



(RESEARCH ARTICLE)



## Nutraceutical from *Tetracapedium conophorum* (Walnut) Protect against cadmium chloride induced hypertension in Albino rats

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### Abstract

The use of natural plant as food, nutraceuticals consumption, phytotherapy and nutritional therapy has recently become widely used in the treatment of diseases and shown to improve health. The study evaluated the hepatoprotective and cardioprotective effect of some nutraceuticals from phytosterol diet such as *Tetracarpidium conophorum* on some parameter as found in albino rats. Cadmium chloride was used for the inducement of hypertension on the albino rats. Each study group was a total number of 10 rats in five different groups made up of the Control Group, Hypertensive Group, Standard group and 2 Test Groups (methanol and n-hexane). Renal function test was done through biochemical analysis, standard methods were used to estimate the serum Urea and Creatinine, Electrolyte and Calcium. Liver function test evaluated was Aspartate Transaminase (AST), Alanine Aminotransferase (ALT) and Alanine Phosphatase (ALP). Some lipid profile was also evaluated. One way ANOVA was used for analysis of the collected data and was expressed as Mean  $\pm$  SEM. The result of Hypertensive group showed significant increase in sodium level ( $p < 0.05$ ) when compared with Control group while significant decrease in Sodium level of Standard drug and Walnut group were observed when compared with the hypertensive Control. *Tetracarpidium conophorum* study groups exhibited significant decrease in Total Protein level, Conjugated Bilirubin level and Total Bilirubin level when compared with the Control, Hypertensive and the Standard groups. The liver and kidney tissues showed histological distorted patchy necrotic areas on the Hypertensive groups while the Negative, Standard and Test groups showed normal histological tissues/cells. This results suggest that *Tetracarpidium conophorum* possesses renoprotective, cardioprotective and hepatoprotective effect on the Wistar rats which implies that nutraceutical of the Walnut protection against Cadmium chloride induced hypertension in the Wistar rats.

**Keywords:** Hypertension; Nutraceutical; *Tetracarpidium conophorum*; Cadmium Chloride

### 1. Introduction

Plants has an abundant supply of good things in forms of flowers, fruits, corn and nuts. These micronutrients enhances healthy living and reduces the risk of disease. The [1] defined traditional medicine as the sum total of knowledge or practices whether explainable or inexplicable used in diagnosing, eliminating or preventing a mental, social or physical disease. Medicinal plants have shown to form the basis of healthcare services throughout the world, right from the earliest days of humanity and are still widely used in the international trade [2]. *Tetracarpidium conophorum* are rounded single seeded stone fruits of the walnut tree of genus *Tetracarpidium* especially the Persian or English walnut, *Juglans regia*. That of the eastern black walnut is called *Tetracarpidium conophorum*. They are high density source of nutrients particularly proteins and essential fatty acids, walnut is locally know in Nigeria as 'Ekporo' by Efik and Ibibio's

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of Cross River and Akwalbom, 'Ukpa' (Igbo), 'Awusa' or 'Asala' (Yoruba), 'Okhue' or 'Okwe' (Edo), 'Gawudibairi' (Hausa) [3]. Walnut (*Tetracarpidium conophorum*) can be taken as snacks when the unshelled nuts are boiled and cracked. A bitter taste is usually observed upon drinking water immediately after eating the nuts. The proximate composition of *Tetracarpidium conophorum* revealed that the nut is rich in protein, fat, Carbohydrate, but low in fibre and ash content [3]. The nuts have also been found to be very good sources of Vitamins A, B1, B2, B6, E, folate, sodium, potassium, manganese, copper, chloride, iron and ascorbic acid. Their green hulls or the immature fruits are good sources of vitamin C [3].

