Ameliorative effect of ethanol extract of *Eragrostis tremula* Hochst. ex Steud. against diazepam-induced amnesia in mice

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Abstract

**Background:** *Eragrostis tremula* Hochst. ex Steud. (Poaceae) is used in ethno-medicine as a memory enhancer. Studies have shown that the whole plant possesses memory enhancing potentials and could be beneficial in the management of amnesia and cognitive deficit.

**Aim:** This study was aimed at investigating the actions of *E. tremula* extract on diazepam-induced amnesia in mice. Acute toxicity profiling was done as stated by the Organization for Economic Co-operation and Development (OECD 425). Oral doses of 125, 250 and 500 mg/kg of *E. tremula* extract were used for the diazepam-induced amnesia studies. Cognitive function was evaluated using elevated plus maze (EPM) and novel object recognition tests (NORT). The brain tissues were evaluated for the concentrations of malondialdehyde (MDA), reduced glutathione (GSH) and superoxide dismutase (SOD) using enzyme-linked immunosorbent assay kits.

**Results:** The oral median toxic dose of *E. tremula* extract was assessed to be > 5000 mg/kg in mice. The extract substantially (*p* < 0.05) reduced the transfer latency of mice during the retention phase of EPM test. In the NORT, *E. tremula* extract at all the doses appreciably (*p* < 0.05) reduced the exploration time on the familiar object. Also, it substantially (*p* < 0.05) improved the recognition index. *E. tremula* extract substantially (*p* < 0.05) reduced the MDA levels, and at doses of 250 and 500 mg/kg, it prevented the cortical and hippocampal tissues from lesions produced by diazepam.

**Conclusions:** *Eragrostis tremula* extract is practically safe after acute administration and possesses anti-amnesic actions.

**Keywords:** Acute toxicity, Amnesia, Diazepam, *Eragrostis tremula*, Oxidative stress

Background

Alzheimer’s dementia is an ongoing neurodegenerative malady and a typical cause of dementia (Silva et al. 2014; Thakur et al. 2018). Approximately, 50 million victims of dementia exist worldwide and the number is expected to grow up to more than 150 million by 2050, increasing especially in low- and middle-income nations where about 65% of people with dementia live (Livingston et al. 2020). Amnesia, cognitive and behavioural dysfunction are the primary symptoms associated with Alzheimer’s dementia (Lane et al. 2018). This negatively affects the daily activities and quality of life because it impairs learning and memory functions, as well as intellectual ability (Sandry 2015). The treatment of this debilitating disease is still far from been established (Mansouri et al. 2016). This is so because apart from the associated adverse effects of the approved drugs, they only mask the symptoms and or delay the progression of the disease but do not provide cure (Cummings et al. 2019; Sharma 2019).
As such, other possibilities including natural products from medicinal plants are continuously investigated to provide a cure against the disease and its associated consequences (Vyas et al. 2019).

Investigation on medicinal herbs has led to the breakthrough of bioactive constituents which act as lead compounds for the development of drugs against many therapeutic targets (Uddin et al. 2018). Accordingly, investigation of bioactive compounds from medicinal plants that will be valuable in the treatment of cognitive dysfunction is also increasing (Perry and Howes 2011; Tewari et al. 2018; Vyas et al. 2019) and at present, many medicinal plants have been shown to prevent cognitive dysfunction and impede the advancement of Alzheimer's disease (Akram and Nawaz 2017; Wightman 2017; Kim et al. 2020). One of such plants found in Nigerian traditional medicine is Eragrostis tremula. Eragrostis tremula Hochst. ex Steud. is an annual grass and a member of Poaceae family. It is commonly called “Love grass” in English, and in Nigerian languages as “Burburwa” (Hausa), “Ariran” (Yoruba), “Dutaleho” (Fulfulde) and “Berberinoa” (Nupe). E. tremula is used in ethno-medicine as a lactation stimulant, aphrodisiac, memory enhancer and antidote to snake bites (Burkill 1985; Poilecot et al. 2007; Nazifi et al. 2019). Previously, the plant extract has been shown to contain important phytochemical constituents (alkaloids, flavonoids, saponins, steroids and triterpenes) with memory enhancing potentials and found useful in the management of cognitive deficit (Nazifi et al. 2019). This study, therefore, was conducted to evaluate the anti-amnesic properties of the whole plant extract of E. tremula in experimental animal models.

