Original Research Article

Effects of Ethanolic Extracts of Fruits of *Dennettiatripetala* on Kidney Function of Male Albino Rats

ABSTRACT

This study examined the effects of ethanolic extracts of fruits of *Dennettiatripetala* on kidney function of healthy male albino rats. *Dennettiatripetala* is commonly also referred to as pepper fruit. Antioxidant, anti-inflammatory, and antidiarrheal characteristics are only a few of the fruit's many known therapeutic benefits.

Fifty healthy adult male albino rats were utilized and were used in this study and were randomly distributed into 5 groups of 10 animals each. The test animals were orally administered ethanolic extracts of fruits of *D. tripetala*(200 mg/kg and 400 mg/kg) for twenty-one days and sacrificed.

The result of kidney function biochemical parameters revealed that sodium increased significantly (p<0.05) in all the test groups, potassium increased significantly in all the test groups except in group 4 which showed no significant alteration, chloride was lowered in all the groups except in group 2 which increased significantly. Urea was lowered significantly in all the test groups, creatinine was significantly elevated in groups 4 and 5, while groups 2 and 3 showed no significant alteration.Photomicrograph of kidney section of normal control rat (group 1) showed normal glomerulus (G), Bowman's capsule (Bc), Bowman's space (Bs) and tubules (T) as well as group 2 and 3, while group 4 and 5 showed slightly shrunken glomerulus (SG) with increased Bowman's capsular space (IBs) and dilated tubules (DT) within the tissue stroma.

This study suggests that both the extracts may possess active ingredients that may aid in electrolyte homoeostasis while the unriped fruit extract contains some chemicals that may cause mild kidney toxicity when consumed for a long duration.

Keywords: *Biochemical parameters,Dennettiatripetala,histology, kidney,medicinal properties, toxicity.*

INTRODUCTION

It is well recognized that medicinal herbs have a variety of benefits to animals, particularly humans [1]. In herbal medicine, the majority of spices are used for disease prevention or management[1, 2].*Dennettiatripetala* is a notable indigenous plant of West Tropical Africa, a member of the *Annonaceae*family [3]. This specieis usually found in Nigeria, Ivory Coast, and Cameroon. It is referred to as "pepper fruit" in English.It is also known as "mmimi" in Igbo,

"nkaika" in Ibibio, "imako" in Urhobo, "ako" in Edo, "opipi" in Idoma, and "igberi" in Yoruba [4, 5].Often used as seasonings or spices in meats, sausages, stews, soups, and vegetables are the fruits and leaves [6]. The leaves and seeds of the tree are used in traditional medicine to treat fever, cough, asthma, catarrh, toothache, diarrhoea, and rheumatism as well as to increase appetite, clear throats, ease coated tongues, and stop nausea[6, 7]. The bark of the tree is used to add flavour to a variety of dishes and the wood is burned as a source of energy [8]. Okoli (2014), examined the ability of ethanolic extract of roots of *D. tripetala* to impede lipid peroxidation in refrigerated heart muscle, and noticed that the extract had a significantly ($p<0.05$) higher antioxidant effect than vitamin C, implying that the root extract of *D. tripetala* is high in antioxidants and can be used to improve the preservation of frozen meat [9].

The kidneys are two bean-shaped and reddish-brown organs of vertebrates [10]. Because the liver takes up a lot of space on the right side, the right kidney is displaced roughly 1.5 cm lower than the left [10]. The adult human kidney weighs around 160 g and is 2.5 cm thick, 11.25 cm long, and 5.5–7.7 cm wide [10-12].

Through filtration, reabsorption and secretion, the kidneys convert plasma into urine [11, 13]. Blood from the circulatory system enters the glomerulus where it is filtered before moving into the Bowman's capsule. Because glomerular filtration is only done base on size, big molecules remain in the blood. Only 1% of the filtrate is converted to urine, but the rest is reabsorbed into the bloodstream [10, 13]. Salts and other molecules are reabsorbed through active transport when the filtrate passes from the Bowman's capsule through the proximal convoluted tubule through the distal convoluted tubule, whereas water, negative ions, and tiny proteins are taken up by proximal tubule cells through pinocytosis [11, 13]. Tubular secretion takes place in the renal tubule where blood is filtered. The filtrate is secreted with creatinine, ammonia, and other waste materials. As a result, the tubular fluid changes composition and hydrogen ions are

release to assists maintain blood pH [10, 11, 13]. Urine is formed when tubular fluid leaves the distal convoluted tubule and enters the collecting duct, urine is then stored in the urinary bladder before being expelled [11].

