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Neuroprotective effects of brown rice consumption in an iron-induced parkinsonism in Drosophila

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ABSTRACT

Objectives: Iron (Fe) accumulation and resultant oxidative stress play a significant role in the neuronal death observed in Parkinson's disease (PD). Brown rice (BR) possesses antioxidant properties able to reduce cellular oxidative damage. Thus, we hypothesized that BR may ameliorate Fe-induced parkinsonism due to oxidative stress.

Methods: Two – to three-day-old male flies were concurrently exposed to Fe (ferrous sulphate, 1 mM) and interventions, divided into eight groups: control; Fe; BR; white rice (WR); L-dopa (1 mM); Fe (1 mM) + BR; Fe (1 mM) + WR; and Fe (1 mM) + L-dopa (1 mM). The flies were exposed for 15 days to their respective diets, and their behavior, relevant biomarkers, and the expression of related genes were evaluated.

Results: Chronic exposure to Fe caused cognitive and locomotor deficits by increasing Fe levels ($p = 0.027$) in flies' heads, as well as heightened aggression and grooming episodes ($p < 0.001$). The elevated iron levels induced changes consistent with oxidative stress, evidenced by increased MDA levels ($p < 0.001$), and reduced activity of catalase ($p < 0.001$) and glutathione peroxidase (GPx) ($p < 0.001$), along with decreased dopamine levels ($p < 0.001$). Additionally, there was dysregulation in the mRNA expression of malvolio, ferritin, Nrf2, DJ-1, GPx, and catalase ($p < 0.05$). BR prevented the Fe-induced effects (Fe + BR group) even more effectively than L-Dopa ($p < 0.001$).

Conclusion: The findings indicate that BR has the potential to mitigate Fe-induced ROS-mediated damage in a Drosophila model of PD-like disease by modulating key players in the Nrf2 signaling pathway.

KEYWORDS
Iron accumulation; Nrf2; oxidative stress; brown rice; Parkinson's disease

Introduction

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease globally after Alzheimer's disease (AD) [1]. The disease affects more than 2% of individuals above 65 years of age and is characterized by degeneration of dopaminergic neurons (DANs) in the substantia nigra, thus, affecting motor coordination [1]. In addition to motor-related symptoms, dementia, anxiety, and aggressiveness have also been reported [2]. Several pathophysiological alterations in dopamine (DA) metabolism, oxidative stress (OS), protein aggregations, and mitochondrial function have been implicated in the development of PD [3]. OS arises from an imbalance between the generation of reactive oxygen species (ROS) and the cellular capacity to counteract their harmful effects through antioxidant mechanisms such as catalase and glutathione peroxidase (GPx) [4]. Meanwhile, iron (Fe), an essential metal vital to biological processes has been implicated in the pathogenesis of PD [5]. Fe increases the production of hydroxyl (OH) radicals via the Fenton reaction leading to OS, reduced antioxidant capacity and cellular damage. Fe reacts with DA to generate a potent oxide, resulting in the production of 6-hydroxydopamine (6-OHDA), which induces the release of Fe from ferritin, thus further complicating OS and causing neurotoxic damage [6].

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Levodopa (L-Dopa) is used to manage PD, but its prolonged use is associated with motor fluctuations and dyskinesias [7]. Antioxidants can neutralize ROS and can be used as natural and cost-efficient addition to current treatments for PD [8]. Accordingly, brown rice (BR) contains various bioactive compounds such as phenolic compounds, flavonoids, and gamma-aminobutyric acid (GABA), which have been shown to ameliorate OS in various diseases [9]. However, little is known about its effects on OS associated with PD. In this study, Drosophila was used as an animal model of PD because like humans, it has a distinct central nervous system with similar age-related phenotypes and dopaminergic clusters [10]. Moreover, it has been effectively used in studying bio-metal homeostasis [11], making it an excellent model to study PD. Thus, this study aimed to investigate the effects of BR in preventing OS associated with Fe overload in a Drosophila model of PD.

Materials and methods

Chemicals and reagents

Ferrous sulphate, $FeSO_4.7H_2O$ (MW-278.02 g/mol) (Kermel, China); L-Dopa (Carbidopa/Levodopa) Sinemet®, Karachi, Pakistan; sucrose, LOBA Chemie, CAS No.: 57-50-1; Fe detection kit, catalase activity assay kit, glutathione peroxidase (GSH-Px/GPx) activity kit, malondialdehyde (MDA) kit, were purchased from Beijing Solarbio Science & Technology Co., Ltd, China; DA ELISA assay kit (Elabscience, China); nucleic acid extraction kit (Hunan Runmei Gene Technology, China); PCR kits TransScript Green One-Step qRT-PCR SuperMix (AQ211) (TransGen Biotech Co, China); primers were synthesized by INQABA Biotech, South Africa; corn flour (Faso Farine Mais Blanc, Ouagadougou, Burkina Faso); agar-agar powder (Bacteriological grade-B1CA1HV01, Titan Biotech Ltd, Rajasthan, India); powdered milk (Peakmilk; FrieslandCampina WAMCO Nigeria Plc., Lagos, Nigeria); methyl paraben (16260, Molychem, Mumbai, India); baker's yeast (STK ROYAL Ltd, Lagos, Nigeria); phosphate buffered saline Tablets, Dulbecco A (PBS, Oxoid) (BR0014G, Selangor, Malaysia); and distilled water (CAMRET, UDUS).

