Protective Potentials of *Vernonia amygdalina* Leaf Extracts on Cadmium-Induced Hepatic Damage

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Abstract

The protective potentials of *Vernonia amygdalina* (bitter leaf) leaf extracts on cadmium-induced hepatic damage were investigated. Thirty five (35) female Wistar rats were completely randomized into 7 groups (A-G) of 5 rats each. Group A received 0.5 ml distilled water only; group B received 10 mg/kg body weight $CdCl_2$; group C received the standard drug, silymarin; groups D and E received 50 and 100 mg/kg body weight of leaf extract of *V. amygdalina* (LEVA) respectively; while groups F and G received 50 and 100 mg/kg body weight of squeezed leaf extract *V. amygdalina* (SLEVA) respectively. The animals in groups C-G were orally administered 10 mg/kg body weight $CdCl_2$ 3 hours after the oral administration of silymarin or the extract for 10 days. There was a significant reduction ($p<0.05$) in the serum total protein and albumin concentrations in the animals intoxicated with cadmium when compared to the normal control group with the pre-administration of silymarin or *Vernonia amygdalina* leaf extracts. There were also significant reduction ($p<0.05$) in the superoxide dismutase activity and reduced glutathione concentrations in the liver of intoxicated rats. The catalase activity and malondialdehyde concentrations in the liver of the intoxicated untreated rats were however, significantly increased ($p<0.05$) compared to the normal control group and the extract-treated groups. The results suggest that the processed *Vernonia amygdalina* leaf extract exhibited protective potentials against liver damage with the SLEVA having the highest potentials. This might be due to the antioxidant properties contained in the extract. It can therefore be concluded from the above results that processed *Vernonia amygdalina* leaf extract have protective effect against cadmium- induced hepatic damage in female albino rats.

Keywords: Hepatic damage, cadmium, antioxidant, *Vernonia amygdalina* leaf

1.0 Introduction

The liver is an important organ performing several metabolic and physiological functions as well being a site of protection against the hazards of harmful drugs and chemicals [1,2]. It is prone to damage by exposure to a range of toxic elements through both endogenous molecules and exogenous substances such as alcohol and toxic chemicals such as cadmium, antibiotics, chemotherapeutic agents, peroxidised oil, aflatoxin, CCl_4 and chlorinated hydrocarbons [3-5]. Cadmium (Cd) is one of such chemicals that can cause liver injury (necrosis). It initially accumulates in the liver and therefore acute exposure to cadmium produces apoptosis and necrosis in the liver [6].

Cadmium (Cd) is one of the non-essential heavy metals known for its non-corrosive nature. It is widely used in the production of paints and dyes, cement and phosphate fertilizers. Cd occurs naturally in the environment in insignificant amounts but its release in the recent past is steadily increasing due to human activities causing pollution of soil and aquatic systems [7]. Cadmium poses a potential environmental hazard due to its increased industrial use [8]. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and by generation of reactive oxidative intermediates in liver [9]. Symptoms of hepatotoxicity may include loss of appetite, weakness, nausea, swelling of feet and/or legs, diarrhea and high level of bilirubin in extracellular fluid [10].

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Treatment of hepatotoxicity is dependent upon the causative agent, degree of liver dysfunction, age and general health of the patient. There is no effective treatment other than stopping the causative medication or removal from the exposure to the causative agent and providing general supportive care [4]. Liver diseases have become a worldwide problem, which is associated with high morbidity and mortality [11]. Liver diseases are most common in Africa, responsible for at least 12% of medical admissions and over 20% of hospital mortality in many parts of Africa [12]. Many synthetic drugs used for the treatment of liver diseases, which often have side effects and also unaffordable also damage the liver [13], thereby calling for alternative remedies for this disease.

Many plant species have been used in managing liver diseases in Nigeria including *Vernonia amygdalina*. The plant is a widely used locally in Nigeria for both therapeutic and nutritional purposes [14]. It is a common vegetable among the people of West Africa [15]. Studies have shown that the leaf extract of *V. amygdalina* and its components have anticancer, antidiabetic, antimalarial, antioxidant, antitumor, immunomodulatory and hepatoprotective properties [16-21]. *V. amygdalina* is commonly called bitter leaf because of its bitter taste. It is a perennial herb belonging to the *Asteraceae* family and it is a small evergreen shrub that grows all over Africa [20, 22].

