International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 11 Nov. 2022, pp: 410-424 www.ijaem.net ISSN: 2395-5252

Effect of Garlic and Coconut extracts on some biochemical parameters of wistar rats with L-NAME-induced hypertension

Ezeonwumelu, E. C.[1], Omeodu, S. I.[2], and Monanu, M. O.[3]
[1.2.3] *Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria.*

Submitted: 05-11-2022 Accepted: 15-11-2022

ABSTRACT: This study evaluated the effect of garlic (Allium sativum) extract and coconut (Cocos nucifera) sap on some biochemical parameters in N-nitro-L-arginine methyl ester (L-NAME)-induced hypertensive Wistar rats. The study compared the blood samples of the normal control, test control, reference drug, and test group subjects. Seventy two adult female wistar rats weighing between 150-200g were divided into twelve groups of six animals each and fed with high fat diet and induced with L-NAME which resulted in hypertension. Qualitative and quantitative analysis was carried out to determine the phytochemical composition of the extracts. Group 1 were fed with standard diet and water ad libitum. Group 2 were induced but not treated. Group 3 were orally administered the Reference Drug (Eplerenone) while Groups 4-12 were administered 100mg/kg, 250mg/kg, and 500mg/kg of the extracts and the mixture of the extracts respectively. Blood serum samples were analyzed using Randox and Agappe kits, and an autoanalyser to test for various parameters. The overall results revealed a significant difference at 95% level of confidence interval ($p<0.05$) in the parameters in a dose-dependent manner. The 500mg/kg body weight dose of the extract was most effective compared to the other body weight doses as seen in the values while there was a significant difference ($p<0.05$) in Eplerenone with values in mmol/l of 4.65 ± 0.13^{a}, 95.50 ± 0.76^{b}, 21.50 ± 0.43^{b}, 4.27 ± 0.12^{a} for potassium ion (K^{+}), chloride (Cl^{-}), bicarbonate ion (HCO_3^{-}), and urea (U) respectively compared to Allium sativum extract values of 4.13 ± 0.08^{c}, 97.00 ± 0.58^{b}, 23.00 ± 0.37^{b}, and 4.02 ± 0.10^{ac} for K^{+}, Cl^{-}, HCO_3^{-}, and U respectively, and Cocos nucifera extract values of 4.00 ± 0.07^{c}, 96.40 ± 0.68^{b}, 22.60 ± 0.51^{b}, and 4.12 ± 0.12^{a} for K^{+}, Cl^{-}, HCO_3^{-}, and U respectively confirming that Eplerenone works best at managing hypertension in comparison to the two plants extracts stated. This study revealed that Eplerenone is the preferred anti-hypertensive substance.

I. INTRODUCTION

Hypertension is defined as either systolic pressure consistently at 140 or higher or a diastolic pressure consistently at 90 or higher (Halliday, 2012). Hypertension is classified according to its causes into three types; primary (essential) accounts for over 90% of cases and is often referred to as idiopathic, since the underlying cause is unknown. This type has an insidious onset with few, if any, symptoms, so it is often not recognised until complications have occurred. Secondary hypertension results from a number of conditions that impair blood pressure regulation, particularly renal, endocrine, vascular, and neurological disorders, hypertensive disease of pregnancy (formally known as toxaemia); and use of estrogen containing oral contraceptive and some other drugs. A sever acceleration form of hypertension, malignant hypertension, results from either type and can cause blood pressure as high as 240/150mmHg, possibly leading to coma and death (National Institutes of health, 2004; Sommers and Johnson, 2007).

Studies over the years by many scientists have shown promising returns for application of numerous plants materials as extracts in the treatment of various ailments (Wang et al., 2007). In recent years, garlic (Allium sativum) has been thought to be effective in the treatment of hypertension.

Coconut (Cocos nucifera) sap comes from the cavity of the fruit of the Cocos nucifera palm, which is grown throughout the world in tropical climates. Cocos nucifera sap is approximately 95 percent water, 4 percent carbohydrates, 0.1 percent fat, 0.02 percent calcium, 0.01 percent phosphorous, and 0.5 percent iron, and contains amino acids, vitamin C, B complex vitamins, and

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 11 Nov. 2022, pp: 410-424 www.ijaem.net ISSN: 2395-5252

minerals, including sodium and magnesium (Jean et al., 2009). Cocos nucifera sap may contain more than 600 mg of potassium per serving, an amount higher than one serving of just about any other food or beverage. Due to its high potassium content, Cocos nucifera sap has been studied for its effects in controlling high blood pressure (Alleyne et al., 2005).

Eplerenone, sold under the brand name Dosterep, is a steroidal antimineralocorticoid of the spirolactone group that is used as an adjunct in the management of chronic heart failure and high blood pressure, particularly for patients with resistant hypertension due to elevated aldosterone. Classed as a selective aldosterone receptor antagonist (SARA), (Delyani, 2000), it is similar to the diuretic spironolactone, though it is much more selective for the mineralocorticoid receptor in comparison (i.e., does not possess any antiandrogen, progestogen, glucocorticoid, or estrogenic effects), and is specifically marketed for reducing cardiovascular risk in patients following myocardial infarction.

1.1 Aim and Objectives of the Study

Aim: The aim of this study is to determine a single and combined effect of Allium sativum extract and Cocos nucifera sap on some biochemical parameters in the treatment of hypertensive cases using L-NAME-induced hypertension model in Wistar rats.

Objectives: The objectives of the study are to:

1. Use the stated experimental model for the study of hypertension in order to further the advancement in this field.
2. Estimate the serum electrolyte levels in order to ascertain the effect of Allium sativum extract and Cocos nucifera sap generated in L-NAME induced hypertensive Wistar rat.
3. Compare the effectiveness of these natural plants source (Allium sativum and Cocos nucifera) with a reference drug (Eplerenone) known for its effectiveness in the treatment or management of hypertension.
4. Use dose variation and synergistic effect as a measure to combat or manage resistance in hypertension.

1.2 Significance of the Study

Over the years, Allium sativum and Cocos nucifera sap have been used as spice and drink respectively, which have generated a lot of interest throughout human history as a medicinal panacea. This research would be particularly relevant in our society where importance of Cocos nucifera sap as drink and Allium sativum as spice in food is underutilized and their cultivations are considered not important (Eric, 2010).

1.3 Scope of the Study/Delimitation

This study would be limited to animal model (use of Wistar rats) and the scope of investigation in the study would include:

(1) Renal function parameters: Electrolytes which are Sodium ion (Na^+), Potassium ion (K^+), Chloride (Cl^-), and Bicarbonate ion (HCO_3^-), Urea, and Creatinine.

(2)

1.4 Study Area

Choba community is located along the East-West road and lies between Longitude 6°45 and 7°30´ East and Latitude 4°31 and 5°00´ North in the South South, Nigeria surrounded by vegetation. Farming, fishing, and trading are the major occupations of the inhabitants of Choba in Obio-Akpor local government area (LGA), a LGA in Port Harcourt, the capital city of Rivers State, Nigeria. Rivers State was formed in 1967 by the Federal Government of Nigeria. Rivers State covers a land area of 21,850 sq. km.

Figure 1: Map of the Study Area (Google)

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 11 Nov. 2022, pp: 410-424 www.ijaem.net ISSN: 2395-5252

II. MATERIALS AND METHODS

1.5 Experimental Design

The following approach was employed in grouping the animals; the animals were divided into three (3) groups: control Groups A and B, and Group C.

Group A: This group consisted of 6 Wistar rats, was fed with standard diet and water ad libitum, and served as normal control.

