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Investigating the Effects of *Gingko Biloba* Extract and L-Ascorbic Acid on Some Hematological, Biochemical, and Hepatic-Architectural Alterations in Mercury Chloride Intoxicated Adult Wistar Rat.

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ABSTRACT

The study investigated the effects of *Ginkgo biloba* and Ascorbic acid (A.A) on mercury chloride ($HgCl_2$) toxicity in Wistar rats. Forty-two adult Wistar rats were randomly divided into seven groups (n=6). Group I served as control. Groups II–VII received 5 mg/kg of $HgCl_2$. Groups III and IV received 100 mg/kg and 500 mg/kg of A.A respectively, while groups V and VI daily received 100 mg/kg and 500 mg/kg of *EGB*761 respectively. Group VII received 100 mg/kg of A.A and *EGB*761. All administration were done orally, once daily for 21 days. Mercury exposure caused significant ($p<0.05$) decreases in RBC (3.62.00±0.75), hemoglobin (8.67±0.14), and PCV (32.93±0.47) in group II when compared to the control group, while significantly ($p<0.05$) increasing white blood cell count (18.57±1.51). Liver function tests significantly ($p<0.05$) increased Alanine transaminase (1.38±1.51), Aspartate Transaminase (10.11±4.21), and Alkaline Phosphatase (1.89±0.51) in group II when compared to Control. Biochemical analysis revealed a significant ($p<0.05$) increase in malondialdehyde levels and a significant ($P<0.05$) decrease in superoxide dismutase (3.86 ± 0.69), glutathione (10.08 ± 2.13), and catalase (3.65 ± 0.2) levels in group II. However, treatment with ascorbic acid and *EGB*761 significantly ($P<0.05$) increased in Groups IV (6.15 ± 0.38); VI (6.28 ± 0.40), and VII (6.19 ± 0.41), SOD levels in Groups IV (6.15 ± 0.38); VI (6.28 ± 0.40) and VII (6.19 ± 0.41) and GSH level when compared to the $HgCl_2$ group. Histological examination showed moderate hepatocyte necrosis in group II, while treatment

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with *EGB*761 and A.A showed moderate healing, binucleate nuclei, and a slight decrease of distorted sinusoids and normalization of liver structure. The study concludes that *EGB761 and* A.A has hepatoprotective effects on mercury-induced hepatotoxicity in a dose-dependent manner. The findings suggest that these antioxidants may be useful in mitigating the harmful effects of mercury exposure on the liver.

Keywords: Mercuric chloride, hepatotoxicity, *Ginkgo biloba* extract, Ascorbic acid.

INTRODUCTION

The toxicity of mercury compounds has been recognized since ancient times and is not in dispute [13] Mercury and its compounds have been used for industrial and medicinal purposes since ancient times, and the toxicity of mercury compounds has long been recognized [13] ,[20] Various users (e.g., skin-lightening creams and soaps, herbal remedies, laxatives, tattooing dyes, fingerpaints, artist paints, and make-up paints), and medicinal products (e.g., thimerosal, an ethyl mercury-containing compound that was used as a preservative in vaccines) that contain mercury or mercury compounds can contribute to consumers' exposure [51], [43], [33], [14]

Mercury exposure can lead to decreased red blood cell count (anemia) due to its toxic effects on bone marrow [24]. It can also lead to reduced hemoglobin levels, contributing to anemia and impairing the oxygen-carrying capacity of the blood [23] Mercury exposure can lead to decreased hematocrit. Mercury can affect white blood cells, which play a crucial role in the immune system [49] Chronic mercury exposure may suppress immune function, leading to reduced white blood cell counts and compromised immune responses [44]. Mercury exposure can interfere with platelet function and may lead to bleeding disorders or impaired blood clotting ability [28] Increased reticulocyte count is observed in response to anemia, as the body tries to compensate for the decreased red blood cell production caused by mercury toxicity [16]

Studies have shown that L-ascorbic acid and *Ginkgo biloba* extract possess protective potentials against biochemical changes induced by toxic substances like mercury ($HgCl_2$) [52], [7] Research has demonstrated that *Ginkgo biloba* extract can safeguard against mercury-induced oxidative tissue damage in rats, possibly due to its antioxidant properties [41] Moreover, *Ginkgo biloba* has been found to attenuate hepatotoxicity resulting from combined exposure to heavy metals and other toxins [46]. Similarly, studies have explored the potential of *Ginkgo biloba* extract in mitigating hematological disorders, oxidative stress, and pathological issues, potentially contributing to improved hematological parameters [27].

lysosomes that keep proton gradient through the membrane and decline renal glutathione peroxidase activity with upregulation of heme oxidase function. Several studies have found increased risk of pulmonary, renal, and CNS systems among dental workers [4] Mercury induces disruption of the cytochrome c oxidase system/ATP energy function [45] and inhibits enzymes needed to change porphyrins to ATP causing progressive porphyrinuria, leading to low energy and digestive injuries [12] Many studies indicated that oxidative stress represents a dangerous event correlated to the neurotoxic effects of $HgCl_2$ [30] The levels of various reactive species are dramatically increased upon HgCl2 exposure [21]. Although it is broadly sulfhydryl reactive, yet signaling cascade implicated in mediating $HgCl_2$-induced liver injury is not fully investigated. This initiates our interest in studying the mechanistic role of $HgCl_2$ hepatotoxicity at the biochemical, hematological and histological level and finding a way to protect against this toxicity using a combination of *Ginkgo biloba* Extract and L- Ascorbic acid powerful protection action against complications of various diseases due to their antioxidant role [9].