**Methods**

**Drugs and chemicals**

Donepezil (Ranbaxy Laboratories Limited, U.S.A.); diazepam (Valium®, Roche, Italy); Ethanol (Sigma-Aldrich, U.S.A.); mouse malondialdehyde (MDA, CK-bio-19577); reduced glutathione (GSH, CK-bio-16863) and superoxide dismutase (SOD, CK-bio-16990) enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Coon Koon Biotech., China).

**Experimental animals**

Swiss Albino male mice (18–22 g) were acquired from the animal house of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The mice were maintained in regular cages with access to water and fodder and kept under natural day and light cycle. Before starting the experiment, the animals were taken to the behavioural room (room temperature 23±3 °C, relative humidity 35–70%) for 1 week to acclimatize to the laboratory environment. The experimental protocols were authorized by Ahmadu Bello University Committee on Animal Use and Care (Approval number: ABUCAUC/2020/68). Animal maintenance and treatment were also performed according to the guidelines set by Animal Research: Reporting of In Vivo Experiments (ARRIVE).

**Plant material**

The intact plant of E. tremula was obtained from Babbar Ruga, Batagarawa Local Government area of Katsina State, Nigeria, in October, 2018. It was ascertained by Mr. Sanusi Namadi in the Department of Botany, Ahmadu Bello University, Zaria. A voucher number (900729) was issued by matching with a reference voucher specimen formerly kept in the herbarium.

**Plant extraction**

The whole plant was air-dried under shade and then milled using mortar and pestle. The pulverized plant material (1840 g) was subjected to cold maceration with 10 L of 70% ethanol (70% absolute ethanol and 30% water) for 14 days. The filtrate obtained was concentrated with the aid of a rotary evaporator and the extractive yield was determined. The extract was labeled as Eragrostis tremula extract (ETE) and then stored in a desiccator until needed for further studies.

**Preparation of stock solutions**

Stock solutions of the extract and donepezil were prepared by dissolving a specified quantity in distilled water followed by serial dilution to acquire the correct concentrations for the studies. Similarly, stock solution of diazepam was prepared by diluting 0.2 mL of 5 mg/mL diazepam with 9.8 mL distilled water to obtain 10 mL of 0.1 mg/mL concentration. The drug solutions were prepared fresh for each day's experiment to preserve their stability.

**Acute toxicity study**

The up-and-down method as described by the Organization for Economic Co-operation and Development (OECD) was adopted to establish the acute oral toxicity profile of ETE in mice (OECD 2001). Two groups of 5 mice each were used (n=5); the first group was designated as control (administered distilled water, 10 mL/kg), while the second group (test group) of mice were sequentially administered a single dose of ETE (5000 mg/kg) by oral gavage (p.o). The mice were deprived of food (but not water for 3–4 h) before dosing and afterward. They were carefully observed during the first 24 h and then daily for two weeks, after which the median lethal dose (LD50) was estimated. Their body weight was recorded weekly, and after the 14 days
observation, they were sedated under soft chloroform and then euthanized. Blood samples were collected for haematology and essential organs (livers, kidneys and hearts) were harvested for histology.

Experimental design for neurobehavioural studies
Neurobehavioural studies to evaluate the effect of ETE was done using diazepam-induced amnesia in mice (Dhingra and Kumar 2012). A total of 42 mice were used which were randomly divided into 6 groups of seven mice each (n = 7). The mice were subjected sequentially to elevated plus maze (EPM) and novel object recognition tests (NORT). For each study, the mice were pretreated with graded doses of ETE (125, 250 and 500 mg/kg, p.o) an hour before each trial, and diazepam (1 mg/kg, i.p.) was given 30 min. before each test (Table 1).

Elevated plus maze test
The EPM test was carried out to assess retention memory (Komada et al. 2008). The EPM paradigm was made up of two open and two enclosed arms of the same size (35 × 5 cm) with 15 cm high wooden walls. The arms were extended from central platform (5 cm × 5 cm) and elevated 60 cm above the floor. The mice were subjected to the EPM test for 2 days. On the first day (acquisition test), each mouse was kept at the end of one open arm, facing away from the centre. The transfer latency (time taken for the mouse to move from the open to the enclosed arms) was documented within 60 s with the aid of a video camera (DCR-PJ5E, Sony Corporation, Japan). An animal is considered to have moved into an arm when its four paws are over the line separating the area and the centre. The transfer test (time taken for the mouse to move from the open to the enclosed arms) was documented within 60 s with the aid of a video camera (DCR-PJ5E, Sony Corporation, Japan). An animal is considered to have moved into an arm when its four paws are over the line separating the area and the centre. The transfer test was conducted each mouse was observed for 60 s. The platform was wiped with a cotton wool dipped in 70% ethanol after each trial to get rid of any olfactory hint.

