Acetonic extract of *Vernonia amygdalina* (Del.) attenuates Cd-induced liver injury: Potential application in adjuvant heavy metal therapy

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ABSTRACT

Exposure to cadmium (Cd), even at low doses, is of serious health concern because it does not undergo metabolic degradation to less toxic metabolite. Liver injury/disease, with a world-wide increasing incidence, is one of the consequences of exposure to Cd toxicity. This study aimed at determining the effects of acetonic extract of Vernonia amygdalina leaf (AEVAL) in a Wistar rat model of Cd-induced liver injury. Phytochemical screening of the extract was carried out and its oral LD$_{50}$ was determined to guide the choice of therapeutic doses. Thereafter, thirty male Wistar rats were recruited for this study. The experimental groups received 4 weeks oral graded doses of the extract (100, 200 and 400 mg/kg) following Cd-induced liver injury. Cd-induced liver injury (5 mg/kg i.p for 5 consecutive days) was characterized by deleterious alterations in the levels of AST, ALT, ALP, total bilirubin and hepatic total protein ($p < 0.05$). Also, deleterious alteration of oxidative stress indicators (GSH, SOD and CAT) and lipid peroxidation index (TBARS) was observed in the liver homogenates. Histopathological examination showed evidence of degenerated hepatocytes as well as inflammation with disseminated steatosis. These conditions were significantly attenuated ($p < 0.05$) following treatment with graded doses of the extract, with the highest dose expressing least therapeutic effects. This study concluded that AEVAL attenuated Cd-induced liver injury and is, potentially, a suitable option in adjuvant therapy for heavy metal toxicity.

1. Introduction

The liver, one of the vital organs in the body that performs homeostatic function through detoxification mechanisms, is a natural chemical factory which neutralizes toxins and aids in the anabolism of complex molecules from simple substances that are absorbed from the gastro-intestinal tract [1,2]. Adverse disturbances of liver function can have deteriorating health consequences which sometimes lead to terminal illness [3] due to biological build-up of toxins. Liver injury can result from exposure to various kinds of exogenous compounds or chemicals, either through job demands or way of life [4].

Cadmium (Cd), one of the known environmental toxins that are detrimental to liver function, is a ubiquitous heavy metal that has found its relevance in several industrial processes such as electroplating, manufacturing of paint pigments, plastic, dyes as well as its use in agriculture for the production of fertilizers [5–7]. Exposure to Cd is of serious health concern because it does not undergo metabolic degradation to less toxic metabolites [8]. Human exposure to this heavy metal is majorly by two main routes, inhalation and ingestion [9]. It is both an environmental and an occupational toxin. Its emission from industrial processes can cause atmospheric, soil, water and food pollution [10,11]. Food consumption is a major source of its exposure because it is readily absorbed by the roots of plants in contaminated soils [12,13]. Through cigarette smoke, Cd is readily absorbed in the body by inhalation [14], thereby exerting its deleterious effects in both active and passive smokers. Unlike most heavy metals, its exposure can induce deleterious health effects at relatively lower doses once it is absorbed in the body [15–17]. It is known to exert its toxic effects by inducing reacting oxygen species (ROS) generation through oxidative damage [18]. These ROS (H_2O_2 and OH^+) initiate reactions with cellular biomolecules, causing lipid peroxidation with consequent disruption of the antioxidant system as well as membrane protein damage [18,19]. We, therefore, hypothesized that a potent antioxidant may inhibit, retard or beneficially alter this basic mechanism of Cd-induced deleterious alterations and possibly ameliorate its toxic effects on hepatic function. Established models of therapy for heavy metal toxicity

(such as the use of dimercaptosuccinic acid) are often very expensive, not readily available and burdened with undesirable side effects [20]. Therefore, this hypothesis was tested using ethno-botanical approach, since plant-derived medicines (besides their easy availability, being relatively cheaper and affordable to the common man) are safe [21] and has been used as a source of inspiration for the development of novel drugs [22].

Vernonia amygdalina, commonly called "Bitter leaf" because of its bitter taste, is a member of the Asteraceae family; a small shrub that grows in tropical Africa [23]. In various regions of Africa, particularly in Nigeria, the plant is a common homestead farming vegetable and is being used as an ingredient to prepare several delicacies [24,25]. Its usage as a medicinal herb is being explored by scientists to date. *Vernonia amygdalina* is reputed to have several medicinal properties such as antibacterial [26,27], anti-parasitic [28,29], anti-plasmodial [30,31], anti-mutagenic [32], anti-inflammatory [33,34], anti-nephrotoxic [35] as well as anti-oxidant [36,37] activities.

Although literatures exist on the experimental evaluation of *Vernonia amygdalina* activity in several models of xenobiotic-induced hepatic injury [38–41], our literature survey revealed that (to date) there is dearth of experimental evidence of its effects on liver function in models of heavy metal toxicity. This study aimed at bridging this gap in knowledge by providing information on the effects of its acetonic extract in a Wistar rat model of Cd-induced liver injury.

2. Materials and methods

2.1. Plant material, chemicals and biochemical kits

Vernonia amygdalina leaves were harvested from a garden at Ife-Ibadan area of Ile-Ife, Osun state, Nigeria and certified by a Taxonomist (Mr. A. Gabriel) at the Department of Botany, Obafemi Awolowo University (OAU), Ile-Ife, Osun state, Nigeria.

Cadmium sulphate ($CdSO_4$) was purchased from Guangzhou Fischer Chemical Co., Ltd, Guangdong – China while acetone (analytical grade) was procured from Crescent Chemical Co. Inc., New York, United States.

Standard laboratory kits (Randox products) for assaying the biomarkers of liver function were purchased from Randox Laboratory Ltd., United Kingdom.