Walnut is rich in beta-sitosterol which is an aspect of phytosterols seen in other plants which is consumed as food, nuts, grains, seed and vegetable as dietary source. [4] in their study reported the effectiveness of beta-sitosterol nutraceuticals in the treatment of benign prostatic and breast cancer, anti-atherogenic and antioxidative role and hypercholesterolemia. A study revealed that beta-sitosterol and beta-sitosterol glucoside and a mixture of both possesses hyperprotective and cardioprotective properties, with possible practical application in management of cardiovascular diseases [5]. Study also showed the phytosterol solubility in triglyceride phase of margarine produces a more effective bioactivity than the crystalline form, indicated a little biological effect of beta-sitosterol [6;7]. The degree of solubility of phytosterol glycosides has raised a lot of question of its solubility by the intestinal bile salt and pancreatic enzymes. [8] in a study reported that fatty acid are cleared from glycosylated phytosterols in vitro by pancreatin. Pathogenesis and progression of cardiovascular diseases is as a result of dyslipidemia and hypertension, which are the two major factors of cardiovascular diseases [9]. The epidemiologic importance of inverse relationship in cardiovascular events and renal function, in sodium, chloride, potassium and other electrolyte handling is important in hypertension and extracellular volume expansion [10]. Therefore changes of hypertension and dyslipidemia associated parameters with nutraceutical could be useful in the control, prevention and treatment of cardiovascular disease. Due to high prevalence and incidence risk of cardiovascular disease, it is of paramount interest that cost-effective, population based, accessible and affordable therapeutics be made available for the populace. The need for the development of a safe and effective way of management of hypertension which for the centuries of years burdened the medical research and diverted attention to the therapeutic uses of medicinal plant/nuts, most especially to the commoners, patients with adverse effects and low-risk individuals [11]. Thus, the research is prompted by the fact that investigation on cardio protective and hepatoprotective effects of *Tetracarpidium conophorum* nutraceuticals, beta-sitosterol on some parameter in hypertensive rats and to compare the activities of all such parameters in all the study group.

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## 2. Material and methods

### 2.1. Identification and Preparation of Plant Materials:

Fresh nuts of *Tetracarpidium conophorum* was purchased from the market and were identified by Dr. Chimezie Ekeke of the Department of Plant Science and Biotechnology, University of Port Harcourt and with plate number given as UPH/V/307. The Walnut hard tusk was peeled off while the seeds of the walnut were harvested, completely drained, separately coarse, cut into small particles and allowed to air-dried at room temperature. They were monitored daily and shuffled to prevent decay. At the end of the three weeks, two kilogram each of all three fully dried leaves and nuts were grinded separately into powdered form using electrical blender.

### 2.2. Extraction of the blended nuts of *Tetracarpidium conophorum*

Rotary Vane Extraction process was done on the grinded *Tetracarpidium conophorum* nuts with solvents methanol and n-hexane extraction was divided into 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 in the next 5 days. The aqueous filtration was also evaporated to semi-solid state using rotary evaporator. Each 500g of blended forms was refluxed in two (2) liter volume of methanol solvent (1:4) (Adienbo et al., 2013) [12].

Basic phytochemical screening was done to detect the presence of alkaloids, flavonoids, tannins, anthraquinone, (Bontragers test), triterpenoid/steroids, fixed oils, carbohydrates, cardenolide, cyanogenic glycosides and saponins in the plants and nut extracts in accordance with [13] established procedures.

### 2.3. Animal experiment

A total number of 50 albino rats (20 males, 30 females) were used for the study. The animals were bred from the animal house of the Department of Human Physiology, Nnamdi Azikiwe University Okofia Campus. They were acclimatized for 14 days, during which they were checked with UgoBasil tail-cuff pressure measurement (this enabled the animals to be used to the equipment to avoid unnecessary agitation of the first entrance of the rat into the cuff), before the inducement

and treatment which lasted for another 35 days. The albino rats were housed in rat cage of room temperature, well ventilated, 12 hour lightened 12 hour darkness cycle. Feed and water were purchased from Pfizer Nigeria limited feeds, Benin throughout the study. The rats were used in accordance with NIH Guide for the care and use of laboratory animals revised Publication and Standard Operation Procedures (SOPs) were applied (Helsinki Declaration) while biochemical parameters and histological study of the heart, kidney and liver were done.

