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Research article

Cucumeropsis mannii seed oil shields against cyclophosphamide-induced hepatorenal toxicity by modulating redox imbalance and iNOS/IL-β1 signalling in rats

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ABSTRACT

This study aimed to determine whether C. mannii seed oil (CMSO) could shield rats from cyclophosphamide-induced hepatorenal toxicity. The focus was on its antioxidant and anti-inflammatory properties. We randomly assigned thirty-six (36) male Wistar rats into six groups of six rats each. Groups A and B served as normal and negative controls, respectively. Group C, the standard control, was administered 300 mg/kg body weight (bw) of Omega 3 oil for 27 days, followed by 100 mg/kg bw of cyclophosphamide on day 28. Group D, E, and F received 5, 2.5, and 1.5 ml/kg b.w. of CMSO for 27 days, then 100 ml/kg b.w. of cyclophosphamide on day 28. We measured the body weights of the experimental rats every week. Rats of all groups were sacrificed on day 30 and collected blood for biochemical analysis using standard methods. The phytochemical constituents were determined by the spectrophotometric method. The phytochemical study of CMSO indicated the relative composition of constituents(mg/100g) as phenols (30 %), tannins (20 %), flavonoids (18 %), terpenoids (15 %), glycosides (10 %), alkaloids (5 %), and HCN (2 %) in Cucumeropsis mannii seed oil. When cyclophosphamide was given to Wistar albino rats, it greatly decreased the activities of catalase (CAT) and superoxide dismutase (SOD), but it increased the activities of iNOS and the levels of MDA and IL-1β. Cyclophosphamide increased ALT (52.3 U/L), AST (48.7 U/L), ALP (46.5 U/L), total bilirubin (1.4 mg/dL), conjugated bilirubin (0.8 mg/dL), creatinine (0.8 mg/dL), urea (45.6 mg/dL), and BUN (15.7 mg/dL),

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with a reduction in the albumin (4.8 g/dL) level. Cyclophosphamide administration also caused hepatocellular necrosis, inflammatory leukocyte infiltration, tubular necrosis, and proteinaceous deposits in Bowman's space in the kidney. However, combining CMSO and Omega 3 oils significantly restored the altered histology architecture, antioxidant, inflammatory markers, and liver and kidney function parameters to levels comparable to the normal group, thereby reducing the harmful effects of cyclophosphamide. This effect did not depend on the dose. These results suggest that CMSO, being an antioxidant and an anti-inflammatory, could potentially aid in preventing and treating hepatorenal toxicity resulting from cyclophosphamide.

1. Introduction

Chemotherapy uses cyclophosphamide, an alkylating agent, to treat various cancer types. It is known to have adverse effects on the liver and kidneys, leading to hepatorenal damage [1]. This damage is due to oxidative stress, which leads to inflammation of the liver and kidneys [2]. The metabolization of cyclophosphamide produces reactive oxygen species (ROS), which in turn cause oxidative stress [3]. These ROS cause damage to cells, proteins, and DNA, leading to inflammation and tissue damage. This can result in hepatorenal damage, including liver fibrosis, kidney tubular damage, and kidney failure [4,5] (see Fig. 23).

Plant-natural antioxidants are known to be effective in mitigating oxidative damage [6,7]. Some researchers suggest including plant-based natural antioxidants in the patient's diet or administering them as dietary supplements to reduce oxidative stress [8]. Natural plant antioxidants such as ascorbic acid, tocopherol, and selenium are known to improve the body's antioxidant capacity and reduce the oxidative stress associated with the use of cyclophosphamide [9]. Additionally, studies have found that plant-derived flavonoids, polyphenols, and carotenoids provide protective effects against cyclophosphamide-induced hepatorenal damage [10]. These compounds can be incorporated into the diet or taken as dietary supplements.

CMSO is a vegetable oil obtained from the seeds of C. mannii, a tropical African plant also known as African white melon [11,12]. It is used as a source of essential fatty acids for both culinary and cosmetic purposes [13,14]. CMSO is rich in oleic acid, linoleic acid, and linolenic acid, as well as other fatty acids [15,16]. According to some studies, the oil possesses anti-inflammatory properties that aid in treating conditions like eczema and psoriasis [17]. This study investigates the protective effects of C. mannii seed oil against hepatorenal toxicity induced by cyclophosphamide, looking at its ability to modulate redox balance and influence iNOS/IL-β1 signaling pathways in a rat model. This objective encompasses evaluating both the antioxidant and anti-inflammatory roles of *C. mannii* seed oil in the context of hepatorenal damage.

2. Materials and methods

2.1. Collection of plant materials and extraction of seed oil

Prof. S. C. Onyekwelu, a taxonomist in the Department of Applied Biology at Ebonyi State University, Nigeria, authenticated the fresh C. mannii seeds we collected from Isi-eke in the Ebonyi Local Government Area of Ebonyi State, Nigeria, in April 2023. The herbarium officer, Dr. Nwankwo Ephraim O, preserved the plant in the Applied Biology Department herbarium at Ebonyi State University in Nigeria and assigned voucher number EBSU-H-396. We extracted the oil from the seeds of C. mannii using Oti and Eze-Ilochi's method, modified by Aja et al. [18].

2.1.1. Phytochemical analysis of CMSO

We used the phytochemical analytical guide by Balamurugan et al. [63] to determine the alkaloids, flavonoids, phenols, terpenoids, steroids, tannins, saponins, glycosides, and HCN.

2.1.2. Experimental animals

The Research, Innovation, and Institutional Ethics Committee of Ebonyi State University, Nigeria (EBSU/BCH/ET/23/012) supervised and approved this work. According to Kalariya et al. [19] and Tusubra et al. [20], we followed the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, amended in 1996) for all animal study protocols. The study used 36 4-week-old male Wistar albino rats from Ebonyi State University's Animal House. We kept them in well-ventilated animal houses with 16 x 9 (144-inch) stainless steel cages. Before the testing, we acclimated them for seven days under a 12-h light/dark cycle and room temperature. All animals were provided with standard rodent feed and water. We sacrificed all animals with halothane.

2.2. Experimental animals and grouping

We randomly assigned six rats to each group (A–F) as shown below.

Group A (normal control)	Received 5 ml/kg b.w of normal saline.