The kidney's other homeostatic functions include detoxification, vitamin D activation, glucose synthesis, erythropoietin production, renin secretion, blood pressure management, and blood oxygen and carbon dioxide pressure control [11,13].

MATERIALS AND METHODS

Plant material used: The*Dennettiatripetala* fresh fruits utilized in this investigation were bought in Nsukka, Enugu State, Nigeria. A manual blender was used to pulverize the healthy fruits after they were air-dried.

Preparation of plant extract: The crude extraction was carried out according to the method of with little modification [14]. The plant materials were air-dried and made into powder. The powder (250g) was soaked in 1000 ml of 70% ethanol in a beaker, stirred rigorously and allowed to stand for 48 hours before filtering twice with cheesecloth and Whatman filter paper (No 1) [14]. The filtrate was concentrated using rotary evaporator at 68°C. Appropriate weights of the filtrate were prepared in normal saline equivalent of the various concentrations used for the experiment. The concentrated extracts were corked in an airtight container, refrigerated at 4°C for further analysis.

Figure 1: Unriped fruits of *Dennettiatripetala*

Figure 2: Riped fruits of *Dennettiatripetala*

Eperimental Animals

Fifty healthy albino rats were purchased from Hema farms federal housing estate BajaburieYola, Adamawa State, Nigeria. The animals were housed in animal house of

Biochemistry Department, Federal University Wukari, Nigeria under standard laboratory conditions and were allowed free access to standard diet and water *ad libitum*. The animals were acclimatized for two weeks before the experiment.

Experimental Design

Fifty (50) healthy adult male albino rats were used for this experiment and were randomly distributed into five groups of ten animals each (table 1). The animals were acclimatized for two weeks before the experiment. The experiment was carried out for a period of 21 days. The plant extracts were administered to the animals as stated in table 1.

Administration of plant extract

The animals were administered single dose of the extracts daily for 21 days (table 1).

Table 1: Administration of plant extract

Group	1	2	3	4	5
Extract administered	Normal Control	Ethanolic extract of riped fruits of *D. tripetala* (200 mg/kgbwt)	Ethanolic extract of riped fruits of *D. tripetala* (400 mg/kgbwt)	Ethanolic extract of uniped fruits of *D. tripetala* (200 mg/kgbwt)	Ethanolic extract of uniped fruits of *D. tripetala* (400 mg/kgbwt)

Animal Sacrifice and Collection of Samples at the end of the Administration of the Extracts

At the end of administration of the plant extract, the rats (seven from each group) were anaesthetized with chloroform vapour. Each rat's blood was collected through cardiac puncture

into different blood collection tubes for determination of concentrations of selected biochemical parameters. The blood collected in sample collection tubes were allowed to stand for ten minute and spun at 3000 rpm for 10 minutes using centrifuge. The serum was collected using Pasteur pipette and then subjected to various biochemical analyses using auto-chemistry analyzer Land wind LW E60B, China. The kidneys were harvested, stored in 10% formalin for histological examinations of the organs.

Determination of Levels of Selected KidneyFunction Biochemical Parameters in Rats Administered Ethanolic Extracts of Fruits of *D. tripetala*

Level of selected biochemical parameters such as creatinine, urea and electrolytes (Na, K and Cl) were determined using auto-chemistry analyzer Landwind LW E60B, China.

Histological Examination of the Kidneysof Rats Administered Ethanolic Extracts of Fruits of *D. tripetala*

The kidneys were harvested and fixed in 10% formalin, then gradually dehydrated in 50-100 percent ethanol, cleaned in xylene, and embedded in paraffin wax. The 5-6 mm thick sections were then prepared with a rotary microtome (Leica RM 2125 RTS, Singapore) and stained with hematoxylin and eosin dye for microscopic study of histological changes in the kidneys.

Statistical Analysis

One-way analysis of variance (ANOVA), further with Duncan's multiple comparison test was used. Results were expressed as mean ± standard deviation of means obtained (N=7). The statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 23 and significance was at $p< 0.05$.

