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# Preliminary toxicology profile of *Dennettia tripetala* (Pepper Fruit) methanolic leaves extract

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## Abstract

**Background:** *Dennettia tripetala* (Pepper Fruit) belongs to the Annonaceae family and is abundant in Nigeria. Its fruit in folklore medicine is used for treatment of varying ailments. While ample research evidence exists on the plants fruit and seed, no current study exists on the toxicological profile of the plant leaves.

**Methods:** qualitative and quantitative phytochemicals and In vitro antioxidant assays were carried out using standard methods. The acute toxicity study indicates that the LD<sub>50</sub> was higher than 2000 mg/Kg body weight. Sub-chronic toxicity studies was carried out using five groups of rats. Group 1 served as control, 2–5 received 100 mg/Kg, 200 mg/Kg, 500 mg/Kg and 1000 mg/Kg body weight orally for 28 days.

**Results:** Post-administration biochemical analysis indicates there was increased weight in rats administered 100 mg/kg and 200 mg/kg while it reduced in the 500 mg/kg group. Significant elevations of liver function markers were reported for 200 mg/kg and 500 mg/kg respectively. Serum and hepatic protein profiles remained unaltered. Renal function analysis revealed elevated serum urea and creatinine for 200 and 500 mg/kg groups, elevated serum Na<sup>+</sup> and Ca<sup>+</sup> and reduced serum Cl<sup>-</sup> for the 500 mg/Kg group. Elevated Kidney K<sup>+</sup> and Ca<sup>+</sup> levels, reduced Cl<sup>-</sup> were significantly observed in 500 mg/Kg group. Significant rise in hepatic and renal lipid peroxidation was observed in 200 and 500 mg/Kg groups. There were observed disarmament of the antioxidant defense systems occasioned by rise and drop in tissue (hepatic, renal, testes, heart) Superoxide dismutase (SOD), Catalase (Cat), Glutathione-s-transferase (GST), Glutathione peroxidase (GPx) activities in the test groups relative to control. Histopathological examination indicated architectural aberrations at 500 and 1000 mg/kg.

**Conclusions:** It concluded that the plant had significant phytochemical and antioxidant properties of medical interest and possessed toxic properties in rats when administered at a dose above 200 mg/Kg over a prolonged period of time.

**Keywords:** Toxicology, *Dennettia tripetala*, Acute toxicity, Chronic toxicity, Antioxidants, Oxidative stress

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## Introduction

Plants have been identified as a major contributor to the sustenance of good health hence their uses have been widely reported [1, 2]. Several pharmaceutical products used in modern medicine for treatment of several ailments are derivatives of plant-based products that have undergone various stages of isolation and purification [3, 4]. In addition, the World Health Organization reported that several populations in developing countries depend on herbal medicine in meeting their health needs [5]. With the increased search for natural products as an alternative to synthetic drugs, several screening processes has been carried out in different plant materials. This had provided useful observations that could lead to further investigation of the chemical and pharmacological potentials of these medicinal plants.

*Dennettia tripetala* (Pepper Fruit) is a plant that belongs to the Annonaceae family. It is abundant in the tropical rain forest region of Nigeria [6, 7]. It bears edible fruits which are green when unripe and pink when ripe with a pungent spicy taste. Evidence based on folklore medicine showed that the seeds are used for treatment of cough, fever, diarrhea and rheumatism [8, 9]. The leaves were also reported to be used by locals as tea. Research evidence shows that studies conducted on the fruit (seed ripe and unripe) have reported it to possess some anti-diabetic, anti-analgesic, anti-inflammatory and neuro-pharmacological effects [9–11]. The seed have also been reported to possess anti-microbial properties due to its high levels of flavonoids, polyphenols and other medically significant phytochemicals [12–15].

Although the plant fruits and seeds have been extensively researched, only scant literatures have been specifically devoted to preliminary phytochemical and antimicrobial screening of *Dennettia tripetala* leaves [8, 16, 17]. At the time of this research, there is no evidence based studies on the in vitro antioxidant properties or toxicity profile of the leaves of this plant on any animal model, and based on the potential therapeutic benefits of the leaves of *Dennettia tripetala*, this study was carried out to access the phytochemical, in vitro antioxidant properties and the preliminary toxicological assessment of the methanol extract of the plant leaves.

## Materials and method

### Collection of plant material

Fresh leaves of *Dennettia tripetala* were harvested from a local farm in Umeghe-Abraka, Delta State, Nigeria. Samples of the leaf were taken to the Department of Botany Faculty of Life Science University of Benin, Benin City and was identified by a taxonomist Dr. Akinnibosun Henry Adewale as *Dennettia tripetala* (Family: Annonaceae). A voucher number UBH-D360 was deposited at the departmental herbarium.

