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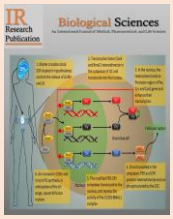

Neuroprotective Effect of Ginkgo biloba and L-Ascorbic Acid on Mercury Chloride ($HgCl_2$)-Induced Oxidative stress and Neuroinflammation in Adult Male Wistar Rats

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ABSTRACT

Mercury chloride ($HgCl_2$) toxicity poses significant health risks upon exposure. Chronic exposure can lead to severe neurological damage. Ginkgo biloba extract (EGB761) enhance cognitive function and memory. Ascorbic acid is a powerful antioxidant that helps protect cells from damage by free radicals. The present study evaluates the effects of EGB761 and L-Ascorbic Acid on HgCl2-Induced Oxidative Stress and Neuroinflammation. 48 adult Wistar rats were randomly divided into 6 groups of 8 rats each. The control group received distilled water. $HgCl_2$ Only received (5 mg/kg $HgCl_2$), $HgCl_2$ + A. A received (5 mg/kg $HgCl_2$ + 100 mg/kg A. A, $HgCl_2$ + A. A received (5 mg/kg HgCl2 + 500 mg/kg A. A, $HgCl_2$ + EGB761 received (5 mg/kg $HgCl_2$ + 100 mg/kg EGB761, and $HgCl_2$+ EGB761 received (5 mg/kg $HgCl_2$ + 500 mg/kg EGB761. All administration was done orally for 21 days. The animals were subjected to a Y maze test and sacrificed on day 22. The brain was excised, and 1 g of tissue homogenized and utilized to assay for (MDA, SOD, CAT, GSH) and (TNF- α, and IL-6) activity. The result showed significant ($p<0.05$) increase in MDA level, TNF- α, and IL-6 activity and a significant ($p<0.05$) decrease in SOD, CAT, and GSH activity in the $HgCl_2$ Only Group. Ascorbic acid (500 mg) and EGB761 (100 mg and 500 mg) treated animals showed significant ($p<0.05$) improvement in alternations, decrease in MDA level, and increase in CAT, SOD level thereby mitigating mercury-induced oxidative stress indicating that EGB761 has neuro-protective property.

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Abbreviations: *$HgCl_2$: Mercury Chloride; EGB 761: Ginkgo biloba Extract; TNF-α: Tumour Necrosis Factor – Alpha; ROS: Reactive Oxygen Species; IL-6: Interleukin – 6; TNFR1: Tumour Necrosis Factor Receptor 1; MDA: Malondialdehyde; CAT: Catalase; SOD: Superoxide Dismutase; GSH: Glutathione; TAE: Total Arm Entry; ALT: Alternation; AD: Alzheimer's disease; PD: Parkinson's disease; MS: Multiple sclerosis; ALS: Amyotrophic lateral sclerosis; NF-κB: Nuclear Factor kappa B; OD: optical density*

Introduction

Mercury (Hg) is considered by the World Health Organization (WHO) to be one of the top 10 chemicals or groups of chemicals of major public health concern. Mercury is a naturally occurring element that is found in air, water, and soil [1]. Exposure to Hg can occur from both natural and artificial sources. Mercury is a highly toxic metal, exposure to mercury even small amounts may cause serious health problems with harmful consequences, with Hg primarily targeting the brain and its components, such as the central nervous system (CNS).

Multiple studies [2, 3, 4] have reported that exposure to $HgCl_2$ promotes the production of reactive oxygen species (ROS), which are the main culprits behind a number of neurological disorders, such as Parkinson's disease (PD) and Alzheimer's disease (AD). Hippocampal neuronal cells are harmed by oxidative stress brought on by mercury chloride exposure [5]. A quantitative imbalance between the production of antioxidants and reactive oxygen species (ROS) results in oxidative stress. Many illnesses, such as neurodegenerative diseases (AD or PD), protein oxidation, or cell damage, can be brought on by this imbalance [6].

According to [7], oxidative stress is a cellular condition that arises from a physiological imbalance between the amounts of antioxidants and oxidants (free radicals or reactive species), with an excess of oxidants. ROS damage proteins, oxidizing their basic structure and side groups [8, 9]. Both in vitro and in vivo studies indicate that exposure to mercury (Hg) can cause OS in the biological system through the production of reactive oxygen species (ROS), the reduction of glutathione (GSH), and the decrease of the sulfhydryl (–SH) protein group [10, 11].

In neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease (AD), depression, and memory loss, oxidative stress has been linked to the death of neuronal cells [12]. As a defense mechanism against foreign infections or injured cells, inflammation occurs. Neuroinflammation is brought on by the overproduction of pro-inflammatory cytokines [14]. Neurotransmitters are means by which astrocytes and other brain cells

communicate. The key players in neuroinflammation are cellular and molecular immune constituents such as macrophages (microglia), cytokines, complement, and pattern recognition receptors [15]. In this respect, low-level neuroinflammation is protective, whereas high-level chronic neuroinflammation is harmful (Figure 1).