(*continued on next page*)

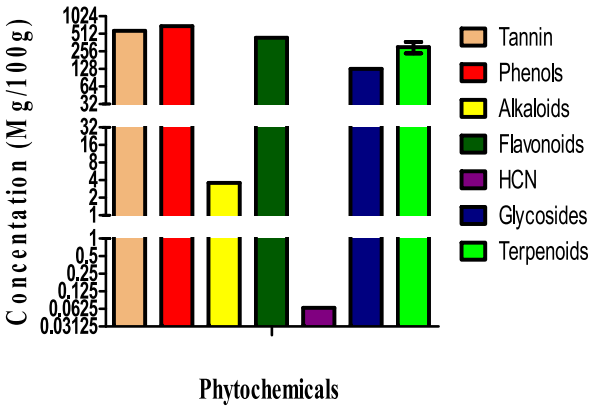

Fig. 1. Phytochemical composition of Cucumeropsis *mannii* seed oil (CMSO). Data are shown as mean ± S.D (n = 3).

(*continued*)

Group B (Negative Control)	Received 5 ml/kg b.w. of normal saline for 27 days and 100 mg/kg cyclophosphamide[42] intraperitoneally on day 28.
Group C (Standard Control)	Got 100 mg/kg b.w. of cyclophosphamide intraperitoneally on day 28 after receiving 300 mg/kg b.w. Omega 3 oil (from fish) orally for 27 days [42].
Group D (Test Group 1)	Received 5 ml/kg b.w. of *C. mannii* seed oil [11,12] for 27 days and 100 mg/kg cyclophosphamide on day 28
Group E (Test Group 2)	Received 2.5 ml/kg b.w. of *C. mannii* seed oil (11,12) for 27 days and 100 mg/kg b.w. of cyclophosphamide intraperitoneally on day 28.
Group F (Test Group 3)	Got 1.5 ml/kg of *C. mannii* seed oil [11,12] via oral intubation for 27 days and cyclophosphamide intraperitoneally on day 28.

Rats' weights were measured weekly. The rats were sacrificed on the 30th day.

2.3. Tissue sample collection

On the 30th day, we sacrificed the rat under mild anesthesia and collected a sample for biochemical analysis. Within 20 min after the sacrifice, we collected the livers and kidneys, placed them in specimen bottles, stored them on ice, and transferred them to the laboratory for sample analyses.

2.4. Determination of biochemical parameters

Malondialdehyde (MDA) level was determined as lipid peroxidation (LPO) markers and the activities of SOD and CAT in the kidney and liver homogenates using the methods of Ohkawa et al. [21], Aebi [22], and Marklund and Marklund [23], respectively. Interleukin-1 and inducible nitric oxide synthase (iNOS) activities in the liver and kidney homogenates were determined by enzyme-linked immune sorbent assay (ELISA) kits. We used the methods of Ngashangva et al. [64] to measure serum albumin, total bilirubin, and total protein. We assayed the activities of Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using the colorimetric methods of Reitman and Frankel [65], while Yakubu et al. [66] used the colorimetric method. Kidney function parameters were determined according to Jovanović et al. [67].

2.5. Histological examination

We conducted histopathological studies on the rats' liver and kidneys using the method of Bhat et al. [68].

2.6. Statistical analysis

All data were analysed using GraphPad Prism 5, version 8. Mean and standard deviation were used. The parameters' means were compared using a post-hoc one-way ANOVA at $p < 0.05$. Groups' mean values were compared to group B (cyclophosphamide only) using Dunnett's multiple comparisons test, revealing significant differences at $p < 0.05$.

3. Results

The phytochemical study of CMSO indicated the relative composition of constituents(mg/100g) as phenols (30 %), tannins (20 %), flavonoids (18 %), terpenoids (15 %), glycosides (10 %), alkaloids (5 %), and HCN (2 %) in Cucumeropsis mannii seed oil as shown in Fig. 1.

As shown in Figs. 2–7, giving cyclophosphamide to rats decreased the activity of SOD and CAT and raised MDA levels in the kidneys

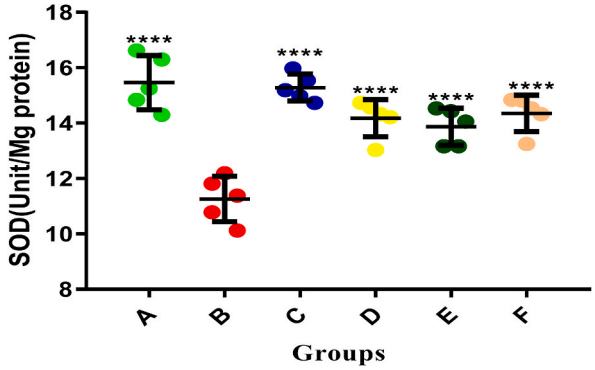

Fig. 2. Effects of *C. mannii* seed oil **on Liver SOD Activity against Cyclophosphamide-induced Hepato-toxicity in albino rats. ****p < 0.0001, ***p < 0.0006, **p < 0.003.** A (Normal saline), B (100 mg/kg cyclophosphamide + Normal saline), C (300 mg/kg b.w. Omega 3 oil +100 mg/kg cyclophosphamide), D (5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), E (2.5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), F (1. 5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide).

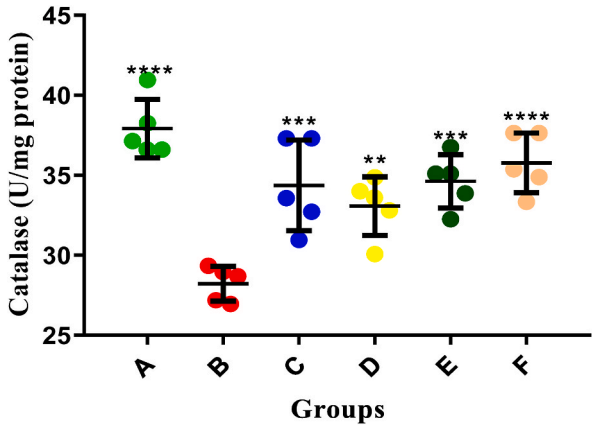

Fig. 3. Effects of *C. mannii* seed oil on Liver Catalase Activity against Cyclophosphamide-induced Hepato-toxicity in albino rats. ****p < 0.0001, ***p < 0.0006, **p < 0.003. A (Normal saline), B (100 mg/kg cyclophosphamide + Normal saline), C (300 mg/kg b.w. Omega 3 oil +100 mg/kg cyclophosphamide), D (5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), E (2.5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), F (1. 5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide).