2.2. Extraction process

Acetonic extraction of *Vernonia amygdalina* (Del.) leaves was carried out as follows; Fresh leaves of the plant were air-dried and pulverized with an electric pulverizer (DIK-2910, Daiki Rika Kogyo Co. Ltd, Tokyo – Japan) and, thereafter, weighed (**W1**). The pulverized leaves were further crushed in 80% acetone (1:2 w/v) using a Waring blender. The resulting mixture was homogenized in a Polyron Homogenizer (Glen Mills Inc., Clifton, NJ) for about 3 min and the homogenate was filtered under vacuum using Buchner funnel and Whatman number 2 filter paper (Whatman PLC, Middlesex, UK). The filtrate was concentrated under vacuum using a Rotary Evaporator (Hahnshin Scientific, HS-2005-N) and freeze-dried in a Lyophilizer (Ilshon Lab. Co. Ltd., Seoul, Republic of Korea). The yield obtained (after the extraction process) was weighed (**W2**) and kept in a desiccator until when needed. This yield was the acetonic extract of *Vernonia amygdalina* leaf (AEVAL).

The percentage yield of AEVAL was calculated as follows;

$$\% \text{ yield} = \frac{W2}{W1} \times 100$$

2.3. Detection and quantification of phytochemicals in AEVAL

The presence of alkaloids was qualitatively determined by the method of Halilu et al. [42] and quantified as described by Harbone [43]. Flavonoids were also qualitatively determined by the method of Halilu et al. [42] but quantified by the method of Obadomi and Ochuko [44]. The presence of Tannins was qualitatively determined by the method of Halilu et al. [42] and quantified by the method of Allen et al. [45]. Saponins were qualitatively determined using Froth test as described by Benmehdi et al. [46] and thereafter quantified by the method of Obadoni and Ochuko [44]. Keller-Kiliani test as described by Anjali and Sheetal [47] was used to qualitatively determine cardiac glycosides while it was quantified by the method of Harbone [43].

2.4. Determination of oral lethal dose (LD$_{50}$) of AEVAL

The oral LD_{50} of AEVAL was determined by a modification of Lorke's method [48]. The modification was the use of 8 rats in the second phase of study, rather than 4 rats as proposed by Lorke. Lorke's method proposed the use of a total number of 13 animals; 9 animals for the first phase and 4 animals for the second phase. However, a total number of 17 adult Wistar rats were used for this study. In the first phase of study, 9 rats were divided into 3 groups of 3 rats each and were administered AEVAL at graded doses of 10, 100 and 1000 mg/kg, orally. The rats were observed for 24 h after which the first phase of study was terminated. In the second phase of study, 8 rats were divided into 4 groups of 2 rats each and were administered AEVAL at 750, 1500, 3000 and 6000 mg/kg, orally. They were also observed for 24 h after which the oral LD_{50} of the extract was determined by the formula below;

$$LD_{50} = \sqrt{a \, x \, b}$$

Where **a** = least dose that killed a rat; and
b = highest dose that did not kill any rat.

2.5. Preparation of AEVAL and Cd solutions

The choice of therapeutic doses that was adopted for this study was guided by the (determined) oral LD_{50} of AEVAL. These doses were taken to be less than 10% of the oral LD_{50}. Hence, doses of 100, 200 and 400 mg/kg of AEVAL were prepared as follows;

1 g of AEVAL was dissolved in 20 ml of distilled water to make a stock solution of 100 mg/kg of AEVAL. Stock solutions of 200 and 400 mg/kg AEVAL were prepared by dissolving 2 g and 4 g of AEVAL each in 20 ml of distilled water, respectively. The rats, therefore, received 0.2 ml/100 g of AEVAL, orally throughout the study period. Fresh samples were prepared every 48 h while left-overs were stored in a deep-freezer after use.

Cd in the form of $CdSO_4$ was administered to the rats at 5 mg/kg for five consecutive days via intraperitoneal route (i.p.). 50 mg of the salt was dissolved in 20 ml of distilled water in order to prepare a stock solution of 5 mg/kg. Therefore, each rat received 0.2 ml/100 g of Cd, intraperitoneally for a period of 5 days.

2.6. Animal management and experimental protocol

All experimental protocols were in strict compliance with the guidelines for animal research, as detailed in the NIH Guidelines for the Care and Use of Laboratory Animals [49] and approved by local institutional Research Committee. Thirty (30) male Wistar rats of about 2–3 months of age, weighing 120–150 g, were used for this study. They were purchased from the Animal Holdings Unit of the College of Health Sciences, OAU, Ile-Ife, Osun State, Nigeria where the study was carried out. They were housed in plastic cages under natural light and dark cycle and allowed access to standard laboratory rat chow (Caps Feed PLC, Osogbo – Nigeria) and water ad libitum.

