

Evaluation of protective potentials of *Vernonia amygdalina* (Bitter leaf) extracts on some selected organs of cadmium chloride-induced hypertensive albino rats

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Abstract

Many food components such as plant metabolites, have been shown to possess antioxidant activities. *Vernonia amygdalina* is widely consumed as vegetable for its rich array of phytochemicals and nutritional components which are attributed to its various health and medicinal benefits. The study evaluated the protective potentials of *Vernonia amygdalina* extracts on selected heart, Liver and Kidney of Cadmium Chloride-induced Hypertensive albino rats. The animals were induced hypertension with cadmium chloride. Total size of 50 albino rats of male and females were grouped according to sex in five different groups of ten rats per group; Control Group (1), Hypertensive Group (2), Standard group (3), methanol group (4) and n-hexane group (5). At the end of the study, blood was collected by cardiac puncture while the organs were harvested for histology test. Liver function evaluated was Aspartate Transaminase (AST), Alanine Aminotransferase (ALT) and Alanine Phosphatase (ALP). Renal function test was done following standard methods for estimation of serum Urea and Creatinine, Electrolyte and Calcium while heart, liver and kidney were subjected to histological technique. One way ANOVA was used for analysis of the collected data and was expressed as Mean \pm SEM. Result of Hypertensive group showed significant increase in sodium level ($p < 0.05$) when compared with Control group. Significant decrease was observed in Sodium values of Standard drug, *Vernonia amygdalina* methanol and n-hexane groups when compared with the hypertensive none treated Control. Significant decrease was observed in AST values of Test groups 4 and 5 while Total bilirubin value showed significant decrease in Group 4, when hypertensive control is compared with all other Groups. Total bilirubin values of Groups 4 and 5 showed significant decrease as compared Standard to all groups. The treatment with bitter leaf resulted in a reversal of values found in untreated hypertensive group compared with the test group. Heart, liver and kidney tissues showed no lesion or inflammation in all the groups except hypertensive none treated group that showed distorted patchy necrotic area in the histology test. The results of this study suggest that *Vernonia amygdalina* possesses protective potential against Cadmium chloride induced hypertension in albino rats.

Keywords: Bitter leaf; Protective; Hypertension; Disease; *Vernonia amygdalina*

1. Introduction

Food spices have numerous functions such as food flavoring, antioxidant, medicinal, and preservative functions [1-2]. Nutraceuticals from *Tetracapedium conophorum* have been reported to protect against cadmium chloride-induced hypertension [3]. *Vernonia amygdalina* (Bitter leaf) is a green leafy vegetable and contains nutrients which are important to the body as a protective and regulatory agents [4]. *Vernonia amygdalina* has been shown to contain

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phosphorus, ascorbic acid, iron, B-carotene, Calcium, fiber, water and other nutrients and also reported to possess phytochemical constituents such as alkaloids, saponins, tannins, steroid, glucosides, flavonoids, glycosides [5]. *Vernonia amygdalina* commonly called bitter leaf is a multipurpose and rapid regenerating shrub of 2 to 10m tall and leaves of about 6mm in diameter which is consumed either as a vegetable for cooking African soups or the aqueous extracts taken as tonics for the treatment of various illnesses [6]. The plant has been called different ethnic names in Nigeria such as Onugbu or Olugbu (Igbo), Kiriologbo (Ijaw), Shiwaka (in Hausa), Ewuro (Yoruba) and also Ndole in Cameroun [7, 8]. *Vernonia amygdalina* is an abundant farming vegetable and fodder tree in Nigeria [9] whose leaves are used as soup condiment and as vegetable. The leaves and roots were reported to have been used in the treatment of fever, kidney disorder, hiccups, stomach discomfort such as increased rat uterine motility, rabbit jejunum contraction and also protect against kidney impairment [10-15]. Some studies have shown that this antioxidant-rich food product have potential bioactive substances that exhibit protective properties [16-22]. Epidemiological findings have shown that antioxidants can keep back the development of degenerative diseases such as hypertension, coronary heart diseases, cancer, obesity, premature aging, type 2 diabetes, and inflammatory diseases [23]. *Vernonia amygdalina* has been reported to also exhibit anti-helmitic and anti-malaria properties [24], as well as anti-tumorigenic properties [25]. *Vernonia amygdalina* has also been used for both therapeutic and nutritional purposes, it has been shown to contain significant quantities of Calcium, iron, potassium, phosphorous, manganese, copper and cobalt. Heart, Kidney and liver diseases are serious health issues, especially for the central roles they play in metabolic homeostasis and xenobiotic transformations. A number of physiological mechanisms are involved in the maintenance of normal blood pressure, and their derangement may play a part in the development of essential hypertension. Report has it that the incidence of cardiovascular diseases can be lowered by application of phytosterols through serum cholesterol levels reducing actions, but that of hypertension has not been shown much report. Two major risk factors of cardiovascular diseases (hypertension and dyslipidaemia) was reported to contribute to the progression and pathogenesis of cardiovascular disease both synergistically and individually [26]. The search for alternative ways of treatment of such derangement using nutraceuticals could be useful in management, prevention, control and treatment of cardiovascular, renal and hepatic diseases [27]. Hence evaluation of nutraceuticals from *Vernonia amygdalina* may possess protective potentials which may as well help to scavenge this free-radicals and offers health benefit to the cadmium chloride-induced hypertensive rats.