- **Group 1:** Control group (CG) were normal rats which were not induced nor treated with any extract but were fed only with animal feed and water on daily basis throughout the study period. **Group 2.** Hypertensive control group (HCG) which were induced with Cadmium chloride, confirmed hypertensive and were not treated throughout the study but were fed only with animal feed and water.
- **Group 3:** Standard drug group (SDG) which was induced with cadmium chloride and were treated with Nifedipine (a known hypertensive drug).
- **Group 4:** Cadmium Chloride induced hypertensive group treated with methanol *Tetracarpidium conophorum* plant extract.
- **Group 5:** Cadmium Chloride induced hypertensive group treated with n-Hexane *Tetracarpidium conophorum* plant extract.

At the end of the 4 weeks of treatment, the animals were sacrificed and blood collected by cardiac puncture into sodium fluoride and EDTA sample bottles for biochemical and haematological 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.

#### 2.4. Measurement of biochemical parameters

The biochemical analysis carried out was endocrine and kidney function test on serum electrolyte test (sodium, potassium, chloride), urea and creatinine, liver function test carried out (AST, ALT, alkaline phosphatase), metabolic system test and total protein, albumin, total bilirubin, conjugate bilirubin was analysed.

#### 2.5. Sodium (spectrophotometric method)

Labelled test tubes as blank, standard, control, test sample. Then pipette 1ml of filtrate reagent to all tubes.

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

##### 2.5.1. 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 and recorded absorbance of all tubes (unit in meg/l)

#### 2.6. Potassium reagent (dpectophotometric method)

Labelled test tubes; standard, control, test sample, blank, then pipette 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, thereafter turned the wavelength of spectrophotometer to 500nm, zero spectrophotometer with reagent blank. Read and recorded the absorbance of all tubes

#### 2.7. Chloride (automated method)

Labelled test tubes blank, calibrator, test sample. Pipette 1.5ml chloride reagent to each tubes. Added 0.01ml of calibrator or sample 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 and recorded the absorbance of all tubes (unit is Meq/l)

#### 2.8. Urea (manual spectrophotometry method)

Labelled test tubes blank, standard, test sample. Pipette (10µl) of urea reagent to reagent tubes.

Mixed and incubated all tubes at the temperature of 37°C for 10 minutes. Spectrophotometer wavelength was fixed at 546nm, temperature to 37°C. 2.5ml of water was pipetted, standard and sample to the cuvette labelled blank, standard and test sample respectively. Mixed immediately and incubated at the temp 37°C for 15minutes. Read and recorded the absorbance of the sample and standard against the blank.

### **2.9. Cratinine (Spectrophotometry Method)**

Labelled test tubes blank calibrator, text sample. 2ml of reagent was pipetted into each tube. Then 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 after 30 seconds read the absorbance  $A_1$  of the standard and sample. At exactly 2minutes, read absorbance  $A_2$  of standard and samples.

### **2.10. AST (Aspartate Aminotransferase): (Spectrophotometry Method)**

Labelled test tubes, blank, standard and test sample tubes. Pipetted 0.1M of sample into all test tubes. Added 0.5ml of reagent (1) buffer to all test tubes mixed and incubated for exactly 30 minutes at 37°C. Spectrophotometer wavelength was fixed at Hg 546nm. Pipetteed 0.5ml of  $R_2$  (2,4 dinitrophenylhydrazine) to the cuvettes test tubes respectively, added 0.1ml sample and mixed. Allowed to stand for exactly 20minutes at 20 °C to 25 °C. Then added 5.0ml of Sodium Hydroxide and mixed. Read the absorbance of the sample ( $A_{\text{sample}}$ ) against the sample blank after 5minutes.

### **2.11. ALT (Alamine Aminotransferase): (Spectrophotometry Method).**

Labelled test tubes; blank, test sample, standard. Pipetted (0.5ml) of reagent into test tubes. Added 0.1ml of 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. Added 5.0ml of sodium hydroxide and mixed, allowed to stand for exactly 20min at 20-25°C. Read the absorbance of sample ( $A_{\text{sample}}$ ) against the reagent blank after 5minutes.