MATERIALS AND METHODS

Ethical Clearance

Approval for the study was given by Animal Use and Care Committee of Bingham University, Karu with approval number of No: BHUCAUC/2023/059. All protocols in this study followed the National Institutes of Health's guidelines for the use and care of laboratory animals and relevant approaches were maintained to ensure that animals were not subjected to excessive stress and discomfort in the course of this study.

Chemicals And Drugs:

Mercuric chloride and Laboratory graded ascorbic acid manufactured by Central Drug House (P) Ltd, Batch. No.038P011, India), were purchased from Covelyn Nigeria Ltd., Suit 2-126 Area 1 shopping center, Abuja Nigeria. Analytically graded *Ginkgo biloba* extract capsule (Bactolac Pharmaceutical Inc. Batch. No. KBPL/GBE/140101, USA Indore, India) was purchased from God is Able Pharmacy, Masaka, Karu, Nigeria. All other reagents and chemicals used were of analytical grade.

Experimental Design:

Forty-two animals weighing 180 to 200g were separated into 7 groups and each group had six animals (n=6). Group I control group received distilled water only all through the period of the study. Group II received 5mg/kg body weight of mercuric chloride for 21 days. Group III received 5mg/kg body weight of mercuric chloride and 100mg/kg body weight of ascorbic acid for 21. Group IV received 5mg/kg body weight of mercuric chloride and 500mg/kg body weight of ascorbic acid for 21 days. Group V received 5mg/kg body weight of mercuric chloride and 100mg/kg body weight of *Ginkgo biloba* for 21. Group VI 5mg/kg body weight of mercuric chloride and 500mg/kg body weight of *Ginkgo biloba* for 21 days. Group VII 5mg/kg body weight

of mercuric chloride and 100mg/kg body weight of ascorbic acid and 100mg/kg body weight of *Ginkgo biloba*.

Biochemical Analysis

At the end of the administration, the animals were subjected to a gradual concentration of CO_2, then sacrificed by decapitation. Blood samples were collected from cardiac puncture was left for 15mins, and sera were separated by centrifugation at 3000 rpm (revolution per minutes) for 5min. The supernatant was removed and then assayed for MDA, SOD, CAT and GSH. Malondialdehyde (MDA) level was estimated in the liver tissue following the method of Mihara and Uchiyam (1978). Reduced glutathione (GSH) level was determined using the method of Ellman (1959) Superoxide dismutase (SOD) activity was evaluated following the procedure of Marklund and Marklund (1974). The livers were also collected; parts of livers were homogenized in phosphate buffer to yield 20% homogenates. Then the homogenates were centrifuged for 5 min at 3000 rpm at 4^0C, and the supernatants were used to assay for ALP (Alkaline Phosphatase), ALT (alanine transaminase), and AST (aspartate transaminase) using spectrophotometric methods at the Department of Chemical Pathology, Ahmadu Bello University Teaching Hospital, Shika Zaria Kaduna State, Nigeria.

Histological analysis.

Liver samples were fixed in 10% formaldehyde, and thinly sliced sections (8 - 10 microns) were cut and the sections were floated on warm water bath to spread the tissue properly prior to mounting on a glass slide. The tissues were then be passed through changes of xylene for dewaxing and then taken to water by passing the slide with the tissue intact through descending grades of alcohol-absolute, 100%, 95%, 90%, and 70%, two changes each for each and finally water. The tissues were then stained using hematoxylin and eosin (H&E) stain at the Department of Human Anatomy, Ahmadu Bello University Zaria Kaduna State, Nigeria.

Statistical Analysis

Data were expressed as mean ± SEM for quantitative measures. The statistical comparisons were performed using one-way analysis of variance (ANOVA), followed by Tukey-Kramer multiple comparisons test. The level of significance was set at $P < 0.05$ and $P < 0.01$. Statistical tests were conducted using GraphPad Prism 9.4.1 (GraphPad Prism, San Diego, California, USA).

RESULT

Ginkgo biloba and L- Ascorbic Acid Retained Hematological Functions in $HgCl_2$-Induced Hematological parameters changes

The current work showed a significant ($p<0.05$) elevation of the activity levels of WBC in group II when compared to the control group; and on the other hand, the result revealed significant

($p<0.05$) decrease in RBC, Hb, and PCV in the group II exposed to $HgCl_2$-Only group when compared to the control group. The treatment with *Ginkgo biloba* and L- ascorbic acid significantly lowered ($p< 0.05$) the activity of WBC in groups IV, VI and VII and, increase RBC in groups IV, VI and VII, increased Hb in groups IV and VI and PCV in groups IV, VI and VII ($p<0.001$) when compared to group II (Table 1).

