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# *Vernonia amygdalina* leaf extract protects against carbon tetrachloride-induced hepatotoxicity and nephrotoxicity: a possible potential in the management of liver and kidney diseases

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## ABSTRACT

The rising prevalence of liver and kidney diseases is worrisome and constitutes a major threat to public health. The present study investigates the medicinal potentials of *Vernonia amygdalina* leaves in the management of liver and kidney diseases. Albino rats were randomly divided into five groups each containing 5 animals. In all groups, except group I (control), animals were exposed to 3 ml/kg bw of $CCl_4$ and then administered further different treatments. Groups III, IV, and V each were treated with 50 mg/kg bw, 100 mg/kg bw of bitter leaf extract, and 100 mg/kg bw of silymarin, respectively. Group II animals were left untreated after exposure to toxicant. Activities of creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), as well as the level of urea, uric acid, and bilirubin were determined in the serum and tissue homogenates. Lipid profile as well as activities of superoxide dismutase (SOD) and catalase (CAT) were also determined. Exposure to $CCl_4$ resulted in a significant increase in CK, AST, ALP, and ALT levels as well as bilirubin, urea, and uric acid when compared to the control. Lipid profile was disrupted, activities of SOD and CAT were markedly inhibited and the level of GSH was significantly depleted. However, treatment with *V. amygdalina* reversed the toxic trend in a dose-dependent manner comparable to animals treated with silymarin. In conclusion, *V. amygdalina* leaf extract restored deranged lipid profile, distorted histoarchitecture as well as liver and kidney function markers. Hence, the plant is a potential candidate for the management of liver and kidney diseases.

## 1. Introduction

Since antiquity, nature has provided man with basic requirements such as healthcare, food, shelter as well as other necessities of human existence. Globally, medicinal plants occupy a central space and have dominated the healthcare system of developing countries where a very large percentage of the population depends on plants for therapy. As it stands, several developed and well-industrialized nations of the world are fast embracing the use of herbal extracts as complimentary therapy (Gurib-Fakim, 2006). Medicinal plants are not just important for healthcare but as an assured hope for the development of future medicines. At the moment, only about one-third of human diseases have readily available and efficacious therapies. There is, therefore, a dire need for medicinal plants with proven potency and safety

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that can serve as panacea to the menace of several diseases (Hamburger & Hostettmann, 1991). The World Health Organization (WHO) has endorsed and promoted the inclusion of herbal medicines in national healthcare programs due to ease of access, affordability, and safety (Singh & Singhi, 1981). Consequently, collaborative research efforts in the area of screening of plant extracts has led to the identification of pharmacologically active agents that can be exploited as drugs for the management of diseases (Rastogi & Meharotra, 1990).

Chronic liver disease (CLD) constitutes a major threat to global public health, causing increased morbidity and mortality worldwide (Marcellin & Kutala, 2018). Between 1980 and 2010, mortality as a result of liver disease increased by 46% globally (Mokdad et al., 2014) with higher prevalence in developing nations including Africa (Stepanova et al., 2017). Similarly, the global disease burden report in 2010 ranked chronic kidney disease as 27$^{th}$ on the list of causes of global death in 1990. However, in 2010, chronic kidney disease rose to the 18$^{th}$ position on the list (Stepanova et al., 2017). This rise in burden was second to HIV/AIDS. According to that report, the overall premature mortality was estimated at 82% which was the third largest coming after diabetes (93%) and HIV/AIDS (96%). Considering these data on various diseases that threaten global health as well as the sustainable development goal (Levin et al., 2017), there is a dire need to leverage on potent medicinal plants that could serve as a complimentary therapy to stem down the tide of these diseases.

*Vernonia amygdalina* (Del.) is a leafy vegetable widely noted for its bitter taste, hence it's popularly called 'bitter leaf'. Its leaves are normally employed in cooking delicious vegetable soup usually recommended for treating several ailments. *V. amygdalina* has been widely studied due to its versatile medicinal relevance such as in the treatment of diabetes, hypertension, and infertility (Kassebaum et al., 2016). Recent scientific evidence has given credence to its numerous medicinal benefits such as anti-obesity (Farombi & Owoeye, 2011), antioxidant (Adesanoye & Farombi, 2010), anticarcinogenic (Wong et al., 2013), antihyperglycemic (Jan Mohamed et al., 2015), anti-sickling (Clement et al., 2014), and in the management of cardiovascular disorders (Abdulmalik et al., 2016). Reports have indicated that leaf extract of *V. amygdalina* protects against brain degeneration thereby enhancing memory (Ebuehi & Ajagun-Ogunleye, 2017). Besides, extract of *V. amygdalina* leaf exhibits anti-helminthic, antiparasitic, and antimicrobial properties (Anibijuwon et al., 2012). In view of the rising burden of liver and kidney diseases, it is clear that there is a dire need to investigate the potential of *V. amygdalina* extract in the management of these diseases, that is, the motivating reason for this study.

## 2. Materials and methods

### 2.1. Plant materials

*V. amygdalina* leaves were obtained from a private farm in Ado Ekiti. The leaves were air dried, pulverized, and stored in an airtight container. Ethical approval for the study was obtained from the Office of Research and Development, Ekiti State University, Ado Ekiti.

### 2.2. Reagents and chemicals

All biochemical kits were of analytical grade and obtained from Randox laboratories, UK.

### 2.3. Extraction procedure

Bitter leaves were air-dried at room temperature and pulverized to obtain fine powder using a blender. 500 g of each of the powdered leaves of *V. amygdalina* was soaked in 5000 ml of distilled water for 72 hours to allow for extraction. It was then filtered using a cheesecloth, and freeze-dried to obtain the dried extract. The extract was kept in a closed container and kept inside the fridge at 4 °C for further studies.

