



***Ginkgo biloba* Extract Ameliorates Cadmium-Induced Hepatotoxicity in Experimental Animals**

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Abstract: The present study investigated the effects of *Ginkgo biloba* extract (GBE) in attenuating cadmium-induced hepatotoxicity. A total of 24 adult Wistar rats with an average weight of 145g were used for this study. The rats were randomly divided into groups of four: groups A (control), B (Cd at 50mg/kg BW), C (Cd at 50mg/kg BW and GBE at 100mg/kg BW) and D (Cd at 50mg/kg BW and GBE at 300mg/kg BW). Liver tissues were excised, homogenized and centrifuged to obtain supernatant for analysis of liver enzyme activities including ALP, AST, and ALT. Other samples were fixed in 10% formal saline for 24 hrs and processed for histological analysis. Statistical analysis of data - one way analysis of variance- was done using GraphPad Prism 5. Results indicated changes in the activities of liver enzymes (ALP and AST) in the treated groups compared to the control group. ALP activity was significantly higher in group B compared to groups C and D. Also, AST activities of group B was significantly higher than the control group, and no significant difference was observed in the activities of ALT across the groups. It was observed that cadmium produced cytotoxic effects in both the liver histoarchitecture and enzyme activities as seen in the increased levels of ALP, AST activities while *Ginkgo biloba* ameliorated alterations in enzyme activities and preserved liver histoarchitecture. The low dose of *Ginkgo biloba* was more effective in ameliorating the hepatotoxic effects.

Keywords: Cytotoxic, *Ginkgo biloba*, Hepatocellular, Cadmium

1. Introduction

The liver, being the largest organ in the body, functions in metabolism and detoxification of toxic or unwanted substances that enter the body through the orogastric route. Nutritive components of digestion coming from the walls of the gastrointestinal tract pass through the liver for adequate filtering and removal of unwanted substances. Because of the activities of the liver, it is vulnerable to damage in cases of high components of unwanted material ready to plaque the body. The disease condition of the liver will leave the body more vulnerable to different agents be it pathogens or toxic element that can affect the tissues of the body. The essence of liver detoxification and elimination is to maintain the homeostasis of the body [1].

Cadmium (Cd), a toxic element, is an industrial and environmental toxicant with many industrial uses. Its presence and emission to the atmospheric, aquatic, and terrestrial environments have increased during the last century [2]. Since Cd is not degraded in the environment, the risk of human exposure to it is always on the increase because it enters the food chain. Humans are exposed to Cd by two main routes; inhalation and ingestion [3]. When taken up by plants, Cd concentrates along the food chain and accumulates in the body of people eating contaminated foods [4]. Cd is also present in tobacco smoke, further contributing to human exposure. By far, the most salient toxicological property of Cd is its exceptional long half-life in the human

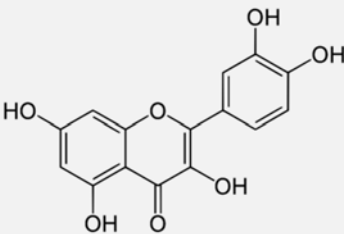
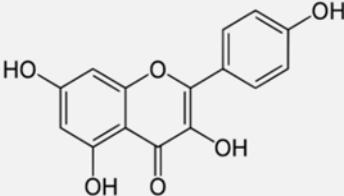
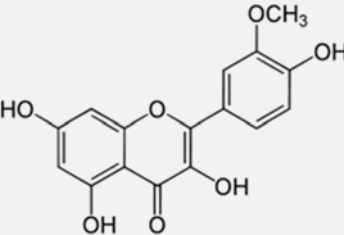
body. Once absorbed, Cd accumulates in the human body, kidneys and other vital organs such as the lungs and the liver [5]. Besides its extraordinary cumulative properties, Cd is also a toxic metal that can disrupt several biological systems at doses that are much lower than most toxic metals [6].