### **2.12. Alkaline Phosphates (Spectrophotometry Method)**

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

### **2.13. Total Protein (TP): (Spectrophotometry Method).**

Labelled test tubes; blank, standard, patient. Pipette into cuvette test tubes 0.02ml of distilled water. Add 0.02ml of standard of CAL to all test tubes. Set the spectrophotometer wavelength of Hg 546nm at the temperature 20 to 25C. Then add 0.02ml of serum. Mix and incubate for 30mintes at the temperature of 20 °C to 25 °C. Then measure the absorbance of the sample ( $A_{\text{sample}}$ ) and of the standard ( $A_{\text{standard}}$ ) against the reagent blank.

### **2.14. Albumin (ALB): (Spectrophotometry Method)**

Label test tubes; blank, standard, patient. Pipette into test tubes 0.01ml of distilled water. Added 0.01ml of standard (cal). Set spectrophotometer wavelength at 630nm at the temperature of 20-25 °C. Then pipette into corresponding cuvette test tubes 0.01ml of serum. Add 3.00ml BCG (bromocresol green) reagent. Mixed and incubated for 5minutes at temperature of 20-25 °C.

Measured the absorbance of the sample ( $A_{\text{sample}}$ ) and of the standard ( $A_{\text{standard}}$ ) against the reagent blank.

### **2.15. Total bilirubin (Spectrophotometry Method)**

Labelled test tubes; blank, standard, test tubes. Pipette into cuvettes of distilled water

Added (R1) sulphanilic acid, added (R2) Nitrite with (R3) caffeine and sample. Allowed to stand for 10 minutes to 25C. Set the spectrophotometer wavelength at 578nm. Then added (R4) tartrate, mixed and allowed to stand for 5-30 minutes at 20-25C. Then read by measuring the absorbance of the sample against the sample blank ( $A_{\text{tb}}$ ).

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

Tissues from harvested heart, liver and kidney were fixed in 10% formalin solution and was embedded in melted paraffin wax. H&E histological techniques was implored while an Olympus light microscope were used for photomicrography on the mounted slide and was captured with Kodak digital camera.

## 3. Results and discussion

### 3.1. Effects of Serum Electrolyte, Urea and Creatinine level

**Table 1** Electrolyte levels between 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	Urea (mEq\L) P-VALUE	Urea (mEq\L) P-VALUE	Urea (mEq\L) P-VALUE	Urea (mEq\L) P-VALUE	Urea (mEq\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	260.70±50.31	0.75	14.64±2.09	0.08	41.10±3.73	0.65	51.14±15.51	0.31	5.75±0.25	0.55	283.34±129.80	0.23
5	364.20±69.62	0.10	15.18±3.187	0.06	35.80±3.90	0.55	139.83±41.33	0.45	5.36±0.96	0.92	91.02±4.56	0.27

There was significant increase in the sodium level in the group 2

**Table 2** Electrolyte levels between the Hypertensive 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 P-Value	Urea P-Value	Urea P-Value	Urea P-Value	Urea P-Value	Urea 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	260.70±50.31	0.01*	14.64±2.09	0.04*	41.10±3.73	0.03*	51.14±15.51	0.00*	4.32±0.75	0.99	80.26±8.62	0.38
5	364.20±69.62	0.30	15.18±3.187	0.06	35.80±3.90	0.55	139.83±41.33	0.12	5.36±0.96	0.03*	91.02±4.56	0.14

There was significant decrease in the sodium level of the groups 3 and 4

**Table 3** Electrolyte levels between 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 P-VALUE	Urea P-VALUE	Urea P-VALUE	Urea P-VALUE	Urea P-VALUE	Urea 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	261.89±57.98	0.66	14.64±2.09	0.06	41.10±3.73	0.74	51.14±15.51	0.99	4.32±0.75	0.99	80.26±8.62	0.02*
5	364.20±69.62	.368	15.18±3.187	.091	35.80±3.90	0.18	139.83±41.33	1.00	5.36±0.96	0.031*	91.02±4.56	0.91

