

Research paper

Mercury chloride causes cognitive impairment, oxidative stress and neuroinflammation in male Wistar rats: The potential protective effect of 6-gingerol-rich fraction of *Zingiber officinale* via regulation of antioxidant defence system and reversal of pro-inflammatory markers increase

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ABSTRACT

This study investigated the effects of 6-gingerol-rich fraction of *Zingiber officinale* (6-GIRIFZO) on mercury chloride (HgCl₂)-induced neurotoxicity in Wistar rats. Thirty-five male Wistar rats weighing between (150–200 g) were divided randomly into five groups (n = 7): group 1: control, received 0.5 mL of normal saline, group 2: received HgCl₂ (5 mg/kg), group 3: received N-acetylcysteine (NAC) (50 mg/kg) as well as HgCl₂ (5 mg/kg), group 4: received 6-GIRIFZO (100 mg/kg) and HgCl₂ (5 mg/kg), group 5: had 6-GIRIFZO (200 mg/kg) and HgCl₂ (5 mg/kg), consecutively for 14 days. On the day 14, the rats were subjected to behavioural tests using a Morris water maze and novel object recognition tests. The rats were then euthanized to obtain brain samples for the determination of biochemical parameters (acetylcholinesterase (AChE), nitric oxide (NO), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione (GSH), tumor necrosis factor- α (TNF- α), nuclear factor kappa-B (NF- κ B), interleukin-1 β (IL-1 β) and interleukin-6 (IL-6)) using standard methods. The result revealed a significant increase in escape latency and a significant decrease in recognition ratio in the rats that were exposed to HgCl₂ only. However, 6-GIRIFZO produced a significant reduction in the escape latency and (p < 0.05) increase in the recognition ratio. Similarly, HgCl₂ exposure caused a significant (p < 0.05) decrease in the brain SOD, GPx, CAT, GSH with increased brain levels of MDA, NO, AChE, TNF- α , NF- κ B, IL-1 β and IL-6. Similarly to the standard drug, NAC, 6-GIRIFZO (100 and 200 mg/kg) significantly (p < 0.05) increased brain SOD, GPx, CAT, and GSH levels with decreased concentrations of MDA, NO, AChE, TNF- α , NF- κ B, IL-1 β and IL-6. Also, pre-treatment with 6-GIRIFZO prevented the HgCl₂-induced morphological aberrations in the rats. This study concludes that 6-GIRIFZO prevents HgCl₂-induced cognitive deficit via reduction of brain inflammation as well as oxidative stress in rats.

1. Introduction

Neurotoxicity is any negative impact on the structure or operation of the central and peripheral nervous system brought on by exposure to biological, chemical, or physical factors (Mohammad et al., 2016). According to Cesario et al. (2018), mercury (Hg) is a hazardous element with well-documented negative health effects. It is widely utilized by humans in a variety of activities such as agriculture, manufacturing, and medicine and is widely disseminated throughout the world and is

thought to be one of the major environmental contaminants (Yang et al., 2020). Various types of mercury, such as elemental mercury (Hg₀), inorganic, as well as organic molecules occur. Mercury is found in the environment as a result of anthropogenic sources as well as natural occurrences (UNEP, 2013). Anthropogenic sources include the industrial use of fossil fuels, the burning of solid waste, topical medication use, production of cement, the use of mercury measurement devices, dental amalgam, cosmetics, and small-scale artisanal gold mining. The most common source of accumulated mercury for human consumption is

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found in seafood (Jha et al., 2019). Exposure may occur orally, inhalation, or topically (Sadeeq et al., 2013). A number of neurological or mental diseases have been linked to mercury exposure, both acutely and over time (Xu et al., 2012). One of the organs most susceptible to mercury toxicity is the central nervous system (CNS) (Cariccio et al., 2019).

Mercury chloride (HgCl_2) is a highly toxic compound composed of mercury and chlorine. It is a water-soluble, odourless, and colourless crystalline substance. HgCl_2 poisoning has been linked to substantial damage to rats' hippocampal formation as well as the cortex and cerebellum (Xu et al., 2012; Owoeye and Farombi, 2015). The precise underlying mechanism of HgCl_2 neurotoxicity is still poorly understood, and the mechanism of HgCl_2 toxicity is still unknown. However, recent research points to a number of processes, with oxidative stress being one that is widely known to be a major factor in neurotoxicity caused by HgCl_2 (Mieiro et al., 2011; Moneim, 2015; Mesquita et al., 2016).

According to research, HgCl_2 has a great attraction for glutathione enzymes by interacting with their thiol (-SH) group (Rao and Purohit, 2011; Mesquita et al., 2016). Because this process depletes intracellular thiols, reduced glutathione, glutathione peroxidase, and glutathione reductase are less effective at fighting free radicals and oxidative damage.

According to Kongsui and Jittiwat (2023), antioxidants have the ability reduce oxidative stress by scavenging or blocking the reactive oxygen species produced to stop cellular damage. Additionally, the use of plant-based, naturally occurring antioxidants is being promoted (Enogieru and Momodu, 2021). There is proof that adding nutritional antioxidants to the diet can help with cognitive impairment and brain damage (Tams et al., 2018). The potent antioxidant qualities of medicinal plants are well-known, and they are still regarded as reliable sources of supplemental medications (Farouk et al., 2021). A popular spice is ginger, also known as *Zingiber officinale* of Zingiberaceae. Ginger has been shown to combat oxidative stress (Benkermiche et al., 2021; Kongsui and Jittiwat, 2023) as well as having a neuroprotective effect (Farombi et al., 2018). Its active component, 6-gingerol, is a fragrant polyphenol. According to Salihu et al. (2016), this active ingredient has been studied for its abilities to fight inflammation, oxidative stress and cancer.

The body is better able to remove mercury from the tissues when chelating agents such as 2, 3-dimercapto-propanesulphonate (DMPS) and dimercaptosuccinic acid (DMSA) are used (Kim et al., 2019). Due to their negative side effects and inability to pass the blood-brain barrier, these medications seem to be of limited benefit (Kim et al., 2019). Despite extensive research into developing novel medications to combat mercurial toxicity, there are still no efficient cures for this toxin.

Z. officinale's 6-gingerol plays a well-documented antioxidant effect (Abolaji et al., 2017; Halawany et al., 2017; Farombi et al., 2018). The neuroprotective benefits of the 6-gingerol-rich fraction of *Z. officinale* (6-GIRIFZO) on HgCl_2 -induced neurotoxicity in male Wistar rats, however, have not been investigated. The goal of the current study was to investigate 6-GIRIFZO's potential protective effects against HgCl_2 -induced neurotoxicity in male Wistar rats.

2. Materials and methods

2.1. Drugs and chemicals

Ketamine Hydrochloride (catalogue number-736, Sigma-Aldrich in Steinheim, Germany) was obtained from Parke-Davies in Freiburg, Germany. HgCl_2 ; catalogue number-104419) and N-acetylcysteine ((NAC); catalogue number-112422) were purchased from Aromokeye Pharmacy in Ilorin, Nigeria.

