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Extract of *Mallotus oppositifolius* ameliorates mercuric chloride-induced neurotoxicity

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Abstract

Background: Mercuric chloride ($HgCl_2$) induces neurotoxicity in both animals and humans, with unclear treatment mechanisms and associated side effects. *Mallotus oppositifolius* leaf extract, known for its pharmacological properties like antidepressant and anti-inflammatory effects, thus suggests potential neuroprotection.

Objective: The study aimed to assess the neuroprotective effect of Mallotus oppositifolius in a mercuric chloride-induced neurotoxicity mouse model.

Methods: Male mice (20 - 25 g) from the Institute of Cancer Research (ICR) were randomly divided into six groups of eight (48 mice). Group 1 received the vehicle (without mercuric chloride) throughout the experimental period. Mice in groups 2, 3, 4, 5 and 6 were pre-treated with $HgCl_2$ orally for 7 days, after which groups 3, 4 and 5 were treated with the oral graded dose of *Mallotus oppositifolius* leaf extract (MOE 10, 30, 100 mg/kg) while group 6 was treated with the reference drug, piracetam (PCT 150 mg/kg, orally). The various groups were evaluated for neurotoxicity using the open field, catalepsy and novel object recognition (NOR) tests. Cresyl Violet staining was used to assess the neurohistological changes caused in the hippocampus of the brain.

Results: $HgCl_2$-induced catalepsy and decreased posture correction time compared to the vehicle group. However, MOE and PCT significantly reversed this effect. In the novel object recognition (NOR) test, $HgCl_2$ reduced the recognition index and novel object time, while MOE and PCT increased both. Locomotor activity, assessed through line crossing, was significantly reduced with $HgCl_2$ but remained unaffected with MOE and piracetam. $HgCl_2$ also reduced time in the central compartment, while MOE and PCT reversed this effect. Neurohistological assessment revealed $HgCl_2$-induced reductions in neuronal count in cornu ammonis areas 1 and 3 (CA1 and CA3) and the dentate gyrus (DG) regions, with varied effects observed in MOE- and PCT-treated groups.

Conclusion: This study demonstrates the neuroprotective effect of *Mallotus oppositifolius* against mercuric chloride-induced neurotoxicity in mice. The leaf extract may have potential in clinical conditions characterised by neurodegeneration.

Keywords: Mallotus oppositifolius; Mercuric chloride; Neurotoxicity; Hippocampus

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INTRODUCTION

Neurotoxicity due to neurotoxicants disrupt normal nervous system activities, causing reversible or irreversible damage [1]. Neurotoxicants may be medications (e.g. ethambutol, isoniazid, vincristine); domestic products used in antidandruff shampoos (pyridinethione); fragrance raw materials (2,6-dinitro-3-methoxy-4-tert-butyltoluene), pyrolysis products in broiled, baked, or fried food (acrylamide), beverages (ethanol), pest-control agents (aldrin), and environmental pollutants (mercury) [1]. In high doses, some physiologically essential heavy metals such as copper, cobalt, iron, nickel, magnesium, molybdenum, chromium,

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selenium, manganese and zinc may cause neurotoxicity [2]. During neurotoxicity, glutamate activates the N-Methyl-D-aspartic acid (NMDA) receptors, resulting in the excessive release of calcium ions (Ca2+) [3]. This increases the stress on the mitochondria, resulting in excessive oxidative phosphorylation and production of reactive oxygen species (ROS) via the activation of nitric oxide synthase, ultimately leading to cell death [4]. In addition, the extracellular signal-regulated kinase (ERK) pathway is disrupted, which normally functions as memory control in the brain. The disruption, therefore, leads to memory dysfunction [5].

In the last two decades, more than a thousand published articles on mercury neurotoxicity have highlighted its relevance in public health discourse [6,7]. In Ghana, mercury contamination is primarily linked to artisanal and small-scale gold mining (legal and illegal), i.e. "galamsey", in which it is used in the gold purification process [8] and ends up in water bodies and in fish. Studies support fish consumption as one of the main means of mercury toxicity [8,9]. The classic symptoms associated with exposure to mercuric toxicity are a combination of renal, gastrointestinal and CNS symptoms, eventually leading to death [10]. Management of mercury toxicity includes GI decontamination, washing of exposed skin, and the use of activated charcoal [10]. Other studies suggest that selenium (i.e. sodium selenite) can elicit neuroprotective effects against mercuric chloride neurotoxicity or brain damage in chicken [11]. These options are, however, fraught with several limitations, prompting the need for alternative options.