Table 1: Effect of Ascorbic Acid and GBE on hematological parameters in mercury chloride Induced intoxicated Rats.

Parameters	**RBC (10^6/mm3)**	**Hb (g/dL)**	**PCV (%)**	**WBC (10^3/mm^3)**
CTRL	7.35±0.61	14.00±0.54	50.33±1.36	8.57±1.27
GRP II	3.62±0.75[a]	8.67±0.14[a]	32.93±0.47[a]	18.57±1.51[a]
GRP III	4.30±0.39[a]	9.39±0.12[a]	34.50±0.72[a]	14.73±1.93[a]
GRP IV	6.37±0.38[bc]	12.33±0.49[bc]	46.31±1.40[bc]	10.63±1.27[bc]
GRP V	4.80±0.41[ad]	9.00±0.23[ad]	36.30±0.90[ad]	13.80±1.44[ad]
GRP VI	6.35±0.52[bc]	13.00±0.12[bc]	47.41±1.70[bc]	10.57±1.61[bc]
GRP VII	5.83±0.38[bc]	11.13±0.21[ab]	45.66±1.20[ab]	12.63±1.70[ab]

The data are presented as the Mean ± SEM (n=6). [a]Significantly different from control [b]Significantly different from GRP II Mercury chloride (toxicant group), [c]Significantly different from GRP III Mercury chloride + Ascorbic Acid 100mg/kg, [d]Significantly different from GRP IV Mercury chloride + Ascorbic Acid 500mg/kg.

Ginkgo biloba and L- Ascorbic Acid Restored Liver Functions in $HgCl_2$-Induced Hepatotoxicity

The current work showed significant $p< 0.05$ increase in the activities of the ALP, AST and ALT in serum of group II ($HgCl_2$) group when compared to the control and the rest of the treated groups. Following the administration of *Gingko biloba* and Ascorbic acid, ALP significantly decreased ($p< 0.05$) in groups IV, VI and VII when compared to control group (Figure 1). The activity of AST in group III, VI and VII were significantly $p<0.001$ lower than group II ($HgCl_2$), this may be that action of *Ginkgo biloba* and Ascorbic Acid may attenuate cellular disturbance in the activities of liver AST caused by mercury induced toxicity. The activity of ALT in group III to VII was significantly $p<0.001$ decreased when compared to the group II following treatment with *Gingko biloba* extract and ascorbic acid (Figure 1).

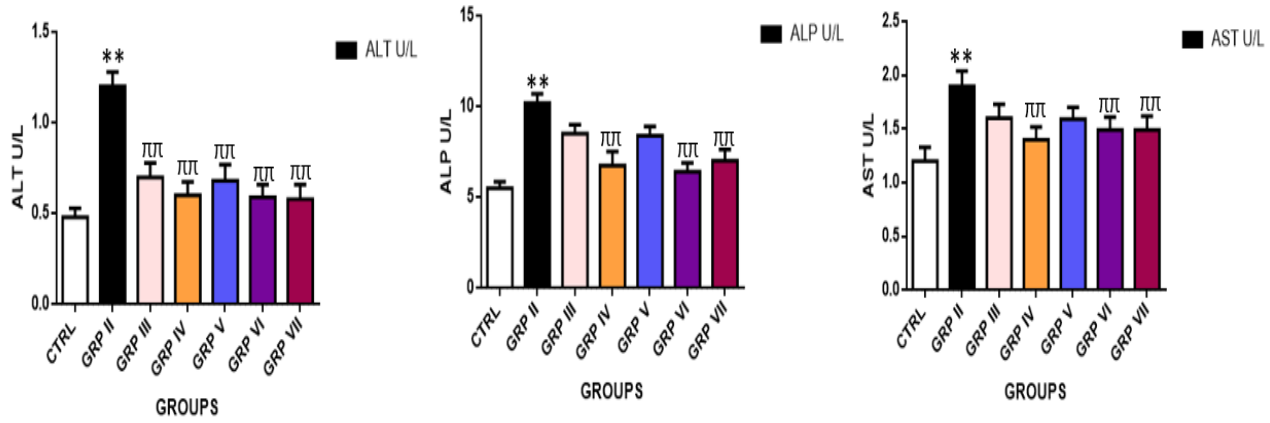

Figure 1. *Ginkgo biloba* Extract, L-Ascorbic Acid and their combination prevents $HgCl_2$-induced liver function alterations. *Ginkgo biloba* Extract, L-Ascorbic Acid reduced serum ALT, AST and ALP in $HgCl_2$-intoxicated rats. The data are presented as the Mean ± SEM (n=6). **Significantly different from control ππSignificantly different from GRP II Mercury chloride (toxicant group).