2.2. Procurement, authentication, extraction of *Zingiber officinale* and isolation of 6-GIRIFZO

Rhizomes of *Z. officinale* were obtained from Ipata Market in Ilorin,

and they were recognized and authenticated by the Plant Biology Department of the University of Ilorin in Ilorin with a voucher number UILH/001/1168/2022. Fresh ginger rhizomes were cleaned with water before being cut into small pieces, minced, dried in the air, ground, and extracted with 95 % ethanol for 72 h (Farombi et al., 2018). The extract was then sieved to separate it. Then, using a rotary evaporator and vacuum, this was concentrated at 45 °C. According to Almada da Silva et al. (2012), the column chromatography was performed.

About 50 g of the product were dissolved in 100 mL ethyl acetate, absorbed over silica gel (70–230 mesh) as the stationary phase, defatted with n-hexane, and isocratically eluted with the solvent combination of n-hexane and ethyl acetate (1:1; 500 mL) as the mobile phase. 10 fractions were produced. Thin-layer chromatography (TLC) was carried out on the fractions.

2.3. High performance liquid chromatography (HPLC)

HPLC was then used on the samples to separate, distinguish, and quantify the various chemicals. By employing an HPLC device (Shimadzu Co. Nexara, MX) and a column (Ubonda pak C18, length: 100 mm, ID: 4.6 mm, thickness: 7 μm), the chemical components of 6-GIRIFZO were identified. The 6-GIRIFZO samples were diluted with acetonitrile and water before being collected in a 25 mL standard flask for HPLC analysis. Using a diode array detector, 6-GIRIFZO was quantitatively detected after being analyzed in a water HPLC system. The stationary phase, C-18, had 7 μm -sized particles and was pre-packed in a column with a 100 mm X 4.6 mm I.D column.

Water and acetonitrile flowed at a rate of 1 mL min⁻¹ as the mobile phase. Pump B carried acetonitrile, while Pump A carried water. The sample was injected using a 10 μL loop rheodyne injector. At room temperature, the analyses were performed. The samples were exposed to a diode array detector (254 nm) after separation through the column, which recognized and measured the different chemicals. There are 14 bioactive substances in 6-GIRIFZO, with 6-gingerol being the most prevalent (51.05 %) as shown in Fig. 1.

2.4. Determination of the *in vitro* antioxidant ability of 6-GIRIFZO

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radicals scavenging property of 6-GIRIFZO was evaluated using the technique outlined by Kamdem et al. (2013). The Oyaizu (1986) approach was used to assess the 6-GIRIFZO's ferric reducing antioxidant property (FRAP). The modified Re et al. (1999) method was used to conduct the 2, 2'-Azinobis (3-Ethylbenzothiazoline-6-Sulphonic Acid) (ABTS) free radical scavenging activity assay.

2.5. Animals

All animal experiments were carried out according to the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). The guidelines for the care of laboratory animals were followed, and the University of Ilorin's Ethical Committee granted its approval with the approval number UERC/ASN/2023/2451. This investigation was conducted at the central research laboratory of the University of Ilorin in Nigeria using 35 male Wistar rats that weighed between 150 g and 200 g. Before administration, the animals spent two weeks in the laboratory to get familiarized to their surroundings. They were put in animal cages with unlimited accessibility to food and water while being kept under conditions of constant temperature, humidity and 12-hour light/dark cycles.

2.5.1. Animal grouping

Thirty-five Wistar rats were grouped in to 5 groups of 7 rats each, they were placed in individual cages and classified as follows (Table 1):

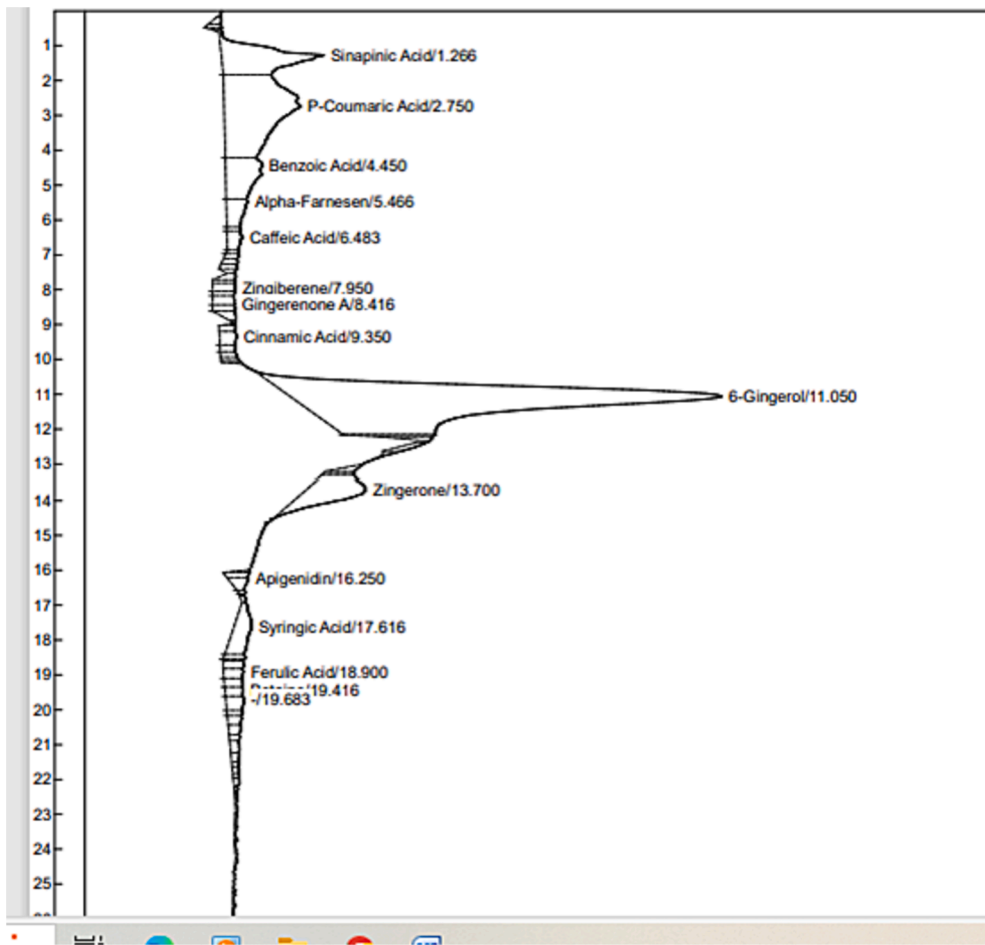


Fig. 1. HPLC Identification and Quantification of 6-GIRIFZO Components.

Table 1
Animal grouping and the administered dosages.

