



## Original Research Article

# PRELIMINARY INVESTIGATION OF THE PHYTOCHEMICAL PROPERTIES OF AQUEOUS AND ETHANOLIC CRUDE EXTRACTS OF *HUNTERIA UMBELLATA* K. (SCHUM) SEEDS AND ITS ANTIHYPERTENSIVE EFFECTS ON SALT INDUCED HYPERTENSION IN WISTAR RATS

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## ABSTRACT

*The aim of this study was to investigate the preliminary phytochemical properties of aqueous and ethanolic crude extracts of Hunteria umbellata seeds and its physiological effects on salt induced hypertension in experimental animals. The phytochemical studies were carried out according to the methods of Association of Official Analytical Chemist (A.O.A.C). Twenty - five (25) female adult Wistar rats were administered with 8 % of NaCl (salt) for 2 weeks and shared into five groups of five animals in each group. The results of the quantitative phytochemical components of aqueous and ethanolic extracts revealed the presence of oxalate, phytate, tannins, flavonoids, saponins, alkaloids, phenols, cyanogenic glycoside and anthraquinones. The administration of the standard drug (propranolol) caused a reduction in high systolic blood pressure of the hypertensive experimental animals from 162.00 mmHg to 134.00 mmHg, while the diastolic pressure was observed to fall from 103.00 mmHg to 73.67 mmHg. The administration of aqueous extract of H. umbellata seeds caused a reduction of the systolic blood pressure of the hypertensive experimental animals from 159.20 mmHg to 136.25 mmHg, while the diastolic blood pressure was observed to fall from 103.80 mmHg to 80.25 mmHg. The administration of ethanolic extract of H. umbellata seeds caused a reduction in systolic blood of the hypertensive experimental animals from 160.20 mmHg to 133.00 mmHg, while the diastolic pressure was recorded to drop from 102.20 mmHg to 76.75 mmHg. The results presented here revealed the effect of ethanolic crude extract of H. umbellata on the management of blood pressure of the animals compared to the control (p<0.05). The histological results of both aqueous and ethanolic extracts of H. umbellata seeds revealed appreciable recovery of the degenerating tissues.*

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## 1. INTRODUCTION

The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed (Adeneye and Adeyemi, 2009). Diversity, flexibility, easy accessibility, broad acceptance in developing countries and increasing popularity in developed countries, relatively low cost, relative low side effects and rising economic importance are some

of the encouraging features of traditional medicines (Shan-ran and Zhong-ming, 1991). Many Africa countries find the idea of research into traditional medicine attractive, for it embodies with the hope that traditional medicines can supplement or even replace the orthodox form of healthcare, which these countries are unable to provide adequately. In recent times, there has been an increased awareness of the importance of traditional/alternative medicine in the healthcare of human and animal population in developing countries and efforts are being made to integrate them with modern orthodox medicine (Adinya *et al.*, 2012). Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Famsworth *et al.*, 1985).

*Hunteria umbellata* K. Schum (Apocynaceae) is a tree, about 15 m - 22 m in height, found in west and central Africa. In Nigeria, it is known as Osu (Edo), erin (Yoruba) and nkpokiri (Ibo). The leaves have been described as broad, abruptly acuminate and broadly lineate. The fruit is about 5 cm - 25 cm and consists of two separate globose mericaps 3 - 6 cm long, yellow, smooth, 8 - 25 seeded embedded in a gelatinous pulp (Keay *et al.*, 1964; Soforowa, 1982). Various parts of the plant have been used in herbal medicine for the treatment of diabetes, peptic ulcers, piles, yaws, dysmenorrhea, fevers, infertility, and helminthic infections (Keay *et al.*, 1964; Soforowa, 1982). Chemical constituents such as saponins, steroids, tannins, volatile oils, phenols and copious amounts of alkaloids have been reported in the fruits of *H. umbellata* (Falodun *et al.*, 2006). For example, crude alkaloids extracted from the stem bark of *Hunteria zeylanica* inhibited acute inflammation in experimental animals, antinociceptive and antipyretic effects of alkaloids extracted from the stem bark of *H. zeylanica* have also been reported (Reanmongkol *et al.*, 1994). The folk use of the fresh leaves as an oxytocic agent was recently validated and its oxytocic action mediated via muscarinic acetylcholine mechanism have been established (Falodun *et al.*, 2006). The antinociceptive and antipyretic effects of the alkaloids extracted from the stem bark of *Hunteria zeylanica* have equally been reported (Reanmongkol *et al.*, 1994). Also, the analgesic and antipyretic effect of the aqueous extract of the fruit pulp of *H. umbellata* have been investigated and proven to be effective in the regulation of pain and fever and these effects were independent of its antibacterial activities (Igbe *et al.*, 2009). *H. umbellata* has also been shown to be very active against microorganisms such as *Escherichia coli*, *Proteus* spp., and *Staphylococcus aureus*. Although the fruit pulp of *H. umbellata* has been used traditionally in the treatment of various fevers by boiling with water and subsequent drinking of the extract, there is no scientific evidence to support this therapeutic use. Since bacteria and other microorganisms cause fevers, it is possible that the usefulness of this extract is dependent on antimicrobial activity.

