDL= 20% w/v fructose solution as drinking fluid + Lycopene 60 $mg.kg^{-1}$BM day^{-1} in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 $mg.kg^{-1}$BM day^{-1} in gelatine cube. Data are expressed as mean ± SD, n = 8 per treatment group.

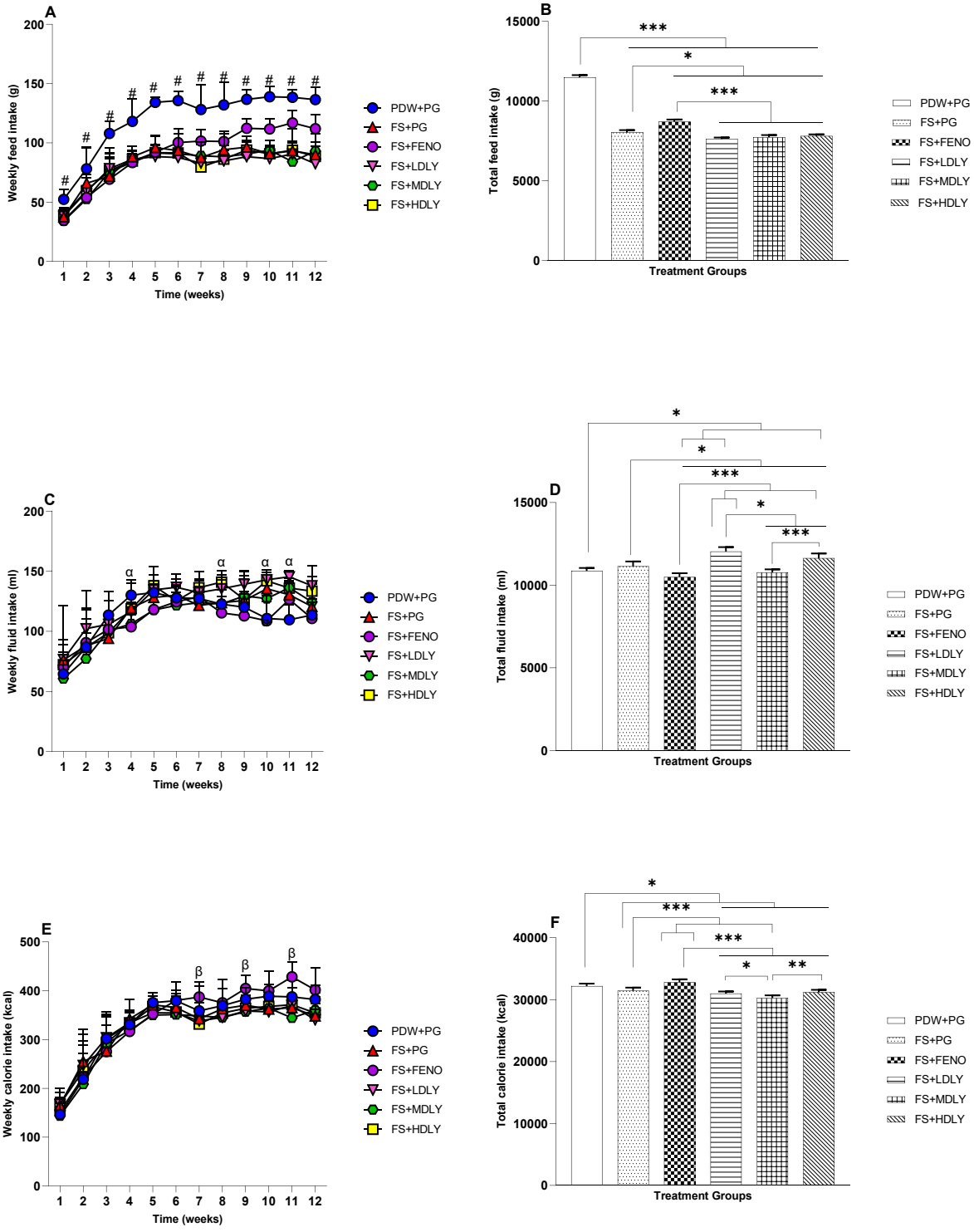

A
Weekly feed intake (g)
Time (weeks)
B
Total feed intake (g)
Treatment Groups
C
Weekly fluid intake (ml)
Time (weeks)
D
Total fluid intake (ml)
Treatment Groups
E
Weekly calorie intake (kcal)
Time (weeks)
F
Total calorie intake (kcal)
Treatment Groups
PDW+PG
FS+PG
FS+FENO
FS+LDLY
FS+MDLY
FS+HDLY

Figure 3. 5: Effect of lycopene on weekly feed (A), total feed (B), weekly fluid (C), total fluid (D), weekly calorie (E), and total calorie intake (F) of male Wistar rats fed a high-fructose diet.

$^{\#, \alpha, \beta}$ $p < 0.05$, *= significantly different at $p < 0.05$, **= significantly different at $p < 0.001$, ***= significantly different at $p < 0.0001$. PDW + PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 $mg.kg^{-1}BM\ day^{-1}$ of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube. Data are expressed as mean ± SD, n = 8 per treatment group.

3.2.3 Long bone indices

Tables 3.1 and 3.2 show the effect of lycopene on the morphometric characteristics of femur and tibia in female and male rats fed a high fructose diet. No significant differences were observed in the lengths, masses, and bone mass to length ratio of the tibiae and femora in the female rats ($p > 0.05$) across treatment groups (Table 3.1). Male Wistar rats fed a high fructose diet and fenofibrate as an intervention had significantly lower ($p < 0.05$) femora mass compared to those fed a high fructose diet with a low dose of lycopene as an intervention. However, the mean femora mass of the male rats fed the control diet, high fructose diet, and high fructose diet supplemented with low, medium, and high lycopene doses, respectively, were similar ($p > 0.05$). The mean tibia length of the male rats fed a high fructose diet with fenofibrate as an intervention was significantly shorter ($p < 0.05$) compared to that of counterparts fed the control diet and a high fructose diet in combination with either of the three lycopene doses. Nonetheless, the mean tibia length of the male rats fed the control, high fructose diet, and high fructose diet with low, medium, and high doses of lycopene as an intervention were similar ($p > 0.05$; Table 3.2).

Table 3. 1: Effect of lycopene on the lengths, masses and mass to length ratio of tibiae and femora in growing female Wistar rats fed a high-fructose diet

Parameter	PDW + PG	FS + PG	FS + FENO	FS + LDLY	FS + MDLY	FS + HDLY	Significance
Tibia							
Mass (mg)	455.00 ± 19.27[a]	462.50 ± 19.09[a]	462.50 ± 26.05[a]	451.30 ± 20.31[a]	460.50 ± 13.09[a]	447.5 ± 26.59[a]	ns
Length (mm)	34.98 ± 0.65[a]	35.58 ± 1.89[a]	34.50 ± 0.51[a]	34.43 ± 0.60[a]	35.37 ± 1.74[a]	34.68 ± 0.64[a]	ns
Mass/length ratio (mg/mm)	13.01 ± 0.58[a]	13.09 ± 0.57[a]	13.41 ± 0.76[a]	13.11 ± 0.51[a]	13.03 ± 0.69[a]	12.89 ± 0.55[a]	ns
Femur							
Mass (mg)	545.00 ± 31.62[a]	548.80 ± 20.31[a]	532.50 ± 23.75[a]	541.30 ± 26.96[a]	526.30 ± 48.97[a]	535.00 ± 34.23[a]	ns
Length (mm)	30.68 ± 0.94[a]	30.45 ± 2.00[a]	29.73 ± 0.53[a]	29.97 ± 0.58[a]	28.89 ± 2.64[a]	30.24 ± 0.54[a]	ns
Mass/length ratio (mg/mm)	17.75 ± 0.54[a]	18.08 ± 1.21[a]	17.92 ± 0.91[a]	18.06 ± 0.91[a]	18.22 ± 0.47[a]	17.68 ± 0.88[a]	ns

[a]Means within rows having the same superscripts are similar at $p > 0.05$, ns = not significant. PDW + PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 $mg.kg^{-1}$BM day^{-1} of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 $mg.kg^{-1}$BM day^{-1} in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 $mg.kg^{-1}$BM day^{-1} in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 $mg.kg^{-1}$BM day^{-1} in gelatine cube. Data are expressed as mean ± SD, n = 8 per treatment group.

Table 3. 2: Effect of lycopene on the lengths, masses and Mass to length ratio of tibiae and femora in growing male Wistar rats fed a high-fructose diet

Parameter	PDW +PG	FS + PG	FS + FENO	FS +LDLY	FS + MDLY	FS + HDLY	Significance
Tibia							
mass (mg)	613.80 ± 35.83[a]	605.00 ± 35.05[a]	572.50 ± 32.84[a]	606.30 ± 28.25[a]	596.30 ± 28.25[a]	597.50 ± 26.05[a]	ns
Length (mm)	39.73 ± 0.75[a]	39.40 ± 0.60[ab]	38.47 ± 0.74[b]	39.85 ± 0.65[a]	39.47 ± 0.47[a]	39.77 ± 0.61[a]	**
Mass/length ratio (mg/mm)	15.44 ± 0.76[a]	15.36 ± 0.87[a]	14.89 ± 0.93[a]	15.21 ± 0.61[a]	15.11 ± 0.69[a]	15.03 ± 0.63[a]	ns
Femur							
Mass (mg)	725.00 ± 37.42[ab]	710.00 ± 31.62[ab]	678.80 ± 36.43[a]	730.00 ± 27.26[b]	718.80 ± 30.91[ab]	716.30 ± 25.04[ab]	*
Length (mm)	34.25 ± 0.77[a]	34.00 ± 0.41[a]	33.07 ± 0.79[a]	30.18 ± 11.98[a]	35.01 ± 0.88[a]	34.32 ± 0.54[a]	ns
Mass/length ratio (mg/mm)	21.16 ± 0.67[a]	20.88 ± 0.89[a]	20.52 ± 0.74[a]	143.70 ± 346.00[a]	20.53 ± 0.75[a]	20.87 ± 0.83[a]	ns

[ab]Means within rows having the different superscripts differ significantly at * $p < 0.05$, ** $p < 0.001$, ns = not significant. PDW + PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 mg.kg^{-1}BM day^{-1} of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 mg.kg^{-1}BM day^{-1} in gelatine cube. Data are expressed as mean ± SD, n = 8 per treatment group.

3.2.4 Visceral organ macro-morphometry

Tables 3.3 and 3.4 show the effect of lycopene on the absolute and relative masses and length (where applicable) of the small and large intestines, stomach, and caecum of female and male rats, respectively, fed a high fructose diet. There were no significant differences in viscera morphometry of female rats ($p > 0.05$) across treatment groups (Table 3.3). The male rats fed the control diet had significantly reduced ($p < 0.05$) absolute and relative stomach masses compared to their counterparts fed a high fructose diet with fenofibrate and or a high fructose diet with a medium and high dose of lycopene as interventions. However, the absolute and relative stomach masses of the rats fed the control, high fructose diet, and high fructose diet with low dose of lycopene as an intervention were similar ($p > 0.05$).

Table 3. 3: Effect of lycopene on absolute and relative masses and length of the visceral organs in growing female rats fed a high-fructose diet

Parameter	PDW +PG	FS + PG	FS + FENO	FS +LDLY	FS + MDLY	FS + HDLY	Significance
Stomach mass (g)	1.34 ± 0.12[a]	1.36 ± 0.14[a]	1.44 ± 0.12[a]	1.49 ± 0.19[a]	1.38 ± 0.20[a]	1.34 ± 0.09[a]	ns
Stomach (%BM)	0.64 ± 0.05[a]	0.64 ± 0.07[a]	0.69 ± 0.08[a]	0.69 ± 0.08[a]	0.64 ± 0.05[a]	0.64 ± 0.05[a]	ns
Caecum mass (g)	1.20 ± 0.29[a]	0.88 ± 0.17[a]	0.91 ± 0.29[a]	0.80 ± 0.20[a]	0.89 ± 0.18[a]	1.03 ± 0.23[a]	ns
Caecum (%BM)	0.52 ± 0.14[a]	0.42 ± 0.08[a]	0.44 ± 0.14[a]	0.39 ± 0.08[a]	0.41 ± 0.08[a]	0.49 ± 0.12[a]	ns
L. Intestine mass (g)	1.32 ± 0.19[a]	1.07 ± 0.41[a]	1.33 ± 0.19[a]	1.24 ± 0.14[a]	1.14 ± 0.14[a]	1.22 ± 0.15[a]	ns
L. Intestine (%BM)	0.63 ± 0.08[a]	0.51 ± 0.20[a]	0.64 ± 0.09[a]	0.58 ± 0.07[a]	0.53 ± 0.06[a]	0.58 ± 0.09[a]	ns
L. Intestine length (mm)	197.30 ± 24.18[a]	176.30 ± 25.46[a]	173.30 ± 69.99[a]	183.8 ± 24.89[a]	182.10 ± 10.58[a]	184.40 ±37.17[a]	ns
S. Intestine Mass (g)	5.36 ± 0.80[a]	5.36 ± 0.80[a]	5.82 ± 0.69[a]	5.90 ± 0.33[a]	5.71 ± 1.13[a]	5.81 ± 0.73[a]	ns
S. Intestine (%BM)	2.55 ± 0.36[a]	2.75 ± 0.30[a]	2.82 ± 0.23[a]	2.67 ± 0.59[a]	2.70 ± 0.28[a]	2.33 ± 0.77[a]	ns
S. Intestine length (mm)	1023.00 ± 36.45[a]	1018.00 ± 36.44[a]	1068.00 ± 74.26[a]	1059.00 ± 34.30[a]	1026.00 ± 45.02[a]	974.90 ± 160.40[a]	ns

[a]Means within rows having the same superscripts are similar at $p > 0.05$, ns = not significant, L = Large, S = Small. PDW +PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 $mg.kg^{-1}BM\ day^{-1}$ of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking

fluid + Lycopene 30 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 mg.kg^{-1}BM day^{-1} in gelatine cube. Data are expressed as mean ± SD, n = 8 per treatment group.

Table 3. 4 Effect of lycopene on absolute and relative masses and length of the visceral organs in growing male rats fed a high-fructose diet.

Parameter	PDW +PG	FS + PG	FS + FENO	FS +LDLY	FS + MDLY	FS + HDLY	Significance
Stomach mass (g)	1.71 ± 0.09^{a}	1.83 ± 0.36^{ab}	2.03 ± 0.15^{b}	1.91 ± 0.12^{ab}	2.00 ± 0.12^{b}	2.01 ± 0.17^{b}	*
Stomach (%BM)	0.49 ± 0.03^{a}	0.54 ± 0.11^{ab}	0.63 ± 0.06^{c}	0.55 ± 0.03^{abc}	0.59 ± 0.03^{c}	0.58 ± 0.04^{c}	***
Caecum mass (g)	1.41 ± 0.18^{a}	1.33 ± 0.33^{a}	1.28 ± 0.46^{a}	1.24 ± 0.27^{a}	1.46 ± 0.23^{a}	1.50 ± 0.31^{a}	ns
Caecum (%BM)	0.40 ± 0.05^{a}	0.38 ± 0.08^{a}	0.39 ± 0.13^{a}	0.36 ± 0.08^{a}	0.43 ± 0.06^{a}	0.44 ± 0.08^{a}	ns
L. Intestine mass (g)	1.78 ± 0.28^{a}	1.68 ± 0.16^{a}	1.72 ± 0.32^{a}	2.28 ± 1.59^{a}	1.70 ± 0.18^{a}	1.79 ± 0.13^{a}	ns
L. Intestine (%BM)	0.50 ± 0.08^{a}	0.49 ± 0.05^{a}	0.53 ± 0.08^{a}	0.67 ± 0.50^{a}	0.50 ± 0.05^{a}	0.52 ± 0.04^{a}	ns
L. Intestine length (mm)	228.10 ± 11.00^{a}	213.10 ± 17.72^{a}	222.90 ± 18.22^{a}	213.10 ± 12.23^{a}	218.80 ± 27.09^{a}	223.80 ± 8.35^{a}	ns
S. Intestine Mass (g)	7.11 ± 0.57^{ab}	7.69 ± 0.70^{ab}	8.01 ± 0.48^{a}	6.48 ± 2.18^{b}	7.60 ± 0.49^{ab}	8.17 ± 0.57^{a}	*
S. Intestine (%BM)	2.02 ± 0.17^{a}	2.23 ± 0.22^{abc}	2.48 ± 0.17^{b}	1.86 ± 0.62^{ac}	2.25 ± 0.16^{abc}	2.36 ± 0.12^{ab}	**
S. Intestine length (mm)	1148.00 ± 40.35^{a}	1211.00 ± 30.33^{a}	1188.00 ± 63.47^{a}	1189.00 ± 45.00^{a}	1219.00 ± 77.85^{a}	1214.00 ± 31.59^{a}	ns

[abc]Means within rows having different superscripts differ significantly at * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$, ns = not significant, L = Large, S = Small. PDW + PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 $mg.kg^{-1}$BM day^{-1} of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 $mg.kg^{-1}$BM day^{-1} in gelatine cube; FS + MDLY = 20% w/v fructose solution

as drinking fluid + Lycopene 60 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 mg.kg^{-1}BM day^{-1} in gelatine cube. Data are expressed as mean ± SD, n = 8 per treatment group.

3.3 Discussion

3.3.1 Growth performance: body mass, feed and fluid intake, and calorie consumption

The study investigated the prophylactic potential of orally administered lycopene against dietary fructose-induced metabolic derangements, focusing on growth performance and gastrointestinal tract visceral macromorphometry in growing weanling Wistar rats in a model mimicking adolescents fed an obesogenic diet. The induction, mean weekly body mass, and terminal body masses of the female rats across treatment groups were similar. However, while the induction body mass of the male Wistar rats was similar across treatments, male rats fed the high fructose diet with fenofibrate and or the medium dose of lycopene had significantly lower mean weekly body masses during weeks 10 to 12 and 3 and 4, for fenofibrate and medium lycopene groups, respectively, compared to controls. These results suggest that both lycopene and fenofibrate can potentially be used to reduce body mass gain. Wang et al. (2019) noted a reduction in body mass gain in mice fed a high-fat diet with lycopene supplementation. Although the induction body masses of both the female and male rats were similar, in both sexes, the rats significantly grew across treatment groups when induction body masses were compared to terminal body masses. This suggest that the treatment protocols did not negatively affect their growth. However, in male rats fed the high fructose diet the administered fenofibrate resulted in decreased terminal body mass when compared to their control counterparts. This finding aligns with Lee et al. (2023), who reported that fenofibrate decreased the body mass, adipose tissue mass and size in ovariectomized mice fed a high-fat diet. The regulation of body mass by fenofibrate is mediated through nuclear peroxisome proliferator-activated receptor α (PPARα), which controls the genes vital for lipid and lipoprotein metabolism by enhancing fatty acid oxidation, promoting the breakdown of triglyceride, and decreasing triglycerides synthesis and secretion (Shin et al., 2021). The terminal body masses of the rats (female and male) fed the control diet was similar to that of counterparts fed the high fructose diet and the high fructose diet with either of the lycopene doses, suggesting that dietary fructose did not mediate increased body mass gain and that both fructose and lycopene did not negatively affect growth during the 12-week intervention. These findings align with Ramos et al. (2017), who noted that feeding Wistar rats a diet supplemented with 20% fructose as drinking fluid did not alter their body mass but led to an augmentation in visceral adiposity. They suggested that the elevated adipogenic potential and relatively low energy content of fructose were accountable for the observed rise in visceral fat without a concurrent increase in body mass. It is

essential to emphasise that while body mass is a crucial marker of an animal's health, its accuracy as a gauge of growth may be compromised by the size of visceral organs, gut fill, and hydration status, thus decreasing its reliability (Borga et al., 2018; Muller et al., 2018; Ekingen et al., 2022).

This study noted a difference in the consumption of feed, fluid, and calories in the female and male rats across treatment groups. In summary, rats reared on the control diet consumed more feed, drank less plain water, and had a lower total calorie intake. Conversely, rats provided with fructose solution as the drinking fluid, drank more fluid, consumed less feed but had a higher total calorie intake. Notably, the administration of fenofibrate resulted in decreased fluid intake. In both sexes, rats fed the control diet significantly increased weekly and total feed consumption compared to their counterparts fed a high fructose diet, a high fructose diet with either fenofibrate and or lycopene as interventions. Long-term consumption of dietary fructose has been reported to decrease feed and energy intake (Smajis et al., 2020), impacting the secretion and function of appetite-regulating hormones like leptin and insulin (Capucho & Conde, 2022). This disruption may lead to irregular hunger signals and increase the risk of metabolic disorders such as obesity and T2DM (Cui et al., 2022). Rats reared on the high fructose diet with fenofibrate as an intervention had high weekly and TFI compared to counterparts fed the plain high fructose diet and as well as those fed the high fructose diet with lycopene as an intervention. This finding suggests the orally administered fenofibrate at 100 mg/kg body mass in adolescent rats fed a high fructose diet mediates increased feed intake which contradicts the observation that it (fenofibrate) decreases intake of a standard rat chow by meditating increased ketogenesis from mobilised body fat (Park et al., 2012). Based on the observation that fenofibrate decreased the body mass of fructose-fed rats compared to control, it is hypothesised that the increase in feed intake might have been a compensatory response to mitigate the fenofibrate-induced decrease in body mass. The dichotomy in these findings could have been attributed to altered metabolic pathways or hormonal responses leading to increased feed intake. Additionally, fenofibrate might have independent effects on appetite or metabolism that contribute to increased feed intake when combined with fructose. These effects could be mediated through various pathways, such as changes in gut hormone secretion or alteration in central nervous system signalling related appetite control. Male Wistar rats fed a high fructose diet had significantly higher TFI compared to their counterparts fed low, medium, and high doses of lycopene. However, in the female rats, this difference in feed

consumption was particularly evident when compared to those administered a high dose of lycopene. Additionally, female rats fed the medium dose of lycopene demonstrated significantly higher total feed intake compared to their counterparts fed the low and high doses of lycopene. Wang et al. (2019), reported that lycopene supplementation significantly inhibits the increase in body mass observed in mice fed a high fat-diet. Similar findings were reported by Zhu et al. (2020). In this current study, high-fructose diet with lycopene supplementation did not negatively affect body mass, similar to control and dietary fructose. This outcome might be attributed to the body mass changes induced by the 20% fructose, as noted in prior studies (Ramos et al., 2017). However, we did notice a decrease in feed consumption. I hypothesise that this reduction in feed intake could be linked to the influence of lycopene on metabolic processes. It's plausible that higher metabolic rates induced by lycopene (Wang, 2012), could lead to decreased feelings of hunger and, subsequently, lower feed consumption. Another explanation could be that lycopene's potential to influence appetite regulation by enhancing leptin sensitivity or reducing ghrelin secretion. Additionally, lycopene might alter taste perception or feed palatability, affecting rats' feed preferences and resulting in reduced consumption.

In this study, female rats administered fenofibrate orally at weeks 6 and 10, showed a decreased weekly fluid intake compared to control and low dose lycopene groups. Similarly, male rats on fenofibrate had reduced weekly fluid intake at week 8 compared to high-dose lycopene group, and at week 10 compared to both low and high doses of lycopene. In both sexes, the total fluid intake of the high fructose diet group was similar to control group. Female and male rats reared on the high fructose diet with fenofibrate as an intervention showed significantly lower total fluid intake compared to control group and those on a high fructose diet, with or without lycopene interventions. Fenofibrate has been shown to alter perception and reduce preference for sweet tastes in adult humans (Davis, 2020; Kochem & Breslin, 2016). Therefore, rats treated with fenofibrate may find the fructose solution less palatable, leading to reduced fluid consumption. Despite decreased fluid intake, total calorie was found to be higher in both female and male rats administered fenofibrate. This could be due to fenofibrate's effects on metabolism, as mentioned earlier. Increased fatty acid oxidation and energy expenditure may necessitate higher calorie intake to meet the body's energy demands. Interestingly, lycopene supplementation significantly increased total fluid intake, however, rats supplemented with low dose lycopene drank more fluid,

consequently had increase total fluid intake compared to either medium or high doses of lycopene. This suggest that different doses of lycopene could have varied effects on metabolic processes, such as digestion or nutrient absorption, which in turn might influence the rats' fluid patterns. Thus, it can be speculated that in the present study, lycopene supplementation, acting as an antioxidant, might interact with fructose to modulate thirst or fluid intake regulatory mechanisms. This interaction could enhance the perceived need for fluid intake, leading to increased consumption compared to rats fed a control diet, fructose alone, or fructose with fenofibrate. Moreover, it could be posited that lycopene supplementation could affect metabolic processes related to fluid regulation or thirst mechanisms in the rats. This could include an alteration in hormone levels or signalling pathways that regulate fluid balance, leading to an increase in total fluid consumption. Findings from the current study also showed that high-dose lycopene resulted in a significant increase in both total fluid and calorie intake compared to medium-dose in male Wistar rats. Conversely, in the female rats, the medium dose significantly increased total calorie intake compared to high-dose lycopene. Additionally, it was observed that in the male rats, lycopene supplementation significantly decreased total calorie intake compared to those on the control diet, while in the female rats, both low and high doses of lycopene significantly increased total calorie intake compared to their control counterparts. Furthermore, in both sexes, dietary fructose significantly increased total fluid intake compared to fenofibrate administration, although fenofibrate notably increased total calorie intake compared to dietary fructose. In the female rats, the total fluid and calorie consumption on a high fructose diet were similar to those in the control group. However, in the male rats, the total fluid intake on a high fructose diet was similar to both control and medium-dose lycopene, while the total calorie intake on fenofibrate was similar to the control group. In this study, it was noted that male rats consumed more feed and fluid, consequently resulting in a greater calorie intake than female rats. A dimorphic response has been reported in feeding, with males having higher intake than females (Maric et al., 2022). It is acknowledged that males and females have varying metabolic pathways that lead to sex-specific dietary-induced modifications (Hyer et al., 2019). Male rodents tend to be larger and heavier than females, requiring more energy to maintain their body mass (Maric et al., 2022). This larger size may contribute to their increased feed and fluid intake. Hormonal fluctuations, such as those associated with the estrous cycle in female rats, can decrease appetite and feed intake (Asarian &

Geary, 2013; Alonso-Caraballo & Ferrario, 2019). In male rats, testosterone has been linked to increased food intake and appetite regulation (Alrabadi et al., 2020).

3.3.2 Long Bone Growth

The longitudinal growth of the femora and tibia, provides a more precise measure of growth performance in growing animals than body mass (Cho et al., 2020). These antigravity bones exhibit a dose-dependent response to growth hormone (Ranke, 2021), which makes them a more reliable indicator of growth performance. Previous research has demonstrated that lycopene can prevent femora loss, increase the number osteocytes and osteoblasts in ovariectomized rats (Semeghini et al.,2022). The current study found no significant difference in femora and tibiae lengths, masses, and bone mass to length ratios across treatment groups in the female rats. This aligns with the findings of Muhammad et al.(2020), who observed no impact on these parameters in growing female Spraque Dawley rats after 10 weeks of dietary fructose and orally administered fenofibrate. This finding suggests that dietary fructose, the orally administered lycopene and fenofibrate did not negatively affect growth performance during the 12-week intervention period. However, in the male Wistar rats, supplementation of varying doses of lycopene significantly increased tibia length when compared to counterparts administered fenofibrate. Additionally, low-dose lycopene significantly increased femora mass compared to fenofibrate. These findings indicate a beneficial impact of lycopene on bone growth. The observed enhancements in tibia length and femora mass in male Wistar rats may be attributed to lycopene's antioxidant properties, which protect bone cells from oxidative damage, enhance osteoblast activity, and thereby contribute to overall bone health, by promoting bone growth and density. The sex-specific response to lycopene supplementation in terms of femora mass and tibiae length observed in this study may stem from variations in sex hormones such as testosterone and oestrogen. These hormones have different effect on bone growth and metabolism between the sexes (Emmanuelle et al., 2021). During the adolescent-to-adulthood transition, testosterone has been shown to promote linear growth (Breehl & Caban, 2023), increase bone mineral density, and enhance bone microarchitecture (Shigehara et al., 2021)**.** Interestingly, lycopene supplementation has been reported to increase testosterone levels in varicocele-induced Sprague-Dawley rats (Antonuccio et al., 2020). These combined effects could potentially account for the observed enhancements in femora mass and tibia length in male rats.

3.3.3 Morphometry of Visceral Organs

Plant-derived bioactive chemical compounds have been demonstrated to affect visceral organ mass (Mir et al., 2019), underscoring the importance of assessing visceral mass as a tool for evaluating the impact of these compounds on overall health and metabolic function (Shaik et al., 2023). In this study, no significant difference was observed in the absolute and relative masses of the stomach, caecum, small intestine, large intestine, and the lengths of the small and large intestine of the female rats across treatment groups. These results suggest that the treatments had no discernible effect on the growth and development of the viscera. Furthermore, the findings indicate that dietary fructose, lycopene, or fenofibrate did not induce atrophy or hypertrophy of visceral organs. In the male Wistar rats, we noted a similarity in the absolute and relative masses of the caecum, and large intestine, and the length of both the small and large intestines across treatment groups. However, the rats fed a high fructose diet with fenofibrate, high fructose diet with medium and or high doses of lycopene had significantly increased absolute and relative masses of the stomach compared to their counterparts fed the control diet. Additionally, rats administered fenofibrate had a significantly heavier relative mass of stomach compared to the control group. A significant increase was observed in both the absolute and relative masses of the small intestine with the administration of fenofibrate and high-dose lycopene, compared to low-dose lycopene. Furthermore, the relative mass of the small intestine significantly increased with fenofibrate administration compared to their control counterparts. These findings suggest that fenofibrate with high fructose diet, high fructose diet with medium to high doses of lycopene may have caused hypertrophy and or hyperplasia in the cells of the stomach and small intestines. Consequently, this could have led to the observed increase in the mass of the stomach and small intestines in male rats. We therefore hypothesise that the increase in stomach and small intestine mass observed with fenofibrate administration and lycopene supplementation could be attributed to several factors: fenofibrate is known to affect lipid metabolism. It can increase fatty acid oxidation and decrease triglyceride levels, which might lead to alterations in the composition of tissues, including the stomach and small intestine. Studies have shown that both fenofibrate and lycopene possess anti-inflammatory properties. Fenofibrate has been shown to reduce inflammation in various tissues (Jin et al., 2023). Lycopene has been noted to alleviate inflammation in conditions like ulcerative colitis and Crohn's disease, which are inflammatory bowel diseases (Glabska et al., 2016; Chen et al., 2023). By reducing inflammation, lycopene and fenofibrate may prevent tissue damage and

promote the integrity of the stomach and small intestine tissues, leading to an increase in mass. Additionally, the small intestine is primarily responsible for nutrient absorption (Basile et al., 2023). Lycopene has been suggested to enhance nutrient absorption and modulate the composition and activity of the gut microbiota by improving intestinal barrier function and promoting the expression of nutrient transporters (Meng et al., 2022; Tu et al., 2023). This improved nutrient absorption and gut microbiota could contribute to improved nutrient use and assimilation in the GIT, which could translate to increased small intestine mass. I hypothesise that lycopene may promote the growth and regeneration of cells lining of the stomach and small intestine, leading to an increase in tissue mass of the male rats. In addition, hormones play a crucial role in regulating GIT function and tissue homeostasis (Norman & Henry, 2015; Farhadipour & Depoortere, 2021). Therefore, it can be speculated that lycopene may alter hormone signalling pathways involved in gastrointestinal health, thus affecting tissue growth and development. However, further research is needed to fully understand the specific mechanisms underlying this phenomenon.