Tumor necrosis factor (TNF-α), growth factors, adhesion molecules, and chemokines are examples of inflammatory chemicals that are released through the mediation of cellular immune components like microglia or astrocytes. Insufficient or excessive expression of pro- or anti-inflammatory molecules can lead to neuroinflammation, which in turn can cause the onset and development of disease [16]. Conventionally stimulating microglia result in the release of various harmful and pro-inflammatory chemicals, including ROS, NO (Figure 2), and cytokines (Figure 1). Astrocytes and microglia gradually become activated upon receiving signals of damage or injury, which results in morphological alterations and the release of pro-inflammatory substances (i.e., cytokines, cytotoxic components, ROS). Accordingly, the continuous exposure of astrocytes and microglia to injury-causing substances and the production of these components causes mutual activation between astrocytes and microglial cells (Figure 2), which in

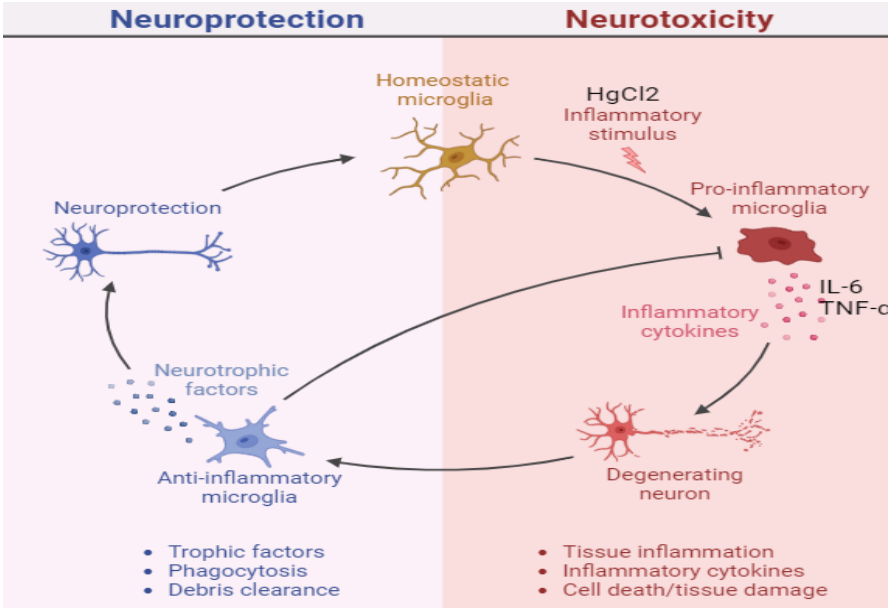

Figure 1. Microglia Activation and Resolution of Inflammation [13].

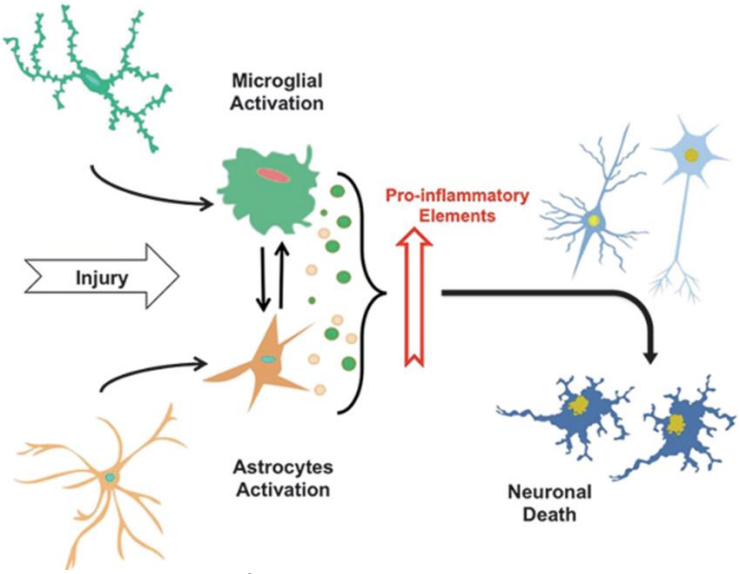

Figure 2. The neuroinflammatory process [19].