In South-West Nigeria, traditional birth attendants or midwives employ the fresh leaves and pulp of fresh fruits of *H. umbellata* in the indication or augmentation of labour in the gravid uterus at term. Hot and cold decoctions made from the plant seeds have also been reported to be highly valued in the local treatments of obesity, hypertension, pain, swellings, anaemia and as an immune booster (Adeneye and Adeyemi, 2009). Previous studies have reported the antihyperglycemic of *H. umbellata* (Igbe *et al.*, 2009), anti-obesity and antihyperlipidaemic (Adeneye *et al.*, 2010) effects of the crude aqueous seed extract of *H. umbellata* in rats. This study was therefore aimed at investigating the antihypertensive effects of aqueous and ethanolic extracts of *Hunteria umbellata* seeds using experimental animals.

## 2. MATERIALS AND METHODS

### 2.1. Sample Collection and Identification

The plant seeds of *H. umbellata* were purchased from a village market in Oshodin Local Government area of Lagos State, Nigeria. The plant seed was transported to Benin City, Nigeria and air dried for three weeks. Thereafter, the epicarp was removed and further air dried for three (Adinya *et al.*, 2012) weeks to obtain a constant weight. The air drying was carried out to protect the bioactive component of the plant

seed. The preliminary identification of the plant was carried out by Dr. E. I. Aigbokhan, Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

## 2.2. Preparation of Sample

The dried plant seeds of *H. umbellata* were pulverized using electronic milling machine grinder, model Lab. Mill, serial No. 4745, Christy and Norris Ltd., England. The pulverized seeds were stored in an airtight plastic container for further use.

## 2.3. Extraction of Plant Material

The extraction was carried out based on the modified method described by Igbe *et al.* (2009), in which 400 g of the powdered seeds were macerated in a sterile grinder. The macerated sample was transferred into a big bottle containing 1.5 liters of ethanol and allowed to soak for 72 h. At the end of extraction, the homogenate was filtered through a Whatman filter paper No. 1 using a glass funnel. The filtrates were labeled accordingly for subsequent use. The filtrates were further concentrated using water bath at a temperature of 80 °C to dryness. The dried aqueous extract was preserved in clean glass containers at 40 °C in a refrigerator until use.

## 2.4. Phytochemical components of *H. umbellata*

The quantitative phytochemical analyses of *H. umbellata* plant seeds were determined using the methods of Association of Official Analytical Chemists (1999). All determinations were carried out in triplicates.

## 2.5. Experimental Animals

Twenty-five (25) healthy young female albino Wistar rats weighing between 100 g and 250 g were used in this study. The rats were housed in cages in a room with proper ventilation and handled in accordance with international principles guiding the use and handling of experimental animals (United States National Institutes of Health, 1985). The experimental animals were allowed to acclimatize for two weeks and fed with pellets (growers feed) and tap water *ad libitum*. The animals were maintained in an ambient temperature between 28 °C and 30 °C, humidity of 55 ± 5 %, and standard (natural) photoperiod of approximately 12 hrs of light and 12 hrs of darkness.

## 2.6. Grouping of Animals

The animals were randomly divided into five groups of five animals each, Group I (control animals), Group II - Group V experimental animals. The body weights of the animals were obtained using a weighing scale (Gallenkamp FA2104A, England).

Twenty-five (25) animals of 5 animals per group were distributed as follows:

Group I - control (non-hypertensive), Group II- Negative control (8 % NaCl without treatment)

Group III- Positive control (hypertensive, 8 % NaCl + propranolol), Group IV- Hypertensive, 8 % NaCl + aqueous crude extract of *H. umbellata* and Group V- Hypertensive, 8 % NaCl + ethanolic crude extract of *H. umbellata*.