V. amygdalina is usually consumed after processing which removes the astringent components of the bitter leaf [23-26]. Several processing techniques are however employed for *V. amygdalina* before consumption including manual squeezing with hands, hand-squeezing with table salt added and sun drying. Several researches have been conducted on hepatoprotective activities of the extract of *V. amygdalina* leaf [17, 19, 27, 28], but the processing methods used traditionally in preparing the plant leaf have not been well documented in relation to its hepatoprotective activity. Therefore, this study seeks to investigate the protective potentials of the processed *V. amygdalina* leaf extract on cadmium-induced hepatic damage in rats.

2.0 Material and Methods

2.1 Plant Material

Fresh leaves of Vernonia amygdalina was obtained from a garden at Hajji Camp Area Ilorin, Nigeria in the month of July, 2015. The plant sample was taken to the Herbarium Unit of the Department of Plant Biology, University of Ilorin for authentication and given the voucher number UIH001/972. A specimen sample was prepared and deposited at the Herbarium for reference.

2.2 Chemicals and Reagents

All Chemicals used for this research were of analytical grade. The assay kits were products of Randox Laboratories Limited, County Artrim, United Kingdom. 5,5'-dithiobis-(-2-nitrobenzoic acid) (DTNB), thiobarbituric acid (TBA), epinephrine, sulphosalicylic acid, trichloroacetic acid (TCA) and cadmium chloride were products of Sigma-Aldrich Inc., St. Louis, USA. Silymarin was a product of Micro Labs Limited, India. Reagents were prepared using distilled water and other appropriate solvents.

2.3 Experimental Animals

Female Wistar rats (n=35) with a mean weight 150.00±5.00 g were obtained from the Animal Holding Unit of Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals were humanely kept in metabolic cages under a 12 hours light/dark cycle in well-ventilated room at 26^{o}C to 27^{o}C. They were fed with standard mouse diet and water ad libitum. The animals were acclimatized for 14 days before treatment commenced.

2.4 Sample Preparation

The collected fresh leaves of *V. amygdalina* were divided into two (2) parts and prepared as follows:

I. In the first part, fresh leaves of *V. amygdalina* were rinsed with clean cold water and sundried.

II. For the second part, the fresh leaves were rinsed with clean cold water and then manually squeezed to remove the foaming juice. The squeezed leaves were then rinsed again with clean cold water, drained and sundried.

The two samples were then pulverized separately using electric blender.

2.5 Sample Extraction

The procedure described by Salawu *et al*. [29] was modified and used for extraction. The powdered samples (100 g each) were weighed separately into 2 different beakers and extracted with a mixture of ethanol and water (70:30). The content in each beaker was shaken properly for even distribution and left for 24 hours for extraction to take place. The content was filtered with the residue soaked again in the same proportion of solvent for another 24 hours and the content filtered again. The filtrate recovered from the first and second extraction were pooled together and then concentrated at a reduced pressure using a rotary evaporator to give a yield of 7.57 g and 15.85 g for LEVA and SLEVA respectively.

2.6 Experimental Design

Thirty five female Wistar rats were divided into 7 groups (A-G) of 5 rats each as follows:-

Group A: Normal control group that received distilled water

Group B: Negative control group which received 10 mg/kg bodyweight of $CdCl_2$

Group C: Positive control group which was pre-administered with silymarin (reference drug) and then 10 mg/kg bodyweight of $CdCl_2$.

Group D: Animals pre-administered with 50 mg/kg body weight of leaf extract of *V. amygdalina* (LEVA) followed by 10 mg/kg bodyweight of $CdCl_2$

Group E: Animals pre-administered with 100 mg/kg body weight of leaf extract of *V. amygdalina* (LEVA) followed by 10 mg/kg bodyweight of $CdCl_2$

Group F: Animals pre-administered with 50 mg/kg body weight of squeezed leaf extract of *V. amygdalina* (SLEVA) followed by 10 mg/kg bodyweight of $CdCl_2$

Group G: Animals pre-administered with 100 mg/kg body weight of squeezed leaf extract of *V. amygdalina* (SLEVA) followed by 10 mg/kg bodyweight of $CdCl_2$.

The animals were pre-treated daily with extracts/reference drug followed by administration of hepatotoxicant ($CdCl_2$) 3 hours later for a period of 2 weeks.