3.4 Conclusion

This study found that prolonged intake of a high-fructose diet reduced total feed intake (TFI) and increased fluid consumption in both female and male rats. Supplemental lycopene similarly decreased TFI and elevated fluid intake. Female rats showed an increase in total calorie intake, while male rats had a decrease. Supplemental lycopene resulted in longer tibias, greater femoral mass, and higher absolute and relative masses of the stomach and small intestine in male rats. As a dietary supplement, lycopene may support bone growth and development, help counteract diet-induced bone mass loss, and enhance nutrient digestion and absorption in males. Nonetheless, additional research is required to clarify the underlying mechanisms responsible for these positive effects on bone and gastrointestinal health.

Having discussed the effects of supplemental lycopene on feed, fluid, calories intake, growth performance and GIT viscera morphometry in rats fed a high fructose diet, the next chapter presents a narrative on its (supplemental lycopene) potential benefits on haematology, circulating metabolic substrates, and metabolism-regulating hormones in growing Wistar rats fed a high-fructose diet.

3.5 References

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CHAPTER FOUR: EFFECT OF LYCOPENE ON BLOOD GLUCOSE, GLYCATED HAEMOGLOBIN, HAEMOGLOBIN, HAEMATOCRIT, SERUM LEPTIN CONCENTRATION AND LIPID PROFILE IN GROWING WISTAR RATS FED A HIGH FRUCTOSE DIET

4.0 Introduction

Excessive consumption of dietary fructose from childhood through adulthood can cause the emergence of metabolic conditions including dyslipidaemia, insulin resistance, obesity, and T2DM (Softic et al., 2020; Codazzi et al., 2023) which are all elements of metabolic syndrome. Metabolic syndrome has affected over a billion people globally (Mohamed et al., 2023), with its prevalence at 2.8%, 4.8% (Noubiap et al., 2022), 32.1%, and 24.7% (Nouri-Keshtkar et al., 2023) in children, adolescents, females and males, respectively. Most of the fructose consumed is metabolised by the liver (Francey et al., 2019). Unlike glucose whose metabolism is controlled by the amount of ATP produced and insulin secretion, particularly at the level of phosphofructokinase (Nakrani et al., 2023), the metabolism of fructose occurs independently of these two factors (Merino et al., 2020). Excessive fructose intake can overwhelm the liver's capacity to efficiently metabolise it, which can lead to the production of triglycerides through *de novo* lipogenesis. This dietary fructose induced *de novo* lipogenesis alters the lipid profile, increases oxidative stress and inflammation, which heightens the likelihood of developing obesity and metabolic syndrome (DiNicolantonio et al., 2018; Yu et al., 2021). In addition, excessive dietary fructose hinders the liver's ability to oxidise fatty acids and reduces the production of high-density lipoprotein cholesterol, leading to accumulation of visceral fat and an overall body mass gain (Guney et al., 2023).

Haematological abnormalities, particularly anemia, are frequently observed in individuals with metabolic syndrome, especially in those who are overweight or obese, or who have T2DM (Bekele et al., 2019; Jeong et al., 2022). Oxidative stress and inflammatory conditions, common features of metabolic syndrome, have been associated with haematological abnormalities (Bissinger et al., 2019). These abnormalities in the haematological profile increase the risk of kidney disease, cardiovascular complications, congestive heart failure, and ischemic stroke in people with metabolic syndrome (Sahay et al., 2017; Shenkut et al., 2024). Furthermore, high blood glucose and cholesterol concentrations are associated with disruptions in blood cell formation (Lee et al., 2018). Leptin, a 16-kDa polypeptide hormone primarily produced and released by white adipocytes, plays a crucial role in regulating food intake by suppressing appetite, controlling energy expenditure, and enhancing sympathetic nervous system activity (Dilworth et al., 2021). It regulates body mass and fat accumulation by activating receptors in the hypothalamus, leading to the suppression of appetite (Vilariño-García et al., 2024). Serum leptin concentrations have been shown to decrease during fasting (Fontana et al., 2023), suggesting that higher concentrations of

leptin would be associated with lower mass and vice versa (Ekmen et al., 2016). However, this is not consistently observed, especially in obese and overweight individuals who consume excessive dietary fructose (Johnson et al., 2023). In such cases, serum leptin concentrations may be more aligned with body fat due to leptin resistance (Picó et al., 2021). Elevated serum leptin negatively affects insulin secretion (Chiriacò et al., 2024), which can counteract its appetite-suppressing effects, potentially leading to weight gain over time and difficulty managing body mass (Rangareddy et al., 2023).

Metabolic disorders pose a global challenge, as the high cost of conventional synthetic medications and inadequate healthcare infrastructure, particularly in developing nations, rendering these medications out of reach for the vast majority (Yenet et al., 2023). In light of these challenges, there is a rising interest in utilising medicinal plants and naturally derived phytochemicals with a wide range of biological effects (Sezer et al., 2024). Lycopene, a fat-soluble compound present in tomatoes and watermelons, is known for its ability to combat oxidative stress and inflammation (Sharma et al., 2024). Studies have demonstrated its ability to enhance haematological parameters (Eze et al., 2019), potential in combating obesity (Zhu et al., 2020), and its hypolipidaemic and antidiabetic properties (Jin & Arroo, 2023). This study therefore investigated the potential benefits of supplemental lycopene on blood glucose, glycated haemoglobin, haemoglobin, haematocrit, serum leptin concentration, and lipid profile in growing rats fed a high fructose diet.

4.1 Materials and methods

4.1.1 Ethical approval

This is as described in chapter 3, subheading 3.1.1.

4.1.2 Chemicals and reagents

The chemicals and reagents utilised are consistent with those described in chapter 3, subheading 3.1.2.

4.1.3 Animals, housing, and general care

The management of experimental animals in this chapter is consistent with the procedures described in chapter 3, under subheading 3.1.3.

4.1.4 Experimental design

The study design is as described in chapter 3, subheading 3.1.4.

4.1.5 Terminal procedures, sample collection and measurements

On postnatal day 106, the rats were fasted overnight but were allowed access to plain drinking water to prevent dehydration. In the morning of termination (postnatal day 107), the body mass of each rat was measured using an electronic balance (Snowrex Electronic Scale, Clover Scale Pty Ltd, Johannesburg). Blood was collected via tail vein puncture (Hattori et al., 2020) to assess fasting blood glucose (FBG), triglyceride, haemoglobin, and haematocrit concentrations. FBG was measured with a Bayer Contour Plus Meter (Isando, Johannesburg, South Africa), while triglyceride concentrations were determined using an Accutrend GCT meter (Roche, Mannheim, Germany). Haemoglobin and haematocrit concentrations were measured using an Insight HCT meter (Woodley Equipment Co. Ltd., Bolton, UK).

Following this, the rats were euthanised using an intraperitoneal injection of sodium pentobarbitone at 200 mg/kg body mass (Euthapent; Kyron laboratories, Johannesburg). Blood was then obtained through cardiac puncture with 20G needles and 10ml syringes. A portion of the blood (1 ml) was transferred to EDTA K_2 blood collection tubes (BD Vacutainer Systems Bigamart, UK) and later used for the assessment of glycated haemoglobin using the A1CNow meter (PTS Diagnostics, Whitestown, USA). The remainder of the blood was placed in SST gel coated blood collection tubes (BD Vacutainer System Bigamart, UK). Subsequently, it (remainder of the blood)

was centrifuged (DLAB-DMO412S, Wolflabs Laboratory Products, Pocklington, UK) at 3000 × g for15 minutes at 20℃ and the serum collected was decanted into Eppendorf microtubes (Abdos Labtech Pvt, Ltd, Uttrakhand, India) and then frozen-stored at -80 ℃ for subsequent analyses of leptin, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and total cholesterol (TC) concentrations.

4.1.6 Determination of serum lipoproteins, and total cholesterol

Fasting serum LDL, HDL, and TC concentrations were measured using a rat-specific enzyme-linked immunosorbent assay (ELISA) kit from Elabscience Biotechnology Inc., Houston, Texas, USA, in accordance with the manufacturer's guidelines.

4.1.7 Determination of glycated haemoglobin concentrations

Glycated haemoglobin (HbA1c) concentrations were determined from blood samples collected into EDTA K_2 blood collection tubes (BD Vacutainer Systems Bigamart, UK). An A1CNow meter (PTS Diagnostics, Whitestown, USA) was used to assess HbA1c concentrations, adhering to the manufacturer's guidelines. Briefly, a small drop of blood was pipetted onto a glass slide. It was then transferred into the collector within the shaker body where it was shaken vigorously about 6 to 8 times to mix with the reaction solution. Following this, the test cartridge was inserted into the A1CNow meter, and the blood sample was dispensed into the cartridge. The results were then displayed on the screen after 5 minutes.

4.1.8 Determination of serum leptin concentration

Serum leptin concentration was assessed using a rat-specific ELISA kit (Elabscience Biotechnology Inc., Houston, Texas, USA) based on the sandwich ELISA method. Standard solutions and diluted serum samples were added to a micro-ELISA plate, followed by the addition of a specific antibody. A biotinylated detection antibody for Rat LEP and an Avidin-Horseradish Peroxidase (HRP) conjugate were then introduced into each well, allowing leptin in the samples to bind to the antibodies. After incubation, unbound substances were removed through washing. A substrate solution was added, causing wells containing the Rat LEP HRP conjugate to turn blue. The reaction was stopped with a stop solution, resulting in a yellow color change. Optical density (OD) was measured at 450 ± 2 nm using a microplate reader (Multiskan Ascent, Lab System,

Model No. 354, Helsinki, Finland). Leptin concentrations were determined by comparing the absorbance values to a standard curve generated from known concentrations.

4.1.9 Statistical analysis

Data analysis was performed using GraphPad Prism version 9.0 (GraphPad Software Inc., San Diego, USA), and results were expressed as mean ± standard deviation. A one-way ANOVA was used to assess parametric data across multiple groups, followed by Tukey's *post hoc* test for mean comparisons. Significance was established at $p < 0.05$.

4.2 Results

4.2.1 Haemoglobin and haematocrit concentrations

Table 4.1 show the concentrations of haemoglobin and haematocrit in female and male rats, respectively. No significant differences ($p > 0.05$) were observed in haemoglobin and haematocrit concentrations across treatment groups in both sexes.

Table 4. 1: Effect of lycopene on haemoglobin and haematocrit concentration in growing female and male Wistar rats fed a high-fructose diet

Parameter	PDW +PG	FS + PG	FS + FENO	FS +LDLY	FS + MDLY	FS + HDLY	Significance
Females							
Haemoglobin (g/dL)	17.31 ± 2.95[a]	17.30 ± 1.50[a]	16.13 ± 2.45[a]	17.56 ± 3.82[a]	17.85 ± 3.21[a]	17.89 ± 2.68[a]	ns
Haematocrit (%)	52 ± 8.70[a]	52 ± 4.38[a]	48.38 ± 7.56[a]	52.75 ± 11.40[a]	53.88 ± 9.60[a]	53.5 ± 8.02[a]	ns
Males							
Haemoglobin (g/dL)	18.55 ± 1.72[a]	16.98 ± 2.87[a]	17.83 ± 1.67[a]	19.74 ± 2.26[a]	19.06 ± 1.54[a]	18.39 ± 2.19[a]	ns
Haematocrit (%)	55.63 ± 5.07[a]	46.96 ± 18.65[a]	53.50 ± 5.10[a]	59.50 ± 6.92[a]	57.13 ± 4.29[a]	55.13 ± 6.66[a]	ns

[a]Means within rows having the same superscripts are similar at $p > 0.05$, ns = not significant. PDW +PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 $mg.kg^{-1}$BM day^{-1} of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 $mg.kg^{-1}$BM day^{-1} in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 $mg.kg^{-1}$BM day^{-1} in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 $mg.kg^{-1}$BM day^{-1} in gelatine cube. Data are expressed as mean ± SD, n = 8 per treatment group.

4.2.2 Fasting blood glucose and glycated haemoglobin concentrations

Figures 4.1 and 4.2 show the fasting blood glucose (A) and glycated haemoglobin (B) concentrations in female and male rats, respectively. Female rats fed the high fructose diet with fenofibrate intervention exhibited significantly higher ($p < 0.05$) fasting blood glucose concentrations compared to their counterparts fed the high fructose diet with medium and high lycopene doses as supplements (Figure 4.1A). Similarly, glycated haemoglobin concentrations were significantly higher ($p < 0.05$) in female rats administered fenofibrate compared to those on the control diet (Figure 4.1B). In male rats, fasting blood glucose levels were significantly elevated ($p < 0.05$) in those fed a high-fructose diet with fenofibrate compared to those in the control group, the high-fructose group, and the high-fructose groups supplemented with low, medium, or high doses of lycopene (Figure 4.3A). However, no significant differences ($p > 0.05$) were observed in glycated haemoglobin concentrations across treatment groups in male rats (Figure 4.3B).

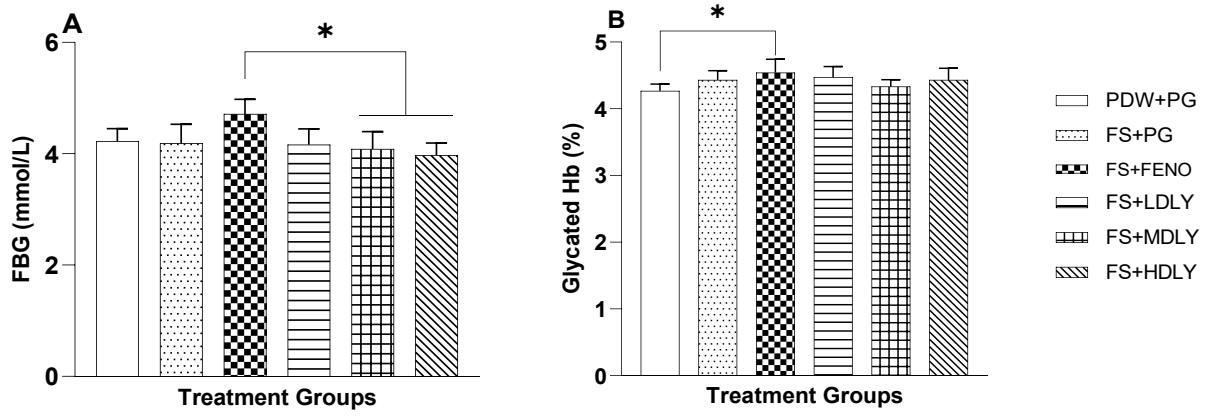


Figure 4. 1: Effect of lycopene on fasting blood glucose (A) and glycated haemoglobin (B) concentration in growing female Wistar rats fed a high-fructose diet.

*= significantly different at $p < 0.05$. PDW +PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 mg.kg^{-1}BM day^{-1} of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 mg.kg^{-1}BM day^{-1} in gelatine cube. Data are expressed as mean ± SD, n = 6-8 per treatment group.

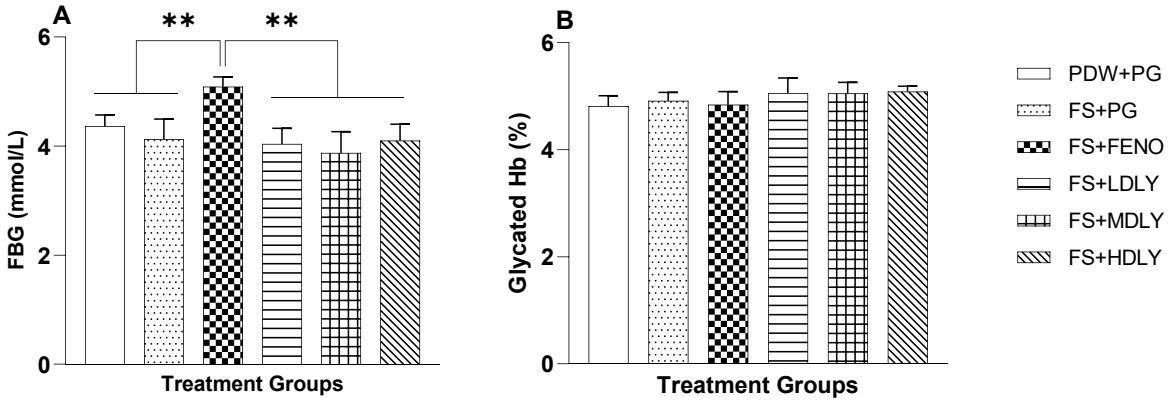


Figure 4. 2: Effect of lycopene on fasting blood glucose (A) and glycated haemoglobin (B) concentration in growing male Wistar rats fed a high-fructose diet.

**= significantly different at $p < 0.001$. No significant difference ($p > 0.05$) in the glycated haemoglobin concentration across treatment groups. PDW +PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 $mg.kg^{-1}$BM day^{-1} of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 $mg.kg^{-1}$BM day^{-1} in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 $mg.kg^{-1}$BM day^{-1} in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 $mg.kg^{-1}$BM day^{-1} in gelatine cube. Data are expressed as mean ± SD, n = 7-8 per treatment group.

4.2.3 Lipid profile

Tables 4.2 show the effect of lycopene on lipid profiles in female and male rats fed a high fructose diet. Female rats fed the high-fructose diet with fenofibrate as an intervention exhibited significantly higher ($p < 0.05$) serum high-density lipoprotein (HDL) concentrations compared to those fed the high-fructose diet supplemented with a low dose of lycopene. However, no significant differences ($p > 0.05$) were observed in the lipid profiles of male rats across treatment groups (Table 4.2).

Table 4. 2: Effect of lycopene on lipid profile in growing female and male Wistar rats fed a high-fructose diet

Parameter	PDW +PG	FS + PG	FS + FENO	FS +LDLY	FS + MDLY	FS + HDLY	Significance
Females							
TG (mmol/L)	1.24 ± 0.28[a]	1.49 ± 0.28[a]	1.71 ± 0.54[a]	1.59 ± 0.46[a]	1.67 ± 0.38[a]	1.41 ± 0.20[a]	ns
LDL (µg/mL)	629.30 ± 140.50[a]	651.00 ± 149.30[a]	734.90 ± 299.40[a]	446.90 ±176.70[a]	571.40 ± 276.80[a]	591.00 ± 232.00[a]	ns
HDL (ng/mL)	31386 ± 5536[ab]	33943 ± 8897[ab]	38597 ± 5577[a]	26419 ± 6207[b]	30420 ± 10269[ab]	30836 ± 3659[ab]	*
TC (mmol/L)	1.132 ± 0.23[a]	1.53 ± 0.40[a]	1.51 ± 0.40[a]	1.32 ± 0.29[a]	1.33 ± 0.28[a]	1.43 ± 0.13[a]	ns
Males							
TG (mmol/L)	1.36 ± 0.62[a]	1.19 ± 0.14[a]	1.57 ± 0.80[a]	1.35 ± 0.32[a]	1.18 ± 0.17[a]	1.15 ± 0.10[a]	ns
LDL (µg/mL)	574.40 ± 269.30[a]	553.20 ± 142.60[a]	738.40 ± 203.20[a]	508.00 ± 86.31[a]	536.20 ± 94.91[a]	710.70 ± 141.70[a]	ns
HDL (ng/mL)	22081 ± 7699[a]	28216 ± 10721[a]	28981 ± 3300[a]	20604 ± 5144a	24860 ± 8427a	29015 ± 5309a	ns
TC (mmol/L)	1.08 ± 0.15[a]	1.25 ± 0.36[a]	1.10 ± 0.31[a]	0.86 ± 0.17[a]	1.13 ± 0.48[a]	1.42 ± 0.50[a]	ns

[ab]Means within rows having the different superscript differ significantly at * $p < 0.05$, ns = not significant. PDW +PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 mg.kg^{-1}BM day^{-1} of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 mg.kg^{-1}BM day^{-1} in gelatine cube. Data are expressed as mean ± SD, n = 6-8 per treatment group.

4.2.4 Serum leptin concentration

Figures 4.3A and 4.3B show the serum concentration of leptin in female and male rats, respectively. In both sexes, the serum concentration of leptin was similar ($p > 0.05$) across treatment groups.

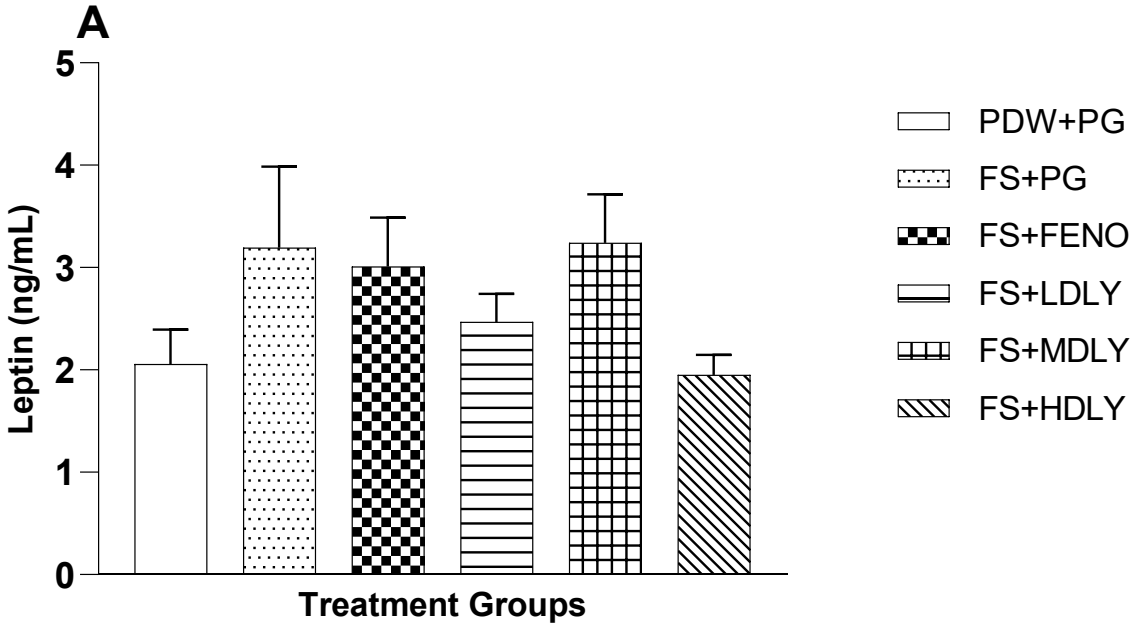


Figure 4. 3A: Effect of lycopene on serum leptin concentration in growing female Wistar rats fed a high-fructose diet.

No significant difference ($p > 0.05$) in the serum leptin concentration across treatment groups in the female rats. PDW +PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 mg.kg^{-1}BM day^{-1} of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 mg.kg^{-1}BM day^{-1} in gelatine cube. Data are expressed as mean ± SD, n = 6-7 per treatment group.

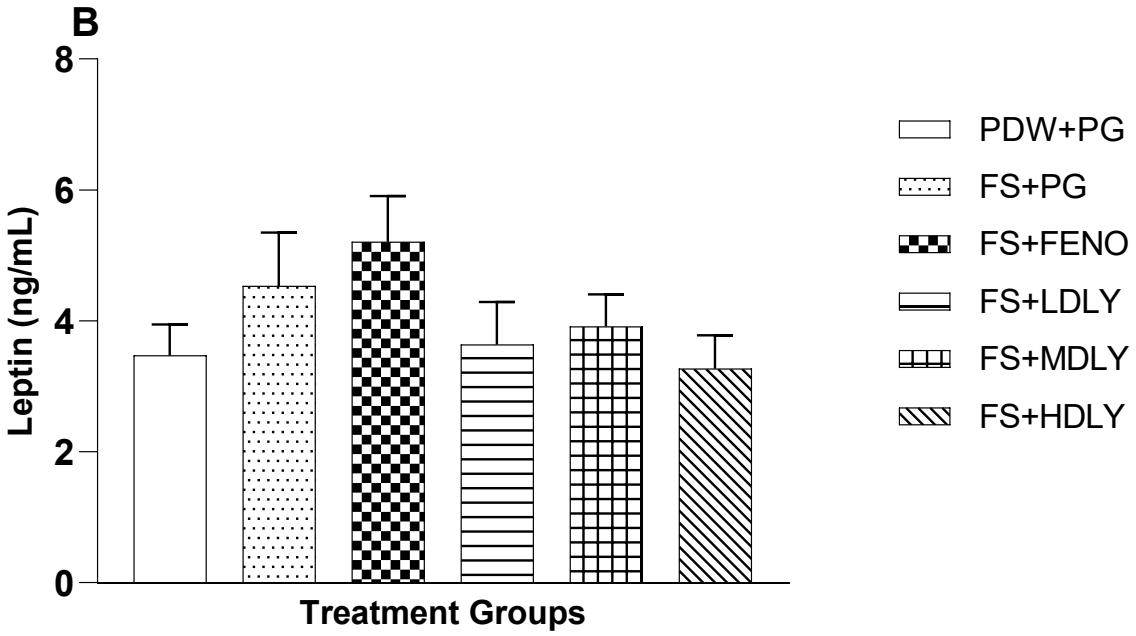


Figure 4. 3B: Effect of lycopene on serum leptin concentration in growing male Wistar rats fed a high-fructose diet.

No significant difference ($p > 0.05$) in the serum leptin concentration across treatment groups in the male rats. PDW +PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 $mg.kg^{-1}BM\ day^{-1}$ of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube. Data are expressed as mean ± SD, n = 6-7 per treatment group.

4.3 Discussion

4.3.1 Haemoglobin and Haematocrit Concentrations

This study investigated the potential benefits of orally administered lycopene against dietary fructose-induced metabolic derangements, focusing on effects on blood glucose, haemoglobin, haematocrit and leptin concentrations and serum lipid profile in growing weanling Wistar rats in a model mimicking adolescents fed a high fructose diet. In this study, no significant differences were observed in the haemoglobin and haematocrit concentrations of the rats across treatment groups. These results suggest that the treatments did not have a detrimental effect on the haemoglobin and haematocrit levels during the 12-week intervention. Studies have shown that lycopene increased red blood cell counts, haemoglobin and haematocrit, with no effect on mean corpuscular volume, mean corpuscular haemoglobin or mean corpuscular haemoglobin concentration in streptozotocin-induced diabetic rats (Eze et al., 2019) and protected against cadmium-induced toxicity in mice (Sharma & Chowdary, 2015). Fructose's direct effects on haemoglobin and haematocrit concentration and other blood cell counts are not extensively documented. Unlike certain compounds or medications known to directly influence blood parameters, the impact of fructose on haematology appears to be more indirect and secondary to its effects on metabolism, inflammation, and oxidative stress (Bissinger et al., 2019). Kelem et al. (2023) reported that haematological abnormalities are common in individuals with type 2 diabetes mellitus who are either overweight or obese. In the current study dietary fructose did not lead to obesity, type 2 diabetes mellitus or dyslipidaemia. Therefore, I hypothesise that the effect of fructose on blood parameters may vary depending on factors such as dosage, duration of exposure, and individual metabolic and health status.

4.3.2 Glucose and Lipid Profile

In this study, female rats fed the high fructose diet with fenofibrate as an intervention showed significantly increased fasting blood glucose concentration compared to their counterparts fed the high fructose diet supplemented with medium and high doses of lycopene. Similarly, rats reared on a high-fructose diet with fenofibrate had higher glycated haemoglobin levels compared to their counterparts fed the control diet. Male Wistar rats fed the high fructose diet with fenofibrate had significantly higher fasting blood glucose concentrations compared to their counterparts fed the control diet, the high fructose diet, or the high fructose diet supplemented with low, medium, or

high doses of lycopene. However, there was no significant difference observed in the glycated haemoglobin concentration across treatment groups in the male rats. The current study found no significant difference in the triglycerides, low-density lipoprotein, high-density lipoprotein, and total cholesterol levels in the male rats across treatment groups. Similarly, in female Wistar rats, those fed the high fructose diet with fenofibrate as an intervention had significantly higher high-density lipoprotein compared to their counterparts fed the high fructose diet with a low lycopene dose as a supplement. These results suggest that dietary fructose did not have negative impact on metabolic markers (fasting blood glucose, HbA1c, triglycerides, LDL, HDL, and total cholesterol) levels in female and male rats. This implies that fructose consumption did not lead to adverse metabolic effects in this study. These findings align with Tillman et al. (2014), who reported that weanling c57BI/6 mice fed a 60% fructose diet showed no significant differences in body mass, plasma glucose triglycerides, and free fatty acids concentrations. Previous studies have demonstrated that long-term consumption of a high-fructose diet can lead to metabolic syndrome in adult rodents. For instance, Farag et al., (2020) found that rats fed a 60% fructose for 10 weeks exhibited signs of metabolic syndrome, such as elevated levels of fasting blood glucose, insulin, triglycerides, and total cholesterol. Koseler et al., (2018) also observed features of obesity, type 2 diabetes mellitus, hyperlipidaemia, and insulin resistance when 30% of fructose was administered for 6 weeks to adult male Wistar rats. Similarly, adult rats fed a 20% fructose diet for 12 weeks showed dyslipidaemia, insulin resistance, visceral fat accumulation and hepatomegaly (Ferreira-Santos et al., 2020). It is important to note that this study targeted adolescents, hence the use of growing weanling Wistar rats. The surge in overweight and obesity has been linked to increased consumption of sugary foods among children and adolescents (Magriplis et al., 2021). The body surface area to volume ratio is greater in young pups and growing rats than in adult rats. This can result in a faster metabolic rate and lead to the metabolism of excess fructose without exhibiting the same adverse effects seen in adult rats (Tillman et al., 2014). I hypothesise that the resistance of young growing rats to metabolic derangements caused by dietary fructose could be due to high energy demand for growth and development. This increased energy demand may help metabolise fructose more effectively, preventing the accumulation of harmful metabolic by-products. The liver plays a crucial role in metabolising fructose (Francey et al., 2019). In young growing rats, liver function may be more efficient, allowing for better clearance and utilisation of fructose compared to adult rats. Additionally, younger humans and rodents typically exhibit higher insulin

sensitivity, which helps regulate blood sugar levels more effectively (Kolb et al., 2023). This can prevent the development of hyperglycaemia associated with fructose consumption (Ehrhardt et al., 2019; Kolb et al., 2023). However, it is essential to note that excessive fructose consumption can still have detrimental effects on health over time if not properly controlled (Song et al., 2021).

Lycopene is known as a potent antioxidant (Leh & Lee, 2022). Previous studies have demonstrated that feeding a high-fat diet for 4 weeks followed by oral lycopene supplementation at 20 mg/kg body mass for 10 weeks decreased blood glucose and glycated haemoglobin levels and ameliorated lipid disorders in adult male Spraque-Dawley rats with streptozotocin-induced diabetes mellitus (Yin et al., 2019). Mulkawar et al. (2015) noted no significant difference in the fasting blood sugar levels and observed decreased lipid activity in adult male New Zealand White rabbits fed a high-fat diet supplemented with 10 mg/kg body mass of lycopene for 6 weeks. Additionally, when 300g of tomatoes were administered to 50 healthy volunteers (16 men and 34 women) daily for one month, there were no changes in their plasma triglycerides, LDL, and total cholesterol levels, but a significant increase in plasma HDL level was observed (Blum et al., 2006). The findings of this study showed that the consumption of a high-fructose diet supplemented with lycopene during the 12-week intervention neither affected blood glucose levels nor lipid profile of the rats. I hypothesise that the consumption of high fructose diet supplemented with lycopene may lack an impact on the rat's glucose and lipid profile levels. This could be due to lycopene interacting with other nutrients in the diet or with the high-fructose content in ways that did not lead to changes in glucose or lipid levels. We speculate that the rats may have had relatively healthy baseline glucose and lipid levels, making it difficult to observe significant changes with the intervention. Additionally, the 12-week duration of the intervention may have been too short to observe any meaningful changes in glucose or lipid metabolism, considering that young rats are resistant to metabolic derangements caused by dietary fructose (Tillman et al., 2014).In the present study, orally administered fenofibrate caused a significant increase in fasting blood glucose and glycated haemoglobin levels in female rats. Similarly, male rats treated with fenofibrate exhibited significantly higher fasting blood glucose concentrations. Nonetheless, it was noted that fenofibrate caused a significant increase in HDL levels in the female rats. These results suggest that orally administered fenofibrate affects glucose metabolism differently in male and female rats, indicating adverse effects on glucose regulation in both sexes, while it has a positive impact on HDL levels in the female rats. Previous studies have demonstrated that fenofibrate, when included

in the diet of female non-obese diabetic mice for 27 weeks, decreased non-fasting blood glucose and elevated fasting blood glucose levels, decreased fasting glucagon levels, and averted fasting hyperinsulinaemia (Holm et al., 2019). Liu et al. (2011) found that orally administered fenofibrate for 12 weeks decreased plasma lipid levels, showed no beneficial effects on glucose homeostasis or insulin sensitivity in obese monosodium glutamate induced male Wistar rats. In this study, insulin sensitivity was not assessed, but normal levels of circulating lipids in fructose-fed rats were observed. Given that high blood fats are linked with insulin resistance and considering that fasting blood glucose and glycated haemoglobin concentrations were normal in these rats, it suggests that the fructose-fed rats in this study may not have been insulin resistant. Fenofibrate, a hypolipidaemic peroxisome proliferator-activated receptor α (PPARα) agonist works by reducing the production of triglycerides and increasing the levels of HDL cholesterol, often referred to as "good" cholesterol (La Fountaine et al., 2019). It is commonly prescribed to treat high levels of cholesterol and triglycerides in the blood (Lee et al., 2023). I hypothesise that fenofibrate may decrease insulin sensitivity in some rats causing their cells to take up less glucose and resulting in elevated blood glucose levels. It could also disrupt the liver's regulation of glucose production, leading to more glucose being released into the bloodstream. In addition, it may affect pancreatic function, including insulin secretion, which could further influence blood glucose levels. Fenofibrate might interfere with different pathways related to glucose metabolism, leading to irregular blood glucose levels.