Group B: This group consisted of 6 Wistar rats, was administered N-nitro-L-arginine methyl ester (L-NAME) for the purpose of inducing hypertension but was not treated, and served as test control.

Group C: This group was divided into 10 sub-groups which consisted of 6 Wistar rats each. For Allium sativum bulb and Cocos nucifera sap extracts, the animals received ethanolic Allium sativum extract (100 mg/kg, 250 mg/kg, and 500 mg/kg of body weights in 28 days using the median lethal dose [LD_{50}]), Cocos nucifera sap extract (100 mg/kg, 250 mg/kg, and 500 mg/kg of body weights in 28 days using LD_{50}), and a mixture of both ethanolic Allium sativum extract and Cocos nucifera sap extract at 100 mg/kg, 250 mg/kg, and 500 mg/kg of body weights in 28 days using LD_{50}.

Acute Toxicity (LD_{50}): LD_{50} of the Allium sativum extract and Cocos nucifera sap extract on female Wistar rats was determined orally using the method described by Alaribe et al., (2012). These Wistar rats were divided into four groups of four wistar rats each weighing between 100g and 200g. The Wistar rats were subjected to 24 hours fasting (with only water) before administration of extracts. The Allium sativum bulb and Cocos nucifera sap extracts were dissolved in 20% between-30 and administered in doses of 100, 250, 500 and 1000mg/kg body weight orally. The fifth group served as the control and received only 20% between-30. The Wistar rats were then observed for toxicity and fatalities within 72 hours.

No mortality was recorded after 72 hours in both categories of extracts and as well all through the four concentrations so chosen, though, certain attributes such as paw licking, restiveness, aggressive behaviors and at times extreme calmness were also observed. Loss of weight associated with reduction in food consumption was observed in groups administered with 500mg/kg and 1000mg/kg doses.

The doses of Allium sativum bulb and Cocos nucifera sap extracts were decided on the basis of LD_{50} (Banerjee et al., 2001).

2.2 Determination of LD_{50}

Table 2.1 This group was divided into 10 sub-groups consisting of 6 Wistar rats each.

S/N	TREATMENT	INDUCTION L-NAME									
		GROUP	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10
1	10mg/kg of Reference Drug (Eplerenone)		YES									
2	100mg/kg of Allium sativum bulb extract			YES								
3	100mg/kg of Cocos nucifera sap extract				YES							
4	100mg/kg of Allium sativum bulb extract + 100mg/kg of Cocos nucifera sap extract					YES						
5	250mg/kg of Allium						YES					

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 11 Nov. 2022, pp: 410-424 www.ijaem.net ISSN: 2395-5252

	sativum bulb extract					
6	250mg/kg of Cocos nucifera sap extract	YES				
7	250mg/kg of Allium sativum bulb extract + 250mg/kg Cocos nucifera sap extract		YES			
8	500mg/kg of Allium sativum bulb extract			YES		
9	500mg/kg of Cocos nucifera sap extract				YES	
10	500mg/kg of Allium sativum bulb extract + 500mg/kg of Cocos nucifera sap extract					YES

The extracts used were Allium sativum bulb and Cocos nucifera sap while the reference drug that was used was Eplerenone which is a standard drug.

2.3 Collection of Test Materials

Allium sativum was purchased from Aluu market, Port Harcourt while Cocos nucifera was harvested from a Coconut tree growing near Faculty of Pharmacy, University of Port Harcourt. Both test materials were identified in the Herbarium of the Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria with Identification numbers UPH/P/262 for Allium sativum and UPH/P/261 for Cocos nucifera.

2.4 Preparation of Plants extract:

2.4.1 Allium sativum extract preparation

(a) Allium sativum was derived, the cloves peeled, sliced into pieces, placed in 40% ethanol, blended to increase surface area, and kept for 12 hours to extract. Afterwards, it was sieved and filtered into a container.

(b) The filtrates were evaporated to a syrupy residue using a rotary evaporator at 40°C. The dishes were then weighed and the weight of extract was calculated as follows:

(i) Weight of extract = weight of evaporating dish after evaporation – weight of evaporating dish before extract addition e.g. extract weight = (71–51)g = 20g.

(ii) The extracts were pooled together into an airtight container and stored at -4°C until required for use.

(iii) The dry concentrate was weighed and dissolved in different volumes of distilled water from which different concentrations of the extract was derived and given to the animals at different time intervals.

2.4.2 Cocos nucifera sap preparation

Mature Cocos nucifera sap would be used for this for the study. Seven (7) dehusked mature Cocos nucifera was found and harvested from a tree growing near the Faculty of Pharmacy, University of Port Harcourt. The mature Cocos nucifera was already dehusked and Cocos nucifera sap was collected through the pores bore from one

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 11 Nov. 2022, pp: 410-424 www.ijaem.net ISSN: 2395-5252

end, the sap was filtered and refrigerated, and ready for use. The relative density of the sap to water was calculated to measure the volume of the sap that was administered to each animal per kg body weight. The known density of a fluid such as water was used to calculate the unknown density of the sap on three dilutions.
Qualitative and quantitative analysis was carried out to determine the phytochemical composition of the Allium sativum bulb and Cocos nucifera sap extracts.

2.5 Phytochemical screening

The Phytochemical screening of the Allium sativum bulb and Cocos nucifera sap extracts were carried out by the following method used by Sofowara (1993). The phytochemical analysis examined the presence of the following bioactive compounds in the plants extracts: alkaloids, anthraquinones, glycosides, flavonoids, saponins, steroids, tannins, and terpenoids. About 0.5-2g ethanolic and sap extracts of Allium sativum and Cocos nucifera respectively were mixed with carious reagents depending on the bioactive compounds to be investigated. The change on colouration determines the presence or absence of certain bioactive compounds.

2.6 Ethical Consideration

Before the commencement of this study, ethical approval was obtained from the University of Port Harcourt research ethics committee (REC) with a given REC number: UPH/CEREMAD/REC/MM74/005

2.7 Experimental Animals:

The Wistar rat (Rattus norvegicus) was used.

2.7.1 Animals care and handling

(a) A total of 72 female Wistar rats (three months old) were purchased from animal house at the University of Port Harcourt, Rivers State.
(b) The animals were weighed. The weight of the Wistar rats ranged from 150-200g. They were housed in stainless steel cages (6 rats per cage), marked for easy identification, and were kept in a well-ventilated room. Habituation condition was 25–32°C and relative humidity of 45±5% with 12 hours light and dark cycle.
(c) The animals were allowed to feed with standard diet (Livestock Feeds Nig. Ltd., Ikeja, Nigeria) and water ad libitium, and acclimatized for 14 days.
(d) Approved standard guidelines for the care and handling of laboratory animals (including applying humane actions during sacrifice) was adhered to.

2.8 Methods

2.8.1 Measurement of Body Weights

A weighing balance was used. Each rat was placed on the weighing balance and the weight displayed on the screen was recorded.

2.8.2 Induction and Administration

The induction and administration was all via oral route and lasted for a period of 56 days (28 days for induction and 28 days for administration [treatment]). At the end of the process, the animals were anaesthetized with chloroform and blood sample was collected through cardiac puncture. The blood sample was used for biochemical analysis.
8 ml of blood sample was obtained from each Wistar rat into lithium heparin bottles for biochemical assays. Sera bottles were separated into plastic vials and refrigerated at 2° to 8°C until required for use.

2.8.3 Duration

Acclimatization lasted for 14 days. The animal research lasted for a minimum of 10 weeks while the entire study which involved feasibility study and preparation of extracts lasted for 5-6 months.