### 2.4. Animals protocol

Twenty-five (25) male Wistar albino rats weighing 180–200 g were acclimatized for two weeks, housed in clean wire meshed cages under standard conditions (24 ± 1 °C), relative humidity, and 12/12-hour light and dark cycle. They were allowed to have free access (ad libitum) to food (commercial palletized diet from Vital Feed Mill) and drinking water daily. The rat beddings were changed and replaced every day throughout the experimental period.

#### 2.4.1. Animal grouping and treatment

Experimental animals were administered different treatments as shown in Table 1.

Table 1. Animal treatment

| Groups | Treatment |
|---|---|
| I | Drinking water only for 14 days |
| II | Single intraperitoneal injection of 3 ml $CCl_4$/kg body weight |
| III | 3 ml $CCl_4$ + 50 mg/kg *V. amygdalina* extract by oral gavage for 14 days |
| IV | 3 ml $CCl_4$ + 100 mg/kg *V. amygdalina* extract by oral gavage for 14 days |
| V | 3 ml $CCl_4$ + 100 mg/kg silymarin by oral gavage for 14 days |

### 2.5. Dissection of rats

The animals were decapitated under very light anesthesia to obtain the liver, kidney, and heart, while whole blood, collected by cardiac puncture, was allowed to stand for 1 hour in an EDTA tube. Serum was prepared by centrifugation at 3000 rpm for 15 min at 25 °C. The clear supernatant was collected and used for the estimation of serum biochemical parameters.

### 2.6. Preparation of homogenates

The liver, heart, and kidney were excised using scissors and forceps. They were trimmed of fatty tissue, washed in distilled water, blotted with filter paper, and weighed. They were then chopped into bits and homogenized in ten volumes of the homogenizing phosphate buffer (pH 7.4) using a Teflon homogenizer. The resulting homogenates were centrifuged at 3000 rpm at 4 °C for 30 mins. The supernatant obtained was collected and stored under 4 °C and then used for biochemical analyses.

### 2.7. Determination of serum creatine kinase (Ck-Mb) activity

Creatine kinase was measured by the method of Vanderlinde (1981). One thousand microliters of imidazole buffer (10 mM, pH 6.6), containing creatine phosphate (30 mM), glucose (20 mM), N-acetyl-cysteine (20 mM), magnesium acetate (10 mM), ethylenediaminetetraacetic acid (2 mM), ADP (2 mM), NADP (2 mM), AMP (5 mM), DAPP (10 µM), G6PDH (≥ 2.0 ku/l) and HK (≥ 2.15 ku/l) was incubated in a thermostatic cuvette at 37 °C after the addition of 50 µl of serum. Absorbance at 340 nm of the resulting mixture was read immediately for 5 min at 30-sec intervals. Change in absorbance per minute was estimated while enzyme activity was determined using the formula given below:

$$CK - Mb\ Activity\ (IU/l) = (\Delta Abs/min)\ \times\ 6667$$

### 2.8. Assay of aspartate aminotransferase (AST) activity

AST activity was determined following the principle described by Reitman & Frankel (1957). Briefly, 0.1 ml of organs' homogenates as well as the serum was mixed separately with phosphate buffer (100 mmol/l, pH 7.4), L-aspartate (100 mmol/l), and α-oxoglutarate (2 mmol/l), and the mixture incubated for exactly 30 min at 37 °C. Five hundred microliters of 2,4-dinitrophenyl hydrazine (2 mmol/l) were added to the reaction mixture and allowed to stand for exactly 20 min at 25 °C. Five milliliters of NaOH (0.4 mol/l) were then added and the absorbance of the mixture was read after 5 min at 546 nm against the reagent blank.

### 2.9. Assay of alanine aminotransferase (ALT) activity

The principle described by Reitman & Frankel (1957) was followed in the assay of ALT using a commercially available assay kit (Randox Laboratories, UK) according to the instructions of the manufacturer. Five hundred microliter of reagent I (R1) containing phosphate buffer (100 mmol/l, pH 7.4), L-alanine (200 mmol/l), and α-oxoglutarate (2 mol/l) was added to 0.1 ml of serum in a test tube and the mixture was incubated at 37 °C for 30 min. Exactly 0.5 ml of R2 containing 2,4-dinitrophenylhydrazine (2.0 mmol/l) was added and the solution was incubated again at 20 °C for 20 min. Finally, 5 ml of NaOH was added and the solution was allowed to stand for 5 min at 25 °C and the absorbance was read at 546 nm. The activity of ALT was obtained from the standard curve provided in the kit.

### 2.10. Assay of alkaline phosphatase (ALP) activity

The assay of serum ALP was based on the method of Englehardt (1970) using commercial assay kits (Randox Laboratories, UK) according to the instructions of the manufacturer. Exactly, 1.0 ml of the reagent (1 mol/l diethanolamine buffer pH 9.8, 0.5 mmol/l $MgCl_2$; substrate: 10 mmol/l *p*-nitrophenol phosphate) was added to 0.02 ml of the serum sample and mixed. The absorbance was taken at 405 nm for 3 minutes at intervals of 1 min. ALP activity was determined using the formula given below:

$$CALP\ activity\ (U/l) = 2760\ x\ \Delta Abs/min$$

### 2.11. Serum lipid profile

#### 2.11.1. Estimation of total cholesterol level

The total cholesterol level was determined based on the method of Trinder (1969) using commercially available kits (Randox Laboratories, UK). Ten microliters (10 µl) of standard and 10 µl serum samples were measured into labeled test tubes. One milliliter (1 ml) of working reagent containing; Pipes buffer (80 mmol/l at pH 6.8), 4-amino antipyrine (0.25 mmol/l), phenol (6 mmol/l), peroxidase (≥ 0.5 U/ml), cholesterol esterase ion (≥ 0.15 U/ml), and cholesterol oxidase (0.10 U/ml) was added into all the tubes. The test tubes were mixed thoroughly and incubated for 10 min at 25 °C. The absorbance of the sample (Asample) was read at 500 nm against the reagent blank. Cholesterol concentration (mg/dl) was calculated using the formula given below:

$$Total\ Cholesterol\ \left(\frac{mg}{dl}\right) = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} x\ Concentration\ of\ standard$$