4.3.3 Leptin concentration

The present study found no significant differences in the fasting serum leptin levels of the female and male rats across treatment groups. These results suggest that the treatment did not have a negative impact on fasting serum leptin concentration in the rats. Previous studies showed that a 20% fructose solution administered orally daily for 8 weeks increased plasma leptin and insulin levels, elevated serum glucose and TG levels, decreased HDL and total cholesterol levels, and showed no significant changes in LDL levels in adult male Sprague-Dawley rats (Eğritağ & Haliloğlu, 2022). Teff et al. (2004) reported that dietary fructose decreased 24-hour leptin and insulin levels, raised triglycerides, and inhibited ghrelin in women. Studies indicate that plasma leptin levels decreased during fasting or energy restriction (Mars et al., 2006), but meal consumption did not lead to an immediate increase in plasma leptin concentrations. This suggests that leptin acts as a regulator of energy balance over the medium to long term (Mendoza-Herrera

et al., 2021). Leptin secretion is controlled by the metabolism of glucose mediated by insulin. Unlike glucose, fructose does not trigger insulin secretion (Baena et al., 2016; Dilworth et al., 2021). I hypothesise that a high-fructose diet would lead to reduced leptin levels in young growing rats. However, despite the study lasting 12 weeks, no significant changes in leptin levels were observed. It is known that leptin responses to dietary changes can take weeks to become apparent. In this study, orally administered fenofibrate and lycopene did not have a negative impact on fasting serum leptin concentrations. However, Jeong et al. (2005) found that a 15% high-fat diet supplemented with fenofibrate for 14 weeks increased body mass, total cholesterol, and triglycerides in C57BL/6J female mice, but did not significantly affect the expression of leptin and TNFα genes. In their study, Nabil et al. (2020) reported that a 20% fructose solution administered for 18 weeks, with fenofibrate given from the 13th to the 18th week, resulted in decreased fasting plasma levels of leptin, TNFα, glucose, triglycerides, total cholesterol, and total lipids in male Sprague-Dawley rats. However, insulin concentrations did not differ significantly. Thus, from the current study it can be speculated that orally administered fenofibrate did not negatively affect fasting serum leptin levels, as fenofibrate primarily targets lipid metabolism and may not directly impact leptin production or secretion. Lycopene was shown to reduce the expression of leptin, resistin and interleukin-6 (IL-6) gene in epididymal adipose tissue, as well as lower plasma concentration in male Wistar rats fed a high-fat diet for 6 weeks (Luvizotto et al., 2013). I hypothesise that the lack of a significant difference in fasting serum leptin levels in lycopene-treated rats may be due to the possibility that lycopene supplementation did not exert a substantial effect on leptin levels in young growing rats during the 12-week intervention period. The young, growing rats used in the study may have different metabolic responses compared to adult rats, thereby minimizing the effects of lycopene supplementation on leptin levels. Further research is warranted to understand the dynamics of lycopene supplementation on leptin levels, considering factors such as dosage, duration, and the specific physio-metabolic conditions, for example, age, and growth stage.

4.4 Conclusion

Supplemental lycopene and dietary fructose did not negatively impact glycated haemoglobin, haemoglobin, haematocrit, blood glucose and serum leptin concentrations and lipid profile in young growing Wistar rats. However, orally administered fenofibrate may exert sex-specific effects on glucose metabolism and lipid profiles. In female and male rats, fenofibrate caused a

notable increase in fasting blood glucose but increased glycated haemoglobin (HbA1c) and serum HDL levels in females only. While orally administered fenofibrate might improve metabolic health by mediating increased serum HDL in females, its chronic use may disrupt blood glucose homeostasis in both sexes.

Having discussed the effects of supplemental lycopene on blood glucose, glycated haemoglobin, haemoglobin, haematocrit, serum leptin concentration, and lipid profile in growing rats fed a high fructose diet, the next chapter explored the potential benefits of supplemental lycopene for kidney health in growing Wistar rats fed a high-fructose diet.

4.5 References

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CHAPTER FIVE: EFFECT OF LYCOPENE ON KIDNEY HEALTH IN GROWING WISTAR RATS FED A HIGH FRUCTOSE DIET

5.0 Introduction

Chronic kidney disease (CKD) has become one of the foremost causes of death globally (Kovesdy, 2022). This condition gradual impairs kidney function over a period of three months or more (Ammirati, 2020), posing significant health challenges and burdens for both individuals and healthcare systems (Lameire et al., 2021). It is estimated that around 850 million people worldwide is affected by CKD, with substantial number residing in low- and middle-income countries (Jager et al., 2019). Many of these individuals do not have access to diagnosis, prevention, or treatment for the condition (Jager et al., 2019). By the year 2040, CKD is expected to rank as the fifth leading cause of death worldwide (Foreman et al., 2018). The growing prevalence of CKD cases is driven by various factors, including the global increase in diabetes, hypertension, chronic inflammation, obesity, dyslipidaemia, proteinuria, aging, high serum uric acid levels, genetic predispositions, socioeconomic and ethnic influences and dietary factors such as high salt intake (Xie et al.,2023; Jairoun et al., 2024; Mallamaci & Tripepi, 2024). All these factors are significant risk contributors to CKD, which, without proper management, can advance to end-stage renal disease (ESRD), which often requires dialysis or a kidney transplant for survival (Liu et al., 2023).

High fructose consumption and sedentary lifestyles contribute to CKD (Xu et al., 2021). Fructose is metabolised in the kidney, although the majority of fructose metabolism occurs in the liver (Dholariya & Orrick, 2022). The kidneys are involved in fructose metabolism through the enzyme fructokinase, which converts fructose to fructose-1-phosphate (Dholariya & Orrick, 2022). Under normal physiological conditions, fructose is metabolised into glucose in the proximal tubular epithelial cells of the kidneys (Faivre et al., 2021). However, prolonged exposure to high dietary fructose levels can trigger fructolysis, causing substantial ATP depletion and inflammation that ultimately lead to injury to the renal tubules (Nakagawa et al., 2020). This metabolic process can impact kidney function, especially in people with diabetes mellitus or metabolic syndrome, where excessive fructose consumption may cause kidney damage or exacerbate existing kidney conditions (DiNicolantonio et al., 2016; Nakagawa & Kang, 2021).

Surrogate markers like serum creatinine, albumin levels, and blood urea nitrogen (BUN) are commonly used to evaluate kidney function and monitor CKD progression (Gounden et al., 2023). However, the glomerular filtration rate (GFR) remains the gold standard for assessing kidney function, offering a thorough evaluation of kidney health (Farrell & Vassalotti, 2024). In contrast,

neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1) provide early detection and specific insights into acute kidney injury by detecting proteins released from injured kidney cells, indicating early renal tubular damage and injury (Westhoff et al., 2017; Treacy et al., 2019). Nonetheless, kidney biopsies and histology provide the most accurate and detailed information about kidney structure and specific disease processes, serving as the gold standard for assessment (Madrazo-Ibarra & Vaitla, 2023; Schnuelle, 2023). This evaluation includes a thorough examination of histopathological features and morphometric changes, such as variations in glomerular tufts, urinary spaces, renal corpuscle areas, and both proximal and distal convoluted tubules (Abd-Eldayem et al., 2024).

Lifestyle adjustments and the use of synthetic medications can reduce the risk and advancement of diet-related kidney disease (Alkhatib et al., 2023). However, synthetic medications do not completely cure the disease and can have adverse side effects (Alhassani et al., 2021). Additionally, the cost of treating advanced stages of kidney disease with dialysis and kidney transplants places a substantial strain on both patients and the healthcare system (Himmelfarb et al., 2020). In response to these challenges, there is growing interest in the use of crude plant extracts and phytochemicals derived from medicinal plants which exhibit health benefitting biological effects (Misra et al., 2024). Lycopene, a naturally occurring carotenoid abundant in red fruits and vegetables possesses antioxidant and anti-inflammatory properties (Sharma et al., 2024). It has been shown to mitigate oxidative stress-induced kidney damage in rats treated with isoniazid and rifampicin (Bedir et al., 2021). Furthermore, the carotenoid has been demonstrated to reduce serum levels of creatinine and BUN in rats with adenine-induced chronic renal failure (Gori et al., 2021) and to decrease KIM-1 and NGAL concentration in rats with diclofenac-induced acute kidney injury (Rasheed et al., 2020). Majority of the research on the efficacy of lycopene have evaluated its potential therapeutic value and its prophylactic potential. Hence this study evaluated the potential of orally administered lycopene to protect kidney health in Wistar rats fed a high fructose diet focusing specifically on kidney macro- and micromorphometry as well as on serum biomarkers reflecting kidney function.

5.1 Materials and methods

5.1.1 Ethical approval

This is as detailed in chapter 3, under subheading 3.1.1.

5.1.2 Chemicals and reagents

The chemicals and reagents utilised are consistent with those detailed in chapter 3, under subheading 3.1.2.

5.1.3 Animals, housing, and general care

The management of experimental animals in this chapter is consistent with the same procedures outlined in chapter 3, under subheading 3.1.3

5.1.4 Experimental design

The study design is as detailed in chapter 3, under subheading 3.1.4.

5.1.5 Terminal procedures, sample collection, and measurements

Following the 12-week intervention period, the rats were fasted overnight but with access to plain drinking water to prevent dehydration. The terminal body mass of each rat was measured using an electronic balance [Snowrex Electronic Scale from Clover Scale (Pty) Ltd, Johannesburg]. The rats were subsequently euthanised using an intraperitoneal injection of sodium pentobarbitone administered at a dose of 200 mg/kg body mass (Euthapent; Kyron laboratories, Johannesburg). Blood samples were obtained through cardiac puncture using 20G needles and 10ml syringes into SST gel-coated blood collection tubes (BD Vacutainer System Bigamart, UK). The blood was then centrifuged (DLAB-DMO412S, Wolflabs Laboratory Products, Pocklington, UK) at 3000 × g for 15 minutes at -20 ℃. The serum collected was decanted into Eppendorf microtubes (Abdos Labtech Pvt, Ltd, Uttrakhand, India) and frozen-stored at -80 ℃ pending assay for serum creatinine, blood urea nitrogen (BUN), BUN/creatinine ratio, KIM-1, and NGAL concentrations. After blood collection, a midline incision was performed in the abdomen of each rat, and kidneys were carefully removed and weighed using an electronic balance (Presica 310M, Instruments, Johannesburg, South Africa). The left kidney was fixed in 10% phosphate-buffered formalin for histological analysis. The mass of both kidneys relative to body mass (%BM) was then calculated as described by Muhammad et al. (2022) using the formula:

$$\text{Relative kidney mass} = \frac{Mass\ of\ kidney\ (g)}{Terminal\ body\ mass\ (g)} x\ 100$$

5.1.6 Determination of kidney function and injury biomarkers

Serum creatinine, BUN, and BUN/creatinine ratio concentrations were measured using a Noahcali-100 automatic LOCMEDT biochemistry analyser (Tianjin LOCMEDT Technologies Co., Ltd., China). Following the manufacturer's guidelines, 120 µl of serum was added to the reagent disc containing lyophilised beads, which was then loaded into the Noahcali-100 analyser. Results were generated within 8-12 minutes. Serum concentrations of KIM-1, and NGAL were determined using a rat-specific Enzyme-Linked Immunosorbent Assay (ELISA) kits from Elabscience Biotechnology Inc., Houston, Texas, USA, following the manufacturer's instructions.

5.1.7 Determination of kidney micromorphometry

The kidney samples preserved in 10% phosphate-buffered formalin were processed using an automatic tissue processor (Micro STP 120, Thermo Fisher Scientific, Waltham, USA) before being embedded in paraffin wax. Sections of 3 µm thickness were cut using a rotary microtome (Leica Biosystems, Buffalo Grove, USA) and mounted onto glass slides. Paraffin was removed from sections using xylene, and the tissues were rehydrated by passing through a series of progressively decreasing alcohol concentrations. The sections were then stained with haematoxylin and eosin. Following staining, the sections were dehydrated by passing through a series of increasing alcohol concentrations. The sections were then cleared in xylene to make the tissue transparent. A coverslip was placed over each tissue section using a mounting medium. The slides were examined under a light microscope and images were taken from various fields using a high definition ICC50W digital camera (Leica Biosystems, Germany) connected to a Leica DM750 microscope (Leica Biosystems, Germany). The images were assessed with the groups blinded to prevent bias. Measurements were obtained using ImageJ software for precise image analysis and quantification. The urinary space area, as well as the epithelial areas of the proximal and distal convoluted tubules, were determined using the following formulas:

(a) Urinary space area (µm²) = Renal corpuscular area (µm²) – Glomerular tuft area (µm²)

(b) Proximal convoluted tubules epithelial area (μm²) = Proximal convoluted tubules outer area (μm²) – Proximal convoluted tubules inner area (μm²)

(c) Distal convoluted tubules epithelial area (μm²) = Distal convoluted tubules outer area (μm²) Distal – convoluted tubules inner area (μm²)

5.1.8 Statistical analysis

GraphPad Prism version 9.0 (GraphPad Software Inc., San Diego, USA) was employed for data analysis, and results were presented as mean ± standard deviation. A one-way ANOVA assessed parametric data across multiple groups, followed by *Tukey's post hoc* test to compare differences between means. A p-value of less than 0.05 was considered statistically significant.

5.2 Results

5.2.1 Kidney mass

Table 5.1 show the effect of lycopene on the absolute and relative kidney masses of female and male Wistar rats. In females, no significant differences ($p > 0.05$) were observed in either absolute or relative kidney masses across treatment groups. In males, although no significant differences ($p > 0.05$) were found in absolute kidney mass across the groups, rats fed a high-fructose diet with fenofibrate intervention had significantly higher ($p < 0.05$) relative kidney masses compared to those fed the control diet or the high-fructose diet with any of the lycopene supplementation.

Table 5. 1: Effect of lycopene on absolute and relative kidney masses in growing female and male Wistar rats fed a high-fructose diet

Parameter	PDW +PG	FS + PG	FS + FENO	FS +LDLY	FS + MDLY	FS + HDLY	Significance
Females							
Kidney (g)	1.50 ± 0.13^{a}	1.43 ± 0.38^{a}	1.66 ± 0.12^{a}	1.53 ± 0.07^{a}	1.58 ± 0.11^{a}	1.45 ± 0.09^{a}	ns
Kidney (%BM)	0.71 ± 0.05^{a}	0.68 ± 0.18^{a}	0.80 ± 0.04^{a}	0.71 ± 0.04^{a}	0.73 ± 0.05^{a}	0.69 ± 0.02^{a}	ns
Males							
Kidney (g)	2.45 ± 0.19^{a}	2.43 ± 0.15^{a}	2.58 ± 0.20^{a}	2.36 ± 0.11^{a}	2.33 ± 0.10^{a}	2.28 ± 0.48^{a}	ns
Kidney (%BM)	0.69 ± 0.04^{a}	0.70 ± 0.04^{ab}	0.80 ± 0.03^{b}	0.68 ± 0.02^{a}	0.69 ± 0.02^{a}	0.65 ± 0.13^{a}	*

^{ab}Means within rows having different superscripts differ significantly at * $p < 0.05$, ns = not significant. PDW +PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 mg.kg^{-1}BM day^{-1} of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 mg.kg^{-1}BM day^{-1} in gelatine cube. % BM = Percentage body mass (relative to body mass). Data are expressed as mean ± SD, n = 8 per treatment group.

5.2.2 Serum biomarker concentrations

Table 5.2 shows the serum concentrations of BUN, creatinine, BUN/creatinine ratio, KIM-1, and NGAL in the rats. No significant differences ($p > 0.05$) were detected in the concentrations of these biomarkers across treatment groups in both sexes.

Table 5. 2: Effect of lycopene on serum concentrations of creatinine, BUN, BUN/creatinine ratio, KIM-1 and NGAL in growing female and male Wistar rats fed a high-fructose diet

Parameter	PDW +PG	FS + PG	FS + FENO	FS +LDLY	FS + MDLY	FS + HDLY	Significance
Females							
BUN (mg/dL)	19.14 ± 2.85[a]	19.94 ± 3.63[a]	19.14 ± 2.45[a]	20.64 ± 2.33[a]	18.38 ± 3.41[a]	20.40 ± 2.03[a]	ns
Creatinine (mg/dL)	0.34 ± 0.04[a]	0.31 ± 0.06[a]	0.33 ± 0.04[a]	0.33 ± 0.03a	0.32 ± 0.07[a]	0.32 ± 0.03[a]	ns
BUN/creatinine ratio	55.99 ± 6.44[a]	65.01 ± 6.01[a]	60.20 ± 12.40[a]	62.56 ± 7.13[a]	59.36 ± 15.96[a]	64.48 ± 8.031[a]	ns
KIM-1 (ng/mL)	1.52 ± 0.47[a]	1.86 ± 0.10[a]	2.19 ± 0.59[a]	1.07 ± 0.68[a]	1.57 ± 1.12[a]	1.82 ± 0.73[a]	ns
NGAL (pg/mL)	32466 ± 16756[a]	55094 ± 29085[a]	63863 ± 21220[a]	38193 ± 15550[a]	55944 ± 42247[a]	60882 ± 25192[a]	ns
Males							
BUN (mg/dL)	23.67 ± 1.27[a]	23.72 ± 11.66[a]	20.73 ± 1.91[a]	21.20 ± 3.13[a]	22.55 ± 2.07[a]	26.19 ± 3.47[a]	ns
Creatinine (mg/d/L)	0.35 ± 0.03[a]	0.33 ± 0.05[a]	0.31 ± 0.03[a]	0.29 ± 0.06[a]	0.29 ± 0.04[a]	0.35 ± 0.03a	ns
BUN/creatinine ratio	67.81 ± 7.76[a]	78.18 ± 48.03[a]	67.62 ± 3.80[a]	74.14 ± 16.17[a]	78.54 ± 8.52[a]	74.88 ± 12.16[a]	ns
KIM-1 (ng/mL)	0.88 ± 0.52[a]	1.78 ± 1.25[a]	1.39 ± 0.52[a]	0.54 ± 0.58[a]	1.60 ± 1.12[a]	1.23 ± 0.55[a]	ns
NGAL (pg/mL)	32916 ± 16570[a]	55491 ± 47379[a]	49216 ± 19611[a]	22840 ± 15022[a]	66128 ± 34323[a]	74428 ± 47699[a]	ns

[a]Means within rows having the same superscripts are similar at $p > 0.05$, ns = not significant, BUN = Blood urea nitrogen, KIM-1 = Kidney injury molecule 1, NGAL = Neutrophil gelatinase-associated lipocalin. PDW +PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 $mg.kg^{-1}BM\ day^{-1}$ of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube; FS +

HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 mg.kg^{-1}BM day^{-1} in gelatine cube. Data are expressed as mean ± SD, n = 5-7 per treatment group.

5.2.3 Kidney histomorphology

Tables 5.3 and 5.4 show the kidney morphometry of the female and male rats respectively. Figures 5.1 and 5.2 represent the kidney histology (H and E staining, 40x magnification) in female and male rats respectively fed a high fructose diet. No significant differences ($p > 0.05$) were observed in the corpuscular area, glomerular tuft area, or the outer, inner, and epithelial areas of the proximal convoluted tubules across treatment groups in the female rats (Table 5.3 and Figure 5.1). Female rats fed the control diet had a significantly larger urinary space area ($p < 0.05$; Table 5.3) compared to those on the high fructose diet supplemented with a low lycopene dose. Similarly, female rats fed the high fructose diet supplemented with a medium dose of lycopene had significantly increased urinary space area ($p < 0.05$) compared to those administered fenofibrate and counterparts whose diets were fortified with low and high lycopene doses. Female rats fed the high fructose diet with fenofibrate intervention had significantly larger ($p < 0.05$) outer, inner, and epithelial areas of the distal convoluted tubules compared to those on the high fructose diet supplemented with a high dose of lycopene. Furthermore, female rats fed the control diet had significantly larger renal distal convoluted tubule outer area ($p < 0.05$; Table 5.3) compared to counterparts fed the high fructose diet supplemented with low, medium, or high doses of lycopene. Female rats fed the control diet had significantly larger renal distal convoluted tubule inner areas ($p < 0.05$) compared to the high fructose diet fed and lycopene-supplemented counterparts. Additionally, the renal distal convoluted tubule epithelial area was significantly larger ($p < 0.05$) in control rats compared to high-dose lycopene-supplemented counterparts (Table 5.3 and Figure 5.1).

Table 5. 3: Effect of lycopene on kidney morphometry in growing female Wistar rats fed a high-fructose diet

Parameter	PDW +PG	FS + PG	FS + FENO	FS +LDLY	FS + MDLY	FS + HDLY	Significance
Corpuscular area (µm²)	5213 ± 1884^{a}	4932 ± 1393^{a}	4364 ± 809^{a}	4650 ± 983^{a}	4958 ± 1846^{a}	4460 ± 925^{a}	ns
Glomerular tuft area (µm²)	4351 ± 1387^{a}	4206 ± 1174^{a}	3761 ± 735^{a}	4108 ± 1000^{a}	3943 ± 1435^{a}	3802 ± 780^{a}	ns
Urinary space area (µm²)	862. ± 608^{ac}	727 ± 434^{dc}	603 ± 283^{dc}	542 ± 425^{d}	1015 ± 541^{ab}	658 ± 465^{cd}	*
PCT outer area (µm²)	950 ± 256^{a}	979 ± 299^{a}	1038 ± 278^{a}	1003 ± 264^{a}	1031 ± 208^{a}	1112 ± 454^{a}	ns
PCT inner area (µm²)	299 ± 123^{a}	306 ± 148^{a}	283 ± 117^{a}	307 ± 151^{a}	336 ± 99^{a}	355 ± 195^{a}	ns
PCT epithelial area (µm²)	651 ± 163^{a}	674 ± 173^{a}	755 ± 182^{a}	696 ± 140^{a}	695 ± 135^{a}	757 ± 277^{a}	ns
DCT outer area (µm²)	953 ± 505^{a}	794 ± 331^{ab}	919 ± 444^{ac}	698 ± 280^{bc}	703 ± 248^{bc}	622 ± 199^{b}	*
DCT inner area (µm²)	236 ± 239^{a}	133 ± 70^{bc}	208 ± 170^{ab}	134 ± 79^{bc}	130 ± 68^{bc}	111 ± 53^{c}	*
DCT epithelial area (µm²)	717 ± 380^{a}	661 ± 294^{ab}	712 ± 302^{a}	565 ± 227^{ab}	574 ± 221^{ab}	511 ± 167^{b}	*

^{abcd}Means within rows having different superscripts differ significantly at * $p < 0.05$, ns = not significant. PCT = Proximal convoluted tubule, DCT = Distal convoluted tubule. PDW +PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution

as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 mg.kg^{-1}BM day^{-1} of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 mg.kg^{-1}BM day^{-1} in gelatine cube. Data are expressed as mean ± SD, n = 4 per treatment group.

Male Wistar rats fed the control diet had significantly larger renal corpuscular and urinary space areas ($p < 0.05$) compared to those fed the high fructose diet supplemented with a medium lycopene dose (Table 5.4). Male rats fed the high fructose diet had significantly larger renal urinary space areas ($p < 0.05$) compared to their lycopene supplemented counterparts. Similarly, the renal urinary space areas of males administered fenofibrate were significantly larger ($p < 0.05$) compared to those males supplemented with a medium lycopene dose. Additionally, the distal convoluted tubule epithelial area of the rats was significantly larger ($p < 0.05$) in the fenofibrate administered rats compared to control, high fructose diet fed and low and high lycopene supplemented counterparts. Furthermore, male rats administered fenofibrate had a significantly larger ($p < 0.05$) distal convoluted tubule outer area compared to the high fructose diet-fed and high-dose lycopene-supplemented counterparts. Male rats supplemented with a medium lycopene dose showed a significant increase ($p < 0.05$) in the proximal convoluted inner area compared to those administered fenofibrate. No significant differences ($p > 0.05$) were observed in the glomerular tuft, outer and epithelial areas of the proximal tubules, or the inner area of the distal tubules across male treatment groups (Table 5.4, Figure 5.2). Additionally, no evidence of histological tubular damage was detected in either sex (Figures 5.1 or 5.2).

Table 5. 4: Effect of lycopene on kidney morphometry in growing male Wistar rats fed a high-fructose diet

Parameter	PDW +PG	FS + PG	FS + FENO	FS +LDLY	FS + MDLY	FS + HDLY	Significance
Corpuscular area (μm²)	6820 ± 2256^{a}	6358 ± 2233^{ab}	6024 ± 1989^{ab}	5879 ± 1534^{ab}	5435 ± 1298^{b}	5847 ± 1070^{ab}	*
Glomerular tuft area (μm²)	5644 ± 1827^{a}	4976 ± 1641^{a}	4804 ± 1600^{a}	5032 ± 1257^{a}	4795 ± 1235^{a}	4978 ± 870^{a}	ns
Urinary space Area (μm²)	1176 ± 649^{ac}	1382 ± 808^{a}	1219 ± 597^{ac}	847 ± 622^{bc}	640 ± 357^{b}	869 ± 588^{bc}	*
PCT outer area (μm²)	1511 ± 487^{a}	1489 ± 419^{a}	1635 ± 457^{a}	1524 ± 499^{a}	1827 ± 740^{a}	1557 ± 548^{a}	ns
PCT inner area (μm²)	521 ± 251^{ab}	528 ± 240^{ab}	495 ± 194^{a}	555 ± 241^{ab}	676 ± 363^{b}	593 ± 259^{ab}	*
PCT epithelial area (μm²)	990 ± 275^{a}	961 ± 244^{a}	1140 ± 292^{a}	969 ± 289^{a}	1151 ± 415^{a}	964 ± 335^{a}	ns
DCT outer area (μm²)	1010 ± 484^{ab}	920 ± 407^{a}	1305 ± 651^{b}	995 ± 503^{ab}	1045 ± 511^{ab}	901 ± 415^{ac}	*
DCT inner area (μm²)	190 ± 147^{a}	186 ± 82^{a}	189 ± 74^{a}	192 ± 120^{a}	191 ± 134^{a}	177 ± 126^{a}	ns
DCT epithelial area (μm²)	819 ± 395^{a}	734 ± 359^{a}	1116 ± 593^{b}	803 ± 414^{a}	854 ± 391^{ab}	723 ± 344^{a}	*

[abc]Means within rows having different superscripts differ significantly at * $p < 0.05$, ns = not significant. PCT = Proximal convoluted tubule, DCT = Distal convoluted tubule. PDW + PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution

as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 $mg.kg^{-1}$BM day^{-1} of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 $mg.kg^{-1}$BM day^{-1} in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 $mg.kg^{-1}$BM day^{-1} in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 $mg.kg^{-1}$BM day^{-1} in gelatine cube. Data are expressed as mean ± SD, n = 4 per treatment group.

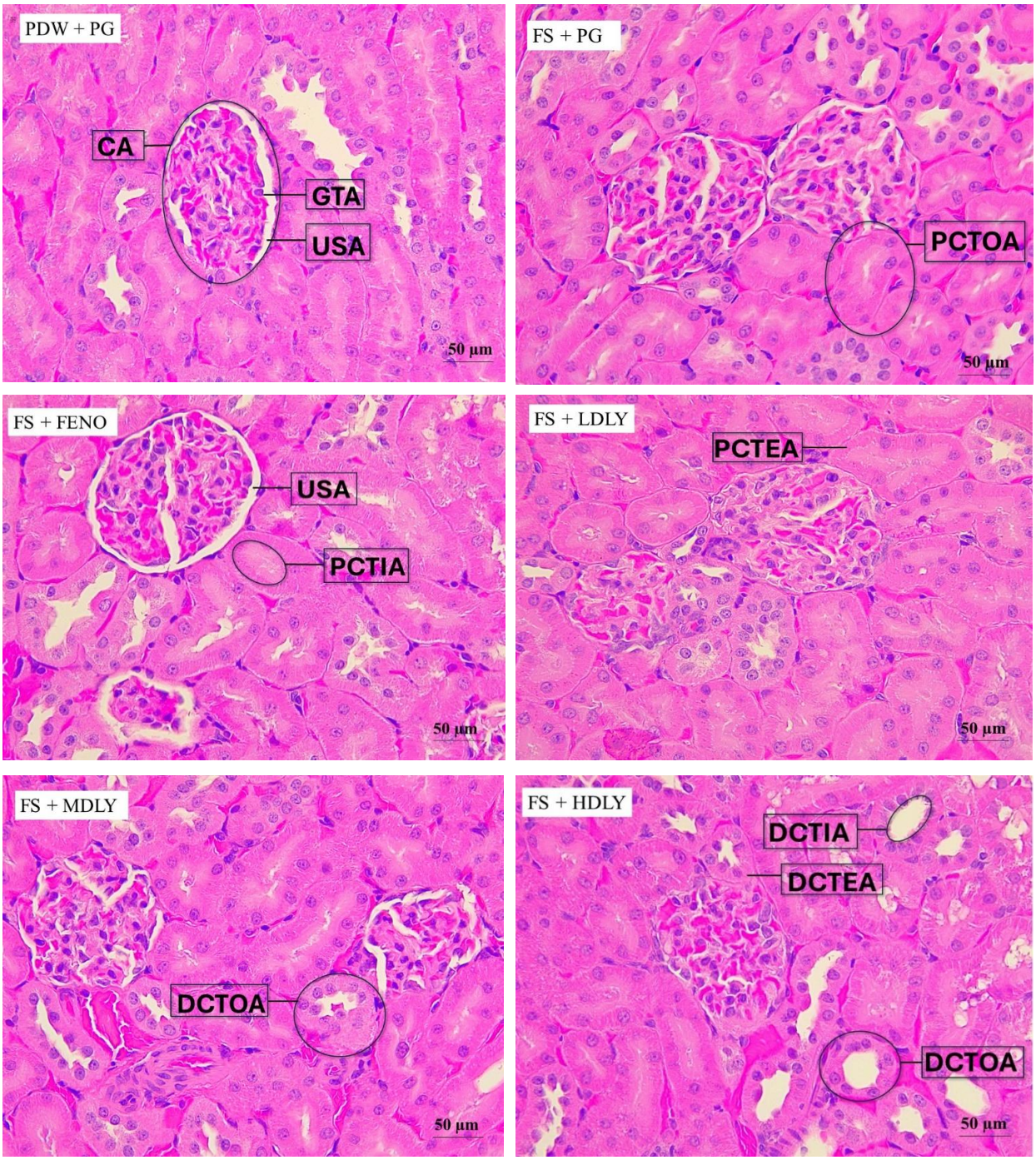


Figure 5. 1 Representative photomicrographs illustrating the effect of lycopene on kidney histology (H&E staining, 40x magnification, scale bar = 50 µm) in growing female Wistar rats fed a high-fructose diet.