Novel object recognition test
The NORT was carried out to assess learning and recognition memory (Ennaceur 2010; Lueptow 2017). The paradigm consisted of a Plexiglas box of 40 cm × 40 cm × 40 cm in dimension. The NORT consist of 3 phases: the habituation, training and testing phases. In the habituation phase (day 1), each mouse was permitted to explore the arena without object for 2 min and then taken back to the home cage. In the second day (training phase), two similar objects were placed in opposite quadrants of the arena 20 cm apart from each other and 5 cm away from the walls of the paradigm. Each animal was permitted to survey the identical objects for 10 min and then returned to the holding cage. In the testing phase (24 h after training), one of the objects was substituted with another one of different colour and size (novel object). One hour after treatment, each mouse was reintroduced into the arena and permitted to explore for 5 min. The behaviour of each mouse was recorded with the aid of a video recorder positioned above the apparatus. The exploration time for novel and familiar objects were taken and the recognition index was computed using the relationship:

\[ \text{Recognition index (RI)} = \left( \frac{b}{e_2} \right) \times 100 \]

where \(b\) = time spent exploring novel object; \(e_2\) = total exploration time on familiar and novel objects during testing phase.

Biochemical analyses
After the behavioural studies, the mice were anaesthetized under light chloroform and then euthanized. The brains were removed, washed with cold isotonic saline solution and then homogenized with 10 times (\(10^\text{th}\) ice cold phosphate buffer (0.1 M, pH 7.4) to produce 10% \(\text{w/v}\) homogenates. The homogenates were centrifuged at 10, 000 rpm for 15 min and the supernatants were used for estimations of MDA, GSH and SOD using mouse specific ELISA kits in line with the manufacturers’ description. Following the instructions for each kit, 50 µL of standard was added into the designated

| Group | Designation      | Treatment                      | Routes |
|-------|------------------|--------------------------------|--------|
| I     | Normal group     | Distilled water 10 mL/kg       | p.o.   |
| II    | Negative control | Distilled water 10 mL/kg + Diazepam 1 mg/kg | p.o/i.p. |
| III   | Positive control | Donepezil 5 mg/kg + Diazepam 1 mg/kg | p.o/i.p. |
| IV    | Test group 1     | ETE 125 mg/kg + Diazepam 1 mg/kg | p.o/i.p. |
| V     | Test group 2     | ETE 250 mg/kg + Diazepam 1 mg/kg | p.o/i.p. |
| VI    | Test group 3     | ETE 500 mg/kg + Diazepam 1 mg/kg | p.o/i.p. |
standard wells. Into the sample wells, 10 µL of samples and 40 µL of diluent were added sequentially and gently mixed. Horseradish peroxidase conjugate (100 µL) was then added to all sample wells. The wells were covered with a membrane and incubated at 37 °C for 60 min. The reaction mixture was discarded and the wells were washed 5 times with the wash solution to remove unbound enzyme and then dried. Chromogen A and B (50 µL each) were added for colour development. The samples were further incubated at 37 °C for 15 min. Thereafter, 50 µL stop solution (0.16 M sulfuric acid) was added to each well to discontinue the reaction. The absorbance were measured at 450 nm and the concentration of each marker was calculated from the regression curve of the standard markers.

Statistical analysis
Data generated were analysed using Statistical Package for Social Sciences (SPSS) software (Version 20). Variations between means were analysed using independent sample t-test (where only two groups are involved) and one-way analysis of variance (ANOVA) followed by Bonferroni’s post hoc test. Values of $p < 0.05$ were considered as significant in all the statistical tests. Data obtained were expressed as mean±standard error of the mean (S.E.M.).

Results

Extractive value
Extraction of 1840 g of the powdered plant material produced 189.8 g of extract. The percentage yield was thus calculated to be 10.31% w/w.

Acute toxicity profile of *E. tremula* extract in mice
The single oral administration of ETE (5,000 mg/kg body weight) in mice did not produce signs of toxicity or death during the 14 days observation period. The oral LD$_{50}$ of ETE was thus determined to be > 5000 mg/kg body weight in mice. Also, there were no substantial changes in skin, behaviour, body weights and relative organ weights of the extract treated mice when compared to control (Fig. 1).