#### 2.11.2. Evaluation of concentration of triglyceride

Triglyceride levels were determined based on the method of Tietz (1995) using commercially available kits (Randox Laboratories, UK). Triglyceride standard (10 µl) and serum (10 µl) were measured into labeled test tubes. One milliliter of the working reagents; R1a (buffer) containing Pipes buffer (40 mmol/l, pH 7.6), 4-chloro-phenol (5.5 mmol/l), magnesium-ion (17.5 mmol/l); R1b (enzyme reagent containing 4-amino phenazone (0.5 mmol/l), ATP (1.0 mmol/l), lipase (≥ 150 U/ml), glycerol-kinase (≥ 0.4U/ml), glycerol-3-phosphate oxidase (≥ 1.5 U/ml), and peroxidase (≥ 0.5 U/ml) was added into all the tubes. The test tubes were mixed thoroughly and incubated for 10 min at room temperature. Absorbance was taken at 546 nm against the blank. Triglyceride concentration (mg/dl) was calculated using the formula given below:

$$Triglyceride\ concentration\ \left(\frac{mg}{dl}\right) = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} x\ Concentration\ of\ standard$$

#### 2.11.3. High-density lipoprotein-cholesterol (HDL-c) assay

High-density lipoprotein cholesterol was determined by the method of Grove (1979) in two stages:

*I. Precipitation*

A reaction mixture containing 200 µl of the serum, 200 µl of the cholesterol standard, and 500 µl of the diluted precipitant R1 (0.55 mM phosphotungstic acid, 25 mM magnesium chloride) was mixed and allowed to stand for 10 min at room temperature. It was then centrifuged for 10 min at 4000 rpm to obtain a clear supernatant. The clear supernatant was separated off within 2 h and the cholesterol content was determined by the CHOD-PAP reaction method.

*II. Cholesterol CHOD-PAP Assay*

One milliliter of cholesterol reagent was added to 100 µl of the sample supernatant in a test tube. The standard test tube contained 100 µl of the cholesterol standard supernatant and 1 ml of cholesterol reagent. The reagent mixture was mixed thoroughly and incubated for 10 min at 25 °C. The absorbance of the sample (A sample) and standard (Astandard) was then measured at 500 nm against the reagent blank within 1 h.

#### 2.11.4. Low-density lipoprotein (LDL)-cholesterol determination

The concentration of low-density lipoprotein in the serum was calculated using the formula of Friedewald et al. (1972) given below:

$$LDL - Cholesterol\ \left(\frac{mg}{dl}\right) = \text{Total cholesterol} - \frac{Triglycerides}{5} - HDL - Cholesterol$$

### 2.12. Antioxidant assay

#### 2.12.1. Determination of catalase activity

This experiment was carried out according to the method described by Sinha (1972). Two hundred microliters of serum and organs' homogenates were mixed separately with 0.8 ml of distilled water to give 1 in 5 dilutions of the sample. The assay mixture contained 2 ml of solution (800 µmol) and 2.5 ml of phosphate buffer in a 10 ml flat bottom flask. Properly diluted enzyme preparation (0.5 ml) was rapidly mixed with the reaction mixture by a gentle swirling motion. The reaction was run at room temperature. 1 ml portion of the reaction mixture was withdrawn and blown into 1 ml dichromate/acetic acid reagent at 60 seconds intervals. The hydrogen peroxide content of the withdrawn sample was determined by the method described below:

$$Catalase\ activity = [H_2O_2]\ Consumed = 800 - [H_2O_2]\ left$$

The concentration of $H_2O_2$ left was extrapolated from the standard curve for catalase activity.

### 2.12.2. Determination of superoxide dismutase (SOD) activity

The level of SOD activity was determined by the method of Misra & Fridovich (1972). A ten-fold dilution of the sample was prepared. An aliquot of the diluted sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in a spectrophotometer. The reaction was initiated by the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer, 0.3 ml of the substrate (adrenaline), and 0.2 ml of water. The increase in absorbance at 480 nm was monitored every 30 seconds for a total period of 150 seconds.

### 2.12.3. Determination of reduced glutathione level (GSH)

The method of Beutler (1963) was followed in estimating the level of reduced glutathione (GSH). Exactly 0.2 ml of supernatant was added to 1.8 ml of distilled water followed by the addition of 3 ml of the precipitating solution and then shaken thoroughly. The mixture was then allowed to stand for 5 min and then filtered. One milliliter of the filtrate was mixed with 4 ml of 0.1 M phosphate buffer at pH 7.4. Finally, 0.5 ml of the Ellman reagent was added. A blank was prepared with 4 ml of the 0.1 M phosphate buffer, 1 ml of diluted precipitating solution (3 parts to 2 parts of distilled water), and 0.5 ml of the Ellman reagent. The absorbance was measured at 412 nm against a reagent blank. The level of GSH in the serum was calculated from the standard curve.