## 2.7. Administration of Normal Saline

The experiment was carried out in line with the guidelines on the use of animals for the experiment as issued by the Physiological Society of Nigeria and Physiological Society of London. Salt loading was done by administering 8 % NaCl diet for 14 days (Sofola *et al.*, 2002; Mojiminiyi *et al.*, 2007). The animals

were considered hypertensive when their blood pressure was recorded to be between 140/90 mmHg and 160/100 mmHg. The blood pressures of the experimental animals were taken before the start of the experiment and it was determined weekly (Simchon *et al.*, 1989).

Cage side examination was performed daily to detect overt signs of toxicity (salivation, lacrimation, chewing jaw movements, ptosis, squinting, writhing, convulsion, tremors, yellowing of fur, loss of hair, stress, erection of fur, vocalization and exophthalmia, behavioural abnormalities and dead rats) (Ratnasooriya *et al.*, 2003). After 24 hrs of the last administration, the animals were anaesthetized with chloroform and the kidneys were excised and fixed in formalin fluid for histological studies.

### **2.8. Determination of the Blood Pressure of the Experimental Animals**

The blood pressures for the experimental animals were measured using the Ugo Basil Biological Research Machine (Model No: 58500, Italy). The animals were allowed to get used to the restrainer before the blood pressure measurements were measured and determined. The animals were allowed to be relaxed in the restrainer of the blood pressure machine for about 20 mins before readings were taken

### **2.9 Determination of Histopathological Parameters of the Experimental Animals**

The hearts and kidneys of the experimental animals were harvested and preserved in 10 % formal-saline for histopathological studies. Serial sections of the formalin organs were cut (5µm thick), fixed on microscope slides, dewaxed and stained with haematoxylin and eosin according to the experimental methods of Ibeh (1998). The sections were mounted in Canada Balsam and examined under light microscope and analysed for architectural defects in the tissues using (× 40).

### **2.10. Statistical Analyses**

Data obtained were analyzed by one-way analysis of variance (ANOVA) using Student T-test to determine the significance differences in group result and Duncan's multiple range tests to locate points of significant differences (Bailey, 1981).

## **3. RESULTS AND DISCUSSION**

### **3.1. Phytochemical Components of *H. umbellata* Seeds**

The results in Table 1 revealed the presence of the phytochemical components such as oxalate, phytate, tannins, flavonoids, saponins, alkaloids, phenols cyanogenic glycoside and anthraquinones. Saponins, alkaloids and phenols were found to be present in high concentrations, which collaborates with the findings of Adeneye and Adeyemi (2009) on the research carried out on the seeds of *H. umbellata*, who reported the presence of tannins, alkaloids, cardiac glycosides, flavonoids, saponins, anthraquinone and reducing sugar with alkaloids present in high concentration. The extraction of bioactive components had led to the discovery of potent compounds with low toxicity.

### **3.2. Physical and Behavioural Observation**

From observation made, there was a difference in behavioural changes noticed between the control group and experimental groups. There were mortality, stress and abnormal behavioural changes observed within the treated groups during the salt loading periods. Overt signs of toxicity such as lacrimation, squinting and exophthalmia were not expressed by the rats throughout the period of study.

Table 1: Quantitative phytochemical constituents of *Hunteria umbellata* seeds

Parameters	Aqueous (mg/100 g)	Ethanol (mg/100 g)
Oxalate	99.0	53.0
Phytate	66.0	33.0
Tannins	2.0	4.0
Flavonoids	0.00	5.00
Saponins	128.0	91.0
Alkaloids	208.0	270.0
Phenols	81.0	379.0
Cyanogenic glycoside	36.0	55.0
Anthraquinones	84.0	110.0

Table 2: Effects of *Hunteria umbellata* on the body weight of the animals during the period of treatments

Group	BWB (g)	BWA (g)	BWT 1 (g)	BWT 2 (g)
I	160.85	169.58	184.50	190.45
II	158.24	142.54	144.98*	154.43*
III	176.00	163.86	166.02	181.07
IV	179.24	165.50	176.08	<sup>a</sup> 195.00
V	169.44	158.10	166.68	<sup>a</sup> 177.00

The values are the Means  $\pm$  SEM for five rats in each group. Significant at  $p < 0.05$ , with respect to normal control,  $^a p < 0.05$  with respect to negative control.