RESULTS AND DISCUSSION

Results

Table 2: Concentration of selected kidney function parameters of male albino rats administered ethanolic extracts of fruits of *D. tripetala*

Parameters	Group 1 (Normal control)	Group 2 (Riped fruit extract of *D. tripetala*: 200 mg/kg. bw)	Group 3 (Riped fruit extract of *D. tripetala*: 400mg/kg. bw)	Group 4 (Unriped fruit extract of*D. tripetala*:200 mg/kg. bw)	Group 5 (Unriped fruit extract of *D. tripetala*:400 mg/kg. bw)
Sodium (mmol/L)	134.64 ± 6.85^{a}	158.12 ± 21.59^{b}	165.62 ± 24.94^{b}	194.53 ± 24.81^{c}	162.44± 8.18^{b}
Potassium (mmol/L)	0.78 ± 0.10^{a}	2.10 ± 0.20^{c}	2.67 ± 0.33^{d}	1.25 ± 0.17^{a,b}	1.65 ± 0.23^{b,c}
Chloride (mmol/L)	134.46 ± 21.67^{c}	202.61 ± 9.27^{d}	101.00 ± 15.93^{b}	83.73 ± 10.98^{a}	77.72 ± 7.28^{a}
Urea (mg/dL)	45.64 ± 2.47^{c}	37.87 ± 3.64^{b}	34.43 ± 3.22^{b}	3.35 ± 0.43^{a}	3.55 ± 0.38^{a}
Creatinine (mg/dL)	0.62 ± 0.10^{a}	0.58 ± 0.12^{a}	0.73 ± 0.17^{a}	0.92 ± 0.03^{b}	1.05 ± 0.21^{b}

Results are expressed as mean ± standard deviation of group results obtained (n=7).

Means in the same row having different superscripts are statistically significant ($p<0.05$).

The results of selected kidney function indices showed that sodium increased significantly ($p<0.05$) in all the test groups when compared to the normal control, potassium increased significantly in all the test groups except in group 4 which showed no significant alteration, chloride was lowered in all the groups, except group 2 which increased significantly. Urea was lowered significantly in all the test groups, creatinine was significantly elevated in groups 4 and 5, while groups 2 and 3 showed no significant alteration when compared with the normal control.

The results of kidney function parameters showed increase in serum sodium (Na^+) concentration in all the test groups. When there is too much sodium in proportion to water, hypernatremia may result. Hypernatremia can be caused by a variety of conditions, such as renal illness, inadequate hydration, and water loss via diarrhea and/or vomiting [15]. When the quantity of bodily water increases relative to sodium, there is a corresponding reduction in sodium concentration (hyponatremia). This occurs with various liver and renal illnesses, congestive heart failure patients, burn sufferers, among many other ailments. This result suggests that consumption of excess unriped fruit extract of *D. tripetala* or a long time, especially in people with renal impairment, may be at high risk of kidney failure and hypertension.

Serum potassium (K^+) was raised significantly ($p<0.05$) in all test groups except test group 4 which showed no significant increase when compared with the control. The kidney maintains K^+ homeostasis by reabsorbing virtually all K^+ by the proximal tubules and then excreting excessive potassium in the urine under the action of aldosterone [16]. When too much potassium is produced (via oral intake or tissue breakdown) or not eliminated properly, hyperkalemia may occur [17]. Massive hemolysis, which involves the excessive destruction of cells and redistribution of K^+ from the intracellular to extracellular compartment, renal insufficiency, which

impairs the body's ability to control serum potassium levels through the kidneys, and exercise have been reported to be associated with its occurrence [18]. In healthy people, severe hyperkalemia is uncommon, but when it does happen, it can affect the neuromuscular, cardiac, and gastrointestinal organ systems. The rise in the equilibrium potential of potassium ions may cause depolarization of cell membrane potentials, which opens voltage-gated sodium channels and causes an increase in inactivation at the same time [19]. The control of potassium homeostasis is influenced significantly by aldosterone, humans are able to counteract an increase in dietary potassium by increasing the renal excretion of this ion, preventing hyperkalemia in healthy persons from occurring[20]. Consumption of fruit extracts of *D. tripetala*in people with history kidney problem may affect renal excretion, which may result to aggregation of ions, which may in turn result to kidney stone if left untreated. This may also affect cardiac muscles and neuromuscular.