The rats were divided into five groups as follows; Group 1 (control) consisted of 5 rats that received distilled water (2 ml/kg) intraperitoneally for 5 consecutive days and thereafter received oral distilled water (2 mg/kg) for 4 weeks before they were sacrificed. Group
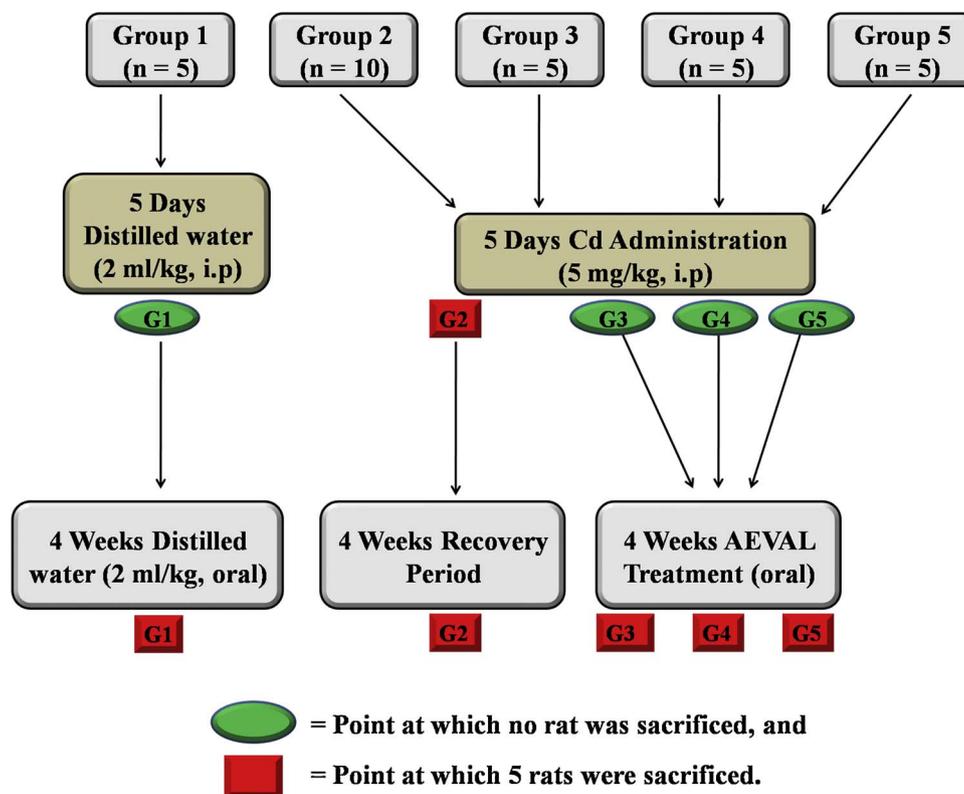
Fig. 1. Experimental Protocol.
G1 = Group 1 (2 ml/kg of distilled water, both i.p and orally); **G2** = Group 2 (*First 5 rats* = 5 mg/kg Cd; *Remaining 5 rats* = 5 mg/kg Cd + Recovery); **G3** = Group 3 (Cd + 100 mg/kg AEVAL); **G4** = Group 4 (Cd + 200 mg/kg AEVAL); **G5** = Group 5 (Cd + 400 mg/kg AEVAL); **n** = number of rats in the group.

2 (toxic) consisted of 10 rats that received Cd (5 mg/kg) intraperitoneally for 5 consecutive days after which 5 rats were sacrificed. The remaining 5 rats were left for a recovery period of 4 weeks, after which they were also sacrificed. Groups 3, 4 and 5 each consisted of 5 rats that were pre-treated as group 2 after which they received graded doses of AEVAL at 100, 200 and 400 mg/kg respectively, orally, for 4 weeks after which they were also sacrificed (Fig. 1). At the end of the study, the rats were euthanized and their blood samples were collected by cardiac puncture into separate EDTA bottles.

Blood samples were centrifuged at 4000 rpm for 15 min at $-4\,°C$ using a cold centrifuge (Centurium Scientific, Model 8881). The plasma obtained was decanted into separate plain bottles using sterile syringes, for the assessment of biochemical markers of liver function. About 1 g of the liver of each rat was excised and kept in a cooler for the preparation of tissue homogenates for the assessment of markers of oxidative stress and lipid peroxidation while the other portion of the liver were fixed in 10% formal-saline solution for histopathological examination using Hematoxylin − Eosin (H & E) staining technique.

2.7. Measurement of body and organ weight

The assessment of weekly body weight was carried out using Hanson digital weighing balance (Hanson, China) while organ weight was determined using Camry sensitive weighing balance (Camry, China). The percentage weight change as well as relative liver weight was determined using the formulae below;

Percentage Weight Change (PWC) (%)
$$= \frac{(\text{Final body weight} - \text{Initial body weight}) \text{ g}}{\text{Initial body weight (g)}} \times 100\% \qquad [50,51]$$

Relative Liver Weight (RLW) (%)
$$= \frac{\text{weight of whole liver (g)}}{\text{Final body weight (at the point of sacrifice)(g)}} \times 100\% \qquad [50,51]$$

2.8. Assessment of biochemical markers of liver function

Assessment of biochemical markers of liver function such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin in the plasma was carried out using Randox standard laboratory kits. The procedures for these tests were as provided in their respective kits. However, total protein was assayed in the liver homogenate according to the method of Lowry and co-workers [52].

2.9. Assessment of indicators of oxidative stress and lipid peroxidation

With the aid of an electric homogenizer (S1601001), 10% homogenate in phosphate buffer (100 mM) was prepared with the tissues at pH of 7.4. The homogenates were centrifuged at 3000 rpm for 20 min and the supernatants were collected for the assessment of the following indicators of oxidative stress and lipid peroxidation;

Reduced glutathione (GSH) level was determined by the method of Beutler and co-workers [53], Super-oxide dismutase (SOD) by the method of McCord and Fridovich [54], catalase by the method of Sinha [55] while Thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation, was determined as described by Ohkawa et al. [56].

2.10. Histopathological examination

The liver of each rat was fixed in 10% formal- saline solution. Thereafter, they were dehydrated in graded alcohol and embedded in paraffin wax. Sections taken (7–8 µm thick) were stained using H & E

Table 1
Percentage Yield of AEVAL (%).