S/ No	Group	Dosages that were administered once daily for 2 weeks
1	Control	0.5 mL of normal saline orally
2.	HgCl ₂ only	5 mg/kg body weight orally (Agbon <i>et al.</i> , 2016)
3.	NAC + HgCl ₂	50 mg/kg body weight orally (Farombi <i>et al.</i> , 2018) + 5 mg/kg body weight orally
4.	6-GIRIFZO (100) + HgCl ₂	100 mg/kg body weight (Farombi <i>et al.</i> , 2018) + 5 mg/kg body weight
5.	6-GIRIFZO (200) + HgCl ₂	200 mg/kg body weight (Farombi <i>et al.</i> , 2018) + 5 mg/kg body weight

2.6. Behavioural testing

The two behavioural (Morris water maze test (MWMT) and novel object recognition test (NORT)) were carried out on the 14th day of administration

2.6.1. Morris water maze test

For this investigation, the approach for spatial memory and learning described by Morris *et al.* (1982), as reported by Drapeau *et al.* (2003), was used. A Morris water maze that measured 1.80 m in circumference and 0.6 m in height was used to test rats. In one of the four quadrants located midway between the wall and the center of the pool, an escape platform was fixedly placed 0.02 m under the water’s surface.

Procedure: The rats were used to the pool every day for five days prior to the test (i.e., before treatment). Animals were trained to find the submerged platform using distant additional maze cues. They

underwent testing for four trials each day, each lasting 90 s with a 30-second inter-trial interval and starting from one of three randomly chosen start positions. At the conclusion of the trial, animals that had not discovered the platform were placed there. It was noted how long it took to get to the platform (latency in seconds). After 14 days of therapy, rats underwent testing. The experiment was captured via a webcam.

2.6.2. Novel object recognition test

To measure cognitive memory, Brown *et al.* (1999) modified the NORT. The NORT measures a mouse’s capacity for recognition memory, which is the capacity to remember a familiar object over a varied period of time. NOR tasks comprise three phases: habituation, familiarization, and test. An animal was exposed to and explored two similar objects (objects A) in the test area to acquire early familiarity.

After a brief interval, the animal was then exposed to the test component, which involved presenting a fresh instance of object A and a brand-new object B in the same setting. An assumption is made that the animal has “recognised” object A if it gravitates toward object B rather than object A, demonstrating a sensitivity to distinguish between the two. On the other hand, it is thought that the animal lacks sufficient memory sensitivity to carry out the recognition discrimination if it examines both objects equally and orients to them both (Kinnavane *et al.*, 2015).

During habituation, rat was given five minutes to roam free in an open field. For the training (T1), the rat was given free reign to explore the arena while two identical objects were positioned diagonally. Five minutes of testing were conducted following T1. Performance on the NORT problem was measured using the recognition ratio (RR). When compared to the animal’s overall exploration time (Ttotal), RR reflects

how long the animal spent exploring the new object (Tnew) as opposed to the old object (Told). The experiment was captured via a webcam.

2.7. Euthanasia of animals and collection of samples

Following behavioural evaluations on the 14th day, rats were given 75 mg/kg intraperitoneal ketamine injection to induce anesthesia. The animal brain samples were then removed, weighed, and homogenized in a 0.1 M phosphate buffer solution (pH 7.4) at a ratio of 1 g tissue to 4 mL (Ige et al., 2011). Homogenization and centrifugation were performed at 0 to 4 °C. After 10 min of centrifugation at 3000 revolution per minute with the homogenate, the supernatant was collected and used for biochemical analysis.

2.8. Determination of biochemical parameters

With the use of the GPx ELISA Kit (WKEA Med Supplies Corp, China), glutathione peroxidase (GPx) was analysed. The approach used in the procedure was developed by Ursini et al. (1995).

SOD ELISA Kit (WKE Med Supplies Corp., China) was used to measure SOD activity according to Okey and Ayo (2015), the procedure was based on the approach used by Martin et al. (1998).

GSH Kit (Sigma-Aldrich, USA) was used to measure the GSH level. The process was based on Ellman's approach (Ellman, 1959).

AchE Activity Assay Kit (Sigma-Aldrich, USA) was used to measure AchE activity according to Okey and Ayo (2015), the procedure was based on the approach used by Ellman et al. (1961).

Malondialdehyde (MDA), which is reactive to thiobarbituric acid, was measured in the samples using a rat MDA ELISA kit (WKEA Med Supplies Corp., China) as a measure of lipid peroxidation. The method developed by Janero (1990) was used as reported by Okey and Ayo (2015).

To measure the nitric oxide (NO) level, the supernatant was poured into a well. By measuring the amount of nitrite present, the quantity of NO in the supernatant was identified. Nitrate was changed into nitrite by vanadium trichloride. Gress reagent, which measures nitrite concentration, was then added. Using a microplate reader, the concentration of nitrite was utilized to determine the concentration of nitric oxide.

A commercially available ELISA kit (Biosciences) was used to measure TNF- α . It was measured in duplicate using a 1:2 dilution of the samples based on the instructions given by the manufacturer (Bancroft and Gamble, 2008). Additionally, the ELISA test was used to measure IL-1 β , IL-6, and NF- κ B.

2.9. Histological assessment

After the experiment, the hippocampus was removed and fixed in a 10 % neutral buffered formalin solution. After fixation, tissues were processed via routine paraffin embedding, and serial sections of 5 μ m thickness were produced on a rotary microtome, and stained using routine Haematoxylin and Eosin (H&E) techniques for the histological appearance of the CA3 regions of the hippocampus.

2.10. Statistical analysis

Data collected using the Windows version of GraphPad Prism 6.0 (GraphPad® Software, San Diego, CA, USA) were statistically analyzed using one-way ANOVA and the Newman-Keuls *post-hoc* multiple comparison test. The mean $\pm$ standard error of the mean (S.E.M.) was used to express the data. At the level of $p \leq 0.05$, values were deemed statistically significant. Linear regression analysis was used to calculate the inhibitory concentration (IC₅₀) values.

3. Results

3.1. Invitro-antioxidant result

In Fig. 2, the DPPH radical scavenging ability of 6-GIRIFZO increases as the concentration of 6-GIRIFZO increases, and this increase compares favourably with the standard antioxidant used (Ascorbic acid). In fact, at 100 μ g/mL and 150 μ g/mL, the percentage of inhibition of 6-GIRIFZO and ascorbic acid are equal. At 50 μ g/mL the inhibition ratio of 6-GIRIFZO is observed to be 0.4, while that of the standard, ascorbic acid is 0.35. Also, the inhibitory concentration (IC₅₀) of 6-GIRIFZO (126.5 ± 6.2 μ g/mL) is lower than that of the standard antioxidant, ascorbic acid (204 ± 9.5 μ g/mL).

Also, in Fig. 3, the ferric reducing antioxidant ability of 6-GIRIFZO increases as the concentration of 6-GIRIFZO increases, at 10 μ g/mL, the ferric reducing power of 6-GIRIFZO is more than that of the ascorbic acid. Also, the inhibitory concentration (IC₅₀) of 6-GIRIFZO (18.98 ± 0.25 μ g/mL) is lower than that of the standard antioxidant, ascorbic acid (22.63 ± 0.87 μ g/mL).

Fig. 4 shows the ABTS free radical scavenging property of 6-GIRIFZO, the percentage of inhibition and the inhibition ratio increase as the concentration of 6-GIRIFZO increases and this increase compares favorably with ascorbic acid at 50 μ g/mL, 100 μ g/mL, and 150 μ g/mL. Also, the inhibitory concentration (IC₅₀) of 6-GIRIFZO (100.5 ± 0.02 μ g/mL) is lower than that of the standard antioxidant, ascorbic acid (101.6 ± 0.15 μ g/mL).