Experimental design

Drosophila (Harwich strain) flies were acquired from the fly laboratory at CAMRET, UDUS. The experimental flies were kept in a temperature-controlled Drosophila incubator (ICB-BII Series, Bioevopeak®, China) at 22 ± 3°C, 60–70% humidity, and 12: 12 h light/dark cycle. The fly groupings and experimental design are shown in Figure 1. The study lasted for 15 days, and the diets were changed every three days to provide fresh food to the flies. In the early hours of the 16th day, behavioral tests were conducted.

Diet preparation

The diet was prepared following the protocol in Table 1 adapted from [12] with slight modification. Briefly, for a 100 mL diet, 150 mL dH_2O was brought to boil, after which mixed 1% w/v agar-agar, 1% w/v powdered milk, 2% w/v sucrose was added and stirred continuously for 5 min. Activated yeast (1% w/v) is then added to the mixture and stirred for 2–3 min. The mixture is cooled and 0.08% w/v methylparaben (already dissolved in absolute alcohol) is added to preserve the diet from microbial growth. The Fe and L-dopa dissolved in dH_20 are later added to a 5-mL diet in a 50 mL Falcon tube for the experiment. Sucrose, yeast, and powdered milk served as sources of carbohydrate and protein. To prepare BR and WR diets, 2% w/v sucrose was halved: 1% sucrose and 1% grinded BR or WR respectively. The choice of the rice varieties (Danboto BR and WR) was based on our earlier study [13] demonstrating high content of antioxidants including phenolic acids, flavonoids, and gamma oryzanol.

Behavioral tests

Negative geotaxis (Climbing assay)

Negative geotaxis was used to assess the locomotion performance of the flies [14]. This entailed observing the flies that can cross the 8 cm mark in 6 sec from the base of a transparent measuring cylinder with a diameter of 1.5 cm. The flies were placed in the cylinder using a pooter, gently tapped to the bottom of the cylinder and the numbers able to cross the 8 cm mark in 6 sec were recorded. Flies were subjected to five repetitions of the test, and a total of 20 flies were selected from each group for evaluation. The collected data was then analyzed based on the average time for three replicates ($n = 60$).

Aversive phototaxis suppression (APS) test

The APS test was used to examine the learning and memory abilities of the flies by testing for quinine (chloroquine) sensitivity. The procedure was adapted from [12] with some modifications. The responsiveness of the flies to light was initially tested (an important factor for the APS test) using phototaxis

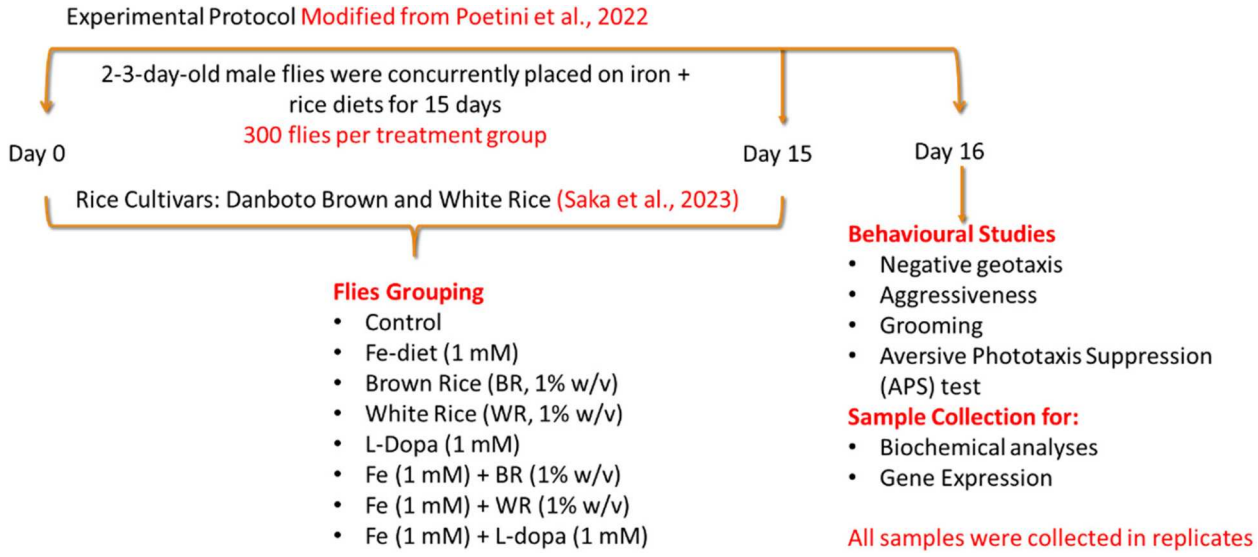

Figure 1. Overview of the experimental design.

behavior on a T-maze. The T-maze device has two arms: dark and fully lit arms connected by a free passage. Ten flies were placed in the dark arm using a pooter to prevent stress associated with anesthesia. The first five flies able to cross to the fully lit arm in 10 s were used for the next test steps.

The next step was to train the flies; a filter paper moistened with 100 µL of 0.1M chloroquine was placed at the end of the illuminated tube close to the light source. Then, the flies were placed in the dark arm, coupled to the T-maze, and allowed 10 s of acclimatization. After putting on the light, the flies were expected to cross to the illuminated arm, while the presence of the chloroquine bitter taste was expected to repel them. This was repeated five times for the flies before the assessment of learning ability and this was recorded as the baseline result (post-conditioning time zero, PC0).

Six hours after the PC0, the same procedure was repeated as post-conditioning at six-hour (PC6). However, to test their short-term memory, the filter paper with chloroquine was removed from the illuminated arm of the T-maze. Flies were awarded a pass if they failed to cross to well-lit arm of the T-maze, meaning they remembered that light repelled them. Flies earlier trained were evaluated for PC6 and recorded as percent pass and this was related to short-term memory. A total of 15 flies were used per group, corresponding to three replicates.