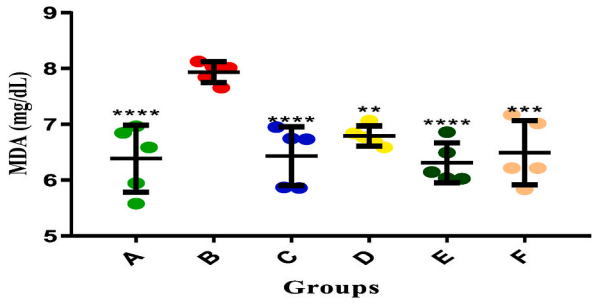

Fig. 4. Effects of *C. mannii* seed oil on Liver MDA Level against Cyclophosphamide-induced Hepato-toxicity in albino rats. ****p < 0.0001, ***p < 0.0006, **p < 0.003. A (Normal saline), B (100 mg/kg cyclophosphamide + Normal saline), C (300 mg/kg b.w. Omega 3 oil +100 mg/kg cyclophosphamide), D (5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), E (2.5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), F (1. 5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide).

and liver. Rats were administered CMSO or Omega-3 oil for 27 days before they were intraperitoneally injected with cyclophosphamide. Cyclophosphamide injection led to greatly decreased MDA levels and increased SOD and CAT activity in the liver and kidneys (Figs. 2–7).

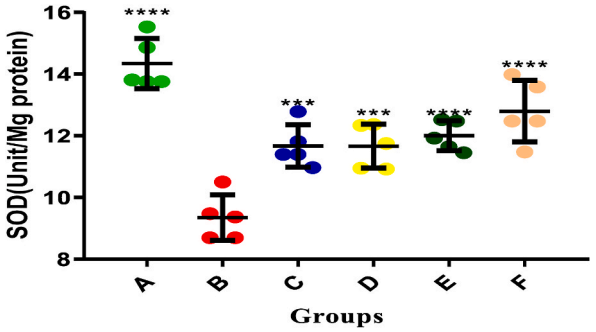

Fig. 5. Effect of *C. mannii* seed oil on Kidney SOD Activity against Cyclophosphamide-induced Nephrotoxicity in albino rats. ****p < 0.0001, ***p < 0.0006, **p < 0.003. A (Normal saline), B (100 mg/kg cyclophosphamide + Normal saline), C (300 mg/kg b.w. Omega 3 oil +100 mg/kg cyclophosphamide), D (5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), E (2.5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), F (1. 5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide).

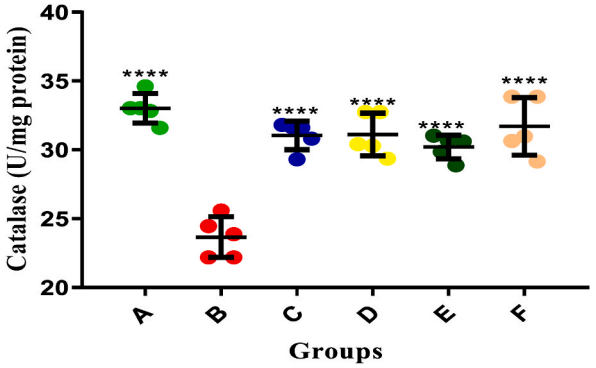

Fig. 6. Effect of *C. mannii* seed oil on Kidney Catalase Activity against Cyclophosphamide-induced Nephrotoxicity in albino rats. ****p < 0.0001, ***p < 0.0006, **p < 0.003. A (Normal saline), B (100 mg/kg cyclophosphamide + Normal saline), C (300 mg/kg b.w. Omega 3 oil +100 mg/kg cyclophosphamide), D (5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), E (2.5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), F (1. 5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide).

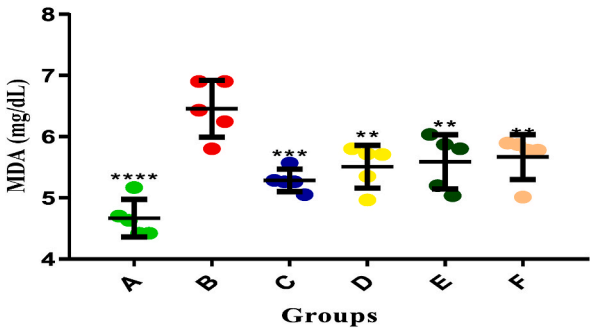

Fig. 7. Effect of *C. mannii* seed oil on Kidney MDA Level against Cyclophosphamide-induced Nephrotoxicity in albino rats. ****p < 0.0001, ***p < 0.0006, **p < 0.003. A (Normal saline), B (100 mg/kg cyclophosphamide + Normal saline), C (300 mg/kg b.w. Omega 3 oil +100 mg/kg cyclophosphamide), D (5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), E (2.5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), F (1. 5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide).

In this investigation, cyclophosphamide significantly increased IL-1β and iNOS activity in the kidneys and liver, as seen in Figs. 8–11. Rats were given CMSO or Omega-3 oil for 27 days before they were given cyclophosphamide showed a great decrease in IL-1β and iNOS activity in the liver and kidneys (Figs. 8–11).

The administration of cyclophosphamide in rats significantly elevated the activities of ALT (52.3 ± 2.4 U/L), AST (48.7 ± 3.1 U/L), and ALP (46.5 ± 3.2 U/L), and levels of total bilirubin (1.4 ± 0.1 mg/dL) and conjugated bilirubin (0.8 ± 0.1 mg/dL) with a reduction in albumin (4.8 ± 0.2 g/dL) level as shown in Figs. 12–17. In contrast, pretreatment with CMSO and omega-3 fatty acid for 27 days

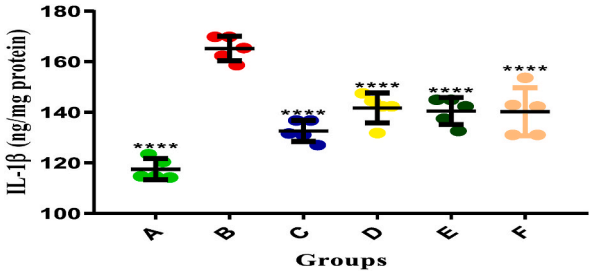

Fig. 8. Effect of *C. mannii* seed oil on Liver IL-1β Level against Cyclophosphamide-induced Hepato-toxicity in albino rats. ****p < 0.0001, ***p < 0.0006, **p < 0.003. A (Normal saline), B (100 mg/kg cyclophosphamide + Normal saline), C (300 mg/kg b.w. Omega 3 oil +100 mg/kg cyclophosphamide), D (5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), E (2.5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), F (1. 5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide).