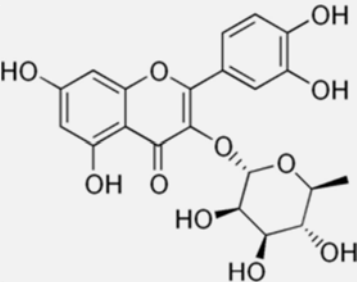
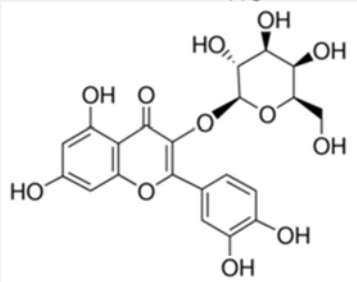
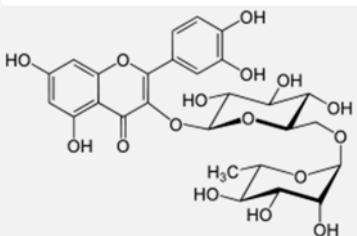
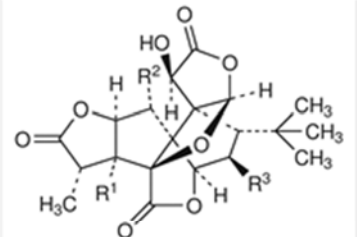
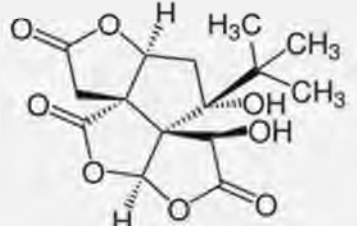
Cd has been implicated in the development of cancer and has been classified as a type I carcinogen by the International Agency for Cancer Research and cadmium is transported through the body by binding to a sulfhydryl group-containing proteins [7]. Some suggest that under condition of chronic exposure to cadmium, complexes of cd-methallothionein (formed in the hepatocytes in response to the uptake of cadmium) are released from necrotic hepatocytes and are delivered through systemic circulation to the kidney where it appears to be taken up and induced proximal tubular injury and death [8]. Cadmium acts as a catalyst in forming reactive oxygen species. It increases lipid peroxidation and depletes antioxidants, glutathione and protein-bound sulfhydryl groups [9]. Hepatotoxicity implies chemical-driven liver damage, drug-induced liver injury is a cause of acute and chronic liver disease. The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Hepatotoxicity may come not only from direct toxicity of the primary compound but also from a reactive metabolite or from an immunologically mediated response affecting hepatocytes, biliary epithelial cells and liver vasculature [10].

The increasing rate at which the use of herbal medicine is gaining approval in both the public and medical world is because there is great improvement in the mechanism of their

actions. One of such herbal product is *Ginkgo biloba*. *Ginkgo biloba* leaf has been used in traditional Chinese medicine to treat various conditions for several years and it is one of the top selling herbs in the United States [11]. *G. biloba* (maidenhair tree) is one of the oldest herbal medicines that have been used as therapeutic agents in modern pharmacology. The healing ability of *Ginkgo biloba* has been reported for thousands of years. At present, it is one of the most extensively researched medicinal plants in the world, used by medical professionals to aid the treatment of problems typical with aging, such as poor circulation, mental confusion and memory loss [12]. *Ginkgo biloba* leaf extract contains flavonoids and flavone glycosides, lactone derivatives (ginkgolides), bilobalide, ascorbic acid, iron-based superoxide, 6-hydroxykinuretic acid, protocatechuic acid, sterols and vanilic acid. The major classes of active ingredients are the ginkgolides and bilobalides (also known as terpenes) and the flavonoid [13-14]. The chemical structure of the active components and their molecular formula are listed in Table 1 [15]. *Ginkgo biloba* is a common plant used as a natural supplement and its extract is well known for its antioxidant properties, this may result from its ability to scavenge free radicals [13]. The role of *Ginkgo biloba* in the treatment of diseases associated with free radicals and oxidative stress has been suggested and tested [16] with various positive results. In the present study, the efficacy of *Ginkgo biloba* against heavy metal poisoning produced by cadmium (Cd) was tested for its ameliorative effects at different doses in cadmium induced hepatotoxicity.