Table 1. Drosophila dietary composition.

Component	Iron-overload/ Control Diet (per 100 mL)	Brown rice diet (per 100 mL)	White rice diet (per 100 mL)
Sucrose	2% w/v	1% w/v	1% w/v
Agar-agar	1% w/v	1% w/v	1% w/v
Baker's yeast	1% w/v	1% w/v	1% w/v
Distilled Water	0.15 L	0.15 L	0.15 L
Methyl paraben	0.08% w/v	0.08% w/v	0.08% w/v
Powdered milk	1% w/v	1% w/v	1% w/v
Brown rice	–	1% w/v	–
White rice	–	–	1% w/v

The diet were prepared by cooking at 100°C.
Abbreviation: w/v: weight per volume.

Grooming behavior

The grooming behavioral test was used to examine anxiety-like symptoms in flies. The procedure for this test was performed as described by [12] with some modifications. Fifteen flies were used per group (3 replicates) and the number of times each fly performed grooming movements was observed and recorded for 2 min. Two separate experiments were conducted per group for the test. The average mean time of grooming episodes of the flies in each group was statistically compared with other groups.

Aggressive behavior

The assessment of aggressive behavior in the flies was conducted by use of a pair of male flies selected from each experimental group following the method by [15]. Prior to the commencement of the experiment, the flies were subjected to a fasting period of 90 min, during which they were placed in empty 50 ml tubes without access to food. After that, they were moved to a 45-mm-radius, 12-mm-high petri dish with a drop of 2% sucrose in it with the aid of a pooter. A period of two minutes was allowed for the flies to acclimatize. The behavioral tests were conducted at a temperature of 22°C. After acclimatization, the flies were observed, and their behavior recorded for 2

min. The aggressive encounters observed in this study encompassed several distinct behaviors. These included instances of leg extension from one fly to another resulting in physical contact, chasing behavior, rapid approach leading to direct orientation, wing raising in response to proximity or approach of another fly, and high box impact interaction involving the front legs of both flies. A total of 15 pairs representing three replicates of five pairs of flies were observed from each group. The average mean time of aggressive behaviors of the flies in each group was statistically compared with other groups.

Sample preparation

After the experiments, flies from each group were collected, anaesthetized by cooling, rinsed in ice-cold phosphate buffered saline (PBS) and their heads removed for biochemical and gene expression analyses. For biochemical analyses, twenty-five fly heads in 3 replicates were used. The heads were transferred into sterile micro-centrifuge tubes and homogenized with a micro-pestle in PBS. The homogenates were centrifuged at 8000 x g for 10 min at 4°C (MX-301 Highspeed, Tomy Kogyo Co., Ltd., Tagara, Japan). For gene expression analyses, thirty fly heads in three replicates were carefully removed after cooling on ice, rinsed, homogenized, and stored in RNA hold (TransGen Biotech Co, China) at −80°C until needed for gene expression analysis.

Biochemical analysis

Serum fe concentration detection

The serum Fe concentration in the head and body of the flies was determined using a commercially available serum Fe concentration detection kit (Beijing Solarbio Science & Technology Co., Ltd, China; Cat No BC1735) following the manufacturer's instructions. The absorbance was measured at 520 nm using a microplate reader (Infitek Microplate Reader, MPR-H200BC, Shandong, China).

Dopamine assay

The commercially available dopamine enzyme-linked immunosorbent assay (ELISA) kit (Elabscience, China; Cat No. E-EL-0046) was used to measure dopamine levels in the fly heads following the manufacturer's instructions. The absorbance was detected at 450 nm using a microplate reader (Infitek Microplate Reader, MPR-H200BC, Shandong, China). A four-parameter (4PL) logistics curve ($y = 0.8925x + 2.221$; $R^2 = 0.997$) was used to determine the concentration of dopamine in the standards and samples.

Catalase activity

Catalase activity was measured using a commercially available CAT activity assay kit (Beijing Solarbio Science & Technology Co., Ltd, China; Cat No BC0200) following the instructions provided by the manufacturer. The change in sample absorbance at 240 nm was measured with a microplate reader (Infitek Microplate Reader, MPR-H200BC, Shandong, China).

Glutathione peroxidase activity

The activity of glutathione peroxidase (GSH-Px/GPx) was assessed using a GSH-Px/GPx assay kit (Beijing Solarbio Science & Technology Co., Ltd, China; Cat No BC1190), following the instructions provided by the manufacturer. The absorbance was measured at 412 nm using a microplate reader (Infitek Microplate Reader, MPR-H200BC, Shandong, China).

MDA assay

Lipid peroxidation was assessed by measuring MDA content using a commercially available assay kit (Beijing Solarbio Science & Technology Co., Ltd, China; Cat No BC0020) following the manufacturer's instructions. The absorbance at 450, 532, and 600 nm was measured using a microplate reader (Infitek Microplate Reader, MPR-H200BC, Shandong, China).

Gene expression analysis

RNA extraction

RNA was extracted from a total of 60 fly heads using a RunMei RNA extraction kit (Hunan Runmei Gene Technology Co., Ltd), following the instructions provided by the manufacturer. The purity of the extracted RNA was assessed using a Bioevopeak Nucleic Acid Analyzer (SP-MUV2000F, Jinan, Shandong, China), and values of 1.8–2.2 were considered for both A260/230 and A260/280.