2. Material and methods

2.1. Identification and Preparation of Plant Materials:

Fresh bitterleaf (*Vernonia amygdalina*) was purchased by the researcher and was identified by Dr. Chimezie Ekeke of the Department of Plant Science and Biotechnology, University of Port Harcourt with identification number UPH/P/308. The leaf was washed, completely drained, separately coarse and was allowed to air-dry at room temperature. It was monitored and at the end of the two weeks, large kilogram of fully dried leaves were grinded into powdered form with the use of electrical blender. Extraction of the blended leaves of *Vernonia amygdalina* was done by the use of Rotary Vane Extraction with solvents methanol and n-hexane. Extraction was done in two stages – first stage involved crude extraction with ethanol and the second stage involved further extraction with Whatman No 1 filter paper. The ethanol were evaporated to semi-solid state at 80°C within two days. The aqueous filtration evaporated to semi-solid state by the use of rotary evaporator. Each 500g of blended forms was caused to flow back in two (2) liter volume of methanol solvent (1:4). Basic phytochemical screening was done on the leaves using standard methods.

3. Animal experiment

Number of animals used in this study were a total of 50 albino rats (30 females, 20 males). The animals were breed at the Animal House of the Department of Human Physiology, Nnamdi Azikiwe University Okofia Campus. They were acclimatized for 14 days, during which UgoBasil tail-cuff pressure measurement was implored to measure their blood pressure (this enabled the animals acclimatized with the equipment avoiding unnecessary agitation of the first entrance of the rat into the cuff) before they were induced and treated. The albino rats were housed in rat cage of normal room temperature, well ventilated with 12 hour lightened 12 hour darkness cycle. Feed and water were purchased from Pfizer Nigeria limited feeds Benin City for the study. The albino rats were used in accordance with NIH Guide for the care and use of laboratory animals revised Publication. Standard Operation Procedures (SOPs) were also applied (Helsinki Declaration). Histological study of the heart, kidney and liver were done while renal and liver function test was also done.

- **Group 1:** Control group were rats which were not induced nor treated with any extract but were only fed with animal feed and water daily throughout the study period.

- **Group 2.** Hypertensive control group were induced with Cadmium chloride, confirmed hypertensive, were not treated throughout the study but were fed with animal feed and water only.
- **Group 3:** Standard drug group were induced with cadmium chloride and were treated with Nifedipine (a known hypertensive drug). The animals were also fed with feed and water.
- **Group 4:** Cadmium Chloride induced hypertensive group treated with methanol *Vernonia amygdalina* leaf extract.
- **Group 5:** Cadmium Chloride induced hypertensive group treated with n-Hexane *Vernonia amygdalina* leaf extract.

At the end of the 4 weeks of treatment, the animals were sacrificed, blood collected by cardiac puncture into sodium fluoride and EDTA sample bottles for biochemical analysis. The organs of each animal in each group were harvested into a plane sample bottle for histopathology analysis on the kidney, heart and liver each study group [3].

3.1. Measurement of biochemical parameters [3]

The biochemical parameters analysed (kidney function test) on serum electrolyte were sodium, potassium, chloride, urea and creatinine. Liver function test were AST, ALT, alkaline phosphatase, metabolic system test and total protein, albumin, total bilirubin, conjugate bilirubin were also analysed. We considered and selected the above indices for hypertension analysis as was recommended by Harrison's Principles of Internal Medicine [28]

3.2. Sodium (spectrophotometric method)

Test tubes were labelled as blank, standard, control, test sample. Then 1ml of filtrate reagent were pipetted to all tubes.

50 µl of sample were added to all tubes and distilled water to the blank, shook all tubes vigorously and mixed continuously for three minutes. Tubes were centrifuged at high speed (1500g) for 10 minutes. The supernatant were tested as described below taking care not to disturb the protein precipitate

3.2.1. Colour developer

Labelled test tubes corresponding to the above filtrate tubes, pipette 1.0ml acid reagent to all tubes, added 50µl of colour reagent to all tubes and mixed, zero the spectrophotometer with distilled water at 550nm, read the absorbance of all tubes and recorded (unit in meg/l)

3.3. Potassium reagent (Dpectophotometric method) [3]

Test tubes were labelled standard, control, test sample, blank, then pipetted 1ml of potassium reagent to all tubes. Added 0.01ml of samples to respective tubes, mixed and allowed to stand at room temperature for 3minutes. Turned the wavelength of spectrophotometer on to 500nm, zero spectrophotometer with reagent blank. Read the absorbance of all tubes and recorded.

