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Andrographis Paniculata: Protects against Hepatorenal in Rats Exposed to Mercury Chloride

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ABSTRACT

Andrographis paniculata is a traditional plant, although little is known regarding its effectiveness against mercury chloride-induced liver and kidney damage**.** The hepatorenal protective properties of the *A. paniculata* extract was assessed in mercury chloride-induced (0.5 mg/kg) hepatorenal toxicity in male Wistar rats (5/group). The aqueous *A. paniculata* extract was administered at 250 and 500 mg/kg doses orally for 28 consecutive days concurrently with the mercury chloride**.** Ascorbic acid (200 mg/kg) was used as the standard drug. After the administration, biochemical parameters on serum were assessed, and histology on H&E-stained liver and kidney sections. The results showed that *A. paniculata* significantly ($p < 0.05$) decreased the levels of serum AST, ALT, ALP, Urea, and Creatinine MDA and significantly ($p < 0.05$) increased the contents of superoxide dismutase, catalase and glutathione peroxidase. Rats given only mercury chloride developed hepatic steatosis and renal glomerulus degeneration. The *A. paniculata* extract and ascorbic acid prevented the degeneration of the cytoarchitecture of the liver and renal glomerulus. Histological results support the biochemical findings. In conclusion, the findings in this study suggest that *A. paniculata* has a protective potential against mercury chloride induced liver and kidney toxicity by preventing the upsurge of liver enzymes markers and oxidative stress.

Keywords: Mercury chloride, *Andrographis paniculata*, Liver, Kidney

INTRODUCTION

Mercury is a highly toxic metal that contributes to pollution in the environment[1]. The cement, electrical, mining, paper, and industries all use mercury[2]. It is also used as a catalyst in the production of plastics, a component in various measurement and control devices such as barometers and thermometers, a dental filling material, and a fungicide in agriculture[3]. Mercury exposure in various forms, such as water, air, soil, and food, poses a severe threat to our health and the environment[4]. Annually, about 2200 metric tons of mercury are estimated to be released into the atmosphere[5].

Mercury salts are inorganic mercury compounds formed when mercury is combined with chlorine, sulfur, and oxygen[2]. They are water-soluble and can enter into the human body via direct contact or diet[6]. Mercury ions accumulate in various organs after exposure, including the intestine, brain, kidney, placenta, and liver[7]. One of the inorganic compounds of mercury, mercury (II) chloride, has been classified as a "violent poison"[8]. $HgCl_2$, one of the most toxic mercury salts, is primarily metabolized in the liver before being stored in the kidneys. As a result, the liver and kidneys are the most affected organs[9]. $HgCl_2$ destroys free radical scavenging systems like catalase, reduced glutathione and superoxide dismutase[10] and boosts reactive species levels, disrupting the prooxidant-antioxidant balance system and oxidative stress[11].

Andrographis paniculata Nees. is a medicinal plant of the genus Andrographis (family Acanthaceae) that overgrows and is easy to cultivate. It has a bitter taste. The plant has long been used for various medicinal and health-related purposes[12]. *A. paniculata* contains a variety of compounds, including labdane diterpenoid lactones, flavonoids, and other compounds. It has a diverse set of pharmacological properties that have been demonstrated [13, 14]. In Asia, America, and Africa, the plant has been used to treat various illnesses[15]. The Indian Pharmacopoeia includes this plant and is used in approximately 26 Ayurvedic formulations[16,17]. *A. paniculata* has been shown to help with antioxidant defences in the past [18,19]. It works by mitigating the free radicals directly. It indirectly affects mitochondrial integrity by inhibiting pro-oxidant enzymes and activating antioxidant enzymes. It can also activate enzymatic and non-enzymatic antioxidants, primarily through the Nrf_2 signalling pathway[20]. Despite *A. paniculata's* medicinal potential, the plant's protective potential against mercury chloride has not been examined. Thus, the objective of this study was to determine if *A. paniculata* could protect adult Wistar rats from mercury chloride-induced hepatorenal toxicity.

