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2 **THE EFFECT OF ETHANOL EXTRACT OF RIPE *DENETTIA TRIPETALA***
3 **(PEPPER FRUIT) ON INDICES OF LIVER AND KIDNEY FUNCTION IN**
4 **MALE ALBINO WISTAR RATS.**

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14 **ABSTRACT**
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Aim: To investigate the effect of oral administration of ethanolic extract of ripe *Denettia tripetala* (DT) fruits some on indices of kidney and liver function in male albino rats.

Study design: Twenty (20) male albino rats weighing between 160 – 210 g were used for the study and randomly assigned into four groups of five animals each. Group 4 served as control while Groups 1, 2, and 3 received orally the ethanol extracts of *Denettia tripetala* at doses of 262.20 mg/kg, 524.40mg/kg and 786.61mg/kg respectively for 30 days.

Place and duration of study: the study was carried out at Department of Pharmacology and Toxicology Laboratory, University of Uyo between May 2016 and June 2016.

Methodology: Serum creatinine, urea, electrolytes, albumin, aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) levels were measured using Randox kits, USA.

Results: The result showed that there was an increase in the level of potassium in Group 2 (6.90 ± 1.64) and Group 3 (7.58 ± 0.39) compared to control (6.32 ± 0.20). However, only Group 3 compared to control was significantly increased at ($P=0.05$). There was no significant difference ($P > 0.05$) in the level of creatinine, urea, sodium and chloride when compared to control. There was a significant increase ($P=0.05$) in the level of ALP in Group 2 when compared to control. There was no significant difference ($P > 0.05$) in total bilirubin, albumin, ALT and AST levels in the treated groups when compared to control.

Conclusion: The study suggests that ethanol extract of ripe *Denettia tripetala* fruit was not toxic to the liver and kidney. However further work is needed to validate the results of the study.

16
17 **Key words:** *Denettia tripetala*, potassium, alkaline phosphatase, liver, kidney.
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20 **1. INTRODUCTION**
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22 The World Health Organization survey estimated that 80% of the world population use medicinal plants in the treatment of
23 disease; in African countries this rate is said to be much higher (1). According to Hammer *et. al.*(1999), plants are the
24 richest resource of drugs for traditional system of medicine, nutraceuticals, food supplements, folk medicines,
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compounds in fruit, vegetables, grains, and other plant foods(3)—have been linked to reductions in the risk of major chronic diseases including cardiovascular disease, diabetes, cancers as well as neurodegenerative disorders(4). Therefore people who consume high quantities of vegetables and fruits may be at reduced risk for some of the diseases caused by biochemical dysfunction (5). Since most plants have medicinal properties, it is of utmost importance that their efficacy and toxicity risks are evaluated.

***Denettia tripetala* G. Baker** is an indigenous fruit tree of the family Annonaceae (6). It is known in Nigeria by the following names: Ako (Edo), Mmimi (Ibo), Ata Igbere (Yoruba) and Nkarika (Ibibio/Efik). It's widely distributed and consumed by the inhabitants of Western Cameroons, Ivory Coast and Southern Nigeria. It is also used in traditional medicine as a remedy for cough, fever, toothache, diarrhea, diabetes, and nausea in pregnant women (6). Particularly, the rural dwellers consume it together with local gin (kaikai or ufofop) as snacks.

DT have received only minimal scientific attention throughout the past years. Phytochemical screening of the ethanolic extract revealed the presence of tannins, alkaloids, steroids, flavonoids, cardiac glycosides, saponins, and terpenoids (7). These constituents suggest the scientific basis for the use of DT in traditional medicine. Ikpi and Nku (8) investigated the effect of the ethanolic extract of DT on hematological parameters in normal healthy rats, the study showed that DT may be hematotoxic to rats at low to moderate dose, but the toxicity was relieved at administration of high dose of the extract. Due to dearth of literature on *in vivo* toxicity of *Denettia tripetala* fruits. It was therefore pertinent to examine the effect of ethanol extract of DT on indices of kidney and liver function.