L- Ascorbic Acid and *Ginkgo biloba* Extract Attenuated Oxidative Stress Induced by $HgCl_2$

Further assessment of the hepatic protective effects of L- Ascorbic acid and *Ginkgo biloba* Extract was conducted by measuring the levels of oxidative markers. Our study revealed that the administration of $HgCl_2$ caused significant increase ($p< 0.05$) in the levels of (MDA) in group II when compared to the control group (Figure 2A), while CAT, SOD activity and GSH level were significantly lowered ($p< 0.05$) in $HgCl_2$ treated group (group II) when compared to the control group (Figure 2B, 2C and 2D) respectively. In the same figure, the administration of L-Ascorbic acid and *Ginkgo biloba* Extract significantly ($p< 0.05$) decreased the toxic effects of $HgCl_2$ on MDA level in groups IV, VI and VII when compared to control group (Figure 2A). The administration of L-Ascorbic acid and *Ginkgo biloba* Extract significantly $p<0.001$ increased the activity of CAT, SOD in group III, V VI and VII when compared to group II ($HgCl_2$). The administration of L-Ascorbic acid and/or *Ginkgo biloba* Extract significantly lowered the $HgCl_2$ induced oxidative stress ($P < 0.05$) and prevent the overexpression of MDA in groups IV, VI and VII.

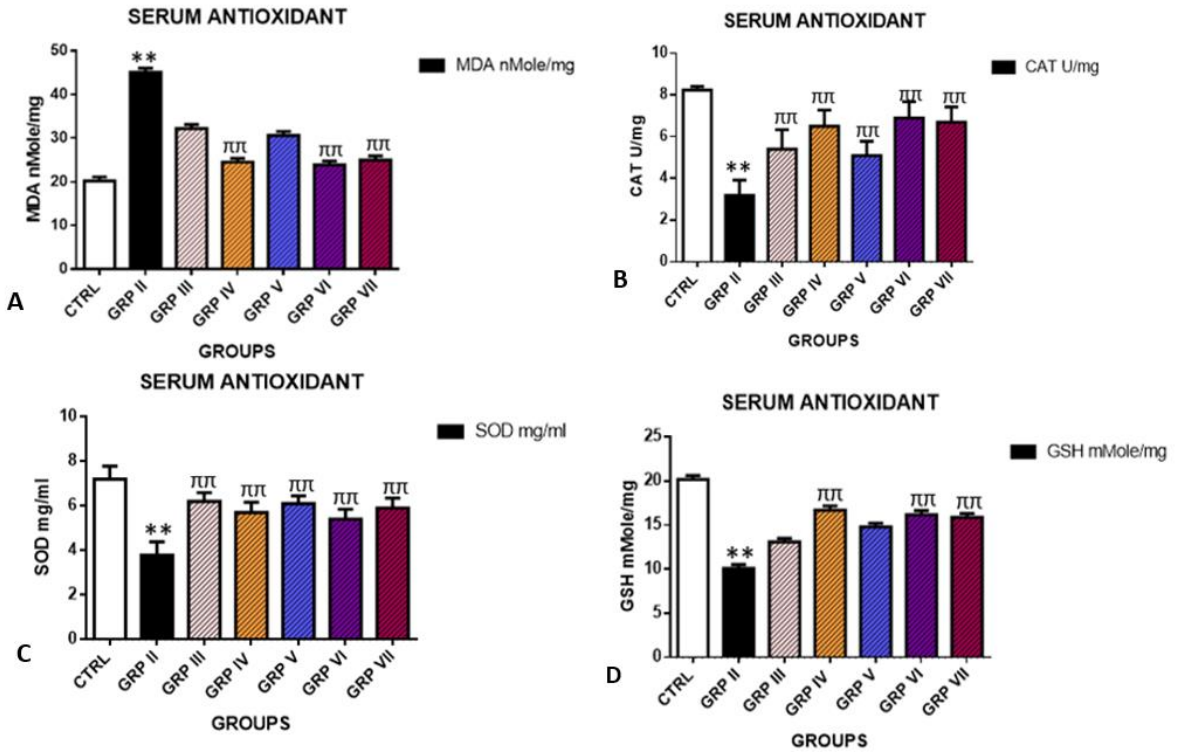

Figure 2. *Ginkgo biloba* Extract, L-Ascorbic Acid and their combination Modulates $HgCl_2$-induced liver injury in rats. *Ginkgo biloba* Extract, L-Ascorbic Acid reduced serum MDA; increases CAT, SOD and GSH in $HgCl_2$-intoxicated rats. The data are presented as the Mean ± SEM (n=6). **Significantly different from control ππSignificantly different from GRP II Mercury chloride (toxicant group).