Table 1. Phytochemical constituents of *ginkgo biloba*.

S/N	ACTIVE INGREDIENT	CHEMICAL COMPOSITION	MOLECULAR FORMULA
Flavonoids found in the leaves of <i>Ginkgo biloba</i>			
1	Quercetin		C ₁₅ H ₁₀ O ₇
2	Kaempferol		C ₁₅ H ₁₀ O ₆
3	Isorhamnetin		C ₁₅ H ₁₂ O ₆

S/N	ACTIVE INGREDIENT	CHEMICAL COMPOSITION	MOLECULAR FORMULA																
Flavonoids found in the leaves of Ginkgo biloba																			
4	Quercitrin		$C_{21}H_{20}O_{11}$																
5	Quercetin-3 β -D-glucoside		$C_{21}H_{20}O_{12}$																
6	Rutin		$C_{27}H_{30}O_{16}$																
Terpenes found in the leaves of Ginkgo biloba																			
7	Ginkgolides	 <table border="1" data-bbox="678 1355 1037 1500"> <thead> <tr> <th></th> <th>R¹</th> <th>R²</th> <th>R³</th> </tr> </thead> <tbody> <tr> <td>ginkgolide A</td> <td>OH</td> <td>H</td> <td>H</td> </tr> <tr> <td>ginkgolide B</td> <td>OH</td> <td>OH</td> <td>H</td> </tr> <tr> <td>ginkgolide C</td> <td>OH</td> <td>OH</td> <td>OH</td> </tr> </tbody> </table>		R ¹	R ²	R ³	ginkgolide A	OH	H	H	ginkgolide B	OH	OH	H	ginkgolide C	OH	OH	OH	$C_{20}H_{24}O_9$
	R ¹	R ²	R ³																
ginkgolide A	OH	H	H																
ginkgolide B	OH	OH	H																
ginkgolide C	OH	OH	OH																
8	Bilobalide		$C_{15}H_{18}O_8$																

2. Materials and Methods

2.1. Experimental Animals

Twenty four (24) adult Wistar rats (*Rattus norvegicus*) with an average body weight of 145g were obtained from the animal house of Babcock University, Ogun State. The rats

were randomly divided into groups of four: groups A, B, C and D. The animals were housed in clean, well ventilated plastic cages at room temperature, under natural light and dark cycles. The rats were left to acclimatize for 8 days and afterwards used for the experiment. All animals were fed with a standard pelletize diet and water ad-libitum and the care and treatment of animals was performed accordingly.

Cadmium sulphate was obtained from Sigma® Chemicals, USA, and *Ginkgo biloba* leaf extract made into powder in capsules form was gotten from Med Plus Limited, Lagos Nigeria. The powdered extract was obtained from the capsule and weighed before it was dissolved in distilled water in the right proportions required for the experiment. Both *Ginkgo biloba* extract and Cadmium sulphate were dissolved in distilled water separately and administered daily to the animals between the hours of 08:00 and 09:00 using suitable oral gavages throughout the period of administration, the dosage and time span chosen for this experiment were based on previous researches and a pilot study that was carried out to arrive at the dosages used.

2.2. Grouping and Treatment of Experimental Animals

The following treatment protocol was used for this experiment:

Groups	Treatment
A	Control, rats received distilled water only
B	Negative control, Cadmium sulphate (50mg/kg body weight) only
C	Cadmium sulphate (50mg/kg body weight) for 7 days and later received Low dose, <i>Ginkgo biloba</i> (100mg/kg body weight) for 7 days consecutively
D	Cadmium sulphate (50mg/kg body weight) for 7 days and later received High dose, <i>Ginkgo biloba</i> (300mg/kg body weight) for 7 days consecutively.