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2. MATERIALS AND METHODS

Denettia tripetala (pepper fruit)

Mature ripe fruits of *Denettia tripetala* (see figure 1) obtained from Oboh market in Etim Ekpo Local Government Area, Akwa Ibom State. The fruit samples were authenticated by a Botanist in Department of Botany and Ecological Studies, University of Uyo, Akwa Ibom State. It was washed, sun dried and grounded into powder with mortar and pestle. It was then taken to the lab for extraction. *Denettia tripetala* was macerated in 80% of ethanol for 3 days and was filtered, the filtrate was placed on the water bath for complete evaporation. The concentrated extract was stored in the freezer at -4 degrees Celsius until further analysis.

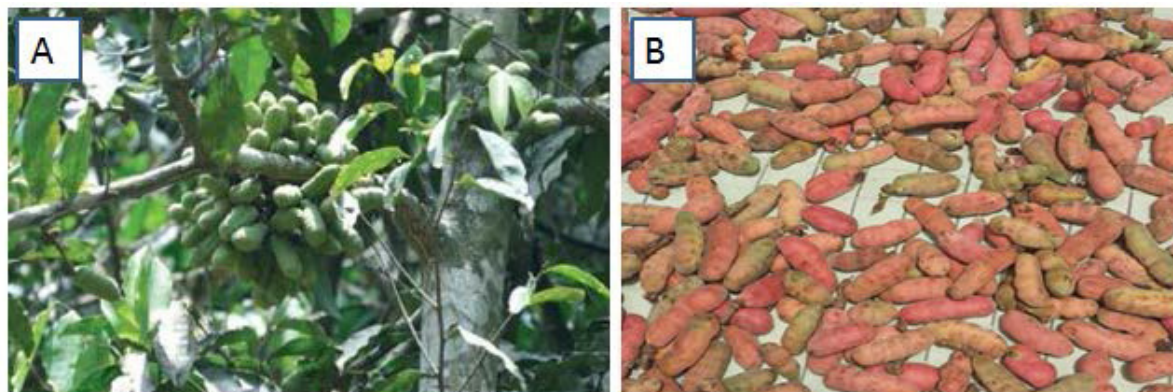


Figure 1. (A) *Denettia tripetala* tree with leaves and unripe fruits. Image courtesy: World Agroforestry Center and Rubber Research Institute of Nigeria (B) Ripe (red) and unripe (green) *Denettia tripetala* fruits.

Twenty male albino rats were bought from University of Calabar and kept in animal house of Department of Biochemistry, Faculty of Basic Medical Science, University of Uyo, Akwa Ibom state. The animals were allowed to acclimatize for 30 days under standard laboratory with free access to commercial food and water. All animals were cared for in accordance with the principle and guidelines of the ethical committee for conduction of animal studies in Department of Pharmacology and Toxicology, University of Uyo, Nigeria.

Acute Toxicity Study

Forty eight (48) male mice weighing 18-30g were used for the evaluation of acute toxicity of ethanolic extract of *Denettia tripetala*. The mice were deprived of food for 3 hours before administration of the extract. The mice were divided into 16 groups of 3 animals each. Each group received the following doses: 100, 200, 400, 600, 800, 1000, 1200, 1500, 2000, 2500, 3000, 3250, 3500, 3750, 4000 and 5000mg of the extract per kilogram body weight of the mice intraperitoneally (I.P). All the mice were observed for general behavioural changes, symptoms of toxicity and mortality. The number of deaths within 24 hours was recorded. The LD₅₀ was determined using the Lorke's method (21)

Experimental design

Twenty male albino Wistar rats weighing 160-210g were obtained from the animal house of Faculty of Basic Medical Sciences, University of Calabar, Cross River State, Nigeria, and was transported to the animal house, Department of

Pharmacology and Toxicology, University of Uyo, Akwa Ibom State, Nigeria. They were allowed to acclimatize for two weeks under standard conditions with free access to water and feed. They were randomly selected into four groups, five animals per group. Group 4 served as control. Groups 1, 2, and 3 received orally a daily dose the ethanol extract of *Denettia tripetala* fruits at doses of 262.20 mg/kg, 524.40mg/kg and 786.60mg/kg respectively for 30 days. After the last administration, the animals were fasted overnight and then sacrificed under chloroform anesthesia. Blood was obtained by cardiac puncture into a sterilized sample bottles. The blood collected was allowed to clot by standing at room temperature for one hour and centrifuge at 2500g for ten minutes. The serum (supernatant) was isolated and stored at -4 degree Celsius.