In this regard, medicinal plants such as *Mallotus oppositifolius* with neuroprotective potentials may be useful [12,13]. *Mallotus oppositifolius* (Geiseler) Müll.Arg. (Euphorbiaceae) is an important and versatile medicinal herb prevalent across various African countries. Known for its diverse pharmacological properties, this plant thrives in multiple habitats, including semi-deciduous forests, wet and dry evergreen forests, grasslands, swamps, and savannahs with forest patches [14]. It is widely distributed in countries such as Ethiopia, Nigeria, Ghana, Cameroon, Madagascar, Senegal, Angola, Malawi, Zambia, Zimbabwe, Mozambique, Uganda, Kenya, and more [14]. Remarkably, *Mallotus oppositifolius* blooms and bears fruit throughout the year. The *Mallotus oppositifolius* leaf extract has demonstrated antidepressant, anticonvulsant and antiaggressive effects in murine models [13,15,16]. Various phytochemicals such as terpenoids, cardenolides, benzopyrans, flavonoids, (+)-α-tocopherol (vitamin E), stigmasterol, bergenin, methyl gallate and vitamin C with possible beneficial neuroactive effects have been identified in the plant.

We recently reported the presence of methyl laurate, ethyl palmitate, ethyl stearate, benzoic acid derivatives and phenolic compounds in the plant [15]. These constituents were associated with reduced nuclear factor κ-B, tumour necrosis factor-α, interleukin-6 levels and inhibition of α-synuclein in rats [17-19]. The reported pharmacological properties suggest that *Mallotus oppositifolius* may have a neuroprotective effect. Thus, this present work investigated the effect of *Mallotus oppositifolius* leaf extract (MOE) on the neurotoxicity caused by mercuric chloride in mice.

MATERIALS AND METHODS

Plant collection and extraction

Leaves from the *M. oppositifolius* (Geiseler) Müll. Arg (Family: Euphorbiaceae) plant was collected at the Centre for Plant Medicine Research (CPMR) in Mampong-Akuapem, Ghana (5°55′05.6″N, 0°08′04.9″W), and authenticated on-site (Voucher Specimen Number: CPMR 4977). Collection methods ensured the plant's well-being remained intact. After a 7-day period of air-drying, the leaves underwent pulverisation with a hammer mill to achieve a fine powder. Emulating traditional practices, cold maceration with absolute methanol was employed for 3 days, hinting at the likelihood of active constituents in a polar medium. The ratio of drug extract to solvent was maintained at 600 g of powdered leaves per 6000 ml of methanol. The resulting extract was concentrated at 60°C under pressure using a rotary evaporator, yielding a syrupy mass. This syrupy material was subsequently dehydrated into a dark brown semisolid mass using a water bath and stored in a desiccator for future use. This product was designated as *M. oppositifolius* leaf extract (MOE).

Chemicals

Piracetam, ketamine and xylazine were obtained from Sigma Aldrich Inc., St. Louis, MO, USA, and mercuric chloride ($HgCl_2$) was from Merck KGaA, Darmstadt, Germany.

Preparation of drugs

All the drugs were prepared just enough for daily use only. About 33.32 mg of the MOE was weighed daily into a container and diluted to 10 mL with distilled water to prepare 100 mg/kg/mL of MOE solution. Serial dilution was done to obtain the 10 mg/kg/mL and 30 mg/kg/mL solution of the MOE. Piracetam was prepared by dissolving the weighed amount in distilled water.

Animals

Male ICR mice were obtained from the Department of Pharmacology, KNUST, Kumasi, Ghana and housed under standard laboratory conditions. Six groups of eight mice were kept in stainless steel cages measuring 34 × 47 × 18 cm^3 and lined with soft wood shavings for bedding. The mice were fed a regular commercial pellet diet (GAFCO, Tema) and given water ad libitum. The laboratory temperature was maintained between 24°C and 25°C, with an average humidity level of 77%. A daylight cycle between 7 am and 2 pm was maintained. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Ethical and Protocol Review Committee, College of Health Sciences, University of Ghana, Korle-Bu.

Experimental design

Group 1 (negative control) received normal saline orally administered using the oral gavage for 7 days, followed by distilled water for 3 days. Groups 2 to 6 received mercuric chloride (5 mg/kg) orally for 7 days. Following this, group 2 (disease control) received distilled water, groups 3, 4 and 5 received graded doses of MOE (10, 30, 100 mg/kg) and group 6 was given piracetam (150 mg/kg) for 3 days.

Catalepsy test

Neurotoxicity-associated motor incoordination and Parkinson-like effects were evaluated using the catalepsy test. Briefly, mice were placed on an elevated horizontal bar, and the time taken for them to descend was recorded. The duration spent on the rod served as an index for assessing motor coordination.