CA = corpuscular area; GTA = glomerular tuft area; USA = urinary space area; PCTOA = proximal convoluted tubules outer area; PCTIA = proximal convoluted tubules inner area; PCTEA = proximal convoluted tubules epithelial area; DCTOA = distal convoluted tubules outer area; DCTIA = distal convoluted tubules inner area; DCTEA = distal convoluted tubules epithelial area. PDW + PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 $mg.kg^{-1}$BM day^{-1} of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 $mg.kg^{-1}$BM day^{-1} in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 $mg.kg^{-1}$BM day^{-1} in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 $mg.kg^{-1}$BM day^{-1} in gelatine cube.

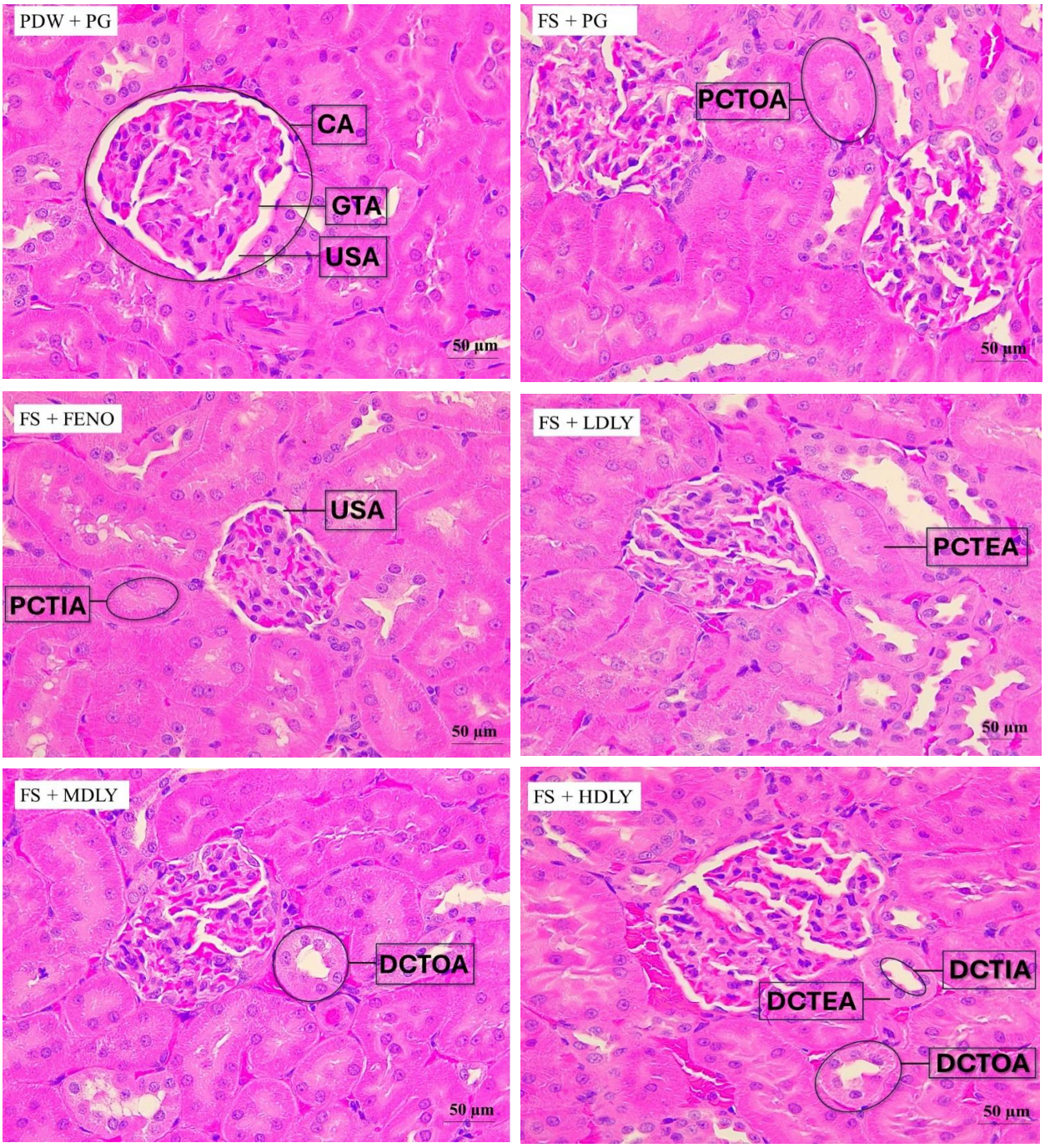


Figure 5. 2: Representative photomicrographs illustrating the effect of lycopene on kidney histology (H&E staining, 40x magnification, scale bar = 50 µm) in growing male Wistar rats fed a high-fructose diet.

CA = corpuscular area; GTA = glomerular tuft area; USA = urinary space area; PCTOA = proximal convoluted tubules outer area; PCTIA = proximal convoluted tubules inner area; PCTEA = proximal convoluted tubules epithelial area; DCTOA = distal convoluted tubules outer area; DCTIA = distal convoluted tubules inner area; DCTEA = distal convoluted tubules epithelial area. PDW + PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 $mg.kg^{-1}$BM day^{-1} of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 $mg.kg^{-1}$BM day^{-1} in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 $mg.kg^{-1}$BM day^{-1} in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 $mg.kg^{-1}$BM day^{-1} in gelatine cube.

5.3 Discussion

5.3.1 Kidney masses

This study evaluated the prophylactic potential of orally administered lycopene against possible dietary fructose-induced kidney derangements in growing weanling Wistar rats in a model mimicking human adolescents fed a high fructose diet. Findings showed no significant differences in the kidney masses (absolute and relative to body weight) of female rats and the absolute kidney mass of male rats across treatment groups. However, male Wistar rats fed a high fructose diet with fenofibrate intervention had significantly heavier relative kidney mass compared to those fed the control diet or the high fructose diet supplemented with lycopene. These results suggest that dietary fructose and lycopene neither compromised nor increased kidney mass in both female and male rats. However, treatment with fenofibrate caused an increase in the kidney mass in male rats through either hypertrophy and or hyperplasia. This increase may reflect structural adaptations in renal tubules. Histological analysis from the present study revealed a reduction in the inner area of the proximal convoluted tubule (PCT), alongside an increase in the outer and epithelial areas of the distal convoluted tubules (DCT). These findings suggest a compensatory response, possibly linked to altered renal workload or PPAR-α-mediated changes in tubular morphology (Kikuchi et al., 2023). Previous studies have also associated fenofibrate with glomerular and tubular modifications, which could contribute to overall increase in kidney mass (Li et al., 2021; Kikuchi et al., 2023). High dietary fructose intake has been associated with the development of metabolic syndrome, obesity, hypertension, and insulin resistance (Giussani et al., 2022). All these metabolic conditions can lead to kidney hypertrophy (Kotsis et al., 2021). However, young rats have been shown to exhibit resistance to metabolic derangements caused by dietary fructose (Tillman et al., 2014)., which may explain the absence of significant changes in kidney masses during the 12-week intervention period. Lycopene is renowned for its potent antioxidant and anti-inflammatory properties (Long et al., 2024), which can protect kidney tissues from oxidative stress and damage (Bedir et al., 2021), potentially counteracting any negative effects of fructose (Ferreira-Santos et al., 2020), thereby helping to maintain kidney mass and function (Abir et al., 2023). Previous studies demonstrated that fenofibrate administered orally for 12 weeks to male mice on a high-fat diet reduced glomerular and tubulointerstitial damage caused by lipotoxicity (Tanaka et al., 2011). According to Yaribeygi et al. (2018), an 8-week oral administration of fenofibrate enhanced renal function by reducing oxidative stress, alleviating inflammation, and preventing apoptosis in

streptozotocin-induced diabetic male Wistar rats. Additionally, a high dose of fenofibrate administered to male Wistar-Han rats for 30 days was shown to activate multiple cellular stress pathways in the kidneys of aged rats (Wrońska et al., 2024). I hypothesise that the sexually dimorphic effect in the increased relative kidney mass with fenofibrate could be due to hormonal and metabolic differences between males and females. In males, testosterone may enhance kidney hypertrophy through interactions with androgen receptors, while differences in metabolism and pharmacokinetics may also account for the distinct responses seen in both sexes (Harvey & Alvarez De La Rosa, 2024; Steiger et al., 2024). In addition, fenofibrate can induce different levels of inflammation or cellular stress in male compared to female rats, potentially leading to increased cellular hypertrophy or hyperplasia in males.

5.3.2 Renal function and injury biomarkers

The present study found no significant differences in fasting serum concentrations of BUN, creatinine, BUN/creatinine ratio, KIM-1 and NGAL in female and male rats across treatment groups. These results suggest that dietary fructose, fenofibrate and lycopene did not negatively affect renal function, as reflected by these biomarkers. While BUN and creatinine concentrations and BUN/creatinine ratio are important indicators of kidney function, the lack of significant changes suggests that these treatments (fructose, fenofibrate and lycopene) did not induce adverse effects on kidney health in young growing rats. BUN reflects the concentration of urea nitrogen in the blood, a byproduct of protein metabolism, produced in the liver and excreted by the kidneys (Jiang et al., 2017). Creatinine, a waste product of muscle metabolism, is a sensitive indicator of glomerular filtration efficiency (Brodsky et al., 2021). The BUN/creatinine ratio is commonly used to differentiate between prerenal and intrinsic renal conditions (Sancho-Martínez et al., 2023). Elevations in these markers are indicative of impaired kidney function (Al Jameil, 2019; Brookes & Power, 2022). High fructose intake has been associated with elevated plasma concentrations of BUN and creatinine (Monteiro et al., 2023), likely due to its role in inducing oxidative stress, promoting uric acid production, and impairing renal perfusion and glomerular filtration (Yokota et al., 2018; Hu et al., 2023; Du et al., 2024). Flisiński et al. (2021) reported no significant changes in serum concentrations of BUN or creatinine clearance in male Wistar rats fed 10% or 60% fructose diets for 8 weeks, while creatinine concentrations remained unchanged only in the 10% fructose group. Similarly, in the present study, serum concentrations of BUN and creatinine and BUN/creatinine ratio remained unaffected in growing Wistar rats fed a 20% fructose solution for

12 weeks. Studies in humans have demonstrated that fenofibrate increases serum concentrations of BUN and creatinine, although these levels generally return to normal once the drug is discontinued (McQuade et al., 2008; Park et al., 2017; Emami et al., 2020). Wrońska et al. (2024) also found that fenofibrate treatment elevated serum creatinine and BUN concentrations in adult male Wistar-Han rats, compared to younger rats. Researchers believe that these changes result from altered renal haemodynamics or mild renal impairment, which are usually reversed after discontinuation of the drug (McQuade et al., 2008; Attridge et al., 2012; Park et al., 2017). However, the degree and reversibility of these effects may vary, depending on factors such as age, dosage, treatment duration, and baseline renal health (Emami et al., 2020; Wrońska et al., 2024). Interestingly, some studies have suggested that fenofibrate might exert protective effects under certain conditions. For instance, in models of kidney injury or metabolic disorders, fenofibrate has been shown to reduce oxidative stress, inflammation, and lipid accumulation, as well as serum creatinine and BUN concentrations, which may mitigate renal damage (Kadian et al., 2013; Balakumar et al., 2014; Chakkarwar & Kawtikwar, 2021; Feng et al., 2021).These dual effects highlight the complexity of fenofibrate's impact on renal physiology and suggest that outcomes may depend on the experimental context (Kostapanos et al., 2013; Med et al., 2018). Lycopene has demonstrated protective effects by reducing serum concentrations of urea, creatinine, uric acid, and KIM-1, alleviating renal function decline associated with 5-fluorouracil induced nephrotoxicity in male rats (Albadrani et al., 2024). Similarly, lycopene has been reported to lower plasma creatinine and urea concentrations in adult female Wistar rats fed a high-fat diet for 8 weeks (Khan et al., 2016). These findings underscore the relevance of early biomarkers like KIM-1 and NGAL, which are recognised in both serum and urine for their high sensitivity and specificity in detecting renal tubular injury and damage (Treacy et al., 2019). High fructose consumption has been reported to cause renal tubular damage (Flisiński et al., 2021), which can result in increased concentrations of KIM-1 and NGAL (Juett et al., 2021). Although dietary fructose did not significantly increase serum KIM-1 and NGAL concentrations to indicate renal tubular injury in this study, it elevated serum KIM-1 concentrations compared to the respective control groups. In female rats, fructose increased KIM-1 concentrations by 22.4%, though this was not statistically significant and was attenuated by supplemental lycopene at low (42.5%), medium (15.6%), and high (2.2%) doses. Similarly, in male rats, fructose caused a 102.3% increase in KIM-1 concentrations compared to control, suggesting potential biological relevance despite the lack of

statistical significance and a tendency towards increased risk of renal tubular injury. Supplemental lycopene mitigated this increase by 69.7%, 10.1%, and 30.9% at low, medium, and high doses, respectively. Additionally, high-fructose diet caused a non-statistically significant increase in serum NGAL concentrations compared to controls in both sexes. In females, dietary fructose increased serum NGAL concentrations by 70%. Supplemental lycopene reduced this increase by 30.7% at low doses but increased it by 1.5% and 10.5% at medium and high doses, respectively. In males, fructose elevated serum NGAL concentrations by 69%. Lycopene reduced this increase by 58.8% at low doses, while medium and high doses increased serum NGAL concentrations by 19.2% and 34.1%, respectively. These findings point to a biological significance, suggesting that while fructose induces an increase in serum NGAL concentrations, supplemental lycopene modulates this effect differently across doses. Lycopene is widely recognised for its potent antioxidant and anti-inflammatory properties (Shinkre et al., 2024), offering protective effects against kidney damage in various models of renal injury (Shalaby & El Shaer, 2019; Adikwu et al., 2021; Xie et al., 2022). Its ability to neutralise free radicals and reduce oxidative stress plays a crucial role in maintaining kidney health (Stojiljkovic et al., 2018). However, while lycopene acts as an antioxidant at low doses, very high doses have been shown to exhibit pro-oxidant behaviour, potentially inducing mild cellular stress (Elvira-Torales et al., 2019). The observed non-significant increase in NGAL levels might reflect this complex dynamic rather than indicating pathological kidney damage. Further investigation is needed to elucidate these effects and establish the optimal dosage for therapeutic benefits.

As a PPARα agonist, fenofibrate primarily functions to lower lipid levels (Jin et al., 2023). It alleviates oxidative stress, reduces apoptotic and inflammatory markers, and decreases KIM-1 and NGAL concentrations in male Wistar rats with vancomycin-induced nephrotoxicity (El-Shoura et al., 2024). Additionally, fenofibrate significantly attenuates high-fat diet induced upregulation of KIM-1 and NGAL mRNA expression in the kidneys, as well as the urinary excretion of these markers, in C57BL/6J male mice fed a high-fat diet for 12 weeks (Sohn et al., 2017). However, in certain conditions such as high-dose treatment or ageing, fenofibrate has also been shown to activate cellular stress pathways, potentially increasing these markers (Wrońska et al., 2024). In the present study, fenofibrate did not cause renal tubular damage, and consequently, no adverse effects were seen in KIM-1 and NGAL concentrations in female and male rats (Table 5.2). Lycopene has potent antioxidant and anti-inflammatory activities (Khongthaw et al., 2022).

Supplementing male Wistar rats on a high-fat diet with 50 mg/kg body mass of lycopene for three months prevented renal damage and led to reductions in creatinine, urea, and NGAL levels (Albrahim & Robert, 2022). Similarly, Rasheed et al. (2020) reported a notable decrease in serum concentrations of creatinine, urea, KIM-1, and NGAL and an increase in the estimated glomerular filtration rate following the administration of 5 mg/kg/day of lycopene for 10 days to male Sprague-Dawley rats with diclofenac-induced acute kidney injury. In the present study, young, growing Wistar rats did not exhibit significant changes in concentrations of BUN and creatinine concentrations, or the BUN/creatinine ratio across treatment groups. Although serum KIM-1 and NGAL concentrations showed slight increases in fructose-treated rats compared to the control group, these changes were not significant, suggesting that no substantial renal injury or oxidative stress occurred. The heightened resilience of young rats to metabolic and oxidative stress, coupled with their adaptable renal systems, likely contributed to these consistent findings across all renal markers.

5.3.3 Kidney histomorphometry

Kidney biopsies and histological analysis offer the most precise and comprehensive insights into kidney structure and specific disease mechanisms, making them the gold standard for evaluation of kidney health (Schnuelle, 2023). In this study, the long-term consumption of a high-fructose diet resulted in sex-specific responses in the kidneys of rats. In females, supplementation with low and high doses of lycopene as well as treatment with fenofibrate reduced urinary space area compared to medium-dose lycopene. Conversely, in males, the high-fructose diet resulted in a significant increase in urinary space area compared to lycopene-treated groups, but this increase was not significant when compared to the control group. Supplemental lycopene at low, medium, and high doses effectively mitigated the fructose-induced urinary space enlargement, reducing it by 39%, 54%, and 37.1%, respectively. Additionally, dietary fortification with the medium-dose lycopene decreased urinary space area in male rats compared to control and fenofibrate treated counterparts (Figures 5.1 and 5.2). An enlarged urinary space area may reflect swelling of the renal interstitium or damage to the tubular or glomerular structures, leading to a disruption in the kidney's normal architecture (Tobar et al., 2013). The findings of the current study suggest that dietary fructose contribute to renal changes in male rats, as indicated by the non-statistically significant but biologically significant increase in urinary space area pointing to potential biological effects. However, supplemental lycopene at various doses significantly reduced urinary

space area, suggesting a protective role against fructose-induced renal enlargement. Medium-dose supplemental lycopene reduced the renal corpuscular area in males compared to the control but with no effect in the glomerular tuft area. However, no treatment regime induced differences were observed in either the renal corpuscular or glomerular tuft areas in female rats. This finding suggests that lycopene can reduce the size of the glomeruli or the overall filtration area in male rats. A reduction in the renal corpuscular area indicates a normalisation or decrease in hypertrophy, which is potential beneficial. An increase in renal corpuscular area is associated with glomerular hypertrophy (Kataoka et al., 2020), which can result from diabetes mellitus, hypertension and/or chronic kidney disease (Poloni & Rotta, 2022). Glomeruli enlargement often indicates stress on the kidneys which can lead to further damage and decreased kidney function over time (Kataoka et al., 2023). Findings from the current study show that dietary fructose did not cause significant difference in the inner, outer, or epithelial areas of the distal convoluted tubule (DCT) in either female or male rats compared to their control counterparts. However supplemental lycopene significantly reduced these areas in females compared to the control group, suggesting a potential protective effect. In contrast, fenofibrate increased these areas compared to high-dose lycopene. Similarly, in males, orally administered fenofibrate increased the outer area of the DCT compared to the high-fructose diet and high-dose lycopene and increased the epithelial area of the DCT compared to the control, high-fructose diet, and low- and high-dose lycopene, with no significant change observed in the inner area (Tables 5.3 and 5.4, respectively). These findings suggests that dietary fructose did not negatively affect DCT areas in either female or male rats. Lycopene may help counteract the enlargement of DCT areas induced by fenofibrate. High fructose intake has been shown to induce renal damage in renal corpuscles, distal tubules, and the interstitium when a 25% fructose diet was fed to adult male Wistar rats for 8 weeks (Elsisy et al., 2021). Thus, it can be hypothesised that a high-fructose diet may not significantly affect DCT areas in young, growing rats, as they may exhibit reduced susceptibility to fructose-induced metabolic and structural changes in kidney function. This could be attributed to their active growth phase, higher metabolic demands, or compensatory renal mechanisms (Kolb et al., 2023). Lycopene has been shown to preserve kidney structure and improve renal function markers in male Sprague Dawley rats treated with adenine for 30 days (Gori et al., 2021). Conversely, orally administered fenofibrate for 6 weeks has been demonstrated to induce inflammation, necrosis, fibrosis, and oxidative stress, leading to detrimental alterations in the kidney's structure and function in adult male albino rats

(Med et al., 2018). These changes included damage to both the proximal and distal convoluted tubules, with observations of dilated bowman's capsule, disrupted tubular lumina, loss of cellular differentiation, and increased interstitial infiltration (Med et al., 2018). The DCT plays a crucial role in maintaining homeostasis: it reabsorbs sodium and calcium to maintain electrolyte balance, regulates pH, and controls blood pressure and fluid levels (Subramanya & Ellison, 2014; Franken et al., 2021; Shrimanker & Bhattarai, 2023). Studies have shown that excessive enlargement of the DCT areas lead to hypertension, hypercalcaemia and hypokalaemia due to increased reabsorption of sodium, calcium, and excessive potassium, respectively (Purbhoo, 2020; Pearce et al., 2022). In contrast, a substantial reduction in DCT areas decreases reabsorption, causing sodium and water loss, which may result in low sodium levels, hypotension, and hypocalcaemia (Purbhoo, 2020; Zieg et al., 2024). This study also that found dietary fructose did not significantly affect the proximal convoluted tubule (PCT) in both sexes across treatment groups. Orally administered fenofibrate reduced the inner area of the PCT compared to medium-dose lycopene in male rats, with no significant changes observed in the outer or epithelial areas. In female rats, no significant changes were noted in the outer, inner, or epithelial areas of the PCT (Tables 5.3 and 5.4, respectively). These results suggest that dietary fructose, orally administered lycopene, and fenofibrate did not negatively affect the inner, outer, or epithelial areas of the PCT in female rats over the 12-week intervention period. In contrast, fenofibrate had a selective impact on the inner area of the PCT in male Wistar rats, with no significant effects observed in other areas. These findings imply that fenofibrate's effects are specific to certain aspects of kidney tubule structure and may be influenced by sex. A reduction in the inner area of the PCT generally leads to impaired reabsorption, potential fluid and electrolyte imbalances, and may signal kidney dysfunction (Dalal et al., 2023). Conversely, an increase in the inner area of the PCT can enhance reabsorption efficiency as part of an adaptive response, though excessive enlargement may indicate pathological changes (Chevalier, 2016). Research has shown that fenofibrate protects proximal tubular cells from apoptosis caused by albumin-bound fatty acids by activating NF-kB in human kidney-2 (HK-2) cells (Zuo et al., 2015). The reduction in the inner area of the PCT observed in male rats with fenofibrate highlights potential sex-specific renal responses. While Zuo et al. (2015) reported protective effects of fenofibrate on proximal tubular cells, the current findings suggest its impact may vary based on sex or physiological context. Further research is needed to determine whether these structural changes reflect adaptive responses or early markers of dysfunction, with particular

emphasis on their effects on electrolyte balance and fluid regulation. Investigating the specific mechanisms of action of both fenofibrate and lycopene is essential for understanding their impact on kidney tubules and overall renal health.

5.4 Conclusion

The consumption of a high-fructose diet and fenofibrate in male rats resulted in increased urinary space area, highlighting a sex-specific response. Lycopene demonstrated protective effects by preventing potential diet induced enlargement of the urinary space and distal convoluted tubule. While caution is advised with fenofibrate use, as it might cause kidney damage, lycopene holds promise as a dietary supplement for improving kidney health and function.

Having discussed the effects of supplemental lycopene on kidney masses, serum BUN, creatinine, KIM-1, NGAL concentrations and BUN/creatinine ratio and renal histomorphometry in growing rats fed a high fructose diet, the next chapter investigated the potential benefits of supplemental lycopene on liver health in growing Wistar rats fed a high-fructose diet.

5.5 References

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CHAPTER SIX: EFFECT OF LYCOPENE ON LIVER HEALTH IN GROWING WISTAR RATS FED A HIGH FRUCTOSE DIET

6.0 Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD), is a growing global health concern, primarily associated with the increasing prevalence of obesity, T2DM and metabolic syndrome (Grander et al., 2023). This condition is characterised by the buildup of fat in the liver in individuals who consume little to no alcohol (Godoy-Matos et al., 2020). Histologically, MASLD includes a spectrum of liver conditions, ranging from simple steatosis (fat accumulation) to non-alcoholic steatohepatitis (NASH), which causes liver inflammation and can progress to fibrosis, cirrhosis, and liver cancer (Huby & Gautier, 2021; Allen et al., 2023). MASLD is one of the most common liver diseases worldwide (Muhamad et al., 2023), affecting about 30% of adults, with higher rates in those with metabolic disorders (Younossi et al., 2023). It impacts 80% to 90% of obese adults, 30% to 50% of those with diabetes mellitus, and over 90% of patients with hyperlipidaemia (Kudaravalli & John, 2023). In children, MASLD is present in 3% to 10% of the population, increasing to 40% to 70% in obese children (Kudaravalli & John, 2023). Projections indicate that MASLD will continue to increase and become a leading cause of liver transplantation by 2030, placing a growing financial burden on healthcare systems (Satapathy et al., 2022; Dong et al., 2024).

This alarming trend is closely linked to excessive fructose consumption, which has been strongly associated with the development of MASLD by promoting fat accumulation in the liver (Federico et al., 2021). This metabolic process primarily takes place in the liver, where fructose is converted into fatty acids, leading to hepatic steatosis, a hallmark of MASLD (Duarte et al., 2019). Over the past 40 years, fructose consumption has significantly increased in children and adults, further increasing the risk of developing MASLD (Mcvicker et al., 2024). Prolonged fructose metabolism in the liver depletes adenosine triphosphate (ATP), leading to elevated uric acid production (Zhang et al., 2022). The subsequent increase in plasma uric acid concentration disrupts mitochondrial function and triggers oxidative stress, leading to further damaging liver cells (Muriel et al., 2021). This worsens inflammation and fibrosis, ultimately accelerating the progression of MASLD (Ma et al., 2021). Individuals with MASLD who consume high amounts of fructose often show elevated serum markers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT), as well as increased total bilirubin (TBIL) and total bile acids (TBA) (Lee et al., 2023; Zhang et al., 2023), which are indicative of impaired liver function. Additionally, the progression of MASLD has been associated with decreased levels of plasma total

protein (TP), albumin (ALB), and increase globulin (GLOB), further highlighting liver dysfunction (Dzordzo & Andreychyn, 2022). Excessive dietary fructose intake contributes to visceral fat pad accumulation by promoting *de novo* lipogenesis in the liver, where fructose is rapidly metabolised into triglycerides (Geidi-Flueck & Gerber,2023). These triglycerides are subsequently stored in visceral adipose tissue leading to increased visceral fat pad size and metabolic disruptions (Hernández-Díazcouder et al., 2019). In male rodents, fructose feeding increases epididymal fat pad mass, further reflecting broader metabolic changes. (Azevedo-Martins et al., 2024). Visceral fat is metabolically active; it secretes pro-inflammatory cytokines and adipokines that impair insulin signalling and fuel systemic inflammation (Azevedo-Martins et al., 2024). The combined effects of visceral and epididymal fat accumulation exacerbate these inflammatory and insulin-resistant states, creating a feedback loop that acerates MASLD progression through heightened hepatic fat deposition and metabolic derangement (Rodrigues et al., 2021; Azevedo-Martins et al., 2024).

Currently, pharmacological agents for treating MASLD, such as metformin, fenofibrate, and N-acetylcysteine, have shown mixed results, often accompanied by side effects and limitations in addressing the full spectrum of the disease (Francque & Vonghia, 2019). These treatments can also be expensive and inaccessible to some communities, further complicating management of MASLD. This highlights the growing need to explore safer, more effective alternatives, particularly those derived from medicinal plants and phytochemicals. Lycopene, a potent antioxidant abundant in tomatoes, is widely recognised for its ability to reduce oxidative stress, lower inflammation, and inhibit hepatic fat accumulation. Its potential to target the metabolic dysfunctions underlying MASLD makes it a promising intervention that can be used to complement traditional therapies and or used as a natural prophylactic agent against lifestyle induced metabolic diseases. This study therefore evaluated the potential prophylactic benefits of supplemental lycopene to protect liver health in Wistar rats fed a high fructose diet by specifically determining effects on liver lipid content hepatic and systemic antioxidant and oxidant status, serum markers of liver function, and histomorphology.

6.1 Materials and methods

6.1.1 Ethical approval

As explained in chapter 3, under subheading 3.1.1.

6.1.2 Chemicals and reagents

The chemicals and reagents utilised are consistent with those detailed in chapter 3, under subheading 3.1.2.

6.1.3 Animals, housing, and general care

The management of experimental animals in this chapter is consistent with the procedures explained in chapter 3, under subheading 3.1.3.

6.1.4 Experimental design

As explained in chapter 3, under subheading 3.1.4.

6.1.5 Terminal procedures, sample collection and measurements

After a 12-week intervention, the rats were fasted overnight with access to plain drinking water to prevent dehydration. Their fasting terminal body mass was recorded using an electronic balance [Snowrex Electronic Scale, Clover Scale (Pty) Ltd, Johannesburg]. The rats were then euthanised via intraperitoneal injection of sodium pentobarbitone (Euthapent; Kyron Laboratories, Johannesburg) at a dose of 200 mg/kg body mass. Blood samples were collected through cardiac puncture using 20G needles and 10 ml syringes, then transferred into SST gel-coated blood collection tubes (BD Vacutainer System, Bigamart, UK). The blood was then centrifuged (DLAB-DMO412S, Wolflabs Laboratory Products, Pocklington, UK) at 3000 × g for 15 minutes at 20°C. The serum collected was decanted into Eppendorf microtubes (Abdos Labtech Pvt, Ltd, Uttrakhand, India) and frozen-stored at -80°C for subsequent analyses of serum markers of oxidative stress, systemic glutathione peroxidase (GPX-1) activity and liver health.

A midline abdominal incision was performed on each rat to extract and weigh the liver, visceral fat, and epididymal fat (in males) using an electronic balance (Presica 310M, Instruments, Johannesburg, South Africa). A liver sample from the medial hepatic lobe was preserved in 10% phosphate-buffered formalin for histological examination. Additionally, another liver sample was collected and stored in sealed ziplock bags at -20°C for the assessment of hepatic oxidative stress (TBARS) and antioxidant enzyme activities (GPX-1, CAT). The remaining portion of the liver was stored in a sealed ziplock bag at -20°C for hepatic lipid content analysis.

6.1.6 Determination of hepatic lipid content

The liver lipid content was measured by solvent extraction using a Soxhlet apparatus (Gebr. Rettberg GmbH, Göttingen, Germany), following the procedure outlined by the Association of Official Analytical Chemists (AOAC, 2005, method number 920.39). Briefly, the liver samples were thawed at room temperature for 30 minutes prior to the assay. Liver samples from the rats, females and males, in each group were combined to form a composite sample. Each group composite sample was then, homogenised, 2 to 3 grams from each homogenised composite sample were weighed into the thimble above the cotton wool and placed into the Soxhlet extractor chamber. An empty distillation flask was weighed, and 200 ml of petroleum ether was added into the distillation flask and placed onto the heating pad. The thermostat was set at 40 ± 10°C, the cooling water supply to the condenser was turned on, and the power supply was switched on. Each subsample was extracted for 4 hours. Thereafter, the excess petroleum ether in the extracted lipid was blown off using a rotary evaporator (LabFriend PTY Ltd, Johannesburg, South Africa) at 50 ± 10°C for 5 to 10 minutes, leaving the extracted lipid in the flask. The distillation flask with the lipid was then placed in an oven (LABOTEC (Pty) Ltd., Johannesburg, South Africa) at 50°C for 30 to 45 minutes to remove any residual petroleum ether from the lipid. Finally, the flask was placed in a desiccator to cool, and the flask with the lipid was weighed again. The total liver lipid content percentage was then calculated using the formula:

$$\%\ \text{lipid} = \frac{\text{Mass of flask with lipid} - \text{Mass of empty flask (g)}}{\text{Mass of the liver sample (g)}} \text{x } 100$$

This extraction process was repeated three times for each treatment group.