L- Ascorbic Acid and *Ginkgo biloba* Extract Improved the Histopathological Changes Induced by $HgCl_2$ Overdose

The hepatoprotective effects of L- Ascorbic Acid and *Ginkgo biloba* Extract was confirmed by the histological examination of liver sections stained with H&E. The liver sections from the control group demonstrate normal central vein, normal arrangement of hepatocytes in sinusoids and regular interspaced around hepatocytes (Figure 3A). The liver section from the group administered 5 mg/kg $HgCl_2$ showed congested central vein, dilated sinusoid, altered hepatocytes characteristics with vacuolization of hepatocytes and pyknotic nuclei (Figure 3B). The liver section from the group administered 100 mg/kg L- Ascorbic Acid with 5 mg/kg $HgCl_2$ showed distorted central vein and sinusoids, irregular and distinct interspaced around hepatocytes, inflamed hepatocytes and binucleated nuclei. a hepatic tissue with normal structure and architecture with dilated congested sinusoids, central vein congestion (Figure 3C). The liver section from the group administered 500 mg/kg L- Ascorbic Acid with 5 mg/kg $HgCl_2$ revealed moderate healing of the necrosis and a slight decrease of the dilated sinusoids, central vein congestion, pyknotic nuclei and

hepatocytes (Figure 3D). The liver section from a rat administered 100 mg/kg *Ginkgo biloba* Extract concurrently with 5 mg/kg $HgCl_2$ shows altered structural changes with moderate healing of the necrosis and a slight decrease of the dilated sinusoids (Figure 3E). The liver section from a rat administered 500 mg/kg *Ginkgo biloba* Extract with 5 mg/kg $HgCl_2$ shows moderate healing of the necrosis and organized sinusoids, central vein congestion with regular interspace around hepatocytes (Figure 3F). Lastly, the liver section from a rat administered a combined therapy of 100 mg/kg L- Ascorbic Acid and 100 mg/kg *Ginkgo biloba* Extract with $HgCl_2$ shows a marked improvement in hepatocyte degeneration with normal sinusoids (Figure 3G).