MATERIALS AND METHODS

Materials: Mercury chloride (BDH Chemicals Ltd, England) was obtained and used as a hepatorenal toxicant. Vitamin C (Ascorbic Acid; 70 mg/tablet; Micro Labs Limited's) was obtained from a reputable pharmaceutical store (M.U.B Pharmaceutical Enterprises Ltd.) Zaria, Kaduna State, and used as a standard antioxidant drug. Seventy per cent ethanol and phosphate-buffered saline (PBS) (Sigma-Aldrich Co. LLC St. Louis, USA). Ketamine hydrochloride was used as an anesthetic (Sigma-Aldrich Co. LLC St. Louis, USA). Colourimetric Diagnostic Kits (Agappe Kits) used to measure ALP, ALT, AST, creatinine, and urea. Assay kits (Biodiagnostic Co., Cairo, Egypt) were used to determine the antioxidant enzyme activities (SOD, CAT, and GSH) and MDA content in blood serum. Haematocrit centrifuge (Denley BS400 centrifuge, England), Automated biochemistry analyzer (Selectra XL, Vital Scientific, Netherlands). Electronic weighing machine (Precision Electronic instrument Company, India), etc.

Plants Collection and Authentication: Aerial parts of *A. paniculata* (Green chireta) were collected in Idoani, Ose Local Government Area, Ondo State, Nigeria. In the Herbarium Section of Department of Botany, Ahmadu Bello University (ABU) Zaria, the plant specimen was identified and authenticated, and voucher number 1331 was assigned for reference.

Plant Extraction: The *A. paniculata* was ground and extracted in a soxhlet apparatus for 10 hours with distilled water, according to the methods described by Association of Official Analytical Chemists procedure[21]. The extract was subjected to preliminary phytochemical screening at the Department of Pharmacognosy and Drug Development, ABU, Zaria, according to the technique described by Trease and Evans[22].

Experimental Animals: Twenty-five male and female Wistar rats were obtained from the Animal House, Department of Pharmacology, Faculty of Pharmaceutical Sciences, ABU, Zaria, and fed commercial grower poultry feed with free access to water. The rats were housed in clean metal cages with twice-weekly bedding changes. The rats were acclimatized for ten days and the rats were cared for following internationally recognized animal welfare standards.

The Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) granted ethical approval for this study (ABUCAUC/2020/57).

Experimental Procedure: The rats were randomly divided into five groups (n=5). The administration of mercury chloride, ascorbic acid, and *A. paniculata* aqueous extract is summarized in Table 1. The mercury chloride dose was adopted from previous research[23]. The mercury chloride, *A. paniculata* and ascorbic acid were administered via oral gavage. The rats were weighed weekly before and during the study.

Experimental Design: The rats were randomly distributed into five groups (n=5). The grouping and administration of $HgCl_2$, ascorbic acid and aqueous extract of A. paniculate is summarized in Table 1.

Table 1: A summary of the experimental design

Groups	Treatment (oral)	Duration
Control	Distilled water at (2 ml/kg)	28 days
$HgCl_2$	$HgCl_2$ (0.5 mg/kg)	28 days
250 AP + $HgCl_2$	*A. paniculata* (250 mg/kg) + $HgCl_2$ (0.5 mg/kg)	28 days
500 AP + $HgCl_2$	*A. paniculata* (500 mg/kg) + $HgCl_2$ (0.5 mg/kg)	28 days
200 AA + $HgCl_2$	Ascorbic Acid (200 mg/kg) + $HgCl_2$ (0.5 mg/kg)	28 days

n = 5; AA= Ascorbic Acid; AP =***Andrographis paniculate*****;** $HgCl_2$ = **Mercury chloride**

Procedure for Sampling: At the end of the experiment, rats were sacrificed humanely under ketamine anaesthesia (75mg/kg: intraperitoneally). Blood samples were collected from each rat in a plain tube by cardiac puncture, and the blood samples were centrifuged at 3000 rpm for 5 minutes to obtain serum for biochemical studies, such as the liver function parameters (ALP, ALT and AST), kidney function parameters (Urea and Creatinine) and oxidative stress bio-markers (SOD, CAT, GSH and MDA). Organs (liver and kidney) were harvested and fixed for subsequent studies.

Biochemical Studies: Alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), for liver functionality, urea, and creatinine level for kidney functionality were determined using commercially available kits and following the manufacturer's instructions. Agappe Laboratories Limited developed the ALT, AST, ALP, urea, and creatinine test kits (United Kingdom). Akanji et al.[24] method was adopted for measuring lipid peroxidation (MDA). The Catalase (CAT) activity was determined using Aebi's [25] method, the Superoxide dismutase (SOD) activity was determined using Fridovich's [26] method, and the Glutathione (GSH) concentration was determined using Rukkumani et al. [27] method.