2.8.4 Sample Collection and Preparation

Whole blood sample collection was by cardiac puncture and the biochemistry samples were collected into plain and heparinized bottles respectively. Both sets of bottle were labeled accordingly. The plain bottles were allowed to stand for 30 minutes to clot to obtain serum. Both sets of bottle were centrifuged at 3,000 rpm for 10 minutes for proper separation, separated into plastic vials and labeled accordingly. These vials were refrigerated until when needed for biochemical analysis.

2.8.5 Determination of Biochemical parameters:

2.8.5.1 Lipid Profile assays

(1) CHOL, TG, and HDL CHOL concentration was determined using Randox reagent kits (Ayakeme et al., 2015) and lipid panel (a simple blood test) method.
(2) LDL CHOL concentration was determined by difference according to the formula described by Friedwald equation (Ayakeme et al., 2015).

2.8.5.2 Renal Function parameters:

(1) Na^+ level was determined using SPECTRUM318 test kit (Egyptian Company for Biotechnology (S.A.E), Egypt). Na^+ was estimated by colorimetric method which resulted in a

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 11 Nov. 2022, pp: 410-424 www.ijaem.net ISSN: 2395-5252

chromophore of directly varying absorbance as the Na^+ concentration in the test sample.
(2) K^+ level was estimated by turbidity method using Elabscience test kit (Elabscience, USA). K^+ was estimated by turbidometric method. The extent of turbidity is proportional to the K^+ concentration and is measured photometrically at 578 nm (Engelbrecht and McCoy, 2002).
(3) Cl^- level was determined using Agappe chloride test kit (Agappe Diagnostics, Switzerland) and colorimetric method.
(4). H_2CO_3 level was determined by titrimetric method. The H_2CO_3 reagent utilizes the enzymatic method (Cheesbrough, 2006).
(5) Urea and creatinine levels were determined using Agappe urea and creatinine test kits, and using the Berthelot method and colorimetric method respectively.

2.9 Histopathology
Histological examination of the organs (the liver and kidney) of the experimental animals was conducted, and the findings obtained as shown below.

2.10 Statistical Analysis
From the obtained experimental data, statistical analysis was carried out to aid in the results interpretation and discussion using the appropriate statistical tools. The statistical analysis was done using:
(a) SPSS version 22.0 (IBM, U.S.A).
(b) Values which were considered significant at $p \leq 0.05$

The data was analyzed using one-way analysis of variance (ANOVA) and significant differences were determined using post Hoc Duncan multiple comparison test ($p<0.05$).

The results were considered significant at 95% confidence level. The values were represented as mean ± standard error of mean (SEM) and data obtained was analyzed using the SPSS. Data was shown as mean + SEM and displayed in tables. Difference in mean of parameters was compared using ANOVA.

III. RESULTS

3.1 Phytochemical composition of Allium sativum and Cocos nucifera extracts

a. Qualitative phytochemistry of the bulbs of Allium sativum and sap of Cocos nucifera

The secondary metabolic content of any food substance are essentially responsible for the therapeutic effects exerted by these food substances. To ascertain the kind of phytochemical constituents present in the Allium sativum and Cocos nucifera extracts, its phytochemistry was analysed and result indicated that the two parts investigated possess varying degree of alkaloids, anthraquinones, glycosides, flavonoids, saponins, steroids, tannins, and terpenoids. Table 3.1a below showed that both the bulbs and sap are low in all the named phytochemicals except for anthraquinone which is absent in the sap of Cocos nucifera. This is an indication of the plants low cytotoxicity abilities.

Table 3.1a: Qualitative phytochemistry of the bulbs of Allium sativum and sap of Cocos nucifera

S/N	Phytochemicals	Qualitative
	Allium sativum bulbs	
1	Alkaloids	+
2	Anthraquinones	+
3	Glycosides	+
4	Flavonoids	+
5	Saponins	+
6	Steroids	+
7	Tannins	+
8	Terpenoids	+
	Cocos nucifera sap	
1	Alkaloids	+
2	Anthraquinones	-
3	Glycosides	+
4	Flavonoids	+
5	Saponins	+
6	Steroids	+
7	Tannins	+
8	Terpenoids	+

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 11 Nov. 2022, pp: 410-424 www.ijaem.net ISSN: 2395-5252

Legend: - = absent; + = present

b. Quantitative phytochemistry of the bulbs of Allium sativum and sap of Cocos nucifera

To further quantify the phytochemical constituents of Allium sativum and Cocos nucifera, quantitative phytochemistry was carried out. Results showed that 3.27 ± 0.03^{a} and 2.37 ± 0.11^{b} of alkaloids were present in bulbs and sap of Allium sativum and Cocos nucifera respectively, the indication that alkaloid is present more in bulbs than in sap. The bulbs of Allium sativum contain 0.48 ± 0.01^{a} of anthraquinone while anthraquinone is absent (0.00 ± 0.00^{b}) in the sap of Cocos nucifera. The glycoside content of the bulbs was 0.98 ± 0.07^{a} while the sap content was 1.15 ± 0.01^{a}. Flavonoids were also higher in the bulbs, 4.60 ± 0.04^{a}, than in the sap, 3.44 ± 0.12^{b}, respectively. 3.70 ± 0.01^{a} and 0.94 ± 0.03^{b} of saponin were present in bulbs and sap of Allium sativum and Cocos nucifera respectively, the indication that saponin is far more present in bulbs than in sap. The bulbs of Allium sativum contain 1.50 ± 0.09^{a} of steroid while steroid content is 2.00 ± 0.07^{b} in the sap of Cocos nucifera. The tannin content of the bulbs was 5.78 ± 0.07^{a} while the sap content was 1.04 ± 0.02^{b}. Terpenoids were also low in the bulbs, 1.07 ± 0.03^{a}, than in the sap, 3.01 ± 0.08^{b}, respectively as shown in Table 3.1b below.

Table 3.1b: Quantitative phytochemistry of the Allium sativum bulbs and the Cocos nucifera sap

Phytochemicals	**Allium sativum bulbs**	**Cocos nucifera sap**
Alkaloids	3.27 ± 0.03^{a}	2.37 ± 0.11^{b}
Anthraquinones	0.48 ± 0.01^{a}	0.00 ± 0.00^{b}
Glycosides	0.98 ± 0.07^{a}	1.15 ± 0.01^{a}
Flavonoids	4.60 ± 0.04^{a}	3.44 ± 0.12^{b}
Saponins	3.70 ± 0.01^{a}	0.94 ± 0.03^{b}
Steroids	1.50 ± 0.09^{a}	2.00 ± 0.07^{b}
Tannins	5.78 ± 0.07^{a}	1.04 ± 0.02^{b}
Terpenoids	1.07 ± 0.03^{a}	3.01 ± 0.08^{b}

Values are means ± Standard Error of Mean (SEM). Values with different superscripts are significantly different at $p<0.05$. Superscript (a,b) compares Cocos nucifera to Allium sativum.
Note: Values were analyzed using Student T test.

Table 3.2 The average percentage mortality of Allium sativum bulb and Cocos nucifera sap extracts administered orally in the Wistar rats during acute toxicity study.

Group	Dose (mg/kg)	Total No. of wistar rats used	No. of Deaths in bulbs	No. of Deaths in sap	Mortality %
1	100	4	0	0	0
2	250	4	0	0	0
3	500	4	0	0	0
4	1000	4	0	0	0

*Group 5 comprising 4 Wistar rats served as control (feed and water), no dose was administered. Total no. of Wistar rats used = 20 where no. represents number.

