

Original Research Article

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

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ABSTRACT

Aim: This study examined the effects of ethanolic extracts of fruits of *Dennettia tripetala* on kidney function of healthy male albino rats. *Dennettia tripetala* 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.

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Study design: 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.

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Methodology: Blood sample was collected via cardiac puncture for kidney biochemical analysis, while the kidneys were harvested for histological examination.

Place and duration of study: Department of Biochemistry, Faculty of Pure and Applied Sciences, Federal University Wukari, between August 2022 and January 2023.

Results: 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.

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

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

1. 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]. *Dennettia tripetala* is a notable indigenous plant of West Tropical Africa, a

member of the *Annonaceae* family [3]. This species is 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].

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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, 11, 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 based 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 released to assist 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].

2. MATERIALS AND METHODS

2.1 Plant material used: The *Dennettia tripetala* 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.

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2.2 Preparation of plant extract: The crude extraction was carried out according to the method of Ezeonwu and Dahiru (2013), with little modification. 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: Unripe fruits of *Dennettia tripetala*



Figure 2: Riped fruits of *Dennettia tripetala*

2.3 Experimental 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.

2.4 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 plant extracts were administered to the animals as stated in table 1. All experiments were conducted in compliance with ethical guide for care and use of laboratory animals of Federal University Wukari.

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Table 1: Administration of plant extract

Group	1	2	3	4	5
Extract administered	Normal Control	Ethanollic extract of riped fruits of <i>D. tripetala</i> (200 mg/kgbw)	Ethanollic extract of riped fruits of <i>D. tripetala</i> (400 mg/kgbw)	Ethanollic extract of uniped fruits of <i>D. tripetala</i> (200 mg/kgbw)	Ethanollic extract of uniped fruits of <i>D. tripetala</i> (400 mg/kgbw)

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2.5 Animal Sacrifice and Collection of Samples

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. The kidneys were harvested, stored in 10% formalin for histological examinations of the organs.

2.6 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.

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

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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.

2.8 Statistical Analysis

One-way analysis of variance (ANOVA), further with Duncan's multiple comparison test was used. Results were expressed as mean \pm 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 $p < 0.05$.

3. 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 <i>D. tripetala</i> : 200 mg/kg. bw)	Group 3 (Riped fruit extract of <i>D. tripetala</i> : 400mg/kg. bw)	Group 4 (Unripped fruit extract of <i>D. tripetala</i> : 200 mg/kg. bw)	Group 5 (Unripped fruit extract of <i>D. D. tripetala</i> : 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).

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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, hyponatremia may result. Hyponatremia 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 unripe fruit extract of *D. tripetalaor* 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. tripetalain* 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 unripped 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 unripped 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 unripped 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 Iseghohi and Orhue (2017), which reported the ameliorative impact of an aqueous extract of *D. tripetala* fruits on rats exposed to carbon tetrachloride. The retention of creatinine in animal test groups 4 and 5 may suggest kidney toxicity.

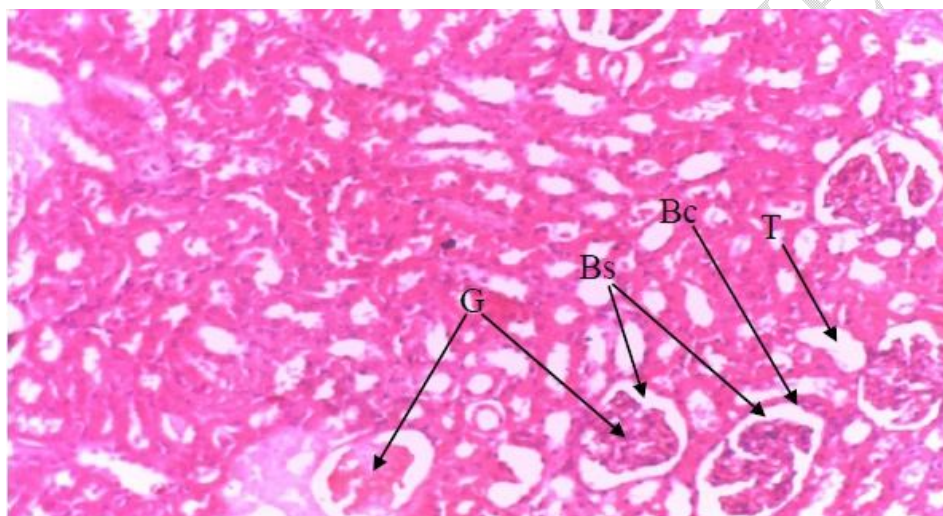


Figure 3: Photomicrograph of kidney section of normal control rat (group 1).

