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Vernonia amygdalina Leaf Extract Protects Against carbon tetrachloride-induced hepatotoxicity and Nephrotoxicity: 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 of 5 animals each. All experimental animals, except group I, were exposed to 3 ml/kg b.w of CCl_4 and administered different treatments. Groups III, IV and V each were treated with 50 mg/kg b.w, 100 mg/kg b.w. of bitter leaf extract and 100 mg/kg b.w. of silymarin respectively. Group II animals were left untreated after exposure to toxicant. Activities of creatine kinase (CK), aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), as well as 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 significant increase in CK, AST, ALP, ALT as well as bilirubin, urea and uric acid relative to the control. Lipid profile was deranged, activities of SOD and CAT were markedly inhibited and level of GSH 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.

Keywords: *Vernonia amygdalina*, animals, biomarkers, carbon tetrachloride, diseases.

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 has 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 (1). 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 has readily available and efficacious therapy. There

is therefore a dire need for medicinal plants with proven potency and safety that can serve as panacea to the menace of several diseases (2). 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 (3). Consequently, collaborative research efforts in the area of screening of plants extracts has led to the identification of pharmacologically active agents that can be exploited as drugs for the management of diseases (4).

Chronic liver disease (CLD) constitutes a major threat to global public health, causing increasing mortality and morbidity worldwide.(5) Between 1980 and 2010, mortality as a result of liver disease increased by 46 % globally (6) with higher prevalence in the developing nations including Africa. (7). Similarly, the global disease burden report of 2010 ranked chronic kidney disease as 27th on the list of causes of global death in 1990. However, in 2010, chronic kidney disease rose to 18th position on the list (7) This rise in burden was second to HIV/AIDS. According to the report, the overall premature mortality was estimated at 82% which was third largest behind diabetes (93%) and HIV/AIDS (396%). Considering this data that threatens global health as well as the sustainable development goal (8), there is a dire need to leverage on potent medicinal plants that could serve as complimentary therapy to stem-down the tide of these diseases.

Vernonia amygdalina is a leafy vegetable widely noted for its bitter taste, hence its 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 (9). Recent scientific evidences have given credence to its numerous medicinal benefits such as anti-obesity (10), antioxidant (11), anticarcinogenic (12), antihyperglycemic (13), anti-sickling (14) and in the management of cardiovascular disorders (15). Reports have indicated that leaf extract of *Vernonia amygdalina* protects against brain degeneration thereby enhancing memory (16). Besides, extract of *V. amygdalina* Del leaf exhibits anti-helmintic, anti-parasitic and antimicrobial properties (17).

I n view of the rising burden of liver and kidney diseases, there is a dire need to investigate the potentials of *V. amygdalina* extract in the management of these diseases. Hence, this study.

Materials and Methods

Plant Materials

*Vernonia amygdalina*leaves was obtained from a private farm in Ado Ekiti, air dried in the laboratory, pulverized and then stored in an air-tight container.

Reagents and chemicals

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

Extraction of the extract

Bitter leaves were air-dried at room temperature and pulverized to obtain fine powder using a blender. 500 g 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 cheese cloth, and freeze-dried to obtain the dried extract. The extract was kept in a closed container and kept inside the fridge at 4^0C for further studies

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 temperature (24 ± 1^{o}C), relative humidity, and 12 / 12-hour light and dark cycle. They were allowed to have free access 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.

Animal grouping and treatment

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

Table 1. Animal treatment

Groups	Treatment
I	Distilled 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 *Vernonia amygdalina* extract for 14 days
IV	3 ml CCl_4 + 100 mg/kg *Vernonia amygdalina* extract for 14 days
V	3 ml CCl_4 + 100 mg/kg silymarin for 14 days

Dissection of rats

The animals were decapitated under very light anesthesia to obtain the liver, kidney and heart, while whole blood was collected by cardiac puncture allowed to stand for 1 hour in an EDTA bottle. 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.

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 4^0C and then used for biochemical analyses.

Determination of serum creatine kinase (Ck-Mb) activity

Creatine kinase was measured by the method of Mattenheimer (18). 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), ethylene diaminetetraacetic 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 interval. Change in absorbance per minute was estimated while enzyme activity was determined as:)

Assay of aspartate aminotransferase (AST) activity

AST activity was determined following the principle described by Reitman and Frankel (19). Briefly, 0.1 ml of organs homogenates as well as 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-dinitrphenylhydrazine (2mmol/L) was added to the reaction mixture and allowed to stand for exactly 20 min at 25 °C. Five milliliter of NaOH (0.4 mol/L) was then added and the absorbance of the mixture read after 5 min at 546 nm against the reagent blank.