Extraction Processes	Weight of Pulverized Leaves (g)	Yield (g)	Percentage Yield (%)
1st Extraction	300	24.50	8.17
2nd Extraction	300	24.80	8.27
3rd Extraction	300	24.20	8.07

Result shows that the percentage yield of AEVAL is 8.17 ± 0.06% (where n = 3).

technique. Photomicrographs were taken using a Leica DM 750 camera microscope at ×400 magnification.

2.11. Statistical analysis

Data obtained were expressed as mean ± standard error of mean using one-way analysis of variance (for multiple comparisons) and thereafter subjected to Tukey's post-hoc test at p < 0.05. Student's *t*-test was used to determine differences between two variables. Data were analysed using Graph Pad Prism 5.03 (Graph Pad Software Inc., CA, USA).

3. Results

3.1. Percentage yield (%), phytochemical screening and oral LD_{50} of AEVAL

After three different extraction processes, the percentage yield of AEVAL is as presented in Table 1.

Important phytochemicals that are present in the extract (AEVAL) are as presented in Table 2.

The determined oral lethal dose (LD_{50}) of AEVAL is as presented in Table 3.

3.2. Effects of AEVAL on percentage weight change (%) and relative liver weight (%) of Wistar rats exposed to Cd toxicity

The AEVAL-treated groups 3, 4 and 5 showed significant restoration of percentage weight change to physiological levels when compared with the control, toxic and toxic recovery groups (Table 4).

Also, the Cd-induced significant increase in relative kidney weight was significantly restored to physiological levels in the AEVAL-treated groups 3, 4 and 5 when compared with the control, toxic and toxic recovery groups (Table 4).

3.3. Effects of AEVAL on hepatic GSH (μg/mg protein), SOD (mM/mg protein), CAT (μmol/min/mg protein) and TBARS (nmol/mg protein) activities in wistar rats with Cd-induced liver injury

Cd-induced reduction in hepatic GSH, SOD and CAT levels was significantly normalized following AEVAL administration. The AEVAL-treated groups 3, 4 and 5 showed no significant difference in these indicators of oxidative stress (at the end of the study) when compared

Table 2
Phytochemical Screening and Quantification of AEVAL.

Phytochemical Constituent	Status	Quantification/Percentage Composition (g/100 g)
Alkaloids	+	10.09 ± 0.38
Flavonoids	+	32.54 ± 0.25
Tannins	+	16.62 ± 0.69
Saponins	+	3.97 ± 0.11
Cardiac glycosides	−	nil

Each value (n = 3) is expressed as mean ± standard error of mean; + = present; − = absent.

Table 3
Acute Oral Toxicity Test (LD_{50}) of AEVAL.

No of rats	Dose (mg/kg)	Mortality
1ST PHASE		
3	175	0/3
3	350	0/3
3	700	0/3
2ND PHASE		
2	625	0/2
2	1250	0/2
2	2500	0/2
2	5000	0/2

Least dose that killed a rat = nil; Highest dose that did not kill any rat = 5000 mg/kg. Therefore, oral LD_{50} of AEVAL is > 5000 mg/kg in Wistar rats.

Table 4
Effects of AEVAL on Percentage Weight Change and Relative Liver Weight of Wistar Rats Exposed to Cd Toxicity.

Groups	Percentage Weight Change (%)	Relative Liver Weight (%)
[1] Control	19.80 ± 0.55	3.25 ± 0.09
[2a] Cd	−18.40 ± 0.60[e]	5.10 ± 0.11[e]
[2b] Cd + Recovery	−7.20 ± 0.48[e,a]	4.65 ± 0.20[e]
[3] Cd + 100 mg/kg AEVAL	9.20 ± 0.45[e,a,b]	3.55 ± 0.11[a,b]
[4] Cd + 200 mg/kg AEVAL	13.30 ± 0.50[e,a,b,c]	3.40 ± 0.09[a,b]
[5] Cd + 400 mg/kg AEVAL	4.50 ± 0.38[e,a,b,c,d]	3.75 ± 0.13[a,b]

Each value represents mean ± standard error of mean (p < 0.05).
  [a] Significantly different from Cd group.
  [b] Significantly different from Cd + recovery group.
  [c] Significantly different from Cd + 100 mg/kg AEVAL.
  [d] Significantly different from Cd + 200 mg/kg AEVAL.
  [e] Significantly different from control group.

with the control, but the levels were recorded to be significantly higher than both the toxic and toxic recovery groups (Table 5).

The significantly elevated level of TBARS that accompanied Cd administration was significantly lowered following AEVAL administration. The AEVAL-treated groups 3, 4 and 5 showed significant reductions when compared with both the toxic and toxic recovery groups, but recorded no significant difference when compared with the control (Table 5).

3.4. Effects of AEVAL on plasma AST (U/L), ALT (U/L) and ALP (U/L) levels in wistar rats with Cd-induced liver injury

The plasma levels of AST, ALT and ALP were significantly elevated following Cd exposure. These levels were, however, restored to physiological levels in the AEVAL-treated groups 3, 4 and 5 when compared with the control, toxic and toxic recovery groups (Figs. 2–4).

3.5. Effects of AEVAL on plasma total bilirubin (μmol/L) and hepatic total protein (mg/mL) levels in wistar rats with Cd-induced liver injury

Cadmium administration was associated with significantly elevated level of plasma total bilirubin level. The total bilirubin levels were significantly lowered in the AEVAL-treated groups 3, 4 and 5 when compared with both the toxic and toxic recovery groups with no recorded significant difference when these treated groups were compared with the control group (Fig. 5).

Following exposure to Cd, hepatic total protein level was significantly lowered. AEVAL treatment significantly restored hepatic total protein levels to physiological levels when compared with the control,

Table 5

Effects of AEVAL on Hepatic Indicators of Oxidative Stress and Lipid Peroxidation in Wistar Rats with Cd-Induced Liver Injury.