There was significant increase in the Creatinine levels of group 4

**Table 4** Comparison of the different Markers Levels between 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	77.40±8.48	58.40±8.28	49.27±0.27	14.41±2.32	5.25±0.22	0.43±0.16*	0.59±0.13*
5	94.00±0.00	67.33±8.95	48.01±0.56	20.77±1.94*	5.77±0.29	0.47±0.17	0.57±0.11

There was significant decrease in the total protein levels of group 5. There was significant decrease in the total bilirubin levels of group 4

**Table 5** Comparison of the different Markers Levels between 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	77.40±8.48	58.40±8.28	49.27±0.27	14.41±2.32	5.25±0.22	0.43±0.16*	0.59±0.13
5	94.00±0.00	67.33±8.95	48.01±0.56	20.77±1.94	5.77±0.29	0.47±0.17*	0.57±0.11

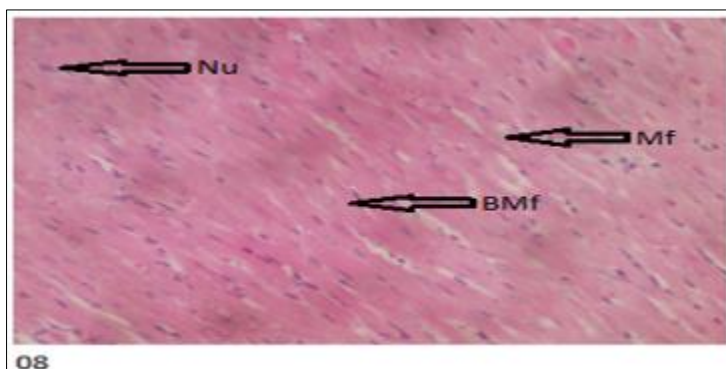
Significant decrease in the total bilirubin levels of groups 4 and 5

**Table 6** Different bio Markers Levels between 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	77.40±8.48	58.40±8.28	49.27±0.27	14.41±2.32*	5.25±0.22	0.43±0.16*	0.59±0.13*
5	94.00±0.00	67.33±8.95	48.01±0.56	20.77±1.94	5.77±0.29	0.47±0.17*	0.57±0.11

Significant decrease in the total bilirubin levels of groups 4 and 5

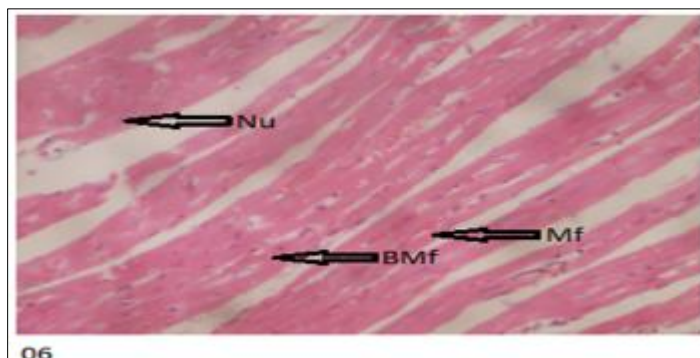
There was significant decrease in the conjugated bilirubin levels of groups



**Figure 1** Photomicrograph of Cardiac muscle (x400), H/E stain for group 1 – Control

Showing histologically normal cardiac muscle

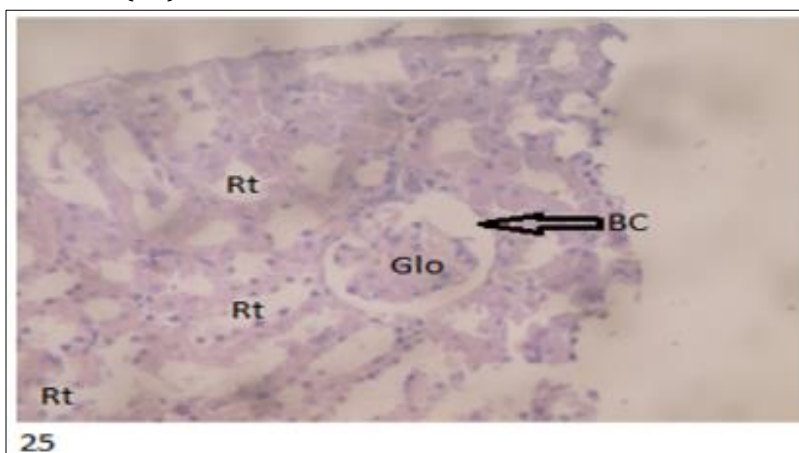
- Homogeneous muscle fiber (mf) diameter, with intact sarcolemma.
- Branching cardiac muscle fiber (Bmf).
- Peripherally placed nuclei (Nu)