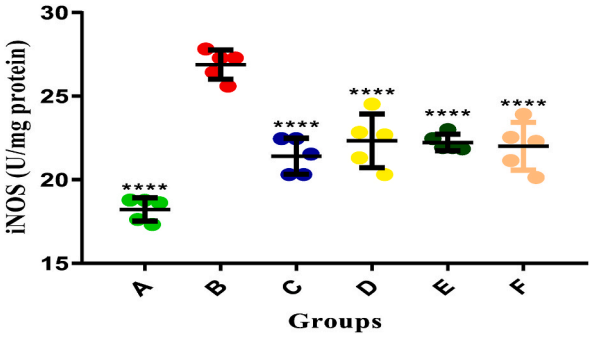

Fig. 9. Effect of *C. mannii* seed oil on Liver iNOS Level against Cyclophosphamide-induced Hepato-toxicity in albino rats. ****p < 0.0001, ***p < 0.0006, **p < 0.003. A (Normal saline), B (100 mg/kg cyclophosphamide + Normal saline), C (300 mg/kg b.w. Omega 3 oil +100 mg/kg cyclophosphamide), D (5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), E (2.5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), F (1. 5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide).

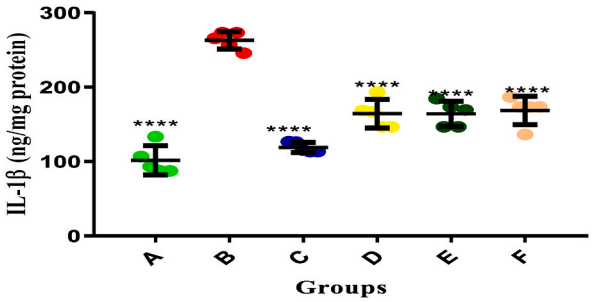

Fig. 10. Effect of *C. mannii* seed oil on Kidney IL-1β Level against Cyclophosphamide-induced Nephrotoxicity in albino rats. ****p < 0.0001, ***p < 0.0006, **p < 0.003. A (Normal saline), B (100 mg/kg cyclophosphamide + Normal saline), C (300 mg/kg b.w. Omega 3 oil +100 mg/kg cyclophosphamide), D (5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), E (2.5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), F (1. 5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide).

significantly reduced ALT (31.2 ± 1.9 U/L), AST (35.4 ± 2.8 U/L), and ALP (32.3 ± 2.1 U/L), levels of total bilirubin (0.9 ± 0.1 mg/dL), and conjugated bilirubin (0.4 ± 0.05 mg/dL) with an increase in albumin (3.9 ± 0.1 g/dL) level (Figs. 12–17). Cyclophosphamide also elevated creatinine (0.8 ± 0.1 mg/dL), urea (45.6 ± 2.5 mg/dL), and BUN (15.7 ± 1.8 mg/dL), whereas pretreatment significantly reduced creatinine (0.5 ± 0.1 mg/dL), urea (36.4 ± 1.9 mg/dL), and BUN (10.2 ± 1.3 mg/dL)(Figs. 18–20)

Cyclophosphamide alone reduced rats' body weight, as illustrated in Fig. 21. In rats, CMSO and omega-3 oil for 27 days before cyclophosphamide increased body weight (Fig. 21). (see Fig. 22).

The study presents a histological analysis of kidney and liver tissues from albino rats treated with cyclophosphamide (Cyclo), either with or without omega-3 oil, or with various doses of CMSO (5, 2.5, and 1.5 ml/kg b.w). In Group A (Control) both the liver and kidney tissues showed normal histoarchitecture. There was severe damage to the liver in Group B (Cyclo only), with multiple areas of hepatocellular necrosis and inflammatory leukocyte infiltration. The kidneys had widespread tubular necrosis and proteinaceous

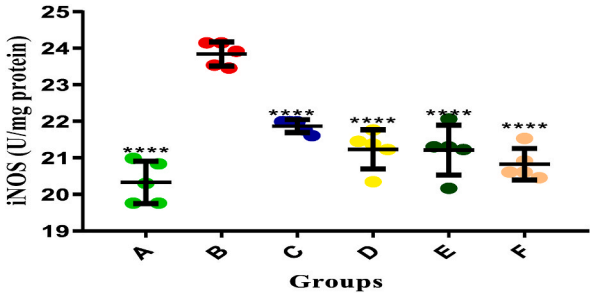

Fig. 11. Effect of *C. mannii* seed oil on Kidney iNOS Level against Cyclophosphamide-induced Nephrotoxicity in albino rats. ****p < 0.0001, ***p < 0.0006, **p < 0.003. A (Normal saline), B (100 mg/kg cyclophosphamide + Normal saline), C (300 mg/kg b.w. Omega 3 oil +100 mg/kg cyclophosphamide), D (5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), E (2.5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), F (1. 5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide).

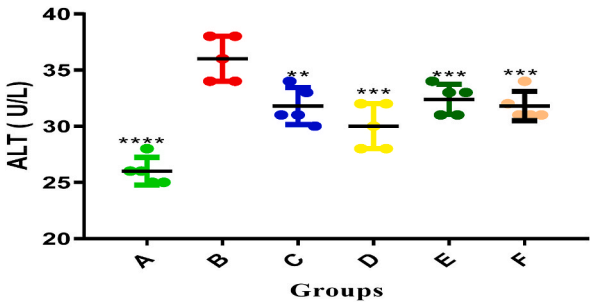

Fig. 12. Effect of CMSO on plasma ALT Activity against Cyclophosphamide-induced hepato-toxicity in albino rats. ****p < 0.0001, ***p < 0.0006, **p < 0.003.A (Normal saline), B (100 mg/kg cyclophosphamide + Normal saline), C (300 mg/kg b.w. Omega 3 oil +100 mg/kg cyclophosphamide), D (5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), E (2.5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), F (1. 5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide).

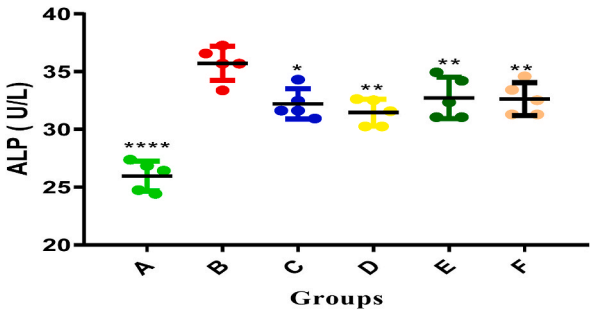

Fig. 13. Effect of CMSO on plasma ALP Activity against Cyclophosphamide-induced hepato-toxicity in albino rats. ****p < 0.0001, ***p < 0.0006, **p < 0.003,*p < 0.015. A (Normal saline), B (100 mg/kg cyclophosphamide + Normal saline), C (300 mg/kg b.w. Omega 3 oil +100 mg/kg cyclophosphamide), D (5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), E (2.5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), F (1. 5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide).

material in Bowman's space. Group C (Cyclo + Omega-3 oils) showed nearly normal liver and kidney histoarchitecture, with minor necrosis in the renal tubule lining. In Groups D, E, and F (Cyclo + CMSO), the liver and kidney tissues had normal histoarchitecture at all CMSO doses. In Group F, there were a few small cases of renal tubule necrosis. The findings suggest that Omega-3 oil and CMSO mitigate cyclophosphamide-induced organ damage, with higher CMSO doses offering better protection.