3.4. Chloride (automated method)

Test tubes were labelled blank, calibrator, test sample. Pipetted 1.5ml chloride reagent to each tubes. Added 0.01ml of calibrator or sample to respective tubes and mixed. Incubated at room temperature for five minutes. Spectrophotometer was fixed for 480nm and zero with reagent blank. Wavelength of 520nm was used. Then read the absorbance of all tubes and recorded in Meq/l.

3.5. Urea (Manual Spectrophotometry Method)

Test tubes were labelled blank, standard, test sample. 10µl of urea reagent was pipetted to reagent tubes. All tubes were mixed and incubated at the temperature of 37°C for 10 minutes. Spectrophotometer wavelength was fixed at 546nm and temperature to 37°C. Pipetted 2.5ml of water, standard and sample to the cuvette labelled blank, standard and test sample respectively. Immediately mixed and incubated at 37°C temperature for 15minutes. Read the absorbance of the sample and standard against the blank and recorded.

3.6. Cratinine (Spectrophotometry Method)

Test tubes were labelled blank, calibrator, text sample. 2ml of reagent was pipetted into each tube. Pipetted 0.1ml of calibrator into cuvette tubes respectively and mixed. Incubated at the temperature of 25-37°C. Spectrophotometer was

fixed at 492nm and zero with reagent, mixed and Absorbance A_1 , standard and sample were read after 30 seconds. Read absorbance A_2 of standard and samples at exactly 2minutes.

3.7. AST (Aspartate Aminotransferase): (Spectrophotometry Method)

Test tubes were labelled, blank, standard and test sample. 0.1M of sample were pipetted into all test tubes. 0.5ml of reagent (1) buffer was added to all test tubes, mixed and incubated for exactly 30 minutes at 37°C. Spectrophotometer wavelength was fixed at Hg 546nm. 0.5ml of R₂ (2,4 dinitrophenylhydrazine) were pipetted to the cuvette test tubes respectively, 0.1ml sample were pipetted and mixed. It was allowed to stand for 20minutes at 20 °C to 25 °C. Then added 5.0ml of Sodium Hydroxide and mixed. After 5minutes, the absorbance of the sample (A_{sample}) were read against the sample blank and recorded.

3.8. ALT (Alamine Aminotransferase): (Spectrophotometry Method)

Test tubes were labelled; blank, test sample, standard. 0.5ml reagent were pipetted into test tubes. Added 0.1ml sample to all tubes, mixed and incubated at 37°C for exactly 30minutes. Spectrophotometer was set at wavelength of Hg 546nm. Pipetted 0.5ml solution into cuvette labeled; blank, test sample, standard. 5.0ml of sodium hydroxide was added, mixed and allowed to stand for 20min at 20-25°C. Read the absorbance of sample (A_{sample}) against the reagent blank after 5minutes.

3.9. Alkaline Phosphates (Spectrophotometry Method)

Brought reagents and the analyzer to 37°C temperature. Labelled test tubes, blank, standard, test sample. 1.0ml of deionized water was pipetted into test tubes. 1 drop of substrate was added into all tubes, mixed and incubated at 37°C for 5minutes. Spectrophotometer wavelength was fixed at 550nm. 0.1ml was pipetted into corresponding test tubes and cuvettes of 1cm thermostat. Mixed, incubated at the temperature of 37°C for 20minutes. Then colored developer was added, allowed to stand at minimum of 1 hour, read and recorded the result.

3.10. Total Protein (TP): (Spectrophotometry Method).

Test tubes were labelled; blank, standard, test sample. 0.02ml of distilled water were pipetted into cuvette test tubes. 0.02ml standard of CAL were added to all test tubes. Set the spectrophotometer wavelength of Hg 546nm at the temperature 20 - 25C. Then 0.02ml of serum was added, mixed and incubated for 30mintes at the temperature of 20 °C - 25 °C. Then measured the absorbance of the sample (A_{sample}) and the standard (A_{standard}) against the reagent blank and record.

3.11. Albumin (ALB): (Spectrophotometry Method)

Test tubes were labekked; blank, standard, test sample. 0.01ml of distilled water was pipetted into test tubes. 0.01ml of standard were added (cal). Spectrophotometer wavelength was set at 630nm at the temperature of 20-25 °C. Then pipetted into corresponding cuvette test tubes 0.01ml of serum, added 3.00ml BCG (bromocresol green) reagent. Mixed and incubated for 5minutes at temperature of 20-25 °C. Measured the absorbance of the sample (A_{sample}) and the standard (A_{standard}) against the reagent blank and recorded.