3.2 - Renal profile of the Wistar rats

3.2.1 – Sodium and Potassium ions

The test control, reference drug, 100mg/kg body weight, 250mg/kg body weight, and 500mg/kg body weight values of the Allium sativum bulb extract, Cocos nucifera sap extract, and mixture of both extracts for the sodium ion (Na^{+}) and potassium ion (K^{+}) was significantly lower and higher ($p<0.05$) respectively than that of the normal control as shown in Table 3.3 below.

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 11 Nov. 2022, pp: 410-424 www.ijaem.net ISSN: 2395-5252

Table 3.3 Sodium and potassium ions for the normal control, test control, reference drug, and test groups

PARAMETER	Sodium ion (Na^+) (mmol/l)			Potassium ion (K^+) (mmol/l)		
GROUP	Allium sativum bulb extract	Cocos nucifera sap extract	Mixture of both extracts	Allium sativum bulb extract	Cocos nucifera sap extract	Mixture of both extracts
Normal Control	139.33 ± 0.71[a]	139.33 ± 0.71[a]	139.33 ± 0.71[a]	3.95 ± 0.08[a]	3.95 ± 0.08[a]	3.95 ± 0.08[a]
Test Control	129.25 ± 1.11[b,c]	129.25 ± 1.11[b,c]	129.25 ± 1.11[b,c]	5.10 ± 0.09[b,c]	5.10 ± 0.09[b,c]	5.10 ± 0.09[b,c]
Reference Drug	134.17 ± 0.65[b,d,e]	134.17 ± 0.65[b,d,e]	134.17 ± 0.65[b,d,e]	4.65 ± 0.13[b,c,e]	4.65 ± 0.13[b,c,e]	4.65 ± 0.13[b,c,e]
100mg/kg body weight	134.40 ± 0.87[b,d,e,g,k]	132.20 ± 0.73[b,c,e,g,k]	134.20 ± 0.66[b,c,e,g,k]	4.66 ± 0.16[b,c,e,g,k]	4.88 ± 0.09[b,c,e,g,k]	4.72 ± 0.15[b,c,e,g,k]
250mg/kg body weight	134.60 ± 0.51[b,d,e,g,i,k]	133.80 ± 0.73[b,d,e,g,i,k]	134.60 ± 0.75[b,d,e,g,i,k]	4.22 ± 0.12[a,d,e,g,i,k]	4.14 ± 0.07[a,d,f,h,i,k]	4.30 ± 0.10[a,d,e,g,i,k]
500mg/kg body weight	135.83 ± 0.65[b,d,e,g,i,l]	136.60 ± 0.75[a,d,e,h,i,l]	137.00 ± 0.37[b,d,e,g,i,l]	4.13 ± 0.08[a,d,f,h,i,l]	4.00 ± 0.07[a,d,f,h,i,l]	4.03 ±0.07[a,d,f,h,i,l]

Values are means ± Standard Error Mean (SEM). Values with different superscripts are statistically different ($p<0.05$). Superscript (a,b) compares test control, reference drug, 100mg/kg body weight, 250mg/kg body weight, and 500mg/kg body weight to Group 1 (normal control) (1st letters) along the column. Superscript (c,d) compares reference drug, 100mg/kg body weight, 250mg/kg body weight, and 500mg/kg body weight to test control (2nd letters) along the column. Superscript (e,f) compares 100mg/kg body weight, 250mg/kg body weight, and 500mg/kg body weight to reference drug (3rd letters) along the column. Superscript (g,h) compares 250mg/kg body weight and 500mg/kg body weight to 100mg/kg body weight (4th letters) along the column, superscript (i,j) compares 500mg/kg body weight to 250mg/kg body weight (5th letters) along the column while superscript (k,l) compares Cocos nucifera sap extract and mixture of both extracts to Allium sativum bulb extract (6th letters) across the row.

3.2.2 – Chloride and Bicarbonate ions

The test control, reference drug, 100mg/kg body weight, 250mg/kg body weight, and 500mg/kg body weight values of the Allium sativum bulb extract, Cocos nucifera sap extract, and mixture of both extracts for the chloride ion (Cl^-) and bicarbonate ion (HCO_3^-) was significantly lower ($p<0.05$) than that of the normal control as shown in Table 3.4 below.

Table 3.4 Chloride and bicarbonate ions for the normal control, test control, reference drug, and test groups

PARAMETER	Chloride ion (Cl^-) (mmol/l)			Bicarbonate ion (HCO_3^-) (mmol/l)		
GROUP	Allium sativum bulb extract	Cocos nucifera sap extract	Mixture of both extracts	Allium sativum bulb extract	Cocos nucifera sap extract	Mixture of both extracts
Normal Control	100.00 ± 0.86[a]	100.00 ± 0.86[a]	100.00 ± 0.86[a]	25.17 ± 0.65[a]	25.17 ± 0.65[a]	25.17 ± 0.65[a]
Test Control	86.50 ± 1.71[b,c]	86.50 ± 1.71[b,c]	86.50 ± 1.71[b,c]	20.00 ± 0.71[b,c]	20.00 ± 0.71[b,c]	20.00 ± 0.71[b,c]
Reference Drug	95.50 ± 0.76[b,d,e]	95.50 ± 0.76[b,d,e]	95.50 ± 0.76[b,d,e]	21.50 ± 0.43[b,c,e]	21.50 ± 0.43[b,c,e]	21.50 ± 0.43[b,c,e]
100mg/kg body weight	93.20 ± 0.66[b,d,e,g,k]	91.80 ± 0.86[b,d,e,g,k]	92.80 ± 0.73[b,d,e,g,k]	20.60 ± 0.60[b,c,e,g,k]	19.60 ± 0.68[b,c,e,g,k]	20.00 ± 0.71[b,c,e,g]
250mg/kg body weight	94.00 ± 0.71[b,d,e,g,i,k]	94.40 ± 0.51[b,d,e,g,i,k]	95.20 ± 0.86[b,d,e,g,i,k]	22.00 ± 0.71[b,c,e,g,i,k]	21.80 ± 0.73[b,c,e,g,i,k]	21.40 ± 0.51[b,c,e,g,i,k]
500mg/kg body weight	97.00 ± 0.58[a,d,e,g,i,l]	96.40 ± 0.68[a,d,e,h,i,l]	97.50 ± 0.67[a,d,e,h,i,l]	23.00 ± 0.37[a,c,e,g,i,l]	22.60 ± 0.51[a,c,e,g,i,l]	22.83 ± 0.95[a,c,e,g,i,l]

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 11 Nov. 2022, pp: 410-424 www.ijaem.net ISSN: 2395-5252

Values are means ± Standard Error Mean (SEM). Values with different superscripts are statistically different ($p<0.05$). Superscript (a,b) compares test control, reference drug, 100mg/kg body weight, 250mg/kg body weight and 500mg/kg body weight to Group 1 (normal control) (1st letters) along the column. Superscript (c,d) compares reference drug, 100mg/kg body weight, 250mg/kg body weight, and 500mg/kg body weight to test control (2nd letters) along the column. Superscript (e,f) compares 100mg/kg body weight, 250mg/kg body weight, and 500mg/kg body weight to reference drug (3rd letters) along the column. Superscript (g,h) compares 250mg/kg body weight and 500mg/kg body weight to 100mg/kg body weight (4th letters) along the column, superscript (i,j) compares 500mg/kg body weight to 250mg/kg body weight (5th letters) along the column while superscript (k,l) compares Cocos nucifera sap extract and mixture of both extracts to Allium sativum bulb extract (6th letters) across the row.