Groups	Indicators of Oxidative Stress			Indicator of Lipid Peroxidation
	GSH (µg/mg protein)	SOD (mM/mg protein)	CAT (µmol/min/mg protein)	TBARS (nmol/mg protein)
[1] Control	5.68 ± 0.30	1.15 ± 0.08	2.60 ± 0.10	35.65 ± 0.80
[2a] Cd	1.07 ± 0.25[c]	0.32 ± 0.07[c]	1.08 ± 0.09[c]	84.90 ± 1.20[c]
[2b] Cd + Recovery	2.00 ± 0.29[c]	0.66 ± 0.07[c,a]	1.49 ± 0.09[c,a]	62.25 ± 1.10[c,a]
[3] Cd + 100 mg/kg AEVAL	4.87 ± 0.18[a,b]	1.01 ± 0.06[a,b]	2.37 ± 0.07[a,b]	36.55 ± 1.00[a,b]
[4] Cd + 200 mg/kg AEVAL	5.35 ± 0.28[a,b]	1.07 ± 0.05[a,b]	2.43 ± 0.10[a,b]	32.33 ± 0.77[a,b]
[5] Cd + 400 mg/kg AEVAL	4.60 ± 0.35[a,b]	0.98 ± 0.04[a,b]	2.20 ± 0.10[a,b]	38.63 ± 1.10[a,b,d]

Each value represents mean ± standard error of mean ($p < 0.05$).

[a] Significantly different from Cd group.

[b] Significantly different from Cd + recovery group.

[c] Significantly different from control group.

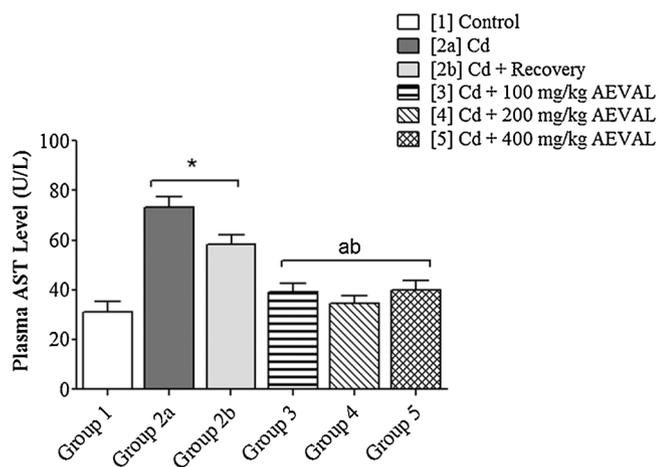[d] Significantly different from Cd + 200 mg/kg AEVAL.

Fig. 2. Graph Showing the Effects of AEVAL on Plasma AST Level of Wistar Rats with Cd-induced Liver Injury.

Each bar represents mean ± standard error of mean ($p < 0.05$).

* = significantly different from control group;

a = significantly different from Cd group; and

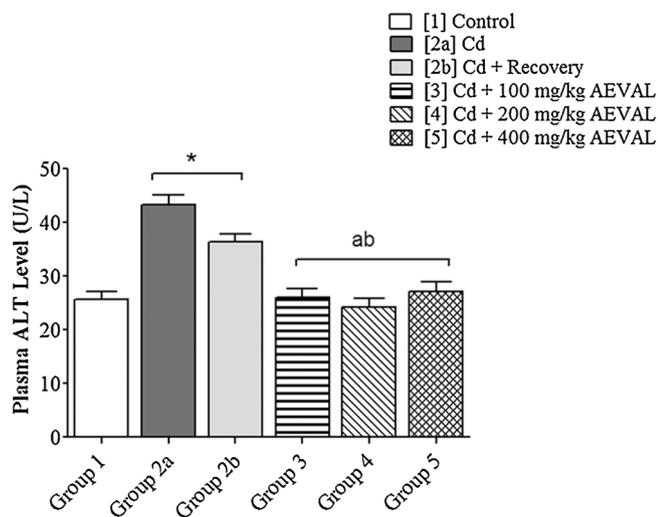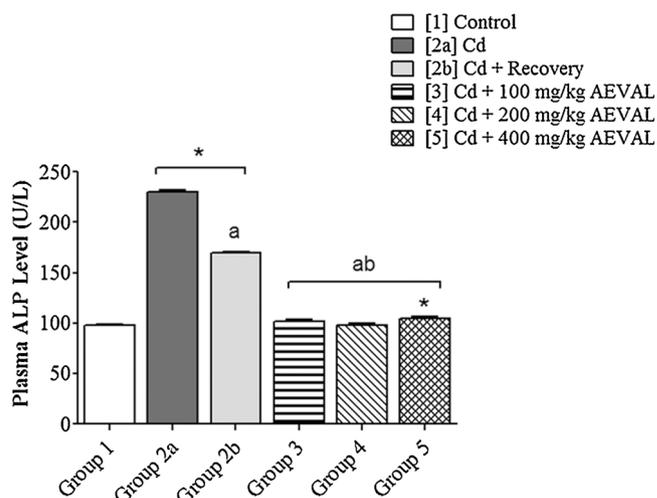b = significantly different from Cd + recovery group.

Fig. 4. Graph Showing the Effects of AEVAL on Plasma ALP Level of Wistar Rats with Cd-induced Liver Injury.

Each bar represents mean ± standard error of mean ($p < 0.05$).

* = significantly different from control group;

a = significantly different from Cd group; and

b = significantly different from Cd + recovery group.

Fig. 3. Graph Showing the Effects of AEVAL on Plasma ALT Level of Wistar Rats with Cd-induced Liver Injury.