2.7 Collection of Serum and Homogenate Preparation

The animals were sacrificed under diethyl ether anesthesia; the jugular veins were exposed and cut with a sharp sterile blade. The blood was allowed to clot for about 15 minutes and centrifuged at 3000 rpm for 10 minutes. The clear serum obtained was collected with a Pasteur pipette and stored frozen. The liver was removed carefully, blotted with tissue paper for determination of liver to body weight ratio by comparing the organ weight to the body weight. A portion of the liver was preserved in 10% buffered formaldehyde solution for histological analysis. Liver homogenate was prepared by homogenizing a known weight of the liver in 0.25 M sucrose solution in a cold medium, and centrifuged at 4000 rpm for 10 minutes. The supernatant was collected using a Pasteurs pipette and stored frozen until required for use [30].

2.8 Assay of Liver Function Indices

The serum was assayed for the liver function indices using standard diagnostic test kits. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed using the method of Reitman and Frankel [31] , alkaline phosphate (ALP) and γ-Glutamyl transferase (GGT) by the method of Rec [32] and Tietz [33] respectively, total protein by the method of Gornall *et al* [34], albumin by Grant *et al*. [35] and bilirubin by the method described by Jendrassik and Grof [36].

2.9 Assay of Superoxide Dismutase (SOD) Activity

The activity of superoxide dismutase was determined using the method described by Mistra and Fridovich [37]. Briefly, 0.2 ml of the sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) and the reaction was started by the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture. The blank contained 2.5 ml buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of water. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds. One unit of enzyme activity is 50% inhibition of the rate of auto-oxidation of adrenaline as determined by change in absorbance/min at 420 nm.

2.10 Assay of Catalase (CAT) Activity

Decomposition of H_2O_2 in the presence of catalase was followed at 620 nm [38]. Phosphate buffer (1 ml, 0.01 M, pH 7.0) was added to 0.4 ml of H_2O_2 (0.2 M) followed by 0.1 ml of sample and gently swirled at room temperature. The reaction mixture was stopped by the addition of 2 ml of dichromate acetic acid reagent (5% of dichromate prepared in acetic acid). The change in the absorbance was measured at 620 nm and recorded after 3 minutes interval.

2.11 Determination of Reduced Glutathione (GSH) Concentration

Reduced glutathione (GSH) concentration was determined by employing the procedure described by Ellman [39]. The homogenate (1.0 ml) was added to 0.1 ml of 25% TCA and the resulting precipitate was removed by centrifuging at 5000 x *g* for 10 minutes. Supernatant (0.1ml) was added to 2 ml of 0.6 mmol/L DTNB prepared in 0.2 mol/L sodium phosphate buffer (pH 8.0). The absorbance was then read at 412 nm.

2.12 Determination of Malondialdehyde (MDA) Concentration

A portion of TBA reagent (2 ml of 0.7%) and TCA (1 ml of 20%) were added to 2 ml of the liver homogenate sample. The mixture was heated in a water bath at 100^{o}C for 20 minutes. It was then cooled and centrifuged at 4000 rpm for 10 minutes. The absorbance of the supernatant was read at 540 nm against a reference blank of distilled water after centrifuging for another 10 minutes.

2.13 Histological Analysis

The portion of liver tissue preserved in buffered formaldehyde was fixed in 10% formalin for histological examination according to the method described by Krause [40]. The tissues were processed into 5 µm thick sections and then stained with hematoxylin-eosin and observed under a photomicroscope set at 400 x magnification.

2.14 Statistical Analysis

Experimental data were presented as mean ± SEM. Statistical analysis was done using computer software SPSS 21.0 version statistical package program (SPSS, Chicago. IL). One way Analysis of Variance (ANOVA) was used to compare the variables among the different groups. Level of significance (Post Hoc comparison) among the various treatments was determined by Duncan's Multiple Range Test. The values were considered statistically significant at $p<0.05$.

3.0 Results

The percentage liver-body weight ratio of rats (Table 1) showed no significant difference ($P>0.05$) in all the groups. The administration of cadmium resulted in significantly increased ($P<0.05$) activities of ALT and AST in the serum, compared to the normal control. The processed extract was able to reduce the activities of these enzymes with the LEVA at 100 mg/kg bw producing activity almost the same with the normal control and the SLEVA at 100 mg/kg bw having activity closer to that of the positive control. Administration of cadmium chloride to the rats resulted in a statistically increased levels of serum ALT and AST activities as seen in the negative control group (Table 2). Pre-treatment with silymarin or the *V. amygdalina* leaf extract was able to reduce the serum ALT and AST activities respectively, in a dose dependent manner.