Primer design and gene expression

The primers were designed to specifically target transcripts of key proteins involved in Fe metabolism, antioxidant system, and *DJ-1* (OS sensor in the DANs) as listed in Table 2. The primers were designed using PrimerQuest, validated using Oligocalc and NCBI Blast (Nucleotide Blast), and subsequently synthesized by INQABA Biotech, South Africa. Real-time polymerase chain reaction (RT-qPCR) experiments were performed using the TransScript® Green one-step quantitative reverse transcription PCR (qRT-PCR) SuperMix kit (AQ211, TransGen Biotech, China) as follows: RNA template (100 ng), forward and reverse primers (0.2uM each), 0.4uL TransScript® Green One-Step

Table 2. List of primer sequences.

Gene products (mRNA)	Strand	Primer (5`−3`)	Annealing temperature (°C)
cat	Forward	CTGCCCTACTGTAGTTAATTCCG	65
	Reverse	GTATATCGAGTGACCACTGTGC	
Nrf2	Forward	GGAAGAAGAAGCAGCGAAGA	60
	Reverse	GATGATGAGACCCGACTACAAC	
GPx	Forward	CGAATATCTGTGCGAGGATGG	60
	Reverse	CTGCTCGTTGGAGATGTAGC	
DJ-1	Forward	CTCGTATCCCTCAATGAAGCC	60
	Reverse	CCTCGACTGGTGATCAGATTG	
mvl	Forward	CGGTCAGTTCTCAATGGAAGG	60
	Reverse	GGCAGAAGGTGGGAATAATGG	
Fer1HCH	Forward	CTGGCTGTTCCTGAGATTACC	60
	Reverse	CATGGCCAAGTACTGGTAGG	
Rpl32	Forward	GGATCGATTCCTGTGAGAGTTC	60
	Reverse	TGGGCAGTATCCATTGAGTTT	

The PCR conditions used were 45°C for 5 mins (reverse transcription), 94°C for 30 sec (pre-denaturation), 40 cycles of 94°C for 5 sec (denaturation), 15 sec of annealing temperature (see table) and 72°C for 10 sec (extension). **Abbreviations:** *cat*: catalase; *Nrf2*: Nuclear factor erythroid 2-related factor 2; *GPx*: Glutathione peroxidase; *DJ-1*: Protein deglycase; *Fer1HCH*: ferritin heavy chain; *mvl*: Malvolio.

RT/RI Enzyme Mix, 10uL TransScript® Green One-step qPCR SuperMix (2×) and RNase-free water to make a final volume of 20uL. The reactions were carried out on a Rotor-Gene Q 5plex HRM thermal cycler (Qiagen, Hilden, Germany). After performing optimization and melt curve analysis, the Cq values were normalized to the reference gene (*Rpl32*) and control sample using the comparative $2^{-\Delta\Delta ct}$ method [16]. The experiments were conducted in triplicates.

Data analysis

The statistical analysis was performed using GraphPad Prism (Version 9.5.1) software. The homogeneity of the collected data was assessed using Shapiro-Wilk's normality test. The parametric data were analysed using one-way analysis of variance (ANOVA) followed by Tukey *post hoc* test for intergroup comparisons. Non-parametric data were analysed using Kruskal-Wallis's analysis, followed by Dunn's multiple comparison tests. Statistical significance was determined at a significance level of $p < 0.05$. The non-parametric data are displayed as box and whisker plots, while the parametric data are presented as mean ± standard deviation (SD).

Results

Behavioral assessment

Effects of fe and BR on locomotor performance, anxiety, and aggression

Exposure to a 15-day chronic Fe overload significantly ($p < 0.001$) decreased the locomotor performance (Figure 2A) and significantly ($p < 0.001$) increased both anxiety (grooming (Figure 2B)) and aggression (Figure 2C) compared to the control. Simultaneously exposing the flies to BR and L-Dopa significantly improved ($p < 0.001$) the locomotor performances and reduced both anxiety and aggressiveness of the flies compared to the Fe group.

Effects of fe and BR on learning and memory using APS test

A 15-day exposure to an Fe overloaded diet resulted in a significant decrease ($p < 0.001$) in the learning capacity (PC0, Figure 3A) and impaired short-term memory (PC6, Figure 3B) of the flies ($p < 0.001$) compared to the control. Simultaneously exposing the flies to BR and L-Dopa (Fe + BR and Fe + L-Dopa) resulted in significant improvements ($p < 0.001$) in both learning and short-term memory abilities compared to the Fe group.

Biochemical parameter

Fe and DA levels

Figures 4(A and B) show the effects of Fe and BR on various biochemical parameters in the flies. Exposure to Fe overload significantly ($p = 0.027$) increased labile Fe levels in the head (Figure 4A), body (Figure 4B), and significantly decreased DA in flies compared to the control ($p < 0.001$). L-Dopa significantly increased Fe levels in the head but not in the body. Conversely, co-exposure with BR and L-Dopa (BR + Fe and L-Dopa + Fe) significantly decreased ($p < 0.01$) Fe levels in head and body as well as increased DA level in the flies with the effect more pronounced in BR + Fe group.

Oxidative stress markers

Flies in the Fe group had an increased lipid peroxidation as measured by MDA level ($p < 0.001$), decreased catalase ($p = 0.049$) and GPx ($p < 0.001$) compared to the control (Table 3). BR co-exposure (BR + Fe) significantly decreased MDA ($p = 0.011$), increased catalase ($p < 0.001$) and GPx ($p < 0.001$) compared to the Fe group. However, L-Dopa co-exposure (L-Dopa + Fe) did not reduce MDA ($p = 0.102$) and catalase ($p = 0.791$) levels significantly but significantly improved GPx ($p < 0.001$) compared to Fe group.