3.12. Total bilirubin (Spectrophotometry Method)

Test tubes were labelled; blank, standard, test tubes. Distilled water was pipetted into cuvettes, added (R1) sulphanilic acid, (R2) Nitrite with (R3) caffeine and sample. Allowed to stand for 10 minutes at 25°C. Spectrophotometer wavelength was fixed at 578nm. Then added (R4) tartrate, mixed and allowed to stand for 5-30 minutes at 20-25C. Read by measuring the absorbance of the sample against the sample blank (A_{tb}) and recorded.

3.13. Histological study: (hematoxylin and eosin (H&E) staining techniques)

Harvested heart, liver and kidney tissues were fixed in 10% formalin solution and was embedded in melted paraffin wax. Hematoxylin and Eosin (H&E) histological techniques were used. An Olympus light microscope were used for photomicrography on the mounted slide and was captured with Kodak digital camera.

4. Results and discussion

4.1. Effects of Serum Electrolyte, Urea and Creatinine levels

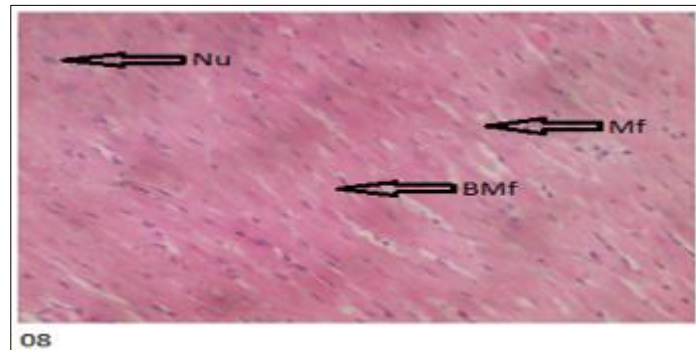


Figure 1 Photomicrograph of Cardiac muscle x400 H&E stained Group 1 – Control [3]

Showing histologically normal cardiac muscle

- Homogeneous muscle fiber (MF) diameter, with intact sarcolemma.
- Peripherally placed nuclei (Nu)
- Branching cardiac muscle fiber (BMF)

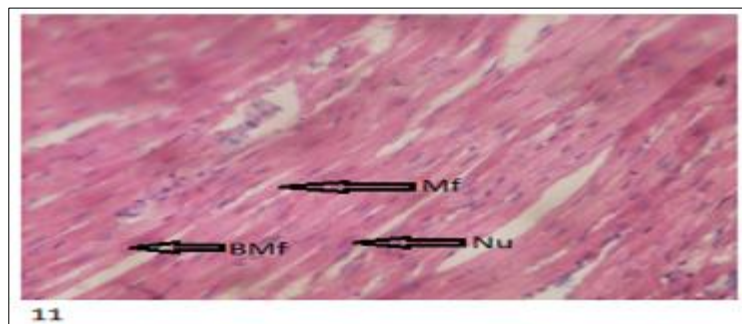


Figure 2 Photomicrograph of Cardiac muscle (x400). H/E stain for group 4 – *Vernonia amygdalina* methanol extract

Showing histologically normal cardiac muscle

- Homogeneous muscle fiber (MF) diameter, with intact sarcolemma.
- Branching cardiac muscle fiber (BMF).
- Peripherally placed nuclei (Nu)

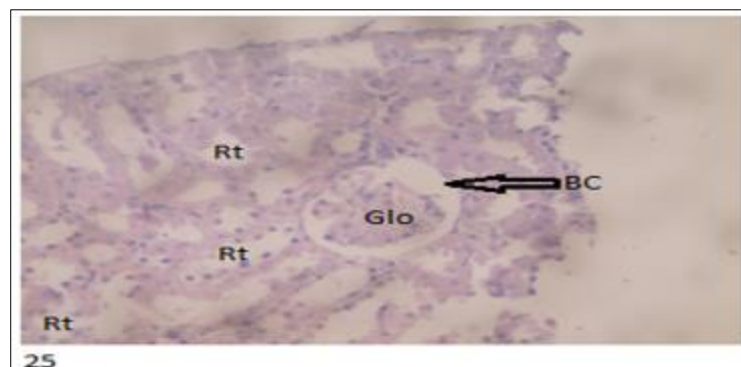


Figure 3 Photomicrograph of Kidney x400, H&E stained Group 1 – Control [3]

Histologically normal kidney showing

- Bowman’s capsular spaces (BC)
- Glomerular tuft (Glo) containing mesengial cells, capillaries and mesengial matrix.
- Renal tubules (Rt).