4. Discussion

The results of the phytochemical study of CMSO revealed that phenols (30 %) have the highest value while the least was HCN (2 %) (Fig. 1). Previous studies have reported that the plant bioactive has myriads of pharmacological functions, perhaps, as antioxidants [43,44]. Antioxidants inhibit the oxidation reaction of other molecules that can produce free radicals thereby modulating metabolic

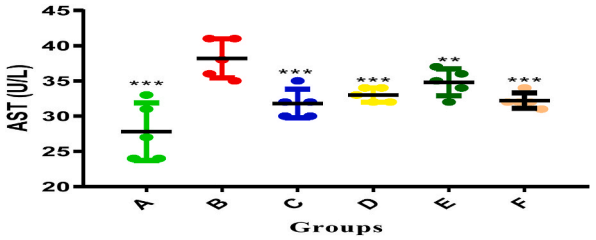

Fig. 14. Effect of CMSO on plasma AST Activity against Cyclophosphamide-induced hepato-toxicity in albino rats. ***p < 0.0006, **p < 0.003. A (Normal saline), B (100 mg/kg cyclophosphamide + Normal saline), C (300 mg/kg b.w. Omega 3 oil +100 mg/kg cyclophosphamide), D (5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), E (2.5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), F (1. 5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide).

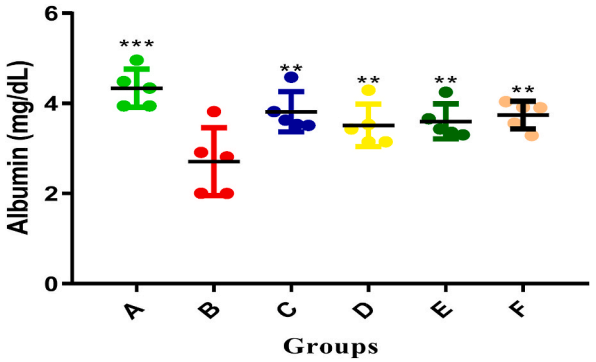

Fig. 15. Effect of CMSO on plasma Albumin Level against Cyclophosphamide-induced hepato-toxicity in albino rats. ***p < 0.0006, **p < 0.003. A (Normal saline), B (100 mg/kg cyclophosphamide + Normal saline), C (300 mg/kg b.w. Omega 3 oil +100 mg/kg cyclophosphamide), D (5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), E (2.5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), F (1. 5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide).

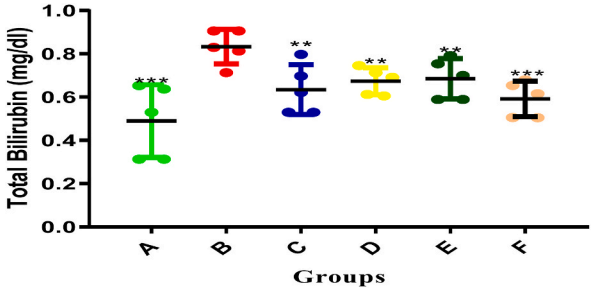

Fig. 16. Effect of CMSO on plasma Total bilirubin Level against Cyclophosphamide-induced hepato-toxicity in albino rats. ***p < 0.0006, **p < 0.003. A (Normal saline), B (100 mg/kg cyclophosphamide + Normal saline), C (300 mg/kg b.w. Omega 3 oil +100 mg/kg cyclophosphamide), D (5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), E (2.5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), F (1. 5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide).

dysregulations [45]. This work is also in agreement with the work of Aja et al.[46[46], which reported the presence of bioactive compounds namely flavonoids, alkaloids, saponins, and tannins respectively in the dry and wet samples of Talinum triangulare (Water Leaf) leaves. This work equally agrees with the findings of Akinwumi and Sonibare [47] reported that phytochemical screening of *Sphenocentrum jollyanum* leaf extract showed the presence of flavonoids, steroids, terpenoids, tannins, and alkaloids. Therefore, these nutraceuticals present in CMSO may have potential pharmacological effects that could modulate fertility hormones, kidney functions, and glucose levels.

Even though natural antioxidants have a wide variety of therapeutic benefits against systematic toxicities, their discovery has become the focus of biological science study. In the current study, giving rats cyclophosphamide significantly ($p < 0.05$) decreased their SOD and CAT activities and increased their MDA levels in their kidneys, and liver investigation corroborated this with findings of reduced SOD levels, which are an indicator of antioxidant defenses. This was also in line with the results of a study conducted by Shokrzadeh et al. [24], which showed that cyclophosphamide-induced oxidative stress was linked to hepatotoxicity by reducing SOD,

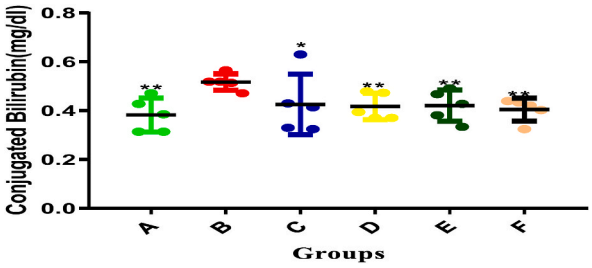

Fig. 17. Effect of CMSO on plasma Conjugated bilirubin Level against Cyclophosphamide-induced hepato-toxicity in albino rats. ***p < 0.0016, **p < 0.04. A (Normal saline), B (100 mg/kg cyclophosphamide + Normal saline), C (300 mg/kg b.w. Omega 3 oil +100 mg/kg cyclophosphamide), D (5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), E (2.5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), F (1. 5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide).