Histological Examination: Harvested organs (kidney and liver) were fixed in 10% neutral buffered formalin, processed for light microscopy, and stained with hematoxylin and eosin (H&E) for general histology[28].

Data Analysis: The mean and standard error of the data were calculated. Statistical Software (GraphPadprism Version 9.2) was used to analyse data; one-way analysis of variance (ANOVA) test followed by a Tukey's post-hoc test. The threshold for statistical significance was set at $p < 0.05$.

RESULTS

Effects of the Extract on the Weight Gain of the Experiment Rats: Rats treated with $HgCl_2$ significantly reduced weight gain ($p < 0.05$) when compared to controls, but rats treated with *A. paniculata* and $HgCl_2$ had a significant weight gain in a dose-dependent manner. No significant difference ($p > 0.05$) between the rats treated with the low dose of *A. paniculate* and the rats treated with only $HgCl_2$ (Table 2).

Table 2: Effect of *A. Paniculata* and Mercury chloride treatments on body weight

Groups	Initial weight (g)	Final weight (g)	Weight gain (g)
Control	138.31 ± 3.11	174.32 ± 2.27	36.01 ± 2.57
$HgCl_2$	139.73 ± 2.94	155.14 ± 3.02$^{\#\#}$	17.23 ± 1.10$^{\#\#}$
250AP + $HgCl_2$	138.86 ± 5.18	160.13 ± 8.19$^{\#}$	20.91 ± 3.42$^{\#}$
500AP + $HgCl_2$	137.43 ± 3.21	168.75 ± 2.13*	30.23 ± 3.22*
200AA + $HgCl_2$	139.74 ± 2.41	162.75 ± 4.23*	22.99 ± 1.26*

n = 5; AA= Ascorbic Acid; AP =***Andrographis paniculate*****;** $HgCl_2$ = **Mercury chloride;** data were analysed with *P<0.033; ** P < 0.002; *** P < 0.0001 indicates a significant difference when compared to Normal control; $^{\#}$P<0.03; $^{\#\#}$ P < 0.002; $^{\#\#\#}$ P < 0.0001, indicates a significant difference when compared to $HgCl_2$ Control group.

Phytochemical Constituents of *Andrographis paniculata* Extracts: Table 3 shows a qualitative phytochemicals study of an aqueous extract of *A. paniculata*. Except for anthraquinone, which was absent in the aqueous extract, the phytochemical screening revealed the presence of saponin, flavonoid, alkaloid, tannin, cardiac glycosides, triterpene/steroid, and carbohydrates.

Table 3: Phytochemical screening of *Andrographis paniculata* aqueous extracts

Phytochemical Constituent	Test Used	Qualitative
Carbohydrates	Molisch's Test	+
Tannin	Ferric chloride Test	+
Saponin	Frothing Test	+
Alkaloid	Wayer Test	+
Anthraquinine	Borntragers Test	-
Cardiac Glycosides	Keller-Kiliani test	+
Flavonoid	Sodium hydroxide Test	+
Triterpene/Steroid	Liebermann-Bucchard Test	+

Key: + = Present, - = Absent

Liver Function Parameters: $HgCl_2$-treated rats were compared to control rats and AP-treated rats, there was a significant increase in ALT, ALP, and AST in the $HgCl_2$-treated rats. The levels of ALT, ALP, and AST in groups treated with AP at all doses decreased significantly ($p < 0.0001$) compared with $HgCl_2$-treated group. The levels of ALP, ALT, and AST in AP-treated and Ascorbic Acid-treated rats did not differ significantly ($p > 0.05$) (See Fig. 1).