Haematologic investigation showed a considerable decrease ($p < 0.05$) in the levels of white blood cells.
(WBC) and lymphocyte counts, while the granulocyte counts were significantly ($p<0.01$) increased when compared to control. However, there were no noticeable changes in the levels of red blood cells (RBC), hemoglobin, hematocrit and platelets after the 14 days observation period (Table 2). Histology of the livers and hearts showed normal features, but, moderate lymphocyte hyperplasia was observed with the kidneys of ETE treated mice (Fig. 2).

**Effect of ETE on transfer latencies of mice on EPM following diazepam-induced amnesia**

The administration of ETE at all the tested doses did not produce significant ($p>0.05$) changes in the transfer latency of mice during the acquisition phase (day 1) of the study when compared to the negative control. In the retention phase (day 2), the administration diazepam significantly ($p<0.05$) increased the mean transfer latency when compared to the negative control. However, pretreatment of mice with ETE at all the tested doses substantially ($p<0.01$) reduced the mean transfer latencies

### Table 2: Effect of acute administration of *Eragrostis tremula* extract on haematological parameters

| Parameters | Units | Treatment          | D/W 10 mL/kg | ETE 5000 mg/kg |
|------------|-------|--------------------|--------------|----------------|
| WBC        | ($\times 10^3/\mu$L) | 5.13 ± 0.03 | 3.70 ± 0.25* |
| LYMP       | (%)   | 50.95 ± 4.51       | 35.97 ± 3.28* |
| GRAN       | (%)   | 31.20 ± 0.12       | 47.08 ± 3.90** |
| RBC        | ($\times 10^6/\mu$L) | 4.88 ± 0.04 | 5.18 ± 0.49 |
| HGB        | (g/dL) | 13.50 ± 0.06       | 12.50 ± 0.92 |
| HCT        | (%)   | 40.27 ± 0.15       | 38.70 ± 2.91 |
| PLT        | ($\times 10^3/\mu$L) | 306.00 ± 0.58 | 341.00 ± 51.83 |

Values are mean ± S.E.M

* = $p < 0.05$, ** = $p < 0.01$ as compared to D/W group—Independent sample t-test, $n = 5$, D/W = distilled water, ETE = *Eragrostis tremula* extract, WBC = white blood cells, PLT = platelets, HCT = hematocrit, LYMP = lymphocytes, HGB = haemoglobin, GRAN = granulocytes, RBC = red blood cells

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**Fig. 2** Photomicrograph of sections of livers, kidneys and hearts of mice. a Liver of control group showing normal hepatocytes, b kidney of control group showing tubules (red arrow) and glomerulus (white arrow), c Heart of control group showing normal cardiac cells, d Liver of ETE (5000 mg/kg) treated group showing normal hepatocytes, e kidney of ETE (5000 mg/kg) treated group showing moderate lymphocyte hyperplasia (white arrow), f Heart of ETE (5000 mg/kg) treated group showing normal cardiac cells, (H&E × 250)
against the negative control. Similarly, the positive control (Donepezil, 5 mg/kg) significantly \((p < 0.05)\) reduced the mean transfer latencies when compared to the negative control (Fig. 3).

**Effect of ETE on object recognition following diazepam-induced amnesia**

In the NORT, diazepam administration significantly \((p < 0.01)\) reduced the exploration time towards the novel object when compared to the negative control. However, the groups treated with ETE (125, 250 and 500 mg/kg) and the positive control (donepezil, 5 mg/kg) spent longer time exploring the novel object. The exploration time towards the familiar object was significantly \((p < 0.05)\) reduced with the extract (125 and 250 mg/kg) and donepezil treated groups when compared to the negative control. The recognition indices for ETE treated groups (125, 250 and 500 mg/kg) were significantly \((p < 0.01, p < 0.05\) and \(p < 0.05)\) respectively when compared to the negative control. Similarly, the recognition index of donepezil treated group was significantly \((p < 0.01)\) increased when compared to the negative control (Fig. 4).

**Effect of ETE on MDA, GSH and SOD levels following diazepam-induced amnesia**

The administration of diazepam produced a remarkable \((p < 0.05)\) increase in the MDA levels when compared to the normal group. On the other hand, the positive control (donepezil, 5 mg/kg) and ETE at all the tested doses significantly \((p < 0.05)\) reduced the MDA levels contrary to the negative control. The levels of GSH and SOD were not significantly altered \((p > 0.05)\) when compared to the normal group. Also, pre-treatment with donepezil and ETE at all the tested doses did not produce significant \((p > 0.05)\) changes in the GSH or SOD levels when compared to the negative control (Fig. 5).