2.3. Sacrifice and Biochemical Analysis

The animals were sacrificed by cervical dislocation method, twenty-four hours after the last administration. Liver tissues were excised from the animals through an abdominal incision. The liver tissues were homogenized and later centrifuged at 4000 revolution for 15 minutes using Gulfex Medical and Scientific Centrifuge, England to get supernatant. The supernatant was separated by decantation and analyzed for liver enzyme activities with investigation of Alkaline Phosphatase (ALP), Aspartate aminotransferase (AST) and Alanine Transaminase (ALT). The analysis of ALP, AST and ALT were determined following strictly the methods described by Ranjna [17]. The remaining liver tissues were fixed in 10% formal saline for 24 hrs and then processed for histological analysis.

2.4. Statistical Analysis

The data obtained were expressed as mean \pm Standard Error of Mean (S. E. M). The statistical significance was evaluated by one way analysis of variance (ANOVA) using Graph Pad Prism 5 (Version 5.03, Graph Pad Inc.). A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Alkaline Phosphatase (ALP) Activities in Rats After Treatment

As shown in figure 1, the activity of ALP in group B (Cd) (0.22 ± 0.00) was significantly higher when compared with the control group (0.20 ± 0.00). ALP activities were significantly lower in groups C (Cd + Ld GBE) (0.14 ± 0.00) and D (Cd + Hd GBE) (0.14 ± 0.00) when compared with that of control group. The ALP activity in group B was significantly higher than that of the groups C (Cd + Ld GBE) (0.14 ± 0.00) and D (Cd + Hd GBE) (0.14 ± 0.00). However, no significant difference was observed between groups C (Cd + Ld GBE) (0.14 ± 0.00) and D (Cd + Hd GBE) (0.14 ± 0.00) when compared with each other.

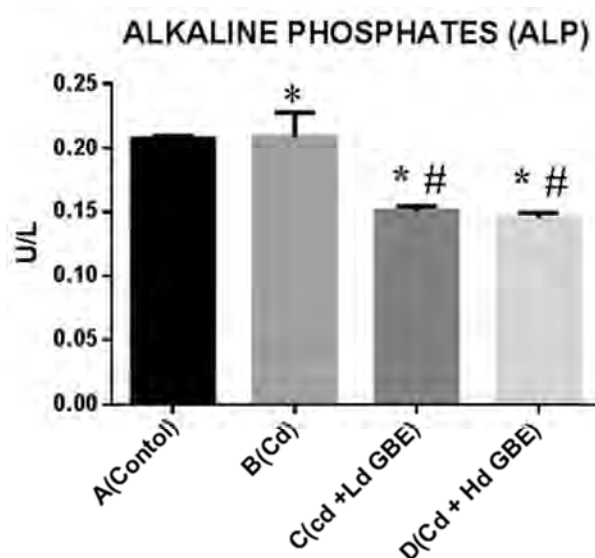


Figure 1. Alkaline Phosphatase activity (ALP) (U/L).

Values are mean \pm SEM of data obtained;

* = significantly different from A (control);

= significantly different from B (Cd) cadmium (Negative control)

C = cadmium + low dose *Ginkgo biloba* (Cd + Ld of GBE),

D = cadmium + high dose *Ginkgo biloba* (Cd + Hd GBE).

3.2. Aspartate Transaminase (AST) Activities in Rats After Treatment

Figure 2 shows the result of Aspartate Transaminase (AST) activities across the groups. The activity of AST in group B (Cd) (0.128 ± 0.001) was significantly higher than that of the control group (0.117 ± 0.002). There was no significant difference between the control group (0.117 ± 0.002) and group C (Cd + Ld GBE) (0.126 ± 0.003). Group D (Cd + Hd GBE) (0.140 ± 0.004) was significantly higher than the control group (0.117 ± 0.002), group B (Cd) (0.128 ± 0.001) and group C (Cd + Ld GBE) (0.126 ± 0.003) respectively. However, no significant difference was observed between the control group (0.117 ± 0.002) and the group C (Cd + Ld GBE) (0.126 ± 0.003).