BWB – Body weight before administration, BWA – Body weight during administration for 2 wks, BWT 1 – Body weight during week one treatment, BWT 2 – Body weight during week two treatment

Group I – Normal control (The animals were only fed with pellets and water during the period); Group II – Negative control (8 % NaCl); Group III – Positive control (Propranolol drug); Group IV – Aqueous extract ; Group V – Ethanol extract

Table 2 shows the results of the effect the extracts of *H. umbellata* seeds on the body weights of animals during the period of treatments. Group I, (Normal Control) had their weight increased from 160.85 g before administration to 190.45 g at the end of treatments, Group II (negative control) had their weight reduced from 158.24 g before administration of 8 % NaCl to 154.43 g after administration, Group III was the standard drug group (propranolol), and their weight increased from 176.00 g to 181.07 g after administration with the conventional drug (propranolol). Group IV treated with aqueous extract had their weight increased from 179.24 g to 195.00 g. Group V treated with ethanolic extract had their increased from 169.44 g to 177.00 g. The results revealed that the seeds of *H. umbellata* possess great health benefit to the test animals and good nourishing components.

Table 3: Results of blood pressure (mmHg) of wistar rats before and after administration of treatments

Groups	Systolic (mmHg)				Diastolic (mmHg)			
	Base line	8% NaCl	Week 1	Week 2	Base line	8% NaCl BPA	Week 1	Week 2
I	125.75	129.00	126.50	127.00	77.00	73.50	77.50	78.75
II	129.20	159.40 <sup>a</sup>	152.80 <sup>a</sup>	145.00 <sup>a</sup>	73.60	102.60 <sup>b</sup>	90.20	80.75
III	128.00	162.00 <sup>a</sup>	151.40 <sup>a</sup>	<sup>c</sup> 134.33	77.20	103.00 <sup>b</sup>	87.40	<sup>d</sup> 73.67
IV	131.80	159.20 <sup>a</sup>	150.20 <sup>a</sup>	<sup>c</sup> 136.25*	76.00	103.80 <sup>b</sup>	94.00	80.25
V	129.60	160.20 <sup>a</sup>	150.60 <sup>a</sup>	<sup>c</sup> 133.00	78.80	102.20 <sup>b</sup>	87.80	<sup>d</sup> 76.75

The values are the Means  $\pm$  SEM for five rats in each group. Significant at  $^a p < 0.0001$ ,  $^* p < 0.01$ ,  $^d p < 0.05$ ,  $^b p < 0.001$  with respect to normal control,  $^c p < 0.003$ ,  $^d p < 0.05$  with respect to negative control. BPA – blood pressure during administration

Salt-induced hypertension was used to assess the antihypertensive effects of ethanolic extract of *H. umbellata* seeds. The results obtained in Table 3 showed that the administration of salt to the experimental animals led to the increase in their blood pressure and resulted to hypertension. This corroborates the

reports of Ogaiharu *et al.* (2002) on the effect of salt on blood pressure. The results obtained in this study revealed that the aqueous and ethanolic extracts were efficient and potent as an antihypertensive agent by significantly preventing the increase of blood pressure and heart rate in salt-induced hypertensive rats. Phytochemicals of glycoside moieties such as cyanogenic glycosides, saponins, anthraquinones (Ajayi and Ojelere, 2013), have been reported to play a significant role in the management of high blood pressure. This agrees with the research carried out by Adeneye and Adeyemi (2009), who reported the water decoction made from dried seeds of *H. umbellata* for the managements and treatments of hypertension, obesity, diabetes mellitus, stomach aches, pains, and swellings. The results in Table 3 revealed that the standard drug (propranolol), aqueous and ethanolic extracts were able to ameliorate the high blood pressure in the tested animals. It has also been proven from other research work that the presence of glycosides in the ethanolic extract can reduce high blood pressure (Nyarko and Addy, 1990). Propranolol inhibits the action of catecholamines, especially of epinephrine, on both  $\beta$ 1- and  $\beta$ 2-adrenergic receptors and decreases its level in the central nervous system (CNS) and in the blood, which results in the fall in blood pressure and suppressing of cardiac arrhythmias (Haddad and Winchester, 1990). Propranolol has been reported to be effective in the managements and treatments of hypertension of varying severity (Frohlich *et al.*, 1968). Hypertension has been reported as a risk factor for the emergence of cardiovascular diseases and is associated with substantial morbidity and mortality, estimated to account for about 35 % of myocardial infarction and stroke, 49 % of heart failure, and 24 % of premature mortality (Padwal *et al.*, 2001). The management of hypertension can be strengthened with the development of hypertensive drugs from *H. umbellata* seeds. It was shown in this study that *H. umbellata* could attenuate the development of salt-induced hypertension in rats due to the presence of phytochemical components present in the seeds of *H. umbellata* (Adeneye and Adeyemi, 2009; Ajayi and Ojelere, 2013). The results of the seeds of *H. umbellata* attenuating the inducement of hypertension as propounded in this study was due to the presence of phytochemicals which is in agreement with the research carried out by (Mojiminiyi *et al.*, 2007) who reported that the aqueous extract of the calyx of *Hibiscus* can attenuate high blood pressure in Wistar rats. Nyarko and Addy (1990) and Adeneye and Adeyemi (2009) reported that the presence of the necessary phytochemical components can cause a drastic fall in high blood pressure. The results from this study give a clear indication of the therapeutic potency of *H. umbellata* seeds.