3.2.3 – Urea and Creatinine

The test control, reference drug, 100mg/kg body weight, 250mg/kg body weight, and 500mg/kg body weight values of the Allium sativum bulb extract, Cocos nucifera sap extract, and mixture of both extracts for the urea (U) and creatinine (Cr) was significantly higher ($p<0.05$) than that of the normal control as shown in Table 3.5 below.

Table 3.5 Urea and creatinine for the normal control, test control, reference drug, and test groups

	Urea (U) (mmol/l)			Creatinine (Cr) (mmol/l)		
PARAMETER GROUP	Allium sativum bulb extract	Cocos nucifera sap extract	Mixture of both extracts	Allium sativum bulb extract	Cocos nucifera sap extract	Mixture of both extracts
Normal Control	2.92 ± 0.25^{a}	2.92 ± 0.25^{a}	2.92 ± 0.25^{a}	67.17 ± 1.19^{a}	67.17 ± 1.19^{a}	67.17 ± 1.19^{a}
Test Control	$5.38 \pm 0.24^{b,c}$	$5.38 \pm 0.24^{b,c}$	$5.38 \pm 0.24^{b,c}$	$96.50 \pm 3.12^{b,c}$	$96.50 \pm 3.12^{b,c}$	$96.50 \pm 3.12^{b,c}$
Reference Drug	$4.27 \pm 0.12^{b,d,e}$	$4.27 \pm 0.12^{b,d,e}$	$4.27 \pm 0.12^{b,d,e}$	$78.33 \pm 1.76^{b,d,e}$	$78.33 \pm 1.76^{b,d,e}$	$78.33 \pm 1.76^{b,d,e}$
100mg/kg body weight	$4.72 \pm 0.17^{b,c,e,g,k}$	$5.04 \pm 0.09^{b,c,e,g,k}$	$4.78 \pm 0.15^{b,c,e,g,k}$	$85.80 \pm 1.66^{b,d,e,g,k}$	$85.40 \pm 1.72^{b,d,e,g,k}$	$84.00 \pm 2.00^{b,d,e,g,k}$
250mg/kg body weight	$4.50 \pm 0.12^{b,d,e,g,i,k}$	$4.40 \pm 0.11^{b,d,e,g,i,k}$	$4.38 \pm 0.12^{b,d,e,g,i,k}$	$79.80 \pm 1.16^{b,d,e,g,i,k}$	$81.40 \pm 1.36^{b,d,e,g,i,k}$	$80.80 \pm 0.86^{b,d,e,g,i,k}$
500mg/kg body weight	$4.02 \pm 0.10^{b,d,e,g,i,l}$	$4.12 \pm 0.12^{b,d,e,h,i,l}$	$4.02 \pm 0.11^{b,d,e,h,i,l}$	$78.33 \pm 0.95^{b,d,e,g,i,l}$	$80.80 \pm 2.24^{b,d,e,g,i,l}$	$80.33 \pm 0.76^{b,d,e,g,i,l}$

Values are means ± Standard Error Mean (SEM). Values with different superscripts are statistically different ($p<0.05$). Superscript (a,b) compares test control, reference drug, 100mg/kg body weight, 250mg/kg body weight, and 500mg/kg body weight to Group 1 (normal control) (1st letters) along the column. Superscript (c,d) compares reference drug, 100mg/kg body weight, 250mg/kg body weight, and 500mg/kg body weight to test control (2nd letters) along the column. Superscript (e,f) compares 100mg/kg body weight, 250mg/kg body weight, and 500mg/kg body weight to reference drug (3rd letters) along the column. Superscript (g,h) compares 250mg/kg body weight and 500mg/kg body weight to 100mg/kg body weight (4th letters) along the column, superscript (i,j) compares 500mg/kg body weight to 250mg/kg body weight (5th letters) along the column while superscript (k,l) compares Cocos nucifera sap extract and mixture of both extracts to Allium sativum bulb extract (6th letters) across the row.

3.2.4 Renal profile of the Wistar rats from all the different groups

The test control, reference drug, 100mg/kg body weight, 250mg/kg body weight, and 500mg/kg body weight values of the Allium sativum bulb extract, Cocos nucifera sap extract, and mixture of both extracts for the Na^{+}, Cl^{-}, and HCO_3^{-} was significantly lower ($p<0.05$) than that of the normal control while the test control, reference drug, 100mg/kg body weight, 250mg/kg body weight, and 500mg/kg body weight values of the Allium sativum bulb extract, Cocos nucifera sap extract, and mixture of both extracts for the K^{+}, U, and Cr was significantly higher ($p<0.05$) than that of the normal control as shown in Table 3.6 below.

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 11 Nov. 2022, pp: 410-424 www.ijaem.net ISSN: 2395-5252

Table 3.6 Renal profile of the normal control, test control, reference drug, and test groups

PARAMETER GROUP	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	HCO_3^- (mmol/l)	U (mmol/l)	Cr (mmol/l)
Normal Control	139.33 ± 0.71[b]	3.95 ± 0.08[c]	100.00 ± 0.86[b]	25.17 ± 0.65[b]	2.92 ± 0.25[c]	67.17 ± 1.19[c]
Test Control	129.25 ± 1.11	5.10 ± 0.09[a]	86.50 ± 1.71[c]	20.00 ± 0.71	5.38 ± 0.24[a]	96.50 ± 3.12[a]
Reference Drug	134.17 ± 0.65	4.65 ± 0.13[a]	95.50 ± 0.76[b]	21.50 ± 0.43[b]	4.27 ± 0.12[a]	78.33 ± 1.76[a]
100mg/kg Allium sativum bulb extract	134.40 ± 0.87	4.66 ± 0.16[a]	93.20 ± 0.66[b]	20.60 ± 0.60	4.72 ± 0.17[a]	85.80 ± 1.66[a]
100mg/kg Cocos nucifera sap extract	132.20 ± 0.73	4.88 ± 0.09[a]	91.80 ± 0.86[b]	19.60 ± 0.68[c]	5.04 ± 0.09[a]	85.40 ± 1.72[a]
100mg/kg Mixture of both extracts	134.20 ± 0.66	4.72 ± 0.15[a]	92.80 ± 0.73[b]	20.00 ± 0.71	4.78 ± 0.15[a]	84.00 ± 2.00[a]
250mg/kg Allium sativum bulb extract	134.60 ± 0.51	4.22 ± 0.12[ac]	94.00 ± 0.71[b]	22.00 ± 0.71[b]	4.50 ± 0.12[a]	79.80 ± 1.16[a]
250mg/kg Cocos nucifera sap extract	133.80 ± 0.73	4.14 ± 0.07[ac]	94.40 ± 0.51[b]	21.80 ± 0.73[b]	4.40 ± 0.11[a]	81.40 ± 1.36[a]
250mg/kg Mixture of both extracts	134.60 ± 0.75	4.30 ± 0.10[ac]	95.20 ± 0.86[b]	21.40 ± 0.51[b]	4.38 ± 0.12[a]	80.80 ± 0.86[a]
500mg/kg Allium sativum bulb extract	135.83 ± 0.65[b]	4.13 ± 0.08[c]	97.00 ± 0.58[b]	23.00 ± 0.37[b]	4.02 ± 0.10[ac]	78.33 ± 0.95[a]
500mg/kg Cocos nucifera sap extract	136.60 ± 0.75[b]	4.00 ± 0.07[c]	96.40 ± 0.68[b]	22.60 ± 0.51[b]	4.12 ± 0.12[a]	80.80 ± 2.24[a]
500mg/kg Mixture of both extracts	137.00 ± 0.37[b]	4.03 ± 0.07[c]	97.50 ± 0.67[b]	22.83 ± 0.95[b]	4.02 ± 0.11[a]	80.33 ± 0.76[a]

Values were expressed as Means ± SEM. Values in the same column with the superscript letter 'a' were significantly higher ($p<0.05$) than that of the normal control. Values with the superscript letter 'b' were significantly higher ($p<0.05$) than that of the test control while values with the superscript letter 'c' were significantly lower ($p<0.05$) than that of the reference drug.