**Figure 2** Photomicrograph of Cardiac muscle (x400). H/E stain for group 4 – Tetracpidium conophorum 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 stain for group 1 – control

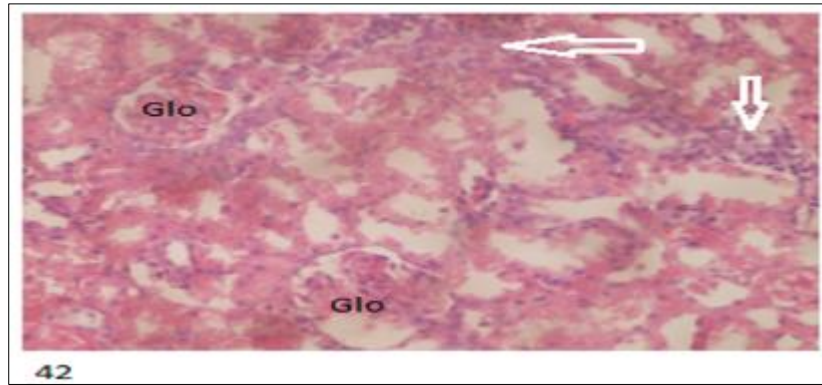
Histologically normal kidney showing

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

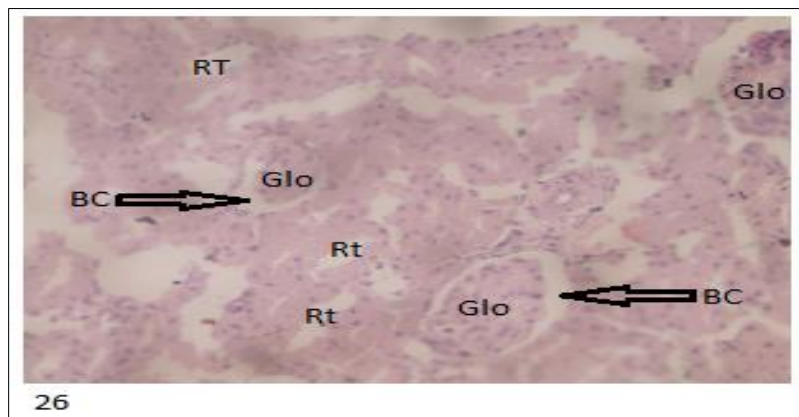
Figure 4 Histologically distorted kidney showing

- Inflammatory cells
- Dilated capillaries





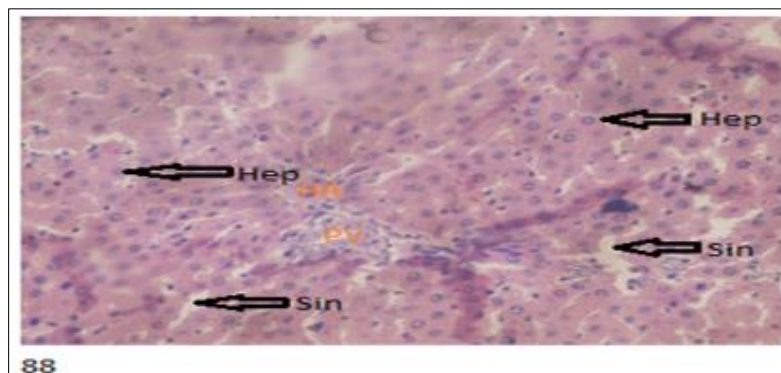
**Figure 4** Photomicrograph of Kidney (x400), H & E stain for group 2 - Hypertensive group