**Effect of ETE on hippocampal and cortical tissues following diazepam-induced amnesia**

Histology of brain sections following diazepam-induced amnesia showed neuronal atrophy and loss of pyramidal cells of the dentate gyrus in the negative control. In the positive control group, examination of the hippocampus showed slight hyperplasia of pyramidal cells. Moderate pyramidal cell necrosis was observed with the extract at 125 mg/kg. However, there were no noticeable pathological changes in the cortical and pyramidal cells of the extract treated groups at doses of 250 and 500 mg/kg (Fig. 6).
Discussion

Eragrostis tremula extract is used in ethno-medicine to enhance memory. Previously, it was reported to possess phyto-constituents with memory enhancing properties and to be useful against amnesia and cognitive deficit (Nazifi et al. 2019). Reports on the effect of E. tremula in amnesic condition were scarce, thus, we investigated the actions of ETE against diazepam-induced amnesia using behavioural animal models to justify its folkloric claim and to serve as basis for further research on novel anti-amnesic agents. The results showed that ETE significantly ameliorated the memory deficit seen in EPM and object recognition tests. Also, it prevented the oxidative stress and histopathological lesions induced by diazepam on cortical and hippocampal tissues.

Acute toxicity evaluation is important in preclinical studies because it assesses the adverse effects that might occur due to deliberate or inadvertent short-term exposure to chemicals (Erhirhie et al. 2018). It also serves as a yardstick in dose selection for efficacy studies. The oral LD$_{50}$ of ETE was estimated to be greater than 5000 mg/kg, and according to the classification of LD$_{50}$ values of chemicals (Loomis and Hayes 1996), the extract was considered practically safe in mice after oral administration.
The haematopoietic system represents an important target for toxic compounds, particularly the bone marrow where the production of RBCs occur (Kifayatullah et al. 2015). As such, haematological indices are universally used markers of toxicity because of their interaction with toxins or their metabolites (Abubakar et al. 2019). Increase in granulocyte (neutrophils) and lymphocyte counts occur under a variety of circumstances, usually related to spontaneous inflammatory disease, stress or secondary to treatment-induced tissue damage in other organs (Greaves 2012). Granulocytes are the most common form of WBCs that increase during bacterial infections, while lymphocytes can increase in cases of viral infections (Merriman 2014). In this study, a significant increase in granulocytes count was observed after acute administration of ETE which translates to possibility of bacterial infection or stress associated with the group.

Benzodiazepines are known to produce anterograde amnesia in both humans and animals (Wang et al. 2011; Ferdousy et al. 2016). They bind to benzodiazepine binding position of gamma amino butyric acid A (GABA<sub>A</sub>) receptors and potentiate GABAergic neurotransmission (Makkar et al. 2010). Enhanced GABA inhibition impairs the role of excitatory synapses, which is necessary for memory and thus causes amnesia (Murakami et al. 2017). The transfer latency into the closed arms of EPM has been used as a parameter to assess retention memory (Dhingra et al. 2004). It has been established to be shorter if the animal had previously entered the closed arms (Morales-Delgado et al. 2018). In this study, diazepam increased the mean transfer latency into the closed arms of the EPM which signifies memory impairment. However, pretreatment with ETE significantly decreased the transfer latencies in the retention phase which indicates an enhancement in retention memory. Therefore, the ability of ETE to prevent diazepam-induced amnesia in the EPM test implies that its cognitive enhancement could be mediated through modulation of GABAergic neurotransmission among other mechanisms.

**Fig. 6** Photomicrograph of brain sections showing the effect of *Eragrostis tremula* following diazepam-induced amnesia in mice. D/W = control group showing normal cortex and pyramidal cells; DZP = Diazepam-alone group showing atrophy of neuron (yellow arrow) and loss of pyramidal cells (blue arrow); DPZ = donepezil treated group showing slight hyperplasia of pyramidal cells; ETE 125 mg/kg = *E. tremula* treated group showing moderate pyramidal cell necrosis (red arrow); ETE 250 and 500 mg/kg = *E. tremula* treated group showing normal features; (H&E × 100)
The NORT is an accepted method for assessing recognition memory in mice based on their natural tendency for exploring novelty (Lueptow 2017). In this study, the groups treated with ETE showed a clear preference for novelty whereas the diazepam alone group failed the novelty preference test indicating memory impairment. Similar effects were observed with the recognition index; the extract and donepezil treated groups significantly improved the recognition index indicating the ability of the animals to retain preference for the novel object. This reveals that the extract ameliorated object recognition memory that was impaired by diazepam. Based on the behavioural tests, it can be inferred that ETE improved retention and recognition memory.