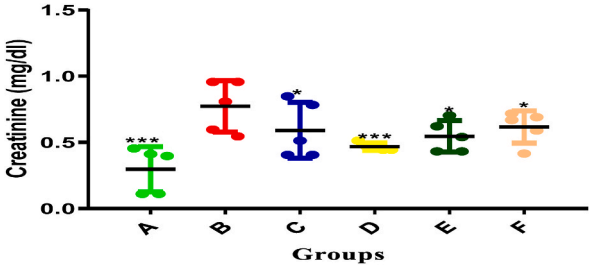

Fig. 18. Effect of CMSO on Creatinine Level against Cyclophosphamide-induced Nephro-toxicity in albino rats. ***p < 0.0005, **p < 0.003, *p < 0.015. A (Normal saline), B (100 mg/kg cyclophosphamide + Normal saline), C (300 mg/kg b.w. Omega 3 oil +100 mg/kg cyclophosphamide), D (5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), E (2.5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), F (1. 5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide).

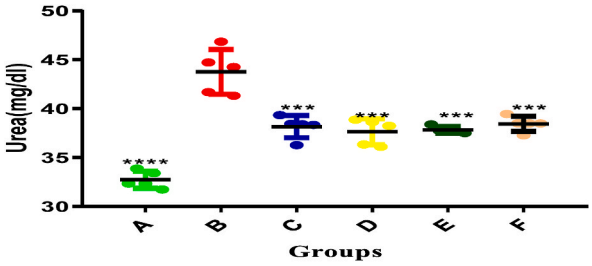

Fig. 19. Effect of CMSO on Urea Level against Cyclophosphamide-induced Nephro-toxicity in albino rats. ****p < 0.0001, ***p < 0.0006. A (Normal saline), B (100 mg/kg cyclophosphamide + Normal saline), C (300 mg/kg b.w. Omega 3 oil +100 mg/kg cyclophosphamide), D (5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), E (2.5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), F (1. 5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide).

CAT, and decreased glutathione levels. Similarly, in circumstances comparable to ours, previous research [25,26] demonstrated a correlation between reduced levels of antioxidant defenses following cyclophosphamide treatment and cardiotoxicity as well as lung damage.

Giving rats CMSO and Omega-3 oil for 27 days before cyclophosphamide dramatically ($p < 0.05$) reduced MDA levels and boosted SOD and CAT activity in the liver and kidneys. In this study, cyclophosphamide increased MDA levels by causing lipid peroxidation, a key cell component change. CMSO treatment effectively prevented membrane lipid peroxidation by restoring MDA concentrations ($p < 0.05$). CMSO may protect against cyclophosphamide-induced oxidative changes in the liver and kidneys because it contains antioxidants, free radical scavengers, and anti-lipoperoxidants, all of which protect the liver [27]. The capacity of the seed oil to scavenge superoxide radicals was demonstrated by the restoration of antioxidant enzyme activity. Their actions, such as a reduction in the generation of free radicals originating from cyclophosphamide or an increase in the antioxidant activity of the bioactive compounds in the seed oil, were responsible for the reversal effects. Following CMSO treatment in the current study, the malondialdehyde levels in the liver and kidney tissues approached the normal range. The seed oil's capacity to replenish antioxidant enzymes may account for its capacity to eradicate toxicity brought on by cyclophosphamide [28,29]. According to some studies, the action of CMSO is brought about by an increase in the liver and kidney's protein content and the activity of antioxidant enzymes like SOD and CAT [30].

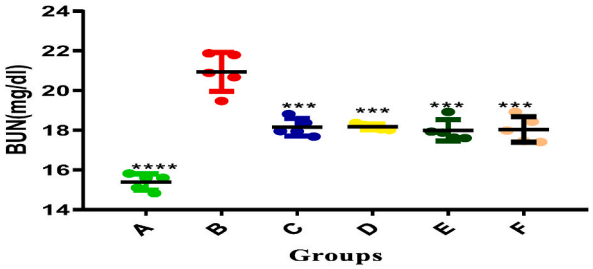

Fig. 20. Effect of CMSO on BUN Level against Cyclophosphamide-induced Nephrotoxicity in albino rats. ****$p < 0.0001$, ***$p < 0.0006$. A (Normal saline), B (100 mg/kg cyclophosphamide + Normal saline), C (300 mg/kg b.w. Omega 3 oil +100 mg/kg cyclophosphamide), D (5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), E (2.5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), F (1. 5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide).

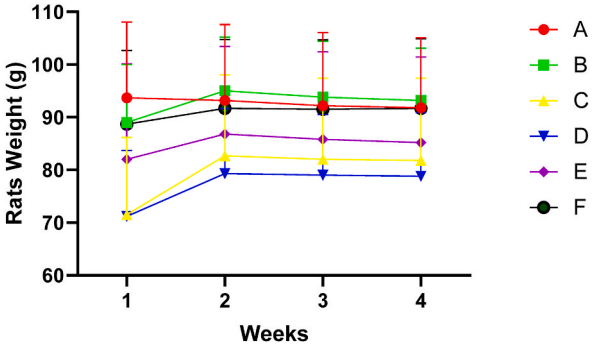

Fig. 21. Effect of *C. mannii* seed oil on Body weight of rats against Cyclophosphamide-induced Nephrotoxicity in albino rats. A (Normal saline), B (100 mg/kg cyclophosphamide + Normal saline), C (300 mg/kg b.w. Omega 3 oil +100 mg/kg cyclophosphamide), D (5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), E (2.5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), F (1. 5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide).

Oxidative stressors set off inflammatory reactions [31]. In this study, cyclophosphamide treatment in rats led to a noteworthy ($p < 0.05$) increase in IL-1β and iNOS activity in the liver and kidneys. On the other hand, rats were administered cyclophosphamide after receiving CMSO and Omega-3 oil for 27 days, and this led to a significant ($p < 0.05$) drop in iNOS activity and IL-1β levels in the kidneys and liver. These findings are in line with the many studies in the field that discuss how oxidative stress, apoptosis, and the pro-inflammatory response interact [32–34].

This finding is similar to what some researchers found. They said that when mice were given an adjuvant to cause arthritis, the levels of IL-1β, IL-6, and TNF-α went up significantly. However, treating the mice with lemon fruit peel and lemon leaf extract had a big opposite effect [35]. According to another study, rheumatoid arthritis is brought on by several inflammatory chemicals, such as those generated by fibroblasts and macrophages. These molecules consist of prostaglandins, reactive oxygen species, IL-1β, IL-6, and TNF-α [36]. Pro-inflammatory cytokines are produced in arthritic joints by both the chondrocytes that remain in the joints and the inflammatory synovium, according to another study [35]. The ameliorative impact of lemon extracts suggests their therapeutic value in the treatment of pain and inflammation [35]. We speculate that CMSO may protect cyclophosphamide-induced liver and renal changes through the antioxidative and acetogenic properties of the seed oil. Likewise, many medicinal herbs have been shown to protect against cyclophosphamide-induced toxicity [37,38].