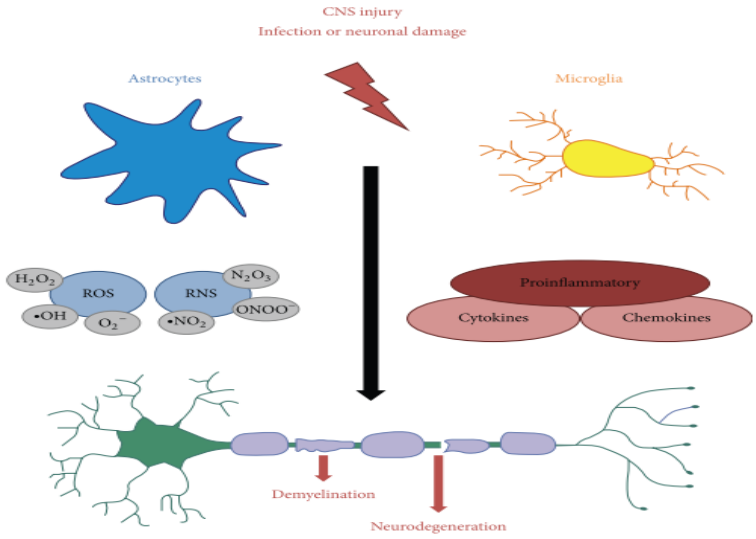

Figure 3. Schematic presentation of the CNS cell-mediated demyelination and neurodegeneration [21].

turn causes a neuroinflammatory process that ultimately results in the death of neurons [17, 18, 19].

In a healthy organism mediator of oxidative stress and inflammation are in balance with the counteracting detoxifying and anti-inflammatory molecules. During disease this balance is shifted towards the oxidative stress and proinflammatory site, leading to DNA and protein damage, inflammation, and finally cell death.

The so-called Pathogen Associated and Danger Associated Molecular Patterns (PAMPs and DAMPs), respectively, are generated during infection or injury and are identified by pattern recognition receptors. Among the key pattern recognition receptors in the central nervous system are the widely expressed Toll-Like Receptors (TLRs) found in microglia and astrocytes [20]. Both microglia and astrocytes, once activated after injury, they can release chemokines, cytokines, and ROS in response to TLR-mediated activation (Figure 3). These molecules can either enhance neuronal survival or, in cases of severe damage such as spinal cord injury or ischemia, may increase inflammation and worsen neuronal damage (Figure 3).

In general, inflammation is a protective response to various cell and tissue injuries to destroy and remove the detrimental agents and injured tissues, thereby promoting tissue repair. However, when inflammation is uncontrolled, it can cause excessive cell and tissue damage ultimately leading to the destruction of normal tissue and chronic inflammation [17]. As a result, the purpose of this study was to investigate how well Ginkgo biloba extract protects the brain of Wistar rats from oxidative stress, inflammation, and neurodegeneration against HgCl2 induced neurotoxicity in comparison to Ascorbic acid, investigate the extent to which EGB 761 can alleviate and mediate repair in mercury chloride-induced neurotoxicity.

Materials and Methods

Ethical Clearance

Ethical approval with Approval number ABUCAUC/2024/039 was obtained from the Ethics Committee on the use of animals of Ahmadu Bello University Zaria. All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals and law for laboratory experimentation.

Experimental Design

Adult male Wistar rats (n=48) were procured from the animal care unit (ACU), Amadu Bello University, Zaria. The animals were kept and maintained in standard laboratory conditions of room temperature, humidity and under 12-hour dark-light cycle in polyester cage with wire gauze. A total of 48 adult male Wistar rats were randomly divided into 6 groups of 8 rats each Table 1.

Neurobehavioral Study

Spontaneous Y- Maze

Table 1. Experimental groups.

Groups	Treatment and Exposure
Control	(Distilled water)1ml/body weight
$HgCl_2$	Mercury 5 mg/kg Only
$HgCl_2$+A. A LD	Mercury 5 mg/kg + 100 mg/kg Ascorbic acid (Low Dose)
$HgCl_2$+A. A HD	Mercury 5 mg/kg + 500 mg/kg Ascorbic acid (High Dose)
$HgCl_2$+EGB 761 LD	Mercury 5 mg/kg + 100 mg/kg Ginkgo biloba Extract (Low Dose)
$HgCl_2$+EGB 761 HD	Mercury 5 mg/kg + 500 mg/kg Ginkgo biloba Extract (High Dose)

All administration was done orally via gavage starting from Day 1 to Day 21.

The spontaneous Y-maze test is a behavioral task commonly used in animal research, particularly in rodents, to assess their spatial working memory and exploration behavior. The Y-maze consists of three arms placed in a Y-shape at a 120° angle from each other, made of opaque material. The test relies on the natural tendency of rodents to explore novel environments and their innate preference for exploring new areas [22]. The maze is usually elevated above the ground to prevent escape and encourage exploration. Before the actual testing began, the animals were placed individually in the Y-maze for a brief habituation period to acclimate them to the new environment. After habituation, the animal was placed at the end of one arm and allowed to freely explore the maze for 3 minutes. During the test phase, the researcher observed and recorded the animal's behaviour, specifically noting the sequence and duration of arm entries, the number of arm entries and the number of triads were recorded in order to calculate the percentage of alternation. An entry was counted when all four paws of the animal entered an arm. The main measure of interest in the spontaneous Y-maze test is spontaneous alternation. It refers to the tendency of the animal to alternate its arm choices. An alternation is defined as entering three different arms consecutively without re-entering any arm. The percentage of alternation is calculated using the formula: (Number of alternations / Total number of arm entries - 2) x 100. The recorded data was analyzed to determine the percentage of spontaneous alternation (% ALT) and other parameters of interest, such as total arm entries (TAE).