Assay of alanine amino transferase (ALT) activity

The principle described by Reitman and Frankel (19) was followed in the assay of ALT using 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 (2.0 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 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. Activity of ALT was obtained

from the standard curve provided in the kit.

Assay of alkaline phosphatase (ALP) activity

Assay of serum ALP was based on the method of Englehardt*et al*. (20) 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 minute. ALP activity was determined as shown

Serum Lipid Profile

Estimation of total cholesterol level

Total cholesterol level was determined based on the method of Trinder (21) 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-aminoantipyrine (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. Absorbance of the sample (Asample) was read at 500 nm against the reagent blank.

Cholesterol concentration (mg/dl) was calculated as follows:

Evaluation of concentration of triglyceride

Triglycerides level was determined based on the method of Tietz (22) 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 (Tietz, 1990).

High Density Lipoprotein (HDL-c)-Cholesterol Assay

High-density lipoprotein cholesterol was determined by the method of Grove (23) in two stages:

Precipitation

Reaction mixture containing 200 µl of the serum, 200 µl of the cholesterol standard, 500 µl of the diluted precipitant R1 (0.55 mM phosphotungstic acid, 25 mM magnesium chloride) was mixed together 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.

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 ($A_{standard}$) was then measured at 500 nm against the reagent blank within 1 h.

Low Density Lipoprotein (LDL) - Cholesterol Determination

The concentration of low-density lipoprotein in the serum was calculated using the formula of Friedwald et al. (1972) (24) :

Antioxidant assay

Determination of catalase activity

This experiment was carried out according to the method described by Sinha (25). Two hundred microliters of serum and organs homogenates was mixed separately with 0.8 ml of distilled H_2O to give 1 in 5 dilution 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. A 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.

Calculation

Concentration of H_2O_2 remaining was extrapolated from the standard curve for catalase activity.

Determination of superoxide dismutase (SOD) activity

The level of SOD activity was determined by the method of Misra and Fridovich (26). 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. 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 substrate (adrenaline) and 0.2 ml of water. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds.

Determination of Reduced Glutathione (GSH) Level

The method of Beutler et al. (27) 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 filtrate was added of 4 ml of 0.1 M phosphate buffer 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 (3parts to 2 parts of distilled water) and 0.5 ml of the Ellman reagent. The absorbance was measured at 412 nm against reagent blank. Level of GSH in the serum was obtained from the standard curve.

Determination of Total Protein (TP) in Serum

The Biuret method described by Weichselbaum (28) 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 sulphate (6 mmol/l) was added to 0.02 ml of the serum sample. The reaction mixture was incubated at 25°C and absorbance measured against the reagent blank at 546 nm.

Statistical Analysis

All values are 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 (Anthony and Richard, 2006). The significance level was set at $p < 0.05$.

Result 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 extracted, while groups III, IV and V animals were exposed to 3ml/kg bw of CCl_4 and treated with 50, 100 mg/kg bw of *V. amygdalina* leaf extract and 100mg/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 level 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). Activity of superoxide dismutase, catalase and creatine kinase were significantly depleted when animals were exposed to CCl_4 but activity were 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).Histomicrographof liver tissue of experimental animals under different

(a) Serum

Parameter	I	II	III	IV	V
T. Chol (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]
Trig (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
T. Chol (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]
Trig. (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
T. Chol (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]
Trig. (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
T. Chol (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]
Trig (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]

treatment is as shown in Figure 1A-D.

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

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; 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

(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]
T. BIL (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]
CK (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 (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]
T. BIL (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]	96.39±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]	64.06±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]	75.27±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 (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±1.5[9]	27.54±0.00[a]	27.54±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]
CK (U/L)	51.88±1.41[a]	83.91±1.17[a]	54±0.01[a]	50.16±0.01[a]	41.45±1.11[a]

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

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; treated with

(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]
TP (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]