Gene expression

As shown in Table 4, chronic exposure to Fe overload significantly upregulated ($p < 0.001$) Fe storage protein

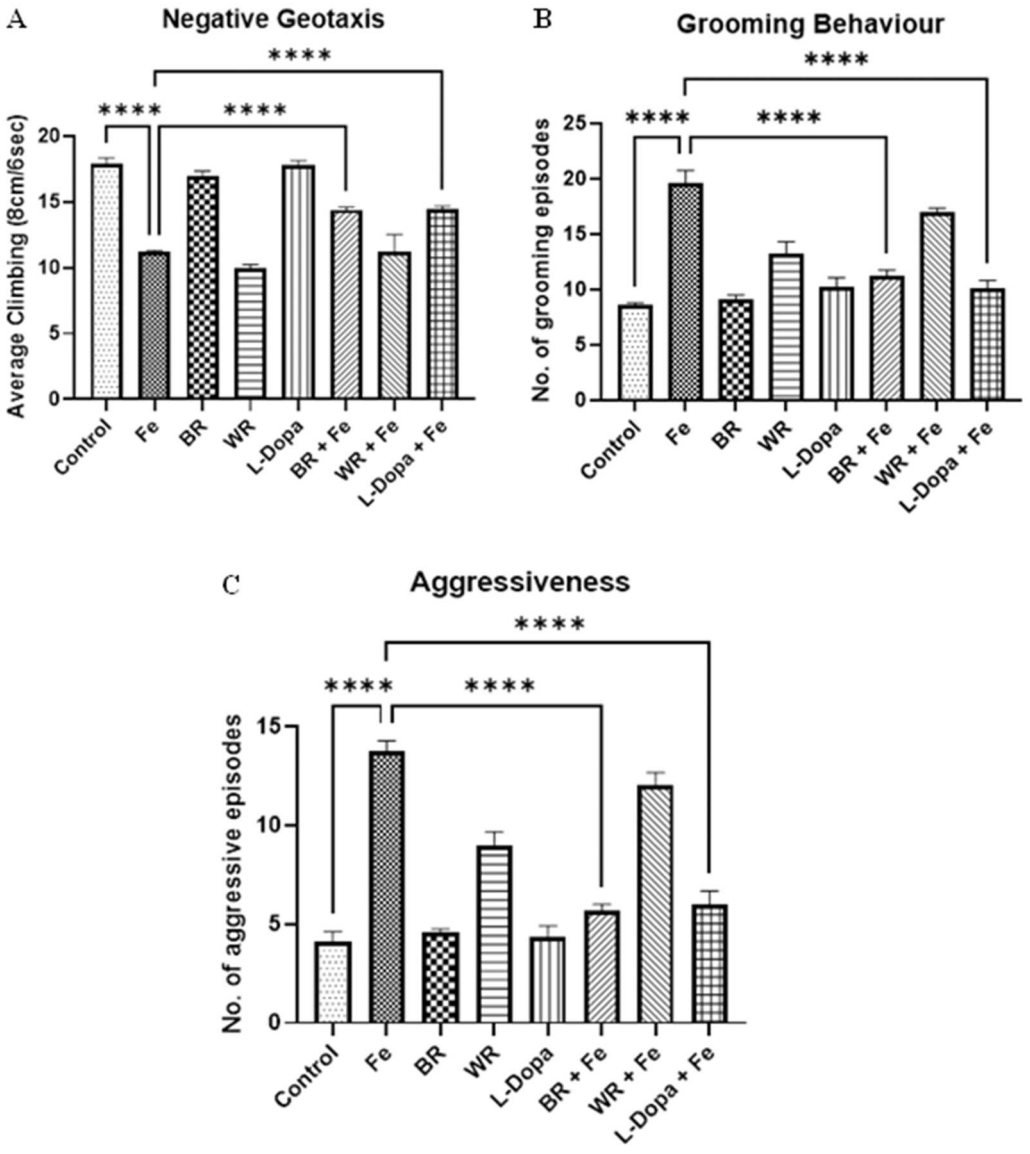

Figure 2. Effect of BR on A. Negative Geotaxis, B. Grooming Behaviour, and C. Aggressive Behaviour in Parkinson disease induced by chronic exposure (15 days) to Iron in Drosophila. Data were analysed using a one-way ANOVA. Values are expressed as mean ± SD. *significantly different compared to the control group. #Significantly different compared to the iron group ($p < 0.05$). Twenty-five fly heads were used per group and included three replicated ($n = 75$). **Abbreviations:** Fe: iron; BR: brown rice; WR: white rice; L-Dopa: levodopa.

(ferritin) mRNA expression, Nrf2 ($p = 0.047$), and DJ-1 ($p < 0.001$) compared to the control but had a comparable result ($p = 0.996$) with the control on enterocyte's Fe importer (*mvl*), catalase, and GPx mRNA expression. There was an upregulated expression of ferritin mRNA in the L-Dopa treated group ($p = 0.959$) and a downregulated expression of *mvl* mRNA ($p = 0.656$) similar to the Fe group. However, co-exposure with BR and L-Dopa (BR + Fe and L-Dopa + Fe) significantly ($p < 0.001$) increased *mvl*, Nrf2, DJ-1, catalase, and GPx and decreased ferritin ($p < 0.001$) mRNA expressions compared to the Fe group with the effects more pronounced in the BR + Fe group compared to the L-Dopa + Fe group.