3.2. Behavioural results

In Fig. 5, HgCl₂-treated group showed a significant increase in Escape Latency (EL) compared with the control group which indicates cognitive impairment (as this group of rats took a longer time to identify the escape platform). Administration of either 100 mg/kg body weight or 200 mg/kg body weight of 6-GIRIFZO caused a significant decrease in the EL when compared with HgCl₂ group ($p < 0.05$). However, there is no significant difference in the EL of the rats treated with NAC and the 6-GIRIFZO.

In Fig. 6, HgCl₂-treated group showed a significant reduction in the recognition ratio (RR) compared with the control group which also indicates cognitive impairment. Administration of either 100 mg/kg body weight or 200 mg/kg body weight of 6-GIRIFZO caused a significant increase in the RR when compared with HgCl₂ group ($p < 0.05$). However, there is a significant increase in the RR of the NAC-treated group when compared with the 6-GIRIFZO-treated groups.

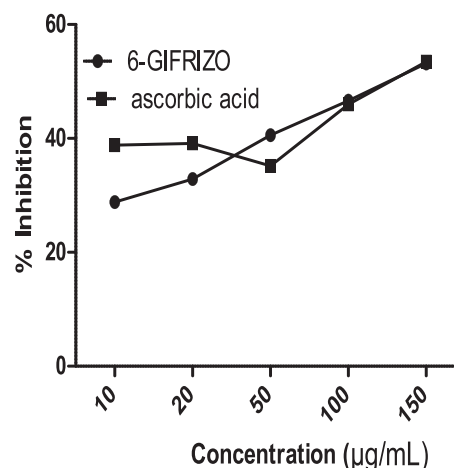


Fig. 2. % DPPH radical scavenging by 6-GIRIFZO and ascorbic acid.

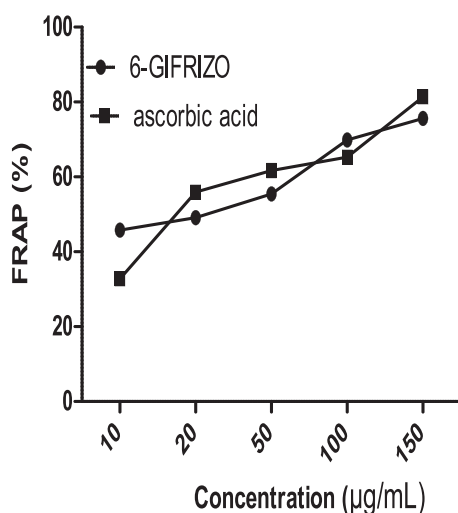


Fig. 3. Ferric Reducing Power of 6-GIRIFZO and ascorbic acid.

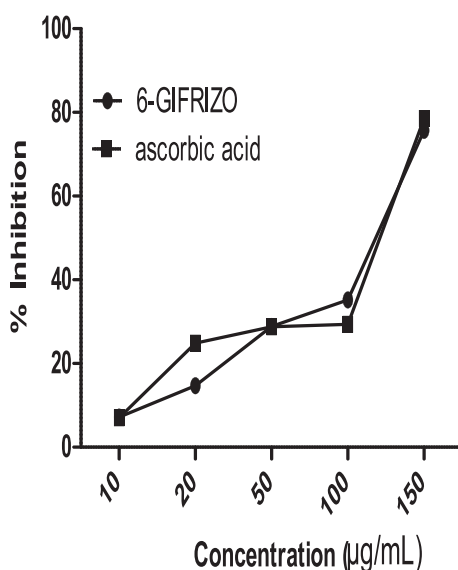


Fig. 4. Inhibition Rate of ABTS by 6-GIRIFZO and ascorbic acid.

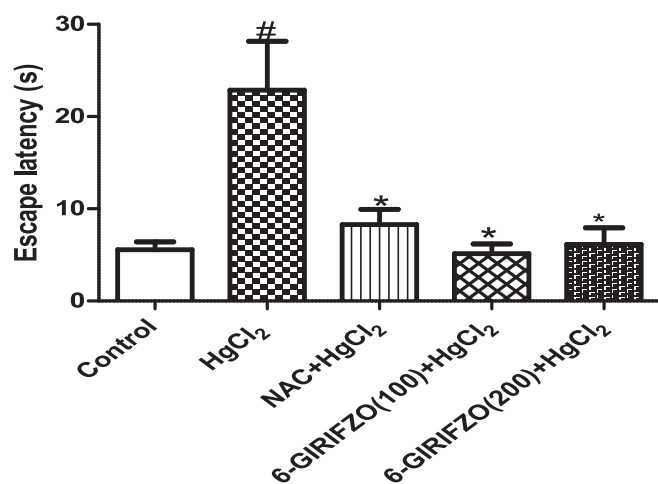


Fig. 5. Effect of 6-GIRIFZO on Morris water maze test. Mean $\pm$ SEM was used to present the data, $n = 5$; [#] $P \leq 0.05$ vs. control; ^{*} $P \leq 0.05$ vs. HgCl₂.

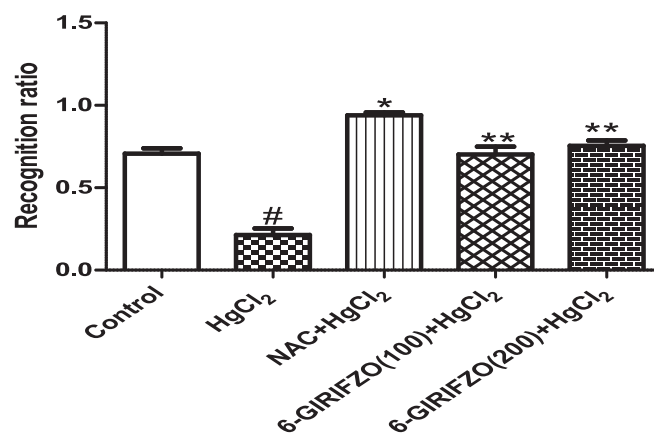


Fig. 6. Effect of 6-GIRIFZO on Novel object recognition test. Mean $\pm$ SEM was used to present the data, $n = 5$; control vs [#] $P \leq 0.05$; HgCl₂ vs ^{*} $P \leq 0.05$, ^{**} $P \leq 0.05$.

3.3. Biochemical results

In Fig. 7, HgCl₂ caused a significant increase ($p \leq 0.05$) in AchE activity in the HgCl₂-treated group compared to the control group, however, administration of 6-GIRIFZO reversed the trend as seen in the decreased AchE activity in 6-GIRIFZO-treated groups compared to the HgCl₂-treated group. This result compares favourably with the NAC-treated group.

Fig. 8 shows that administration of HgCl₂ causes an increase in lipid peroxidation as shown by the significant increase ($p \leq 0.05$) in the MDA level of the HgCl₂-treated group compared to the control, however, administration of 6-GIRIFZO significantly reduced ($p \leq 0.05$) the MDA level in the 6-GIRIFZO groups compared to the HgCl₂-treated group. There is a significant reduction in the MDA level of NAC-treated group compared to the 6-GIRIFZO-treated groups.