### Plant extraction

The authenticated plant samples were transported to the laboratory at the Department of Medical Biochemistry and air dried for a period of 2 weeks after which pulverization into fine powder was carried out using a Warring blender (Quilink QBL-20 L40 model). Extraction of the methanol extract was carried out as described by the method of Achuba [18] by soaking 500 g of powdered leaves in 70% methanol and allowed to ferment for 72 h. At the end of the fermentation period, filtration was achieved using cotton wool and Wattman filter paper which was eventually concentrated using a rotary evaporator and later evaporated to dryness to obtain a finely separated crude extract powder in a water bath. The aqueous extraction employed the hot water extraction method previously described by George et al., [19] by boiling 500 g of the powdered leaf at a temperature of 100 °C for 10 min then allowed to cool. This was eventually filtered and the fine dry powder of the extract was obtained by same procedure described for the methanol extract. The yield obtained was 78.75 g for the methanol extract and 56.23 g for the aqueous extract. This amounted to a total yield of 15.75% and 11.25 % respectively of the ground powder.

### Phytochemical screening

#### Qualitative phytochemical screening

Determination of the presence of flavonoids, saponins, reducing sugar, and steroids employed the methods of Sofowora [20]. The method described by Trease and Evans [21] were used for determination of alkaloids, cardiac glycosides and tannins.

#### Quantitative phytochemical screening

The quantitative determination of the phytochemicals used the methods that were reported by Harborne [22] for alkaloid, cardiac glycosides, reducing sugar and saponin contents. Method of Sofowora [20] was used for tannin contents estimation with tannic acid serving as standard.

### In vitro antioxidant assay

The in vitro antioxidant capacities of the crude plant powder, aqueous extract and methanol extract were examined with four parameters using standard methods. The parameters and methods used were as follows; DPPH radical scavenging activity [23]; Nitric oxide radical scavenging activity [24], Ferric reducing antioxidant power [25] as reported by Iserhierhien and Okolie [26]. The lipid peroxidation (TBARS) inhibition assay was carried out using the method previously reported [27]. The methanol plant extract with the most in-vitro antioxidant capacity was evaluated for acute and sub-chronic toxicity.

### Ethical considerations

Study protocol was in consonance with the guidelines and declarations of Animal Research Ethics [28] and the World Medical Association [29] on animal use in biomedical research and it conformed to the animal rights law of the Delta State University (REC/FBMS/DELSU/19/078).

### Preparation of extracts for animal administration

A stock solution of the plant extract was prepared by dissolving 2 g of the crude methanolic plant extract in 20 ml of water to yield 100 mg/ml. Individual dose was administered based on the dose per kilogram body weight of the animals.

### Acute toxicity test and lethal dose determination

At the end of the in vitro antioxidant evaluation, the methanol leaf extract was reported to have a potent antioxidant potential compared to the aqueous extract. Based on this observation, the 16 rats shared into four groups of four animals each were subjected to acute toxicity test at a single dose of 500 mg/Kg body weight, 1000 mg/Kg body weight, 1500 mg/Kg body weight and 2000 mg/Kg body weight and monitored for 48 h. During this period, the rats were observed to show mild signs of moodiness and inactiveness at doses of 1500 mg/Kg and 2000 mg/Kg body weights, respectively. There were no deaths observed within the 48 h period which is indicative of the fact that the LD<sub>50</sub> was higher than the dose of 2000 mg/Kg body weight. Analysis of haematological parameters indicated a significant drop in PCV, HB and RBC values and rise in WBC values for rats administered the 1500 mg/Kg and 2000 mg/Kg body weights respectively. A further examination of serum liver function markers (ALT and AST) indicated an elevation of these parameters while the liver histopathology results for those administered 1500 mg/Kg and 2000 mg/Kg body weight indicated there were mild ballooning of the liver tissues. It was concluded that at the dose of 1500 mg/Kg and 2000 mg/Kg, the plant extract exhibited some level of toxicity. These results are attached as [supplementary data](#) in support of the current study.

### Chronic toxicity evaluation experimental design

#### Animal characteristic and acclimatization

Following the outcome of the acute toxicity study, thirty male albino rats with weights between 140 and 160 g of about 12 weeks old were purchased from the animal house of Emma Mariae Research Laboratories and Consultancy, Abraka Nigeria. The animals were transported using rectangular perforated plastic cage (30x32x68 cm) to the animal house of the College of Health Sciences, Delta State University, Abraka Nigeria. They were allowed to acclimatize to the new conditions and stored

in well-dressed wooden cages (30x40x80 cm) with a top opening covered with wire mesh to allow for easy ventilation and aeration. This was free of sharp edges and projections capable of causing injuries or accidental entrapment of their appendages. The environmental conditions comprised of an ambient temperature between 28 ± 1 °C, relative humidity 92%, 12:12 light and dark cycles and acclimatization lasted for 5 days.