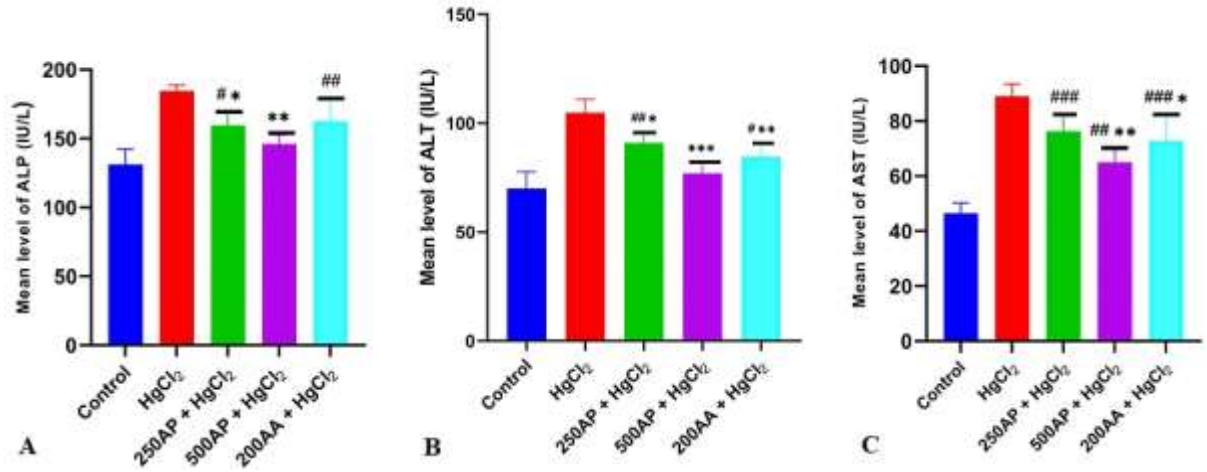

Figure 1: The liver enzymes parameters (a) ALP, (b) AST, and (c) ALT). Twenty-eight days after the experiment, data were analysed with *P<0.033; ** P < 0.002; *** P < 0.0001 indicates a significant difference when compared to Normal control; #P<0.03; ## P < 0.002; ### P < 0.0001, indicates a significant difference when compared to $HgCl_2$ Control group.

Parameters of Kidney Function: The serum levels of urea and creatinine in rats treated only with $HgCl_2$ increased significantly ($p < 0.0001$) when compared to control and AP/Ascorbic acid-treated rats. There was no significant difference ($p > 0.05$) in creatinine levels between 250 mg of AP-treated rats and Ascorbic Acid-treated rats (200 mg/kg) (Fig. 2).

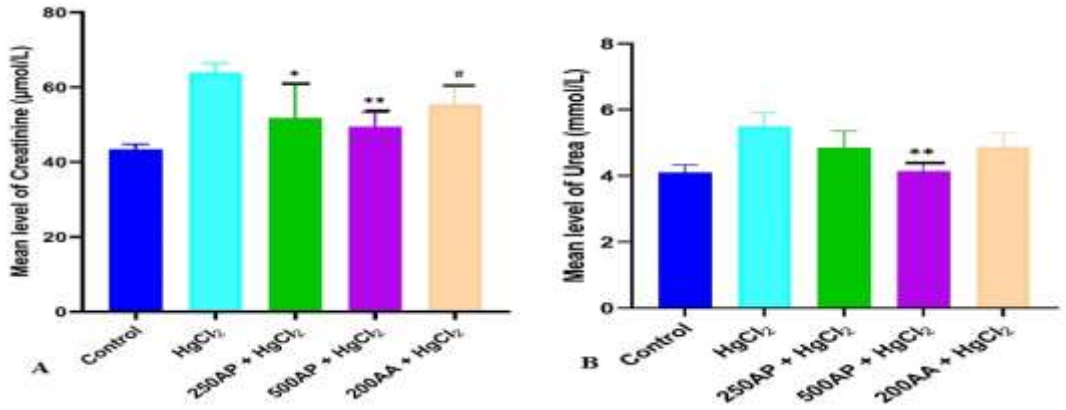

Figure 2: Kidney function parameters (a) Urea, and (b) Creatinine). Twenty-eight days after the experiment, data were analysed with *P<0.033; ** P < 0.002; *** P < 0.0001 indicates a significant difference when compared to Normal control; #P<0.03; ## P < 0.002; ### P < 0.0001, indicates a significant difference when compared to $HgCl_2$ Control group.

Oxidative Stress Biomarkers: The levels of catalase, superoxide dismutase, and reduced glutathione in the serum of $HgCl_2$ treated rats were significantly lower ($p < 0.0001$) than those of control and AP-treated rats. The levels of catalase, superoxide dismutase, and reduced glutathione in control and AP (500 mg/kg) treated rats did not differ significantly ($p > 0.05$) (Fig. 3). Rats that were given AP (500 mg/kg) and ascorbic acid, their MDA levels dropped significantly ($p < 0.05$) when compared to $HgCl_2$ control rats (See Fig. 3).