### 2.12.4. Determination of total protein (TP) in serum

The Biuret method described by Weichselbaum (1946) was employed in the determination of total protein in the serum using commercially available kits (Randox Laboratories, UK). One milliliter of Reagent R1 containing sodium hydroxide (100 mmol/l), Na-K-tartrate (18 mmol/l), potassium iodide (15 mmol/l), and cupric sulfate (6 mmol/l) was added to 0.02 ml of the serum sample. The reaction mixture was incubated at 25 °C and absorbance was measured against the reagent blank at 546 nm. Total protein concentration (mg/ml) was calculated using the formula given below:

$$Total\ Protein\ Concentration\ \left(\frac{mg}{ml}\right) = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard}\ x\ Concentration\ of\ standard$$

### 2.13. Statistical analysis

All values were expressed as mean ± SD. Statistical evaluation was done using One Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) by using SPSS 11.09 for Windows. The significance level was set at $p < 0.05$.

## 3. Results and discussion

Table 1 shows the treatment protocol adopted for the experimental animals. Animals in group II were exposed to 3ml/kg bw of $CCl_4$ but not treated with the extracts, while the animals in groups III, IV, and V were exposed to 3ml/kg bw of $CCl_4$ and treated with 50, 100 mg/kg bw of *V. amygdalina* leaf extract and 100 mg/kg bw of silymarin, respectively. Group I animals received distilled water only and were not exposed to the toxicant at all. Exposure to $CCl_4$ caused a marked derangement in lipid profile (cholesterol, triglyceride, HDL-c, and LDL-c) regardless of the organ involved (Table 2A-D). Treatment with *V. amygdalina* resulted in the restoration of the lipid profile in a dose-dependent fashion comparable to animals treated with silymarin. Serum levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), as well as bilirubin, were significantly raised relative to control animals (Table 3A-D). However, treatment of intoxicated animals with graded doses of *V. amygdalina* led to a restoration of the marker enzymes in a manner comparable with animals treated with silymarin (Table 3A-D). Urea, and uric acid as well as total bilirubin were elevated following exposure to $CCl_4$ (Table 3A-D). Administration of the leaf extract of *V. amygdalina* reversed the trend to a level comparable with animals that were not exposed to the toxicant (Table 3A-D). The activity of superoxide dismutase, catalase, and creatine kinase was significantly depleted when animals were exposed to $CCl_4$ but activity was restored to levels comparable with animals treated with silymarin following treatment with leaf extract of *V. amygdalina* (Table 4A-D). Reduced glutathione (GSH) was markedly decreased in the serum of animals exposed to $CCl_4$. However, GSH level was restored in the exposed animals following treatment with graded doses of *V. amygdalina* leaves extract. Administration of $CCl_4$ caused a marked distortion in hepatic, renal, and cardiac histoarchitecture but the distortion was reversed by treatment with *V. amygdalina* leaves extract (Table 4A-D). Photomicrograph of liver tissue of experimental animals under different treatments is as shown in Figure 1A-D.

The ever-increasing global burden of liver and kidney diseases calls for concerted efforts at stemming the tide which may threaten public health if left unchecked. Attainment of the sustainable development goal (SDG) on health requires not only conventional drugs but the complementary effort of herbal medicines. Animal models of liver and kidney diseases have been routinely used to assess the therapeutic potentials of medicinal plants in managing such diseases. Derangement of lipid profile is a potent toxicity mechanism of several toxicants. Hyperlipidemia has been identified as a major culprit in the onset and progression of cardiovascular diseases (Ugwu Okechukwu et al., 2013). Routinely, notable signs of toxicity include high cholesterol, high triglycerides, high LDL, and depleted HDL in exposed animals (Khafar & Kakey, 2020). In the present study, exposure of experimental animals to carbon tetrachloride ($CCl_4$) (Table 1) caused a marked derangement in the lipid profile regardless of the organ involved (Table 2A-D). Specifically, there was a surge in cholesterol levels in the liver, kidney heart, and serum following exposure to $CCl_4$. This observation implies that $CCl_4$ triggered a derangement in critical membrane lipids leading to a compromise in membrane function. However, treatment with *V. amygdalina* leaf extract restored the cholesterol level to a level comparable with animals treated with silymarin. Undoubtedly, such an effect could be linked to the flavonoids and other polyphenols present in the extract. Triglyceride levels in the serum and organs' homogenates were significantly increased following exposure of experimental animals to $CCl_4$ (Table 2A-D). However, treatment with *V. amygdalina* leaf extract reversed the trend in a manner that was dose-dependent and comparable to animals treated with silymarin. This further suggests the potential of the plant as an efficacious alternative in the management of multiple organ diseases. The level of high-density lipoprotein has been used to predict the antioxidant status and overall well-being of the animals. In the present study, $CCl_4$ exposure caused a marked depletion in the level of HDL in the serum, liver, kidney, and heart homogenates of experimental animals. This observation points to the free radical-induced depletion of antioxidants in the animals. Administration of *V. amygdalina* extract relieved the oxidative stress on the organs as the level of HDL was restored to normal. Detailed phytochemical contents of the leaf extract of *V. amygdalina* have been reported. The relief experienced by experimental animals following treatment with the plant extract can be attributed to the flavonoid and polyphenolic content of the plant. On the other hand,

low-density lipoprotein (LDL) otherwise called 'bad cholesterol' was increased following exposure to $CCl_4$. Administration of *V. amygdalina* extract brought the LDL level back to a basal level comparable to silymarin. Invariably, the surge in LDL was due to increased free radicals triggered by the toxicant. Treatment of intoxicated animals with *V. amygdalina* extract relieved the toxicity imposed by $CCl_4$, further buttressing its potential in the management of diseases related to these organs. Treatment of $CCl_4$ exposed animals with *V. amygdalina* leaves resulted in a dose-dependent reversal of total cholesterol, triglycerides, and LDL-cholesterol in a manner comparable to the negative control and animals treated with the standard drug (Table 2A-D). This may be due to the presence of bioactive ingredients such as flavonoids in the extract.