Biochemical Analysis: Oxidative stress and Neuroinflammation

Oxidative Stress

Animals were sacrificed and the brains were quickly removed. The brain tissues containing cerebral cortex and cerebellar cortex were collected, and then 1 g of tissues immediately homogenized. The tissue homogenates were centrifuged at appropriate centrifugal force for 10 min and the supernatant was utilized for different estimations according to the method of Bradford [23]. The supernatant was used to assay MDA, SOD, CAT and GSH using spectrophotometric methods at the Department of Chemical Pathology, Ahmadu Bello University Teaching Hospital, Shika Zaria Kaduna State, Nigeria.

Neuroinflammation

Determination of Tumor Necrotic Factor-Alpha (TNF-α) Utilizing the approach outlined by Zong, TNF- α activity was determined [24]. Principle: The TNF-a ELISA kit uses a technique called sandwich ELISA. A pre-coated macro-ELISA plate that has been immunosensitized with a rat TNF- α specific antibody is included in this kit. Both the standard or sample to be tested and the appropriate antibody combined in individual wells of a micro-ELISA plat. After incubation, each well received an addition of an avidin-horseradish peroxidase (HRP) combination and a biotinylated detection antibody against rat TNF-α. substrate solution was this applied to each well. A blue colour was formed only in wells containing Rat TNF-α, Avidin-HRP conjugate, detection antibody. The color changes back to yellow when the enzyme-substrate reacting is halted. The OD is measured at a wavelength of 450 nm ± 2 nm using a spectrophotometer. The OD value is proportional to the concentration of Rat TNF- α. In order to qualify the concentration of rat TNF- α in a sample, the optical density (OD) is compared to that of a standard curve

Interleukin-6 (IL-6)

The method published by Faulkner for measuring IL-6 activity was used [25]. Principle: The Sandwich-ELISA method is used for the determination of interleukin-6 in this study. Included in this kit is a micro-ELISA plate that has been pre-coated with an antibody specific to Rat IL-6. All that needs to be done is mix the standard or sample with the appropriate antibody and put it to a well on a micro-ELISA plate. In the following step, a biotinylated detection antibody against Rat IL-6 and HRP conjugate are applied to each micro plate well and incubated. The free elements are flushed away. Each well receives a dose of the substrate solution. The

only blue wells were those with Rat IL-6. biotinylated detection antibody, and Avidin-HRP conjugate. A bright yellow color is formed when the enzyme substrate reaction is being halted using a stop solution. Using a meno-plate reader, the optical density (OD) of each well was immediately determined at 450mm. A higher OD value indicates a higher concentration of Rat IL-6. Rat IL-6 concentrations are calculated by comparing the observed absorbance value (OD) of a sample to the corresponding OD standard curve.

Data Analysis

Descriptive analyses were expressed in terms of mean ± standard error (SEM). Normality of data distributions was analyzed using the Shapiro Wilk test and boxplot. Analysis of variance (one-way) was used to test for difference in brain weight, oxidative stress and inflammatory across treatment groups followed by John Tukey's Honest Significant Difference test for pairwise comparison of other groups against the comparator (control). Repeated measures analysis of variance was used to test for difference in body weight and spatial memory within the subject factors, time points and between-subject factors (treatment groups). Statistical analyses were conducted using Statistical Package for Statistical Product and Service Solutions (IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp) and GraphPad Prism 9.4.1 (GraphPad Prism, San Diego, California, USA). All tests were two-tailed with a p-value <0.05 set as the limit of statistical significance.

Results

Effects of L-Ascorbic Acid and Ginkgo Biloba extract on spatial learning and memory

Animals that received $HgCl_2$ showed significant ($p<0.05$) impairment in spatial learning in the form of less number Alternation, less percentage alternations and less Total Arm Entry (TAE) in comparison with control group which received Distilled water. Ascorbic acid (500 mg) treated animals showed significant improvement in the spatial learning deficits in comparison with the HgCl2 group. From the results of the present study, it is evident that Ascorbic acid can protect the brain from several neurotoxins, especially $HgCl_2$. The Ginkgo biloba (100 mg and 500 mg) treated group showed more alternations, a greater number of alternations, higher number in total arm entry and higher percentage alternation compared to the HgCl2 group (Table 2)