Discussion

In this study, we showed that chronic Fe consumption led to increased Fe levels in flies' heads. Consequentially, there were alterations in flies' behaviors, decreased DA levels, increased lipid peroxidation as evident by a significantly higher MDA level, and decreased antioxidant capacities. These findings have implications for PD as they indicate a possible

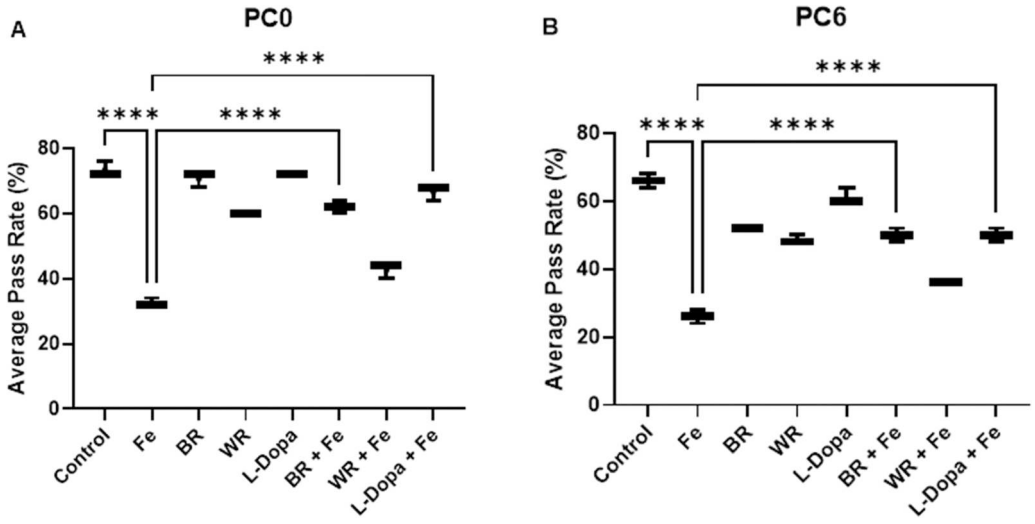

Figure 3. Effect of BR on learning (PC0) [Figure A] and short-term memory (PC6) [Figure B] in Parkinson disease induced by chronic exposure (15 days) to Fe in Drosophila. Kruskal-Wallis's test was used to determine the significance among groups. Values are shown as the median (interquartile range). *Statistically different compared to the control group. #Different statistically compared to the Fe group ($p < 0.05$). Twenty-five fly heads were used per group and included three replicated (n = 75). **Abbreviations:** Fe: iron; BR: brown rice; WR: white rice; L-Dopa: levodopa.

mechanistic link between high levels of Fe, OS, and the degeneration of DANs. Fe accumulates in the brain, especially within the basal ganglia, during ageing, partly due to the increased permeability of the blood – brain barrier and mutation in the gene encoding the protein alpha-synuclein [6]. Fe accumulation in the brain has been implicated in the degeneration of DANs, contributing to motor and non-motor impairments characteristic of PD [17].

The behavioral assessment in this study revealed a significant decline in locomotor activities, impaired learning and memory, increased grooming, and aggressiveness in Drosophila subjected to chronic Fe consumption consistent with previous studies [11, 12], and groups exposed to WR. Previous studies have suggested that BR possesses phytochemicals that may contribute to its neuroprotective effects [9, 13]. Particularly, BR is rich in GABA (see supplementary information), an inhibitory neurotransmitter, which may have regulated neuronal excitability with implications on the PD-like symptoms. Moreover, activation of GABA receptors has been found to exert inhibitory control over pathways involved in anxiety and stress responses, which may result in a reduction in grooming and aggressive episodes [9]. Also, the observed improvements in cognitive function may be influenced by the modulation of GABAergic activity, since GABAergic signaling have been implicated in cognitive function and synaptic plasticity, important for learning and memory [18]. Similarly, L-Dopa, a precursor to DA, has been a mainstay in PD treatment, and its positive impact on behavior aligns with established therapeutic effects [7]. The synergistic improvement observed with BR and L-Dopa co-exposure suggests a potential additive or complementary effect in mitigating PD-like behavioral deficits. Meanwhile, WR has low beneficial phytochemicals [13], leading to symptoms similar to those observed in the Fe group.

We saw an elevated Fe levels in the L-Dopa group in the present study which contradicts the findings of [12], these differences may be due to differences in duration of exposure; 15 days in the present study as opposed to 10 days in that study. Consequently, we noticed a reduction in DA levels in L-Dopa exposed groups (L-Dopa and L-Dopa + Fe), showing that chronic L-Dopa administration could reduce DA levels. L-Dopa has been reported to bind to siderocalin (Scn) protein in the presence of Fe to form Scn-L-Dopa-Fe^{3+} complex that reduces the availability of L-Dopa for the synthesis of DA resulting in DA depletion and DANs' death leading to PD or L-Dopa-induced dyskinesia [19]. Meanwhile, we noticed a slight improved antioxidant capacity in both L-Dopa and L-Dopa + Fe compared to the Fe-group suggesting that L-Dopa may not possess antioxidant capacity as reported by [12].