Table 4: Weight of organs (heart, left and right kidneys) of wistar rats after sacrificing

Groups	Heart (g)	Left kidney (g)	Right Kidney (g)
I	0.66±0.04	0.68±0.11	0.07±0.11
II	0.74±0.03	0.64±0.40	0.56±0.04
III	0.68±0.02	0.61±0.05	0.54±0.03
IV	0.71±0.05	0.68±0.05	0.67±0.05
V	0.63±0.02	0.61±0.03	0.71±0.05

The values are the Means  $\pm$  Standard Error of Mean (SEM) for the organs of five rats in each group.  $p > 0.05$

The results in Table 4 reveals the organ weights (hearts and kidneys) of the rats after sacrificing compared to normal control. The results revealed that the weights of the organ of the induced animals were not significantly different from that of the control group. The result is in agreement with a study carried out by Ibeh *et al.* (2007) where he carried out an experiment using aqueous and ethanol extracts of *H. umbellata* on rabbits. It was observed that the extract had no effect on the organs of the rabbits, which is in conformity with this study.

### 3.3. Histological Studies on the Experimental Animals

The results of Plate 1 shows a cut section of a normal heart tissue while Plate 2 negative control, shows several architectural distortions arising from the treatments with NaCl, such as moderate vascular intimal ulceration, lumina stenosis, mural infiltrations of chronic inflammatory cells and focal myocardial degeneration. The results presented in Plate 3, treatments with standard drug (propranolol) shows recuperation from the architectural

defects noticed in Plate 2. The results presented in Plates 4 and 5, shows the tissues administered with aqueous and ethanolic extracts of *H. umbellata* seeds revealed an appreciable degree of recuperation from the histoarchitectural defects noticed in Plate 2.

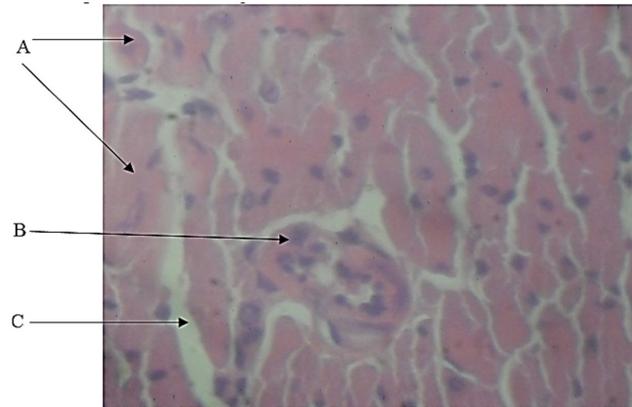


Plate 1: Control Rat heart composed of A, bundles of myocardia fibres, B, coronary vessel and C, interstitial space (H&E x 400)