3.5 Histopathology

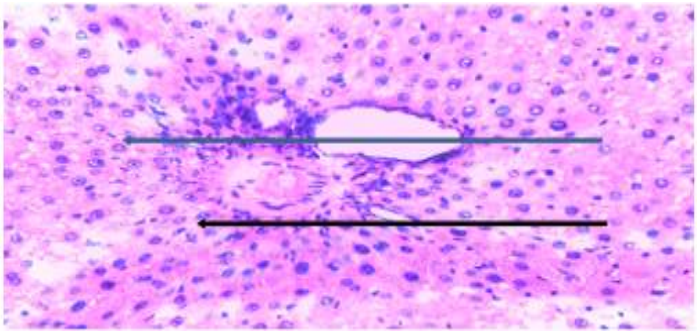

Plate 3.1 (H & E ×20)

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 11 Nov. 2022, pp: 410-424 www.ijaem.net ISSN: 2395-5252

Kidney architecture of normal control rat for day 28
Histology: Sections of the kidney show normal glomeruli (blue) and normal tubules (black). Normal kidney.

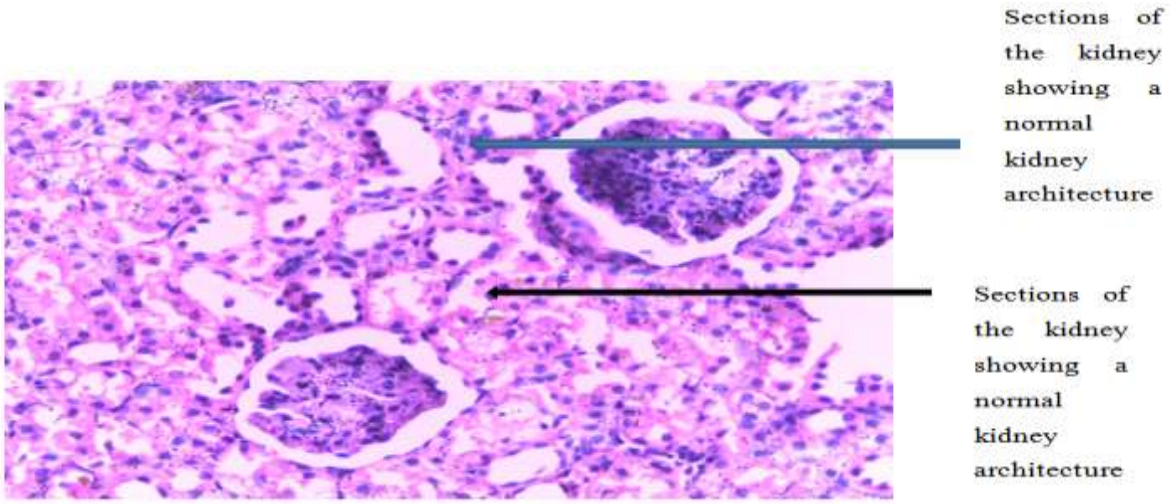

Plate 3.2 (H & E ×20)

Kidney architecture of test control rat for day 28
Histology: Sections of the kidney show collapsed glomeruli (blue) and tubular necrosis (black).
Diagnosis: severe kidney damage

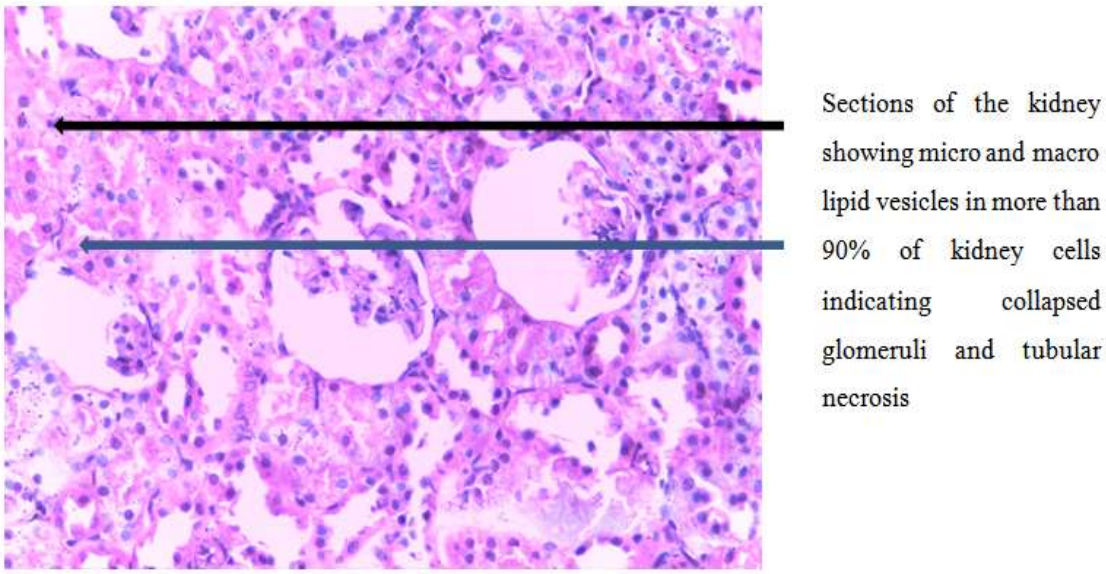

Plate 3.3 (H & E ×20)

Kidney architecture of rat treated with Reference drug for day 28
Histology: Sections of the kidney show collapsed glomeruli (blue) and tubular necrosis (black).
Diagnosis: severe kidney damage

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 11 Nov. 2022, pp: 410-424 www.ijaem.net ISSN: 2395-5252

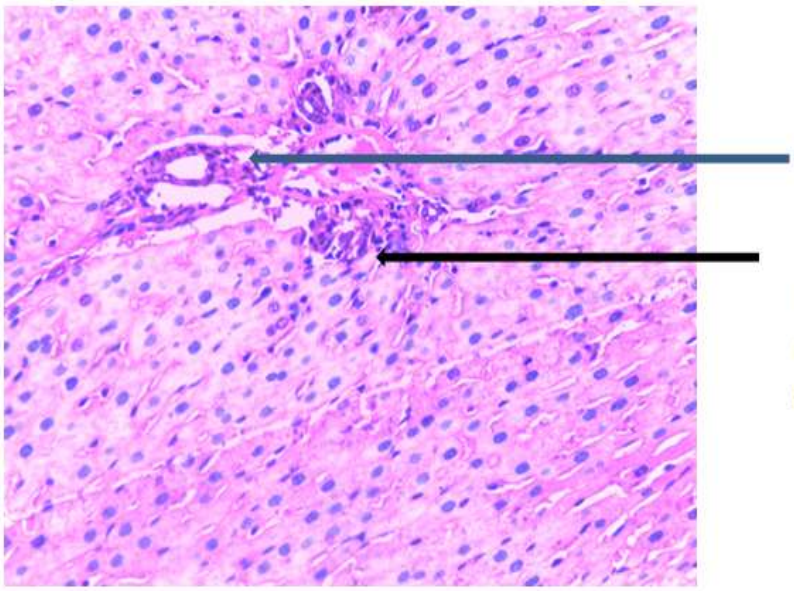

Plate 3.4 (H & E ×20)