Open field test

The effect of treatments on the locomotor activity and anxiety behaviour of mice was evaluated using the open-field test [16]. Mice were placed in an open field box with an enclosed floor and walls, measuring 60 × 60 × 25 cm^3, with an open top. The centre region of the box floor was defined offline as a 20 × 20 cm^2 area, but it was not marked. Each box was placed on the floor of the experiment room and was dimly illuminated. Each mouse was gently placed in the centre of the box and allowed to walk freely. Locomotor activity was scored as the total distance travelled (marked by the number of lines crossed) and anxiety behaviour as the time spent in the centre during the 6-minute period using video tracking software (Boris v 7.9.6 - 2019). Scoring was done by a blinded experimenter.

Novel object recognition (NOR) test

Neurotoxicity affects cognitive function; thus, the effect of mercuric chloride on exploratory learning and recognition memory was assessed using the NOR test. The procedure used was as described by [20]. Mice were randomly assigned to eight groups (n = 8) and administered drugs as outlined in the experimental design. The test involved 3 phases: habituation, familiarisation, and testing. In the habituation phase, the mice were placed in an open field (33 × 33 × 20 cm^3) for 5 minutes twice daily, with a 6-hour interval, for three consecutive days. The familiarisation phase was carried out 24 hours after the last day of habituation. Two identical objects (in terms of shape, colour, and size) were placed 20 cm apart in the open field. The mice were placed in the centre of the field for 10 minutes and allowed to freely explore the objects. Behavioural assessment of each mouse was done for 10 minutes. This phase lasted for three days. Exactly 24 hours after the familiarisation phase, the testing phase was performed, which lasted for 3 days. One hour before each test day, the mice were treated with MOE (10, 30, and 100 mg/kg p.o.), piracetam (150 mg/kg p.o.), or the vehicle (saline, 10 ml/kg p.o.). The test was conducted for 5 minutes, with one of the identical objects replaced by a new object. Environmental cues were hung in the study environment to facilitate the exploration of the novel object and enhance discrimination against the familiar one [21]. Behavioural assessments were done with JWatcher, version 1.0 (University of California, Los Angeles, USA, and Macquarie University, Sydney, Australia) by a blinded observer. The time spent with the novel object and recognition index (RI) were assessed. RI was calculated to demonstrate the level of discrimination against the familiar object, using the formula according to [22]:

$$RI = \frac{(\%\text{ exploration of familiar object during training} - \%\text{ of exploration of familiar object during test})}{\%\text{ exploration of familiar object during training}}$$

Sacrificing of animals and harvesting of the brain

After the study period of 10 days, mice were euthanised with 100 µL of a 10:1 mixture of ketamine (100 mg/ml) and xylazine (10 mg/ml) and brains harvested. The harvested brain samples were placed in a solution of 4% formaldehyde until it was analysed using the cresyl violet stain technique.

Sectioning and cresyl violet staining

Each brain sample was divided into three coronal sections and placed in histological cassettes (Rotilabor embedding cassettes; K114.1, Carl Roth GmbH, Germany). The sections were subsequently processed through a graded ethanol series: 70% ethanol for 1 hour, 95% ethanol for 1.5 hours, and 100% ethanol twice for 2 hours each. Following this, the tissues were immersed in molten paraffin wax for 3 hours, embedded in the wax, and stored in a refrigerator at 4°C until sectioning. The refrigerated tissue blocks were sectioned at 50 µm using a microtome (Leica RM 2235) and placed in water at 60°c. The floating sections were carefully picked up with a brush and mounted onto gelatin-coated slides. Excess moisture was blotted with tissue paper, and moderate downward pressure was applied with the heel of the palm to ensure the sections adhered firmly to the slides. The slides were then dried in the dark for 3 days. Following the drying process, the sections were dehydrated through a graded series of alcohol solutions, starting with 70% ethanol, followed by 95% ethanol, and finally 100% ethanol. The sections were then immersed in a cresyl violet acetate solution for 15 minutes, allowing the dye to bind to the Nissl substance within the neurons. After staining, the sections were briefly rinsed in distilled water to remove excess dye. The sections were treated with 95% ethanol to achieve the desired contrast, highlighting the neuronal cell bodies against a clear background. The sections were rapidly dehydrated in 100% ethanol and then cleared in xylene to enhance tissue transparency. To clear the sections, they were transferred to xylene and then mounted with DPX (dibutyl phthalate in xylene). Once the sections were mounted, a coverslip was placed over the tissue, and the slide was made ready for examination under the light microscope.

Data analysis

Data were analysed using one-way or two-way analysis of variance (ANOVA), followed by Tukey or Bonferroni post-hoc tests to determine the differences between the different treatment groups. The level of significance was set at $p < 0.05$.

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RESULTS

Catalepsy test

The study measured the time mice spent on the horizontal rod. Compared to the vehicle naïve group (VEH), there was an increase in time spent on the rod in the $HgCl_2$-treated mice (Figure 1). Conversely, MOE (10 - 100 mg/kg), just as piracetam (PCT), produced a significant ($p < 0.001$) increase in time spent on the rod (Figure 1). The results from the MOE and PCT group were comparable to VEH group.