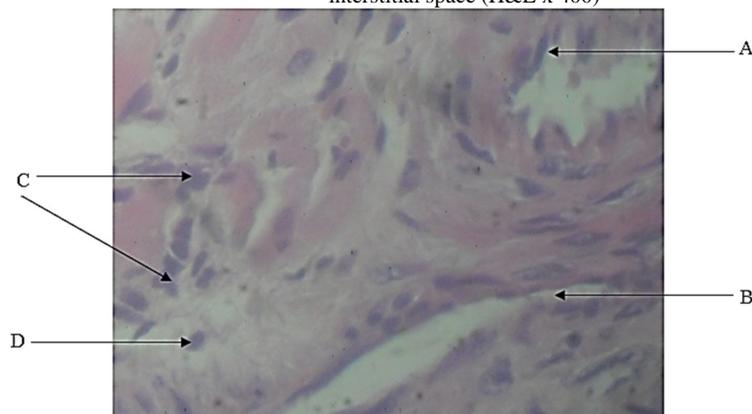


Plate 2: Rat heart induced with 8 % NaCl showing A, moderate vascular intimal ulceration, B, luminal stenosis, C, mural infiltrates of chronic inflammatory cells and D, focal myocardia degeneration (H&E x 400)

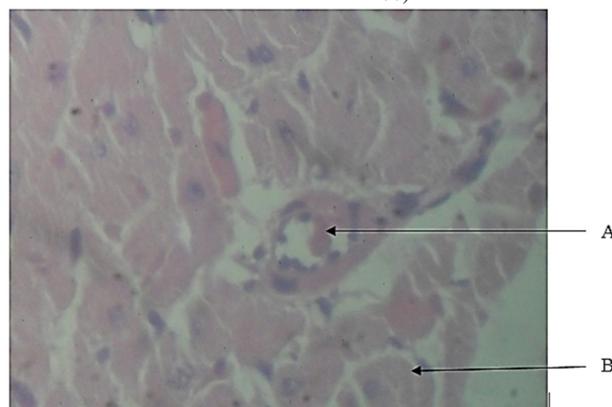


Plate 3: Rat heart induced with hypertension and treated with propranolol showing A, mild vascular congestion and B, normal myocardium (H&E x 400)

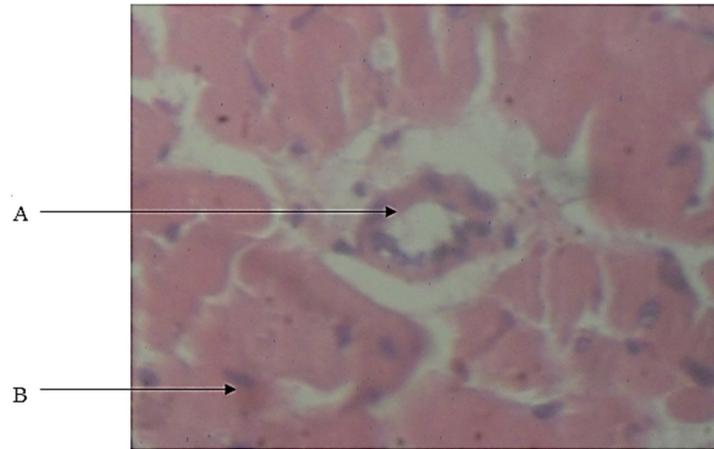


Plate 4: Hypertensive rat heart treated with aqueous extract of *H. umbellata* showing A, mild vascular dilatation and B, normal myocardium (H&E x 400)

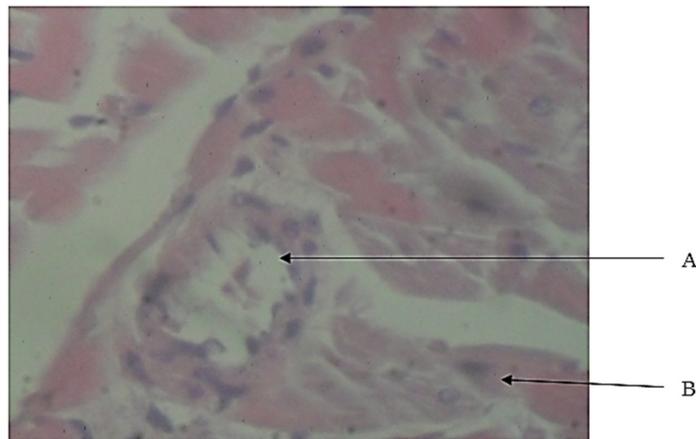


Plate 5: Hypertensive rat heart treated with ethanolic extract of *H. umbellata* showing A, moderate vasodilatation and B, normal myocardium (H&E x 400)