**Figure 5** Photomicrograph of Kidney, (x400) H & E stain for group 4 - *Tetracarpidium conophorum* methanol

Histologically normal kidney showing

- Glomeruli (Glo)
- Renal tubules (Rt).
- Bowman's capsular spaces (BC)

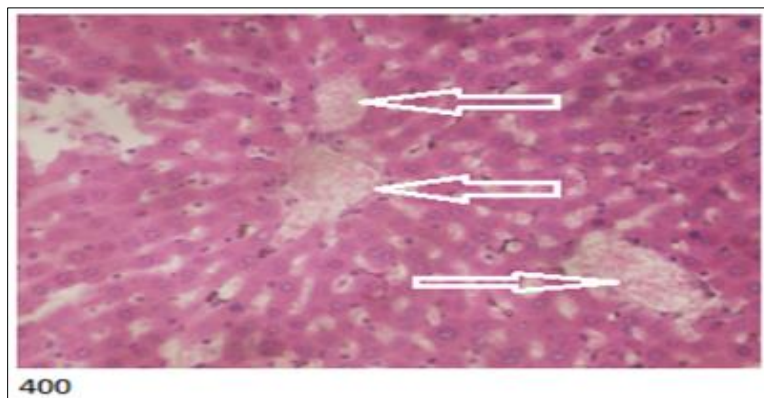


**Figure 6** Photomicrograph of Liver (x400), H & E stain for group 1 - control

Histologically normal Liver showing

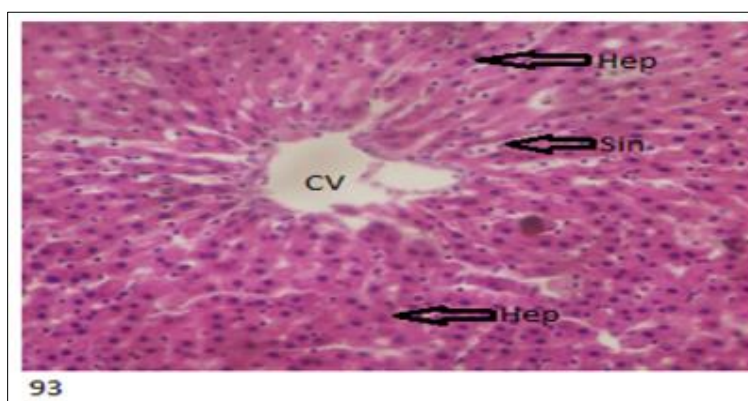
- Cords of normal hepatocytes (Hep)
- Sinusoids (sin) containing capillaries and Yon Kupffer cells
- Hepatic artery (HA) and portal vein (PV)





**Figure 7** Photomicrograph of Liver magnification  $\times 400$ , H&E stain for group 2– hypertensive group

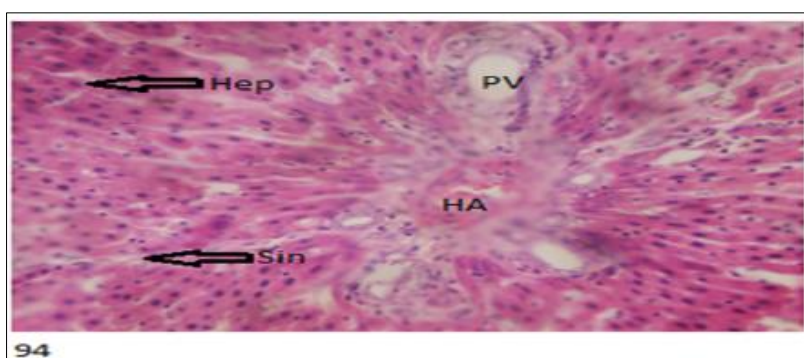
Histologically distorted liver showing patchy necrotic areas arrowed



**Figure 8** Photomicrograph of Liver, magnification  $\times 400$  H&E stain for group 3 – standard drug

Histologically normal liver showing

- Patent central vein
- Normal Hepatocytes
- Sinusoid (Sin): increased inflammatory cells



**Figure 9** Photomicrograph of Liver, magnification  $\times 400$  H&E stain of group 5 – *Tetracarpidium conophorum* n-hexane

Histologically normal liver showing

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

- Sinusoids (Sin) containing capillaries and Kupffer cells.