Low concentration of chloride associated with administration of unripe fruit extract may result to hypochloremia that can be link to chronic respiratory acidosis, which is usually cause by vomiting. Elevated levels of chloride associated with administration of the riped fruit extract (200 mg/kg bw) may result to hyperchloremia, which is kidneys inability to remove or balance adequate saline fluids in the body. Some of the signs of this disease include fatigue, muscle weakness, increased thirst, dry mucous membranes and high blood pressure. People that consume excess unriped fruit extract of *D. tripetala*for a long time may develop respiratory acidosis.

The significantly low levels of serum urea in all the animal test groups showed that urea was highly excreted as a result of administration of the plant extracts, especially, the unriped fruit extract. The significantly elevated levels of serum creatinine in animal test groups 4 and 5 may confer toxic effect on the kidney. It suggests that the unriped fruit extract may negatively influenced the level of creatinine excretion by the kidneys. Because the kidney is in charge of

filtering urea and creatinine out of the blood, urea and creatinine are frequently employed as indicators of renal function [20]. A high concentration of these metabolites in the serum is a sign of renal impairment. The result obtained for urea and creatinine in test groups 2 and 3 is in tandem with the report of a researcher in 2017 that reported an ameliorative impact of an aqueous extract of *D. tripetala* fruits on rats exposed to carbon tetrachloride [20]. The retention of creatinine in animal test groups 4 and 5 may suggest kidney toxicity.

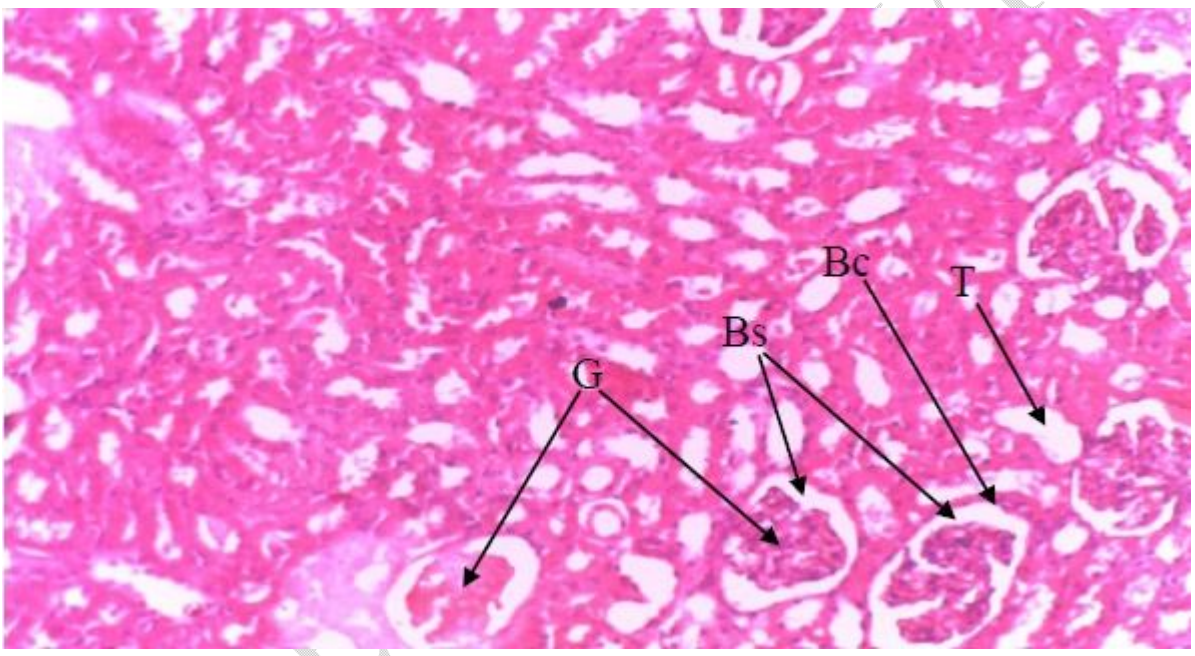

Figure 3: Photomicrograph of kidney section of normal control rat (group 1).

Legend: G: glomerulus, Bc: Bowman's capsule, Bs: Bowman's spaceand T: tubules.