Each bar represents mean ± standard error of mean ($p < 0.05$).

* = significantly different from control group;

a = significantly different from Cd group; and

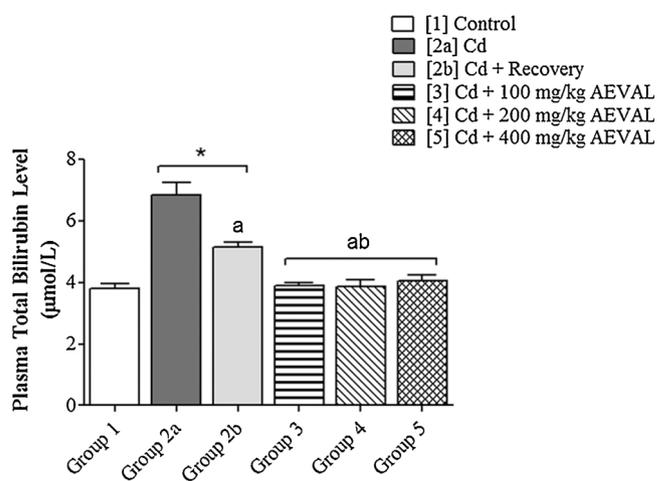b = significantly different from Cd + recovery group.

Fig. 5. Graph Showing the Effects of AEVAL on Plasma Total Bilirubin Level of Wistar Rats with Cd-induced Liver Injury.

Each bar represents mean ± standard error of mean ($p < 0.05$).

* = significantly different from control group;

a = significantly different from Cd group; and
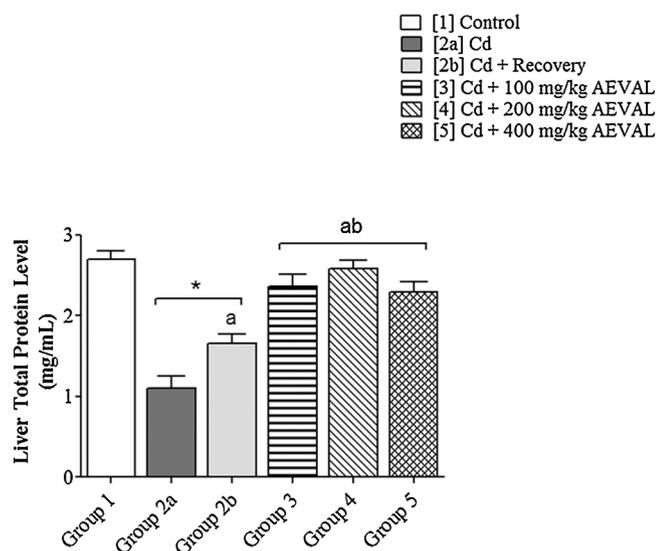
b = significantly different from Cd + recovery group.

Fig. 6. Graph Showing the Effects of AEVAL on Liver Total Protein Level of Wistar Rats with Cd-induced Liver Injury.

Each bar represents mean ± standard error of mean (p < 0.05).

* = significantly different from control group;

a = significantly different from Cd group; and

b = significantly different from Cd + recovery group.

toxic and toxic recovery groups (Fig. 6).

3.6. Histological effects of AEVAL on the liver of Wistar rats exposed to Cd toxicity

The photomicrograph of the control group [Plate 1] showed evidence of apparently normal liver histoarchitecture that was characterised by intact lamellar pattern of the hepatocytes and Kupffer cells. However, the toxic group [Plate 2a] showed evidence of hepatocyte and kupffer cell degeneration with peri-portal infiltration by inflammatory cells. The abnormal morphological disruption was also characterised by well disseminated focal areas of steatosis. The toxic recovery group [Plate 2b] also showed evidence of steatosis with aggregate of inflammatory cells. The AEVAL-treated groups [Plates 3, 4 and 5] showed evidence of ameliorative effects against the Cd-induced disruption in liver histoarchitecture. This was characterised by a milder disseminated steatosis, fewer distribution of inflammatory cells and apparent restoration of the lamellar pattern of both hepatocytes and Kupffer cells when compared with Plates 2a and 2b (Fig. 7).

4. Discussion

This study investigated the hepatic effects of four weeks oral administration of acetonic extract of *Vernonia amygdalina* (Del.) leaf (AEVAL) in a Wistar rat model of cadmium-induced liver injury. Based on the indices that were assayed, the overall finding of this study showed that AEVAL administration at graded doses restored homeostasis of the antioxidant system as well as liver function biomarkers. However, these effects were not dose-dependent; the least effective dose being the highest dose of the extract (400 mg/kg). Also, administration of the extract was associated with improved liver histoarchitecture following Cd-induced injury.

The associated decrease in percentage weight change (PWC) that followed exposure to Cd is consistent with the findings of Merali and Singhal [57]. By way of physical examination, the rats became lethargic and ate less after exposure to Cd toxicity. Consequently, their feed was left, almost, untouched. Therefore, the reduction in body weight can be attributed to the reduction in food consumption since weight gain or loss is determined by a balance between dietary intake and energy

expenditure [58]. Furthermore, the hypothalamus is known to be the "key controller" for the maintenance of energy homeostasis within neural circuitry [58]. Cadmium has been reported in literature to bioaccumulate in the brain by easily crossing the blood-brain barrier [59,60] and exerting undesirable effects on the hypothalamus [60,61]. This study suggests that Cd administration is associated with the suppression or inhibition of hypothalamic centre for food consumption and energy expenditure. This also explains the decreased desire for food consumption with a corresponding decrease in body weight. Following exposure to Cd toxicity, the administration of the extract may have modulated the hypothalamic centre for food consumption to bring about normal homeostasis in feeding pattern with a consequent increase in body weight. This study, therefore, suggests a possible appetite-stimulating effect of the extract; demonstrating its potential as an adjuvant Cd therapy.