The combined effects of oxidative stress and inflammation reflect body weight. In our study, we observed an insignificant ($p > 0.05$) loss of body weight in the group of rats that received cyclophosphamide alone without treatment (Fig. 11). However, oral administration of CMSO or Omega-3 oil prevented the weight loss, albeit at a significantly higher ($p > 0.05$) rate than in the cyclophosphamide group (Fig. 11). A few studies have found that anticancer drugs such as methotrexate (MTX) intoxication exert body weight decrease in animal models [39]. Researchers have linked the observations to stress and MTX-induced changes in physiological responses, which may promote appetite loss. It is similar to what another study found that Moringa oleifera seed oil can do to change redox imbalance and the iNOS/NF-kB/caspase-3 signalling pathway to protect rats' kidneys from the harmful effects of the cancer drug 5-fluorouracil [34]. The results we got from measuring body weight also back up other studies on how adding virgin coconut oil to a diet can help protect against the harmful effects of chemotherapy and methotrexate on the kidneys of rats [40,41].

The study's results also revealed that the test group receiving cyclophosphamide injections had higher levels of total bilirubin and conjugated bilirubin than the control group, as well as increased activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). There was also a decreased level of albumin. The current study validates a previous study that found a significant increase in serum activities of liver markers after CPA-induced hepatotoxicity [48]. El-Karim and

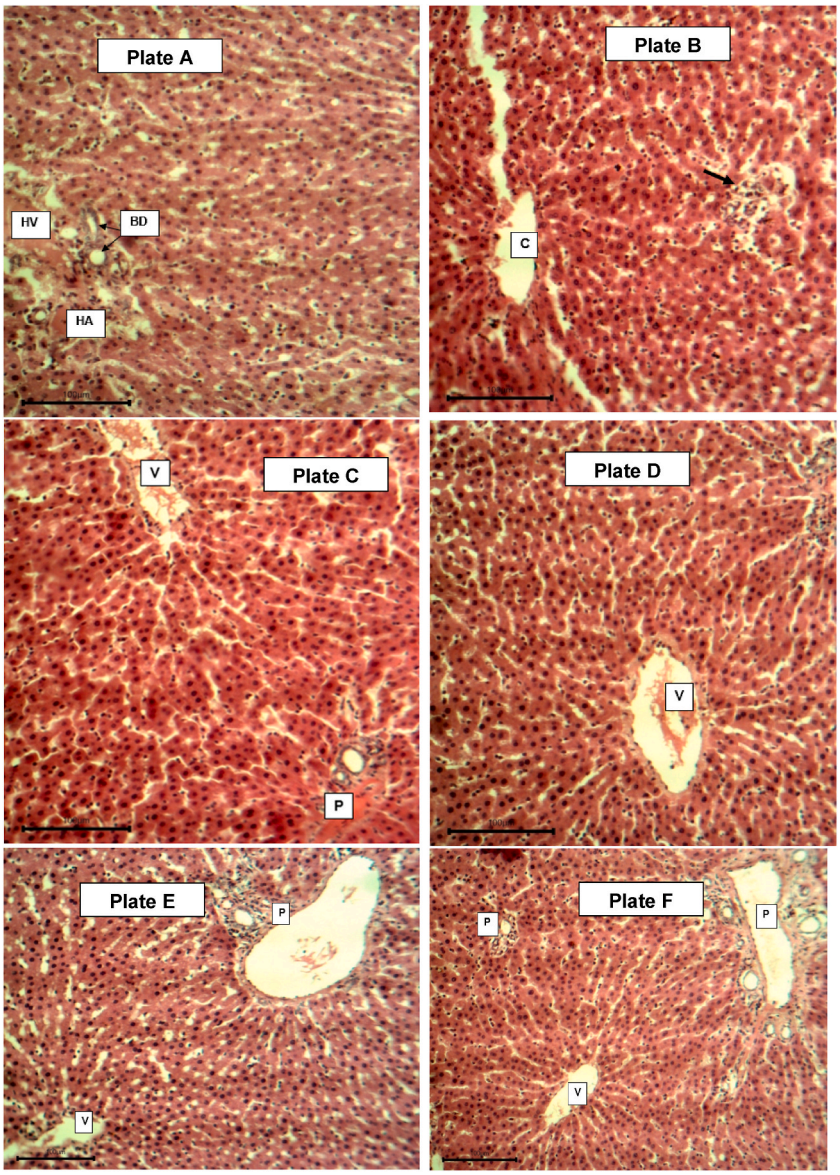

Fig. 22. Photomicrograph of the Liver (A–F) (H&Ex160). Hepatic vein- HV; hepatic artery—HA; bile duct- BD). Central vein (V); Portal area (P). A (Normal saline), B (100 mg/kg cyclophosphamide + Normal saline), C (300 mg/kg b.w. Omega 3 oil +100 mg/kg cyclophosphamide), D (5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), E (2.5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), F (1. 5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide).

El-Amrawi [49] also said that a low dose of CPA (200 mg/kg body weight) given intravenously can damage the liver by raising the activity of liver enzymes in the blood and changing other markers of liver function in a bad way. The damaged structural integrity of the liver is responsible for the rise in serum activities of these markers. Often, this occurs due to their cytoplasmic location, which releases them into circulation following cellular damage. The body contains enzymes, which are proteins that speed up chemical reactions. The liver contains enzymes that carry out these functions. The most common liver enzymes are aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), which serve as useful biomarkers of liver injury in patients with intact liver function [51].

We administered CMSO and omega-3 fatty acids to the rats for 27 days before administering cyclophosphamide. This considerably ($p < 0.05$) reduced the levels of total bilirubin and conjugated bilirubin, as well as the activities of AST, ALT, and ALP, while increasing albumin levels. CMSO's oil contains a variety of flavonoid compounds [54]. These substances may have induced the observed effect on liver enzymes [52]. We recognize flavonoids for their antioxidant properties, radical scavenging abilities, and anti-lipo-peroxidant effects, which contribute to hepatoprotection. Because seed oil is beneficial for antioxidants and acetogenins, it may help explain how CMSO protects the liver from changes caused by cyclophosphamide. Likewise, Oyagbemi et al. [53] and Doustimotlagh et al. [54]