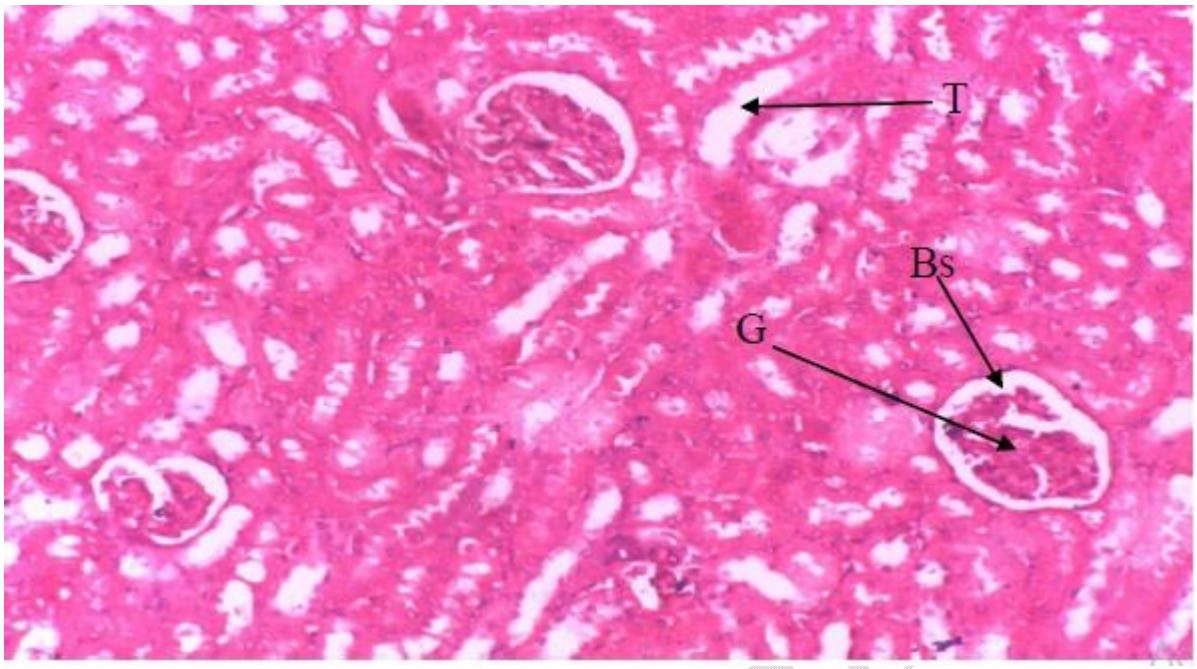

Figure 4: Photomicrograph of kidney section of rat administered ethanolic extract of riped fruit of *Dennettiatripetala* (200mg/kg) (group 2) showing normal kidney histology