Effects of L-Ascorbic Acid and Ginkgo Biloba extract on selected endogenous antioxidant markers (SOD, GSH, MDA, CAT)

The Consumption of mercuric chloride led to a significant ($p< 0.05$) decrease in the activity of superoxide dismutase (SOD) in $HgCl_2$ group when compared to controls after 3 weeks of treatment (Figure 4b). At the same time, tissue catalase (CAT) (Figure 4d) and GSH activity decreased significantly ($p< 0.05$) in HgCl2 group when compared to control rats (Figure 4c). On the other hand, tissue malondialdehyde (MDA) level increased significantly ($p< 0.05$) in $HgCl_2$ group when compared to the control rats (Figure 4a). Ascorbic acid (500 mg/kg) being a potent antioxidant caused significant decrease in MDA level, significant increase in CAT, SOD level thereby mitigating mercury-induced oxidative stress in adult Wistar rats. Ginkgo Biloba Extract administration significantly reduced the harmful effects of HgCl2 on the previously measured parameters, suggesting that both ascorbic acid and Ginkgo biloba extract have neuroprotective qualities.

Effects of L-Ascorbic acid and EGb 761 extract on some inflammatory markers: TNF-α and IL-6 in the brain of rats following HgCl2-induced toxicity

EGB761 on TNF-α and IL-6 in the brain of rats following $HgCl_2$-induced toxicity. The levels of inflammatory markers (TNF-α and IL-6) were significantly increased ($p<0.05$) in the $HgCl_2$-induced group when compared to the control group. The administration of L-Ascorbic acid and 761 extract considerably led to a significant

Table 2. Effects of L-Ascorbic Acid and Ginkgo Biloba extract on spatial learning and memory.

GROUPS	n	TAE	Number of Alternations	% Alternation
Control	6	14.75 ± 2.87	9.13 ± 1.81	87.28 ± 22.94
5 mg/kg $HgCl_2$ only	6	5.11 ± 2.87**	3.00 ± 2.27**	63.00 ± 27. 09**
5 mg/kg $HgCl_2$ + 100 mg A. A	6	7.29 ± 0.93	4.80 ± 0.76	77.78 ± 25.09
5 mg/kg $HgCl_2$ + 500 mg A. A	6	11.90 ± 1.80 ππ	5.98 ± 1.22 ππ	84.50 ± 25.00 ππ
5 mg/kg $HgCl_2$ + 100 mg EGB 761	6	13.43 ± 2.07	7.67 ± 1.27	83.33 ± 28.87
5 mg/kg $HgCl_2$ + 500 mg EGB 761	6	13.86 ± 1.46 ππ	8.00 ± 0.82 ππ	85.71 ± 0.00 ππ

Mean ± SEM (n=6). **p< 0.05 versus control, ππ p< 0.001 versus HgCl2-treated groups. HgCl2 = Mercury Chloride; A.A = Ascorbic Acid; EGB 761 = Ginkgo biloba Extract; TAE = Total Arm Entry.