BR possesses abundant phenylalanine and tyrosine (see supplementary information) suggesting that it may contribute to the synthesis of DA. Unexpectedly, DA was reduced in the BR group in this study. As earlier discussed, the BR used in this study had higher

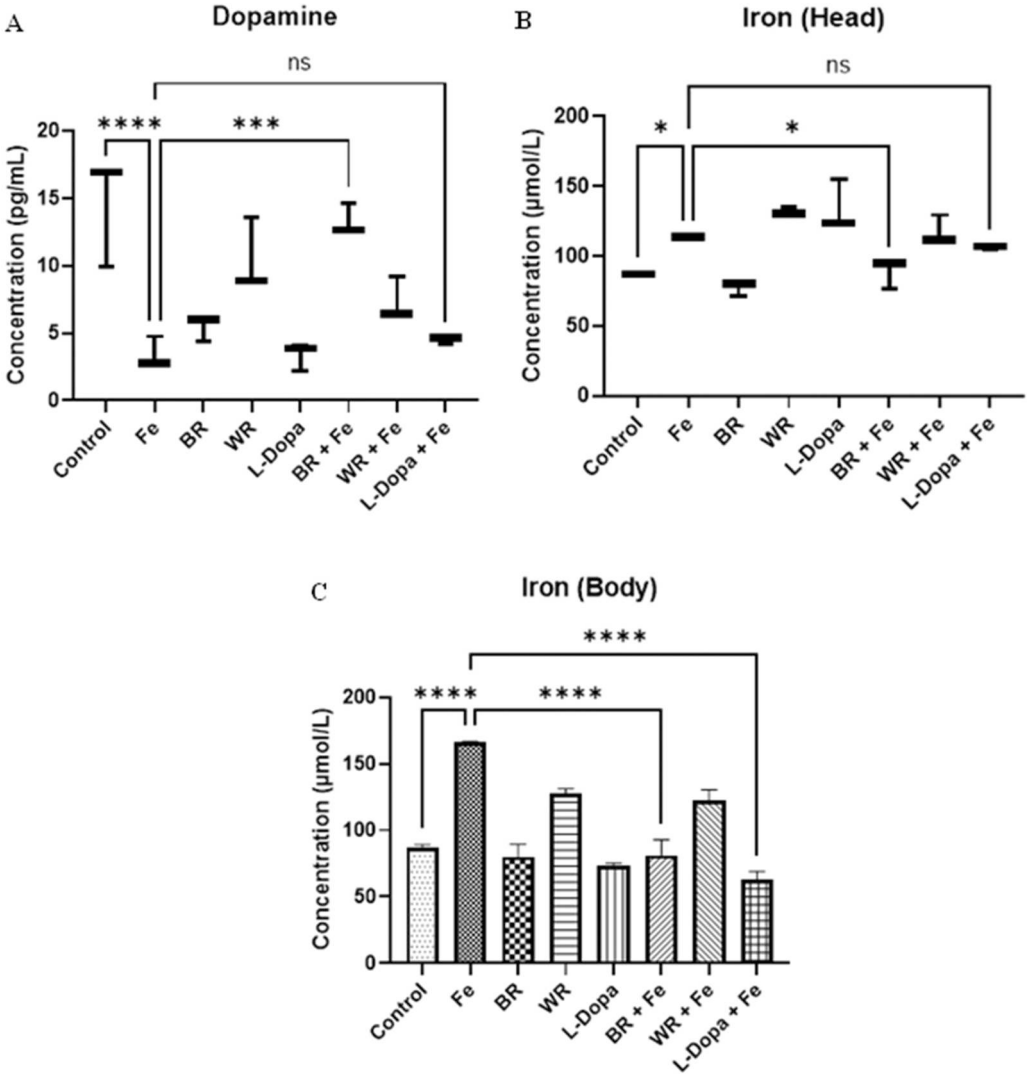

Figure 4. Effects of Iron and BR on Iron level in flies' head (Figure A), body (Figure B), and Effects of Iron and BR on DA (Figure C) levels in Parkinson-like disease induced by chronic exposure (15 days) to Fe in Drosophila. Data were analysed using Kruskal-Wallis's test (A and B) and one-way ANOVA (C). Values are expressed as mean ± SD. *significantly different compared to the control group. #Significantly different compared to the Fe group ($p < 0.05$). Twenty-five fly heads were used per group and included three replicated (n = 75). Abbreviations: Fe: iron; BR: brown rice; WR: white rice; L-Dopa: levodopa.

Table 3. Effects of Fe and BR on OS markers.

Group	MDA (nmol/mL)	Catalase (U/mL)	GPx (U/mL)
Control	0.609 ± 0.295	2.712 ± 0.000	18.98 ± 1.802
Fe	1.570 ± 0.369*	1.808 ± 0.391*	10.52 ± 1.873*
BR	0.869 ± 0.015	4.746 ± 0.000	18.88 ± 1.352
WR	1.528 ± 0.059	1.808 ± 0.391	11.11 ± 1.363
L-Dopa	0.732 ± 0.105	2.034 ± 0.001	15.44 ± 1.192
BR + Fe	0.839 ± 0.142#	3.842 ± 0.391#	16.81 ± 1.022#
WR + Fe	1.534 ± 0.238	2.712 ± 0.000	10.62 ± 1.533
L-Dopa + Fe	1.039 ± 0.208	2.260 ± 0.283	18.09 ± 0.681

Twenty-five fly heads were used per group and included three replicated (n = 75). Data were analysed using a one-way ANOVA. Values are expressed as mean ± SD. *significantly different compared to the control group. #Significantly different compared to the Fe group ($p < 0.05$). **Abbreviations:** Fe: iron; BR: brown rice; WR: white rice; L-Dopa: levodopa.

GABA content and this may have contributed to reduce DA release despite its synthesis. This is because high GABA concentration has been shown to inhibit DA release in the SNpc thereby lowering the DA levels [18]. Nevertheless, there was significant improvement in the DA levels in the BR + Fe group suggesting that BR was able to increase DA synthesis and release under OS condition. Furthermore, exposure to BR (BR only and BR + Fe) improved the antioxidant capacities of the flies through catalase and GPx to reduce the OS (MDA) induced by Fe overload. The data indicate a strong antioxidant ability linked to BR. A previous study from our laboratory [13] has

Table 4. Effects of Fe and BR on mRNA levels key genes.