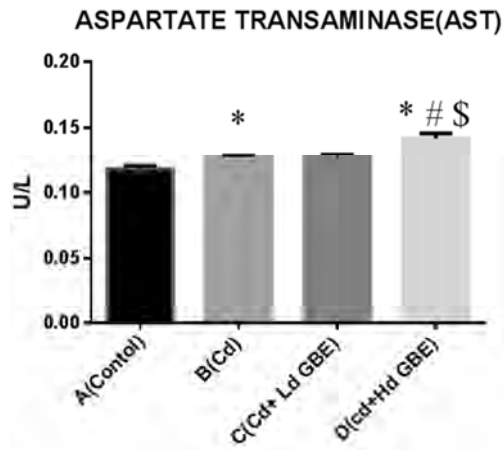


Figure 2. Aspartate Transaminase (AST) activity (U/L).

Values are mean \pm SEM of data obtained;

* = significantly different from A (control);

= significantly different from B (Cd) cadmium (Negative control)

\$ = significantly different from C (Cd + Ld GBE)

C = cadmium + low dose Ginkgo biloba (Cd + Ld of GBE),

D = cadmium + high dose Ginkgo biloba (Cd + Hd GBE).

3.3. Alanine Transaminase (ALT) Activities in Rats After Treatment

Figure 3 shows the result of Alanine Transaminase activities across the groups. No significant difference was observed between the control group (0.0785 ± 0.002) when

compared with the rest of the treated groups B(Cd) (0.0922 ± 0.008), C (Cd + Ld GBE) (0.0780 ± 0.005), and D (Cd + Hd GBE) (0.0723 ± 0.051). However, it was observed that the group B was slightly higher than the control (0.0785 ± 0.002) as well as groups C (Cd + Ld GBE) (0.0780 ± 0.005) and D (Cd + Hd GBE) (0.0723 ± 0.051)

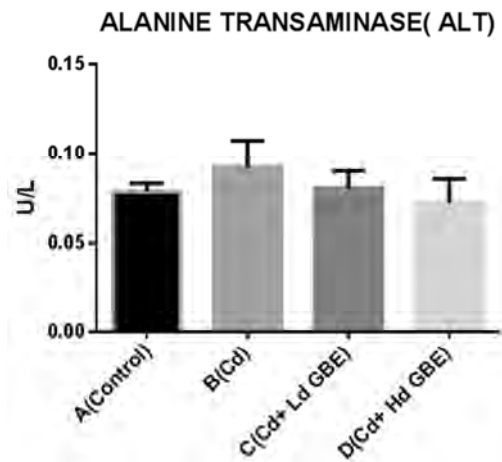


Figure 3. Alanine Transaminase (ALT) activity (U/L).

Values are mean \pm SEM of data obtained;

* = significantly different from A (control);

= significantly different from B (Cd) cadmium (Negative control)

C = cadmium + low dose Ginkgo biloba (Cd + Ld of GBE),

D = cadmium + high dose Ginkgo biloba (Cd + Hd GBE).