6.1.7 Determination of serum and hepatic oxidative stress and antioxidant status

Fasting serum and hepatic GPX-1, hepatic CAT, and serum and hepatic TBARS were measured using a rat-specific enzyme-linked immunosorbent assay (ELISA) kit from Elabscience Biotechnology Inc., Houston, Texas, USA, following the manufacturer's guidelines.

6.1.8 Determination of serum surrogate markers for liver function

The serum activities of Alanine aminotransferase (AST), Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), and Gamma-glutamyl transferase (GGT), along with the concentrations of globulin, total protein (TP), albumin, total bilirubin (TBIL), total bile acids (TBA), and uric acid (UA), were determined using a Noahcali-100 automatic LOCMEDT biochemistry analyser (Tianjin LOCMEDT Technologies Co., Ltd., China), according to the manufacturer's instructions. Briefly, 120 µl of serum was added to the reagent disc containing lyophilised beads. The disc was then inserted into the Noahcali-100 analyser, and results were obtained after 8-12 minutes.

6.1.9 Liver histology assessment

Liver samples were fixed in 10% phosphate-buffered formalin and processed using an automatic tissue processor (Micro STP 120, Thermo Fisher, Waltham, USA). They were then embedded in paraffin wax, sectioned to a thickness of 3 µm using a rotary microtome (Leica Biosystems, Buffalo Grove, USA), and placed onto glass slides. Following paraffin removal with xylene, the sections were rehydrated, stained with haematoxylin and eosin, dehydrated, and cleared in xylene to enhance transparency. A mounting medium was used to apply coverslips. The slides were examined under a Leica DM750 microscope, with images captured via a high-definition ICC50W digital camera (Leica Biosystems, Germany). The haematoxylin and eosin-stained sections were semi-quantitatively evaluated for steatosis, lobular inflammation, and hepatocyte hypertrophy using criteria from Kleiner et al. (2005) and Liang et al. (2014). Micro- and macro-steatosis and hepatocyte hypertrophy were graded within each 40x magnification field based on the percentage of affected area: grade 0 for <5%, grade 1 for 5-33%, grade 2 for 33-66%, and grade 3 for >66% (Liang et al., 2014). Lobular inflammation was scored by counting inflammatory foci in the liver parenchyma: grade 0 for no foci, grade 1 for <2 foci, grade 2 for 2-4 foci, and grade 3 for >4 foci per field (Kleiner et al., 2005).

6.1.10 Statistical analysis

Data were analysed using GraphPad Prism version 9.0 (GraphPad Software Inc., San Diego, USA). Parametric data from multiple groups were assessed using one-way ANOVA and presented as mean ± standard deviation, with Tukey's *post-hoc* test used for mean comparison. Non-parametric data (steatosis, hypertrophy, and inflammation scores) were analysed with the Kruskal-Wallis test

(non-parametric one-way ANOVA) and presented as median ± range, followed by Dunn's *post-hoc* test for median comparisons. Statistical significance was defined as $p < 0.05$.

6.2 Results

6.2.1 Liver, visceral fat pad, and epididymal fat pad masses

Tables 6.1 and 6.2 show the liver and visceral fat masses of the rats, as well as the epididymal fat mass of male rats. Both female and male rats fed a high fructose diet with fenofibrate intervention had significantly heavier ($p < 0.05$) liver masses (absolute and relative to body mass) compared to control, fructose-fed and lycopene-supplemented counterparts (Tables 6.1 and 6.2). Additionally, female and male rats fed the high fructose diet supplemented with a low lycopene dose had significantly heavier livers ($p < 0.05$) compared to livers of the control diet fed counterparts. Female rats on the high fructose diet had significantly higher ($p < 0.05$) relative liver masses compared to the relative liver masses from control diet fed counterparts. In males, the epididymal fat mass was similar across treatment regimes.

Table 6. 1: Effect of lycopene on absolute and relative masses of the liver and visceral fat pads of growing female Wistar rats fed a high-fructose diet

Parameter	PDW +PG	FS + PG	FS +FENO	FS +LDLY	FS + MDLY	FS + HDLY	Significance
Liver mass (g)	5.96 ± 0.35^{a}	6.69 ± 0.43^{ac}	9.15 ± 0.81^{b}	6.80 ± 0.60^{c}	6.63 ± 0.39^{ac}	6.24 ± 0.34^{ac}	*
Liver (% BM)	2.83 ± 0.12^{a}	3.16 ± 0.19^{b}	4.36 ± 0.31^{c}	3.16 ± 0.19^{b}	3.09 ± 0.13^{ab}	2.97 ± 0.12^{ab}	*
Visceral fat pad mass (g)	6.20 ± 1.25^{a}	7.14 ± 2.78^{a}	7.49 ± 1.63^{a}	8.33 ± 1.44^{a}	8.27 ± 1.23^{a}	6.75 ± 1.61^{a}	ns
Visceral fat pad (% BM)	2.94 ± 0.56^{a}	3.34 ± 1.18^{a}	3.55 ± 0.67^{a}	3.86 ± 0.51^{a}	3.84 ± 0.49^{a}	3.20 ± 0.71^{a}	ns

[abc]Means within rows having the different superscript differ significantly at *$p < 0.05$, ns = not significant, $P > 0.05$. PDW +PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 $mg.kg^{-1}$BM day^{-1} of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 $mg.kg^{-1}$BM day^{-1} in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 $mg.kg^{-1}$BM day^{-1} in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 $mg.kg^{-1}$BM day^{-1} in gelatine cube; % BM = Percentage body mass (relative to body mass). Data are expressed as mean ± SD, n = 8 per treatment group.

Table 6. 2: Effect of lycopene on the absolute and relative masses of the liver, visceral fat pads, and epididymal fat pads of growing male Wistar rats fed a high-fructose diet

Parameter	PDW +PG	FS + PG	FS +FENO	FS +LDLY	FS + MDLY	FS + HDLY	Significance
Liver mass (g)	10.12 ± 0.43^{a}	10.55 ± 0.69^{ac}	14.49 ± 0.87^{b}	11.13 ± 0.37^{c}	10.48 ± 0.55^{ac}	10.96 ± 0.65^{ac}	*
Liver (% BM)	2.88 ± 0.07^{a}	3.05 ± 0.13^{ac}	4.48 ± 0.41^{b}	3.21 ± 0.10^{c}	3.09 ± 0.14^{ac}	3.16 ± 0.09^{ac}	*
Visceral fat pad mass (g)	9.21 ± 1.70^{a}	8.45 ± 1.80^{a}	9.11 ± 2.20^{a}	8.50 ± 2.33^{a}	8.31 ± 1.89^{a}	9.09 ± 1.34^{a}	ns
Visceral fat pad (% BM)	2.62 ± 0.47^{a}	2.43 ± 0.38^{a}	2.79 ± 0.59^{a}	2.45 ± 0.67^{a}	2.44 ± 0.52^{a}	2.62 ± 0.38^{a}	ns
Epididymal fat pad mass (g)	3.27 ± 1.61^{a}	3.86 ± 0.97^{a}	3.55 ± 0.94^{a}	3.76 ± 1.46^{a}	3.57 ± 1.38^{a}	4.15 ± 1.33^{a}	ns
Epididymal fat pad mass (% BM)	0.92 ± 0.43^{a}	1.12 ± 0.30^{a}	1.09 ± 0.244^{a}	1.08 ± 0.40^{a}	1.05 ± 0.39^{a}	1.19 ± 0.34^{a}	ns

[abc]Means within rows having the different superscript differ significantly at *$p < 0.05$, ns = not significant, $P > 0.05$. PDW +PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 $mg.kg^{-1}$BM day^{-1} of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 $mg.kg^{-1}$BM day^{-1} in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 $mg.kg^{-1}$BM day^{-1} in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 $mg.kg^{-1}$BM day^{-1} in gelatine cube; % BM = Percentage body mass (relative to body mass). Data are expressed as mean ± SD, n = 8 per treatment group.

6.2.2 Liver lipid content

Figures 6.1A and 6.1B present the liver lipid content of female and male rats, respectively. Female rats fed the high fructose diet had significantly higher ($p < 0.05$) liver lipid content compared to fructose-fed but fenofibrate treated as well as the low- and high-dose lycopene supplements counterparts (Figure 6.1A). Similarly, male rats fed the high fructose diet showed significantly elevated ($p < 0.05$) liver lipid content compared to fructose-fed but fenofibrate treated as well as the medium- and high-dose lycopene supplements counterparts (Figure 6.1B).

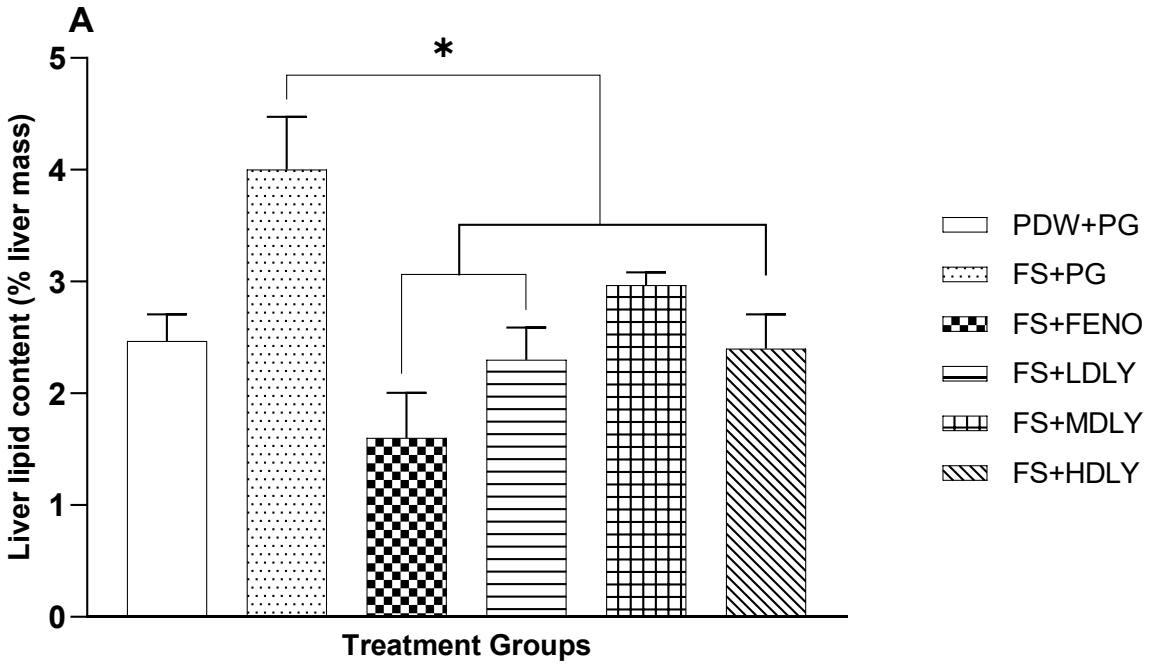


Figure 6. 1A: Effect of lycopene on liver lipid content in growing female Wistar rats fed a high-fructose diet.

*= significantly different at $p < 0.05$. PDW + PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 $mg.kg^{-1}BM\ day^{-1}$ of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube; FS + MDL=

20% w/v fructose solution as drinking fluid + Lycopene 60 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 mg.kg^{-1}BM day^{-1} in gelatine cube. Data are expressed as mean ± SD, n = 3 per treatment group.

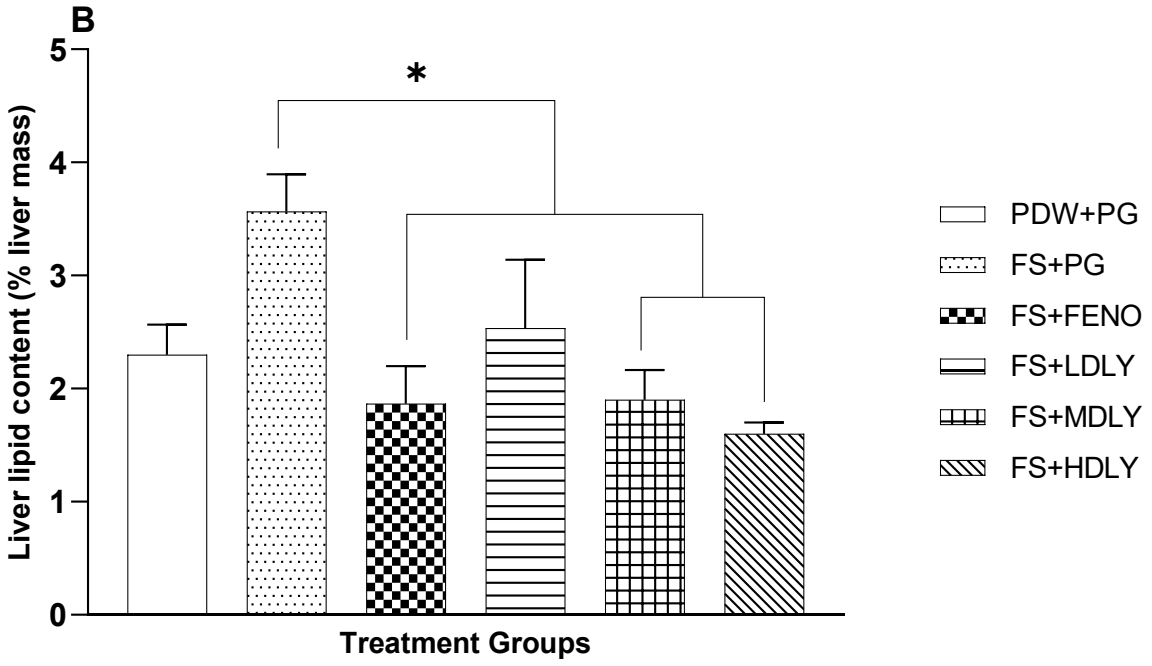


Figure 6. 1B: Effect of lycopene on liver lipid content in growing male Wistar rats fed a high-fructose diet.

*= significantly different at $p < 0.05$. PDW + PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 mg.kg^{-1}BM day^{-1} of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + MDL= 20% w/v fructose solution as drinking fluid + Lycopene 60 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 mg.kg^{-1}BM day^{-1} in gelatine cube. Data are expressed as mean ± SD, n = 3 per treatment group.

6.2.3 Systemic and hepatic oxidative stress and antioxidant status

Figures 6.2 and 6.3 show the effect of lycopene on serum TBARS concentration (A), and GPX-1 activity (B) and hepatic TBARS (C) concentration, GPX-1 (D), and CAT (E) activities in female, and male rats fed a high fructose-diet, respectively. Serum TBARS concentration and GPX-1 activity as well as hepatic TBARS concentration and hepatic CAT levels were similar ($p > 0.05$) in female rats across treatment groups (Figures 6.2A, B, C, and E). However, fenofibrate-treated female rats had significantly higher hepatic GPX-1 activity ($P < 0.05$) compared to control diet-fed and lycopene supplemented counterparts (Figure 6.2D). Similarly, serum TBARS and GPX-1 activity, and hepatic TBARS concentration of male rats was similar ($P > 0.05$) across treatment groups (Figures 6.3A, B, and C). However, fenofibrate-treated male rats had significantly higher hepatic GPX-1 activity ($p < 0.05$) compared to control diet-fed or low- and medium-dose lycopene-supplemented counterparts (Figure 6.3D). Additionally, fenofibrate treated and high-dose lycopene supplemented male rats had significantly higher hepatic GPX-1 activity ($p < 0.05$) compared to the control diet fed counterparts (Figure 6.3D). Male rats whose diet was supplemented with a high-dose of lycopene had significantly higher ($p < 0.05$) hepatic CAT activity compared to control, high-fructose diet fed, fenofibrate-treated and low and medium lycopene supplemented counterparts (Figure 6.3E).

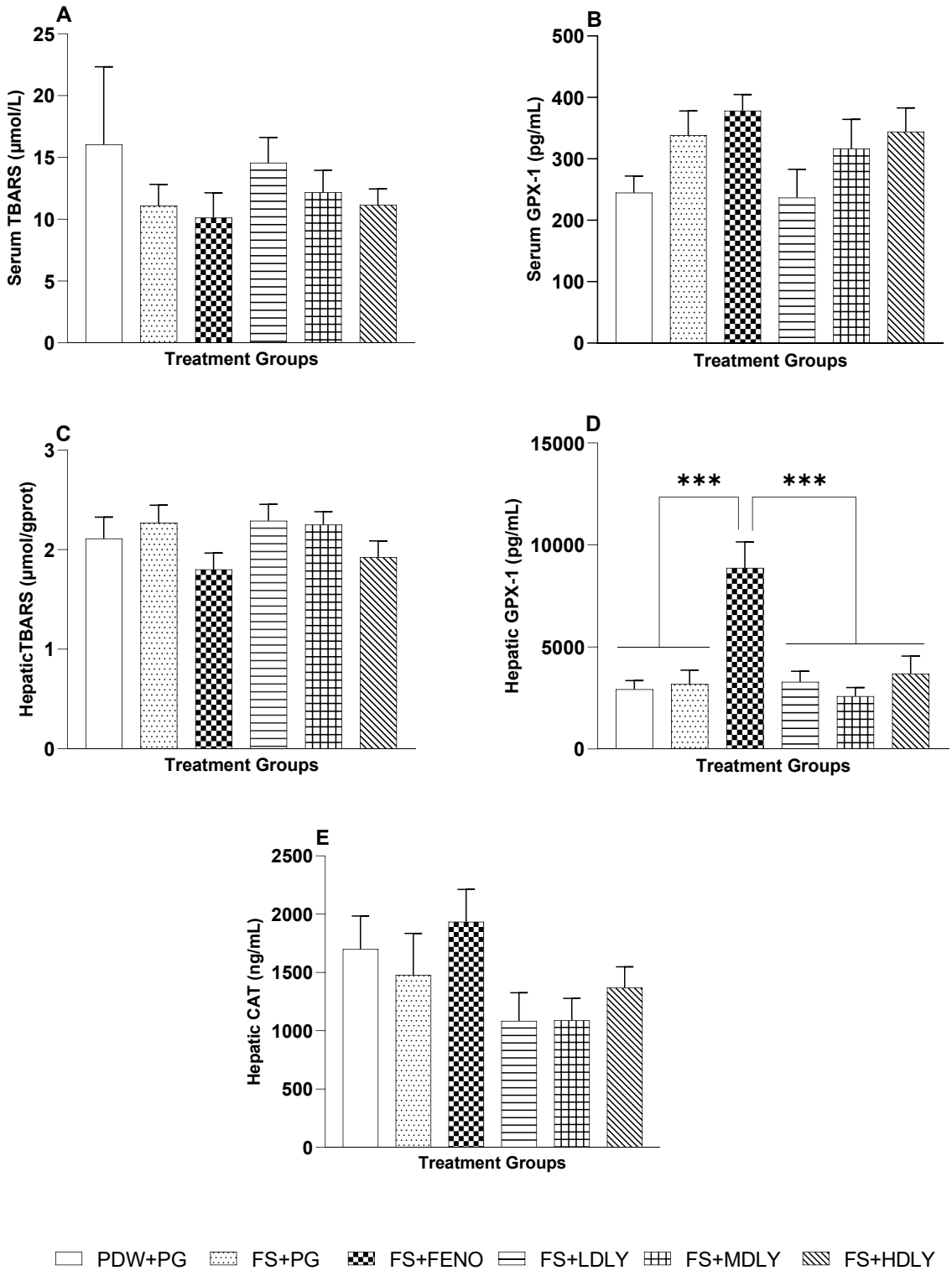

A
Serum TBARS (µmol/L)
25
20
15
10
5
0
Treatment Groups
B
Serum GPX-1 (pg/mL)
500
400
300
200
100
0
Treatment Groups
C
HepaticTBARS (µmol/gprot)
3
2
1
0
Treatment Groups
D
Hepatic GPX-1 (pg/mL)
15000
10000
5000
0
***
***
Treatment Groups
E
Hepatic CAT (ng/mL)
2500
2000
1500
1000
500
0
Treatment Groups
PDW+PG
FS+PG
FS+FENO
FS+LDLY
FS+MDLY
FS+HDLY

Figure 6. 2: Effect of lycopene on serum TBARS (A) concentration and GPX-1 (B) activity and hepatic TBARS (C) concentration, GPX-1 (D) and CAT (E) activities in growing female Wistar rats fed a high-fructose diet.

***= significantly different at $p < 0.0001$. PDW + PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 $mg.kg^{-1}$BM day^{-1} of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 $mg.kg^{-1}$BM day^{-1} in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 $mg.kg^{-1}$BM day^{-1} in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 $mg.kg^{-1}$BM day^{-1} in gelatine cube. Data are expressed as mean ± SD, n = 6-7 per treatment group.

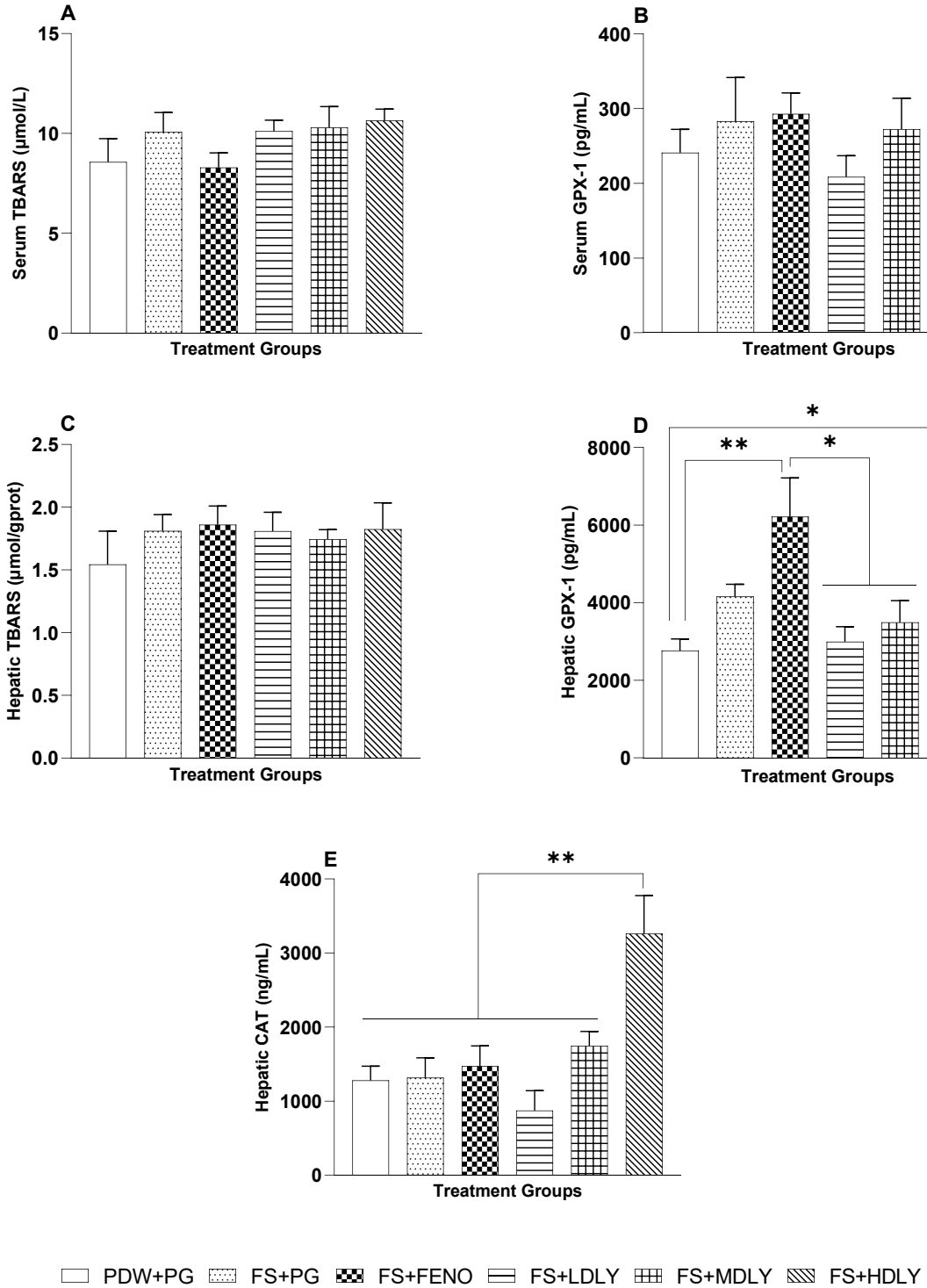

A
Serum TBARS (µmol/L)
15
10
5
0
Treatment Groups
B
Serum GPX-1 (pg/mL)
400
300
200
100
0
Treatment Groups
C
Hepatic TBARS (µmol/gprot)
2.5
2.0
1.5
1.0
0.5
0.0
Treatment Groups
D
*
**
*
Hepatic GPX-1 (pg/mL)
8000
6000
4000
2000
0
Treatment Groups
E
**
Hepatic CAT (ng/mL)
4000
3000
2000
1000
0
Treatment Groups
PDW+PG
FS+PG
FS+FENO
FS+LDLY
FS+MDLY
FS+HDLY

Figure 6. 3: Effect of lycopene on serum TBARS (A) concentration and GPX-1 (B) activity and hepatic TBARS (C) concentration, GPX-1 (D) and CAT (E) activities in growing male Wistar rats fed a high-fructose diet.

*= significantly different at $p < 0.05$, **= significantly different at $p < 0.001$. PDW + PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 $mg.kg^{-1}BM\ day^{-1}$ of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube. Data are expressed as mean ± SD, n = 6-7 per treatment group.

6.2.4 Serum markers of hepatic function

Tables 6.3 and 6.4 present the serum concentrations of surrogate markers of liver health in the rats. In female rats, serum albumin, TP, globulin, TBA, and uric acid concentrations as well as GGT, ALT, and AST activities were similar ($p > 0.05$) across treatment groups. However, the fenofibrate-treated female rats had significantly higher ALP activity ($p < 0.05$) compared to control diet-fed, high fructose diet-fed and lycopene-supplemented groups (Table 6.3). In male rats, dietary fructose caused significantly higher serum concentrations of albumin, total protein, and globulin ($p < 0.05$) compared to control diet-fed, fenofibrate-treated, or lycopene-supplemented groups. Additionally, fenofibrate-treated male rats had significantly higher ALP activity ($p < 0.05$) compared to control diet-fed, high fructose diet-fed and lycopene-supplemented groups. Furthermore, fenofibrate-treated male rats showed significantly higher serum total bile acids ($p < 0.05$) compared to the control group. Male rats fed a high fructose diet also had significantly increased serum uric acid concentrations ($p < 0.05$) compared to control counterparts. However, serum activities of GGT, ALT, AST, and total TBIL concentrations were similar ($p > 0.05$) across treatment groups in male rats (Table 6.4).

Table 6. 3: Effect of lycopene on serum markers of hepatic function in growing female Wistar rats fed a high fructose diet

Parameter	PDW +PG	FS + PG	FS +FENO	FS +LDLY	FS + MDLY	FS + HDLY	Significance
Albumin (g/L)	31.20±0.84[a]	35.33±2.34[a]	35.83±1.84[a]	32.83±1.94[a]	5.50±4.93[a]	34.67±3.01[a]	ns
Globulin (g/L)	29.40±1.52[a]	33.50±4.32[a]	30.00±2.53[a]	30.67±2.81[a]	33.50±5.47[a]	34.83±4.40[a]	ns
TP (g/L)	60.60±1.52[a]	68.83±6.56[a]	65.83±3.71[a]	63.50±3.83[a]	69.00±10.26[a]	69.50±7.34[a]	ns
TBIL (g/L)	2.80±0.84[a]	4.50±0.55[ab]	4.17±1.17[ab]	5.17±1.33[b]	5.00±1.10[b]	4.50±0.84[ab]	*
TBA (µmol/L)	29.60±9.45[a]	33.67±9.65[a]	32.50±5.17[a]	29.67±4.23[a]	33.33±6.31[a]	31.50±4.93[a]	ns
UA (µmol/L)	77.40±39.05[a]	87.00±22.81[a]	74.67±40.86[a]	73.00±36.86[a]	82.17±34.15[a]	103.3±12.16[a]	ns
GGT (U/L)	7.80±1.92[a]	6.00±0.63[a]	5.83±1.33[a]	7.17±3.06[a]	6.67±1.03[a]	6.67±0.82[a]	ns
ALP (U/L)	64.80±10.26[a]	64.33±18.00[a]	109.70±14.07[b]	56.33±15.40[a]	56.50±10.93[a]	72.50±8.64[a]	***
AST (U/L)	230.80±98.79[a]	336.70±181.10[a]	354.20±213.20[a]	416.00±265.80[a]	349.20±158.10[a]	212.50±8.64[a]	ns
ALT (U/L)	72.40±46.93[a]	62.00±14.27[a]	102.30±49.84[a]	119.70±81.67[a]	78.33±37.55[a]	56.00±10.62[a]	ns

[ab]Means within rows having the different superscript differ significantly at * $p < 0.05$, *** $p < 0.0001$, ns = not significant, $p > 0.05$. TP = Total protein, TBIL = Total bilirubin, TBA = Total bile acids, GGT = Gamma-glutamyl transferase, ALP = Alkaline phosphatase, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, UA = Uric acid. PDW +PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 $mg.kg^{-1}BM\ day^{-1}$ of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube. Data are expressed as mean ± SD, n = 6 per treatment group, except for the control group which had n = 5.

Table 6. 4: Effect of lycopene on serum markers of hepatic function in growing male Wistar rats fed a high fructose diet

Parameter	PDW +PG	FS + PG	FS +FENO	FS +LDLY	FS + MDLY	FS + HDLY	Significance
Albumin (g/L)	31.50±0.55[a]	44.83±8.91[b]	35.67±2.07[a]	34.33±1.21[a]	32.33±1.21[a]	34.67±3.67[a]	**
Globulin (g/L)	31.50±1.76[a]	47.50±11.74[b]	27.67±2.42[a]	34.17±2.23[a]	32.00±1.27[a]	33.67±4.08[a]	**
TP (g/L)	63.00±2.10[a]	88.67±17.56[b]	63.33±4.08[a]	68.50±3.27[a]	64.33±2.07[a]	68.33±7.63[a]	**
TBIL (g/L)	3.67±1.03[a]	5.00±1.27[a]	4.17±1.47[a]	4.17±1.17[a]	3.50±0.55[a]	4.33±1.21[a]	ns
TBA (µmol/L)	31.83±15.79[a]	47.17±7.22[ab]	52.83±11.99[b]	36.17±10.74[ab]	34.67±4.72[ab]	42.00±8.17[ab]	*
UA (µmol/L)	75.00±64.32[a]	162.20±37.50[b]	83.50±40.62[ab]	121.30±68.11[ab]	100.00±37.62[ab]	94.50±23.05[ab]	*
GGT (U/L)	5.33±0.82[a]	7.17±0.98[a]	5.67±0.82[a]	13.83 ±20.20[a]	5.50±0.55[a]	5.83±0.92[a]	ns
ALP (U/L)	91.17±38.09[a]	133.50±24.09[a]	259.70±61.48[b]	104.80±19.42[a]	94.67±11.08[a]	104.70±26.55[a]	***
AST (U/L)	310.00±223.80[a]	438.30±300.30[a]	270.20±71.78[a]	398.20±178.70[a]	284.30±83.70[a]	314.00±97.68[a]	ns
ALT (U/L)	319.50±390.00[a]	159.20±98.14[a]	82.00±20.13[a]	147.00±68.40[a]	104.30±28.29[a]	108.80±54.44[a]	ns

[ab]Means within rows having the different superscript differ significantly at * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$, ns = not significant, $P > 0.05$. TP = Total protein, TBIL = Total bilirubin, TBA = Total bile acids, GGT = Gamma-glutamyl transferase, ALP = Alkaline phosphatase, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, UA = Uric acid. PDW +PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 $mg.kg^{-1}BM\ day^{-1}$ of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube. Data are expressed as mean ± SD, n = 6 per treatment group.