Table 2. Effect of *V. amygdalina* leaf extract on lipid profile in the serum, liver, kidney, and heart of $CCl_4$-exposed rat

A. Serum

| Parameter | I | II | III | IV | V |
|---|---|---|---|---|---|
| Total Cholesterol (mg/dl) | 73.28 ± 1.84[a] | 140.21 ± 0.00[b] | 79.61 ± 0.25[a] | 69.17 ± 0.31[a] | 77.76 ± 0.96[a] |
| Triglyceride (mg/dl) | 23.05 ± 1.30[a] | 46.22 ± 1.18[b] | 27.17 ± 1.39[a] | 26.25 ± 0.11[a] | 25.35 ± 0.81[a] |
| HDL (mg/dl) | 15.91 ± 0.68[a] | 10.10 ± 0.04[b] | 10.38 ± 0.20[b] | 11.87 ± 0.05[a] | 13.35 ± 0.28[a] |
| LDL (mg/dl) | 54.10 ± 4.53[a] | 120.87 ± 0.46[b] | 13.74 ± 0.31[a] | 12.05 ± 0.38[a] | 59.34 ± 1.22[a] |

B. Liver

| Parameter | I | II | III | IV | V |
|---|---|---|---|---|---|
| Total Cholesterol (mg/dl) | 72.96 ± 1.01[a] | 119.75 ± 1.52[b] | 83.67 ± 1.26[a] | 95.48 ± 1.77[a] | 86.63 ± 1.95[a] |
| Triglyceride (mg/dl) | 1.40 ± 0.16[a] | 25.8 ± 0.27[b] | 8.57 ± 1.63[a] | 1.07 ± 1.12[a] | 1.20 ± 0.92[a] |
| HDL (mg/dl) | 53.62 ± 0.11[a] | 34.19 ± 0.10[b] | 40.13 ± 0.08[a] | 49.42 ± 0.30[a] | 36.29 ± 0.08[a] |
| LDL (mg/dl) | 6.53 ± 1.06[a] | 67.44 ± 3.41[b] | 27.82 ± 1.26[a] | 13.84 ± 1.69[a] | 6.26 ± 1.76[a] |

C. Kidney

| Parameter | I | II | III | IV | V |
|---|---|---|---|---|---|
| Total Cholesterol (mg/dl) | 30.58 ± 0.50[a] | 57.46 ± 0.76[b] | 42.55 ± 1.13[a] | 42.11 ± 1.58[a] | 34.65 ± 0.95[a] |
| Triglyceride (mg/dl) | 17.34 ± 0.09[a] | 36.17 ± 0.20[b] | 32.01 ± 0.38[a] | 33.79 ± 15.00[a] | 34.81 ± 0.44[a] |
| HDL (mg/dl) | 11.27 ± 0.19[a] | 8.31 ± 0.01[b] | 9.61 ± 0.24[a] | 10.13 ± 0.09[a] | 10.47 ± 0.23[a] |
| LDL (mg/dl) | 16.11 ± 0.57[a] | 41.93 ± 0.74[b] | 28.01 ± 1.03[a] | 25.22 ± 4.18[a] | 19.19 ± 0.75[a] |

D. Heart

| Parameter | I | II | III | IV | V |
|---|---|---|---|---|---|
| Total Cholesterol (mg/dl) | 23.96 ± 1.80[a] | 38.49 ± 0.65[b] | 28.89 ± 0.00[a] | 22.11 ± 0.79[a] | 23.08 ± 2.53[a] |
| Triglyceride (mg/dl) | 7.75 ± 0.65[a] | 11.6 ± 0.50[b] | 9.65 ± 0.20[a] | 9.89 ± 0.08[a] | 8.22 ± 0.04[a] |
| HDL (mg/dl) | 6.40 ± 0.07[a] | 3.57 ± 0.11[b] | 4.71 ± 0.29[a] | 4.91 ± 0.02[a] | 5.70 ± 0.14[a] |
| LDL (mg/dl) | 16.01 ± 1.71[a] | 32.6 ± 0.68[b] | 24.25 ± 0.28[a] | 15.95 ± 0.01[a] | 15.73 ± 2.53[a] |

Data represent mean ± SEM of experiment performed in triplicate. I: Administered water only, II: Administered $CCl_4$ only, III: Treated with *V.amygdalina* at 50 mg/kg bw after exposure, IV: Treated with *V.amygdalina* at 100 mg/kg bw after exposure, V: Treated with silymarin at 100 mg/kg bw after exposure, 'b' represents significant difference from the control 'a' at $p < 0.05$.

Monitoring the integrity of the liver and kidney involves the assessment of specific biomarkers such as ALP, ALT, and AST (Khafar & Kakey, 2020). Whenever there is an unusual increase in the level of these biomarkers in the blood, hepatic injury can be inferred (Udem et al., 2010). In the present study, the significant increase in ALP in the serum and organs homogenates following exposure to $CCl_4$ toxicity (Table 3A-D) could be a result of an obstruction in bile flow, heart failure, dehydration, and a decrease in renal blood flow (Whitby et al., 1984). The most fundamental explanation is that $CCl_4$ deranged the membrane lipid profile, hence, these biomarkers that are normally compartmentalized within the membrane-surrounded cell, leaked into the bloodstream, leading to an elevation in their level in the serum. Treatment with *V. amygdalina* leaves extract caused a dose-dependent restoration of these biomarkers suggesting a curative effect on the organ injury caused by the toxicant. This effect can be linked to the presence of antioxidant phytochemicals present in the extract. Bilirubin is a product of heme degradation in the spleen, liver, and bone marrow. Under normal circumstances, bilirubin is conjugated with glucuronic acid to form a soluble product that is excreted. Unusually high bilirubin is a type of an injured liver or too high a level of heme degradation. Serum and liver bilirubin levels of experimental rats exposed to $CCl_4$ toxicity were significantly increased relative to the control. This, perhaps, suggests that the toxicant upregulates heme degradation causing a derangement in the process. It can also be traced to the free radical-induced oxidative injury on the hepatocytes. Administration of *V. amygdalina* leaves extract relieved the toxicity imposed by the toxicant restoring the bilirubin level to that comparable with animals that were not exposed at all (Table 3A-D). This indicates the potential of the plant as a therapeutic remedy for liver diseases.