3.4. Histological Results

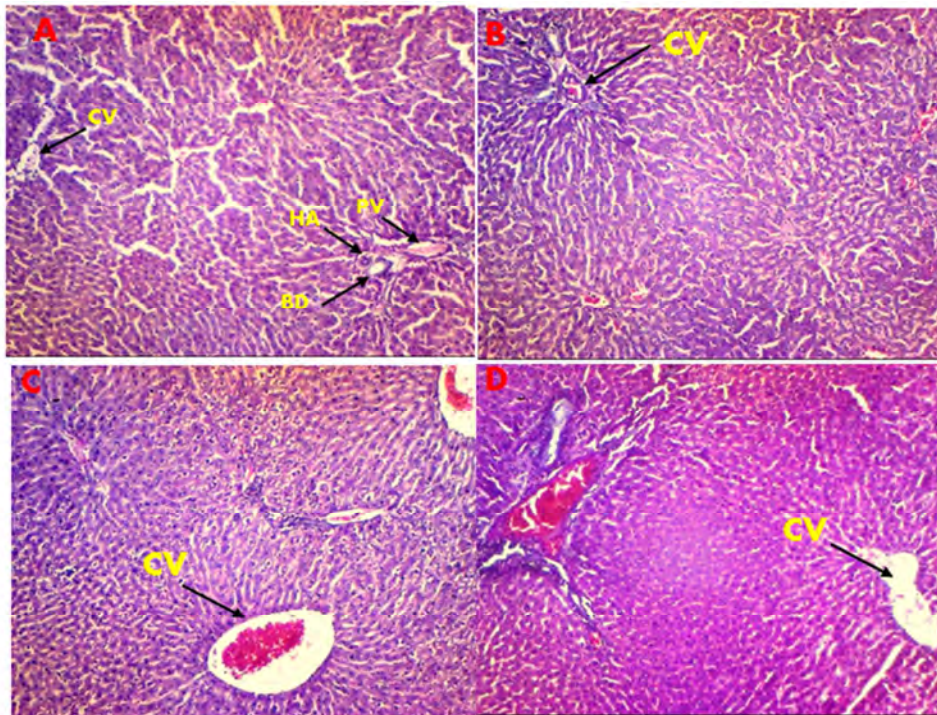


Figure 4. Photomicrograph sections of the Liver. A (Control), B (Cd) Cadmium (Negative control) C = cadmium + low dose Ginkgo biloba (Cd + Ld of GBE), D = Cadmium + high dose Ginkgo biloba (Cd + Hd GBE); CV- central vein, PV- portal vein, HA – hepatic artery, BD –bile duct, H- hepatocytes. H&E X100.

Figure 4A shows the features of a normal liver histology with a clearly shown central vein, hepatocytes with the centrally located rounded nucleus and portal triad which includes the portal vein, hepatic artery and the bile duct.

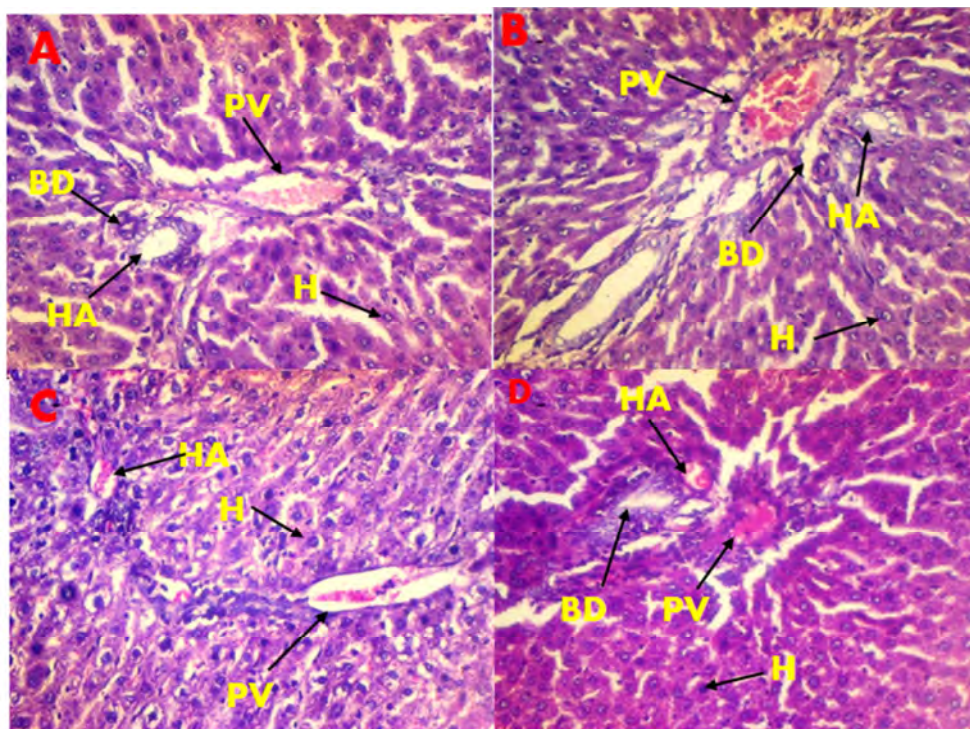


Figure 5. Photomicrograph sections of the Liver. A (Control), B (Cd) Cadmium (Negative control) C = cadmium + low dose *Ginkgo biloba* (Cd + Ld of GBE), D = Cadmium + high dose *Ginkgo biloba* (Cd + Hd GBE); CV- central vein, PV- portal vein, HA – hepatic artery, BD –bile duct, H- hepatocytes. H & E 400.