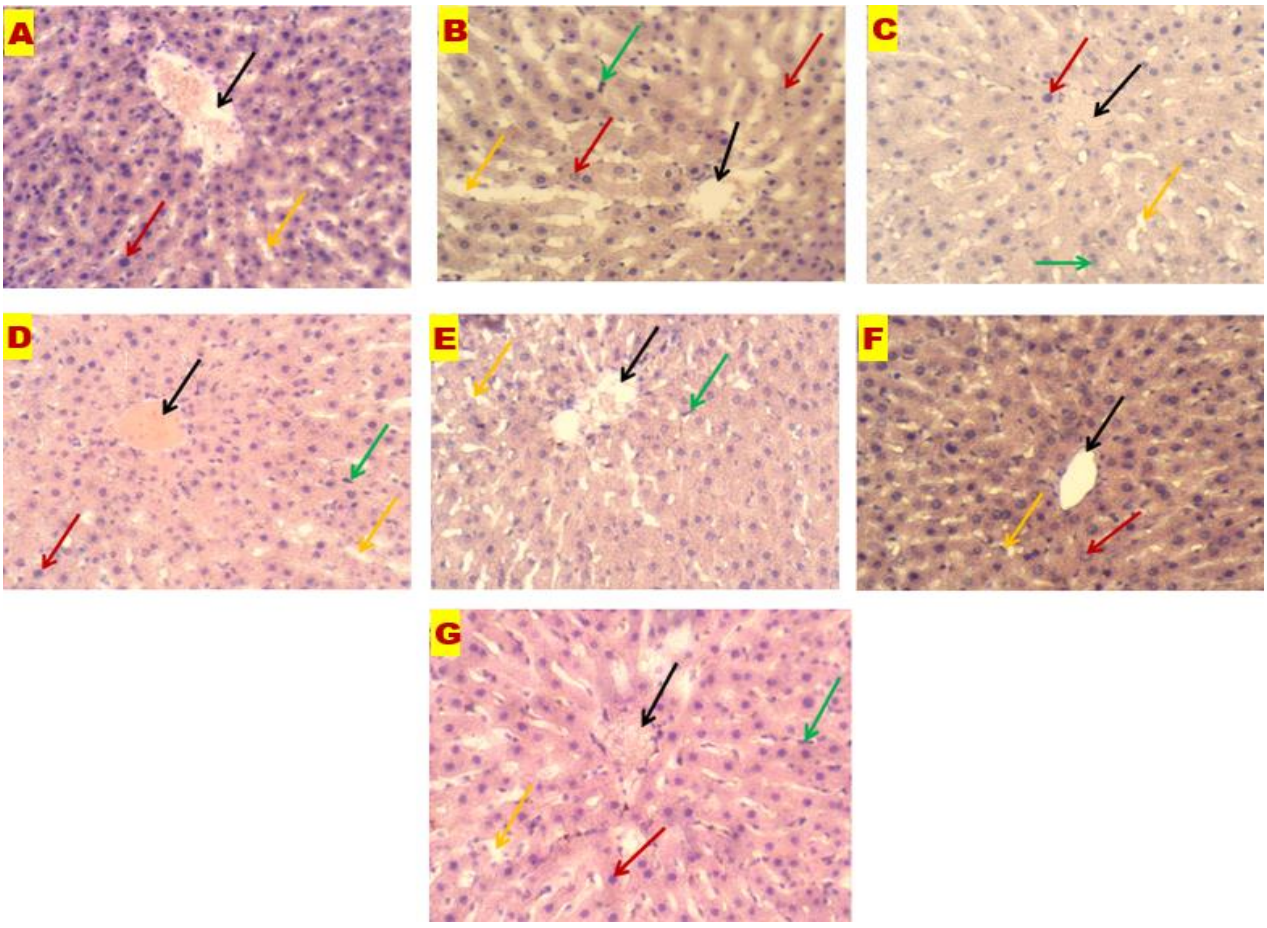

Figure 3. Photomicrographs of liver sections stained with H&E (Magnification 400X). (A) The liver section from the control rat shows normal Central vein (Black arrow), and normal hepatocytes (Red arrow) with normal sinusoids (yellow arrow). (B) The $HgCl_2$-only group shows moderate hepatocytes (Red arrow). (C) The 5 mg/kg $HgCl_2$ and 100 mg/kg Ascorbic acid show moderately inflamed Hepatocytes (Red arrow), Binucleate nuclei (Green arrow), and slightly decreased distorted sinusoids (Yellow arrow). (D) The 5 mg/kg $HgCl_2$ and 500 mg/kg Ascorbic acid showed normal architecture with slightly decreased dilated congested sinusoids (Yellow arrow), central vein slight congestion (Black arrow) pyknotic nuclei (Green arrow). (E) The 5

mg/kg $HgCl_2$ and 100 mg/kg Gingko Biloba Extract show central vein (Black arrow) pyknotic nuclei (Green arrow) slightly distorted sinusoids (Yellow arrow). (F) The 5 mg/kg $HgCl_2$ with 500 mg/kg *Ginkgo biloba* Extract shows marked improved architecture with sinusoids (Yellow arrow), central vein slight congestion (Black arrow) hepatocytes (Red arrow) with regular interspace around hepatocytes. (G) The 5 mg/kg $HgCl_2$ and 100 mg/kg Ascorbic Acid and 100 mg/kg *Ginkgo biloba* Extract shows a marked improvement in hepatocyte degeneration (Red arrow) with slightly distorted sinusoids (Yellow arrow), central vein slight congestion (Black arrow).

DISCUSSION

The roles of the liver in metabolic activities and in providing the body with the energy it needs cannot be overemphasized. It regulates the production, storage, and release of sugar, fats, and cholesterol. Human may be exposed to $HgCl_2$ poisoning by multiple routs including oral, inhalation or skin exposures, since it is widely distributed in the environment. The environmental levels of mercury are rising because of the expulsion from hydroelectric, mining and paper industries. It can be found in some skin lightening products and the filling of dental amalgam [39] The main objective of this study was to investigate the effects of mercury chloride on the activities of some liver enzymes and cytoarchitecture in mercury chloride-induced hepatotoxicity as well as the ameliorative properties of Ginkgo biloba extract and Ascorbic acid in attenuating mercury chloride effects.

Effects of mercury on the hematological system in animals have been evaluated following acute- and intermediate-duration oral exposure to mercuric chloride and intermediate- and chronic-duration oral exposure to inorganic mercury salts [22]. Available data suggesting impaired clotting, small decreases in RBC counts, and increased WBC counts in rodents exposed to mercuric chloride are of uncertain biological relevance [18] Result from the current work showed a significant elevation of the activity levels of WBC in group II when compared to the control; and on the other hand, decrease in RBC, Hb and PCV in rat exposed to $HgCl_2$ only (group II) when compared to the control group. Our results are consistent with the outcome of previous studies which revealed increase in WBC activities (Dieter et al., 1983; Kim et al., 2003) and decreasing activities of RBC [29]; Kim et al., 2003; [32] following exposures of Wistar rats to mercuric chloride. In a chronic study conducted in rats, phenylmercuric acetate given in water at a dose of 4.2 mgHg/kg/day caused decreases in haemoglobin, hematocrit, and RBC counts (Solecki et al., 1991). Following the administration of *Gingko biloba* and Ascorbic acid, RBC, Hb and PCV were significantly increased in groups IV, VI and VII when compared to control group. This is an indication of the attenuating nature of Ginkgo biloba extract and Ascorbic acid on mercury chloride-induced hepatotoxicity. The administration *Ginkgo biloba* and L- Ascorbic acid concurrently with $HgCl_2$ significantly lowered the activity of WBC and increased RBC and Hb in the treated groups

Alkaline Phosphatase (ALP) is an enzyme found in the liver, bile ducts, and the bones. High levels of ALP may indicate liver damage, blockage of the bile ducts, or a bone disease. The activities of Alanine Transaminase (ALT), Aspartate Transaminase (AST) and Alkaline Phosphatase (ALP) in