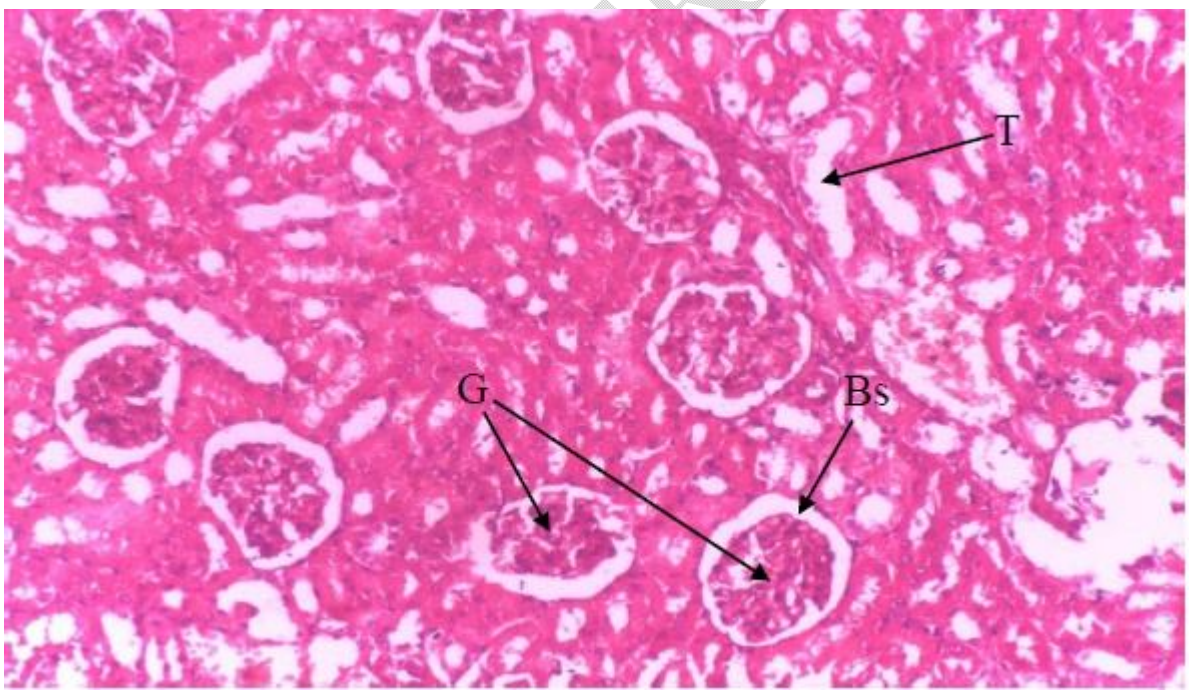

Figure 5: Photomicrograph of kidney section of rat administered ethanolic extract of riped fruit of *Dennettiatripetala* (400mg/kg) (group 3) showing normal kidney histology

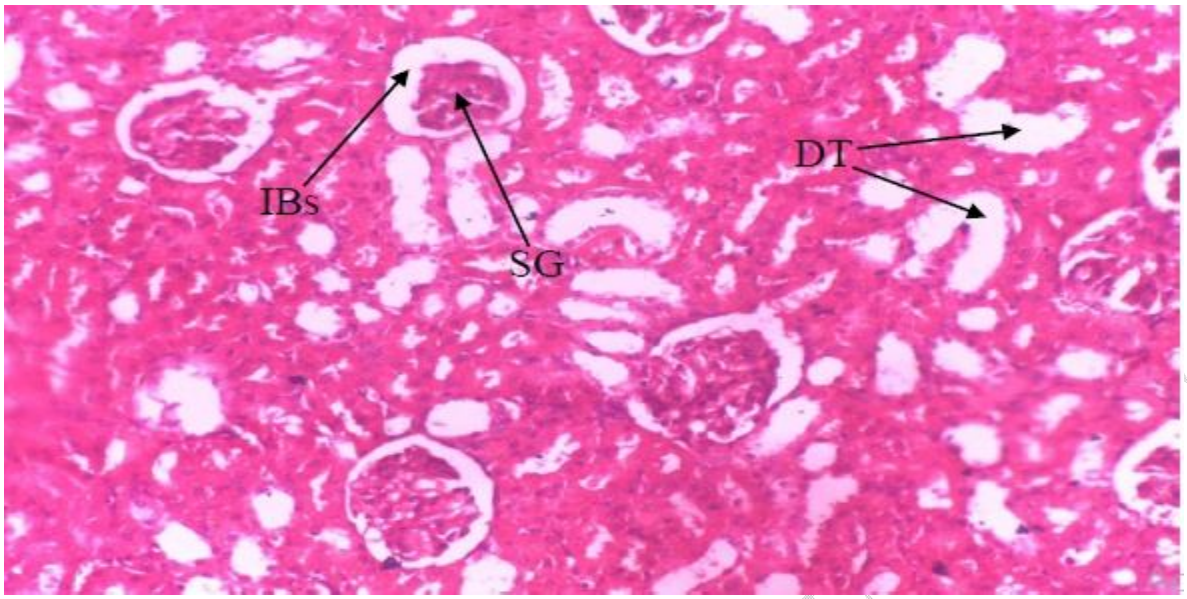

Figure 6: Photomicrograph of kidney section of rat administered ethanolic extract of unriped fruit of *Dennettiatripetala* (200mg/kg) (group 4) showing slightly shrunken glomerulus (SG) with increased Bowman's capsular space (IBs) and dilated tubules (DT) within the tissue stroma