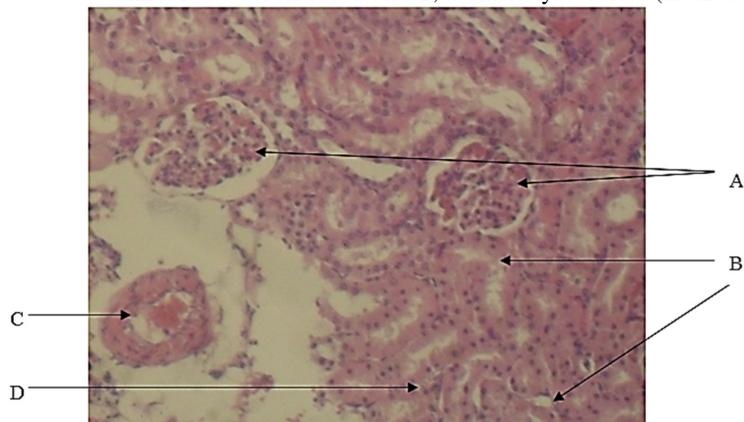


Plate 6 Control: Rat kidney composed of A, glomeruli, B, tubules, C, intercalated artery and D, interstitial space (H&E x 100)

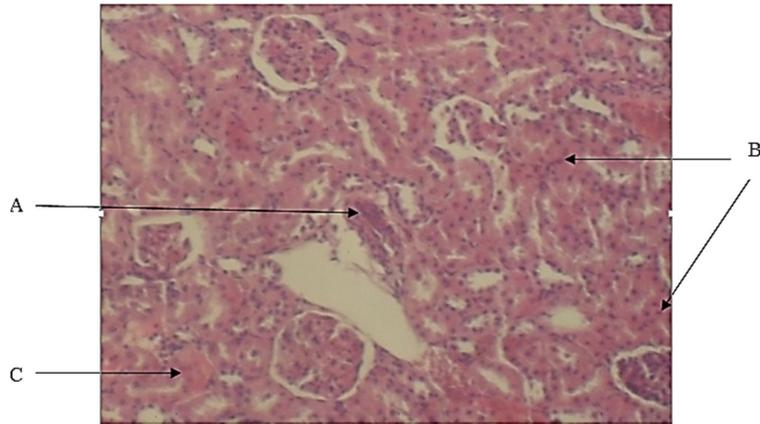


Plate 7: Rat kidney induced with 8% NaCl showing A, moderate vascular stenosis, B, patchy tubular necrosis and C, moderate interstitial congestion (H&E x 100).

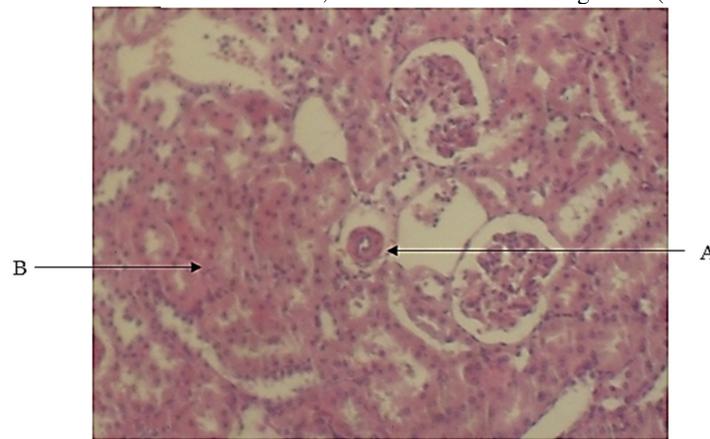


Plate 8: Hypertensive rat kidney treated with propranolol showing A, normal intercalated artery and B focal tubular necrosis (H&E x 100).