Open field test

We show that $HgCl_2$ treatment caused a significant decrease in time spent in the central compartment of the open field and the number of line crossings when compared to the VEH group (Figure 2A and B). MOE, just as PCT, significantly ($p < 0.01$) increased the time spent at the centre of the open field when compared to the $HgCl_2$ group (Figure 3A). The time spent in the centre by mice given the highest dose of MOE (100 mg/kg) was comparable to the VEH group. Moreover, MOE produced a modest increase in the number of line crossing relative to the $HgCl_2$ group, with MOE 30 mg/kg and PCT causing a significant ($p < 0.01$) increase in the number of line crossing (Figure 2B).

Novel object recognition test

From the time-course curve, mice spent the same time when the objects were similar. However, after changing one of the objects, $HgCl_2$ treatment significantly reduced the time spent with the novel object throughout the experimental period when compared to the vehicle naïve group ($p < 0.01$). MOE (30 and 100 mg/kg) and PCT (150 mg/kg) significantly increased the time spent with the novel object when compared to VEH and $HgCl_2$ ($p < 0.05$). MOE's effect began on the second day of treatment and was sustained till the end of the experiment (Figure 3A). Moreover, MOE and PCT reversed the $HgCl_2$-associated decrease in total time spent (calculated as AUC) exploring the new object (Figure 3B). $HgCl_2$ decreased the recognition index while MOE and PCT significantly ($p < 0.0001$) reversed the $HgCl_2$-induced decline in recognition index (Figure 3C). Interestingly, PCT and MOE (10 and 100 mg/kg) produced a greater recognition index than the VEH.

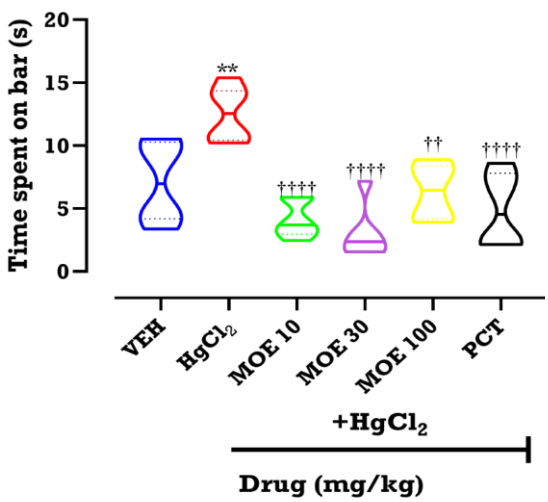

Figure 1. Effects of MOE and piracetam (PCT) on duration of the forelimb on the bar in the catalepsy est. Data are presented as group Means ± SEM (n=8). Significantly different from vehicle: **p < 0.01 (One-way ANOVA followed by Tukey's post hoc test). ††††p <0.0001, †††p < 0.001, ††p < 0.01, †p < 0.05; significantly different from untreated $HgCl_2$ group (One-way ANOVA followed by Tukey's post hoc test).

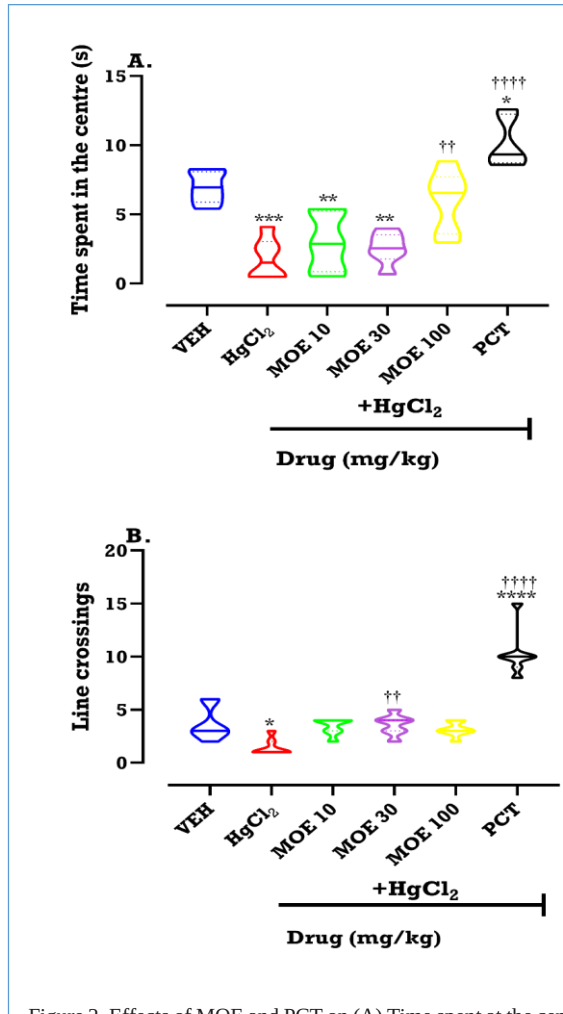

Figure 2. Effects of MOE and PCT on (A) Time spent at the centre and (B) Lines crossed in the OFT. Data are presented as group Means ± SEM (n=6). Significantly different from saline (VEH) group: *p < 0.05, **p < 0.01 and ***p < 0.001 and ****p < 0.0001; significantly different from untreated HgCl2 group: ††p < 0.01, †††p < 0.001 and ††††p <0.0001 (One-way ANOVA followed by Tukey's post hoc test).