Group	Malvolio (*mvl*)	Ferritin	DJ-1	Nrf2	Catalase	GPx
Control	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000
Fe	1.103 ± 0.054	68.59 ± 2.122*	8.519 ± 0.310*	10.56 ± 0.000*	1.283 ± 0.000	0.491 ± 0.231*
BR	2.343 ± 0.264	0.223 ± 0.001	12.79 ± 1.110	27.46 ± 6.722	2.969 ± 0.036	7.111 ± 0.000
WR	0.693 ± 0.014	51.62 ± 5.32	6.749 ± 1.310	4.972 ± 0.983	0.894 ± 0.306	0.692 ± 0.151
L-Dopa	0.847 ± 0.000	67. 65 ± 0.532	14.69 ± 0.010	29.22 ± 6.524	2.544 ± 0.590	6.122 ± 1.211
BR + Fe	5.463 ± 0.594#	11.23 ± 0.017#	49.19 ± 0.110#	54.16 ± 0.000#	9.122 ± 1.056#	15.31 ± 0.000#
WR + Fe	0.423 ± 0.064	54.94 ± 0.000	16.22 ± 0.000	5.353 ± 0.871	0.971 ± 0.176	0.055 ± 0.001
L-Dopa + Fe	2.213 ± 0.424	14.41 ± 2.751	20.29 ± 0.210	52.36 ± 0.000	5.579 ± 0.000	11.76 ± 1.831

Thirty fly heads were used per group and included three replicated (n = 90). Data were analysed using a one-way ANOVA. Values are expressed as mean ± SD. *significantly different compared to the control group. #Significantly different compared to the Fe group ($p < 0.05$). **Abbreviations:** Fe: iron; BR: brown rice; WR: white rice; L-Dopa: levodopa, Nrf2: nuclear factor erythroid 2 related factor 2; DJ-1: protein deglycase 1.

shown that the Danboto BR variety is rich in phenolic compounds compared to WR of the same variety, which may have contributed to the improved activities seen in the BR + Fe group. Moreover, the therapeutic properties of various polyphenols in multiple neurological diseases have been reported [20]. Polyphenols have been reported to preserve DA neurons and recover the hindered movement activity caused by Fe and/or paraquat (PQ) challenges in the Drosophila PD model [14]. However, the opposite was observed in the groups treated with WR (WR only and WR + Fe). Generally, WR is low in antioxidants due to the removal of the bran layer after polishing [21], thus, the increased OS might have contributed to the oxidation of DA leading to the generation of hydroxyl radicals that react with Fe^{3+} molecules and further Fe accumulation, degeneration of DANs and manifestation of PD clinical symptoms [22].

Lastly, exposure to Fe overload, L-Dopa, and WR led to an upregulation of ferritin expression and downregulation of *mvl* expression highlighting the disruption of Fe storage in PD. Fe is tightly regulated, in Fe overload, iron regulatory proteins (IRPs) are prevented from binding to iron-responsive elements (IREs) as its active sites are blocked by 4Fe-S resulting in low *mvl* and increased ferritin mRNA expression and a reverse case under reduced iron levels [23]. Significantly, the simultaneous exposure to BR + Fe resulted in a significant reduction in the expression of ferritin as well as an increased *mvl* expression, indicating that BR plays a regulatory function in the storage of Fe and corroborating the existing literature on the chelating property of polyphenols [24]. On the other hand, the simultaneous exposure to BR + Fe resulted in a notable upregulation of antioxidant genes, such as Nrf2 and DJ-1 with subsequent upregulation of catalase and GPx. Nrf2 is a known primary regulator of the antioxidant response and is responsible for coordinating the activation of multiple genes that protect cells against damage [25]. Research findings indicate that DJ-1 plays a role in stabilizing Nrf2 by inhibiting the process of Nrf2 ubiquitination. Upon phosphorylation, Nrf2, stabilized by DJ-1, translocate to the nucleus to increase the expression of various antioxidant genes including catalase and GPx [25]. We propose that BR prevented the development of Parkinson-like disease in Drosophila by enhancing the antioxidant system via Nrf2/DJ-1/ARE pathway.

Conclusion

Given the association between elevated Fe levels and oxidative damage in the brain and the development of PD, as well as the effectiveness of the fly model in studying Parkinson-like disease, we utilized a Drosophila model to induce Parkinson-like disease by exposing the flies to chronic Fe consumption, BR, WR, and L-Dopa. As a result, BR (BR + Fe) enhanced DAergic function, by decreasing Fe levels in the brain. This, in turn, suppressed the progression of motor impairment (locomotor activity) as well as non-motor dysfunctions such as memory deficits, aggression, and anxiety-like behaviors. Furthermore, BR enhanced the antioxidant activities of catalase and GPx through the Nrf2/DJ-1 pathway in flies, surpassing the effectiveness of the group treated with L-dopa.

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Data statement

Data is available upon request from the corresponding author.

CRediT authorship contribution statement

Yaaqub Abiodun Uthman: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Software; Writing – original draft; Writing – review & editing. **Kasimu Ghandi Ibrahim:** Project administration; Supervision; Visualization; Writing – review & editing. **Murtala Bello Abubakar:** Project administration; Supervision; Visualization; Writing – review & editing. **Ismail Sulaiman:** Investigation; Methodology; Visualization; Writing – original draft**. Mustapha Umar Imam:** Funding acquisition; Methodology; Project administration; Resources; Supervision; Validation; Writing – review & editing.

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