Table 1: Percentage liver-body weight ratio of cadmium-intoxicated rats pre-administered with *Vernonia amygdalina* leaf extract

Groups	Liver-body weight ratio (%)
Normal control	3.45 ± 0.21^{a}
10 mg/kg bw $CdCl_2$	3.19 ± 0.13^{a}
Silymarin + $CdCl_2$	3.86 ± 0.35^{a}
50 mg/kg bw LEVA + $CdCl_2$	3.52 ± 0.11^{a}
100 mg/kg bw LEVA + $CdCl_2$	3.72 ± 0.25^{a}
50 mg/kg bw SLEVA + $CdCl_2$	3.06 ± 0.22^{a}
100 mg/kg bw SLEVA+ $CdCl_2$	3.36 ± 0.03^{a}

Values are expressed as means of 3 replicates ± SEM. Values carrying the same superscripts along the same column are not significantly different ($p>0.05$).

Table 2: Specific activities of liver function enzymes in the serum of cadmium-intoxicated rats pre-administered with *Vernonia amygdalina* leaf extract

Groups	ALT (Ul^{-1} mg protein^{-1})	AST (Ul^{-1} mg protein^{-1})	ALP (nmol min^{-1} mg protein^{-1})	GGT (nmol min^{-1} mg protein^{-1})
Normal control	0.51 ± 0.02^{a}	1.30 ± 0.03^{a}	36.18 ± 1.58^{a}	0.26 ± 0.05^{a}
10 mg/kg bw + $CdCl_2$	2.04 ± 0.09^{b}	4.86 ± 0.06^{b}	81.82 ± 0.91^{b}	3.87 ± 0.27^{b}
Silymarin + $CdCl_2$	0.67 ± 0.03^{c}	2.16 ± 0.08^{c}	35.12 ± 1.37^{c}	1.44 ± 0.16^{c}
50 mg/kg bw LEVA + $CdCl_2$	1.01 ± 0.05^{d}	1.90 ± 0.03^{d}	50.85 ± 1.22^{d}	1.07 ± 0.14^{d}
100 mg/kg bw LEVA + $CdCl_2$	0.54 ± 0.03^{a}	1.41 ± 0.06^{a}	42.98 ± 0.37^{e}	0.75 ± 0.05^{e}
50 mg/kg bw SLEVA + $CdCl_2$	1.07 ± 0.01^{d}	1.96 ± 0.07^{d}	51.58 ± 1.05^{d}	1.29 ± 0.05^{f}
100 mg/kg bw SLEVA + $CdCl_2$	0.68 ± 0.02^{c}	1.47 ± 0.05^{a}	48.84 ± 0.56^{f}	1.06 ± 0.04^{d}

Values are expressed as means of 3 replicates ± SEM. Values carrying different superscripts along the same column are significantly different ($p<0.05$).

The total protein and albumin levels were significantly reduced, while the bilirubin levels increased significantly ($P<0.05$) in the negative control group as presented in Table 3. These results also showed that the LEVA at 100mg/kg bw compares favourably with the normal control, except for the direct bilirubin, where the SLEVA at 100 mg/kg bw which had lower serum concentration than the LEVA at 100 mg/kg bw.

The SOD activity showed that the negative control had a significantly lowered ($p < 0.05$) activity. However, the LEVA and SLEVA at 100 mg/kg bw had activities which compared favourably with the control. The CAT activity on the other hand, showed a significantly elevated ($p<0.05$) activity in the negative control. The raw leaf extract was able to increase and reduce the activities of SOD and CAT respectively, with the LEVA at 100 mg/kg bw comparing favourably with the normal control, while the SLEVA at 100 mg/kg bw compared favourably with the positive control (Table 4).

The GSH concentration was significantly reduced ($p<0.05$) in the negative control group. However, the extracts were able to increase the GSH levels with the LEVA at 100 mg/kg bw producing the highest concentration. Similarly, the negative control group had an elevated MDA concentration, which was attenuated by the extract. Also, the LEVA and SLEVA at 100 mg/kg bw had comparable MDA concentrations (Table 5).