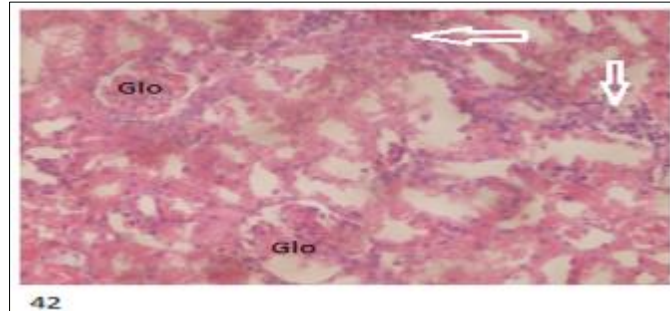


Figure 4 Photomicrograph of Kidney x400, H&E stained Group 2-Hypertensive group [3]

Histologically distorted kidney showing

- Dilated capillaries
- Inflammatory cells

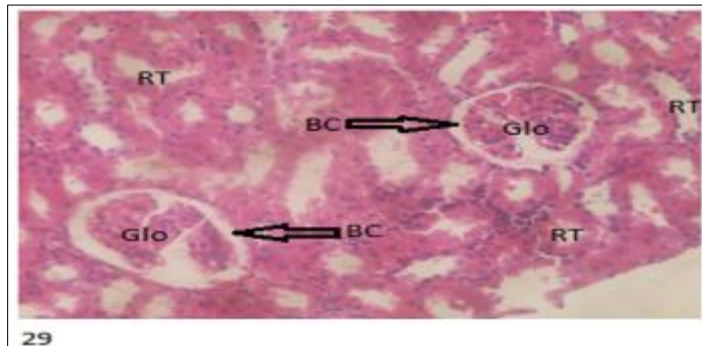


Figure 5 Photomicrograph of Kidney x400, H&E stained Group 3-Standard Drug Group [3]

Histologically normal kidney showing

- Glomerular tuft (Glo) containing mesengial cells, capillaries and mesengial matrix.
- Bowman’s capsular spaces (BC)
- Renal tubules (Rt)

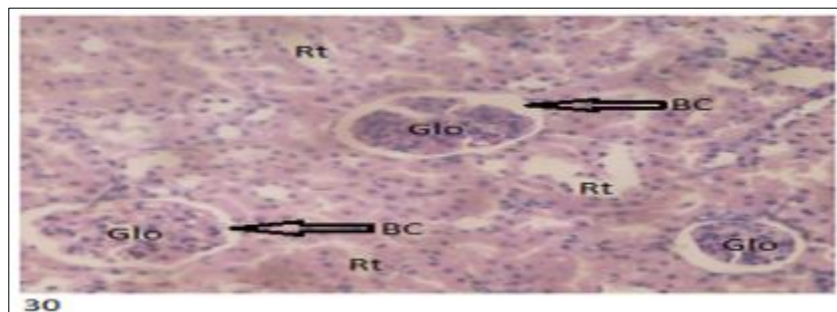


Figure 6 Photomicrograph of Kidney, x400 H&E stained Group 4 -Vernonia amygdalina methanol

Histologically normal kidney showing

- Glomerular tuft (Glo) containing mesangial cells, capillaries and mesangial matrix.
- Renal tubules (Rt).
- Bowman's capsular spaces (BC)

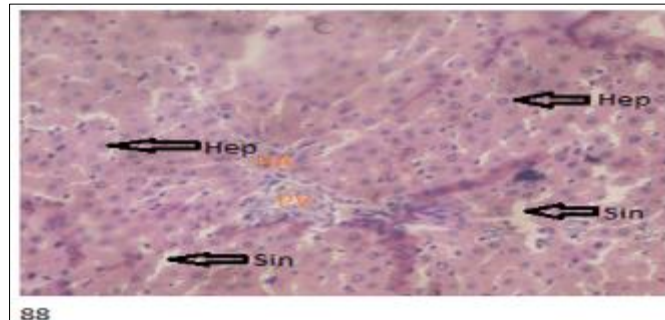


Figure 7 Photomicrograph of Liver x400, H&E stained Group 1 – Control [3]

Histologically normal Liver showing

- Cords of normal hepatocytes (Hep)
- Hepatic artery (HA) and portal vein (PV).
- Sinusoids (sin) containing capillaries and Yon Kupffer cells

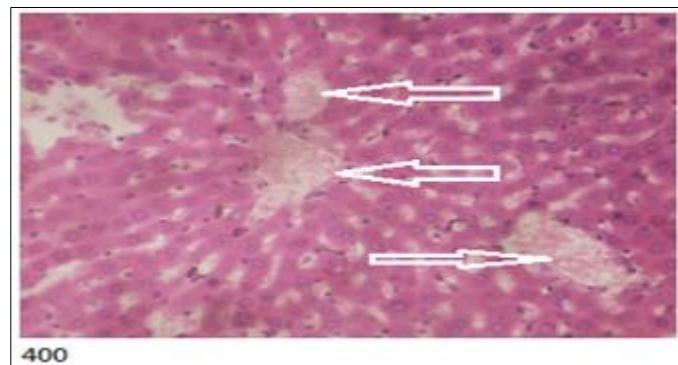


Figure 8 Photomicrograph of Liver x400, H&E stained Group 2- Hypertensive Group [3]

