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The Effect of Ethanol Extract of Ripe *Denettia tripetala* Fruit (Pepper Fruit) on Indices of Liver and Kidney Function in Male Albino Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author UME designed the study. Authors EIA and UEB performed the statistical analysis. Author EIA wrote the protocol, first draft of the manuscript and managed the literature searches. Author OEE managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate the effect of oral administration of ethanolic extract of ripe *Denettia tripetala (*DT*)* fruits 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 were 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* administered daily at doses of 262.20 mg/kg, 524.40 mg/kg and 786.61 mg/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 assayed using Randox kits, USA.

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Results: The result showed that there was an increase in the level of potassium in Group 2 and Group 3 compared to control. 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 confirm the results of the study.

Keywords: Denettia tripetala; liver function test; kidney function test; ethanol extract.

1. INTRODUCTION

An estimate by the World Health Organization (WHO) survey stated that 80% of the world population use medicinal plants in the treatment of disease; this rate is higher in African Countries [1]. According to Hammer et. al. (1999), plants are the richest resource of drugs for traditional system of medicine, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Many studies have proven the link between consumption of fruits, vegetables, grains, and other plant foods which are rich in phytochemicals [2] to reductions in the risk of major chronic diseases including cardiovascular disease, diabetes, cancers as well as neurodegenerative disorders [3]. Consequently, people who consume high quantities of vegetables and fruits may be at reduced risk for some of the diseases caused by biochemical dysfunction [4]. Since most plants have medicinal properties, it is of utmost importance that their efficacy and toxicity risks are evaluated.

Denettia tripetala (DT) G. Baker is an indigenous fruit tree of the family Annonaceae [5]. It is known in Nigeria by the following names: Nkarika (Ibibio/Efik), Ako (Edo), Mmimi (Ibo), and Ata Igbere (Yoruba) [6]. It's widely distributed and consumed by the inhabitants of Western Cameroons, Ivory Coast and Southern Nigeria [7]. 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.

Phytochemical screening of the ethanolic extract of DT revealed the presence of tannins, alkaloids, steroids, flavonoids, cardiac glycosides, saponins, and terpenoids [7]. These phytochemicals 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 non-toxic at high dose of the extract. Isoghohi and Orhue (6) also reported that the aqueous extract of DT ameliorates liver and kidney damage caused by multiple exposures to carbon tetrachloride [9]. 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.

2. MATERIALS AND METHODS

2.1 *Denettia tripetala* (Pepper Fruit)

Mature ripe fruits of *Denettia tripetala* (Fig. 1) was 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, air dried at room temperature and grounded into powder with mortar and pestle. The powdered fruit was macerated in 80% of ethanol for 3 days and 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.

2.2 Experimental Animals

Twenty male albino rats were obtained from University of Calabar and kept in animal house of Department of Pharmacology and Toxicology laboratory, 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.

Fig. 1. (A) *Dennettia tripetala* tree with leaves and unripe fruits. (B) Ripe (red) and unripe (green) *Dennettia tripetala* fruits. Adapted from Isoghohi [6]

2.3 Acute Toxicity Study

Forty eight (48) male mice weighing 18-30 g were used for the evaluation of acute toxicity of ethanol fruit 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 5000 mg of the extract per kilogram body weight of the mice intraperitioneally (I.P). All the mice were observed for general behavioral changes, symptoms of toxicity and mortality. The number of deaths within 24 hours was recorded. The LD_{50} was determined using the Lorke's method [10].

2.4 Experimental Design

The animals (weighing between 160-210 g) were randomly selected into four groups, five animals per group. Group 4 served as control. Groups 1, 2, and 3 received orally the ethanol extract of *Denettia tripetala* fruits at a daily dose of 262.20 mg/kg, 524.40 mg/kg and 786.60 mg/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 2500 g for ten minutes. The serum was obtained and used for biochemical assay.

2.5 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.

2.6 Statistical Analysis

Data were presented as mean ± 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.

3. RESULTS

3.1 Acute Toxicity Study

The LD_{50} of the extract of the fruits of *Denettia tripetala* according to Lorke's method was calculated to be 3496 mg/kg body weight.