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Cresyl violet staining of the hippocampus and neuronal count

Figure 4 shows representative images of the hippocampus from mice treated with $HgCl_2$ or saline. While the $HgCl_2$ group showed loss of neurons in various parts of the hippocampus, the VEH and drug-treated groups showed preserved hippocampal neuronal architecture (Figure 4). From Figure 5, MOE (10 and 100 mg/kg) significantly ($p < 0.0001$) reversed the neuronal loss in the dentate gyrus (DG) caused by $HgCl_2$. The effect of MOE 30 mg/kg was not statistically significant, giving rise to a U-shaped effect. It is worth noting that MOE could not reverse the neuronal loss to the state of the vehicle naïve group (VEH). It can also be observed that the PCT could not reverse the $HgCl_2$-associated neuronal loss in the DG when compared to VEH.

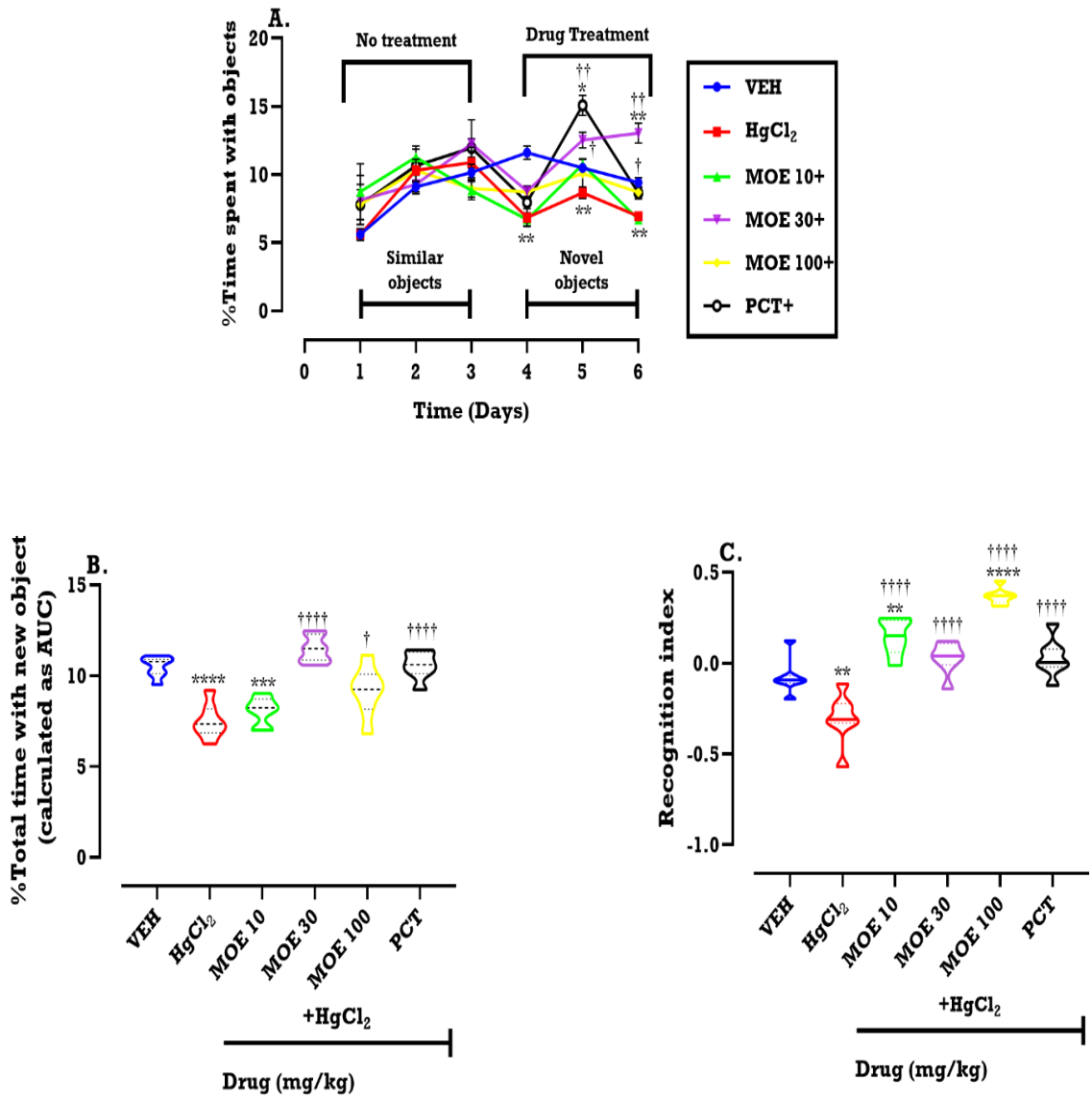

Figure 3. Effects of MOE (10, 30 and 100 mg/ kg) and PCT (150 mg/kg) treatment on the (A) Percentage time spent with the novel object (B) Total time with new object (C) Recognition index in the NOR test. Data are presented