Figure 5A shows a clearly observable sinusoids that are line with the Kupffer cells. The cytoplasm of the hepatocytes are well demonstrated with abundant eosinophilic cytoplasm. Figures 4B and 5B show features of distorted portal triad and the hepatocytes cytoplasm appears degenerated with some features of disorganized hepatocyte cords without the normal lobular architecture that is seen in group A. Cadmium toxicity caused disruption to the histological architecture of the liver as indicated by disrupted plates of hepatocytes and sinusoids that appear widened in figure 5B. Higher dose could not significantly ameliorate Cadmium effects as treated animals liver cells show signs of cell and tissue damage. Low dose of *Ginkgo biloba* produced better ameliorative effects as tissue disruption was mild and cells are relatively preserved in terms of their morphologies.

4. 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. The main objective of this study was to investigate the effects of cadmium sulphate on the activities of some liver enzymes and cytoarchitecture in cadmium-induced hepatotoxicity as well as the ameliorative properties of *Ginkgo biloba* extract in attenuating cadmium effects.

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 [18].

In the present study, the activities of the ALP in homogenate of group B (Cd) animals was significantly higher compared to the control and the rest of the treated groups. This is an indication that cadmium sulphate produced hepatocellular damage in the animals in group B that was administered with cadmium this was also corroborated by the histological findings. This is seen in the higher activities in the ALP enzyme in group B. This is in accordance with the findings of Alhazza [19] who reported observable increase in ALP activities following the administration of cadmium in rats. This is in contrast with the study of Samir [20] who reported significant decrease in ALP activities and Kobayashi [21] who reported no observable changes in Liver ALP activity in the Cd-exposed rats. Many studies have established various adverse effects of cadmium, such as its influence on mitochondrial function, enhancement of lipid peroxidation, and breakage of DNA chain [19, 22-24]. Following the administration of *Ginkgo biloba* in groups C (Cd + Ld GBE) and D (Cd + Hd GBE), ALP activities were significantly decreased in groups C (Cd + Ld GBE) (0.14 ± 0.00) and D (Cd + Hd GBE) (0.14 ± 0.00) when compared with that of group B (Cd), the negative control

group. This is an indication of the attenuating nature of *Ginkgo biloba* extract on Cd induced hepatotoxicity. This is supported by the findings of Atef [25] who reported an attenuating nature of *Ginkgo 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 an efficacy of ameliorating or preventing diseases associated with free radicals [26].

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 [27]. 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 [28]. In the present study, the activity of AST in group B (Cd) (0.128 ± 0.001) was significantly higher than that of the control group (0.117 ± 0.002). This is an indication that cadmium sulphate produced a hepatocellular damage in group B that was administered with cadmium, this is supported by the result of the activities of the ALP. However, Group D (Cd + Hd GBE) (0.140 ± 0.004) was significantly higher than the control group (0.117 ± 0.002), group B (Cd) (0.128 ± 0.001) and group C (Cd + Ld GBE) (0.126 ± 0.003) respectively, this may be that high dose of *Ginkgo biloba* may induce cellular disturbance in the activities of liver AST, this also may be confirm by the histological result as seen in plates 2D which shows some features of distorted portal triad and the hepatocytes cytoplasm appears degenerated with some features of disorganized hepatocyte cords without the normal lobular architecture that is seen in group A, this needs to be further investigated.