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

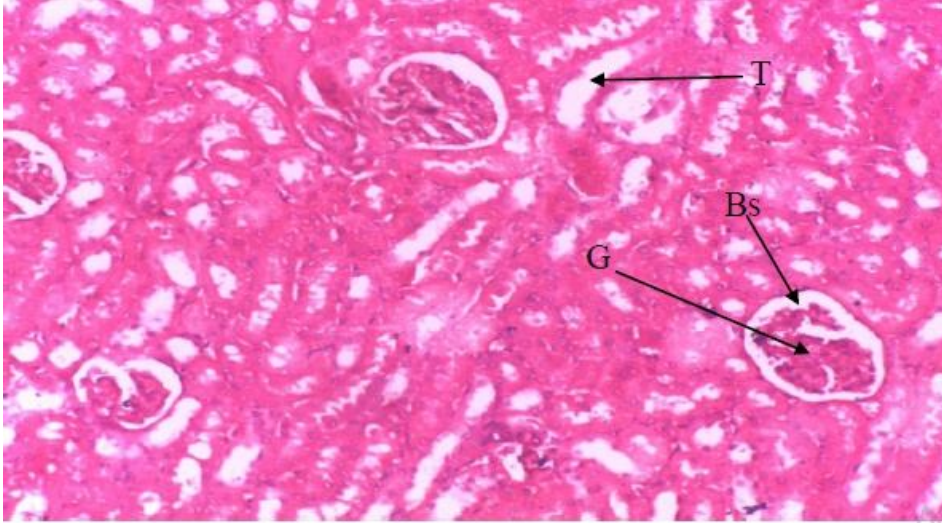


Figure 4: Photomicrograph of kidney section of rat administered ethanolic extract of riped fruit of *Dennettia tripetala* (200mg/kg) (group 2). Legend:

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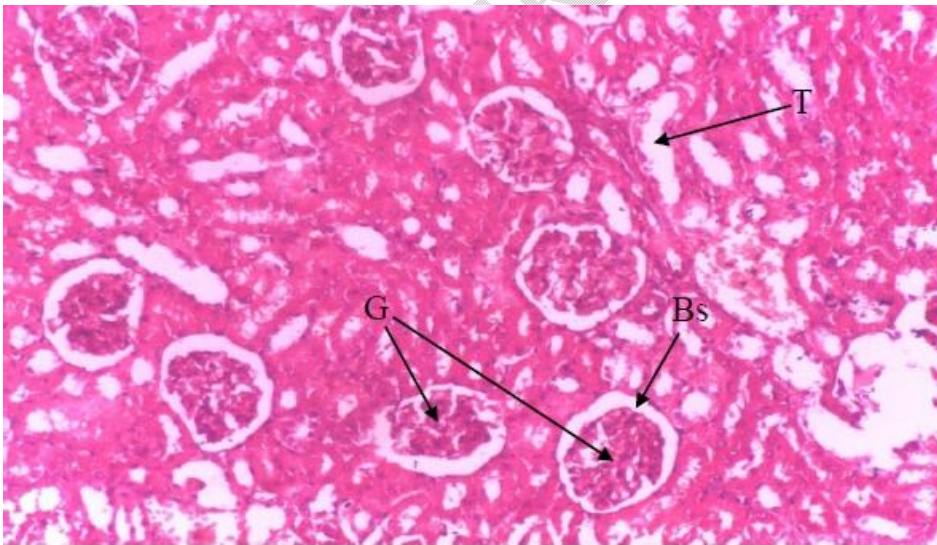


Figure 5: Photomicrograph of kidney section of rat administered ethanolic extract of riped fruit of *Dennettia tripetala* (400mg/kg) (group 3). Legend:

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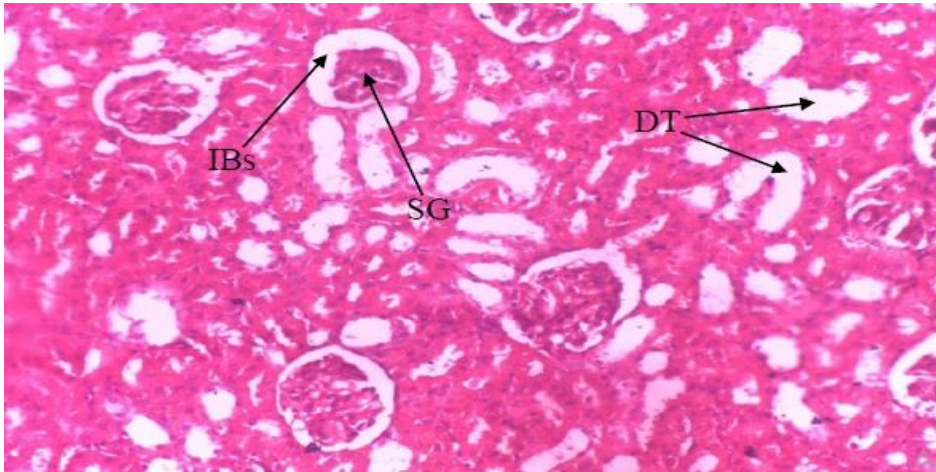


Figure 6: Photomicrograph of kidney section of rat administered ethanolic extract of unripe fruit of *Dennettia tripetala* (200mg/kg) (group 4). Legend:

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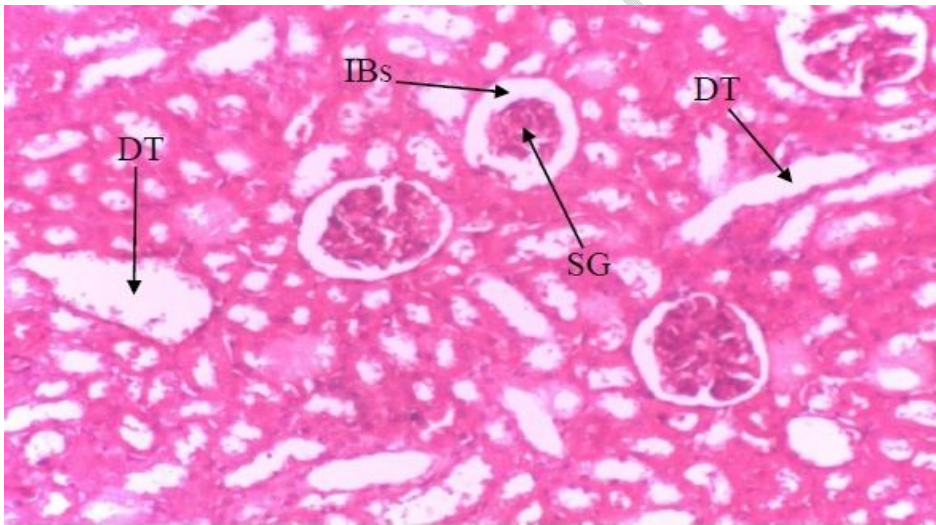


Figure 7: Photomicrograph of kidney section of rat administered ethanolic extract of unripe fruit of *Dennettia tripetala* (400mg/kg) (group 5). Legend:

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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 photomicrograph of kidney sections of groups 2 and 3, while groups 4 and 5 showed slightly shrunken glomerulus (SG) with increased Bowman's capsular space (IBs) and dilated tubules (DT) within the tissue stroma. Photomicrograph of the kidneys agreed with the result of kidney function biochemical parameters such as urea (groups 2 and 3), creatinine (groups 2 and 3), potassium, chloride and sodium, which suggest absence of toxicity. This implies that consumption of riped fruit extract of *D. tripetala* may not have toxic effect on the kidney, whereas consumption of theunripped fruit extract over a prolonged period may cause kidney toxicity which may in turn affect kidney histology.

4. CONCLUSION

This study showed that administration of both riped and unripped fruit extracts of *D. tripetala* may have some therapeutic potential. However, administration of theunripped fruit extract for a long period of time may have a mild toxic effect on the kidneys, which may possibly affect normal kidney functions. Consumption of fruits of *D. tripetala* (especially unripped fruit) for a long period of time should be discouraged among people with history of kidney problem so as to prevent aggravating their ill condition.

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