Biochemical analysis

Creatinine, urea, sodium, potassium, chloride, total protein and albumin concentrations as well as aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP), activities were assayed using their respective Randox kits, USA, according to manufacturer's instructions.

Statistical analysis

Data are presented as mean $\pm$ standard error of the mean and were analyzed using one-way analysis of variance (ANOVA) followed with a *post-hoc* test (least significance difference) using SPSS version 15.0 (SPSS Inc. Chicago, IL 60606-9653, Nov. 2006) and Excel for windows (Microsoft Corporation 2010, Washington.). $P = .05$ was considered as statistically significant.

2. RESULTS AND DISCUSSION

Acute toxicity study

Forty-eight mice weighing between 18-30 g were used to carry out acute toxicity study on the extract of the fruits of *Dennettia tripetala* according to Lorke's method. The LD₅₀ was observed to be 3496 mg/kg body weight. 7.5, 15 and 22.5 percent of the LD50 were used for the study.

The concentration of kidney function markers in rats orally administered with *D. tripetala* fruits are presented in Table 1 below. There was an increase in the concentration of potassium in Group 2 (6.90 ± 1.64) and Group 3 (7.58 ± 0.39), while group 1 was decreased compared to control (6.32 ± 0.20). However, only Group 3 compared to control group was significantly increased at ($P = .05$). There was no significant difference ($P > .05$) in the level of creatinine, urea, sodium and chloride, total protein and albumin when compared to control.

The concentration of liver function markers in rats orally administered with *D. tripetala* fruits is shown in Table 2 below. There was a significant increase ($P = .05$) in the level of ALP in Group 2 (60 ± 3.91) when compared to control (46.2 ± 3.83). The concentration of ALP in group 2 and group 3 were not significantly altered when compared to group 4 (control). There was no significant difference ($P > .05$) in total bilirubin, ALT and AST levels in the treated groups compared to control.

Table 1: Concentration of kidney function markers in rats orally administered with *D. tripetala* fruits

Groups/ Parameters	Urea (mg/dl)	Creatinine (mg/dl)	Sodium (mg/l)	Potassium (mg/l)	Chloride (mg/l)	Total Protein (g/l)	Albumin (g/l)
Group 1 (262.20mg/kg)	5.80 ± 0.57	84.80 ± 3.99	145.00 ± 1.62	6.10 ± 0.28	100.80 ± 0.49	72.20 ± 2.29	38.20 ± 1.69
Group 2 (524.40mg/kg)	6.00 ± 0.61	84.40 ± 4.53	147.60 ± 3.43	6.90 ± 0.36	101.00 ± 1.64	77.80 ± 5.03	40.60 ± 2.94
Group 3 (786.61mg/kg)	4.70 ± 0.30	90.60 ± 3.35	147.00 ± 1.48	7.58 ± 0.34a	102.40 ± 1.29	72.00 ± 4.01	38.80 ± 2.89
Group 4 (control)	4.76 ± 0.23	84.00 ± 1.82	145.90 ± 1.28	6.32 ± 0.20	99.80 ± 2.63	73.20 ± 2.31	39.80 ± 1.02

Values presented as Mean ± SEM: a = significantly different at P = .05 when compared to Group 4 (control).