Table 3: Concentrations of total protein, albumin, total bilirubin and direct bilirubin in the serum of cadmium-intoxicated rats pre-administered with *Vernonia amygdalina* leaf extract

Groups	Total protein (mg/ml)	Albumin (mg/ml)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)
Normal control	74.60± 0.47^{a}	50.84± 0.38^{a}	0.58± 0.10^{a}	4.32 ± 0.30^{a}
10 mg/kg bw $CdCl_2$	41.30± 0.62^{b}	28.90± 0.54^{b}	4.90±0.22^{b}	17.23± 0.35^{b}
Silymarin + $CdCl_2$	67.30± 0.28^{c}	47.77± 0.19^{c}	0.90±0.19^{c}	4.37 ± 0.25^{a}
50 mg/kg bw LEVA + $CdCl_2$	55.60± 0.48^{d}	38.12± 0.37^{d}	2.81±0.22^{d}	6.34 ± 0.25^{c}
100 mg/kg bw LEVA + $CdCl_2$	66.99± 0.68^{c}	49.50± 0.59^{e}	2.09± 0.20^{e}	4.85 ± 0.37^{d}
50 mg/kg bw SLEVA + $CdCl_2$	52.70± 0.47^{d}	36.90± 0.35^{d}	2.93± 0.35^{d}	5.42 ± 0.51^{e}
100 mg/kg bw SLEVA + $CdCl_2$	65.80± 0.26^{e}	47.12± 0.18^{c}	1.73± 0.16^{f}	4.80 ± 0.19^{f}

Values are expressed as means of 3 replicates ± SEM. Values carrying different superscripts along the column are significantly different ($p<0.05$).

Table 4: Activities of superoxide dismutase (SOD) and catalase (CAT) in the liver of cadmium-intoxicated rats pre-administered with *Vernonia amygdalina* leaf extract

Groups	SOD ($nmolmin^{-1}mg\ protein^{-1}$)	CAT ($nmolmin^{-1}mg\ protein^{-1}$)
Normal control	20.55± 0.70^{a}	29.27± 0.18^{a}
10 mg/kg bw $CdCl_2$	8.61 ± 0.19^{b}	48.98± 0.77^{b}
Silymarin + $CdCl_2$	26.63± 1.90^{c}	35.77± 0.52^{c}
50 mg/kg bw LEVA + $CdCl_2$	16.77± 0.24^{d}	39.59± 0.73^{d}
100 mg/kg bw LEVA + $CdCl_2$	20.23± 0.45^{a}	30.88± 0.73^{a}
50 mg/kg bw SLEVA + $CdCl_2$	17.38± 0.31^{d}	42.20± 0.81^{d}
100 mg/kg bw SLEVA + $CdCl_2$	19.27± 0.88^{a}	35.63± 0.19^{c}

Values are expressed as means of 3 replicates ± SEM. Values carrying different superscripts along the same column are significantly different ($p<0.05$).

Table 5: Concentrations of reduced glutathione (GSH) and malondialdehyde (MDA) in the liver of cadmium-intoxicated rats pre-administered with *Vernonia amygdalina* leaf extract

Groups	GSH($\mu g/ml \times 10^{-4}$)	MDA($nmolml^{-1} \times 10^{-5}$)
Normal control	18.44± 2.33^{a}	16.49± 0.05^{a}
10 mg/kg bw $CdCl_2$	13.20± 5.33^{b}	38.12± 0.08^{b}
Silymarin + $CdCl_2$	17.82± 2.75^{a}	13.83± 0.07^{c}
50 mg/kg bw LEVA + $CdCl_2$	17.56± 3.49^{a}	20.92± 0.06^{d}
100 mg/kg bw LEVA + $CdCl_2$	19.98± 3.17^{c}	14.74± 0.04^{e}
50 mg/kg bw SLEVA + $CdCl_2$	16.68± 2.33^{d}	20.97± 0.08^{d}
100 mg/kg bw SLEVA + $CdCl_2$	19.05± 0.88^{a}	15.10± 0.04^{e}

Values are expressed as means of 3 replicates ± SEM. Values carrying different superscripts along the column are significantly different ($P<0.05$).

The finding in respect of the structural integrity of the liver of experimental animals is as depicted in Figures 1a to 1g. The histological studies revealed that the negative control group had severe distortions in cellular morphology as well as perivenular piecemeal necrosis. These effects were reduced in the groups pre-administered with the extract in a dose dependent manner.