In Fig. 9, administration of HgCl₂ causes a significant increase ($p \leq 0.05$) in NO level of the HgCl₂-treated group compared to the control group. However, administration of 6-GIRIFZO reversed the trend. More so, there is a significant reduction in the NO level of the 6-GIRIFZO (200 mg/kg)-treated group when compared with the NAC-treated group and 6-GIRIFZO (100 mg/kg)-treated group.

In Fig. 10, administration of HgCl₂ significantly decreased the GSH level in HgCl₂-treated group compared to the control group. However administration of 6-GIRIFZO significantly increased ($p \leq 0.05$) the GSH level in 6-GIRIFZO groups compared to the HgCl₂-treated group, and

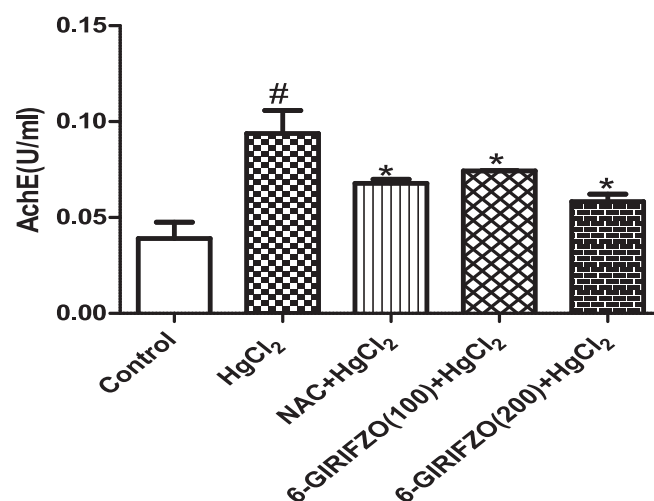


Fig. 7. Effect of 6-GIRIFZO on AchE activity. Mean $\pm$ SEM was used to present the data, $n = 5$; vs. control vs [#] $P \leq 0.05$; HgCl₂ vs ^{*} $P \leq 0.05$.

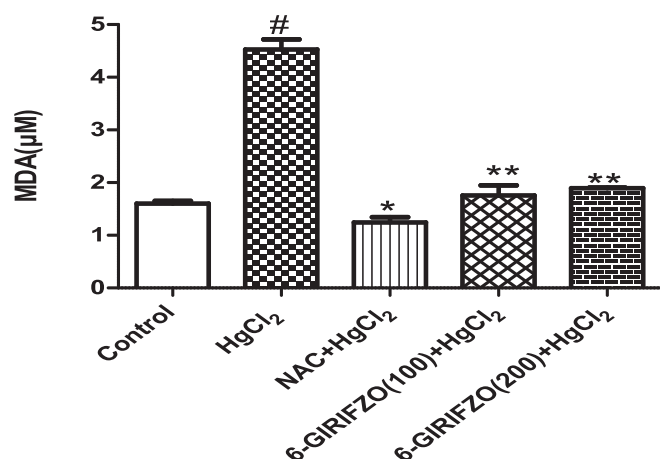


Fig. 8. Effect of 6-GIRIFZO on MDA level. Mean $\pm$ SEM was used to present the data, $n = 5$; control vs. $^{\#}P \leq 0.05$; HgCl₂ vs. $^{*}, **P \leq 0.05$.

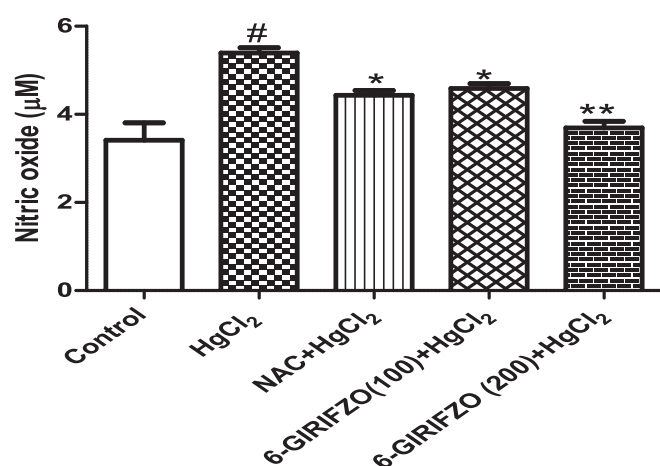


Fig. 9. Effect of 6-GIRIFZO on NO level. Mean $\pm$ SEM was used to present the data, $n = 5$; control vs. $^{\#}P \leq 0.05$; HgCl₂ vs. $^{*}, **P \leq 0.05$.

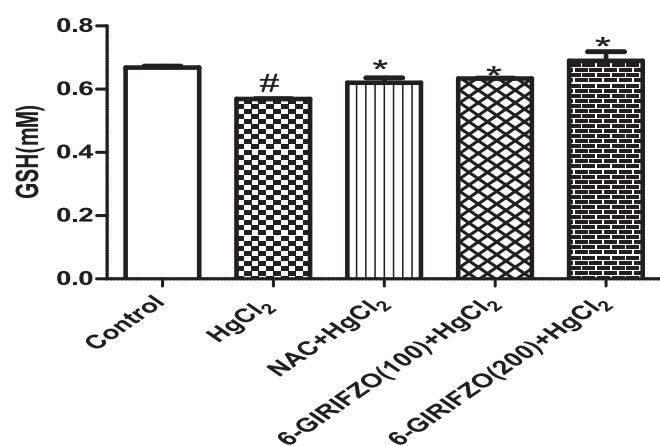


Fig. 10. Effect of 6-GIRIFZO on GSH level. Mean $\pm$ SEM was used to present the data, $n = 5$; control vs. $^{\#}P \leq 0.05$; HgCl₂ vs. $^{*}P \leq 0.05$.

this result compares favourably with the standard drug group.

In Fig. 11, administration of HgCl₂ causes a significant decrease ($p \leq 0.05$) in the catalase activity of the HgCl₂-treated group compared to the control. Administration of 6-GIRIFZO causes a significant increase ($p \leq 0.05$) in the catalase activity in the 6-GIRIFZO groups compared to the

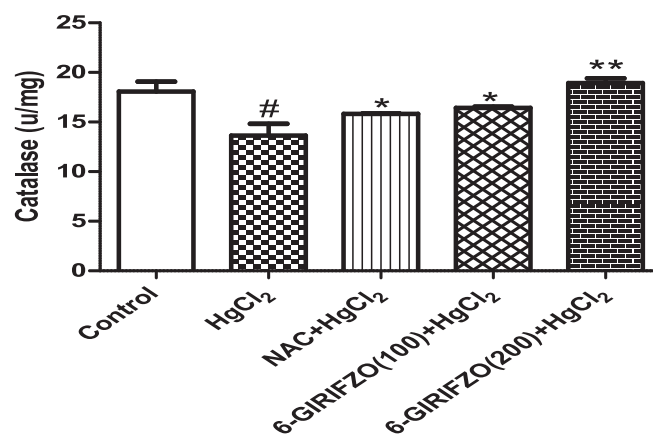


Fig. 11. Effect of 6-GIRIFZO on Catalase activity. Mean $\pm$ SEM was used to present the data, $n = 5$; vs. control vs. $^{\#}P \leq 0.05$; HgCl₂ vs. $^{*}, **P \leq 0.05$.