Table 2: Concentration of liver function markers in rats orally administered with *D. tripetala* fruits

Groups/ Parameters	ALT (U/L)	AST (U/L)	ALP (U/L)	Total Bilirubin (µmol/L)
Group 1 (262.20mg/kg)	29.80 ± 3.94	95.40 ± 7.57	47.60 ± 3.50	8.74 ± 1.28
Group 2 (524.40mg/kg)	34.00 ± 2.49	90.60 ± 3.37	60.00 ± 3.91 ^a	9.10 ± 1.47
Group 3 (786.61mg/kg)	31.20 ± 3.76	87.40 ± 4.05	40.20 ± 2.69	7.60 ± 1.45
Group 4 (control)	36.00 ± 4.09	106.80 ± 9.60	46.20 ± 3.82	6.30 ± 1.26

Values represent mean ± SEM;

a = significantly different at P = 0.05 when compared to Group 4 (control)

Discussion

Potassium ion (K⁺) is found predominantly in the intracellular fluid and it functions in the regulation of neuromuscular excitability, contraction of heart muscles and maintenance of ICF volumes and hydrogen ion concentration. Kidneys are important in the regulation of K⁺ balance by reabsorbing nearly all K⁺ by the proximal tubules and then excreting the additional or excess potassium in the urine under the influence of aldosterone (9).

Hyperkalemia develops when there is excessive production (oral intake, tissue breakdown) or ineffective elimination of potassium (10). It is reported to arise in states characterized by excessive destruction of cells with redistribution of K⁺ from

the intracellular to extracellular compartment in massive hemolysis (11), renal insufficiency thereby decreasing the body's ability to regulate serum potassium via the kidneys (12) and exercise (13).

The significant increase in the level of potassium in Group 3 compared to control is not surprising because, Okaka and Okaka (14) reported that *Denettia tripetala* fruits extracts contain high amount of potassium (2.48%), calcium (1.80%), sodium (0.72%), while zinc, copper, manganese, cobalt, nickel and cadmium are available in trace quantities.

Severe hyperkalemia is rare in normal individuals, but when it occurs, it can lead to impairment of neuromuscular, cardiac and gastrointestinal organ system. This may be due to depolarization of membrane potentials of cells due to increase in the equilibrium potential of potassium ions; as a result, voltage-gated sodium channels are open and there is increase inactivation at the same time. Since the depolarization due to concentration change is slow, action potential is not generated resulting in accommodation. Above a certain level of potassium, the depolarization may inactivate sodium channels and open potassium channels rendering the cell refractory (15).

Aldosterone plays an important role in regulating potassium homeostasis. Humans can adapt to an increase in dietary potassium by an increase in the renal excretion of this ion; thereby ensuring that high dietary intake of potassium does not lead to hyperkalemia in normal subjects.

Consequently, ALP is one of the enzymes reflecting liver cell damage. The major function of alkaline phosphatase is transporting across cell membranes (22). It has widespread tissue distribution including liver, bone, placenta and gastrointestinal tract (16). Damage to these tissues causes release of ALP into the bloodstream as seen in cholestasis, extrahepatic biliary obstruction, and primary biliary cirrhosis (20). Also, bone disease with increased osteoblastic activity shows increased ALP level in the serum as seen in Paget's disease (18). The significant increase of ALP in Group 2 and subsequent decrease in group 3 when compared to control is quite unclear. Moreover, other liver indices were not significantly altered; therefore, minor elevation in ALP may have been due to the presence of artifacts during experiment.

The elevated level of potassium in Group 3 compared to control is not sufficient to conclude that ethanol extract of DT fruits was toxic to the kidneys since other kidney indices like urea and creatinine levels were not significantly raised. Similarly, the extract is not hepatotoxic since AST and ALT were not significantly raised; also ALP is not a specific marker for liver damage.

3. CONCLUSION

The study of the effect of ethanolic extract of ripe *Denettia tripetala* extract on liver and kidney *function indices* in male albino rats suggested that the extract was not toxic to liver and kidney. I recommend that further studies should be done to ascertain this effect.

ETHICAL STATEMENT

This study was carried out with the principle and guidelines of the ethical committee for conduction of animal studies in Department of Pharmacology and Toxicology, University of Uyo, Nigeria

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COMPETING INTERESTS

No competing interest

AUTHORS' CONTRIBUTIONS

Author A' designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. 'Author B' and 'Author C' managed the analyses of the study. 'Author D' managed the literature searches. All authors read and approved the final manuscript."

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