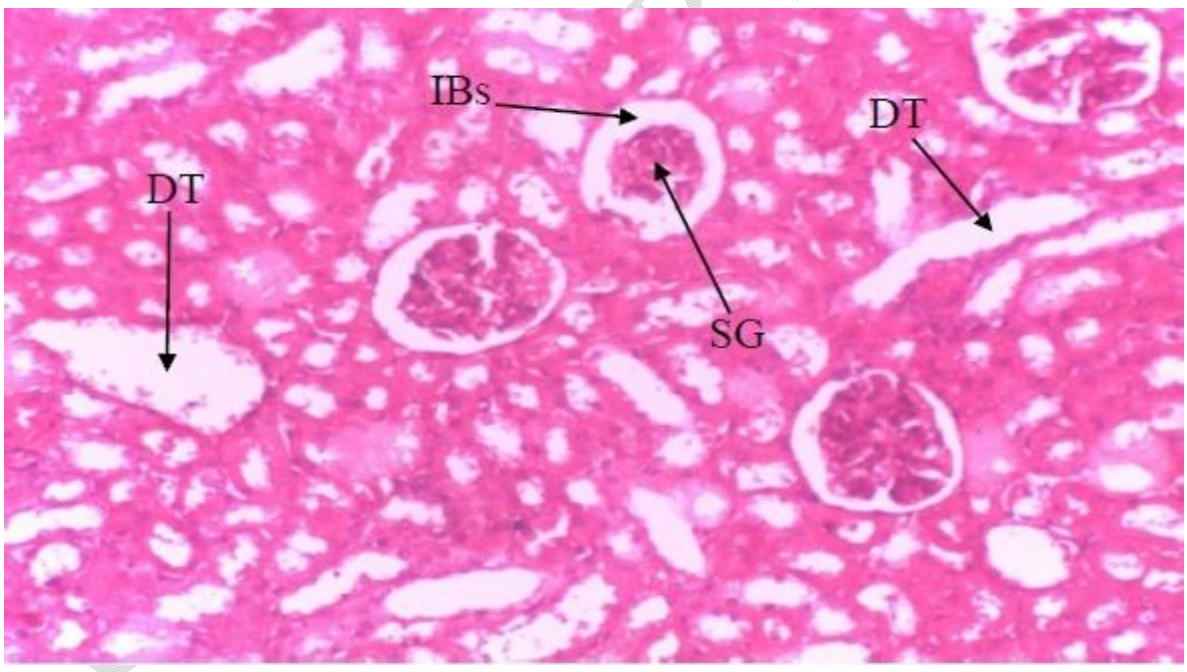

Figure 7: Photomicrograph of kidney section of rat administered ethanolic extract of unriped fruit of *Dennettiatripetala* (400mg/kg) (group 5) showing slightly shrunken glomerulus (SG) with increased Bowman's capsular space (IBs) and dilated tubules (DT) within the tissue stroma

Administration of ripedethanolic extract of fruits *D. tripetala* does not show any toxicity to the kidney as shown in photomicrograph of kidney sections of groups 2 and 3, while administration of unripedethanolic extract of fruits *D. tripetala* showed mild kidney toxicity as shown groups 4 and 5.Photomicrograph of the kidney sections of rats administered ripedethanolic extract of fruits *D. tripetala* agreed with the result of research conducted in 2019 [21].

CONCLUSION

This study showed that administration of both riped and unripedfruit extracts of *D. tripetala*may have some therapeutic potential. These findings support a few of the conventional medical use of fruits of *D. tripetala*. Additionally, our research demonstrates that eating riped *D. tripetala* fruits as a source of nutrients is save. However, consumption of theunriped fruits of *D. tripetala*for a period of 21 days or beyond may cause a mild toxic effect on the kidneys, which may possibly affect normal kidney functions.

Ethical Approval

All experiments were conducted in compliance with ethical guide for care and use of laboratory animals of Federal University Wukari.