HgCl₂-treated group. Moreover, there is a significant increase in the catalase activity of the 6-GIRIFZO (200 mg/kg)-treated group when compared with the NAC-treated group and 6-GIRIFZO (100 mg/kg)-treated group.

Fig. 12 shows that there is a significant decrease ($p \leq 0.05$) in GPx activity of the HgCl₂-treated group compared to the control. However, there is a significant increase ($p \leq 0.05$) in GPx activity of the 6-GIRIFZO groups compared to the HgCl₂-treated group. This result conforms favourably with the NAC group.

In Fig. 13, there is a significant decrease ($p \leq 0.05$) in the SOD activity of the HgCl₂-treated group compared to the control. However there is a significant increase ($p \leq 0.05$) in the SOD activity of the 6-GIRIFZO groups compared to the HgCl₂-treated group. This result conforms favourably with the NAC-treated group.

In Fig. 14, administration of HgCl₂ causes a significant increase ($p \leq 0.05$) in the TNF- α level of the HgCl₂-treated group compared to the control. However, administration of 6-GIRIFZO causes a significant decrease ($p \leq 0.05$) in the TNF- α level of the 6-GIRIFZO groups compared to the HgCl₂-treated group. Also there is a significant decrease in the TNF- α level of the 6-GIRIFZO-treated groups compared to the NAC-treated group.

In Fig. 15, administration of HgCl₂ causes a significant increase ($p \leq 0.05$) in the level of IL-1 β of the HgCl₂-treated group compared to the control group. Administration of 6-GIRIFZO causes a significant reduction ($p \leq 0.05$) in the level of IL-1 β of the 6-GIRIFZO groups compared to the HgCl₂-treated group. There is a significant reduction in the level of IL-1 β of the 6-GIRIFZO (100 mg/kg)-treated group when compared with

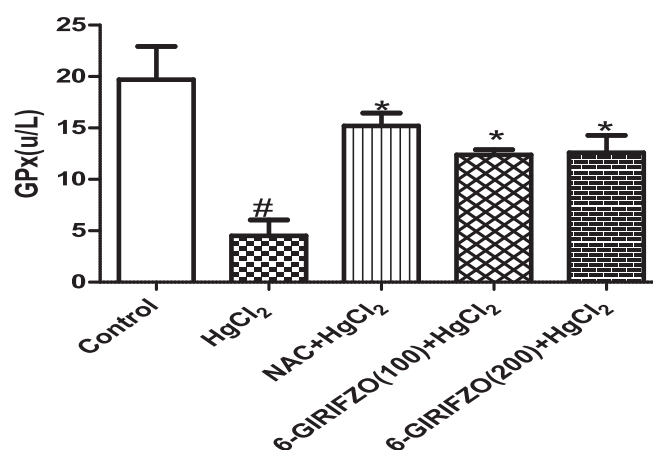


Fig. 12. Effect of 6-GIRIFZO on GPx activity. Mean $\pm$ SEM was used to present the data, $n = 5$; control vs. $^{\#}P \leq 0.05$; HgCl₂ vs. $^{*}P \leq 0.05$.

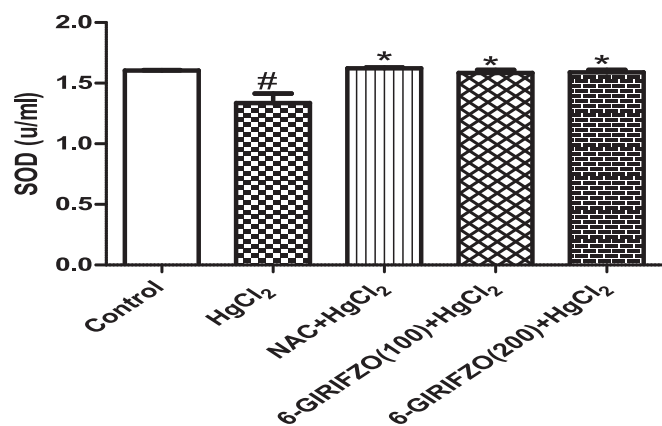


Fig. 13. Effect of 6-GIRIFZO on SOD activity. Mean $\pm$ SEM was used to present the data, $n = 5$; control vs. $\#P \leq 0.05$; HgCl₂ vs. $*P \leq 0.05$.

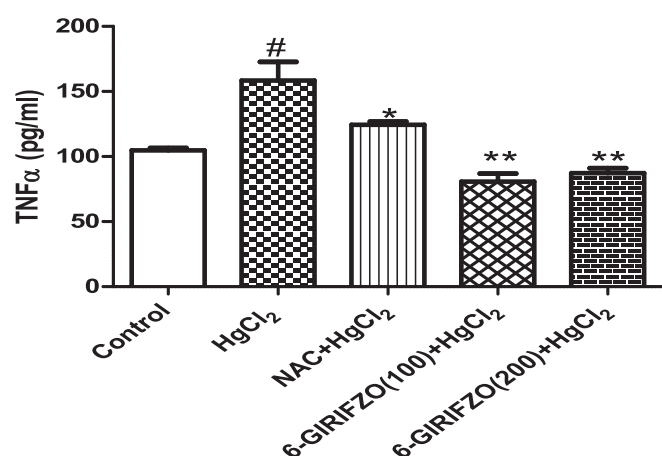


Fig. 14. Effect of 6-GIRIFZO on TNF-α level. Mean $\pm$ SEM was used to present the data, $n = 5$; control vs. $\#P \leq 0.05$; HgCl₂ vs. $*P \leq 0.05$, $**P \leq 0.05$.

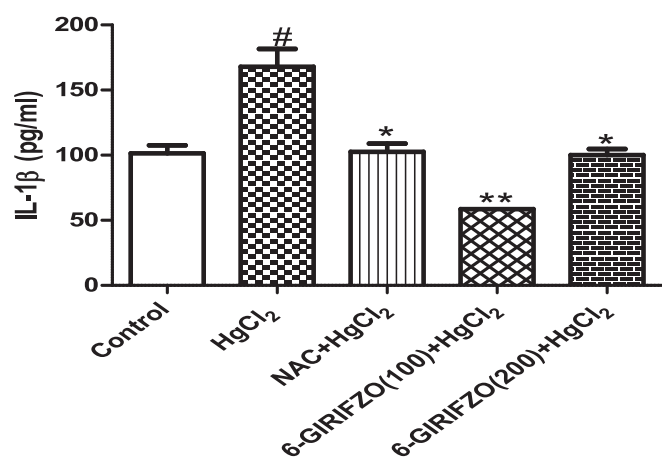


Fig. 15. Effect of 6-GIRIFZO on IL-1β level. Mean $\pm$ SEM was used to present the data, $n = 5$; control vs. $\#P \leq 0.05$; HgCl₂ vs. $*P \leq 0.05$, $**P \leq 0.05$.

the NAC-treated group and 6-GIRIFZO (200 mg/kg)-treated group.