the serum or homogenate are examined as indicators for hepatic function (Brzoska et al., 2003). In the present study, the activities of the ALP in serum of group II ($HgCl_2$) animals were significantly ($p<0.05$) increased compared to the control and the rest of the treated groups. This is an indication that mercury chloride produced hepatocellular damage in the animals in group II that was administered 5 mg/kg of mercuric chloride, this was also corroborated by the histological findings. This finding agrees with the work of [34] who reported an observable increase in ALP activities following the administration of mercury chloride in Wistar rats. The administration of *Gingko biloba* and Ascorbic acid, ALP were significantly decreased in groups IV, VI and VII when compared to the control group. This is an indication of the attenuating nature of *Ginkgo biloba* extract and Ascorbic acid on mercury chloride-induced hepatotoxicity. This is supported by the findings of [6] who reported an attenuating nature of *Gingko biloba* leave extracts in the activities of ALP following Liver Fibrosis induced by thioacetamide in Mice. It was reported that *Ginkgo biloba* extract possessed antioxidant properties with the efficacy of ameliorating or preventing diseases associated with free radicals [53] Aspartate aminotransferase (AST) is an enzyme found in several parts of the body, including the heart, liver, and muscles cells. When the liver is damaged, AST is released into the bloodstream. AST is an enzyme that is associated with liver parenchymal cells and it is raised in acute liver damage. The ratio of AST to ALT, is mostly useful in differentiating between causes of liver damage. When AST and ALT are both over 1000 IU/L, the differential can include acetaminophen toxicity, shock, or fulminant liver failure [36] When AST and ALT are greater than three times normal but not greater than 1000 IU/L, the differential can include alcohol toxicity, viral hepatitis, drug-induced level, liver cancer, sepsis, Wilson's disease, post-transplant rejection of liver, autoimmune hepatitis, and steatohepatitis (nonalcoholic). AST/ALT levels elevated minorly may be due to rhabdomyolysis, among many possibilities [37] In the present study, the activity of AST in group II ($HgCl_2$) was significantly ($p<0.05$) increased when compared to the control group. This is an indication that $HgCl_2$ produced a hepatocellular damage in group II, this is supported by the result of the activities of the ALP. This also may be confirmed by the histological result as seen in Figure 4B which shows some features of distorted sinusoids, altered hepatocytes characteristics, hepatocytes cytoplasm appears degenerated with some features of disorganized hepatocyte cords without the normal lobular architecture that is seen in Figure 3A. However, the activity of AST group III, VI and VII decreased significantly ($p<0.05$) when compared to group II ($HgCl_2$), this may be that action of *Ginkgo biloba* and Ascorbic Acid may attenuate cellular disturbance in the activities of liver AST caused by mercury-induced toxicity. ALT is an enzyme that helps to process proteins and it occurs in large amounts in liver cells. When the liver is injured or inflamed ALT usually rises in the blood level. However, in the present study the activity of ALT in group II ($HgCl_2$) was significantly higher than that of the control group indicating that $HgCl_2$ produced hepatocellular damage in group II. This may be due to the inability of the liver cells to degrade toxic substances. However, treatment with Gingko Biloba extract and ascorbic acid were significantly decreased ALT in group III to VII this might be the as a result of the antioxidant properties of *Ginkgo biloba* and ascorbic acid to ameliorate the effect of mercuric chloride on the liver against oxidative stress. The ameliorative properties of

Ginkgo biloba can be largely attributed to its high flavonoid content, which provides potent antioxidant effects. These flavonoids play a critical role in combating oxidative stress induced by hepatotoxicants, thereby protecting liver cells from damage and supporting overall hepatic health [37].The increase in liver enzyme markers ALT, AST, and ALP in mercury chloride-treated groups signifies hepatocellular injury and dysfunction. This is accompanied by structural changes in the liver, including inflammation, necrosis, and potential fibrosis, which collectively alter hepatocyte characteristics and impair liver function. In this study, we found that $HgCl_2$ cause a significant increase of serum ALT, ALP and AST activities. These enzymes are regarded as critical and initial markers in the diagnosis of liver injury, as these enzymes could be distributed into the circulating blood directly due to hepatic cell injury [26] *Ginkgo biloba* successfully lessened the ALT and AST liver enzyme levels in hepatic disease [54]. Our results were consistent with the outcomes of previous studies which revealed the increased liver enzyme activities following exposure of $HgCl_2$ [26], [11]

Antioxidant enzymes play a critical role in protecting the body's cells from oxidative stress, which can be caused by the accumulation of reactive oxygen species [3] These ROS are highly reactive molecules that can damage cellular components such as DNA, proteins, and lipids, ultimately leading to various diseases and contributing to the aging process [25]. Antioxidant enzymes are a primary defense mechanism against oxidative stress, which helps to prevent cellular damage and contributes to overall health [42]. These enzymes work in concert with nonenzymatic antioxidants, such as vitamins A, C, and E, to maintain a balance between the production of ROS and the antioxidant defenses. This balance is crucial for preventing oxidative damage and maintaining cellular health and function (Price & Preedy, 2020). An increase in malondialdehyde levels and a decrease in the activity of catalase, superoxide dismutase, and levels of glutathione are significant indicators of oxidative stress and potential cellular damage. Our study revealed that the administration of $HgCl_2$ caused a significant increase in the levels of (MDA) in group II when compared to the control, while GSH level and SOD activity were lowered in $HgCl_2$ only treated group. It has been revealed that when $HgCl_2$ is build up within the hepatic cells, this activates oxidative stress and subsequent liver injury, and it is believed that the principal mechanism of hepatotoxic effect of $HgCl_2$ is the emission of free radical and production of reactive oxygen species (ROS) which is an important factor known to be sensitive to oxidative stress [8] [11] The administration of *Ginkgo biloba* Extract could reduce the $HgCl_2$-oxidative stress and prevent the overexpression of MDA. This finding is in line with the report of [17] 2022 who reported that *Ginkgo biloba* mitigates mercury-induced liver damage by restoring oxidative balance, improving serum parameters, caused by mercury chloride toxicity in Wistar rats. It has been demonstrated that *Ginkgo biloba* extract has proved to be an effective antioxidant, this effect could be due to the flavonoids present in ginkgo leaves [8] 2018; [11] Mercury-induced toxicity of the liver was annulled by *Ginkgo biloba* extract by altering the biochemical and oxidative damage that occurred in the liver [46] Chemical-induced fibrosis and oxidative injury of the liver were ameliorated by *Ginkgo biloba* extract [15] *Ginkgo biloba* and ascorbic acid could potentially work synergistically to combat oxidative stress by both direct free radical scavenging and by supporting endogenous