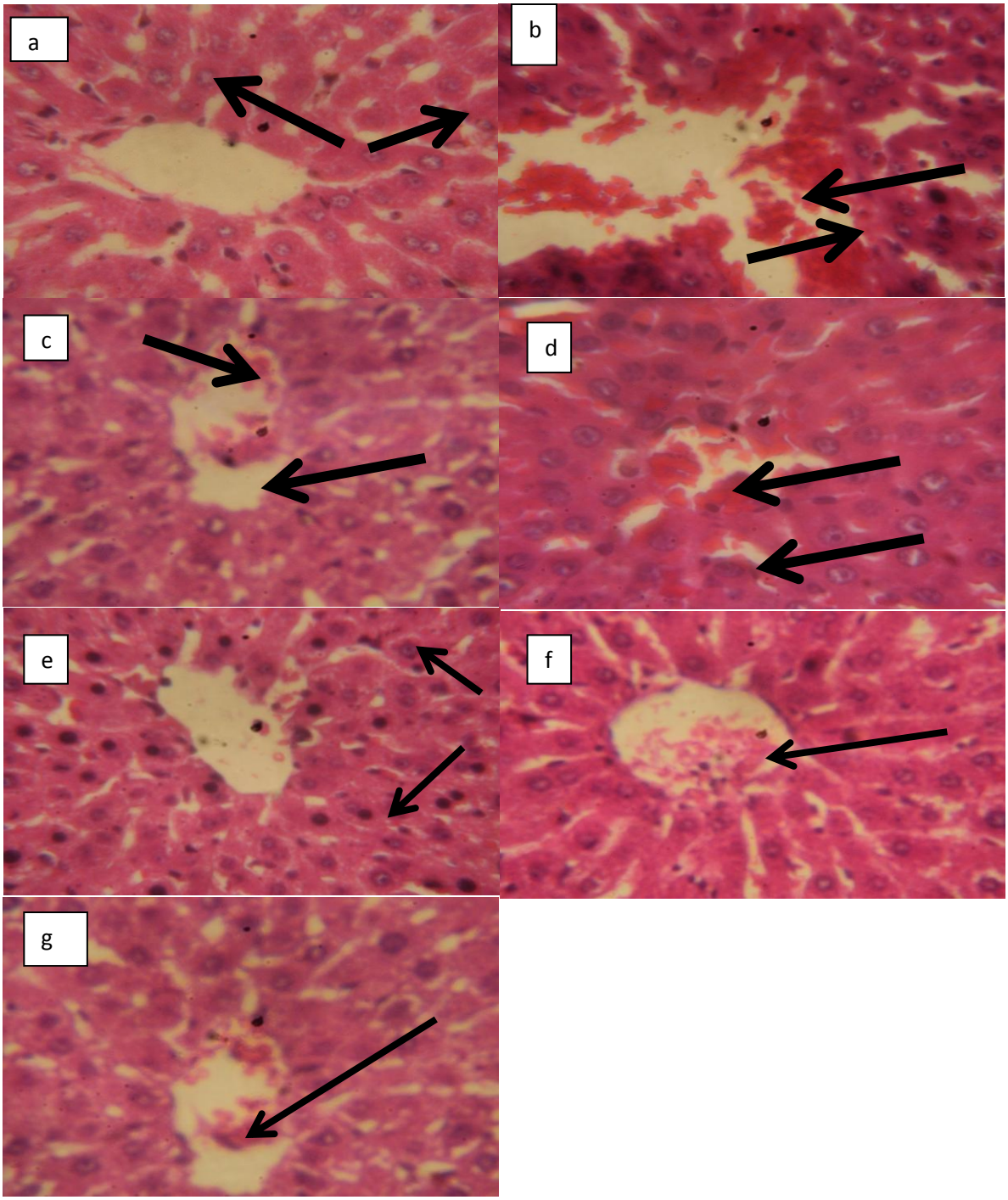

Figure 1: Photomicrograph of cross section of rat liver intoxicated with cadmium chloride for 10 days (Magnification: x400). (a) rat administered with distilled water with normal hepatocytes. (b) intoxicated untreated rat with severe distortion of cellular morphology and perivenular piecemeal necrosis. (c) intoxicated rat administered with silymarin with mild congestion of the central vein. (d) intoxicated rat administered with 50 mg/kg bodyweight LEVA with slight distortion of cellular morphology and lobular inflammation. (e) intoxicated rat administered with 100 mg/kg bodyweight LEVA with lobular inflammation. (f) intoxicated rat administered with 50 mg/kg bodyweight SLEVA with congestion of the central vein. (g) intoxicated rat administered with 100 mg/kg bodyweight SLEVA with mild congestion of the central vein.

4.0 Discussion

Changes in the organ body- weight ratio as suggested by Moore and Dalley [41] may be an indication of cell constriction or inflammation since the cells are the unit components of the organs. The constriction in the organ may occur as a result of loss of fluid from the organ due to damage, while increase in organ-body weight ratio may suggest inflammation. The liver to body weight ratio of the animals as observed in this study suggests that administration of the extract may not have resulted into constriction or inflammation of the cells.