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 as shown in Figure 3, no significant difference was observed between the control group (0.0785 ± 0.002) when compared with the rest of the treated groups B(Cd) (0.0922 ± 0.008), C (Cd + Ld GBE) (0.0780 ± 0.005), and D (Cd + Hd GBE) D (Cd + Hd GBE) (0.0723 ± 0.051) across the groups in ALT activities But observable increase was seen in the group B (0.0922 ± 0.008) when compared to the control (0.0785 ± 0.002) and group C (Cd + Ld GBE) (0.0780 ± 0.005), and D (Cd + Hd GBE) (0.0723 ± 0.051). This result is in contrast with the works of Alhazza and Samir [17, 18] who all reported significant difference in the levels of ALT enzyme activities. This might

be as a result of resilient nature of the liver cells in the degradation of toxic substances. *Ginkgo biloba* may act as antioxidant to ameliorate the liver against oxidative stress. This ameliorative property of *Ginkgo biloba* could be associated to the high concentration of flavonoids it contains because they possess the antioxidant property of the plant which help to fight off the free radicals produced by hepatotoxicant [29].

The histology of the liver was demonstrated using the Haematoxylin and Eosin technique with emphasis on the general cytoarchitecture. Figures 4 and 5 show the photomicrographs of the liver at both X100 and X400 magnifications. Figures 4A and 5A show the features of a normal liver histology with a clearly shown central vein, hepatocytes with the centrally located rounded nucleus and portal triad which includes the portal vein, hepatic artery and the bile duct. Figure 5A shows a clearly observable sinusoids that are line with the Kupffer cells. The cytoplasm of the hepatocytes are well demonstrated with abundant eosinophilic cytoplasm with fine basophilic granules that represent the rough endoplasmic reticulum.

Figures 4B and 5B show features of distorted portal triad and the hepatocytes cytoplasm appears degenerated with some features of disorganized hepatocyte cords without the normal lobular architecture that is seen in group A. Also, figure 5B shows features of degenerated hepatocytes as seen with sparsely nuclei within the cytoplasm. Numerous Kupffer cell are clearly observable with the sinusoid separating the cords of hepatocytes. This was noticed in the groups B (Cd) and D (Cd + Hd GBE). This may be due to the fact that Kupffer cells respond actively to many types of injury by proliferation and enlargement that was caused by cadmium. Also in figure 5B, the majority of hepatocytes appeared clustered together forming eosinophilic syncytial masses. The hepatocytes appeared large with light and foamy cytoplasm that is filled with numerous vacuole-like spaces and in some, the nuclei condenses into shrunken basophilic masses. The hepatocytes appeared irregularly arranged with disorganization of hepatic architecture, indicating destruction of hepatocyte integrity in group B by cadmium.

Figures 4C & 5C shows the features of a normal liver histology with a clearly shown central vein, hepatocytes with the centrally located rounded nucleus and portal triad which includes the portal vein, hepatic artery and the bile duct. Figure 5C shows a clearly observable sinusoids that are line with the Kupffer cells. The hepatocytes cytoplasm are well demonstrated with intact nuclei. Also, features of multinucleated hepatocytes were observed in figure 5C which is an indication of regeneration. Multinucleated hepatocytes appears to be more prominent in the lower dose (group C) of *Ginkgo biloba* than in the higher dose (group D). This might be an indication that *Ginkgo biloba* at low dose was able to ameliorate cadmium toxicity than was observed at the high dose of *Ginkgo biloba*.

Figure 5D shows some features of mild distortions that were observed in group B, however the observable features

were not as prominent as the features that were observed in group B (Cd). Cadmium toxicity caused disruption to the histological architecture of the liver as indicated by disrupted plates of hepatocytes and disrupted sinusoids that appear widened as seen in group B(Cd). Low dose of *Ginkgo biloba* produced better ameliorative effects as tissue disruption was mild and cells are relatively preserved in terms of their morphologies. However, higher dose 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 low dose of *Ginkgo biloba* compared to the high dose. It can be suggested from the findings of the present study that *Ginkgo biloba* has ameliorative effects on cadmium-induced hepatotoxicity with the low dose proving more effective in restoring liver functions and cytoarchitecture.

In conclusion, it was observed that cadmium produced cytotoxic effects in both the liver histoarchitecture and enzyme activities as seen in the increased levels of ALP, AST activities while *Ginkgo biloba* ameliorated alterations in enzyme activities and preserved liver histoarchitecture. The low dose of *Ginkgo biloba* was more effective in ameliorating the hepatotoxic effects.

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