as mean ± SEM (n = 6) for the time course graph A and analyzed by Two-way ANOVA followed by Bonferroni's test. The total times spent with the new object are presented as the areas under the curve (AUC) (B) (One-way ANOVA followed by Tukey's multiple comparison test). Significantly different from the saline (VEH): *$p < 0.05$, **$p < 0.01$; significantly different from untreated $HgCl_2$: †$p < 0.05$, ††$p < 0.01$. The recognition index is presented (C) (One-way ANOVA followed by Tukey's multiple comparison test). Significantly different from the saline (VEH): **$p < 0.01$, ****$p < 0.0001$; significantly different from untreated $HgCl_2$: ††††$p < 0.0001$.

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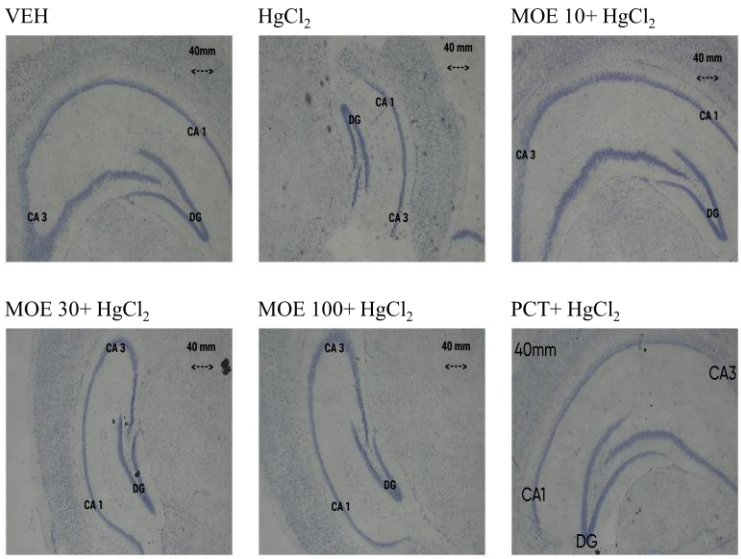

Figure 4. Representative images of Cresyl Violet stained Dentate Gyrus (DG), Cornu Ammonis (CA1), Cornu Ammonis (CA3) regions of the hippocampus of the brain of the mice for each experimental group. VEH = Vehicle-treated group; $HgCl_2$= $HgCl_2$ untreated group; MOE 10, 30, 100 = *M. oppositifolius* leaf extract-treated groups; PCT= Piracetam-treated group.