Histologically distorted liver showing

- patchy necrotic areas arrowed

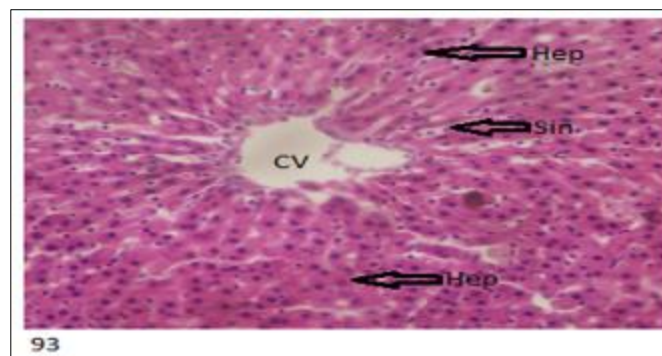


Figure 9 Photomicrograph of Liver, x400 H&E stained Group3 – Standard Drug [3]

Histologically normal liver showing

- Patent central vein
- Sinusoid (Sin)
- Normal Hepatocytes

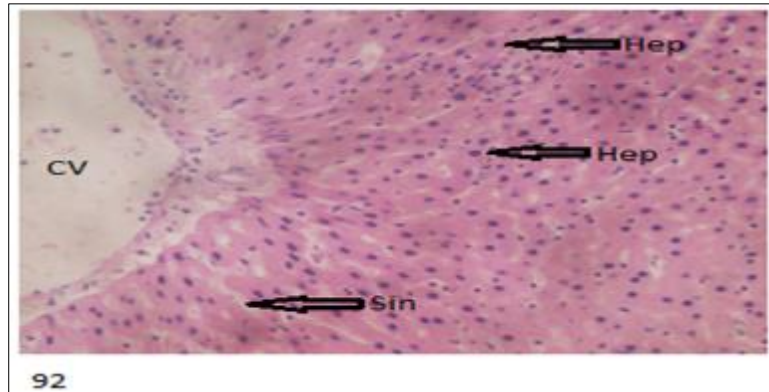


Figure 10 Photomicrograph of Liver, × 400 H&E stained Group 4 -Vernonia amygdalina-methanol

Histologically normal liver showing

- Patent central vein (CV)
- Cords of normal hepatocytes (Hep)
- Sinusoids (Sin)

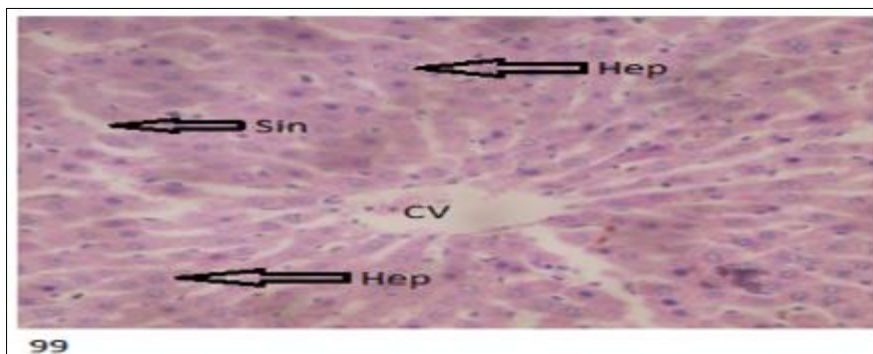


Figure 11 Photomicrograph of Liver, × 400 H&E stained Group 5 -Vernonia amygdalina- n-hexane

Histologically normal liver showing

- Sinusoids (Sin) containing capillaries and Kupffer cells.
- Patent central vein (CV)

Cords of normal hepatocytes (Hep)

Table 1 Electrolyte values of the Control Group and the different Test Groups

	Sodium (mEq\L) Mean±SEM	P-VALUE	Potassium (mEq\L) Mean±SEM	P-VALUE	Bicarbonate	P-VALUE	Chloride (mEq\L)	P-VALUE	Urea (mEq\L)	P-VALUE	Creatinine (µmol\L)	P-VALUE
1	236.33±47.55		8.52±2.17		38.83±2.28		101.29±10.67		5.28±0.35		184.84±99.05	
2	443.96±34.95	0.01*	21.80±1.44	0.00*	30.72±4.56	0.10	216.63±55.48	0.02*	4.26±0.63	0.20	212.42±47.52	0.73
3	293.73±67.95	0.46	21.36±2.73	0.01*	42.73±2.44	0.45	127.86±18.86	0.60	4.62±0.63	0.41	81.55±5.04	0.22
4	280.44±38.90	0.56	3.42±0.98	0.15	34.92±3.87	0.43	160.66±28.32	0.23	5.42±0.32	0.85	77.09±1.01	0.19
5	261.89±57.98	0.74	13.59±3.58	0.15	38.51±3.41	0.95	190.79±167.50	0.07	4.32±0.75	0.23	80.26±8.62	0.21