Kidney architecture of rat treated with 100mg/kg Allium sativum + Cocos nucifera for day 28
Histology: Sections of the kidney show normal appearing glomeruli (blue) and normal tubules (black).
Diagnosis: normal kidney

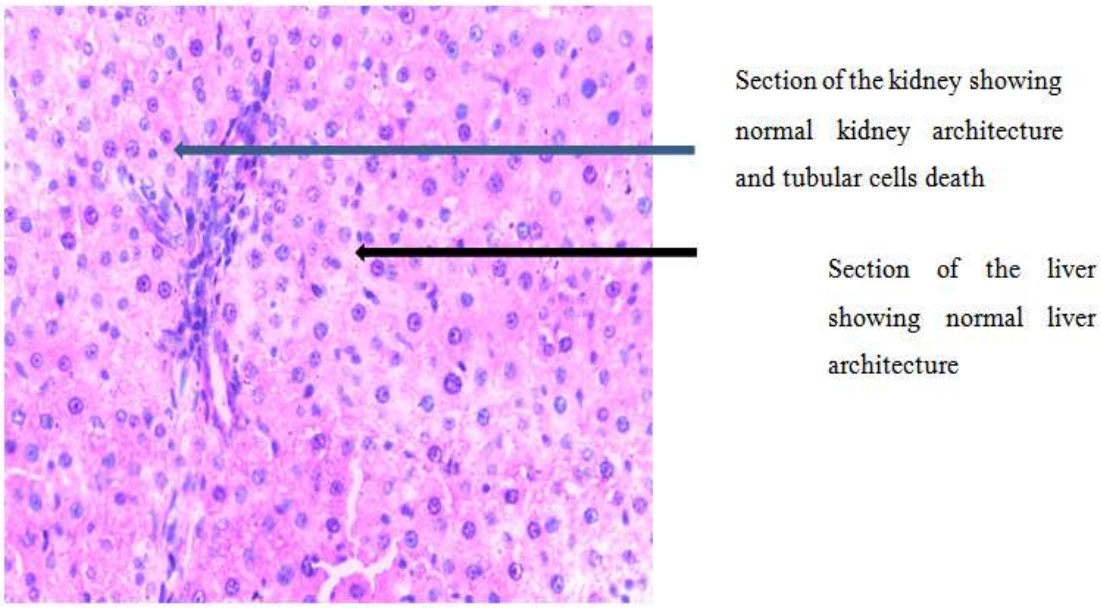

Plate 3.5 (H & E ×20)

Kidney architecture of rat treated with 250mg/kg Allium sativum + Cocos nucifera for day 28
Histology: Sections of the kidney show normal glomeruli (black) and focal death of tubular cells (blue).
Diagnosis: moderate kidney damage

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 11 Nov. 2022, pp: 410-424 www.ijaem.net ISSN: 2395-5252

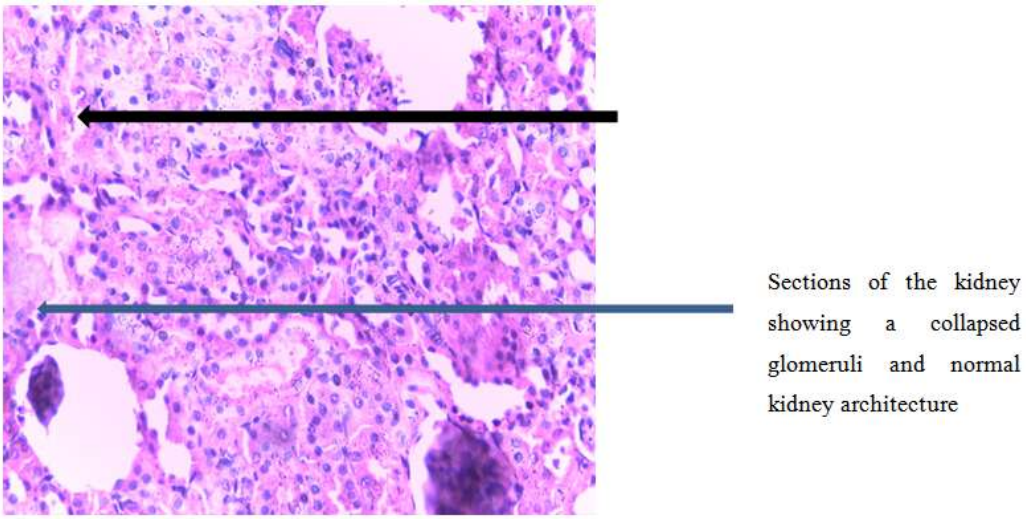

Plate 3.6 (H & E ×20)

Kidney architecture of rat treated with 500mg/kg Allium sativum + Cocos nucifera for day 28
Histology: Sections of the kidney show collapsed glomeruli (black) and normal tubules (blue).
Diagnosis: severe kidney damage

Key
For Kidney,

1. Arrow shows lipid vesicles in kidney cells
2. Less than 5% kidney cell involvement = normal kidney
3. 5% to 33% kidney cell involvement = mild kidney damage
4. 34% to 66% kidney cell involvement = moderate kidney damage
5. Greater than (>) 66% kidney cell involvement = severe kidney damage

IV. DISCUSSION

As a result of the complexity in ascertaining an individual predisposed to hypertension, it likewise, gets arduous to interpret the L-NAME animal model of inducing hypertension. According to Lerman et al. (2019), the use of rat model of hypertension is never questionable, and a clear experimental model of hypertension should be considered. In view of this, this study is aimed at investigating the effect of garlic bulb and coconut sap extracts on stated biochemical parameters of wistar rats induced with hypertension using L-NAME. There is a correlation between hypertension and atherosclerosis (also known as arteriosclerosis and hardening of the arteries) of subjects (Lerman et al., 2019). Atherosclerosis happens when arteries become narrow and hard due to a buildup of plaque around the artery wall. The physical stress of hypertension on the arterial wall also results in the aggravation and acceleration of atherosclerosis, particularly of the coronary and cerebral vessels. Moreover, hypertension appears to increase the susceptibility of the small and large arteries to atherosclerosis (Lerman et al., 2019). Due to the fact that high blood pressure increases the risk of atherosclerosis, it raises the chance of developing all the complications of atherosclerosis, such as: Heart attack, stroke, and peripheral arterial disease. Blood pressure itself is continuous, but the measurement of it would appear to be discrete because of the limit on accuracy which can be recorded (Lerman et al., 2019). Overweight or obese subjects are more likely to have a high systolic blood pressure. Though previous research has suggested high blood pressure may be more dangerous for thinner subjects, a new study finds the cardiovascular disease risks are similar - and high – for the lean, overweight and the obese (Doyle, 2015). Other risk factors such as diabetes, heredity and gender are also correlated to hypertension but were not considered in this work. Hypertension has a direct correlation with an increased risk of visceral fat deposition (Sironi et al., 2004) and visceral fat reduction (Guo et al., 2019). This is in line with our research.

There are a number of factors which can contribute to becoming obese such as eating a high calorie diet (high fat diet), not getting enough physical exercise, genetics, medical conditions and being on medications. Obesity is also thought to trigger changes to the metabolism of the body (Bray, 2004).