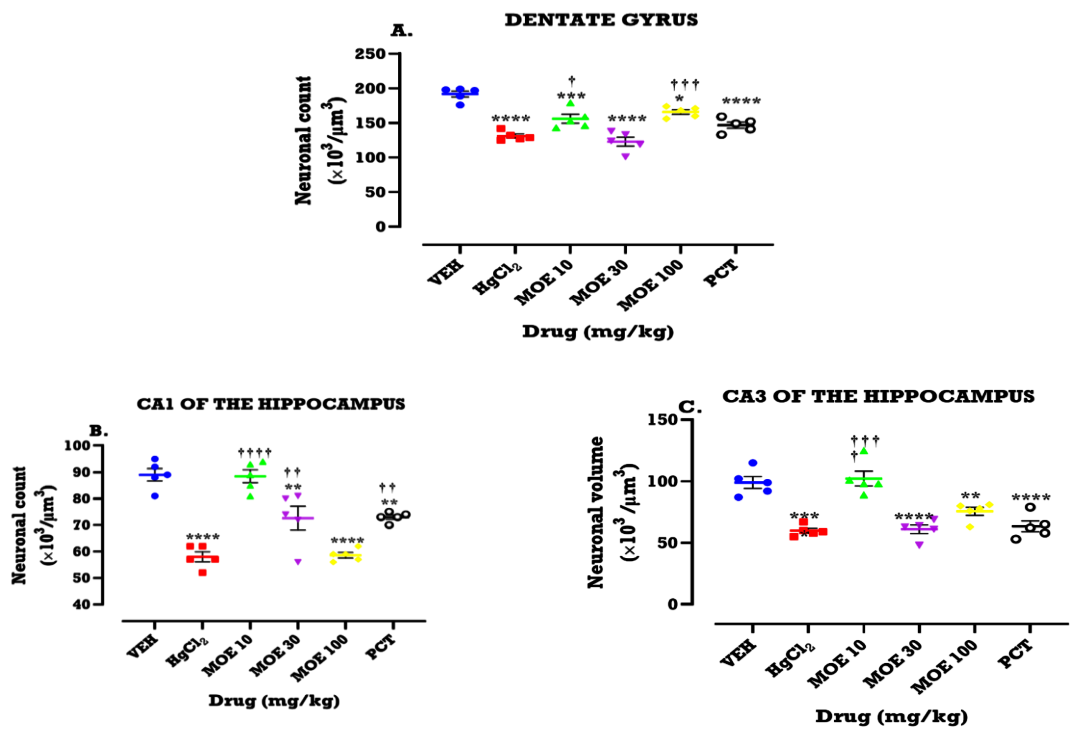

Figure 5. Quantification of neurons. Effect of MOE, $HgCl_2$ and PCT on neuronal count in the A. dentate gyrus (DG), B. CA1 and C. CA3 regions of the hippocampus of mice. The results are expressed as absolute values and expressed as mean ± SEM (n =5). Significantly different from saline (VEH) group: *p < 0.05, **p < 0.01 and ***p < 0.001 and ****p < 0.0001; significantly different from untreated $HgCl_2$ group: †p < 0.05, ††p < 0.01, †††p < 0.001 and ††††p <0.0001 (One-way ANOVA followed by Tukey's post hoc test).

From Figure 5, it was evident that $HgCl_2$ caused a significant ($p < 0.0001$) reduction in the neuron count of the Cornu Ammonis 1 (CA1) region of the hippocampus when compared to the vehicle naïve group (VEH). In contrast, MOE (10 and 30 mg/kg) significantly reversed the $HgCl_2$-associated neuronal loss in the CA-1 of the hippocampus. Surprisingly, MOE (100 mg/kg) could not reverse the $HgCl_2$-induced neuronal loss. It can also be observed that the PCT increased the neuronal count at the CA-1 relative to the untreated $HgCl_2$ group ($p < 0.01$). From the results in Figure 5, $HgCl_2$ caused a significant reduction in the neuronal count of the CA3 region of the hippocampus when compared to VEH. This effect was, however, reversed by MOE (10 mg/kg). MOE (30 and 100 mg/kg) failed to reverse the neuronal loss caused by $HgCl_2$. PCT treatment also failed to reverse the $HgCl_2$-induced neuronal loss in the CA3.

DISCUSSION

The study provides evidence that MOE ameliorates the neurotoxic effects of mercuric chloride in mice. Here, we provide insight into the efficacy of the extract in mitigating mercury-related neurotoxicity, a significant concern in environmental and occupational health due to widespread exposure to it. Catalepsy, NOR, OF tests, and cresyl violet staining were used to assess the protective effect of the MOE. Catalepsy is a condition characterised by muscle rigidity and inability to correct an externally imposed posture. Catalepsy is associated with Parkinson's disease, catatonic schizophrenia, as well as brain damage involving the basal ganglia or cerebellum [23]. Ordinarily, when a healthy animal finds itself in an unfamiliar posture, it instinctively adjusts its position within a matter of seconds. However, a cataleptic animal remains in the imposed posture for an extended duration, often spanning several minutes. This has been used as a model for extrapyramidal symptoms. In addition, it is associated with dopamine D2 receptor antagonism and 5-HT1A receptor dysfunction [24]. Previous studies showed that mercury neurotoxicity is associated with disruption in neurotransmitter release and binding, especially dopamine and 5-HT. Considering the role of these neurotransmitters in catalepsy, it is not surprising that mercuric chloride produced a pronounced cataleptic effect in mice. Even more interesting is the fact that MOE attenuated the mercury-induced catalepsy. The MOE-associated effect may be related to its effect on 5-HT and catecholamines [13].

Coupled with catalepsy, mercury intoxication is associated with anxiety behaviour and impaired locomotor activity [25]. The open field test model was used to assess locomotor activity and exploratory behaviour (line crossing) as well as anxiety (time spent at the centre of the field) in mercury-exposed mice [26]. MOE significantly reversed the $HgCl_2$-induced locomotor impairment and anxiogenic effect [27], suggesting a possible anxiolytic effect. The putative anxiolytic effect of MOE is supported by its GABA and serotoninergic-enhancing effects previously reported [28,13]. Piracetam has been reported to influence the release, synthesis, and receptor binding of neurotransmitters such as acetylcholine, glutamate, and GABA. This may be responsible for its ability to attenuate the mercuric chloride-related effects in the open field test. The novel object recognition test was utilised to evaluate cognitive function and memory retention in the mice. The behavioural tests employed in this investigation aided in the assessment of exploratory work, recognition, and reference memories. Exploratory learning establishes a relationship between existing knowledge and new content or concepts, while working memory involves temporarily holding information and using it in cognitive tasks.