Oxidative stress results due to disparity between cellular generation of reactive oxygen species (ROS) and the power of cells to eliminate them using endogenous antioxidant defense mechanisms (Pizzino et al. 2017). The brain is more responsive to damage by oxidative pressure owing to its high oxygen content (Ma et al. 2018). Indeed, brain injury as a result of oxidative stress can cause impairment in learning and memory abilities (Fukui et al. 2002; Cheignon et al. 2018). In the same perspective, diazepam-induced memory deficit in rodents is associated with increased brain lipid peroxides (such as MDA) and reduced brain repository of antioxidants like GSH and SOD (Ferdousy et al. 2016; Sevastre-Berghian et al. 2017). MDA is an outcome of lipid peroxidation and a measure of free radical generation. Lipid peroxidation is a vital pointer of brain neurodegeneration which affects the wholeness of neuronal membrane and function (Shichiri 2014). GSH is a natural antioxidant found in all animal cells in varying concentrations and an indicator of oxidative stress (Birk et al. 2013). Neuronal protection against H₂O₂, which is the most harmful molecule to the brain, is largely mediated by the glutathione system. Also, lipid peroxidation may increase because of diminution of GSH stores in the brain (Ansari and Scheff 2010; Lee et al. 2020). On the other hand, SOD is an important antioxidant enzyme that performs a vital function in clearing superoxide anions, which otherwise injures the cell membranes and macromolecules (Kurutas 2016). In the present study, diazepam significantly enhanced the MDA levels, while the GSH and SOD levels were not significantly changed. Pre-treatment with ETE did not significantly alter the levels of GSH and SOD. However, it substantially reduced the MDA levels which implies antioxidant activity. This shows that the anti-amnesic activity of ETE could be attributable to its antioxidant activity.

Histological examination of the cortex and hippocampus of the diazepam treated group showed neuronal atrophy and loss of pyramidal cells; however, such changes were not observed with the extract treated groups especially at 250 and 500 mg/kg. The absence of pathological lesions on the brain tissues further supports the ameliorative activity of ETE against diazepam-induced amnesia.

Conclusions

The ethanol extract of *Eragrostis tremula* ameliorated diazepam-induced amnesia and the observed activity could be mediated in part by its antioxidant property. This further supports its ethno-medical use in the management of amnesia.

**Abbreviations**

ANOVA: Analysis of variance; D/W: Distilled water; ELISA: Enzyme-linked immunosorbent assay; ETE: *Eragrostis tremula* extract; EPM: Elevated plus maze; GABA: Gamma amino butyric acid; GSH: Glutathione reductase; GRAN: Granulocytes; HCT: Haematocrit; H&E: Haematoxylin and eosin; HGB: Haemoglobin; LD₅₀: Median lethal dose; LYPM: Lymphocytes; MDA: Malondialdehyde; NORT: Novel object recognition test; OECD: Organization for Economic Co-operation and Development; PLT: Platelets; RBC: Red blood cells; RI: Recognition index; ROS: Reactive oxygen species; SEM: Standard error of mean; SOD: Superoxide dismutase; SPSS: Statistical package for social sciences; WBC: White blood cells.

**Acknowledgements**

The authors appreciate the Department of Pharmacology and Therapeutics, Ahmadu Bello University, for providing us with Neurobehavioural Facility as well as the technical assistance of Aliyu Ahmed and Salihu Abdullahi.

**Author contributions**

ABN designed the research, conducted the experiment, data analysis and drafted the manuscript. MGM, MA and NMD participated in the research design and supervised the experiments. ABN, AA, MGM, MA and NMD revised and approved the submission of the final manuscript. All authors read and approved the final manuscript.

**Funding**

No funding was received.

**Availability of data and materials**

All data related to this study are included herein, otherwise available on request.

**Declarations**

**Ethics approval and consent to participate**

The experimental protocols were authorized by Ahmadu Bello University Committee on Animal Use and Care (Approval number: ABULCAUC/2020/68) and performed according to the guidelines set by Animal Research: Reporting of In Vivo Experiments (ARRIVE).

**Consent for publication**

Not applicable.

**Competing interests**

The authors have no competing interests to disclose.

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Received: 19 March 2022  Accepted: 8 April 2022  Published online: 18 April 2022
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