In Fig. 16, administration of HgCl₂ causes a significant increase ($p \leq 0.05$) in the NF-κB level of the HgCl₂-treated group compared to the control. However administration of 6-GIRIFZO causes a significant reduction ($p \leq 0.05$) in the NF-κB level of the 6-GIRIFZO groups compared to the HgCl₂-treated group. This result conforms favourably

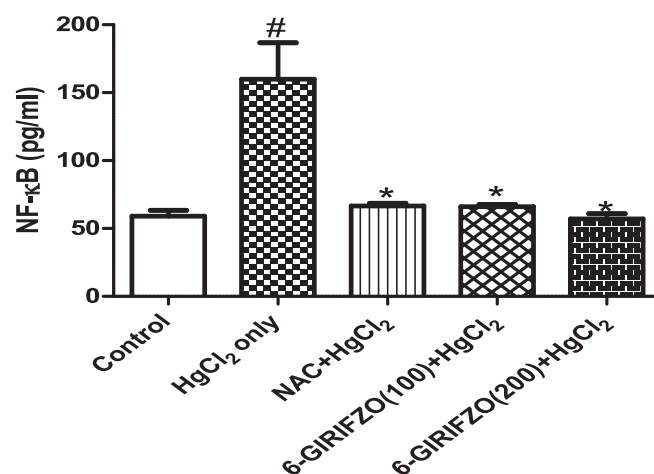


Fig. 16. Effect of 6-GIRIFZO on NF-κB level. Mean $\pm$ SEM was used to present the data, $n = 5$; control vs. $\#P \leq 0.05$; HgCl₂ vs. $*P \leq 0.05$.

with the NAC-treated group.

Fig. 17 shows that there is a significant increase ($p \leq 0.05$) in the IL-6 of the HgCl₂-treated group compared to the control. However, there is a significant reduction ($p \leq 0.05$) in the IL-6 level of the 6-GIRIFZO groups compared to the HgCl₂-treated group. Also, this result conforms favourably with the NAC-treated group.

3.4. Histological result

The impact of 6-GIRIFZO on the CA3 region of the hippocampus is depicted in Fig. 18. The hippocampus tissue in the control group is normal and it is made up of preserved neuronal bodies encased in fibrillary glial matrix. In the hippocampus, the molecular and granular layers are still there. Only a few neurons were seen in the HgCl₂-treated group, indicating degenerative alterations or inflammation. The 6-GIRIFZO and NAC groups display healthy brain tissue with preserved neuronal bodies encased in fibrillary glial matrix. In the hippocampus, the molecular and granular layers are still there. There are no signs of severe inflammation or degenerative changes. The results are in line with typical histology.

4. Discussion

We investigated the antioxidant and free radical scavenging abilities of 6-GIRIFZO using DPPH, ABTS and FRAP assays. The 6-GIRIFZO was

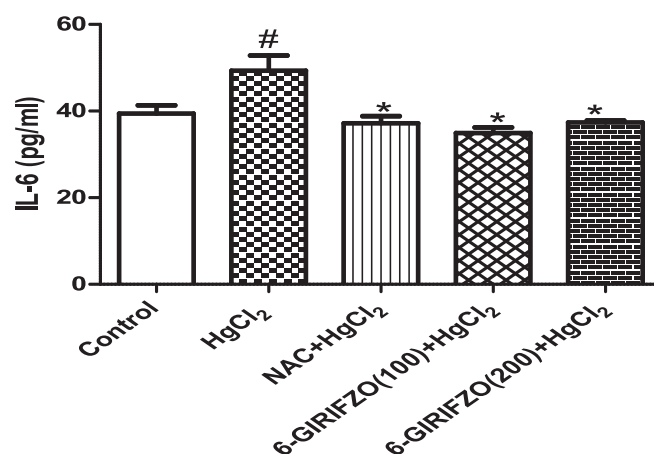


Fig. 17. Effect of 6-GIRIFZO on IL-6 level. Mean $\pm$ SEM was used to present the data, $n = 5$; control vs. $\#P \leq 0.05$; HgCl₂ vs. $*P \leq 0.05$.