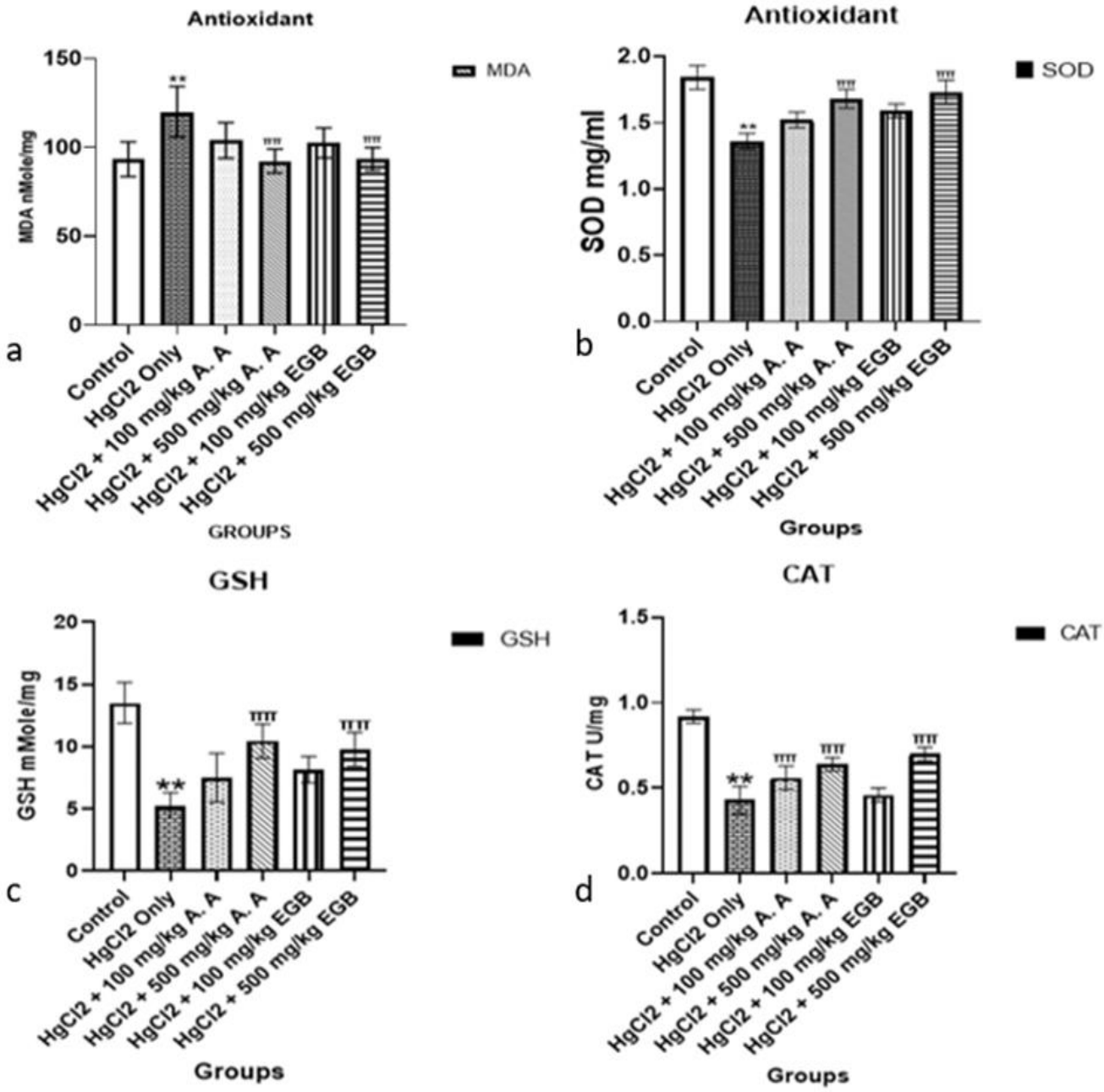

Figure 3. Effects of L-Ascorbic Acid and Ginkgo Biloba extract on spatial learning and memory. Mean ± SEM (n=6). **p< 0.05 versus control, ππ p< 0.001 versus $HgCl_2$-treated groups. $HgCl_2$ = Mercury Chloride; A.A = Ascorbic Acid; EGB 761 = Ginkgo biloba Extract; TAE = Total Arm Entry.

Table 3. Effects of L-Ascorbic acid and EGb 761 extract on some inflammatory markers: TNF-α and IL-6 in the brain of rats with $HgCl_2$-induced toxicity.

Groups	TNF-α (pg/ml) Mean ± SD	IL-6 (pg/ml) Mean ± SD
Control	292.67 ± 24.57	42.47 ± 5.70
$HgCl_2$ Only	789.65 ± 108.98[a]	83.96 ± 7.70[a]
$HgCl_2$+A. A LD	461.42 ± 91.17	53.81 ± 5.77
$HgCl_2$+A. A HD	324.58 ± 18.65[b]	43.75 ± 3.20[b]
$HgCl_2$+EGB 761 LD	348.58 ± 30.78[b]	49.09 ± 3.19[b]
$HgCl_2$+EGB 761 HD	337.44 ± 61.54[b]	45.06 ± 3.19[b]

[a]Significantly different from Control, [b]Significantly different from $HgCl_2$ Only Mercury chloride (toxicant group). The data are presented as the Mean ± SEM (n=6). a p<0.05 versus control, and b p< .001 versus $HgCl_2$-treated groups. $HgCl_2$ = Mercury Chloride, A.A = Ascorbic Acid, EGB = Ginkgo biloba extract, LD = Low Dose, HD = High Dose

decreased ($p<0.05$) TNF-α and IL-6 levels. Following treatment with L-Ascorbic acid and EGb 761 extract, there was observable decrease in TNF-α and IL-6 levels, the administration of $HgCl_2$+ 500 mg A. A (HD), $HgCl_2$ + 100 mg EGB 761 (LD) and $HgCl_2$ + 500 mg EGB 761 (HD) caused significant decrease in TNF-α and IL-6 level.