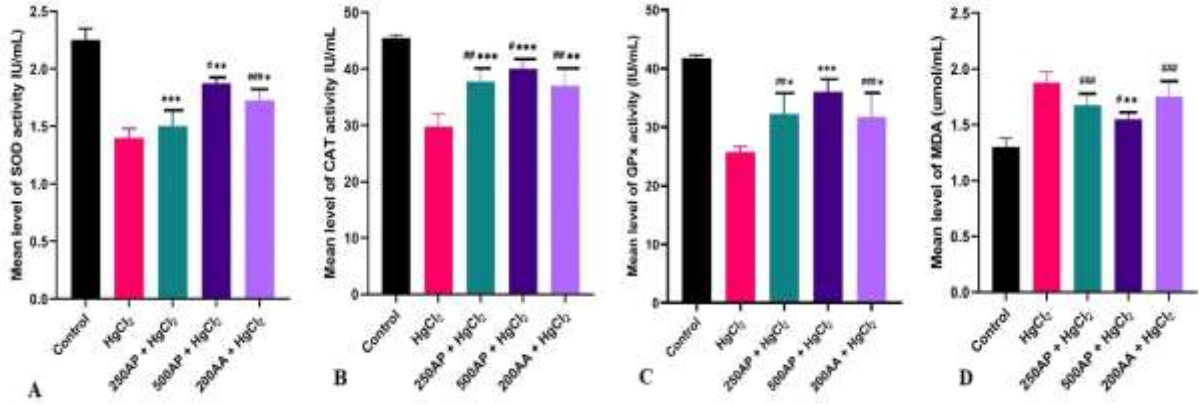

Figure 3: Oxidative stress parameters ((a) SOD, (b) CAT, (c) GPx and (d) MDA). Twenty-eight days after the experiment, data were analysed with *P<0.033; ** P < 0.002; *** P < 0.0001 indicates a significant difference when compared to Normal control; #P<0.03; ## P < 0.002; ### P < 0.0001, indicates a significant difference when compared to $HgCl_2$ Control group.

Histological Study: Normal hepatocytes, central veins, and sinusoids were visible in the liver sections of the control rats (Fig.4a). Histological changes including, steatosis and fat hepatocellular vacuoles were observed in the liver sections of rats exposed only to $HgCl_2$ (Fig4b). $HgCl_2$ and AP treated groups revealed mild steatosis and microvesicular fatty droplet formation in the livers sections (Fig 4c, & d). The rats that were co-administered with $HgCl_2$ and ascorbic acid (200 mg/kg) had mild liver inflammation (Fig. 4e).