REFERENCES

1. Imo C, Arowora KA, Ezeonu CS, Ikwebe J, Yakubu OE, Imo NG,*et al.* Biochemical and Histological Effects of Ethanolic Extracts of Fruits of *Xylopiaaethiopica* and Seeds and Leaves of *Piper guineense* on Liver and Kidney Function in Male Albino Rats. *Future Journal of Pharmaceutical Sciences*, 2021; *7*: 1-12.
2. Imo C, Zaku JS. Medicinal Properties of Ginger and Garlic: A Review. *Current Trends of Biomedical Engineering and Bioscience, 2019; 18*(2): 47-52.
3. Omage SO, Orhue NE, OmageK. Evaluation of the Phytochemical Content, *in vitro* Antioxidant Capacity, Biochemical and Histological Effects of *Dennettiatripetala* Fruits in Healthy Rats. *Food Science & Nutrition*, 2019: *7*(1), 65-75.
4. Ejechi BO, Nwafor OE, OkokoFJ.Growth Inhibition of Tomato-rot Fungi byPhenolic Acids and Essential Oil Extracts of Pepper fruit (*Dennetiatripetala*). *Food Research International, 1999; 32:* 395-399.
5. Egharevba HO, Idah E A. Major Compounds from the Essential Oil of the Fruit and Comparative Phytochemical Studies of the Fruits and Leaves of *Dennettiatripetala*Barker F. Found in North Central Nigeria. *International Journal of Pharmacognosy and Phytochemical Research, 2005; 7*(6): 1262-1266.
6. Ejechi BO, Akpomedaye D. Activity of Essential Oil and Phenolic Extract of Pepper Fruits, *Dennettiatripetala*G. Baker Against Some Food-born Microorganisms. *African Journal of Biotechnology, 2005; 3:* 258-261.
7. Oyemitan IA, Iwalewa EO, Akanmu MA, Asa SO, Olugbade TA. The Abusive Potential of Habitual Consumption of the Fruits of *Dennettiatripetala*G. Baker (*Annonaceae*) Among the People in Ondo Township (Nigeria). *Nigerian Journal of Natural Products Medicine, 2006; 10:* 55-62.
8. Khadijah S. Anthelmintic Efficacy of Crude Methanol Extract of *Dennetiatripetala.* Baker Fruits with its Various Chemical Fractions in Mice Experimentally Infected with *Heligmosomoidesbakeri. A Thesis Submitted to Ahmadu Bello University, Zaria, Nigeria*, 2015; 65-70.
9. Okolie NP, Falodun A, Davids,O. Evaluation of the Antioxidant Activity of Root Extract of Pepper Fruit (*Dennetiatripetala*), and its Potential for the Inhibition of Lipid Peroxidation. *African Journal of Traditional and Complementary Alternative Medicine, 2014; 11:*221-227.

10. Van De Graff Human Anatomy, Sixth Edition,Brian Black. Medical Anatomy and Physiology.Unit 11: Urinary System. 2001; pp:1-5.
11. Saladin KS. The Types of Blood Circulation. *Human Anatomy* 3rd ed. New York: McGraw-Hill. 2011; p. 540.
12. Liu C, Wang X. Clinical Utility of Ultrasonographic Evaluation in Acute Kidney Injury. *Translational Andrology and Urology*, 2020; *9*(3): 1345-1355.
13. Scanlon VC, Sanders T. The Function of Kidney. *Essentials of Anatomy and Physiology*. FA Davis. 5th Edi. 2007; pp. 436-450.
14. Ezeonwu VU, Dahiru D. Protective Effect of Bi-herbal Formulation of *Ocimumgratissimum* and *Gongronemalatifolium* Aqueous Leaf Extracts on Acetaminophen-induced Hepato-nephrotoxicity in Rats. *American Journal of Biochemistry*, 2013; ***3***(1): 18-23.
15. Walker HK, Hall WD, Hurst JW. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd ed. Boston: Butterworths 1990; Available from: https://www.ncbi.nlm.nih.gov/books/NBK201/.
16. Akpakpan EI, Bassey UE, Etim OE, Ekanemesang UM. The Effect of Ethanol Extract of Ripe *Denettiatripetala* Fruit (Pepper Fruit) on Indices of Liver and Kidney Function in Male Albino Wistar Rats. *International Journal of Biochemistry Research and Review*, 2017; *16*(3): 1-7.
17. Desai A. Hyperkalemia Association with Inhibitors of the Renin-angiostensin Aldosterone System Balancing Risk and Benefit, *Circulation*, 1996; *118*(16): 1609-11.
18. Lindinger MI. Potassium Regulation During Exercise and Recovery in Humans: Implications for Skeletal and Cardiac Muscle. *Journal of Molecular and Cellular Cardiology*, 1995; *27*(4): 1011-1022.
19. Kovesdy CP. Management of Hyperkalemia: an Update for the Internist. *The American Journal of Medicine*, 2015; *128*(12): 1281-1287.
20. Iseghohi SO, Orhue NE. Ethanolic Extract of *Dennettiatripetala*Fruits Protects the Liver and Kidney of Rats from Damage Induced by a Single Exposure to Carbon Tetrachloride. *International Journal of Pharmacology, Phytochemistry and Ethnomedicine,* 2017; 68-16.
21. Omage SO, Orhue NE, Omage K. Evaluation of the phytochemical content, in vitro antioxidant capacity, biochemical and histological effects of Dennettiatripetala fruits in healthy rats. *Food Science & Nutrition*, 2019; *7*(1): 65-75.