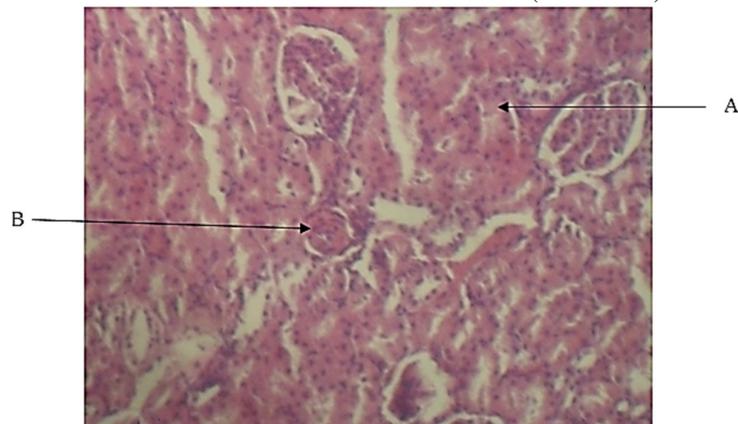


Plate 9: Hypertensive rat kidney treated with aqueous extract of *H. umbellata* showing A, focal tubular necrosis and B, moderate vascular hypertrophy (H&E x 100).

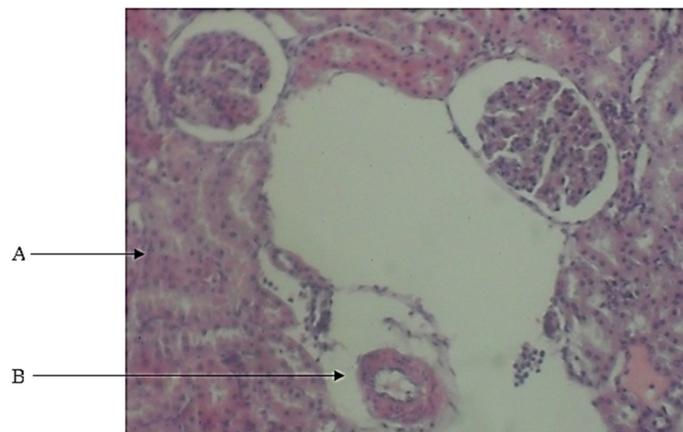


Plate 10: Hypertensive rat kidney treated with ethanolic extract of *H. umbellata* showing A, focal tubular necrosis and B, normal intercalated artery (H&E x 100).

This could be attributed to the effects of the phytochemical components present in the extracts. This is in agreement with the report of Han *et al.* (2007), who reported the significant roles of the phytochemical components such as phenols known to possess biological properties such as antiaging, antiapoptosis, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial. The results presented in Plate 6 showed the normal cut section of the kidney tissues while Plate 7 shows the kidney tissues with histoarchitectural defects such as moderate vascular stenosis, patchy tubular necrosis, and moderate interstitial congestion arising from the treatments with NaCl. The treatments with propranolol shows the repairs of distorted tissues due to salt loading (Plate 8) while Plates 9 and 10 showed the treatments with aqueous and ethanolic extracts, which revealed the repairs and recuperation of distorted tissues. The necrosis experienced by both heart and kidney tissues were not induced by intrinsic stimuli to the cells as observed in programmed cell death, but by an abrupt environmental disturbance, such as salt loading in this study, thereby causing deviations from the normal physiological conditions and functions. Gradual loss of the physiological functions of kidney over time can lead to chronic diseases like acute tubular necrosis, acute and chronic renal failure (Oladiji *et al.*, 2005). The histopathological alterations observed in this study as characterized by histoarchitectural damage to the kidneys and heart of the wistar rats were as a result of the normal saline administered to the experimental animals. This investigation confirmed that oral administration of the aqueous and ethanolic seed extracts of *H. umbellata* possessed no toxic and disruptive interference, instead helped for the regeneration of the disrupted tissues. The healing of the tissues could be attributed to the presence of the phytochemicals in the seeds of *H. umbellata* as reported by Nyarko and Addy (1990).

#### 4. CONCLUSION

Medicinal plants contain some organic compounds which are able to produce definite physiological action on the human body. The results obtained in this study revealed the presence of medicinally active constituents in *H. umbellata* seeds. The results showed that *H. umbellata* seeds were able to ameliorate hypertension induced with NaCl in rats. Both aqueous and ethanolic extracts of *H. umbellata* seeds were effective in ameliorating the induced hypertension in the experimental animals. The result further showed that the organs (hearts and kidneys) treated with aqueous and ethanolic extracts of *H. umbellata* seeds experienced encouraging recuperation just like those treated with standard drug (propranolol). There was a clear indication of the therapeutic potency of *H. umbellata* seeds. Therefore its continuous usage should therefore be encouraged, while further work should be carried out to isolate, purify and possibly characterize the active constituents responsible for its activity.

## 5. CONFLICT OF INTEREST

There is no conflict of interest associated with this work

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