Electrolyte balance is important in the normal function of the body cells and organs. The measurement of electrolyte parameters such as sodium, potassium, chloride, and bicarbonate play major role in the treatment and monitoring of some health conditions such as liver markers and kidney function and disease such as hypertension, cardiovascular diseases, heart failure etc and their progression. Significant increase in sodium as seen in Table 1 (the hypertensive rats group) when compared negative control with all groups is expected and may have been due to sodium retention and volume overload resulting in the expression of hypertensive phenotype (Blaustein et. al., 2006) [14]. Hypertensive group when compared to the other groups in Table 2 showed significant decrease in sodium levels in both the standard drug group (Group 3) and methanol *Tetracarpidium conophorum* Group, unlike in the table 1, there showed a positive response from the groups treated with a known hypertensive drug and the nuts of *Tetracarpidium conophorum*. Agada and Braide, 2009 [15] noted significant independent relationships in several serum cation and hypertension.

The hypertension induce mechanism of action might occur through the tubular injury pathway as Photomicrograph of Kidney (x400), H & E stain for group 2 – Hypertensive group exhibited histologically distorted kidney with inflammatory cells and dilated capillaries. This also resulted in a significant increase in the Creatinine levels of *Tetracarpidium conophorum* group, which indicates nephrotoxic effect. [16] in their report stated that build-up of cholesterol in the blood vessel walls leads to atherosclerosis that progresses with age.

Comparison of Liver marker between the Control group and other Groups showed significant decrease in total protein level of group 5 (*Tetracarpidium conophorum* N-hexane group) and a significant decrease in Total bilirubin level of group 4 (*Tetracarpidium conophorum* methanol group). So also, when the hypertensive control is compared with other groups, both groups 4 and 5 (*Tetracarpidium conophorum* methanol group and *Tetracarpidium conophorum* N-hexane group) showed significant decrease in Total bilirubin. These liver markers as seen in the above Tables 4, 5, and 6 have shown its nutraceutical and therapeutical properties on lowering the Conjugated bilirubin, Total bilirubin and Total protein levels of the study groups. The result above in Figure 6 showed histological normal Liver with cords of normal hepatocytes (Hep), sinusoids (sin) containing capillaries and Yon Kupffer cells and hepatic artery (HA) and portal vein (PV). A study reported that phytosterols reduced significantly in triglyceride level in hypertensive condition. On the other hand, Figure 7 which is the photomicrograph of Liver magnification × 400, H&E stain for group 2- hypertensive group presented histologically distorted liver showing patchy necrotic areas arrowed. The damaged showed on the hypertensive tissue without any such damage on the negative control and standard control Figure 8 shows histologically normal liver with patent central vein, normal hepatocytes and Sinusoid (Sin): increased inflammatory cells suggest that direct target of Cadmium toxicity to be in the liver and kidney. [17] hence reported that heart and kidney section as the specific target and toxicity of Cadmium chloride.

The test group in this study as seen in figure 9 (photomicrograph of Liver) *Tetracarpidium conophorum* n-hexane shows histological normal liver with patent central vein (CV), cords of normal hepatocytes (Hep) and sinusoids (Sin) containing capillaries and Kupffer cells.

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#### 4. Conclusion

The kidney and Liver section in the treatment group of this study has no visible lesion from the above results which indicates that *Tetracarpidium conophorum* may have hepatoprotective and cardioprotective properties in the management of hypertension.

The findings of this study suggest that *Tetracarpidium conophorum* (Walnut) have some biological activities comparable to Nifedepem which served as the standard drug as was used in the study for the treatment of hypertension.

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#### Compliance with ethical standards

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##### *Disclosure of conflict of interest*

There is no conflict of interest from the both authors.

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