6.2.5 Liver histomorphology

Figures 6.4 and 6.5 show liver histology photomicrographs at 40x magnification (H&E stained) for female and male rats, respectively, from each treatment group. In both female and male rats fed a high-fructose diet, there was marked fatty infiltration, including micro- and macro-vesicular steatosis (Figures 6.4 and 6.5). Lycopene supplementation and fenofibrate treatment prevented fructose-induced steatosis in both sexes. However, fenofibrate-treated rats showed increased hepatic hypertrophy, which was mitigated by supplemental lycopene (Figures 6.4 and 6.5).

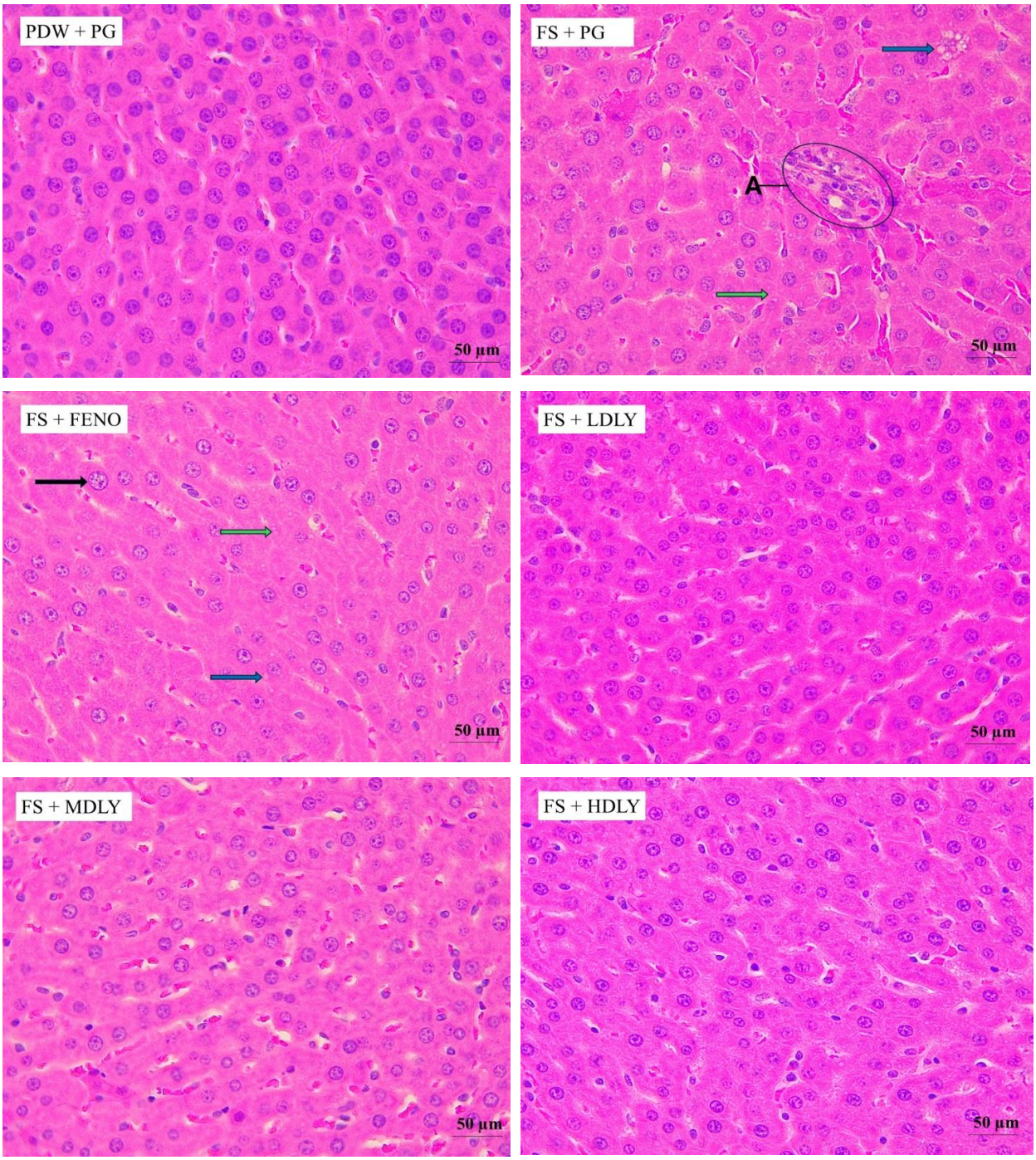


Figure 6.4: Representative photomicrographs showing the effects of lycopene on liver histology in growing female Wistar rats fed a high-fructose diet (H&E staining, 40x magnification, scale bar = 50 µm).

The green arrows point to micro-steatosis, the blue arrows point to macro-steatosis, the black arrow points to hypertrophy and circle A marks foci of lobular inflammation. PDW + PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 $mg.kg^{-1}BM\ day^{-1}$ of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube.

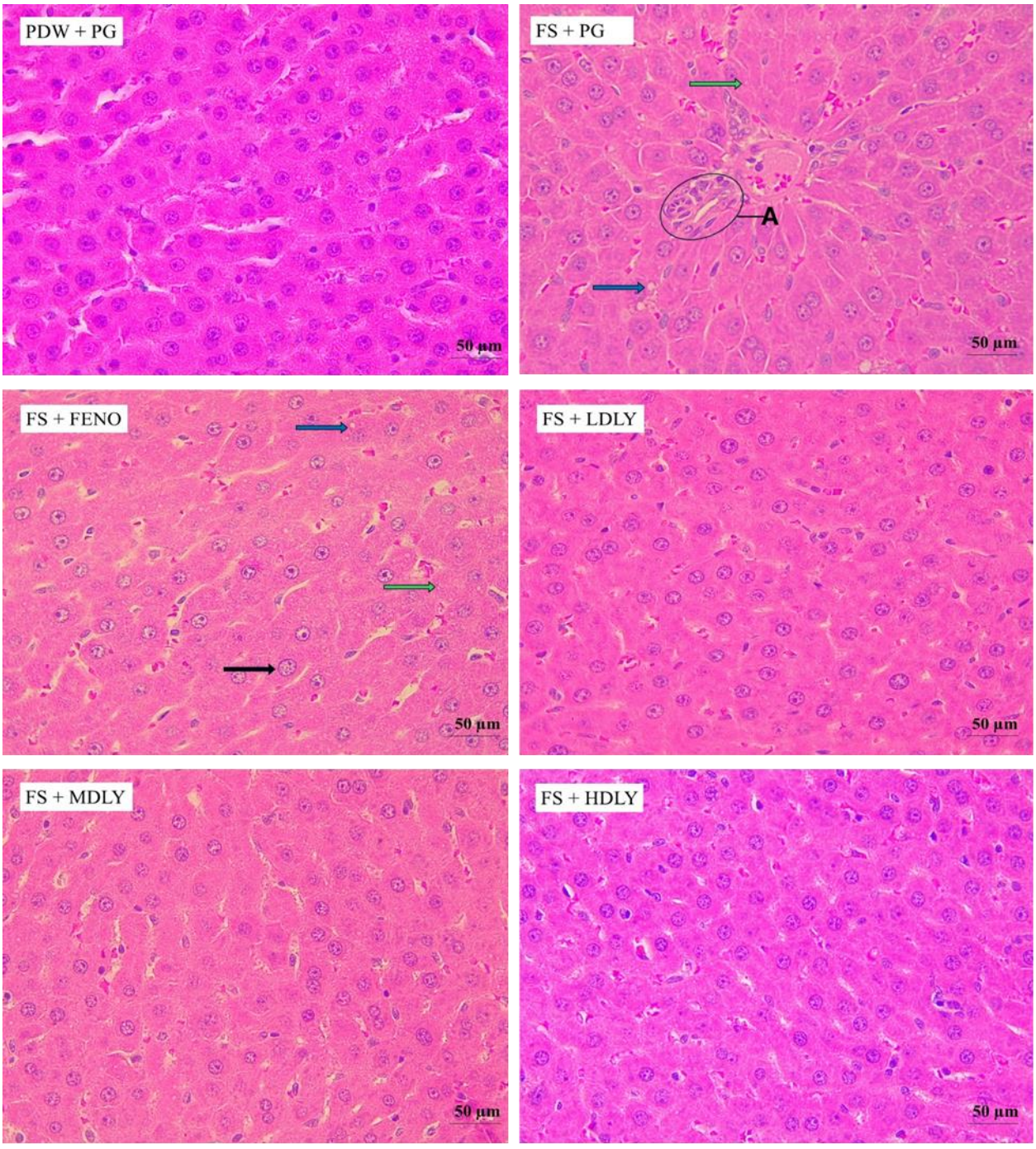


Figure 6.5: Representative photomicrographs showing the effects of lycopene on liver histology in growing male Wistar rats fed a high-fructose diet (H&E staining, 40x magnification, scale bar = 50 µm).

The green arrows point to micro-steatosis, the blue arrows point to macro-steatosis, the black arrow points to hypertrophy and circle A marks foci of lobular inflammation. PDW + PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 $mg.kg^{-1}BM\ day^{-1}$ of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 $mg.kg^{-1}BM\ day^{-1}$ in gelatine cube.

Tables 6.5 and 6.6 present scores for micro- and macro-steatosis, lobular inflammation, and hepatocyte hypertrophy in the treatment groups. No significant differences ($p > 0.05$) were noted in lobular inflammation scores across treatment groups for both female and male rats (Tables 6.5 and 6.6). However, in both sexes, rats fed a high-fructose diet showed significantly increased micro- and macro-steatosis ($p < 0.05$) compared to those on the control diet, fenofibrate-treated, and lycopene-supplemented counterparts. Additionally, fenofibrate-treated rats exhibited significant hypertrophy ($p < 0.05$) in both female and male groups, with no hypertrophy observed in the control, high-fructose diet, or lycopene-supplemented groups (Tables 6.5 and 6.6).

Table 6. 5: Effect of lycopene on liver steatosis, lobular inflammation, and hepatocyte hypertrophy scores in growing female Wistar rats fed a high-fructose diet

Parameter	PDW +PG	FS + PG	FS +FENO	FS +LDLY	FS + MDLY	FS + HDLY	Significance
Micro-steatosis	1 (1, 2)^{a}	2 (1.3, 2)^{b}	1 (1, 1)^{a}	1 (0.3, 1)^{a}	1 (1, 1.8)^{a}	1 (0, 1)^{a}	***
Macro-steatosis	0 (0, 1)^{a}	2 (1.3, 2)^{b}	0.5 (0, 1)^{a}	0 (0, 1)^{a}	1 (1, 1)^{a}	0.5 (0, 1)^{a}	***
Lobular inflammation	0 (0, 1)^{a}	1 (0.3, 2)^{a}	1 (0, 1)^{a}	1 (0.3, 1.8)^{a}	1 (0, 1.8)^{a}	1 (0.3, 1)^{a}	ns
Hepatocyte hypertrophy	0 (0, 0)^{a}	0 (0, 0)^{a}	1 (0, 1)^{b}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	**

^{ab}Means within rows having the different superscript differ significantly at ** $p < 0.001$, *** $p < 0.0001$, ns = not significant, $p > 0.05$. PDW +PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 mg.kg^{-1}BM day^{-1} of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 mg.kg^{-1}BM day^{-1} in gelatine cube. Data are expressed as mean ± SD, n = 4 per treatment group.

Table 6. 6: Effect of lycopene on liver steatosis, lobular inflammation, and hepatocyte hypertrophy scores in growing male Wistar rats fed a high-fructose diet

Parameter	PDW +PG	FS + PG	FS +FENO	FS +LDLY	FS + MDLY	FS + HDLY	Significance
Micro-steatosis	1 (0, 1)^{a}	1 (1, 2)^{b}	0 (0, 1)^{a}	0 (0, 1)^{a}	0 (0, 1)^{a}	0 (0, 0.8)^{a}	***
Macro-steatosis	0 (0, 1)^{a}	2 (1, 2)^{b}	0 (0, 1)^{a}	1 (0, 1)^{a}	0 (0, 1)^{a}	0 (0 ,0)^{a}	***
Lobular inflammation	1 (0, 1)^{a}	1 (0, 2)^{a}	1 (0, 1.8)^{a}	0 (0, 1)^{a}	1 (0, 2)^{a}	1 (0, 1)^{a}	ns
Hepatocyte hypertrophy	0 (0, 0)^{a}	0 (0, 0)^{a}	1 (0, 1)^{b}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	**

^{ab}Means within rows having the different superscript differ significantly at ** $p < 0.001$, *** $p < 0.0001$, ns = not significant, $p > 0.05$. PDW +PG = plain drinking water + plain gelatine cube; FS + PG = 20% w/v fructose solution as drinking fluid + PG; FS + FENO = 20% w/v fructose solution as drinking fluid + 100 mg.kg^{-1}BM day^{-1} of fenofibrate in gelatine cube; FS + LDLY = 20% w/v fructose solution as drinking fluid + Lycopene 30 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + MDLY = 20% w/v fructose solution as drinking fluid + Lycopene 60 mg.kg^{-1}BM day^{-1} in gelatine cube; FS + HDLY = 20% w/v fructose solution as drinking fluid + Lycopene 100 mg.kg^{-1}BM day^{-1} in gelatine cube. Data are expressed as mean ± SD, n = 4 per treatment group.

6.3 Discussion

This study investigated the potential of orally administered lycopene to protect against high fructose diet-induced hepatic metabolic derangements in growing weanling Wistar rats. It focused specifically on effects on hepatic fat content, oxidative stress and antioxidant enzyme activity, hepatic histomorphology and serum markers of hepatic function.

6.3.1 Abdominal fat and liver mass

No significant differences were observed in the visceral fat mass of the rats or the epididymal fat mass in males across treatment groups. Together, the results suggest that dietary fructose, the orally administered lycopene and fenofibrate did not negatively affect visceral and epididymal fat pad masses during the 12-week intervention period. This finding aligns with Ibrahim et al. (2022), who reported no impact on these parameters in growing female and male Spraque Dawley rats administered 20% fructose as drinking fluid for 9 weeks. Tillman et al. (2014) similarly found that young rat pups and growing rats demonstrate greater resistance to fructose-induced visceral adiposity compared to adult rats. Female rats fed the high fructose diet had relative heavier liver masses compared to control counterparts, suggesting sex-specific effects. Notably, fenofibrate-treated female and male rats had significantly heavier liver masses compared to control, high-fructose and lycopene-supplemented counterparts. Additionally, low-dose lycopene supplemented female and male rats had heavier liver masses compared to control counterparts. These findings suggest that, in female and male rats, fenofibrate induced hepatomegaly through either hypertrophy and or hyperplasia. The heavier liver masses observed in rats that had low dose supplemental lycopene compared to those of control counterparts might suggest that lycopene may positively influence liver growth. Collectively, these results suggest that supplemental lycopene could potentially be used to mitigate liver hypertrophy and/or hyperplasia induced by fructose and fenofibrate. Excessive fructose intake has been shown to increase liver mass through fat accumulation, enhanced lipogenesis, oxidative stress, and inflammation (García-Berumen et al., 2019; Novelle et al., 2021). Fenofibrate has been similarly associated with liver enlargement primarily through increased fatty acid metabolism, peroxisome proliferation, and mitochondrial biogenesis, which may collectively drive hypertrophy and potentially hyperplastic changes in the liver (Lakhia et al., 2018; Fan et al., 2024). Oxidative stress drives liver damage in MASLD by generating excess ROS, causing cellular damage, inflammation, mitochondrial dysfunction, and

fibrosis, leading to hepatomegaly (Smirne et al., 2022). Lycopene, with its antioxidant properties directly combats oxidative stress by neutralizing free radicals, thereby protecting liver cells from damage that can lead to inflammation, fibrosis, and liver growth (Zhao et al., 2021; Kulawik et al., 2024).

6.3.2 Hepatic lipid yield

Though not statistically significantly different, it is important to note that female and male rats fed the fructose enriched diet had 62% and 55.2%, respectively, more hepatic fat content compared to control counterparts pointing to a biologically significant increase in hepatic fat with fructose feeding. Findings from this study showed that female rats fed a high-fructose diet had significantly more hepatic lipid compared to fenofibrate treated as well as low and high dose lycopene supplemented counterparts. Furthermore, male rats fed a high-fructose diet had significantly more hepatic lipid compared to fenofibrate treated as well as medium and high dose lycopene supplemented counterparts. These findings demonstrate that dietary fructose promotes hepatic lipid accretion in growing female and male Wistar rats which (lipid accretion) might eventually cause liver diseases. In contrast, treatment with fenofibrate and fortifying a high-fructose diet with supplemental lycopene attenuated the diet-induced increase in hepatic lipid build-up. Excessive fructose intake contributes to undesirable changes in liver fat levels, that potentially lead to steatosis and other metabolic complications related to high liver lipid content (Yu et al., 2021). This lipid accumulation reflects an imbalance in hepatic lipid metabolism, with fructose promoting hepatic lipogenesis than the liver can adequately clear (Lodge et al., 2024). Fenofibrate, a peroxisome proliferator-activated receptor alpha (PPARα), significantly reduced hepatic lipid content in male C57BL/6J mice fed a high-fat diet for 14 weeks (Zhang et al., 2023). This lipid-lowering effect is attributed to fenofibrate's activation of PPARα, which enhances fatty acid oxidation and reduces lipogenesis in the liver, ultimately decreasing lipid storage (Mahmoudi et al., 2022). Similarly, lycopene markedly lowered hepatic lipid accumulation in male C57BL/6J mice fed for 10-weeks on a high-fat and fructose-enriched diet (Wang et al., 2020). In the current study, hepatic lipid content was notably elevated in both female and male rats fed a high-fructose diet, by 62% and 55.2% respectively, when compared to controls. Supplemental lycopene attenuated this increase, with reductions in hepatic lipid accumulation of 43%, 26%, and 40% for low, medium, and high doses in females, and 29.1%, 47%, and 55.2% in males. Interestingly, supplemental lycopene showed a sex-specific response: in females, the low dose supplemental

lycopene was most effective yet in males, it induced a dose-dependent hepatic lipid reduction. These findings underscore lycopene's antioxidative and anti-inflammatory effects in reducing liver lipid accumulation by alleviating oxidative stress and inflammation, key contributors to hepatic lipid build-up (Delli Bovi et al., 2021). Thus, it could be hypothesised that the observed sex-specific response to lycopene may be influenced by hormonal and metabolic differences between males and females.

6.3.3 Oxidative stress and systemic antioxidant response

Excessive fructose intake contributes to obesity, increases ROS production, induces oxidative stress, and elevates lipid peroxidation (Hernández-Díazcouder et al., 2019). The thiobarbituric acid reactive substances (TBARS) assay measures lipid peroxidation in biological samples by detecting MDA, a key byproduct of lipid oxidation. MDA reacts with thiobarbituric acid (TBA) to form MDA-TBA_2 adducts, known as TBARS, which serve as markers of oxidative stress (Aguilar Diaz De Leon & Borges, 2020). Current study findings showed similarities in serum and hepatic TBARS concentrations and serum GPX-1 activity in the rats across treatment groups. Likewise, hepatic CAT activity was similar in females across treatment groups. Fenofibrate-treated females had significantly increased hepatic GPX-1 activity compared to control, high-fructose diet-fed, low, medium, or high lycopene-supplemented counterparts, while fenofibrate-treated males had significantly higher hepatic GPX-1 levels compared to control, low, or medium-dose lycopene-supplemented counterparts. However, the high dose lycopene supplemented males had higher hepatic GPX-1 activity compared to control males. Furthermore, the same high-dose lycopene-supplemented males also had higher hepatic CAT activity compared to control, high fructose diet-fed as well as low and medium dose lycopene supplemented counterparts. These findings demonstrate that in growing Wistar rats, chronic 12 week high-fructose diet neither causes systemic nor hepatic oxidative stress as supported by similarities in TBARS concentrations across treatment groups. Similarly, the 12-week dietary fructose insult did not activate endogenous antioxidant enzymes (GPX-1 and CAT) activity. These current study findings align with Nestorov et al. (2014), who observed that in growing male Wistar rats a 9-week insult with a 10% fructose in drinking water neither stimulated increased hepatic TBARS concentration nor increased CAT or GPX activity pointing to no effects on oxidative stress and endogenous antioxidant enzymes activities. Similar observations were noted by Glban et al. (2015) in 21-day old male Wistar rats challenged with a 60% fructose solution for a 9-week period. Studies have demonstrated that

young, growing rats exhibit better antioxidant capacity to combat oxidative stress than adult rats (Cao et al., 1996; Lee et al., 2024). This enhanced capacity may explain the lack or minimal impact of fructose on oxidative stress and the systemic antioxidant enzyme response observed in this study. At this developmental stage, young rats maintain redox balance, keep ROS levels within safe limits, and efficiently shield cells from oxidative damage (Glban et al., 2015).

In this study, orally administered fenofibrate and high-dose lycopene exerted distinct antioxidant effects in hepatic tissue as demonstrated by mediating increased GPX-1 activity in female and male rats treated with fenofibrate. The increased hepatic GPX-1 activity indicates its effectiveness in enhancing hepatic antioxidant defences across sexes. Supplemental high-dose lycopene mediated, increased GPX-1 and CAT activities in males. It can, therefore, be inferred that these findings suggest that while fenofibrate supports hepatic antioxidant capacity, particularly GPX-1, regardless of sex, the high-dose supplemental lycopene may offer additional antioxidant benefits that appear more pronounced in male rats, possibly due to sex-specific metabolic responses to lycopene at higher doses. Lycopene has been reported to increase MDA concentrations and to significantly elevate SOD, GSH, and CAT activities in human liver cells (HepG2) exposed to acrylamide- and glycidamide-induced toxicity, indicating its protective role against oxidative damage (Reshmitha & Nisha, 2021). Fenofibrate significantly lowers circulating conjugated dienes, a lipid peroxidation marker, decrease MDA concentrations, and increase GPX activity in patients with dyslipidaemia (Tkáč et al., 2006). Findings from the current study demonstrate that lycopene and fenofibrate reduce oxidative stress and boost antioxidant defences, likely through activation of enzymes like GPX-1 and CAT. However, the exact mechanisms by which they mediate these effects remain unclear and require further investigation to fully understand their potential as prophylactic and therapeutic agents, respectively.

6.3.4 Serum biomarkers of hepatic function

This study observed no significant variations in serum GGT, ALT, and AST activities of female and male rats across treatment groups. However, serum ALP activity was elevated in fenofibrate-treated female and male rats compared to their control, high fructose diet-fed, and lycopene-supplemented counterparts. These findings suggest that the treatments did not damage hepatocytes and cause leakage of ALT, AST and GGT into systemic circulation. However, the increased serum ALP activity with fenofibrate treatment suggest that it (fenofibrate) damaged cells lining the biliary

system resulting in leakage of ALP, which is found in high concentrations in cells that line the bile canaliculi and bile ducts (Reshetnyak & Maev, 2023), into systemic circulation. Current study findings demonstrate that supplemental lycopene attenuated the fenofibrate-treatment induced damage of biliary cells. Elevated activities of AST, GGT, and ALT are commonly used as markers of liver damage, particularly in hepatocytes (Krisnamurti et al., 2022; Thakur et al., 2024). Fenofibrate-induced elevation of serum ALP aligns with evidence linking its use to cholestasis and liver injury (Zubrzycki et al., 2020; Vanasco & vanSonnenberg, 2023). Beyond the hepatic effect, elevated ALP is also observed in osteomalacia, a bone metabolic condition characterised by defective mineralisation (Zhao et al., 2021; Cianferotti, 2022). This suggests that the increase in serum ALP with fenofibrate treatment may reflect impacts on both hepatic and skeletal systems. Moreso, in Chapter 3 (under subheading 3.2.3 long bone indices, pages 127-129), fenofibrate treatment was associated with femoral bone loss and reduced tibia length in males, further supporting the notion of its skeletal impact. This study reports similarities in serum albumin, total protein, globulin, total bile acids, and uric acid concentrations across treatment groups in the female rats but females that had supplemental low- and medium-dose lycopene had higher serum total bilirubin concentrations compared to controls. While there were similarities in serum total bilirubin concentration in males across treatment groups, male rats on a high fructose diet exhibited significantly elevated serum albumin, total protein, and globulin concentrations compared to the control, fenofibrate-treated, and lycopene supplemented counterparts. Both dietary fructose and fenofibrate treatment increased serum uric acid and total bile acids concentrations, respectively, in males compared to control males. The findings suggest that the treatments (dietary fructose, fenofibrate, and supplemental lycopene) elicit sex-specific responses. In female rats, these treatments did not adversely affect hepatic synthetic and excretory capacity as demonstrated by similarities serum albumin, total protein, globulin, total bile acids, and or uric acid concentrations. However, low- and medium-dose supplemental lycopene may have interfered with the liver's ability to process or excrete bilirubin. In male rats, similarities in serum total bilirubin concentrations across treatments, suggest that dietary fructose, fenofibrate, and supplemental lycopene did not impair the liver's ability to process and excrete bilirubin. Bilirubin, a byproduct of red blood cell breakdown, is primarily conjugated and excreted into bile by hepatocytes (Kumbhar et al., 2024). Elevated serum bilirubin concentrations can signal liver dysfunction or altered hepatic metabolism (Guerra Ruiz et al., 2021). It is noteworthy that some current research

contends that elevated bilirubin concentrations might play a protective role in preventing the development of diabetes mellitus and metabolic syndrome (Nikouei et al., 2024). Bilirubin, at physiological concentrations, acts as a powerful endogenous antioxidant (Jansen & Daiber, 2012). It neutralises ROS by undergoing oxidation to form biliverdin, which is converted back into bilirubin through the action of biliverdin reductase, creating a continuous antioxidative cycle (Jansen & Daiber, 2012). This recycling mechanism allows nanomolar bilirubin concentrations to protect cells against oxidants present at up to 10,000-fold excess in cell culture studies (Stocker et al., 1987). It can be hypothesised that the increased serum total bilirubin observed in female rats that had low- and medium-dose supplemental lycopene represents an adaptive response to changes in oxidative balance. As a potent antioxidant, lycopene may influence the liver's redox state, triggering mechanisms that enhance bilirubin production or recycling. This could involve shifting the oxidative-antioxidative equilibrium, prompting the liver to produce or retain more bilirubin to complement lycopene's antioxidant activity. Additionally, lycopene may upregulate enzymes such as biliverdin reductase, facilitating the conversion of biliverdin back to bilirubin and increasing circulating levels. The observed increase in serum total bilirubin concentrations in female rats may reflect a coordinated response to maintain redox homeostasis rather than a marker of dysfunction. Nevertheless, further research is needed to validate this hypothesis and determine whether the effect is protective or indicative of oxidative stress.

In the current study, a high fructose diet led to increased serum albumin, total protein, and globulin concentrations, suggesting an impact on hepatic plasma protein synthesis. Additionally, dietary fructose increased serum uric acid concentrations. Treatment with fenofibrate resulted in increased serum total bile acids concentrations, indicating potential adverse effects on liver function in male rats. Notably, the high fructose-induced increases in serum albumin, total protein, and globulin concentrations, as well as the fenofibrate-induced increase in serum total bile acids, were mitigated by supplemental lycopene, demonstrating its protective role in maintaining hepatic homeostasis. The liver serves as the primary site for plasma protein (albumin and globulin) synthesis, and it plays a pivotal role in amino acid metabolism and detoxification (Paulusma et al., 2022; Yu et al., 2022). Insulin resistance impairs these hepatic processes, disrupting the regulation of protein synthesis and degradation, processes that contribute to metabolic dysfunction (Ren et al., 2017; Ruegsegger et al., 2018). Excessive fructose intake disrupts hepatic function by promoting oxidative stress, which indirectly alters protein metabolism and plasma albumin, globulin, and

total protein concentrations (Softic et al., 2019; Duvall et al., 2023). Importantly, chronic fructose consumption contributes to insulin resistance, reduces hepatic and muscle amino acid uptake, further disrupting protein metabolism (Tappy, 2021; Zeng et al., 2024). Additionally, excessive fructose consumption has been associated with hyperuricemia (Zhang et al., 2020; Lubawy & Formanowicz, 2023). High plasma uric acid concentrations contribute to the onset of gout and renal calculi, and are widely recognised as a significant risk factor for both cardiovascular disease and metabolic syndrome (De Oliveira & Burini, 2012). Consistent with these findings, the current study findings show dietary fructose-induced increased serum uric acid concentrations in male rats by 116.3% increase compared to controls. However, this dietary fructose-induced uric acid increase was mitigated by supplemental lycopene in a dose-dependent manner, with reductions of 25.2%, 38.4%, and 41.7% for low, medium, and high doses, respectively suggesting that fortification of obesogenic diets with lycopene could potentially protect against cardiovascular and renal diseases and metabolic syndrome. Indeed, in animal and human models' lycopene has been shown to effectively reduces serum uric acid concentrations (Basuny et al., 2009; Han & Liu, 2017) which is congruent with findings from the current study. In the liver, fructose is metabolised more rapidly than glucose by unregulated fructokinase, which converts it to fructose-1-phosphate: a process that depletes ATP (Nakagawa et al., 2020). This process increases ADP concentrations, activates AMP deaminase, and breaks down purine nucleotides into uric acid, thereby increasing serum uric acid concentrations (Dewdney et al., 2020). Beyond generating uric acid as a byproduct, this pathway also contributes to metabolic disturbances and liver dysfunction (Zhang et al., 2022). Fenofibrate, known for its effectiveness in managing hyperlipidaemia (Deerochanawong et al., 2024), has been shown to increase total bile acid concentrations. Wang et al. (2023) demonstrated that male C57BL/6 mice treated with 125 mg/kg of fenofibrate daily for 3 weeks exhibited elevated serum total bile acid concentrations. Similarly, in the current study, fenofibrate-treated male rats registered a 66% increase in serum total bile acids concentration compared to the control counterparts. This increase was mitigated by supplemental lycopene, which reduced bile acid levels by 32%, 34.4%, and 21% at low, medium, and high doses, respectively. The increase in serum total bile acid concentrations observed in male Wistar rats may result from fenofibrate's activation of PPARα, which upregulates cholesterol 7α-hydroxylase (CYP7A1) activity; driving bile acid synthesis from cholesterol in the liver (Liu et al., 2014; Sun et al., 2024). Fenofibrate's effects on lipid metabolism may alter bile acid homeostasis by increasing synthesis and secretion

to facilitate lipid digestion and absorption (Jia et al., 2021; Ye et al., 2022). Additionally, fenofibrate may modulate the farnesoid X receptor (FXR), a key regulator of bile acid synthesis and transport, contributing to the elevated plasma bile acid concentrations (Pavlović et al., 2018). In contrast, lycopene's antioxidant properties are well-documented in mitigating oxidative stress and preserving liver function (Abdel-Rahman et al., 2018; Khan et al., 2024). When administered at 10 mg/kg orally for 18 days, lycopene reduced serum total bile acids, bilirubin concentrations, and AST, ALT, ALP, and GGT activities but increased hepatic albumin and total protein concentrations in male albino rats with ethinylestradiol-induced cholestasis (Wadie et al., 2021). These findings support lycopene's protective role against dietary fructose and/or fenofibrate-induced adverse effects on hepatic health, warranting further investigation into its underlying mechanisms.