Urea, one of the products of nitrogen metabolism in mammals has been employed as a routine marker for chronic kidney diseases (Borghi et al., 2020). In the present study, intoxicated animals that were treated with graded doses of *V. amygdalina* showed signs of recovery as indicated in the reversal of urea to levels comparable with animals treated with silymarin (Table 3A-D). This suggests the presence of antioxidant phytochemicals such as flavonoids and other polyphenols in the extract which produced the observed effect. It also implies the possible therapeutic relevance of the plant in the management of kidney diseases.

Uric acid, the product of purine degradation in humans has been suggested as central to the development of gout. Recent reports have suggested an intricate link between high serum levels of uric acid and certain pathological conditions such as hypertension, diabetes, obesity, and renal insufficiency (Xiong et al., 2019). In the present study, exposure of experimental animals to $CCl_4$ caused a surge in the serum level of uric acid relative to the control. *V. amygdalina* showed a potent ameliorative effect when administered

to animals under the toxic effect of $CCl_4$. The uric acid level dropped back to levels comparable to animals that were not exposed to the toxicant, following treatment with *V. amygdalina* leaves extract (Table 3A-D). This suggests the potential of the plant in the management of chronic kidney disease.

**Table 3.** Effect of *V. amygdalina* leaf extract on selected biomarkers in organs of $CCl_4$ exposed rat

**A.** Serum

| Parameter | I | II | III | IV | V |
|---|---|---|---|---|---|
| ALP (U/l) | 85.37 ± 0.00[a] | 145.96 ± 0.00[b] | 109.61 ± 0.00[a] | 92.46 ± 1.07[a] | 96.39 ± 0.00[a] |
| ALT (U/l) | 66.09 ± 0.88[a] | 106.69 ± 1.72[b] | 79.51 ± 0.65[a] | 69.69 ± 2.25[a] | 66.92 ± 0.46[a] |
| AST (U/l) | 75.19 ± 1.06[a] | 113.01 ± 1.08[b] | 78.20 ± 0.87[a] | 69.41 ± 1.44[a] | 73.36 ± 0.49[a] |
| Total Bilirubin (mg/dl) | 63.73 ± 0.43[a] | 102.48 ± 0.36[b] | 78.34 ± 0.38[a] | 72.33 ± 0.88[a] | 68.11 ± 0.67[a] |
| Urea mg/dl) | 42.80 ± 0.68[a] | 99.61 ± 0.66[b] | 72.69 ± 0.00[a] | 57.12 ± 0.82[a] | 50.77 ± 0.57[a] |
| Creatine kinase (U/l) | 41.73 ± 1.41[a] | 62.93 ± 2.34[b] | 46.18 ± 1.86[a] | 42.29 ± 1.44[a] | 13.93 ± 2.10[a] |
| Uric acid (mg/dl) | 28.40 ± 0.10[a] | 50.80 ± 0.23[b] | 33.77 ± 0.66[a] | 26.35 ± 0.14[a] | 31.11 ± 0.53[a] |

**B.** Liver

| Parameter | I | II | III | IV | V |
|---|---|---|---|---|---|
| ALP (U/l) | 55.08 ± 0.00[a] | 112.91 ± 0.00[b] | 97.08 ± 1.60[a] | 60.59 ± 34.98[a] | 65.41 ± 1.38[a] |
| ALT (U/l) | 44.99 ± 3.23[a] | 116.55 ± 3.18[b] | 89.32 ± 1.10[a] | 83.21 ± 0.39[a] | 66.03 ± 0.96[a] |
| AST (U/l) | 69.07 ± 1.55[a] | 104.59 ± 4.32[b] | 77.57 ± 0.83[a] | 75.02 ± 1.01[a] | 68.64 ± 1.74[a] |
| Total Bilirubin (mg/dl) | 24.85 ± 1.28[a] | 46.48 ± 0.18[b] | 37.20 ± 0.576[a] | 28.67 ± 0.061[a] | 26.65 ± 1.15[a] |

**C.** Kidney

| Parameter | I | II | III | IV | V |
|---|---|---|---|---|---|
| ALP (U/l) | 85.37 ± 0.00[a] | 145.96 ± 0.00[b] | 14.70 ± 41.31[a] | 93.64 ± 0.00[a] | 6.39 ± 0.00[a] |
| ALT (U/l) | 56.14 ± 0.94[a] | 158.71 ± 7.56[b] | 64.20 ± 0.87[a] | 58.43 ± 2.25[a] | 4.06 ± 0.97[a] |
| AST (U/l) | 69.58 ± 1.28[a] | 99.74 ± 1.08[b] | 93.03 ± 1.55[a] | 89.12 ± 1.06[a] | 5.27 ± 1.47[a] |
| Urea mg/dl) | 52.69 ± 0.67[a] | 93.08 ± 0.00[b] | 71.83 ± 50.18[a] | 65.77 ± 0.00[a] | 58.85 ± 0.00[a] |
| Uric acid (mg/dl) | 24.36 ± 0.29[a] | 50.48 ± 0.33[b] | 41.63 ± 0.44[a] | 32.50 ± 0.41[a] | 25.55 ± 0.55[a] |