Reference memory is a form of long-term memory that utilises two aspects of episodic memory, which are content and place dimensions of activity [29,30]. Recognition memory comprises recollection and familiarity [31]. Impaired recognition memory is a common consequence of neurotoxicity. This test sought to determine whether the leaf extract could mitigate cognitive deficits caused by mercuric chloride exposure. Mercuric chloride induced cognitive deficits by impairing learning and memory in the novel object recognition (NOR) test. Both the acquisition of information and the retention of memory were disrupted by mercuric chloride, as evidenced by the reduction in the time spent with both the novel object and recognition index. As expected, piracetam, a nootropic agent, significantly increased both the time spent with the novel object and the recognition index. Similarly, the MOE reversed the deleterious effect of mercuric chloride on learning and memory. It is worth noting that the MOE- and piracetam-associated improvement in learning and memory was higher when compared to vehicle mice. The result suggests that MOE may have a nootropic effect. This result is supported by previous findings that showed that MOE rapidly reverses depressive symptoms without impairing spatial learning and memory [28]. A high percentage of time spent with the new object indicates improvement in exploratory learning and recognition memory [21,32]. In addition, the hippocampus, prefrontal cortex, cingulate cortex, neostriatum, entorhinal cortex, perirhinal cortex, and N-methyl-D-aspartate (NMDA) receptor function have been implicated in mice behaviour in the NOR test (D'Hooge & De Deyn, 2001; Vorhees & Williams, 2006). Indeed, a previous study has suggested that MOE inhibits the glycine co-stimulatory site of the NMDA receptor [28]. Moreover, MOE prevented loss of dendritic spine density and contributed to neurogenesis and/or neuroplasticity in the prefrontal cortex of mice [16]. It is plausible that the cognitive-enhancing effects of MOE may involve any of the above-mentioned structures or systems.

Furthermore, limitations in learning and memory typically affect quality of life, reduce creativity, slow academic performance, especially in children, and may increase the risk of developing attention-deficit hyperactivity disorder (ADHD), dyslexia, and Alzheimer's disease [33]. Since MOE improved exploratory learning, working, recognition,

and reference memory in mice, further studies exploring their efficacy in these conditions is required.

In order to prove that mercuric chloride caused neurotoxicity, the cresyl violet staining technique was used to assess the neuronal count in the hippocampus (CA1, CA3 and DG) [34]. The hippocampus is one of the brain structures affected during neurotoxicity, and considering its role in behaviour and cognitive processes, it was selected for the histological assessment. In this study, mercuric chloride significantly reduced the neuronal count in the CA1, CA3 and DG regions of the hippocampus. This may suggest mercuric chloride-associated increased neuronal death and/or inhibition of neurogenesis in these hippocampal regions. Both the MOE 10 and 30 mg/kg were able to reverse the toxic effects of mercuric chloride in all the regions assessed. The effect of MOE may be due to increased neurogenesis and/or suppression of mercuric chloride-induced neuronal apoptosis [14,35]. Further studies may be needed to elucidate the mechanism underlying the beneficial effects of MOE. Similarly, the standard drug, piracetam, was able to prevent the neuronal damage in the CA1 from the mercuric chloride. Our study proffers that MOE reverses the behavioural, cognitive and histological changes associated with mercury toxicity.

Conclusion

We have shown for the first time that *Mallotus oppositifolius* leaf extract ameliorates mercuric chloride-induced increased catalepsy, anxiogenesis, impaired locomotion and learning and memory deficits. The behavioural effects of the leaf extract were associated with increased hippocampal neuronal count. The present study suggests that *Mallotus oppositifolius* may have neuroprotective potential against mercury chloride in mice.

DECLARATIONS

Ethical consideration

The animal study was reviewed and approved by the Ethical and Protocol Review Committee, College of Health Sciences, University of Ghana, Korle-Bu (CHS-Et/M.7 – P4.2/2022-2023).

Consent to publish

All authors agreed on the content of the final paper.

Funding

None

Competing Interest

The authors declare no conflict of interest.

Author contribution

KKE conceptualised the study. BEO, KKE, and KKA designed the methodology and conducted the investigation. KKE and BEO collected and analysed the data. KKE and BEO drafted the original manuscript. KKE, BEO, PA, and KKA reviewed and edited the manuscript. All authors have accepted responsibility for the entire content of this submitted manuscript and approved the submission.

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Availability of data

Data is available upon request to the corresponding author.

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