Eplerenone has been known to reduce high blood pressure (Suzuki et al., 2012). Increasing evidence suggests that disturbed acid-base transport function may lead to clinically

relevant changes in blood pressure and contribute to hypertension development (Boedtkjer and Aalkjaer, 2013). Sodium, an electrolyte (mineral) found mostly in the body fluids outside the cells, is very important for maintaining blood pressure (Grillo et al., 2019). In our result, a decrease in blood sodium level was observed as we moved from normal control to test control subjects while an increase in blood sodium level was observed as we moved from test control, to reference drug, and to test subjects, and both increase and decrease were statistically significant at $p<0.05$. Potassium levels are also altered in hypertension. In general acidemia (reduced blood pH) is associated with increased plasma potassium concentration (hyperkalemia), whilst alkalemia (increased blood pH) is associated with reduced plasma potassium concentration (hypokalemia) (Lee-Hamm et al., 2013). This was observed in this study. Hypertension increases the risk of heart disease, stroke, and other cardiovascular disease (American Heart Association, 2019). Bicarbonate (H_2CO_3) degrades to carbon (IV) oxide and water, and anion gap acidosis results. This is observed in the significantly lower Bicarbonate levels in the test control, reference drug, and test groups. The chloride values also follow the same trend. The same pattern of electrolyte imbalance was seen in the rat subjects. In general, hypertensive subjects are at increased risk of assay-based disturbance and electrolyte disturbances. The increased risk is due to the diseased state of hypertension itself and the associated disruptions in sodium and potassium homeostasis, drugs used to treat hypertension, and the organ damage associated with hypertension (Grillo et al., 2019). The urea and creatinine levels of the test control, reference drug, and test groups were higher than that of the normal control. There was an observed increase in urea and creatinine in the hypertensive Wistar rats. This is in agreement with other studies which reported that hyperglycaemia is one of the major causes of progressive renal diseases (Bamanikar et al., 2016). There was also an improvement with the extracts of Allium sativum and Cocos nucifera, and with the reference drug in different concentrations though to a lesser degree. In this study, we found that sodium was decreased significantly ($p<0.05$) in the test control, reference drug, and test groups compared to the normal control while the potassium was increased significantly ($p<0.05$) in the test control, reference drug, and test groups compared to the normal control. This is expected and in line with other studies as it is known that sodium reduction is a major risk factor and predicts hypertension (He et al., 2013). The hall-mark of hypertension (resistant hypertension) is an abnormally high blood pressure that is unresponsive or only slightly responsive to regulation and aggressive medical treatment (Whelton et al., 2018). In this work, urea also followed the same pattern, being significantly increased in the test control, reference drug, and test groups compared to the normal control. Creatinine was also significantly increased in the test control, reference drug, and test groups compared to the normal control. However, it did not follow a linear pattern as urea and creatinine as the chloride and bicarbonate was significantly reduced in the test control, reference drug, and test groups compared to the normal control. This may reflect life style changes as subjects that were already known hypertensive may already be taking intervention measures to ameliorate the hypertensive condition. Treatment with the standard reference drug, Eplerenone, led to improvement in all indices. Treatment with the extracts of Allium sativum and Cocos nucifera and their mixture also led to improvements in the indices albeit to a less degree.

Treatment of the test groups with the standard drug and plants extracts led to a reduction in hypertension as measured by the lipid profile levels. This also resulted in an improvement in the adverse effects of the disease condition as shown by the improvement in the Renal Profiles. Treatment with the plants extracts also led to improvements in these indices.

The results were related to the histopathology in that the normal control group of rats had normal liver and kidney, the test control group of rats had severe steatosis and kidney damage; the Reference drug, Allium sativum bulb, and Cocos nucifera sap groups had mild steatosis and kidney damage to normal liver and kidney, the 100mg/kg groups had severe to moderate steatosis and kidney damage, the 250mg/kg groups had moderate to mild steatosis and kidney damage, while the 500mg/kg groups had mild steasosis and kidney damage showing the positive effect of the reference drug and plants extracts as the dose concentration increased and the negative effect of lack of treatment of hypertensive subjects.

V. CONCLUSION

The animal model (L-NAME) was designed and used to assay various biochemical parameters and the findings largely corroborated previous studies. The serum electrolyte levels ascertained the effect of the extracts generated in the L-NAME-induced hypertensive Wistar rat which is a positive effect (their ability to reduce high blood pressure). Both the plants extracts

(Allium sativum bulb and Cocos nucifera sap) and the reference drug (Eplerenone) were effective in the management of hypertension though Eplerenone was slightly more effective. Dose variation and synergistic effect of the plants extracts were effective as the highest dose (500mg/kg) proved most effective while the mixture of the extracts was effective in the management of resistance in hypertension.

RECOMMENDATIONS

It is recommended that this research should be further carried out using larger population of animal subjects. The study should also be carried out in several geographical locations as variations in different locations affect the genetic factor and limit the generalization of the study findings. Also, further study should be conducted on these plants extract and their effects not only as it relates to hypertension but also their involvement in some of the intermediary metabolic pathways.

CONTRIBUTION TO KNOWLEDGE

The reference drug, Eplerenone works best at managing hypertension in comparison to the two plants extracts stated. The return of some biochemical parameters assayed through the administration of varying doses of the extracts to the Wistar rats helped in the control of hypertension by ameliorating its effect.

REFERENCES

[1]. Alaribe, C. S. A., Coker, H. A. B., Shode, F. O.,
[2]. Ayoola, G., Adesegun, S. A., Bamiro, J., Anyim, E. I., and Anyakora, C. (2012). Antiplasmodial and Phytochemical Investigations of Leaf Extract of Anthocleista vogelii (Planch). Journal of Natural Products, **5**: 60-67.
[3]. Alleyne, T., Roache, S., Thomas, C., and Shirley, A.
[4]. (2005). The control of hypertension by use
[5]. of coconut sap and mauby: two tropical food drinks. West Indian Medical Journal, **54**(1): 3-8.
[6]. American Heart Association (2019). Clinical
[7]. Implications. Early Onset Hypertension and Organ Damage. Hypertension, **74**: 217.
[8]. Bamanikar, S. A., Bamanikar, A. A., and Arora, A.
[9]. (2016). Study of serum urea & creatinine in patients in a tertiary teaching hospital. The Journal of Medical Research, **2**(1), 12-15.
[10]. Banerjee, S. K., Maulik, M., Manchanda, S. C.,
[11]. Dinda, A. K., Das, T. K., and Maulik, S. K. (2001). Garlic-induced alteration in rat liver and kidney morphology and associated changes in endogenous antioxidant status. Food and Chemical Toxicology, **39**: 793-797.
[12]. Boedtkjer, E. and Aalkjaer, C. (2013). Disturbed
[13]. acid-base transport: an emerging cause of hypertension. Frontiers in Physiology, **4**: 388.
[14]. Bray, G. A. (2004). Medical consequences of obesity.
[15]. Journal of Clinical Endocrinology & Metabolism, **89**: 2583-2589.
[16]. Cheesbrough, M. (2006). District laboratory practice
[17]. in tropical countries. 2nd Edition. Part 2. Cambridge University Press, United Kingdom. pp. 243-244.
[18]. Chuku, L. C., Uwakwe, A. A., and Chinaka, N. C.
[19]. (2012). Liver Enzymes in Normal and Sickle Cell Subjects. Journal of Natural Sciences Research, **2**:7.
[20]. Delyani, J. A. (2000). "Mineralocorticoid receptor
[21]. antagonists: the evolution of utility and pharmacology". Kidney International, **57**(4): 1408–1411.
[22]. Doyle, K. (2015). High blood pressure danger equal
[23]. for slim and obese alike. Healthcare and Pharma, **11**(4): 1-6.
[24]. Engelbrecht, R. M. and McCoy, F. A. (2002).
[25]. Determination of potassium by a tetraphenylborate method. Analytical Chemistry, **28**(1): 10-21.
[26]. Eric, B. (2010). The chemistry of garlic and onion.
[27]. Scientific American, **252**: 114-119.