antioxidant defense systems, which may mitigate the effects of mercury chloride on the liver's antioxidant enzymes.

The histology of the liver was demonstrated using the Haematoxylin and Eosin technique with emphasis on the general cytoarchitecture. Figures 3 show the photomicrographs of the liver at x400 magnifications. Figure 3A shows the features of a normal liver histology with a clearly shown central vein, hepatocytes with the centrally located rounded nucleus, and clearly observable sinusoids that are in line with the Kupffer cells. The cytoplasm of the hepatocytes was well demonstrated with abundant eosinophilic cytoplasm with fine basophilic granules that represent the rough endoplasmic reticulum. Also, in figure 3B, the majority of hepatocytes appeared scantly arranged, dilated sinusoids, central vein congestion and Pyknotic nuclei. The nuclei condense into shrunken basophilic masses. The hepatocytes appeared irregularly arranged with disorganization of hepatic architecture, indicating destruction of hepatocyte integrity in group II by mercury chloride. Figures 3D, 3E and 3F shows the features of a normal liver histology with a clearly shown central vein, hepatocytes with intact nuclei and observable sinusoids that are in line with Kupffer cells. Also, features with of binucleated hepatocytes were observed in figure 3C and 3F which is an indication of regeneration. Multinucleated hepatocytes appear to be more prominent in low dose of Ascorbic acid group III and high dose of gingko biloba extract group VI. This might be an indication that Ascorbic acid at low dose and Gingko biloba at high dose was able to ameliorate. Figure 3G showed mild distortions that were observed in group II; However, the observable features were not as prominent as features observed in group II. Mercury chloride toxicity disrupted the histological architecture of the liver as indicated by disrupted plates of hepatocytes and disrupted sinusoids that appear dilated and distorted as seen in group II ($HgCl_2$). This work is in accordance with the findings of [5] who reported histological observations such as inflammation, central vein enlargement, distorted sinusoids, and hemorrhage, indicating destruction of hepatocyte integrity due to mercury chloride exposure. [2] also reported irregular arrangement and disorganization of hepatocytes in the mercury chloride group which indicated destruction of hepatocyte' integrity due to mercury chloride exposure. Low and High doses of Ascorbic acid (500 mg/kg body weight) and high doses of *Ginkgo biloba* (500 mg/kg body weight) produced better ameliorative effects as tissue disruption was mild and cells are relatively preserved in terms of their morphologies. However, low dose of combined therapy could not significantly ameliorate effects as treated animals' liver cells show signs of cells and tissue damage. The Liver tissue was better persevered with the high dose of Ascorbic acid and *Ginkgo biloba* than the low doses. This finding is in line with the report of [40].who reported ginkgo biloba mitigates mercury-induced liver damage by restoring and reversing histopathological alterations caused by mercury chloride toxicity in Wistar rats. It can be suggested from the present study's findings that Ascorbic acid and Ginkgo biloba has ameliorative effects on mercury chloride-induced hepatotoxicity with the high dose proving more effective in restoring liver functions and cytoarchitecture. Hence, using those antioxidants improved liver morphology.

Conclusion

This study suggested that Wistar rats exposed to mercury chloride might have led to interference and change in the dynamics of hematological, biochemical, and liver function parameters. However, the Supplementation of L-Ascorbic acid and/or *Ginkgo biloba* Extract to mercury chloride intoxicated group significantly attenuated hematological, biochemical, and liver function parameters. L-Ascorbic acid and/or *Ginkgo biloba* extract has the potential to protect the liver from the toxicity induced by $HgCl_2$, attenuate and reverse $HgCl_2$-induced oxidative stress injury in liver tissue hence holding potential for the treatment of mercury chloride toxicity. Treatment with *Ginkgo biloba* extract and ascorbic acid restored the normal levels of the previously mentioned parameters and improved hepatic architecture.

Author's Contributions

MIA conceived the idea of the study, participated in its design and coordination; MIA, UWO and OEM carried out the animal experimentation, hematological and biochemical analyses; MIA analyzed the data; MIA and COA drafted the manuscript and all authors read and approved the final manuscript.

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Declaration of Conflicting Interests

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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