**D.** Heart

| Parameter | I | II | III | IV | V |
|---|---|---|---|---|---|
| ALP (U/l) | 23.87 ± 1.59[a] | 38.56 ± 0.00[b] | 34.43 ± 1.15[a] | 27.54 ± 0.00[a] | 7.54 ± 0.00[a] |
| ALT (U/l) | 2.18 ± 0.49[a] | 15.05 ± 0.39[b] | 11.48 ± 0.72[a] | 6.13 ± 0.26[a] | 4.56 ± 0.47[a] |
| AST (U/l) | 11.97 ± 0.77[a] | 27.09 ± 1.44[b] | 21.86 ± 1.57[a] | 14.34 ± 0.00[a] | 9.88 ± 1.05[a] |
| Creatine kinase (U/l) | 51.88 ± 1.41[a] | 83.91 ± 1.17[a] | 54.00 ± 0.01[a] | 50.16 ± 0.01[a] | 41.45 ± 1.11[a] |

Data represent mean ± SEM of experiment performed in triplicate. I: Administered water only, II: Administered $CCl_4$ only, III: Treated with *V.amygdalina* at 50 mg/kg bw after exposure, IV: Treated with *V.amygdalina* at 100 mg/kg bw after exposure, V: Treated with silymarin at 100 mg/kg bw after exposure, 'b' represents significant difference from the control 'a' at $p < 0.05$.

**Table 4.** Effect of *V. amygdalina* leaf on reduced glutathione, total protein and selected antioxidant enzymes activity in $CCl_4$ exposed rats

**A.** Serum

| Parameter | I | II | III | IV | V |
|---|---|---|---|---|---|
| SOD (U/mg protein) | 8.47 ± 0.49[a] | 5.02 ± 0.35[b] | 5.17 ± 1.68[a] | 6.20 ± 0.95[a] | 7.80 ± 1.04[a] |
| CAT (U/mg protein) | 4.36 ± 0.18[a] | 1.84 ± 0.05[b] | 1.92 ± 0.62[a] | 2.44 ± 0.14[a] | 3.77 ± 0.60[a] |
| GSH (mM/g tissue) | 6.81 ± 1.10[a] | 4.53 ± 1.22[b] | 5.27 ± 0.65[a] | 5.88 ± 0.32[a] | 6.04 ± 0.87[a] |
| Total protein (mg/ml) | 3.75 ± 0.20[a] | 1.67 ± 0.60[b] | 2.73 ± 0.27[a] | 3.02 ± 0.81[a] | 2.59 ± 0.18[a] |

**B.** Liver

| Parameter | I | II | III | IV | V |
|---|---|---|---|---|---|
| SOD (U/mg protein) | 2.04 ± 0.10[a] | 1.42 ± 0.42[b] | 1.37 ± 0.17[a] | 1.79 ± 0.29[a] | 2.11 ± 0.32[a] |
| CAT (U/mg protein) | 1.14 ± 0.13[a] | 0.26 ± 0.21[b] | 0.28 ± 1.21[a] | 0.61 ± 0.67[a] | 0.97 ± 0.22[a] |
| GSH (mM/g tissue) | 1.93 ± 0.03[a] | 0.39 ± 0.01[b] | 0.92 ± 0.18[a] | 1.16 ± 0.01[a] | 1.79 ± 0.03[a] |
| Total protein (mg/ml) | 1.88 ± 0.13[a] | 1.07 ± 0.09[b] | 1.18 ± 0.21[a] | 1.44 ± 0.34[a] | 1.58 ± 0.43[a] |

**C.** Kidney

| Parameter | I | II | III | IV | V |
|---|---|---|---|---|---|
| SOD (U/mg protein) | 5.26 ± 0.02[a] | 2.28 ± 0.12[b] | 2.95 ± 0.63[a] | 3.46 ± 0.44[a] | 3.62 ± 0.21[a] |
| CAT (U/mg protein) | 1.36 ± 0.11[a] | 0.64 ± 0.07[b] | 0.77 ± 0.19[a] | 1.05 ± 0.31[a] | 1.47 ± 0.60[a] |
| GSH (mM/g tissue) | 2.52 ± 0.17[a] | 0.93 ± 0.02[b] | 1.56 ± 0.87[a] | 1.93 ± 0.09[a] | 1.95 ± 0.04[a] |
| Total protein (mg/ml) | 2.97 ± 0.02[a] | 2.14 ± 0.01[b] | 2.22 ± 0.13[a] | 2.37 ± 0.17[a] | 2.74 ± 0.11[a] |

Table 4. *Continues*

D. Heart

| Parameter | I | II | III | IV | V |
|---|---|---|---|---|---|
| SOD (U/mg protein) | 4.31 ± 1.47[a] | 2.08 ± 0.75[b] | 2.35 ± 0.49[a] | 3.63 ± 1.27[a] | 4.22 ± 0.43[a] |
| CAT (U/mg protein) | 3.16 ± 0.10[a] | 1.90 ± 0.15[b] | 2.27 ± 0.19[a] | 2.89 ± 0.41[a] | 3.02 ± 0.23[a] |
| GSH (mM/g tissue) | 3.89 ± 0.03[a] | 1.05 ± 0.05[b] | 2.15 ± 1.40[a] | 3.24 ± 1.84[a] | 2.65 ± 0.02[a] |
| Total protein (mg/ml) | 6.47 ± 1.21[a] | 2.43 ± 0.68[b] | 2.77 ± 1.01[a] | 3.37 ± 1.17[a] | 5.43 ± 1.84[a] |

Data represent mean ± SEM of experiment performed in triplicate. I: Administered water only, II: Administered $CCl_4$ only, III: Treated with *V.amygdalina* at 50 mg/kg bw after exposure, IV: Treated with *V.amygdalina* at 100 mg/kg bw after exposure, V: Treated with silymarin at 100 mg/kg bw after exposure, 'b' represents significant difference from the control 'a' at $p < 0.05$.