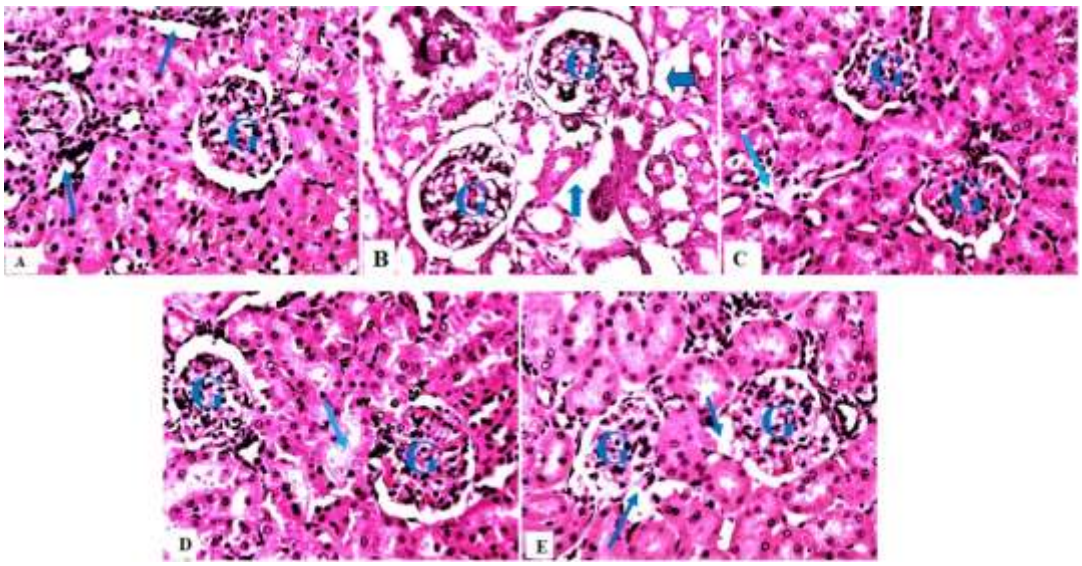

Figure 4: Composite photomicrographs of sections of the liver (H & E X 250).; The control (a) showing normal hepatocytes (arrow) and central vein (CV), $HgCl_2$ treated micrograph (b) distorted hepatocyte (arrow), and fat hepatocellular vacuoles (Asterix). AP extract 250 mg/kg + 0.5 mg/kg $HgCl_2$ (c) mildly inflamed hepatocyte (arrow head) and mild dilatation of the sinusoid (blue arrow), AP extract 500 mg/kg + 0.5 mg/kg $HgCl_2$ (d) normal hepatocyte and mild dilation of the sinusoid(arrow), Ascorbic acid treated group (e), some dilatation of the sinusoid (blue arrow), and macro-vesicular fat droplet (Asterix).

The kidneys sections of the normal control rats had a typical histological structure with renal tubules and glomeruli (Fig. 5a). Sections of $HgCl_2$-treated rats' kidneys showed focal renal tubular degeneration (Fig. 5b). A mildly obliterative form of a glomerulus was observed in the kidneys of rats given $HgCl_2$ + AP (Fig. 5c, & d). The glomerulus in the kidneys of ascorbic acid-treated rats was relatively normal compared with rats in control group (Fig. 5e).

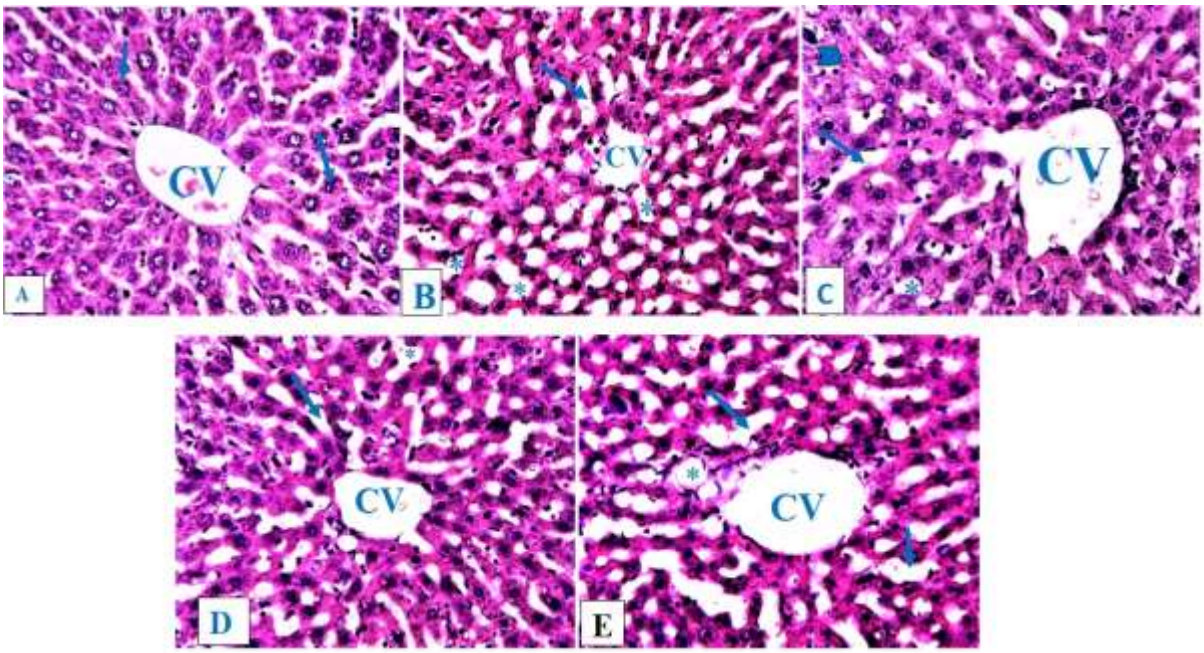

Figure 5: Composite photomicrographs of sections of the kidney (H & E X 250); The control **(a)** normal glomerulus **(G)** and renal tubules, $HgCl_2$ treated micrograph **(b)** distorted and dilated renal tubules **(arrow),** glomerulus degeneration and spontaneous lipid vacuolation (G), AP extract 250 mg/kg + 0.5 mg/kg $HgCl_2$ **(c)** mild obliterative form of glomerular space (G) with mildly distorted renal tubule **(Arrow**), AP extract 500 mg/kg + 0.5 mg/kg $HgCl_2$ (**d)** normal rental tubules (**Arrow)** and a normal glomerulus (**G**), Ascorbic acid treated group **(e)**, showing mild obliterative form of glomerulus with lipid vacuolation (**G**) (H & E, X 250).