Discussion

In the Y-maze test the $HgCl_2$ group of rats showed significant difference in the form of less number alternation, less percentage alternations and less Total Arm Entry (TAE) in comparison with control animals which received Distilled water indicating memory impairment. According to several studies, $HgCl_2$ exposure causes neurological damage and behavioural alterations in experimental animals that could impair learning and memory [2, 26]. Result from this study reveals that $HgCl_2$ exposed rats showed lower number of TAE, ALT and % ALT, which is considered to be an index of learning and memory impairment. The results of the present study are consistent with previous study by [5] who reported that $HgCl_2$ intoxication causes anxiety, learning and memory impairment. The deficit was however not recorded in rats treated with Ginkgo biloba and Ascorbic Acid. It's interesting to note that different doses of the Ginkgo biloba (100 mg/kg body weight and 500 mg/kg body weight) treated group of animals showed a greater number of alternations, more number in total arm entry and higher percentage alternation compared to the $HgCl_2$ treated animals. Better spatial working memory is indicated by higher spontaneous alternation percentages. EGb 761 can significantly improve cognitive performance in patients with dementia and mild cognitive impairment by crossing the blood-brain barrier [27]. According to [28], ginkgo biloba improved memory and learning deficits brought on by fluoride exposure. Tomino et al. [29] reported that EGb 761 potential benefits for cognition may stem from its capacity to enhance cerebral blood flow, in addition to its anti-inflammatory and antioxidant characteristics. According to Le et al. [30], EGb 761 appears to be a safe and useful treatment for dementia patients, enhancing their social and cognitive functioning. Using standardized tests to assess learning, memory, attention and focus, mental status, and expressive language, Solomon et al. [31] also documented the effects of Ginkgo biloba on memory augmentation and improvements in the aged population. Compared to the $HgCl_2$ group of rats, animals treated with ascorbic acid (500 mg) exhibited a considerable improvement in their deficiencies related to spatial learning. The findings from this current study clearly show that ascorbic acid can shield the brain from a variety of neurotoxins, particularly $HgCl_2$. By preventing oxidative stress, ascorbic acid shields the brain against a variety of neurodegenerative conditions [32]. Ascorbic acid treatment was reported to correct learning and memory deficits observed in an Alzheimer's disease-modeling mouse [32]. Ascorbic acid supplementation, both short- and long-term, showed a facilitative effect on memory and passive avoidance learning [33]. Due to its neuromodulatory and cognitive-enhancing qualities, ascorbic acid may facilitate cholinergic transmission and improve the cognitive impairment, it may also function as an acetylcholine esterase inhibitor, which would lessen the impairments in spatial learning caused by mercury chloride [32]. Exogenous administration of Ascorbic acid has metal chelation properties, and it can bind with mercury ions and form stable complexes, thereby reducing the availability of free mercury ions and limiting their toxic effects in the hippocampus.

Oxidative stress has been associated to neuronal cell loss in neurodegenerative diseases. According to Dias et al. [34] and Lovell et al. [35], oxidative stress is regarded as a significant direct or indirect factor linked to the death of neuronal cells in neurodegenerative illnesses including Parkinson's disease and Alzheimer's disease. Reactive oxygen species (ROS) produced by $HgCl_2$ are known to induce oxidative stress [36], which leads to lipid peroxidation and subsequent instability and disintegration of the cell membrane. Similar to the findings published by Rizzetti et al. [37] which showed an increase in MDA in the brain and plasma, it was shown that chronic exposure to inorganic Hg promoted increased levels of malondialdehyde (MDA) and nitrites and decreased total antioxidant capacity by promoting oxidative stress in the hippocampus. The result obtained from this study by measuring the levels of oxidative markers revealed that the administration of mercury chloride induces oxidative stress. The significant increase ($p<0.05$) of MDA level (Figure 4) in $HgCl_2$ only treated group may therefore cause biochemical and functional changes in the brain tissues via increasing lipid peroxidation. On the other-hand decreased or reduced GSH following $HgCl_2$ administration are linked to $HgCl_2$ toxicity. GSH serves as an intracellular antioxidant and mercury carrier because of the thiol (-SH) group. Actually, GSH serves as the cell's initial line of defense against substances that contain hexachlorocl 2. Because $HgCl_2$ binds to glutathione and then removes it from the cell, the amount of GSH in the cell and its antioxidant capacity are decreased. According to the current study, rats given $HgCl_2$ had significantly ($p<0.05$) lower levels of brain GSH. Our research demonstrates that treatment with EGB 761 shielded rat brain tissue from $HgCl_2$-induced oxidative damage and significantly ($p<0.05$) restored the elevated MDA, and lowered GSH levels. This may be due to EGB 761's outstanding antioxidant qualities,