The concentration of kidney function markers in rats orally administered with ethanol extract of DT fruits are presented in Table 1. 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 ethanol extract of DT fruits is shown in Table 2. 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 (262.20 mg/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 (524.40 mg/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 (786.61 mg/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 (µ mol/L)
Group 1 (262.20 mg/kg)	29.80 ± 3.94	95.40 ± 7.57	47.60 ± 3.50	8.74 ± 1.28
Group 2 (524.40 mg/kg)	34.00 ± 2.49	90.60 ± 3.37	60.00 ± 3.91[a]	9.10 ± 1.47
Group 3 (786.61 mg/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)

4. DISCUSSION

The present study was aimed at evaluating the effects of ethanol extract of DT fruits on liver and kidney function. Acute toxicity testing showed a very high LD_{50} value (3623 mg/kg), suggesting that the extract has a very high safety margin. This result is contrary to the work of Ikpi and Nku [8] who reported a very low LD_{50} value of 251.19 mg/kg body weight when ethanol extract of DT was administered intraperitoneally to mice. Anosike et. al. [11] reported an estimate of above 5000 mg/kg LD_{50} value, indicating that the mice did not die after 24hours of administration of ethanolic extract of DT seeds. Also, an LD_{50} of 1120 mg/kg was reported by Anaga and Colleagues [12], when ethyl acetate extract of DT was administered intraperitoneally to mice.

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 electrolyte balance (sodium, chloride, potassium and bicarbonates). The significant increase in the level of potassium in Group 3 compared to control is not surprising because, Okaka and Okaka [13] 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. However, there was no significant difference in the level of sodium and chloride in this study [9].

The kidney regulates 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 [14]. Hyperkalemia develops when there is excessive production (oral intake, tissue breakdown) or ineffective elimination of potassium [15]. 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 [16], renal insufficiency thereby decreasing the body's ability to regulate serum potassium via the kidneys [17] and exercise [18]. Severe hyperkalemia is rare in normal individuals, but when it occurs, it can lead to impairment of neuromuscular, cardiac and gastrointestinal organ system. The mechanism of action of potassium toxicity 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 [19].

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 [9].

Urea and creatinine are commonly used as markers of kidney function because the kidney is responsible for filtration of urea and creatinine from blood [9]. High level of metabolites in the serum is an indicative of kidney problem. In this study the concentration of urea and creatinine were not significantly altered by the extract; this suggest that that extract was not toxic to the kidney. Iseghohi and Orhue [9] reported on the ameliorative effect of aqueous extract of DT fruits on rats exposed to carbon tetrachloride by evaluating some kidney and liver function indices.

There was no significant difference in the concentration of total protein in all the groups when compared to the control, indicating that there was no damage to the liver and kidney which may have caused leakage of enzymes and other proteins in the liver, kidney and possibly other organs into the serum. Similarly, there was no significant difference in the concentration of albumin in all the groups when compared to the control this indicates that the synthetic function of the liver was not compromised by the extract.

Consequently, ALP is one of the enzymes reflecting liver cell damage. The major function of alkaline phosphatase is transportation across cell membranes [20]. It has widespread tissue distribution including liver, bone, placenta and gastro intestinal tract [21]. Damage to these tissues causes release of ALP into the bloodstream as seen in cholestasis, extra hepatic biliary obstruction, and primary biliary cirrhosis [22]. Also, bone disease with increased osteoblastic activity shows increased ALP level in the serum as seen in Pagets disease [23]. The concentration of ALT and AST were decreased

when compared to control but the decrease were not significant. AST and particularly ALT is a true marker for liver damage [24] than ALP, therefore, it is insufficient to conclude that the extract was hepatotoxic. The minor elevation in ALP is unclear; it may have been due to the presence of artifacts during experiment. The extract did not affect Bilirubin concentration; this further confirms that the extract is not hepatotoxic.

5. CONCLUSION

The study of the effect of ethanolic extract of ripe fruits of *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

Authors have declared that no competing interests exist.

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