DISCUSSIONS

The most common routes are contact, inhalation, and oral consumption, after which $HgCl_2$ is absorbed into the body and causes oxidative stress, cellular damage, mitochondrial depolarization with ATP depletion, singlet oxygen generation, and cell death; this occurs most frequently in the liver tissue and kidney, as the liver is the primary site of mercury metabolism[29]. After $HgCl_2$ administration, hepatic and renal functions were altered, resulting in hepatorenal dysfunction as evidenced by significant increases in AST, ALT, ALP enzyme activities, and urea and creatinine levels. Joshi *et al*.[30] and Elblehi *et al*.[31] also reported similar findings who indicated in their work that the $HgCl_2$ administration increased various biochemical parameters, including ALP, AST, ALT, urea and creatinine. Increase in serum liver enzyme activity indicates hepatocyte damage and necrotic lesions, which result in a loss of hepatocyte membrane strength, allowing enzymes to leak into the bloodstream and hepatic tissue dysfunction and disruption in enzyme biosynthesis [32]. This is consistent with the findings of the histology of the liver as macrovesicular fat droplet was observed. Treatment with *A. paniculata*, on the other hand, ameliorated $HgCl_2$-induced hepatotoxicity by Okhuarobo et al., [15] indicating that *A. paniculata* has a hepatoprotective property. This study demonstrated that kidney damage is associated to changes in renal function markers, as evidenced by significant increases in urea and creatinine levels following $HgCl_2$ administration. Treatments with *A. paniculata* reduced elevated levels of renal function markers and protected the organ from $HgCl_2$-induced impairment. These findings support previous work by

Singh *et al*. [33], who states that *A. paniculate* has the potential to mitigate renal toxicity. Furthermore, hepatorenal protective activities of the antioxidant Andrographolide, one of the major chemical constituents of the plant *A. paniculata*, could be attributed to these positive preventive properties [34].

The production of reactive oxygen species (ROS) by mercuric chloride causes oxidative stress, which leads to the destabilization and disintegration of the cell membrane due to lipid peroxidation[35]. According to the findings in this study, the acute administration of mercuric chloride resulted in a significant increase in MDA levels. Thus, an increase in LPO caused by $HgCl_2$ may result in biochemical and functional changes in the membrane. On the other hand, $HgCl_2$ toxicity is linked to the production of superoxide radicals and the reduction of glutathione. Because of the thiol (-SH) group, glutathione is an intracellular antioxidant and a mercury carrier. GSH acts as a first line of defence for cells against Hg compounds. Mercury's binding to glutathione and subsequent elimination of intracellular glutathione lowers the level of GSH in the cell, lowering the cell's antioxidant potential [36]. In this study, it was discovered that $HgCl_2$ reduced the activities of the antioxidant enzymes; SOD and CAT and GSH, whereas the end product of lipid peroxidation (MDA levels) was significantly higher than in the control group. Several experiments have reported similar findings [36, 37].

In contrast, coadministration of *A. paniculata* + $HgCl_2$ resulted in a significant increase in SOD and CAT activities and normalization of GSH and MDA levels. Previous studies have demonstrated that *A. paniculata* has hepatorenal protective properties [33,38]. This could be because *A. paniculata* contains andrographolide and arabinogalactan metabolites, which have the potential to be hepatorenal protective agents due to their glucoside or hydroxyl moieties. These hydroxyl moieties can react directly with chain-carrying radicals, halting their propagation and providing protective action [33].

CONCLUSION

The findings show that aqueous extract of the plant of *A. paniculata* protects rats from $HgCl_2$-induced hepatic damage. *A. paniculata*'s ability to stabilise cell membranes, restore hepatic and kidney cells, and increase production of antioxidant enzymes like catalase, superoxide dismutase, and glutathione peroxidase may explain its hepatoprotective effect against $HgCl_2$-induced liver and kidney damage. Metabolites like andrographolide and arabinogalactan might be responsible for the hepatorenal protective and antioxidant properties of whole plant extract.

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Disclosure Statement

The authors report no conflict of interest

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