There was significant increase in the sodium value in the group 2; There was significant increase in the potassium value of group 3

Table 2 Electrolyte values of the Hypertensive Group and the different Test Groups

	Sodium (mEq\L) Mean±SEM	P-Value	Potassium (mEq\L) Mean±SEM		Bicarbonate	P-Value	Chloride (mEq\L)	P-Value	Urea	P-Value	Creatinine (µmol\L)	P-Value
2	443.96±34.95		21.80±1.44		30.72±4.56		216.63±55.48		4.62±0.63		81.55±5.04	
3	293.73±67.95	0.05*	21.36±2.73	0.89	42.73±2.44	0.01*	127.86±18.86	0.08	5.42±0.32	0.99	77.09±1.01	0.11
4	280.44±38.90	0.03*	3.42±0.98	0.00*	34.92±3.87	0.39	160.66±28.32	0.25	5.55±0.47	0.99	62.97±4.88	0.09
5	261.89±57.98	0.01*	13.59±3.58	0.02*	38.51±3.41	0.11	190.79±167.50	0.59	4.32±0.75	1.00	90.93±5.52	0.10

There was significant decrease in the sodium values of the groups 3, 4 and 5; There was significant decrease in the potassium levels of groups 4 and 5

Table 3 Electrolyte values of the Standard Drug Group and the different Test Groups

	Sodium (mEq\L) Mean± SEM	P-VALUE	Potassium (mEq\L) Mean± SEM	P-VALUE	Bicarbonate	P-VALUE	Chloride (mEq\L)	P-VALUE	Urea		Creatinine (µmol\L)	P-VALUE
3	280.44±38.90		21.36±2.73		42.73±2.44		127.86±18.86		5.42±0.32		77.09±1.01	
4	220.49±55.57	0.86	3.42±0.98	0.00*	34.92±3.87	0.12	160.66±28.32	0.99	5.55±0.47	0.99	62.97±4.88	0.957
5	189.69±53.12	0.68	13.59±3.58	0.03*	38.51±3.41	0.40	190.79±167.50	0.01*	4.32±0.75	0.99	90.93±5.52	0.98

There was significant decrease in the potassium values of groups 4 and 5; There was significant decrease in the chloride value of group 5

Table 4 Comparison of the Markers of Control Group and the Different Test Groups

	ALT	AST	ALK.PHOS (U/L)	TOTAL PROTEIN	ALBUMIN	TOTAL BILIRUBIN	CONJ Bilirubin
1	53.56±11.17	64.00±10.09	48.97±0.27	21.05±1.93	5.45±0.16	0.62±0.15	0.45±0.09
2	83.00±4.41	76.20±8.70	48.83±0.24	21.69±2.16	5.24±0.31	0.88±0.15	0.36±0.11
3	72.44±7.83	64.22±8.03	49.21±0.20	22.57±1.99	5.60±0.26	1.09±0.13	0.30±0.03
4	85.90±5.03	49.90±4.99	48.36±0.40	20.24±1.91	5.50±0.22	0.30±0.06	0.63±0.17
5	73.30±10.27	44.20±9.88	49.20±0.17	21.29±1.99	5.14±0.39	0.71±0.06	0.39±0.08

Table 5 Comparison of Markers of the Hypertensive Group and the Different Test Groups

	ALT	AST	ALK.PHOS (U/L)	TOTAL PROTEIN	ALBUMIN	TOTAL BILIRUBIN	CONJ Bilirubin
2	83.00±4.41	76.20±8.70	48.83±0.24	21.69±2.16	5.24±0.31	0.88±0.15	0.36±0.11
3	72.44±7.83	64.22±8.03	49.21±0.20	22.57±1.99	5.60±0.26	1.09±0.13	0.30±0.03
4	85.90±5.03	49.90±4.99*	48.36±0.40	20.24±1.91	5.50±0.22	0.30±0.06*	0.63±0.17
5	73.30±10.27	44.20±9.88*	49.20±0.17	21.29±1.99	5.14±0.39	0.71±0.06	0.39±0.08

There was significant decrease in the groups 4 and 5 in the AST values significant decrease in the total bilirubin values of groups 4

Table 6 Comparison of Markers of the Standard Drug Group and the Different Test Groups