A significant difference in the organ weight of experimental animals without any apparent morphological changes can be a sensitive indicator of the effect of a chemical agent [62]. However, in order to avoid any complication that may arise as a result of differences in the body weight between experimental groups, the ratio of body weight to organ weight (generally described as relative organ weight) is commonly used for analysis [62]. The observed Cd-induced increase in relative liver weight (RLW) may have resulted from inflammatory response by the liver. Micrographic evidence of the toxic group showed features of inflammation with well disseminated focal areas of steatosis. Although the liver is reputed to have a self-regenerating potential (as supported by the finding of this study), self-restoration to its physiological size was impossible without the intervention of a potent agent (like AEVAL). Tannins and flavonoids, important phytochemicals of the extract, are reputed for their anti-inflammatory and anti-oxidant properties [63–65]. Apparently, this study demonstrated an anti-inflammatory potential of the extract as micrographic evidence of the AEVAL-treated groups showed features of milder distribution of inflammatory cells, scanty disseminated steatosis and an apparent restoration of the lamellar pattern of hepatocyte when compared with that of the toxic and toxic recovery groups. This explains the resulting restorative effect of the extract against Cd-induced increase in relative liver weight. The finding of this study, therefore, suggests that AEVAL potentiates restoration of relative liver weight to physiological levels through anti-oxidant and anti-inflammatory mechanisms.

While GSH is a non-enzymatic index of oxidative stress [50,51,66], both SOD [67] and CAT [68,69] are important enzymatic indices of oxidative stress. On the other hand, TBARS is an important index of lipid peroxidation [35,50,51,65]. Increased activity of TBARS is in direct proportion to the increasing degree of injury in biological tissues; hence, it is an index of lipid peroxidation [35,50]. The deleterious reduction in the activities of GSH, SOD and CAT in the liver following exposure to Cd toxicity was an indication of oxidative stress due to generation of free radicals. This can be attributed to an increased use of these enzymatic and non-enzymatic biomarkers by the liver to scavenge free radicals in an attempt to restore homeostasis of the antioxidant system and (or) reduced ability of the liver to sustain these lines of defence following ROS generation due to Cd toxicity. These culminated in increased TBARS level which was reflective of a high degree of Cd-induced liver injury. The AEVAL-treated groups demonstrated attenuating effects against the Cd-induced oxidative stress and lipid peroxidation. This shows that the plant is a good source of potent antioxidant activities; a finding that supports existing literatures [35–37,50]. It therefore implies that AEVAL restores homeostasis of the antioxidant system by conjugating and excreting toxic cellular molecules, detoxification of ROS, sustenance of cellular integrity as well as a possible enhancement of tissue regeneration (as depicted by the micrographic evidences). This scientific assertion was further buttressed with the fact that the group that was left to self-recover could not reverse the alterations in the antioxidant system to physiological levels, although some measures of recovery was recorded. These pharmacological
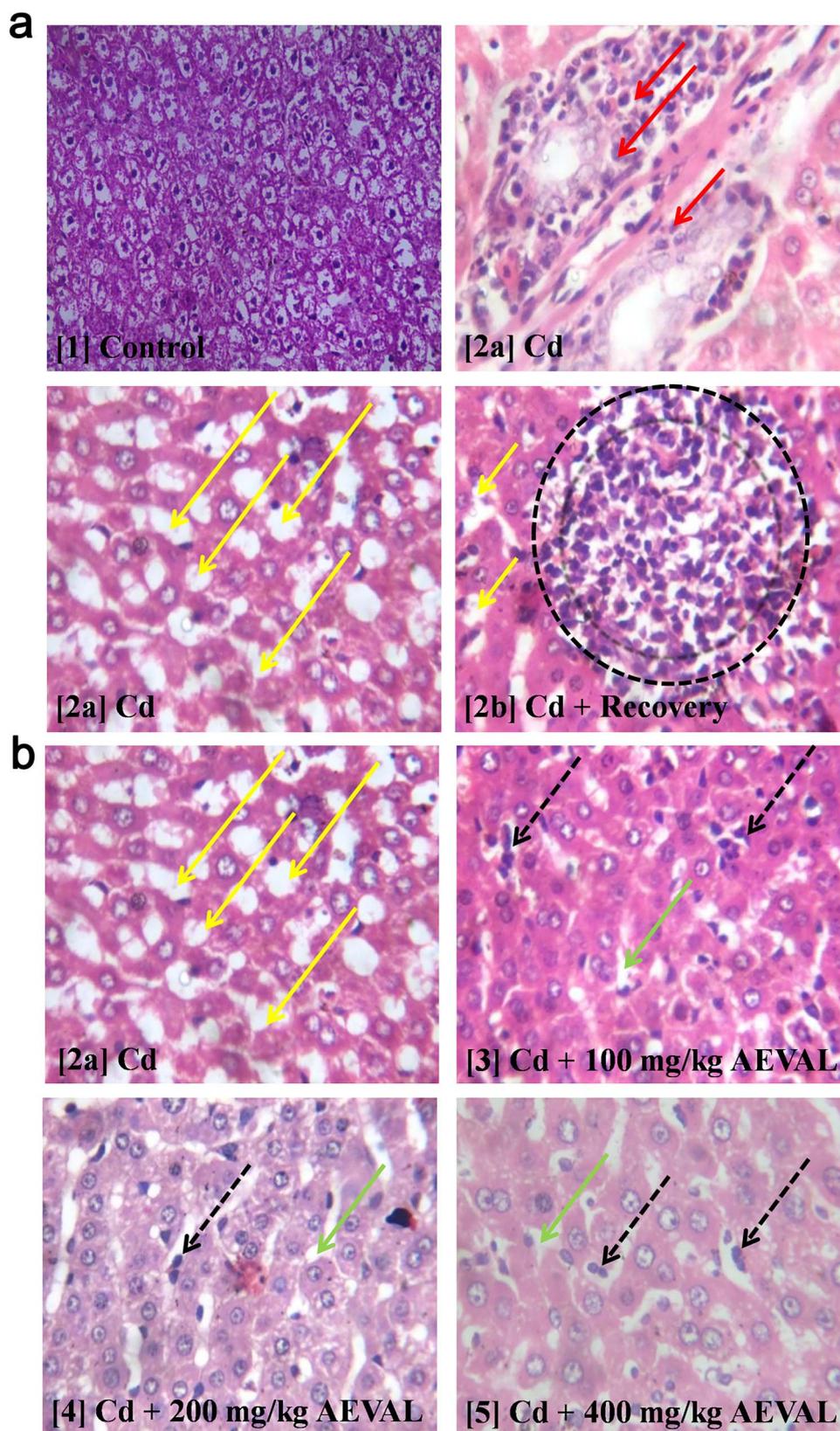
Fig. 7. Photomicrographs Showing the Histological Effects of AEVAL on the Liver of Wistar Rats Exposed to Cd Toxicity.
Red arrow = peri-portal infiltration by inflammatory cells; **Yellow arrow** = focal area of steatosis; **Black dotted circle** = aggregate of inflammatory cells; **Black broken arrow** = mild distribution of inflammatory cells; **Green arrow** = mild disseminated steatosis.