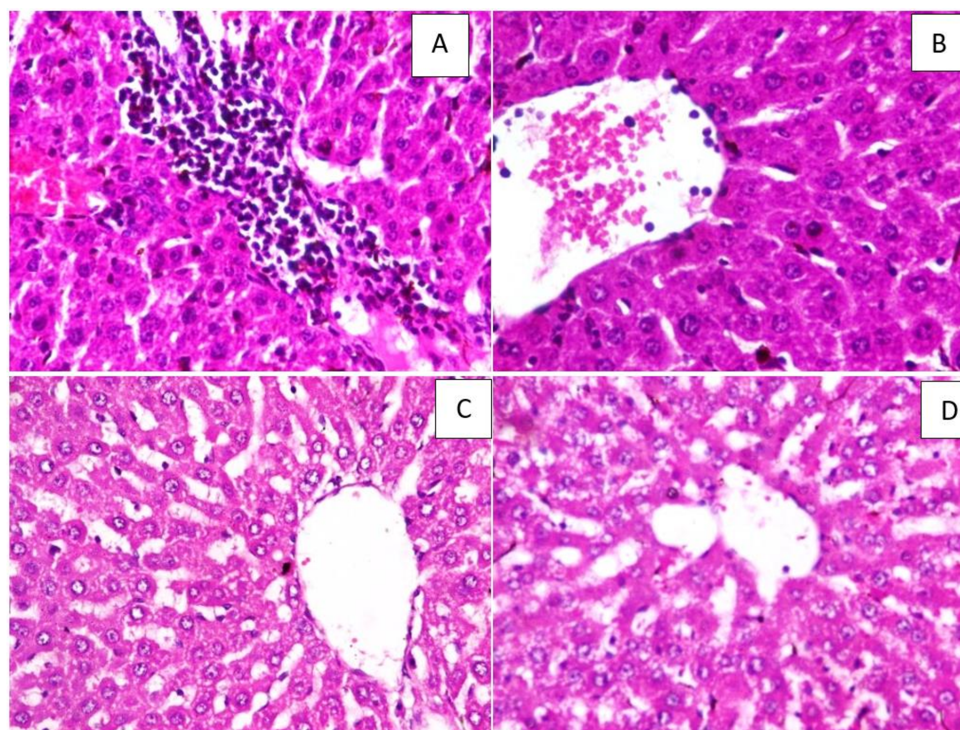


Figure 1. Histoarchitecture of liver tissue slices of experimental animals under different experimental treatments at 400x magnification

A. Photomicrograph of liver slices of animals that were not exposed to carbon tetrachloride toxicity at all. It showed no sign of histological distortion of the hepatocytes. Tissue histomorphology was normal and the nuclei was rightly located in the cytoplasm.

B. Photomicrograph of liver slices of animals exposed to $CCl_4$ (3 ml/kg bw) without treatment. It showed fatty liver with cholestasis.

C. Photomicrograph of liver slices of animals treated with *V. amygdalina* (100 mg/kg bw) after exposure to $CCl_4$. It showed no sign of distorted hepatic histoarchitecture.

D. Photomicrograph of liver slices of animals treated with silymarin at 100 mg/kg bw after initial exposure to $CCl_4$. It showed unperturbed hepatic histomorphology without any sign of histopathological distortion.

Antioxidant enzymes such as superoxide dismutase and catalase (CAT) are critical in shielding the physiological system from the menace of free radicals (Nandi et al., 2019). In terms of this mechanism, SOD dismutates superoxide radicals converting them to hydrogen peroxide which is scavenged by catalase (Zhang & Feng, 2018). In the present study, exposure of experimental animals to $CCl_4$ resulted in depletion in the level of SOD and catalase in the serum and organs homogenates (Table 4A-D). This is probably due to the surge in the level of superoxide anion radicals occasioned by the toxicant. However, treatment with *V. amygdalina* extract caused a restoration of SOD and CAT levels to that comparable with normal animals (Table 4A-D). This implies that certain phytochemicals in the extract exhibited potent antioxidant activity in scavenging both superoxide anions and breaking hydrogen peroxide viz-a-viz the activation of SOD and catalase respectively. This observation can be attributed to the flavonoids and polyphenols present in the extract.

Reduced glutathione GSH is a non-enzymic thiol often used to measure the health status of the organism. Any species that depletes the GSH level of an organism is toxic. In the present study, administration of $CCl_4$ depleted the GSH level in the serum and organs homogenates suggesting the multiorgan toxicity of $CCl_4$ (Table 4A-D). However, treatment with *V. amygdalina* leaf extract restored the GSH level in a dose-dependent manner comparable to animals treated with silymarin. This suggests that the potential of *V. amygdalina* leaves in the management of multiorgan disorders.

Hepatic histoarchitecture distorted by exposure to $CCl_4$ was restored by treatment with *V. amygdalina* leaf extract (Figure 1A-D). This is an indication that the oxidative injury inflicted on the liver by $CCl_4$ was healed by phytochemicals present in the extract, suggesting the medicinal potential of the plant in the treatment of liver diseases.

## 4. Conclusions

*V. amygdalina* leaf extract restored deranged lipid profile, reactivated inhibited antioxidant enzymes, and reversed oxidative injury to the liver, kidney, and heart. Distorted histoarchitecture of the hepatic, renal, and cardiac tissues was also restored following treatment with *V. amygdalina* (Figure 1A-D). Hence, this plant is a potential therapeutic agent that can be exploited in the management of diseases relating to these critical organs.

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## Conflict of interest

The authors confirm that there are no known conflicts of interest.

## Statement of ethics

Ethical approval for this study was obtained from the Office of Research and Development, Ekiti State University, Ado Ekiti (Date: August 23, 2021, Number: ORD/AD/EAC/19/0081).

## Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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## Supplementary File

None.

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