which have already been demonstrated in this study by the findings from other studies. ROS and oxidative damage are prevented by the enzymatic antioxidant system, which comprises CAT, GSH and SOD. The recovery of these biochemical variables demonstrated EGB 761'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 EGB 761'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. Mercuric chloride toxicity has been linked to elevated MDA levels in a variety of tissues and plasma, according to studies by Agarwal et al. [38] and Aslanturk et al. [39]. It has been revealed that the antioxidant defense system of the cell, which includes SOD and CAT, regulates the potentially detrimental effects of free radicals produced by mercuric chloride (Faix et al., [40]. According to Mao et al. [41], the enzyme superoxide dismutase (SOD) catalyzes the conversion of superoxide radicals into hydrogen peroxide and molecular oxygen. Catalase converts H_2O_2 to H_2O and oxygen, protecting cells from oxidative damage [42]. This study demonstrated the ability of mercuric chloride to induce oxidative stress by increased lipid peroxidation as shown in decreasing tissue SOD and CAT activities and increasing tissue MDA. This may be correlated with decreased SOD and CAT activities in mercuric chloride-treated rats in brain tissue Teixeira et al. [43]. In the other hand, the administration of Ginkgo biloba Extract markedly ameliorated the toxic effects of HgCl2 on the previous parameters (Figure 4b, Figure 4c and Figure 4d) indicating also that Ginkgo biloba Extract has neuro protective property. This is in line with previous work done by Yu et al. [44] who reported that standardized extract of Ginkgo biloba, EGb 761, have neuroprotective effects in several central nervous system and neurodegenerative diseases. Specifically, flavonoids are defined as chemicals that exhibit substantial bioactivity in brain processes, exhibiting favorable effects on neuronal activity and synaptic plasticity. Ginkgo biloba is one of the most widely used bioactive phytochemicals in the world [44]. While terpene lactones shield mitochondrial membranes from free radical damage, flavonoids scavenge free radicals [45]. EGb 761 has been described to have antioxidant properties playing an important role as a free radical scavenger [46]. EGb 761 has demonstrated that the antioxidant activity, as a "radical scavenger", is due to its superoxide dismutase-like activity that enables it to scavenge hydroxyl radicals [47]. Ginkgo biloba extract also has the capacity to regulate the oxidative stress. According to Zhou et al The levels of glutathione, malondialdehyde, superoxide dismutase and nitric oxide, increased after a treatment with EGb [48].

Ascorbic acid, a powerful water-soluble potent antioxidant which is involved in numerous cellular functions. It acts by scavenging free radicals, reactive oxygen species and by inhibiting lipid peroxidation [12]. By preventing oxidative stress, ascorbic acid shields the brain against a variety of neurodegenerative conditions. By neutralizing lipid hydroperoxyl radicals, ascorbic acid prevents oxidative stress and caused cellular damage, according to Berger et al. [49]. According to Kumar et al. [50], ascorbic acid has a neuroprotective role. It also has neuromodulatory properties and directly neutralizes substances that cause oxidative and nitrosative stress. The result from this study revealed that Ascorbic acid (500 mg/kg), being a potent antioxidant caused a significant ($p< 0.05$) decrease in MDA level, a significant ($p< 0.05$) increase in CAT, SOD level thereby mitigating mercury-induced oxidative stress in the adult Wistar rats. The result from this study is in line with El-Sharkawy and EL-Nasir, [51] who reported that Ascorbic acid can scavenge ROS, reduce oxidative damage, and protect hippocampal cells against mercury-induced oxidative stress.

Administration of $HgCl_2$ causes a significant increase ($p< 0.05$) in the TNF-α level of the HgCl2-treated group compared to the control. However, administration of EGB 761 and Ascorbic acid causes a significant reduction ($p< 0.05$) in the TNF-α level of the EGB 761 and Ascorbic acid groups compared to the HgCl2-treated group. This result favorably agrees with According to Takahashi et al. [52], mercuric chloride increases the expression level of vascular endothelial growth factor in brain tissue, which facilitates the non-selective influx of inflammatory and cytotoxic blood cells into brain tissue, thereby promoting damage to neurons and the blood-brain barrier (BBB). It was studied whether EGB 761 has a neuroprotective impact on $HgCl_2$-induced neuronal cell death because inflammation is linked to neurodegeneration during the AD process. The results showed that EGB 761 considerably reduced the amounts of TNF-α and IL-6 produced in response to $HgCl_2$. This illustrated that $HgCl_2$- activated microglia were able to induce neuron death by secreting pro-inflammatory cytokines. The cytokine tumor necrosis factor (TNF), a master regulator of the immune system, plays an important role in the propagation of inflammation due to the activation and recruitment of immune cells via its receptor TNF receptor 1 (TNFR1)

Conclusion

Mercury chloride has detrimental effect on the biochemical and neurochemical features. Treatment with EGB 761 reduced neuron death. Ginkgo biloba extract exhibits anti-inflammatory effects by interfering with the release of inflammatory

mediators like TNF-α. and IL-6. The anti-inflammatory action of EGB 761 observed in this study might be due to the blockage of the activation of NF-κB transcription factor, thus preventing the production of downstream pro-inflammatory cytokines such as TNF-α and IL-6, and the free radicals, such as ROS.

Contribution of authors

AMI., conceived the idea of the study and carried out the animal experimentation, SAM, SBO, ZMB and AMD participated in its design and supervision; AMI., analyzed the data, drafted the manuscript and all authors read and approved the final manuscript.

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Conflict of Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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