(c) Kidney

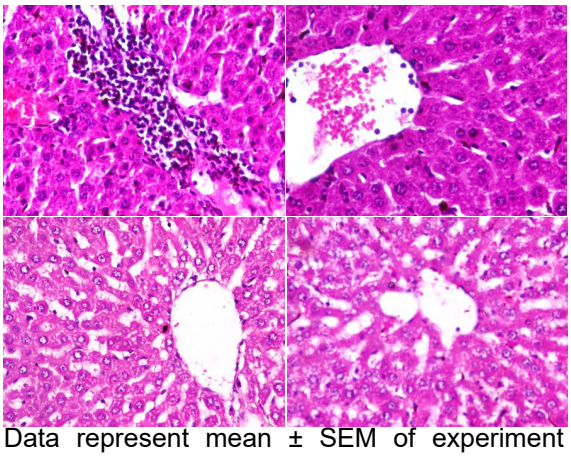
Parameter	I	II	III	IV	V
SOD (U/mg protein)	5.26±0.02[a]	2.28±0.12[b]	2.95±0.63[a]	3.46±0.44[a]	3.62±0.21[a]
CAT (U/mg protein)	1.36±0.11[a]	0.64±0.07[b]	0.77±0.19[a]	1.05±0.31[a]	1.47±0.60[a]
GSH (mM/g tissue)	2.52±0.17[a]	0.93±0.02[b]	1.56±0.87[a]	1.93±0.09[a]	1.95±0.04[a]
TP (mg/ml)	2.97±0.02[a]	2.14±0.01[b]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

(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]
TP (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]

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**

Vernonia amygdalina leaf extract protects against carbon tetrachloride-induced hepatotoxicity



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; treated with V.amygdalina at 100 mg/kg bw after exposure; V- treated with silymarin at 100 mg/kg bw after exposure. 'b' represent significant difference from the control 'a' at P=0.05

Figures 1A-D: Histoarchitecture of liver tissue slices of experimental animals under different experimental treatments at a magnification (x400).

1A: Photomicrograph of liver slices of animals that were not exposed to carbon tetrachloride toxicity at all: It shows no sign of histological distortion of the hepatocytes. Tissue histomorphology was normal and nuclei rightly located in the cytoplasm.

1B: Photomicrograph of liver slices of animals exposed to CCl_4 3 ml /kg bw without treatment. It showed fatty liver with cholestasis.

1C: 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.

1D: 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

DISCUSSION

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 the conventional drugs but the complementary effort of herbal medicines. Animal models of liver and kidney diseases has 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 (29). Routinely, notable signs of toxicity include: high cholesterol, high triglycerides, high LDL and depleted HDL in exposed animals (29). In the present study, exposure of experimental animals to carbon tetrachloride (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 level in the liver, kidney heart and serum following exposure to CCl_4. This observation implies that CCl_4 triggered a derangement in critical membrane lipid leading to a compromise in membrane function. However, treatment with *V. amygdalina* leaf extract restored the cholesterol level back to a level comparable with animals treated with silymarin. Undoubtedly, such effect could be linked with the flavonoid and other polyphenols present in the extract. Triglyceride level in the serum and organs homogenates was 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. Level of high-density lipoprotein has been used to predict the antioxidant status and overall well-being of an 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* has been reported. The relief obtained 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 basal 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 is due to the presence of bioactive ingredients such as flavonoids in the extract.

Monitoring the integrity of liver and kidney involves the assessment of specific biomarkers such as ALP, ALT and AST (30). Whenever there is an unusual increase in the level of these biomarkers in the blood, hepatic injury can be inferred (31). 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 as a result of obstruction in bile flow, heart failure, dehydration and decrease in renal blood flow (32). The most fundamental explanation is that CCl_4 deranged the membrane lipid profile, hence, these biomarkers that are normally compartmentalized within membrane bound cell, got 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 circumstance, bilirubin is conjugated with glucuronic acid to form a soluble product that is excreted. An unusually high bilirubin is a typical of an injured liver or too high 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 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 (33). 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 (34). Recent reports have suggested an intricate link between high serum level of uric acid and certain pathological conditions such as hypertension, diabetes, obesity and renal insufficiency (34). 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. 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.

Antioxidant enzymes such as superoxide dismutase and catalase (CAT) are critical in shielding the physiological system from the menace of free radicals (35). In terms of mechanism, SOD dismutates superoxide radicals converting it to hydrogen peroxide which is scavenged by catalase (36). 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 organism. Any specie 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* leaves extract restored the GSH level in a dose dependent manner comparable to animals treated with silymarin. This suggests that 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 phytochemical present in the extract, suggesting the medicinal potential of the plant in the treatment of liver diseases.