Liver function tests (LFTs) are commonly used in clinical practice to screen for liver disease, monitor the progression of a known disease and determine the effects of potentially hepatotoxic drugs [42]. Alanine transaminase (ALT) and Aspartate transaminase (AST) are enzymes found in the liver and have been widely used for diagnostic purposes, as indicators of liver damage [43]. AST is present in both the mitochondria and cytosol of liver cells, while ALT is found in the cytosol only [44]. The observed increase in serum ALT and AST activities seen in the $CdCl_2$ treated group may be due to damage done to the membranes of the liver by cadmium , thereby causing alteration in hepatic function, and consequent release of ALT and AST into the extra hepatic circulation, as suggested by Navarro *et al*. [45]. The biochemical alterations that occur prior to morphological changes in the organs and changes in certain enzyme levels in the extracellular fluids may be a reflection of the extent of cadmium- induced damage in target organs [46]. The reduction in the serum ALT and AST activities in the groups pre-treated with *V. amygdalina* leaf extracts may be due to natural antioxidants such as vitamin A and vitamin E that are contained in the plant, which boost the defense system of the animals against the assault caused by cadmium [47-49].

Alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) are important enzymes in assessing obstructive liver injury [50, 51]. Elevated serum activity of ALP may be attributed to damaged structural integrity of hepatic cells [52]. The reduction in the serum ALP activity may be an indication that the extracts were able to reduce the damaging effects of the cadmium by repairing the hepatic cells. This may be through the action of secondary metabolites (such as flavonoids and alkaloids) found in the bitter leaf. Hence the liver could perform the various functions expected of it, and consequently preventing leakage into the blood stream. This observation agrees with previous study which linked hepatocyte function to ALP activity [53]. This suggests that increase in serum ALP activity may be attributed to increased synthesis in the presence of increased biliary pressure.

GGT is a more specific and sensitive marker for cholestasis damage than ALP. It is important for the metabolism of foreign substances as well as involved in cell growth and differentiation. The enzyme is over expressed in tumor cells resistant to therapeutic drugs [54]. The elevated serum GGT activities in the $CdCl_2$ treated group indicated that damage may have been done to the liver biliary duct resulting in liver dysfunction. Pre-administration of the extracts was able to reverse this in a dose dependent fashion. The observed increased serum ALP and GGT activities agree with previous studies using CCl_4 as the toxicant [55]. The restoration of the ALT, AST, ALP and GGT activities to normal or almost normal state by the extracts is an indication that *V. amygdalina* has protective effects against cadmium. This may be attributed to the ability of the plant to stabilize cell membrane and prevent enzyme leakages [28] as well as restoring the hepatocytes and regenerating the hepatic parenchyma [56].

Total protein and albumin are markers of liver biosynthetic ability [57]. Proteins are synthesized in response to environmental insults from exogenous or endogenous substances, thereby, adapting the cells to fight back. Thus proteins are synthesized to protect cells, tissues and organs; and to rebuild worn out ones [58]. Albumin is the major osmolar component of the blood serum and is produced by the liver [4]. It maintains the isotonic environment of the blood so that cells of the body do not gain or lose water in the presence of body fluids. It is the most abundant protein in human plasma, representing 55-65% of the total protein. It is synthesized in the liver at a rate that is dependent on protein intake subject to feedback regulation by the plasma albumin level [44]. Cadmium has been reported to cause reduction in serum protein and tissue protein concentration [59]. However, *V. amygdalina* leaf extracts was able to restore serum levels of total protein and albumin in a dose-dependent manner. This is an indication that the extract has the ability to boost immune system of the body and consequently fight back cadmium assault. The extracts were able to prevent the damage caused by cadmium by maintaining albumin concentrations near normal, which may be an indication of stabilization of the endoplasmic reticulum, thereby assisting protein synthesis [60]. This also agrees with the observations of Al- Hashem *et al*. [44] that cadmium ingestion resulted in significant decreased serum albumin levels, which was able to be restored to normal level following oral administration of camel's milk. Reduction in the serum albumin level by cadmium, may be due to protein adduct formation as well as poor liver functions [61] or impaired synthesis either primarily as liver cells damage or secondary to diminished protein intake and reduced absorption of amino acids caused by a malabsorption syndrome or malnutrition.