6.3.5 Hepatic microarchitecture

Excessive intake of dietary fructose is known to contribute to the development of steatosis, metabolic dysfunction-associated steatotic liver disease (MASLD), inflammation, and hepatic hypertrophy (DiStefano & Shaibi, 2020; Li et al., 2024). In the liver, fructose metabolism bypasses key regulatory steps, leading to unregulated lipogenesis and the accumulation of triglycerides within hepatocytes, which are hallmark features of steatosis (Skenderian et al., 2020). Over time, this excessive lipid deposition may progress to MASLD, a condition closely linked to metabolic syndrome and obesity (Pereira et al., 2017). Histologically, MASLD is defined by fatty infiltration into hepatocytes, with steatosis affecting more than 5% of liver cells in histological sections (Brunt et al., 2021). In the present study, no significant differences in hepatic inflammation were observed in either female or male rats across treatment groups. However, dietary fructose notably increased both micro- and macro-vesicular steatosis in both sexes compared to rats fed the control diet, fenofibrate-treated, or supplemented with low, medium and high doses of lycopene. Additionally, orally administered fenofibrate significantly induced hepatocyte hypertrophy in female and male rats compared to control, high-fructose-fed, and lycopene-treated groups. These findings suggest that dietary fructose, supplemental lycopene, and fenofibrate did not cause hepatic inflammation during the 12-week intervention period. Similar to the current study findings, chronic fructose intake has been reported not to induce hepatic inflammation in female Sprague-Dawley rats given a 10% fructose solution as drinking fluid for seven months (Sangüesa et al., 2019). Fenofibrate and lycopene are recognised for their anti-inflammatory properties (Mahmoudi et al., 2022;

Mirahmadi et al., 2023). Although fenofibrate induced hepatocyte hypertrophy in the present study, it has been demonstrated that this increase in hepatocyte size is a physiological adaptive response associated with its lipid-lowering effects, as observed in wild-type C57BL/6 male mice (Fan et al., 2024). This hypertrophy results from fenofibrate's role as a PPAR-α agonist, which enhances fatty acid β-oxidation in peroxisomes and mitochondria, leading to increased metabolic demands on hepatocytes (Jiang & Yang, 2014). These demands necessitate a larger cell size to support elevated metabolic and biosynthetic activity (Jiang & Yang, 2014). Additionally, fenofibrate may promote protein synthesis and organelle proliferation, including an increase in peroxisomes and mitochondria, further contributing to hepatocyte enlargement (Bougarne et al., 2018). Unlike pathological hypertrophy, which is often linked to cellular damage or inflammation (Hora & Wuestefeld, 2023), the hypertrophy recorded in the current study could serves as an adaptive response. However, various factors, such as species, sex, age, and the administered fenofibrate dose can influence the extent of hepatocyte hypertrophy, highlighting its context-dependent nature (Maronpot et al., 2010). Lycopene has been reported to attenuate fructose-induced hepatic steatosis (Ferreira-Santos et al., 2020). Furthermore, lycopene has been demonstrated to prevent the progression of lipotoxicity-induced non-alcoholic steatohepatitis (NASH) by reducing oxidative stress in mice (Ni et al., 2020). In this study, while a high-fructose diet induced steatosis, lycopene effectively mitigated excessive hepatic fat accumulation.

The absence of inflammation in both male and female rats, despite the presence of steatosis in the fructose-fed group and hypertrophy in the fenofibrate-treated group, suggests that the rats in this study did not progress to advanced fatty liver disease, which is commonly marked by steatohepatitis and damage to parenchymal tissues (Peng et al., 2020).

6.4 Conclusion

Dietary fructose elicited sexually dimorphic responses in growing female and male Wistar rats. In males, fructose increased serum albumin, total protein, globulin, and uric acid levels, while no such changes were observed in females. Lycopene effectively mitigated these effects. Both sexes exhibited elevated serum alkaline phosphatase (ALP) levels following fenofibrate treatment, but lycopene protected against fructose-induced lipid accumulation and prevented liver mass increase. Fenofibrate caused hepatomegaly, while high-dose lycopene enhanced hepatic GPX-1 and CAT

activity in male rats. Additionally, fructose induced micro- and macro-vesicular steatosis in both sexes, which lycopene attenuated, while fenofibrate was associated with hepatocyte hypertrophy.

These findings underscore the protective role of lycopene against fructose- and fenofibrate-induced hepatic alterations. By mitigating lipid accumulation, oxidative stress, and steatosis, lycopene offers a promising prophylactic approach to diet-induced liver dysfunction in a sex-dependent manner, highlighting its potential to prevent diet-induced liver pathologies.

Having explored the potential benefits of supplemental lycopene in protecting liver health in Wistar rats fed a high fructose diet, with a focus on hepatic oxidant and antioxidant status, lipid content, hepatic and serum markers of liver function, and liver histomorphology in this chapter, chapter 7 summarises the study's key findings and major conclusions, highlights some of the study's limitations, and proposes recommendations for future research.

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CHAPTER SEVEN: CONCLUSIONS, LIMITATIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

7.0 Introduction

This study evaluated whether orally administered lycopene could protect growing Wistar rats from metabolic derangements caused by dietary fructose, focusing specifically on its effects on:

- growth performance as determined by body mass and long bone indices and gastrointestinal macromorphometry.
- some haematology components, serum metabolic substrate, lipid profile and leptin concentration.
- kidney macro- and micro-morphometry and serum surrogate biomarkers of kidney health.
- liver macro- and micro-morphometry, liver lipid and epididymal (males) and visceral fat, hepatic oxidant and antioxidant status and serum biomarkers of liver health.

7.1 Summary of key findings, limitations, and recommendations

The 12-week chronic fructose consumption decreased total feed intake which (feed intake) was further reduced in rats supplemented with lycopene, but it increased the rats' fluid intake. Supplemental lycopene caused sex-specific effects on total calorie intake (TCI) by increasing TCI in females but decreasing it in males. Femora from male rats treated with fenofibrate were lighter compared to those of counterparts supplemented with low-dose lycopene, and their tibiae shorter compared to those fed the control diet and lycopene-supplemented counterparts. Supplemental lycopene resulted in longer tibiae compared to fenofibrate treated males and heavier femora in low dose-supplemented males compared to fenofibrate-treated counterparts. Furthermore, the medium and high dose supplemental lycopene resulted in heavier stomach masses, but low dose supplemental lycopene caused decrease intestinal mass. It can be inferred that supplemental lycopene may enhance bone growth and development, protect against diet-induced bone mass reduction, enhance gastric digestion and secretion but its low dose could negatively affect nutrient digestion and absorption in the small intestines of males. Therefore, lycopene can be used to fortify diets to support bone health and improve nutrient assimilation, especially in populations exposed to diet-induced bone loss and dietary nutrient imbalances. It is imperative, however, that further studies be conducted to clarify underlying mechanisms of the protective effects of lycopene on bone health and enhance gastric and small intestinal development. Despite its potential positive

effects on bone health and the small intestines and stomach, there is need to further explore its sex-specific effects, potential interactions with obesogenic diets and therapeutic agents including fenofibrate to optimise its utility.

Neither the 12-week fructose insult nor supplemental lycopene adversely affected haematocrit, haemoglobin, HbA1c, fasting blood glucose (FBG) and leptin concentrations and lipid profile of growing Wistar rats. However, fenofibrate produced distinct sex-specific effects on glucose metabolism and lipid profiles by increasing FBC concentrations in females and males and HbA1c in females only. Liu et al. (2011) reported that fenofibrate disrupts glucose metabolism, an observation that aligns with the current study. Importantly, in females, fenofibrate increased serum HDL concentrations, whose increased concentration positively impact cardiovascular health by mopping up cholesterol from the periphery to the liver where it is metabolised and excreted into bile (Perswani et al., 2024). Despite fenofibrate's potential cardiovascular benefits in females, its chronic use poses risks to glucose regulation in both sexes, highlighting the need for caution for long term use. It can be deduced from the study findings that in growing Wistar rats, both dietary fructose and dietary fortification with lycopene may have minimal adverse metabolic effects, if any, but use of fenofibrate can potentially benefit cardiovascular health in females by mediating increases in serum HDL concentration. However, its adverse effects on blood glucose homeostasis warrants caution. Future studies should focus on investigating the potential of lycopene to mitigate fenofibrate-induced metabolic disturbances, which could enhance its therapeutic and prophylactic applications.

Compared to control and lycopene-supplemented rats, fenofibrate-treated males had heavier relative kidney mass but had no effect of the females' kidney masses. Treatment regimens had no effect on serum KIM-1, NGAL, BUN, creatinine concentrations and the rats' BUN/creatinine ratio. Both dietary fructose and fenofibrate increased the urinary space area, enlarged the outer and epithelial areas of the distal convoluted tubule and reduced the inner area of the proximal convoluted tubule of males only indicating a sex-specific effect. In females, fenofibrate caused an enlargement of the inner, outer, and epithelial areas of the distal convoluted tubule. Supplemental lycopene mitigated the dietary fructose-induced changes, demonstrating its health benefits against high-fructose induced renal changes. It thus can be deduced that supplemental lycopene can potentially safeguard both function and structure from dietary fructose induced renal disturbances.

Although fenofibrate positively impacts the lipid profile in patients suffering with MASLD (Martin et al., 2022) and has cardiovascular protective effects in females (d'Emden et al., 2014), its chronic use must be with caution as it can induce adverse renal effects. To mitigate these risks, lycopene-rich foods such as tomatoes or dietary supplements may serve as a beneficial strategy to safeguard kidney health against fenofibrate- and diet-induced kidney alterations. Future studies should explore the molecular mechanism of lycopene's renoprotective effects, with particular focus on its mitigatory effects on increased renal urinary space enlargement and tubular changes. Optimal doses and durations of lycopene supplementation should also be evaluated to ensure its efficacy and safety as a dietary intervention for kidney health.

Regarding the current study's last objective, findings showed sex-specific metabolic responses to dietary fructose and the protective potential of lycopene. In male rats, dietary fructose caused an increase in serum concentrations of globulin, albumin, and total protein compared to the other treatment regimens, and resulted in higher serum uric acid concentration. Although it does not point to a specific condition, higher than normal blood protein concentration is indicative of chronic inflammation. Supplemental lycopene mitigated these alterations, underscoring its protective role. Fenofibrate-treated female and male rats exhibited increased serum ALP activity and hepatomegaly. Fenofibrate-induced hepatomegaly has been confirmed in previous studies (Oscarsson et al., 2018; Fan et al., 2024). Additionally, fructose-induced lipid accretion has been shown in previous research (Coronati et al., 2022). Lycopene, however, prevented fructose-induced liver mass enlargement and lipid accumulation. Notably, the high-dose supplemental lycopene significantly enhanced hepatic GPX-1 and CAT (antioxidant enzymes) activities in males, a further indication of its potential protective role via upregulation the activity of systemic antioxidant system. Additionally, the fructose-induced hepatic micro- and macro-vesicular steatosis in both sexes was attenuated by supplemental lycopene but, fenofibrate caused hepatocyte hypertrophy. These findings suggest that lycopene may be exploited to fortify diets to protect against fructose- and fenofibrate-induced liver disturbances. Future research should aim to clarify the underlying mechanism of lycopene's hepatoprotective effects, particularly its role in enhancing antioxidant defences and mitigating hepatic lipid accumulation. Molecular studies could provide deeper insights into the pathways through which lycopene prevents hepatic lipid accretion, attenuates steatosis, and counteracts oxidative stress. Further exploration of lycopene's

interactions with dietary fructose and fenofibrate across varying doses and durations will help establish its efficacy and safety in both dietary and therapeutic applications. The exclusion of a lycopene-only group from the study design restricted our ability to fully understand its broader health benefits. Future research should address this gap by incorporating a lycopene-only group to assess its protective effects across various organs and systems, contributing to its potential application for promoting overall health and wellness.

7.2 Overall conclusion

Findings from this study demonstrate that dietary fructose induced significant sex-specific metabolic and hepatic and renal macro-and micro-morphometrical changes. Supplemental lycopene effectively mitigated diet-induced hepatic lipid accumulation, and it enhanced the systematic antioxidant enzymes activity thus mitigating oxidative stress. Additionally, supplemental lycopene improved stomach and small intestine growth and development, which may contribute to more efficient nutrient digestion and absorption. It also supported long bone integrity in male rats, further underscoring its positive effects on the skeletal system. Based on these findings, supplemental lycopene demonstrates potential in mitigating high-fructose-induced metabolic disturbances in growing female and male Wistar rats, potentially contributing to sustained health advantages over time.

7.3 References

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APPENDICES

Appendix 1: Animal ethics approval certificate

ANIMALS RESEARCH ETHICS COMMITTEE (AREC)

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

STRICTLY CONFIDENTIAL

CLEARANCE CERTIFICATE NUMBER: 2022/03/02/C

APPLICANT: Mrs M Shafe
School: School of Physiology; Department: N/A; Location: WRAF

PROJECT TITLE: Lycopene: Protective Potential Against Diet-Induced Metabolic Derangements in Wistar Rats
Category: C; Species and Numbers involved: *48X 21 day old weaning pups, male, Wistar Rats and 48X 21 day old weaning pups, female, Wistar Rats*

Approval is hereby given for the use of animals for the research project named above and described in the application reviewed by a quorate meeting of the AREC held on 29 Mar 2022. This approval remains valid until 8 May 2024 and is conditional to the following (if blank there are no special conditions):

Condition 1	Condition 2	Condition 3	Condition 4

All material changes to the approved research must be reported to the AREC before they are implemented. Failure to do so will invalidate this clearance certificate.

An annual progress report must be provided to the AREC.

The use of these animals is subject to AREC guidelines on the use and care of laboratory animals, is limited to the procedures described in the application and is subject to additional conditions listed below:

I, the Chair of the AREC (or my designated representative) am satisfied that the proposed research is ethical as judged by local law, international standards and University policy.

Signed: _____ _____ Date: __10/05/2022_____

(Chairperson of the AREC)

I am satisfied that the persons listed in this application are competent to perform the procedures described in the application, in the context of Section 23 (1) (c) of the veterinary and Para-veterinary Professions Act (19 of 1982).

CC: Student supervisor: «Title1» «Initials1» «Supervisor_surname»
Director Wits Research Animal Facility (WRAF): Dr Kim Jardine

Signed: __ _____ Date: __10 May 2022_____

(Registered Veterinarian)

CC: Student supervisor: «Title1» «Initials1» «Supervisor_surname»
Director Wits Research Animal Facility (WRAF): Dr Kim Jardine

Appendix 2: First amendment to ethics clearance

Please note that only type written applications will be accepted.

UNIVERSITY OF THE WITWATERSRAND
ANIMAL ETHICS SCREENING COMMITTEE
MODIFICATIONS AND EXTENSIONS TO EXPERIMENTS

a. Name: Mercy Shafe (2404174)
b. School and email address: School of Physiology. 2404174@students.wits.ac.za
c. Experiment to be modified / extended **AESC NO**

Original AESC number	**2022**	**03**	**02C**
Other M&Es:			nil

d. Project Title: **Lycopene: Protective Potential Against Diet-Induced Metabolic Derangements in Wistar Rats.**

		No.	**Species**
e.	Number and species of animals originally approved:	96	Wistar Rats (Female: 48, Male: 48)
f.	Number of additional animals previously allocated on M&Es:	0	
g.	Total number of animals allocated to the experiment to date:	10	
h.	Number of animals used to date:	10	

i. Specific modification / extension requested:

A new MSc Med student, Ms. Motlhale Gomotsegang (student no: 2643348; email address: 2643348@students.wits.ac.za) has registered with the School of Physiology, University of Witwatersrand, under the supervision of Prof. Eliton Chivandi (Wits) and Dr. Nontobeko Gumede (UL). Currently Ms. Gomotsegang is attached, as a co-worker, to aforementioned project. She has written and successfully presented her MSc. Medicine (Physiology) protocol with the study titled: **Effects of Lycopene on Bone, kidney, and Pancreatic Health of growing Wistar Rats fed an Obesogenic Diet.**

Ms. Gomotsegang Motlhale will conduct assays on the pancreata (fat content, lipid profile, histology, oxidant and antioxidant markers), kidneys (fat content, lipid profile, surrogate biomarkers, histology, oxidant and antioxidant markers) and bone (bone indices, bone breaking strength and histology) samples. Motlhale will also assay for serum surrogate markers (amylase and lipase) of pancreatic health and serum concentrations of hormones (osteocalcin, C-telopeptide of type I collagen and parathyroid hormone) that regulate bone health. Additionally, serum inflammatory and anti-oxidant biomarkers will also be analysed.

Motivation for modification / extension:

The animal study has just commenced and the tissues have not yet been collected. I am requesting your good office to allow Ms Motlhale Gomotsegang to use the stated tissues from the rats under my ethical clearance as this will be in conformity with the principle of the 3Rs and help reduce the number of animals (rats) by avoiding the need to repeat the study with new rats.

Date: 11/08/2022 Signature: 

Recommendations
Addition of a co-worker and addition of tissue samples as stated above.
Condition: the co-worker requires to assist to the WRAF induction training.

Signature: FMichel

Date: 12/08/2022

Chairman, AESC

Appendix 3: Second amendment to ethics clearance

Please note that only type written applications will be accepted.

UNIVERSITY OF THE WITWATERSRAND
ANIMAL ETHICS SCREENING COMMITTEE
MODIFICATIONS AND EXTENSIONS TO EXPERIMENTS

a. Name: Mercy Shafe (2404174)
b. School and email address: School of Physiology. 2404174@students.wits.ac.za
c. Experiment to be modified / extended **AESC NO**

Original AESC number	**2022**	**03**	**02C**
Other M&Es:			nil

d. Project Title: **Lycopene: Protective Potential Against Diet-Induced Metabolic Derangements in Wistar Rats.**

		No.	**Species**
e.	Number and species of animals originally approved:	96	Wistar Rats (Female: 48, Male: 48)
f.	Number of additional animals previously allocated on M&Es:	0	
g.	Total number of animals allocated to the experiment to date:	75	
h.	Number of animals used to date:	75	

i. Specific modification / extension requested:

We request to add additional co-workers to the AREC approved research project titled "**Lycopene: Protective Potential Against Diet-Induced Metabolic Derangements in Wistar Rats.**" The approved Ethics clearance was given to Mrs. Mercy Shafe (Student number: 2404174; a current PhD student in the Wits School of Physiology; Animal ethical clearance certificate number 2022/03/02/C) under the supervision of Prof. Eliton Chivandi (Wits), Dr. Trevor Nyakudya (UP) and Dr. Nontobeko Gumede (UP). The animal study is currently ongoing. We request that Dr. Jaclyn Johnson and Olukemi Daramola of the Wits School of Anatomical Sciences be attached as co-workers to the aforementioned project. The requested additional co-workers and ourselves will evaluate the effect of lycopene on the hearts, testes, epididymides, ovaries and uteri in Wistar rats fed an obesogenic diet in order to optimize the utility of animal use in research and thus fulfill the requirements for the 3Rs. Histology, antioxidant and molecular assays will be measured on the said tissues in order to evaluate if lycopene protects the organs against potential high-fructose diet induced metabolic derangements and histological alterations.

Motivation for modification / extension:

The animal study is currently ongoing and the tissues have not yet been collected. I humbly request that the two be added as co-workers and collaborate with me and my supervisors and

thereby following the principle of reduction by avoiding the need to repeat the study with new rats.

Date: 23/10/2022 Signature: 

Addition of coworkers (Drs Johnson and Daramola - School of Anatomical Sciences).

Signature: FMichel

Date: 14/12/2022

Chairman, AESC

Appendix 4: Published review article (Chapter 2)

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WILEY

Review Article

Lycopene: A Potent Antioxidant with Multiple Health Benefits

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Lycopene is a naturally occurring carotenoid predominantly found in tomatoes and tomato-based products. Like other phytochemicals, it exhibits health beneficial biological activities that can be exploited when it is used as a dietary supplement. *In vitro* and *in vivo*, lycopene has been demonstrated to mitigate oxidative stress-induced metabolic dysfunctions and diseases including inflammation, obesity, and diabetes mellitus. Lycopene has been shown to alleviate metabolic diseases that affect the bone, eye, kidney, liver, lungs, heart, and nervous system. This review presents the state of the art regarding lycopene's health benefits and its potential applications in health system delivery. Furthermore, lycopene's protective effects against toxins, safety in its use, and possible toxicity are explored.

1. Introduction

The use of medicinal plants has deep historical roots, ingrained in the traditional healing practices of diverse cultures worldwide [1]. Throughout centuries, indigenous communities and ancient civilizations have harnessed the therapeutic properties of plants, passing down invaluable knowledge through generations [2]. Ethnomedicine, a field dedicated to study traditional medicinal practices, has played a crucial role in documenting this wealth of wisdom. The effectiveness of ethnomedicinal plants in disease management is attributed to their constituent bioactive phytochemicals, such as carotenoids, which are known to have multiple health benefits [3]. Lycopene, a fat-soluble carotenoid, is one of the most abundant and important carotenoids [4]. It has potent antioxidant activity [5]. This carotenoid, a bioactive organic pigment, is found in pink grapefruit, papaya, guava, apricot, watermelon, and vegetables but is highly concentrated in tomatoes and tomato-derived products [6]. It has been reported to be one of the strongest antioxidants among carotenoids [7]. As one of the most potent antioxidants, its capacity to neutralise singlet oxygen is double that of β-carotene, ten times greater than that of α-tocopherol, and one hundred and twenty-five times more effective than glutathione [5]. Lycopene, isolated from *Lycopersicum esculentum* (tomato) in 1903, was named after the fruit from which it was isolated [8]. More than 85% of the lycopene in the diet is derived from tomatoes and tomato-based products [8]. In addition to fruits and vegetables, lycopene is also found in some food ingredients, as shown in Table 1 [9, 10]. While overall tomatoes are a good source of lycopene, research has demonstrated that different tomato and other fruit varieties have different lycopene content [7]. In addition to varietal differences, the microenvironment in which the tomato and or other lycopene-containing fruit are grown, for example,

Table 1: Lycopene concentration in fresh fruits and processed food products.

Fruit/processed food product	Lycopene content (mg/100 g)
Apricot and fresh tomatoes	0.11–5.3
Carrot	0.65–0.78
Cooked tomatoes	3.70
Fresh tomatoes	0.72–4.2
Ketchup	9.90-13.44
Papaya	0.11–5.3
Pink grapefruit	0.35–3.36
Pink guava	5.23–5.5
Pumpkin	0.38–0.46
Rosehip	0.68–0.71
Sweet potato	0.02–0.11
Tomato paste	5.40–150
Tomato sauce	6.20
Watermelon	2.30–7.20

Source: [9, 10].

temperature, humidity, edaphic conditions, and fruit maturity status at harvest also influence lycopene content [11]. Where the soil microbiome has favourable microbes, a 36% increase in lycopene has been reported [11].

Several studies have investigated the potential of lycopene to mitigate risk factors for obesity, type 2 diabetes mellitus, and cardiovascular diseases, conditions characterised by dyslipidaemia, oxidative stress, and inflammation [12]. These studies have shown that lycopene improved outcomes of these metabolic diseases [13]. Lycopene, known for its antioxidant properties, has been found to reduce oxidative stress, a significant contributor to the development of metabolic diseases [14]. In addition, it has been shown to mitigate inflammation and dyslipidaemia, thereby reducing the risk of cardiovascular diseases and insulin resistance [15, 16]. Research suggests that regular consumption of lycopene as a dietary supplement can potentially remediate insensitivity to insulin, hypertension, and obesity-related metabolic complications [17, 18].

2. Lycopene: Biochemistry and Physical Properties

In nature, over 750 carotenoids have been identified [19]. About 40 to 50 are found in the human diet, and lycopene is the sixth most common carotene in food products [20, 21]. Two main categories of carotenoids exist: hydrocarbon carotenoids and xanthophylls. Hydrocarbon carotenoids such as α-, β-, and γ-carotene lycopene are made up of hydrogen and carbon, while xanthophylls, for example, lutein, β-cryptoxanthin, and zeaxanthin, contain oxygen along with carbon and hydrogen [4, 22]. Lycopene, as an aliphatic straight-chain hydrocarbon, contains two unconjugated double bonds and 11 conjugated bonds [23]. Its conjugated double bonds are subject to isomerization through heat, light, and chemical reactions [20]. Lycopene is found in *trans*- and *cis*-isomers, but the *cis*-isomers are better absorbed and have greater bioavailability than *trans*-lycopene [24, 25]. All-*trans*, 5-*cis*, 9-*cis,* 13-*cis*, and 15-*cis* are the most common forms of lycopene isomers, and the 5-*cis* isomer is the most stable isomer [26, 27]. The molecular structure and physical properties of lycopene are shown in Figure 1 [28] and Table 2, respectively [8, 29].

3. Lycopene: Absorption, Transportation, and Distribution

Following ingestion, lycopene released from the food matrix combines with micelles-containing bile salts, cholesterol, and fatty acids [30] and is then absorbed. Due to its hydrophobicity, the dissolution of lycopene within micelles in the small intestines facilitates its absorption [5] through the passive diffusion of lipids across the unstirred water layer in the enterocytes [31]. Inside the absorptive enterocyte, lycopene, together with free fatty acids, monoglycerides, and fat-soluble vitamins, is packaged into chylomicrons and released into the lymphatic system for transportation into the bloodstream and liver [23]. A fibre-rich diet has been proven to decrease the absorption of lycopene. Such fibrous diets also mediate the absorption of lycopene, resulting in over 40% reduction in plasma lycopene [32]. Several factors, among these, alcohol, smocking, gender, age, hormonal status, and other dietary elements, affect the absorption of lycopene [32]. As healthy individuals grow older, the bioavailability of lycopene tends to decrease, possibly due to age-related structural changes in the gastrointestinal tract that result in reduced absorptive efficiency [33]. Humans absorb about 10% to 30% of dietary lycopene; the rest is excreted through faeces [8, 33]. The lycopene in heated and processed tomato products is better absorbed compared to that from fresh, unprocessed tomatoes [20]. Thermal exposure during cooking and processing of lycopene-containing foods breaks the food matrix and converts the natural (all-trans) lycopene structure to its *cis* geometric isomer, which is 2.5 times better absorbed from the gastrointestinal tract [34, 35]. Following its absorption from the small intestines, lycopene is distributed to the various body tissues [33]. The assimilation of lycopene by the tissues from lipoproteins is mediated by certain membrane receptors known as scavenger receptor class B type 1 (SR-B1) and cluster of differentiation 36 (CD36) [4]. In humans, the concentration of lycopene in the testes is ten times greater than that found in other tissues [8]. This high concentration in the testes is followed by its concentration in the adrenal gland, liver, prostate, breast, pancreas, skin, colon, ovary, lung, stomach, kidney, adipose tissue, and cervix [8]. However, *cis*-lycopene is mainly distributed in the liver and adipose tissue [24]. Table 3 illustrates the concentration of lycopene in various human tissues [36, 37]. Lycopene, the primary carotenoid found in human plasma, exhibits a half-life of approximately 2 to 3 days. Its concentration in plasma and tissues ranges between 0.2–21.4 nmol/g and 0.15–21.36 nmol/g, respectively [8, 36]. In their study, Zaripheh et al. [38] reported that in rats, lycopene was most concentrated in the liver, adipose tissue, adrenal tissue, and spleen.

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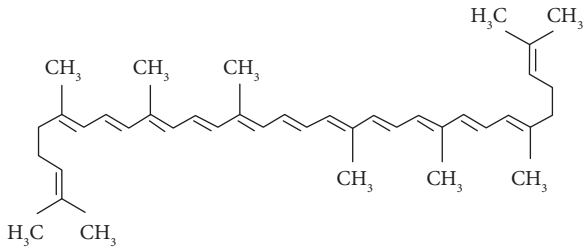


Figure 1: Molecular structure of lycopene.

Table 2: Physical properties of lycopene.

Property	Value/normal range
Boiling point	660.9°C at 760 mmHg
Crystal form	Long red needles separate from a mixture of carbon disulfide and ethanol
Density	0.889 gm/cm^3
Flash point	350.7°C
Main hazards	Combustible
Melting point	172–175°C
Molecular weight	536.85 Da
Powder form	Dark reddish-brown
Refractive index	1.531
Solubility	Soluble in chloroform, hexane, benzene, carbon disulfide, acetone, petroleum, tetrahydrofuran, carbon disulfide, ether, and oil; insoluble in water, ethanol, and methanol
Stability	Sensitive to light, oxygen, high temperature, acids, catalyst, and metal ions
Vapour pressure	1.33·10−16 mmHg (25°C)

Source: [8, 29].

Table 3: Lycopene concentration in some human tissues.

Tissue	Lycopene (nmol/g wet weight)
Adipose	0.2–1.3
Adrenal	1.9–21.6
Brainstem	Non detectable
Breast	0.8
Colon	0.3
Liver	1.3–5.7
Lung	0.2–0.6
Ovary	0.3
Prostate	0.8
Skin	0.4
Stomach	0.2
Testis	4.4–21.4

Source: [36, 37].

4. Lycopene Autoxidation

Known to be thermolabile, lycopene undergoes autoxidation when exposed to both light and oxygen [23]. The heat-, light-, and oxygen-induced lycopene degradation gives rise to acetone, methyl-heptenone, laevulinic aldehyde, and glycoxal, a colourless compound that produces a grass-like smell [23]. In addition to the attractive colour of the final lycopene degradation products, their biodegradation also affects their flavour and nutritive value [39].

5. Biological Activities of Lycopene

The meta-analyses and clinical trials of lycopene in human studies are shown in Table 4.

5.1. Antiobesity Effects. Obesity results from an excessive buildup of body fat. It has a detrimental effect on a person's metabolic health and overall well-being [66]. The development of obesity is influenced by a variety of factors with complicated origins that involve psychological, environmental, socioeconomic status, and biological components [67–69]. The risk of cardiovascular diseases, cancer, depression, dyslipidaemia, type 2 diabetes mellitus, nonalcoholic fatty liver diseases (NAFLD), and hypertension is heightened in obese individuals [70–73]. Obesity elevates the prevalence of oxidative stress by disrupting the balance between oxidants and antioxidant activity [74], which leads to the presence of "unpaired mitochondria" (individual mitochondria within a cell that have not fused or aligned with others to form interconnected networks) and an upsurge in reactive oxygen species [75]. Consequently, the normal functioning of the adipose tissue is disrupted, resulting in an increased production of adipocytokines and a reduction in adiponectin levels, which contribute to the occurrence of metabolic syndrome [76, 77]. Numerous studies have reported on the health beneficial antioxidant activity of lycopene. In male Wistar rats exposed to a high-fat diet for 12 weeks, supplementation with lycopene at 25 mg/kg body weight for a period of 4 weeks was shown to reduce plasma interleukin 6 (IL-6), tumour necrosis factor alpha (TNF-α), leptin, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and total cholesterol (TC), but it elevated plasma high-density lipoprotein (HDL) levels [78]. The supplemental lycopene also reduced malondialdehyde (MDA) concentration but increased hepatic superoxide dismutase (SOD) and catalase (CAT) activities in the liver tissue, demonstrating that it (lycopene) potentially is a potent antioxidant that decreases hepatic oxidative stress by increasing systemic antioxidant and enzyme activities [78]. Pre- and/or postweaning supplementing Sprague–Dawley rat pups whose dams were fed a high-fat diet with lycopene at 1% improved the offspring's brown adipose tissue (BAT) development, reduced accumulation of white adipose tissue (WAT), and enhanced serum antioxidant capacity and blood glucose homeostasis [79]. In mice fed a high-fat diet, lycopene was shown to improve glucose and lipid metabolism and decrease body weight gain by stimulating WAT browning and activating BAT through modulation of peroxisome proliferator-activated receptor gamma (PPARG) [24]. In another study, where lycopene was administered at 25 and 50 mg/kg body weight for 3 months to male Wistar rats, results showed increased HDL, improved antioxidant, and oxidant biomarkers, decreased triglycerides (TG), LDL, apolipoprotein-B (Apo-B), and β-hydroxybutyrate, but boosted hepatic PPARG levels [80]. Furthermore, tomato oleoresin, which contains 10 mg/kg body weight of lycopene, when orally administered to male Wistar rats for 6 weeks, mediated a significant increase in the expression of

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Table 4: Meta-analyses and clinical trials of lycopene in human studies.