activities of the extract can be attributed to the presence of mostly flavonoids, tannins and alkaloids (reputed for their anti-oxidant and anti-inflammatory properties). The potent anti-oxidant and anti-

inflammatory capacities of the extract may be the basis for its pharmacological activities since increased ROS generation and inducing cellular inflammation are the basic biological mechanisms of Cd-

induced cellular injury.

The assay for the activities of blood levels of AST, ALT and ALP as important biomarkers of liver function is of clinical relevance [70–72]. These enzymes are located in the liver cells and are released into the plasma in response to liver cell injury or damage. Following Cd administration, the activities of these liver enzymes were significantly elevated beyond physiological levels. AST and ALT enzymes are greatly concentrated in the liver [73]. Whereas AST is both mitochondrial (about 80% of total activity) and cytosolic (about 20% of total activity) in location, ALT is solely cytoplasmic [73]. Therefore, the expressed potential of the extract to attenuate the Cd-induced derangements in both AST and ALT activities is suggestive of its cytoplasmic, cotosolic and mitochondrial effects to bring about the prevention of membrane fragility and reduced leakage of hepatic enzymes into the blood. Since ALP is a cholestatic index [73,74], the AEVAL-enhanced attenuating effects against elevated ALP levels can be attributed to the extract's potential to reverse cholestatic mechanism(s). This is because cholestasis (obstruction of bile flow) enhances both synthesis and release of hepatic ALP from cell surfaces [73,74].

Total bilirubin is used as an index of bile duct lesion [75] or hepatic injury due to bile duct obstruction [76]. This study recorded an elevated level of plasma total bilirubin after exposure to Cd toxicity. The apparent normalization of the total bilirubin level that was associated with AEVAL treatment can be attributed to the potential of the extract to ameliorate bile duct obstruction or lesions, possibly, via its antioxidant and tissue regeneration mechanisms; a potential that may have been enhanced by the synergetic effects of its important phytochemical constituents.

A contributory self-healing mechanism to liver regeneration process is the stimulation of protein synthesis [77]. A chemical agent can induce adverse changes in the process of protein synthesis; hence, the level of hepatic total protein content can be an important index for the determination of chemically-induced liver injury or dysfunction [74]. Although the self-recovery group showed increased level of total protein level when compared with the toxic group, this level was not within the physiological range. The extract's potential to restore physiological levels of liver total protein is indicative of its tissue-regenerative ability (as supported by their micrographic evidences); an ability that was least expressed in the group that received the highest dose of the extract.

Worthy of note is the fact that the attenuating effects of the extract were not dose-dependent. Almost all the measured parameters showed least therapeutic effects of the extract at the highest dose (400 mg/kg). A note of caution should, therefore, be taken during administration of the extract or during a further experimental evaluation of the acetonic extract of this plant as this study indicates a possible high risk profile of liver dysfunction at high doses. A limitation of this study, worthy of further investigation, is the assessment of blood level of Cd during/after treatment with an intervention; since this heavy metal does not undergo metabolic degradation to less toxic metabolite. Another possible limitation of this study is the low number of rats that was used in appraising the extract's therapeutic effects. It is not unlikely to pool larger data which may provide more information about the extract's therapeutic effects if a larger number of rats were recruited for the study. In addition, this study recommends that cadmium-exposed subject should resort to prompt and efficacious treatment/management therapy. This is because the group that were left untreated (self-recovery), although showed some measure of regenerative and self-healing attributes, did not restore the biomarkers of liver function as well as indicators of oxidative stress to physiological levels.

5. Conclusion

It was concluded that acetonic extract of *Vernonia amygdalina* (Del.) leaf attenuates cadmium-induced liver injury through antioxidant, anti-inflammatory, membrane-stabilizing as well as tissue-regenerating

mechanisms. The pharmacological activities of the extract, attributed to its important phytochemical constituents, potentially make it a suitable option in adjuvant heavy metal therapy.

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