Bilirubin is the yellow breakdown product of normal haem catabolism. It is excreted in the bile and urine. Bilirubin can be conjugated with a molecule of glucuronic acid, which makes it soluble in water and facilitates its excretion into bile [4]. Bilirubin is a marker for hepatobiliary disease and a useful test to substantiate the functional integrity of the liver and severity of necrosis. The direct bilirubin is made up of both the conjugated bilirubin and ɤ-bilirubin. ɤ-bilirubin appears in the serum when hepatic excretion of conjugated bilirubin is impaired in patients with hepatobiliary disease [62]. Total bilirubin is a measure of all the bilirubin in circulation, increased level cause jaundice [63]. Serum bilirubin is considered a true test of liver function as it reflects the liver's ability to take up process and secrete bilirubin into the bile. Elevation in serum bilirubin indicates liver damage because small amount of bilirubin circulates normally in the blood [64]. The elevated levels in the total and direct serum bilirubin concentrations as observed in the negative control may be due to destruction of the hepatic cells leading to hepatic dysfunction. Rana *et al*. [65] reported that hyperbilirubinaemia might have resulted from the decrease of liver uptake, conjugation and increase bilirubin production from haemolysis.

Free radicals induce oxidative state which can cause cellular membrane injury with the consequent alteration in metabolic processes. Reactive oxygen species (ROS) play an important role in the pathogenesis of various degenerative human diseases and have been implicated in liver disorders [66]. Cells have a variety of defense mechanism that intercept free radicals to prevent or limit intracellular damage and ameliorate the harmful effect of ROS. These include low-molecular weight antioxidants (ascorbic acid, vitamin E and glutathione); antioxidant enzymes (thioredoxin, SOD and CAT) as well as non-enzyme antioxidant e.g. reduced glutathione [67].

SOD protects tissues against oxygen free radicals by converting the superoxide radical into hydrogen peroxide and molecular oxygen (i.e. dismutation reaction), while CAT catalyses the detoxification of hydrogen peroxide, preventing damage to cell membranes and other biological structures [68]. The present findings contradict those of other researchers [19, 44] that CAT showed a significantly decreased activity in intoxicated untreated rats. The increase in CAT activity observed in the negative control may imply that there is increased production of the radicals generated (i.e. hydrogen peroxide concentration), which would be mopped up or scavenged by the antioxidant defense system. The increase in free radicals may be due to damage of antioxidant defense system, as a result of increased production and/or decreased destruction of reduced glutathione (GSH) as well as reduced activities of CAT and SOD [69]. GSH is an important detoxifying protein of many environmental chemicals and mutagens. It can directly react with and inactivate toxic electrophiles when consumed thus enhancing the detoxification of reactive metabolites by conjugation with GSH [70]. The reduced concentration of GSH is an indication that the free radicals generated have overwhelmed the defense mechanism of the antioxidant system.

Antioxidants have been observed to exert their action *in vivo* by inhibiting the generation of ROS by directly scavenging free radicals or by raising the levels of endogenous antioxidant defenses [71,72]. Malondialdehyde (MDA) concentration is an indication of increased lipid peroxidation due to generation of reactive oxygen species [73]. Cadmium may induce oxidative damage in different tissues by enhancing peroxidation of membrane lipids in tissues and altering the antioxidant system of the cell [74]. The most practical way to combat degenerative diseases is to improve body antioxidant status, which could be achieved by increasing consumption of vegetables and fruits [75]. The antioxidative effect demonstrated by the extract of *V. amygdalina* may be attributable to the role of the extracts in mopping up the free radicals generated by the cadmium induction and consequently protecting the liver cells.

Histological changes in liver are late manifestation of chemical, physical, mechanical or inflammatory assault on the tissue [76]. Cadmium-induced hepatotoxicity may be manifested by occurrence of inflammatory state or by direct toxic action of cadmium on liver cells [44]. The absence of necrotic lesions in liver of extract-treated animals suggests that the hepatoprotective action may be due to membrane stabilizing effects in hepatic cells [77]. These findings agree with those of Sree Ramamurthy and Srinivasan [78], where it was reported that the pre-treatment of rats with *Tephrosia purpurea* offered hepatoprotection via membrane stabilizing effect in hepatic cells.

5.0 Conclusion

It can be deduced from the results of this study that the pre-treatment of rats with *Vernonia amygdalina* leaf extract protected the liver cells from the damaging effects of cadmium with the LEVA exhibiting higher protective potential when compared to that of SLEVA.

6.0 References

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