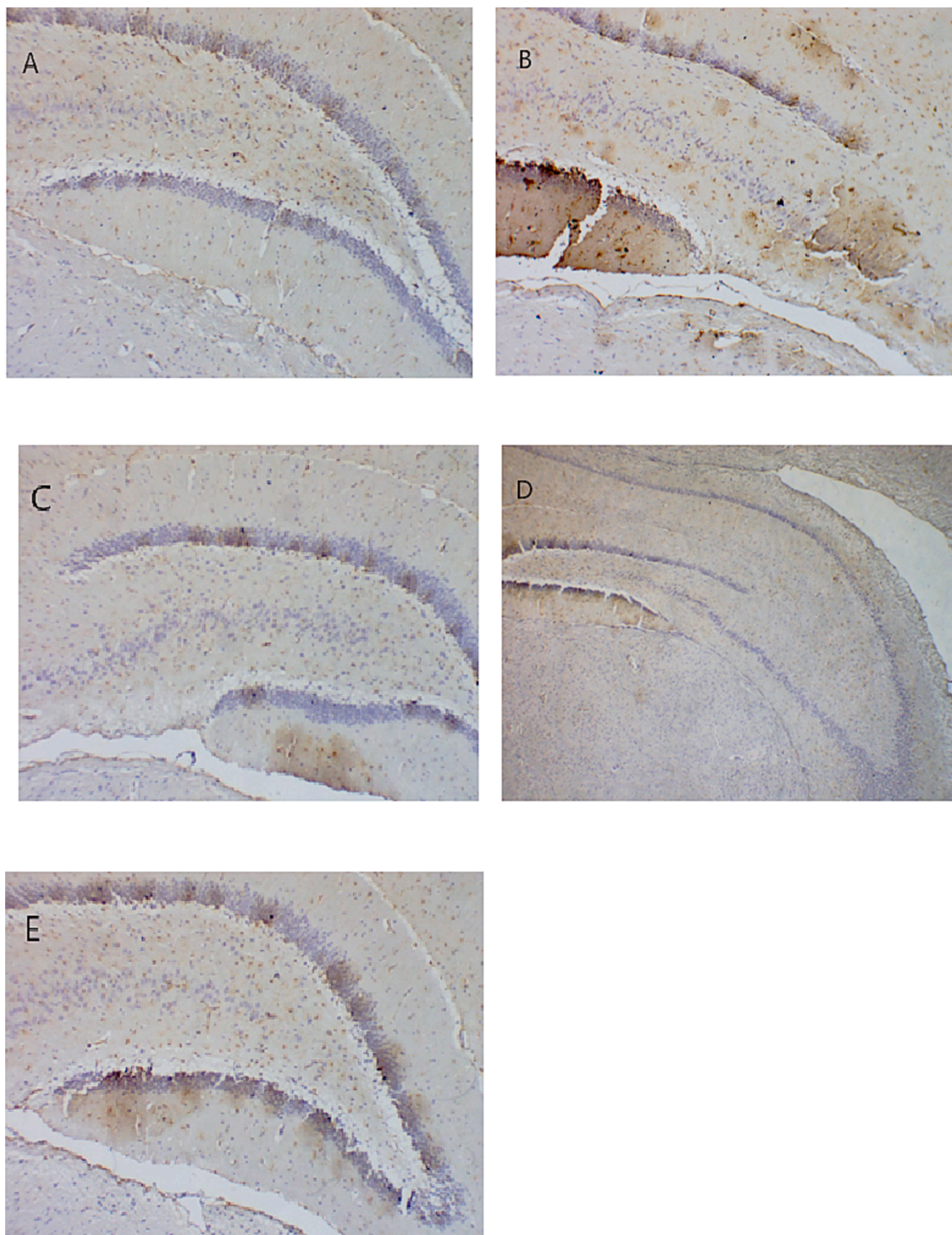


Fig. 18. Effect of 6-GIRIFZO on the CA3 of the Hippocampus. H&E stain; $\times 400$ magnification; 100 μm scale bar. A, control; B, HgCl_2 ; C, NAC + HgCl_2 ; D, 6-GIRIFZO (100) + HgCl_2 ; E, 6-GIRIFZO (200) + HgCl_2 .

able to convert the stable radical DPPH in the DPPH assay into the yellow diphenyl-picrylhydrazine (DPPH-H). This is predicated on 6-GIRIFZO's capacity to donate hydrogen to decrease DPPH in alcoholic solution, which resulted in the reaction's creation of the non-radical form DPPH-H (Gulcin et al., 2007). In general, 6-GIRIFZO and the reference antioxidant (Ascorbic acid) both showed an increase in DPPH scavenging activity with increasing doses, indicating an improvement in the 6-GIRIFZO capacity to donate hydrogen ions to the radical. This is consistent with the findings of Adebisi et al. (2017) who investigated the total flavonoid and phenolic levels, and *in vitro* antioxidant property of ethanol extract of *Grewia carpinifolia* stem and leaf.

The results of the ABTS assay further demonstrate 6-GIRIFZO's potent antioxidant properties by showing its capacity to successfully scavenge the ABTS free radical.

From this study, FRAP assay demonstrated 6-GIRIFZO's potent reducing capacity by converting ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}), a process that becomes more effective as 6-GIRIFZO concentration rises. In all the assays, the maximum inhibitory concentrations (IC_{50}) of 6-GIRIFZO is less than that of the standard showing its antioxidant potency.

MWMT and NORT are used for the assessment of spatial learning and memory by measuring the EL and RR of the tested rats respectively. HgCl_2 has the ability to cause cognitive decline and behavioral deficit in Wistar rats, as evidenced by a significant increase in the EL and a significant reduction in the RR of the group treated with it (Owoeye and Arinola, 2017).

It's interesting to note that different doses of GIRIFZO (both 100 mg/kg and 200 mg/kg body weight) significantly protected against the neurobehavioural impairments caused by HgCl_2 that were seen in this investigation. This may be due to 6-GIRIFZO's ability to pass through the blood brain barrier and exerts its effect on the brain as reported by Simon et al. (2020).

Exposure to HgCl_2 may cause cholinergic or cognitive deficits by elevating the AChE activity (Ishola et al., 2017). The capacity of 6-GIRIFZO to prevent HgCl_2 from enhancing AChE activities may account for the protective effect of 6-GIRIFZO against HgCl_2 -induced cognitive loss observed in this investigation.

The generation of reactive oxygen species (ROS) by HgCl_2 is known to cause oxidative stress, which in turn causes the cell membrane to become unstable and disintegrate due to lipid peroxidation. Our research shows that the administration of HgCl_2 caused MDA levels to significantly rise. HgCl_2 may therefore cause biochemical and functional changes in the brain tissues via increasing lipid peroxidation. On the other hand, reduced GSH and the production of superoxide radicals are also linked to HgCl_2 toxicity. Due to the thiol (-SH) group, GSH functions as an intracellular antioxidant and mercury carrier. In actuality, GSH acts as the first line of defense for cells against HgCl_2 -containing substances. The quantity of GSH in the cell and the cell's antioxidant capacity are reduced as a result of HgCl_2 's binding to glutathione and subsequent removal of glutathione from the cells. According to the current study, rats given HgCl_2 had significantly lower levels of brain GSH. Joshia et al. (2017) noted a marked decline in GSH levels in the tissues of the kidney and liver. Our research demonstrates that pre-treatment with 6-GIRIFZO shielded rat brain tissue from HgCl_2 -induced oxidative damage and significantly restored the elevated MDA and lowered GSH levels. This may be due to 6-GIRIFZO's outstanding antioxidant qualities, which have already been demonstrated in this study by the findings from *in vitro* antioxidant experiment. ROS and oxidative damage are prevented by the enzymatic antioxidant system, which comprises CAT, GPx and SOD. The recovery of these biochemical variables demonstrated GIRIFZO's capacity to protect against brain damage brought on by exposure to HgCl_2 in the experimental rats. Our results might be explained by the GIRIFZO's high antioxidant activity, which can be seen in its ability to scavenge a variety of free radicals, protect the lipid cell membrane from oxidation, reduce lipid peroxidation, and boost the levels of antioxidant enzymes (Farombi et al., 2018).

According to Cui et al. (2018), antioxidants are known to inactivate

NF- κ B, and reduce the levels of pro-inflammatory mediators like TNF- α . The anti-inflammatory action of 6-GIRIFZO observed in this study might be due to the blockage of the activation of NF- κ B transcription factor, thus preventing the production of downstream pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6, and the free radicals, such as ROS and NO.

The CA3 sections of the hippocampus showed neuronal degeneration in rats treated with only HgCl_2 , along with certain neurons having pyknotic nuclei or karyolysis. These findings are similar with earlier research by Owoeye and Farombi (2015). Rats treated with only HgCl_2 in the cerebellum had Purkinje cells that completely lost all of their nuclear material, proving the toxicity of HgCl_2 (Uma et al., 2012). The morphological abnormalities in the rats caused by HgCl_2 were however, prevented by pre-treatment with 6-GIRIFZO.

5. Conclusion

Rats exposed to HgCl_2 experienced behavioral deficits, changes in their antioxidant defenses, an upsurge in lipid peroxidation, and an increase in inflammation, all of which led to neurotoxicity. This study makes a case for 6-GIRIFZO's function in preventing neurotoxicity brought on by HgCl_2 in the brain. 6-GIRIFZO as natural compound can be a unique therapeutic candidate for the treatment of neurodegenerative illnesses. This would surely be a better option than some synthetic medications that are now on the market, which can be expensive and have negative side effects.

CRediT authorship contribution statement

Abraham Olufemi Asuku: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Maryam Tayo Ayinla:** Writing – review & editing, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Ayodeji Johnson Ajibare:** Visualization, Validation, Software, Project administration, Data curation. **Olajide Tobiloba Samuel:** Visualization, Methodology, Investigation, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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