Conclusion

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

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References

1. Fakim, A.G. Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Molecular aspects of medicine*, **2006**, 27, 1-93.

2. Hamburger, M. and Hostettmann, K. Bioactivity in plants: the link between phytochemistry and medicine. *Phytochemistry*, **1991** 30, 3864-3874.

3. Singh, P. and Singh, C. L. Chemical investigations of *Clerodendraonfragrans*. *Journal of Indian Chemical Society*, **1981**, 58: 626-627.

4. Rastogi, P. R. and Meharotra, B. N. In Compendium of Indian Medicinal Plants. Vol. I, 339; a) (**1993**) III: 194. PID, CSIR, New Delhi, India.

5. Marcellin, P.; Kutala B.K. Liver diseases: a major, neglected global public health problem requiring urgent actions and large-scale screening. *Liver Int***2018**,38 (Suppl 1):2–6. https://doi.org/10.1111/liv.13682.

6. Mokdad, A.A.; Lopez, A.D.; Shahraz, S.; Lozano, R.; Mokdad, A.H.; Stanaway, J. Liver cirrhosis mortality in 187 countries between 1980 and 2010: a systematic analysis. *BMC Med***2014**;12:145, PMID: 25242656.

7. Stepanova, M.; De Avila, L.; Afendy, M.; Younossi, I.; Pham, H.; Cable, R. Direct and indirect economic burden of chronic liver disease in the United States. *Clin Gastroenterol Hepatol.,* **2017**, 15. 759–766.e5 https://doi.org/10.1016/j.cgh.2016.07.020

8. Levin, A.; Tonelli, M.; Bonventre, J.; Coresh, J; Donner, J.A. Fogo, A.B ISN Global Kidney Health Summit participants. Global kidney health 2017 and beyond: a roadmap for closing gaps in care, research, and policy. *Lancet.***2017**, 390(10105):1888–917. doi: http://dx.doi.org/10.1016/S0140-6736(17)30788-2 PMID: 28434650

9. Kassebaum, N. J.; Arora, M.; Barber, R.M.; Bhutta, Z.A.; Brown J, Carter A.; GBD 2015 DALYs and HALE Collaborators. Global, regional, and national disability-adjusted life-years (DALYs) for 315 diseases and injuries and healthy life expectancy (HALE), 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet*. **2016** 10 8;388(10053):1603–58. doi: http://dx.doi.org/10.1016/S0140-6736(16)31460-X PMID: 27733283

10. Farombi, E. O.; Owoeye, O. Antioxidative and Chemopreventive Properties of Vernonia amygdalina and Garcinia biflavonoid. *International Journal of Environmental Research and Public Health*, **2011,** 8, 2533–2555.

11. Adesanoye, O. A; Farombi, E. O. Hepatoprotective Effects of Vernonia amygdalina (Astereaceae) in Rats Treated with Carbon Tetrachloride. *Experimental and Toxicologic Pathology*, 2010, 62(2), 197–206.

12. Wong, F. C.; Woo, C. C.; Hsu, A; Tan, B. K. H. The Anti-Cancer Activities of Vernonia amygdalina Extract in Human Breast Cancer Cell Lines Are Mediated through Caspase-Dependent and p53-Independent Pathways. *PLOS ONE*, **2013**, 8(10): 1–15.

13. Jan Mohamed, H. J.; Yap, R. W.; Loy, S. L.; Norris, S. A.; Biesma, R, A. -H. J. Prevalence and Determinants of Overweight, Obesity, and Type 2 Diabetes mellitus in Adults in Malaysia. *Asia Pacific Journal Public Health*, **2015**, 27(2): 123–135.

14. Egharevba, C.; Osayemwenre, E.; Imieje, V.; Ahomafor, J.; Akunyuli, C.; Udu-Cosi, A. A.; Theophilus, O.; James, O.; Ali, I.; Falodun, A. Significance of Bitter Leaf (Vernonia amgdalina) In Tropical Diseases and Beyond: A Review. *Malaria Chemotherapy Control and Elimination*, **2014**, 3(1), 1–10.

15. Abdulmalik, O.; Oladapo, O.O.; Bolaji, M.O. Effect of aqueous extract of Vernonia amygdalina on atherosclerosis in rabbits. ARYA *Atherosclerosis*, **2016**, 12(1), 35.