	ALT	AST	ALK.PHOS (U/L)	TOTAL PROTEIN	ALBUMIN	TOTAL BILIRUBIN	CONJ Bilirubin
3	72.44±7.83	64.22±8.03	49.21±0.20	22.57±1.99	5.60±0.26	1.09±0.13	0.30±0.03
4	85.90±5.03	49.90±4.99	48.36±0.40	20.24±1.91	5.50±0.22	0.30±0.06*	0.63±0.17*
5	73.30±10.27	44.20±9.88	49.20±0.17	21.29±1.99	5.14±0.39	0.71±0.06*	0.39±0.08

Significant decrease in the total bilirubin levels of groups 4 and 5; There was significant decrease in the conjugated bilirubin levels of groups 4

5. Discussion

A number of physiological mechanisms are involved in the maintenance of normal blood pressure, and their derangement may play a part in the development of essential hypertension. In this study, an evaluation of protective potentials of *Vernonia amygdalina* methanol and n-hexane extracts was evaluated on some selected heart, liver and kidney of cadmium chloride-induced hypertensive albino rats, putting into consideration the fact that utilization of natural plants (leaves, nuts, seeds, and herbs) may meet the growing need for value addition regarding the maintenance of physiological condition, prevention of disease and health care promotion. Measurement of electrolyte parameters, liver markers and kidney function are some of the sure ways of diagnosis of cardiovascular diseases. The result in this study showed significant increase of sodium value in Table 1 for the hypertensive (untreated) group as the control was compared with all groups. This may have been due to volume overload and sodium retention which reached its climax as hypertension [3; 29]. Table 2 shows the comparison of Hypertensive group to other groups with significant decrease in sodium level of the Standard drug, *Vernonia amygdalina* methanol and *Vernonia amygdalina* n-hexane Groups (Groups 3, 4 and 5). This significant decrease suggest that *Vernonia amygdalina* have reversal potentials on sodium volume overload and sodium retention as observed in cadmium chloride induced hypertensive group [6]. Table 2 also showed significant decrease in potassium in the test groups 4 and 5 which signifies hypokalaemia. *Vernonia amygdalina* is seen to cause loss of potassium maybe in urine (excessive urination known as water pill/diuretics). This type ameliorate heart disease or high blood pressure, but too much potassium in the blood can damage the heart and cause a heart attack. *Vernonia amygdalina* extract showed similar strong Hepatoprotective effect as seen in Nifedipine (a drug widely known for its blood pressure lowering effect). It is further worthy of note that *Vernonia amygdalina* methanol and n-hexane extracts significantly decreased serum total bilirubin and serum conjugated bilirubin values in the experimental models as compared to negative control and standard value as seen in the study. It could be that *Vernonia amygdalina* possesses antioxidant and flavonoids capabilities.

Histological study of the kidney and liver in the control (Figure 3) showed normal kidney containing Bowman's capsular spaces, Glomerular tuft (Glo) containing mesengial cells, capillaries and mesengial matrix and Renal tubules (Rt). The liver also showed Cords of normal hepatocytes (Hep), Hepatic artery (HA) and portal vein (PV) and Sinusoids (sin) containing capillaries and Yon Kupffer cells [3, 27]. *Vernonia amygdalina* have shown its protective potential on the tested liver and kidney. The cadmium chloride-induced hypertensive untreated group in figure 4 on the other hand shown a distorted kidney showing dilated capillaries and inflammatory cells. This implies that hypertension induced mechanism of action might occur through the tubular injury pathway [3]. Figure 5 and 6 shows histologically normal kidney for the control group and *Vernonia amygdalina* methanol group as seen in figures 8, 9 and 10 respectively.

Liver markers as comparing the Control group showed no significant difference in Table 4, but in Table 5 there was significant decrease in AST and Total bilirubin for Groups 4 and 5 (*Vernonia amygdalina* groups) and significant decrease in Total bilirubin when compare the hypertensive group with other groups. There was also significant decrease in Total bilirubin of Groups 4 and 5 as seen in Table 6 when the standard drug is compared with other groups. These liver markers as seen in the above Tables 5, and 6 as compared with the control, hypertensive and standard drug groups have exhibited its protective, therapeutic and neutraceutical potentials in lowering Total bilirubin values of the study groups treated with *Vernonia amygdalina*. The test group which was treated with *Vernonia amygdalina* had no visible damage, this implies that treatment brought about tissue repair, hence methanol/n-hexane extracts of *Vernonia amygdalina* is capable of improving tissue damage in the condition of renal injury, left ventricular injury or endothelial injury that may result from hypertention [30, 3]

6. Conclusion

Vernonia amygdalina may have healing properties and potentials in the management of hypertension as shown in the treatment groups of the affected organs (heart, kidney and Liver). [27, 30, 31]

The findings of this study suggest that some biological activities of Nifedipine (standard drug) as seen in the study can be compared with that seen in *Vernonia amygdalina* for the treatment of hypertension.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest between the authors.

Statement of ethical approval

Ethical approval was sought and received from the Departmental Head and Committee.

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