Biological effects	Mechanisms of action	References
Anticancer	Suppressed cell proliferation, induced cell cycle arrest, and increased apoptosis in breast cancer cell lines	[40]
	Decreased insulin-like growth factor-1 (IGF-1) and increased apoptosis in prostate cancer cell	[41]
Cardioprotection	Enhanced endothelial function and decreased triglyceride levels in patients with ischemic heart failure	[42]
	Increased flow-mediated dilation and total oxidative status decreased	[43]
	Increased HDL, paraoxonase-1 (PON-1), lecithin cholesterol acyltransferase (LCAT), decreased serum amyloid A (SAA), and cholesteryl ester transfer protein (CETP) activities	[44]
Antidiabetic	Reduced levels of fasting blood glucose in patients with type 2 diabetes mellitus	[45]
	Decreased glycated haemoglobin (HbA1c) levels and fasting blood glucose concentration	[46]
Anti-inflammatory	Inhibited NF-kB and c-Jun N-terminal kinase (JNK) activation. Suppressed the expression of COX-2, iNOS, TNF-α, IL-1β, and IL-6	[47]
Antioxidant	Increased bone mineral density	[48]
	Increased SOD, GSH-px, and decreased MDA	[49]
Sperm quality enhancement and fertility promotion	Decreased lipid peroxidation and fragmentation of sperm DNA	[50]
	Increased arachidonic acid to docosahexaenoic acid ratio	[51]
	Reduced oxidative stress and enhanced sperm quality	[52]
Hepatoprotection	Protection against steatosis and liver damage	[53]
	Regulated oxidative stress and liver enzyme levels in individuals with metabolic syndrome	[54]
Antiobesity	Decreased body weight, BMI, waist circumference, total cholesterol, and increased HDL levels	[55]
Renoprotection	Elevated levels of serum lycopene reduce the risk of mortality in individuals with CKD	[56]
	Increased consumption of lycopene decreased the occurrence of CKD in women	[57]
Lung protection	Decreased airway neutrophil influx and decreased activity of neutrophil elastase in sputum	[58]
	Increased SOD and CAT and decreased MDA, TNF-α, IL-1β, and IL-6 levels in chronic obstructive pulmonary disease (COPD)	[59]
Neuroprotection	Elevated serum levels of lycopene are associated with a decreased risk of Alzheimer's disease (AD) mortality in adults	[60]
	Enhanced cognitive function in middle age	[61]
Gastroprotection	Decreased bleeding index and reduction in the percentage of gingivitis	[62]
	Increased consumption of lycopene improved bowel function and helped prevent chronic constipation	[63]
Osteoprotection	Stimulated WNT/β-catenin and ERK1/2 pathways, increased the expression of RUNX2, alkaline phosphatase, and COL1A, and decreased RANKL in Saos-2 cells	[64]
	Increased total antioxidant capacity, decreased lipid peroxidation, protein oxidation, and N-telopeptide of type 1 collagen	[65]

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messenger RNA (mRNA) of adiponectin, forkhead box 01 (Fox01), fatty acid translocase/cluster of differentiation 36 (FAT/CD36), and sirtuin 1 (SIRTI), but downregulated PPARG expression in the adipose tissue of obese rats [81].

5.2. Antioxidant Effects. Oxidative stress is recognised as a significant contributing factor to an increased risk of cancer, the onset and progression of various metabolic and chronic disorders [82]. The concept of oxygen radicals has been established for the past 50 years; however, its role in the advancement of diseases was discovered in the past two decades [83]. In several biological processes that are vital for life, free radicals play an important role, such as the destruction of intracellular bacteria by phagocytes such as macrophages and granulocytes [82]. Excessive production of reactive oxygen species (ROS) causes protein, deoxyribonucleic acid (DNA), and lipid damage [84]. Damage to these cellular molecules leads to tissue injury and interruption in vital cellular processes [85]. Consuming diets rich in antioxidants or supplementing with bioactive molecules such as vitamins, tannins, and carotenoids may offer protection against oxidative damage [86]. Carotenoids such as lycopene are potent antioxidants that inhibit or hinder the advancement of diverse disorders triggered by ROS [5]. Carotenoid antioxidant activity has been investigated in multilamellar liposomes by measuring the inhibition of the formation of thiobarbituric acid-reactive substances. Lycopene was shown to be the most potent antioxidant in the sequence: lycopene, Υ-tocopherol, astaxanthin, canthaxanthin, α-carotene, β-carotene, bixin, zeaxanthin, lutein, α-tocopherol, glutathione, cryptoxanthin, crocin, and lipoic acid [8, 87]. Lycopene attenuates ROS effects through radical addition or adduct formation, electron transfer to the radical, and allylic hydrogen abstraction [6], and radical addition and allylic hydrogen abstraction contribute to its antioxidant effects [88]. Lycopene has been reported to enhance the status of enzymatic (catalase, superoxide dismutase, and peroxidase) and nonenzymatic antioxidants such as vitamins C and E from their radicals by increasing the cellular antioxidant defence system [33]. In addition, lycopene acts as an antioxidant in systems that produce singlet oxygen but behaves as a pro-oxidant in systems that create peroxide [89]. In low doses, it acts as an antioxidant, but at high doses, it acts as a pro-oxidant [90]. Factors such as lycopene concentration, tissue oxygen tension, and interaction with other antioxidants have been reported to influence the pro-oxidant potency of lycopene [6]. In situation where there is an imbalance between antioxidant defences and ROS production, such as during inflammation or exposure to environmental toxins [91], lycopene may switch from its antioxidant role to a pro-oxidant role [89]. Under these conditions, lycopene radicals may contribute to oxidative stress by reacting with cellular components and promoting further ROS generation [92]. Studies have suggested that under conditions of low oxygen levels, its antioxidant properties predominate [93, 94].

5.3. Hypocholesterolaemic Effects. An imbalance in the level of cholesterol in the body results in a lipid disorder known as hypercholesterolemia, a notable risk factor for atherosclerosis and related conditions such as coronary and cerebrovascular diseases [95, 96]. Several animal and human trials have investigated the association between lycopene and cholesterol. Male broiler chickens fed a standard grower diet supplemented with lycopene at 100 mg/kg body weight for 3 weeks had significantly reduced serum total cholesterol, triglyceride, very low-density lipoprotein, and increased high-density lipoprotein content compared to counterparts fed the control diet [97]. In apolipoprotein E knockout mice fed a high-fat diet and lycopene supplementation at 60 mg/kg body weight daily for 14 weeks, the administered lycopene significantly decreased both total cholesterol and triglycerides, beginning from the sixth week to the end of the experiment [98]. Similarly, male Wistar rats given a high-fat diet and 50 mg/kg body weight of lycopene daily for 3 months had significant reductions in plasma total cholesterol, triglycerides, and low-density lipoprotein levels but increased high-density lipoprotein cholesterol compared to the group given a high-cholesterol diet [99]. The reported cholesterol-lowering effects of lycopene are attributed to reduce cholesterol synthesis through the inhibition of the expression and activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and the modulation of LDL receptor activity [100]. The findings obtained from human studies have been inconsistent. In a systematic review and meta-analysis of 12 and 11 trial arms consisting of 781 and 854 participants, respectively, supplementation of lycopene significantly increased HDL-cholesterol levels when compared to the control group; however, no significant difference was observed in the triglyceride levels [101]. The conflicting findings observed from human studies could be attributed to the differences in the study design, characteristics of the populations under investigation, and the source and dose of lycopene utilised [16, 102].

5.4. Hepatoprotection. In a healthy human adult, the liver weighs approximately 1.5 kg and is the largest gland and visceral organ [103]. It plays a vital role in metabolic processes such as bile production, digestion, detoxification of xenobiotics, metabolism of lipids, proteins, carbohydrates, immune regulation, and storage of vitamins [104, 105]. Among the major causes of global mortality is liver disease [106]. Liver diseases may be caused by several factors, viral infections, ischemia, alcohol-induced damage, autoimmune diseases, and genetic defects such as alpha-1 antitrypsin deficiency, hereditary hemochromatosis, citrin deficiency, hereditary fructose intolerance, cystic fibrosis, cholesteryl ester storage disease, type IV glycogen storage disease, and Wilson disease [107–109]. Nonalcoholic fatty liver disease (NAFLD) is the most prevalent liver disease [110]. Globally, the prevalence of NAFLD is about 25%, in Africa, it is 13% while in Europe, the rate is 23% and the highest at 32% in the Middle East [111]. This disease is characterised by the accumulation of macrovesicular steatosis in ≥5% of hepatocytes without secondary causes such as alcohol intake, drugs,

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or liver diseases [111, 112]. Patients with type 2 diabetes, dyslipidaemia, and obesity are at increased risk of developing NAFLD [113]. Recent studies have shown that consumption of carotenoids such as lycopene can remarkably reduce the chances of developing liver diseases such as NAFLD [90]. In their study, Li et al. [114], using beta-carotene-15,15′-oxygenase and beta-carotene-9′,10′-oxygenase double knockout mice, the oral administration of lycopene at 2.3 mg/g for 24 weeks resulted in significantly decreased severity of hepatic steatosis and triglyceride levels but significantly increased sirtuin 1 and fatty acid oxidation compared to control counterparts fed a high-fat diet. Furthermore, lycopene mediated a decrease in inflammation. In a tramadol-induced hepatotoxicity rat model, supplemental lycopene at 15 mg/kg body weight for 15 days mitigated the hepatotoxicity by increasing antioxidant activity, reducing fatty acid breakdown and necrosis, lipid peroxidation, inhibiting DNA fragmentation, and apoptosis [115]. Lycopene administered at 5, 10, and 20 mg/kg body weight for 6 weeks in a rat model of NAFLD was shown to mediate hepatoprotective effects, as seen with reduced activities of aspartate transaminase and alanine transaminase and concomitant reductions in malondialdehyde, free fatty acids, and LDL-cholesterol concentrations [116]. These findings were associated with elevated hepatic superoxide dismutase and glutathione concentrations, but with reduced cytochrome P450 2E1 and tumour necrosis factor-alpha expression and decreased hepatic fat [116]. The abovementioned experimental studies provide a clear insight that the administration of lycopene not only inhibits ROS but also improves the activity of antioxidant enzymes, thereby providing beneficial effects against NAFLD.

5.5. Renoprotection. Chronic kidney diseases (CKD) have become a global public health issue, affecting more than 200 million people worldwide [117]. Chronic kidney disease is a common term used to describe different disorders that permanently affect the structure and function of the kidneys for over a period of 3 months [118]. This can be diagnosed when the abnormalities in the kidney or glomerular filtration rate are lower than 60 ml/min/1.73 m^2 and albuminuria is characterised by an albumin to creatinine ratio above 30 mg/g [119]. Patients with CKD are more prone to develop end-stage renal disease, a condition that requires expensive management by either dialysis or kidney transplantation [76]. Patients suffering from CKD commonly display a high incidence of arrhythmias, venous thromboembolism, heart failure, and ischemic heart disease, which significantly increases mortality [120, 121]. The increase in the prevalence of cardiovascular disease (CVD) in CKD patients is associated with oxidative stress, chronic inflammation, and vascular endothelial dysfunction [122]. These three factors create an intricate cycle, resulting in pathological variations and playing a crucial role in the initiation and progression of CVD in CKD patients [123, 124]. Among these factors, oxidative stress is a key mediator in the intricate pathways linked to the progression of CKD [124]. As a result, the utilisation of antioxidant therapy is one of the significant approaches to avert and mitigate the advancement of CKD [56]. Lycopene is a potent antioxidant and an efficient free radical scavenger that has been investigated and shown to protect the kidney against chemically induced damage [125, 126]. In female Wistar rats fed a high-fat diet, the supplementation of 200 ml of lycopene extract twice a week for 8 weeks significantly reduced plasma creatinine, urea, serum angiotensin-converting enzymes, renal tissue malondialdehyde, and C-reactive protein levels but increased total protein and tissue antioxidant enzyme levels [127]. Tabrez et al. [128] observed that lycopene protected against the advancement of diabetic nephropathy and improved renal function by inhibiting the advanced glycation product and its receptors' (AGE-RAGE) pathway. Lycopene has shown to inhibit LDL-cholesterol peroxidation, which can damage the kidneys [56]. Furthermore, supplemental lycopene has shown to decrease MDA, RAGE, and TNF-α levels in the kidneys of male Wistar rats fed a high-fat diet for 6 weeks [129], and similarly, lycopene orally administered at 25 and 50 mg/kg body weight daily for 3 months protected the kidneys of male Wistar rats fed a high-fat diet by inhibiting the expression of nuclear factor kappa-B, interleukin 1 beta, tumour necrosis factor alpha, decreasing oxidative stress, increasing nuclear factor erythroid 2-related factor 2, and stimulating B-cell lymphoma 2, hence shielding the kidney tissue against damages [66].

5.6. Osteoprotection. Oxidative stress caused by reactive oxygen species influences the activity of both osteoclasts and osteoblasts [130]. This is thought to impact the pathogenesis of skeletal system disorders, including osteoporosis, the most common skeletal metabolic disease [131]. Osteoporosis often develops in older adults and is characterised by an alteration of the bone microarchitecture, typified by a decline in bone mineral density, which contributes to an elevated risk of fractures [132]. Such bone fractures notably occur at the distal forearm, vertebral column, and proximal femur [133]. Complications associated with osteoporosis, particularly hip fractures, result in a mortality rate that is 4 times higher in the global adult population [132]. Despite its preponderance in the elderly, osteoporosis has shown to impact individuals of various age groups, but postmenopausal women are at high risk [134, 135] due to a decrease in estrogen production which results in increased oxidative stress and osteoclast-induced bone resorption [136]. Studies have shown that children born to parents with a history of osteoporosis and fractures are more prone to the development of osteoporosis [137]. In addition to genetic predisposition, poor nutrition, excessive alcohol consumption, smocking, caffeine intake, and medication side effects, for example, glucocorticoids, can cause the development of osteoporosis [138–141]. Lycopene has shown to have an advantageous effect on the skeletal health [142]. It has shown to play a vital role in protecting postmenopausal women from experiencing bone loss by upregulating alkaline phosphatase, type 1A collagen, runt-related transcription factor 2, triggering the activation of the wingless-related integration site/beta-catenin and extracellular signal-regulated kinase 1/2 pathways, and downregulating receptor activator

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of nuclear factor kappa-B ligand [143]. In mice fed a high-fat diet, supplemental lycopene at 15 mg/kg body weight for 10 weeks increased serum levels of total antioxidant capacity (T-AOC), SOD, and reduced the levels of MDA and AGEs, RAGE, and NF-kB expressions in the tibias and femurs [144]. In male albino rats, orally administered lycopene at 30 mg/kg body weight once daily over an 8-week period mitigated glucocorticoid-induced osteoporosis [145], and in diabetic male rats, lycopene suppressed bone resorption, enhanced osteopreotegerin and RANKL expression ratios by preventing oxidative damage and reducing inflammation [146]. These research findings demonstrate that lycopene has osteoprotective properties.

5.7. Anti-Inflammatory Effects. Inflammation is an immune response mechanism that is triggered when exposed to various harmful stimuli, such as damaged cells, microorganisms, poisonous, and allergenic substances [147]. It serves as a crucial stage in the process of tissue regeneration, repair, and remodelling, as well as the restoration of tissue haemostasis in impaired areas [148]. Inflammatory mediators include the cytokines interleukin (IL)-1, IL-5, IL-6, IL-12, IL-1β, TNF-α, and interferon γ [149], and chemokines such as IL-8, monocyte chemoattractant protein 1, cyclooxygenase, vascular cell adhesion molecule 1, matrix metalloproteinase, free radicals, growth factors, and prostaglandins serve as regulatory mediators in the process of inflammation [150]. On stimulation, these mediators activate endothelial cells, causing increased vascular permeability and the deployment of neutrophils, eosinophils, monocytes, and mask cells to the injury site, which helps eliminate the harmful agents and facilitate the healing process [151]. However, inflammation is known to contribute to the development and progression of various diseases, including but not limited to CKD, cancer, diabetes mellitus, cardiovascular disease, NAFLD, obesity, asthma, rheumatoid arthritis, osteoporosis, autoimmune, and neurodegenerative disorders [152–154]. The consumption of natural antioxidants for maintaining human health has become popular, especially in developed nations [155]. In a study using female Wistar rats, lycopene was shown to alleviate palmitic acid-induced neuroinflammation by reducing oxidative stress and inhibiting the toll-like receptor 4 (TLR4) and nuclear factor kappa-B p65 (NF-kB p65) signalling pathways [156]. Lycopene supplementation mitigated metalaxyl-induced liver damage in male albino rats by restoring antioxidant status, improving liver function, and alleviating liver injury-associated complications [157]. In lycopene-treated endothelial cells, lycopene inhibited the activation of TNF-α but enhanced the expression of heme oxygenase-1 (HO-1) through the upregulation of nuclear factor erythroid 2-related factor 2 signalling pathways [158]. Another experimental study reported that in male albino rats, orally administered lycopene at 10 mg/kg body weight for 21 days effectively protected the colon epithelial mucosa against acetic acid-induced colitis and oxidative injury [159]. In C57BL/6 mice chronically exposed to cigarette smoke for 60 days, lycopene has shown to restore redox status and mitigate hepatic inflammation [160]. In addition, Li et al. [161] reported that lycopene mitigated the dysregulation of lipid metabolism and the inflammatory response induced by lipopolysaccharide in the rat testes. Thus, evidence is plentiful demonstrating the anti-inflammatory effects of lycopene both *in vitro* and *in vivo*.

5.8. Antidiabetic Effects. Diabetes mellitus (DM) causes hyperglycaemia and, if inadequately managed, can result in damage to the heart, eyes, and kidneys [162]. The global prevalence of diabetes is approximately 9.3%, which corresponds to about 463 million individuals. However, it is predicted to rise by 25% in 2030 and 51% in 2045 [163]. Diabetes mellitus is classified into three major types: type 1 (insulin-dependent), type 2 (noninsulin-dependent), and gestational diabetes mellitus [164]. Among these, type 2 diabetes mellitus predominates and accounts for about 90% in all cases worldwide [162].

Scientific evidence shows that lycopene can potentially be used to prevent and treat diabetes mellitus [24]. In streptozotocin-induced diabetes model, dietary fortification with lycopene mediated increased serum insulin concentrations, decreased urine and blood sugar concentrations, and reduced diabetes-induced pancreatic injury [165]. In diabetic Wistar rats, orally administered lycopene at 40 mg/kg body weight significantly decreased serum MDA, cortisol, and blood glucose concentration but increased SOD, CAT, and glutathione peroxidase (GSH-Px) activities at 10, 20, and 40 mg/kg body weight [166]. Furthermore, supplemental lycopene has shown to attenuate renal damage in diabetic rats [167]. In STZ-induced diabetic rats, at 4 mg/kg body weight, lycopene-ameliorated B-cell lymphoma-extra-large, and B-cell lymphoma 2 (Bcl-2) concentrations and reduce the expression of Bcl-2-associated X-protein (BAX) in the hippocampus [168]. Interestingly, orally administered lycopene has shown to increase SOD and GSH-Px activities and lower MDA concentrations in rat pancreatic tissue [169], but it mediated increased plasma insulin concentrations and reduced blood and liver lipid content, fasting blood glucose and glycosylated haemoglobin concentration, and homeostatic model assessment for insulin resistance in diabetic rats [169].

5.9. Anticancer Effects. Cancer is a major global health challenge and is the second primary reason for mortality in the United States [170]. The ingestion of tomatoes and tomato-based products has been associated with a reduced occurrence of different types of cancer [171]. *In vivo* and *in vitro* research has demonstrated that lycopene hinders the growth and multiplication of prostate cancer cells, inhibits the cell cycle, and induces apoptosis [172]. Dietary supplementation with lycopene mitigated the growth of breast cancer cells by suppressing the activity of the insulin-like growth factor 1 receptor (IGF-1R) signalling pathway [151]. While research shows that the consumption of a lycopene-rich diet could be beneficial in reducing the risk of pancreatic cancer [131]. In a rat model, the consumption of lycopene has shown to reduce the progression and proliferation of

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ovarian cancer [173], and in human studies, cisplatin-based chemotherapy in combination with lycopene supplementation enhanced cervical cancer treatment [174]. Furthermore, in animal models of hepatocellular carcinoma, administered lycopene suppressed the onset and development of cancer [175]. In human colorectal adenocarcinoma cell line, treatment with lycopene has shown to exhibit genotoxicity, antiproliferative, and apoptotic effects [176], a demonstration of its anticancer effects.

5.10. Gastroprotection. The incidence of peptic ulcer disease (PUD) has substantially increased, affecting approximately 5 to 10 percent of the general population [177]. The corrosive effects of acid and pepsin on the gastroduodenal mucosa cause peptic ulceration through exposure of the mucosa's lining to gastric acid and digestive enzyme actions [178]. Peptic ulcer disease is primarily caused by the extensive use of nonsteroidal anti-inflammatory drugs (NSAIDS) and *Helicobacter pylori* infection [179]. Other contributing factors include surgery, severe illness, burns, Zollinger–Ellison syndrome, excessive alcohol intake, smoking, and psychological and physical stress [180–182]. The excessive production of ROS is the major factor in stress-induced ulcers [183]. Thus, the utilisation of strong antioxidants may be beneficial in the management of ulcers [184]. In male Albino rats, lycopene administered at 200 mg/kg body weight for 10 days has shown to protect against ethanol-induced mucosal injury [185]. In their study, Chen et al. [186] found that supplemental lycopene at 10, 50, 100, and 150 mg/kg body weight reduced gastric juice secretion in adult male Kunming mice when compared to the gastric injury control group. However, at high doses (150 mg/kg body weight), lycopene exacerbated absolute ethanol-induced acute gastric mucosal injury. In addition to mediating for protection against alcohol-induced gastrointestinal tract mucosal injury, lycopene has shown to suppress gastric acid secretion and combat infection by *Helicobacter pylori* [130].

5.11. Neuroprotection. Neurodegenerative diseases (NDs) are characterised by gradual loss of neurons and are associated with the formation of protein aggregates [187]. These diseases are considered a major medical challenge as it affects millions of patients globally [188]. Alzheimer's, Parkinson's, Huntington's, prion and motor-neural diseases, amyotrophic lateral sclerosis, spinocerebellar ataxia, and spinal muscular atrophy are common NDs [187, 189, 190]. Despite age being the leading factor in the onset of all neurodegenerative disorders, recent discoveries indicate that the combination of a person's genetic makeup and environmental influences can contribute to an elevated risk of developing NDs [191]. Regardless of the various factors causing these NDs, a key feature common to all is the onset and development of neuronal cell death [192]. The progression of NDs is characterised by increased ROS production, which causes oxidative stress [193]. Administered lycopene has shown to attenuate memory loss due to age, cognitive impairments, neuronal damage, and synaptic dysfunctions in the brain [194]. In addition, lycopene was observed to mitigate age-related oxidative stress by suppressing lipid peroxidation and enhancing GSH, SOD, and CAT activities [194]. Dietary fortification with lycopene was demonstrated to decrease age-related neuroinflammation by attenuating microgliosis and combating inflammation [194]. Furthermore, lycopene mediated the reduction in the accumulation of amyloid beta 1–42 in the brains of aged CD-1 mice [194] and when used as a supplement, it upregulated the mitogen-activated protein kinase (MARK)/extracellular signal-regulated kinase (ERK) signalling pathway, inhibited oxidative stress and neuronal apoptosis, and protected against bisphenol-induced neurotoxicity in the hippocampi of adult male rats [195]. It has also shown to decrease palmitic acid-induced brain oxidative stress and neuroinflammation and to inhibit the toll-like receptor 4 (TLR4)/nuclear factor kappa-light chain enhancer of activated B cells p65 (NF-kB-p65) pathway in female rats [156]. In mice with Alzheimer's disease induced by β amyloid, lycopene reduced oxidative stress, decreased neuronal loss, improved synaptic plasticity, and inhibited neuroinflammation [196].

5.12. Cardioprotection. Globally, cardiovascular diseases (CVDs) stand at the forefront as the leading cause of human mortality [16]. Studies have shown that in 2019, CVDs caused 17.8 million fatalities, and this trend is projected to increase by 2030 to 23 million [197]. Several epidemiological studies have confirmed the significance of lycopene in preventing CVDs [198]. For instance, lycopene supplementation has shown to reduce C-reactive protein levels, interleukin-6, pulse wave velocity, blood pressure, and intercellular adhesion molecule 1 and enhance vascular health through flow-mediated dilation of the endothelium [199]. Lycopene supplementation at a dosage of 5 mg/kg body weight for 21 days has shown to confer protection against atrazine-induced cardiotoxicity in mice [200]. In Brown–Norway/Lewis rat model, lycopene treatment was demonstrated to have the potential to mitigate vascular arteriosclerosis in allograft transplantation by inhibiting Rho-associated kinases and by regulating the expression of nitric oxide/cyclic guanosine monophosphate signalling pathways [201], which indicates that lycopene has the potential to alleviate vascular arteriosclerosis. In another study, lycopene administered for 4 weeks at 10 mg/kg body weight reduced inflammation and apoptosis during postmyocardial infarction remodelling by suppressing the NF-KB signalling pathway in mice [202]. In addition, supplemental lycopene improves endothelial function in individuals suffering from CVDs [203].

5.13. Lung Protection. In male C57BL/6 mice, dietary lycopene supplementation at 25 or 50 mg/kg body weight mitigated cigarette smoke-induced pulmonary emphysema [204]. The literature shows that lycopene or matrine treatment alone offered minimal protection against lipopolysaccharide-induced acute lung injury in mice, but when coadministered, significant mitigatory effects were observed [205]. These results indicate that lycopene and matrine in combination may function as an alternative to

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glucocorticoid therapy in treating acute lung injury [205]. In a study conducted by Mustra Rakic et al. [206], supplemental lycopene at 90 mg/kg body weight for 22 weeks effectively suppressed tobacco carcinogen/cigarette smoke (NNK/CS)-induced emphysema, chronic bronchitis, and preneoplastic lesions. Furthermore, dietary lycopene significantly decreased NNK/CS-induced buildup of total cholesterol and upregulated mRNA expression of peroxisome proliferator-activated receptor alpha (PPARα), ATP-binding cassette (ABC) transporters ABCA1 and ABCG1, and liver X receptor alpha (LXRα) in the lungs of the ferret model. These findings suggest that lycopene could act as a preventative agent against the adverse effects of tobacco smoke on lung health and lipid metabolism.

5.14. Sperm Quality Enhancement and Fertility Promotion. Infertility is a prevalent health problem that affects roughly 48 million couples and 186 million individuals globally [207]. ROS-induced oxidative stress is a primary contributor to various reproductive complications [208]. In varicocele-induced rats, supplemental lycopene has shown to protect sperm against DNA damage by mediating upregulation of antioxidant responses that quenched ROS, which manifested with improved sperm viability, Johnson's score, membrane integrity, and the expression of B-cell lymphoma 2-associated X-protein (BAX) [209]. Similarly, in men with oligozoospermia, supplemental lycopene for 12 weeks at 25 mg/kg body weight attenuated oxidative stress and improved sperm quality [52]. In their study, Yamamoto et al. [210] observed that the consumption of tomato juice with 30 mg of lycopene for a duration of 12 weeks increased plasma lycopene concentration and sperm motility and decreased the white blood cell count in the seminal plasma of the tomato juice group compared to the control group of infertile men. Dietary supplementation with lycopene at 20 mg per day for 3 months prior to the scheduled *in vitro* fertilization (IVF) treatment increased the arachidonic acid to docosahexaenoic acid ratio in the seminal fluid and resulted in 7 natural pregnancies in addition to 15 pregnancies following the IVF procedure [51]. In methotrexate-induced ovarian damage, pretreatment with lycopene at 5 mg/kg body weight for 5 days prevented infertility and has shown to mediate increased GSH activity as well as decreased MDA and myeloperoxidase concentrations [211]. These findings suggest that lycopene alleviates imbalances in polyunsaturated fatty acids and can serve as a preventive agent against infertility.

5.15. Protection of Skin Health. The skin, constituting approximately 15% of the total body weight [20], plays a vital role in preventing excessive water loss from the body and maintaining the body temperature within an optimal range [212]. It provides protection against toxic substances, free radicals, physical damage, and ultraviolet radiation [213]. The latter causes the development of skin conditions and diseases through sunburn, photoaging, and excessive ROS production within the skin, which damages DNA and causes skin cancer [213–215]. Lycopene is extensively used as an ingredient in cosmetic products due to its demonstrated ability to protect the skin from aging and photodamage [215]. Anbualakan et al. [216] showed that lycopene can prevent and/or treat sunburn and photoaging and that it could potentially be effective against UV-induced skin cancers. As a dietary supplement, lycopene has been demonstrated to improve skin appearance and pigmentation and mitigate erythema [217].

5.16. Protective Effect on Vision. Age-related ophthalmic conditions, inclusive of macular degeneration, glaucoma, cataracts, and diabetic retinopathy, are key contributors to gradual and permanent vision loss [218]. In diabetic patients, serum lycopene concentration has been observed to be lower than normal [114]. Importantly, due to its consistent lower levels in diabetics, it has been suggested that serum lycopene concentration might serve as a diagnostic tool for diabetic retinopathy [114]. Using ARPE-19 cells derived from human retinal pigment epithelium, Gong et al. [219] demonstrated that lycopene suppressed growth of human RPE cells against oxidative stress-induced cell loss findings which suggests that it (lycopene) may protect against RPE proliferative disease and old-age related macular degeneration. Oxidative stress and inflammation have been shown to be associated with the pathogenesis of eye-related conditions [220]. As a dietary supplement, lycopene has been proven to mitigate the risk of developing eye diseases associated with old age [221]. This could be due to its demonstrated ability to prevent cataract formation both *in vitro* and *in vivo* [131].

6. Lycopene: Protective Effects against Toxins

Toxins are natural and harmful chemical substances that adversely impact health [222]. They cause specific organ toxicity, for example, skin, eye, kidney, liver, blood, cardiovascular, respiratory, reproductive, endocrine, immune, and nervous system damage [222, 223]. Through their actions, toxins disrupt homeostasis, alter gene expression, and cancer-related metabolic signalling pathways [224]. Research has demonstrated that lycopene as a dietary supplement effectively mitigates the deleterious effects of myco-, bacterial, and chemical toxins [225] [125, 226, 227], fungicides [228], pesticides [229], herbicides [230], and fluoride [231]. It is hypothesised that lycopene mediates protection against toxins through its potent antioxidant, chelating, and antiapoptotic properties [224].

7. Lycopene: Safety and Potential Toxicity

There is no specified daily prescription for dietary lycopene intake, but epidemiological studies have recommended an intake of 2 to 20 mg daily of lycopene [93]. It has shown that consumption of up to 100 mg of lycopene daily does not elicit adverse outcomes [5]. In a toxicological study conducted on rats, feeding a diet fortified with lycopene at 1% (w/w) did not elicit any side effects [232]. Similarly, using lycopene at 200 mg/kg body weight per day as a dietary supplement has also been shown not to negatively impact animals [233]. Generally, it is asserted that lycopene can be

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used as a safe dietary supplement during pregnancy and lactation [234]. Although in pregnant women, high dietary intake of lycopene has shown to mitigate the risk of developing preeclampsia [235]. Imran et al. [7] reported that excessive chronic consumption of tomato juice, a rich source of lycopene, caused lycopenemia. Findings from both animal and human studies suggest that although lycopene could generally be used as a safe dietary supplement, some caution must be exercised against excessive intake.

8. Conclusion

The extensive studies carried out on lycopene highlight its exceptional potential to promote overall health and well-being. Its varied spectrum of benefits places it as a potent natural compound which can contribute to the promotion of health either as a prophylactic or ore therapeutic agent against metabolic diseases. In order to fully exploit its potential and increase its utility in health delivery, it is crucial to undertake additional research to comprehensively elucidate the health beneficial mechanisms underlying lycopene's medicinal properties. Furthermore, in order to enjoy optimal utility from the use of lycopene, there is a need to evaluate and recommend effective dosages for efficacy and prevention of possible side effects of abnormally high doses.

Data Availability

The data that support this systematic review come from studies and datasets that were previously reported and cited in this article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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