16. Osaretin, A. T.; Ebuehi, M.; Olufemi, A. Neurochemical Impact of the Aqueous Extract of *Vernonia amygdalina* and *Talinum triangulare*on Learning and Memory in Male Wistar Rats.*International Journal of Brain and Cognitive Sciences*, **2017**, 6(5), 81-88. DOI: 10.5923/j.ijbcs.20170605.01

17. Anibijuwon, I. I.; Oladejo, B. O.; Adetitun, D. O.; Kolawole, O. M. Antimicrobial Activities of Vernonia amygdalina against Oral Microbes. *Global Journal of Pharm*acology, **2012**, 6(3), 178–185.

18. Mattenheimer, H. Urinary enzyme measurements in the diagnosis of renal disorders. *AnnClin Lab Sci*., **1981**,11(3),189–201.

19. Reitman, S.; Frankel, S. Glutamic – pyruvate transaminase assay by colorimetric

method. *Am J Clin Pathol.***1957**, 28, 56–60.

20. Englehardt, A. Measurement of alkaline phosphatase. *Aerztl Labor*,1**970**, 16, 42.

21. Trinder, H. A. simple Turbidimetric method for the determination of serum cholesterol. Ann din Biochem., **1969**,6, 165.

22. Tietz, N.W. Clinical guide to laboratory tests. 3rd ed. Philadelphia: Saunders and Co. Publishers; **1995**.

23. Grove, T.H. Effect of reagent pH on determination of high-density lipoprotein cholesterol by precipitation with sodium phosphotungstate-magnesium. *Clin Chem*., **1979**, 25(4), 560–4.

24. Friedewald, W. T.; Levy, R.I.; Fredrickson, D.S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of their preparative ultracentrifuge. *Clin Chem*. **1972**,18, 499–502.

25. Sinha, A.K. Colorimetric assay of catalase. *Anal Biochem*., **1972**, 47, 89–94.

26. Misra, H.P. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem*., **1972**, 247(15), 3170–5.

27. Beutler, E.; Duron, O.; Kelly, B.M. Improved method for the determination of blood glutathione. *J Lab Clin Med*., **1963**, 61, 882–90.

28. Weichselbaum, T. E. An accurate and rapid method for the determination of protein in small amount of blood, serum. *Am J Clin Pathol*., **1995**, 16, 40.

29. Ugwu, P.C.; Okechukwu, F.C.; Nwodo, O. E.; Joshua Parker, E.; Odo Christian, C; Ossai Emmanuel, Effect of Ethanol Leaf Extract of Moringa Oleifera on Lipid Profile of Mice, *Res. J. Pharm. Biol. Chem. Sci*. **2013**, 4 (1), 1324–1332.

30. Kaniaw, R.K.; Esmail, S.K. Hepatorenal function markers alteration in aged and aged related disease in human. *Indian J Public Health Res Dev*., **2020,**11, 844–9.

31. Udem, S.C.; Obidoa, O.; Asuzu, I.U. Acute and chronic toxicity studies of erythrinasenegalensis stem bark extract in mice*, Comp. Clin. Pathol*. **2009,** 19, 275–282.

32. Whitby, L.G.; Percy-Robb, I.W.; Smith, A.F. Enzymes test in diagnosis, Lecture notes On Clinical Chemistry, 3rd ed., Blackwell Sci. Publication, London, **1984**, pp. 138–168 .

33. Claudio, B.; Enrico, A.; Richard, J.; Jan, T.K.; Empar, L.; Giuseppe, M.; Josep, R.; Stack, A.G.; Konstantinos P.T. Hyperuricaemia and gout in cardiovascular, metabolic and kidney disease. *Eur J Intern Med.***2020**,80, 1–11 ISSN 0953–6205. https://doi.org/10.1016/j.ejim.2020.07.006.

34. Xiong, Q.; Liu, J.; Xu, Y. Effects of uric acid on diabetes mellitus and its chronic complications. *Int J Endocrinol.***2019**. https://doi.org/10.1155/2019/9691345.

35. Nandi, A.; Liang-Jun, Y.; Chandan, K.J; Nilanjana D. Role of catalase in oxidative stress- and age-associated degenerative diseases. *Oxidative Med Cell Longev*. **2019**. https://doi.org/10.1155/2019/9613090.

36. Zhang, L.B.; Feng, M.G. Antioxidant enzymes and their contributions to biological control potential of fungal insect pathogens. *Appl Microbiol Biotechnol*. **2018**,102,4995–5004. https://doi.org/10.1007/s00253-018-9033-2.