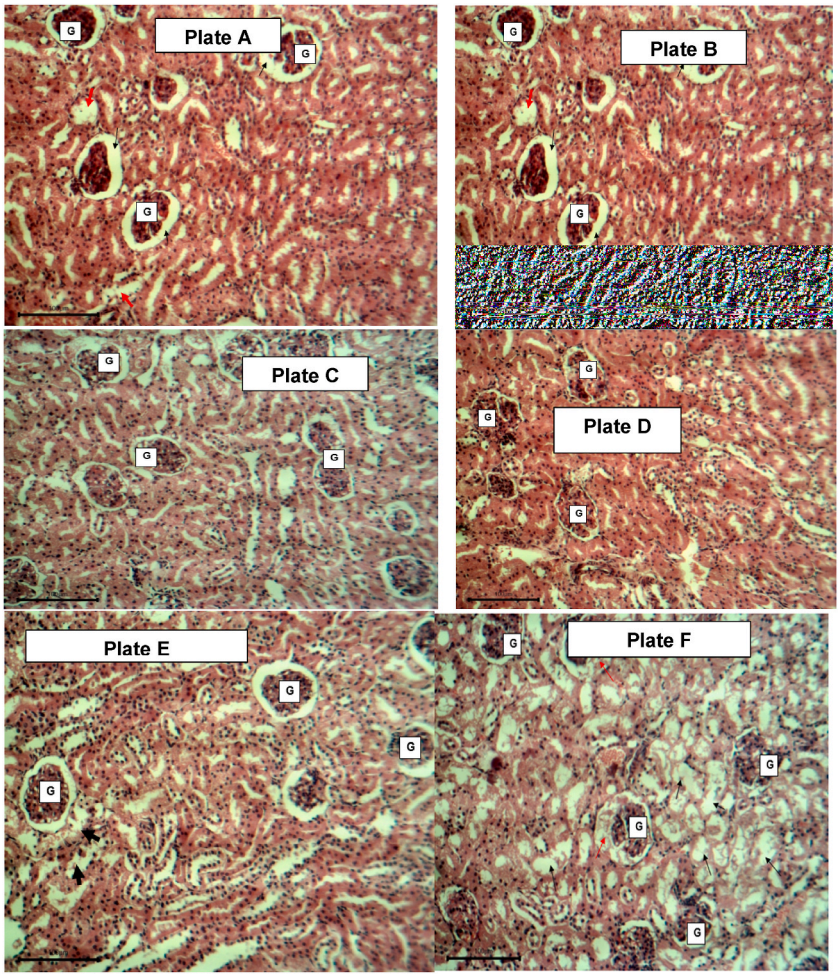

Fig. 23. Plate 3: Photomicrograph of Kidney (A–F) (H&Ex160**).** Glomeruli (G). A (Normal saline), B (100 mg/kg cyclophosphamide + Normal saline), C (300 mg/kg b.w. Omega 3 oil +100 mg/kg cyclophosphamide), D (5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), E (2.5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide), F (1. 5 ml/kg C. mannii seed oil +100 mg/kg cyclophosphamide).

documented the hepatoprotective properties of several medicinal herbs against CPA-induced toxicity.

In this study, it was shown that administration of cyclophosphamide in rats significantly ($p < 0.05$) elevated the level of creatinine, urea, and BUN. However, administration of CMSO and omega-3 fatty acids for 27 days before cyclophosphamide in rats significantly ($p < 0.05$) reduced the levels of creatinine, urea, and BUN. Increasing serum creatinine and urea levels are important indicators of poor glomerular filtration and have been significant clinical markers for renal dysfunction and loss of renal integrity [56]. Creatinine could be a matter of muscle creatine, whose quantity in blood serum is proportional to the body's muscle mass. Sometimes, the amount of creatinine remains constant, and higher levels signify a reduction in nephritic activity, as the kidneys simply eliminate it [57]. Increased proteolysis sustains gluconeogenesis by releasing free glucogenic amino acids that circulate in plasma, deaminate in the liver, and increase urea in the blood [58]. Our results are similar to those that showed that *Terminalia catappa*, coconut water, and *V. amygdalina* extract significantly lowered creatinine and urea levels in rats, which protected the kidneys [59,60].

Plasma concentrations of waste substances such as urea and creatinine, as well as electrolytes, are the most commonly used indices to evaluate renal function [61]. The kidney eliminates waste materials and regulates fluid, electrolyte, and acid-base equilibrium [62]. Renal injury often results in the accumulation of waste substances in the blood as well as altered fluid homeostasis and acid-base balance. The administration of cyclophosphamide significantly elevated the urea and creatinine concentrations in the present study. The balance between their rate of synthesis and excretion determines the plasma levels of creatinine and urea, subject to a multitude of variables [62]. Kidney diseases therefore affect and alter their concentration, as the kidney eliminates them.

In conclusion, we evaluated CMSO for its potential to attenuate cyclophosphamide-induced hepatorenal toxicity. The results of the study revealed that CMSO reduced the levels of MDA, nitric oxide, and bilirubin in the rats, indicating an antioxidant and anti-inflammatory effect. Furthermore, CMSO elevated the activities of SOD and CAT in the rats, further confirming its antioxidant properties. We therefore propose CMSO as a potential therapeutic agent for protecting against cyclophosphamide-induced hepatorenal toxicity.

CRediT authorship contribution statement

Amaka Cecilia Agbara: Project administration, Methodology, Funding acquisition. **Ejike Daniel Eze:** Validation, Funding acquisition. **Christian Emeka Offor:** Supervision. **Adam Moyosore Afodun:** Resources, Investigation, Funding acquisition. **Ezebuilo Ugbala Ekpono:** Writing – original draft, Methodology. **Peter Chinedu Agu:** Visualization, Project administration. **Chinyere Aloke:** Validation, Software. **Nkeiru Nwaamaka Ezeani:** Visualization, Resources. **Emmanuel Orire Ikuomola:** Funding acquisition, Data curation. **Ekom Monday Etukudo:** Writing – original draft, Validation. **Ilemobayo Victor Fasogbon:** Writing – original draft, Data curation. **Angela Mumbua Musyoka:** Software, Resources. **Patrick Maduabuchi Aja:** Writing – review & editing, Formal analysis, Data curation, Conceptualization.

Data availability statement

Data supporting the findings of this study are available from the corresponding author [ASA] on request.

Disclosure statement

No conflict of interest was reported by the authors.

Funding

No funding was received for the conduct of this study.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Aja, Patrick Maduabuchi Aja reports equipment, drugs, or supplies was provided by Ebonyi State University Faculty of Biological Sciences. Aja, Patrick Maduabuchi reports a relationship with Kampala International University - Western Campus that includes: employment. Patrick Maduabuchi Aja has patent pending to Aja, Patrick Maduabuchi. There is no other information on conflict of interest. Thank you If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

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