#### Animal storage facility and distribution

The end of the acclimatization period was followed by the determination of the initial weight of the animals and random distribution into five groups of six rats each. The acclimatization conditions were sustained through the experiment and sawdust provided for use as beddings were changed daily so as to remove food debris and fecal droplets as well as minimize accumulation of moisture for optimal animal health. Free access to commercial rat chow and water were also maintained through the experiment. The first group served as the control and comprised of animals that were not treated with any dose of the methanol extract of *Dennettia tripetala* while the second, third, fourth and fifth groups comprised rats administered 100 mg/Kg, 200 mg/kg, 500 mg/kg and 1000 mg/Kg body weights respectively (see Tables 1 and 2). Treatment of the animal was done once daily for a period of 4 weeks (28 days). In the course of the experimental period, visible signs of weakness were observed in rats administered 1000 mg/Kg body weight this was followed by death of two rats in the group on the 12th day while further two died at the 18th day of the experiment. This observation was immediately followed by a sacrifice of the remaining two rats in the groups and their liver tissues harvested for histopathological analysis.

#### Sample collection

On the 29th day after an overnight fast, rats in the other groups were sacrificed following determination of their final weight. After cervical dislocation, the rats were dissected and blood samples collected via cardiac puncture

**Table 1** Chronic Toxicity Experimental Timeline and Design

Group	1–28 Days Drug Administration		Sacrifice	
		Death		
Control	–	–		Day 29
100 mg/Kg	+(1–28)	–		
200 mg/Kg	+(1–28)	–		
500 mg/Kg	+(1–28)	–		
1000 mg/Kg	+(1–18)	Day 12 + 2	Day 18 + 2	Day 18

+ Signs indicates the presence of a factor while – sign indicates its absence

**Table 2** Qualitative analysis of secondary metabolites of *Dennettia tripetala* leaves extracts

Phytochemicals	Crude leaf Sample	Aqueous extract	Methanol extract
Alkaloids	+	+	+
Saponins	+	+	+
Tannin	+	+	+
Cardiac glycoside	+	+	+
Reducing sugar	+	+	+
Flavonoids	+	+	+
Steroids	+	+	+

+ = Present

while vital organs (Liver, Kidney, Heart and Testes) were harvested, weighed and transferred to labelled containers.

#### Preparation of tissue homogenate

The tissue homogenate of each organ was prepared in pre-chilled pestle and mortar using 0.5 g tissue per 4 ml of cold normal saline solution. The homogenate was subjected to centrifugation at 5000 g for 10 min as previously reported [30] and the supernatant obtained was used for analysis. The serum was obtained by centrifugation of the clotted blood at 3000 g and the serum collected was stored at  $-4^{\circ}\text{C}$  before biochemical analysis was carried out.

#### Biochemical assays

Following sample collection, serum and liver ALT, AST, ALP were assessed using a commercial diagnostic kit Teco diagnostics USA. Serum and kidney urea, creatinine and electrolytes were done using the commercial Randox diagnostic kit according to the manufacturers' instruction.

Assay for lipid peroxidation was done following method described [31], tissue antioxidants were done by the following standard methods highlighted; GSH [32], Catalase [33], Super oxide dismutase (SOD) [34] Glutathione-S-transferase (GST) [35] and Glutathione peroxidase GPx [36].

#### Histopathological examination of liver

Examination of hepatic architecture was carried out as previously described [30]. Harvested liver tissues were rinsed with chilled cold normal saline and was cut to a size of (5 mm) and then fixed with 10% formalin. Sectioning using a microtome was done following a 90 min dehydration period through 70%, 90% and 95% absolute ethanol. Use of xylene for the clearing process was done for 2 h and followed by embedment with paraffin wax for another 2 h before sections of about  $7\ \mu$  thickness was cut, stained with hematoxylin and eosin (H&E) and mounted using cover slips. Specimen examination

followed by photographing was done under a light microscope.

#### Method of data analysis

Data analysis was carried out using analysis of variance (ANOVA) while group comparison was done using bonferroni at  $P \leq 0.05$ . Results were however presented in bar charts and tables as Mean  $\pm$  SD.

## Results

### Qualitative phytochemicals screening of *Dennettia tripetala* leaves

Results in Tables 1 and 2 presents the qualitative phytochemical screening of crude *Dennettia tripetala*, aqueous extract and methanol extract. All tested bioactive were present in all the samples of *Dennettia tripetala* leaves.

### Quantitative phytochemical screening of *Dennettia tripetala*

Table 3 presents the result of the quantitative phytochemical screening of *Dennettia tripetala* leaves extract. Details of the result revealed that Flavonoids had the highest mean value of 6.09 mg/g, 4.76 mg/g and 5.89 mg/g in all three samples of the leave extracts respectively. Steroids were the least for crude leaf sample (0.36 mg/g), and the methanol extract (0.11 mg/g) while saponins were the least in the aqueous extract (0.09 mg/g).

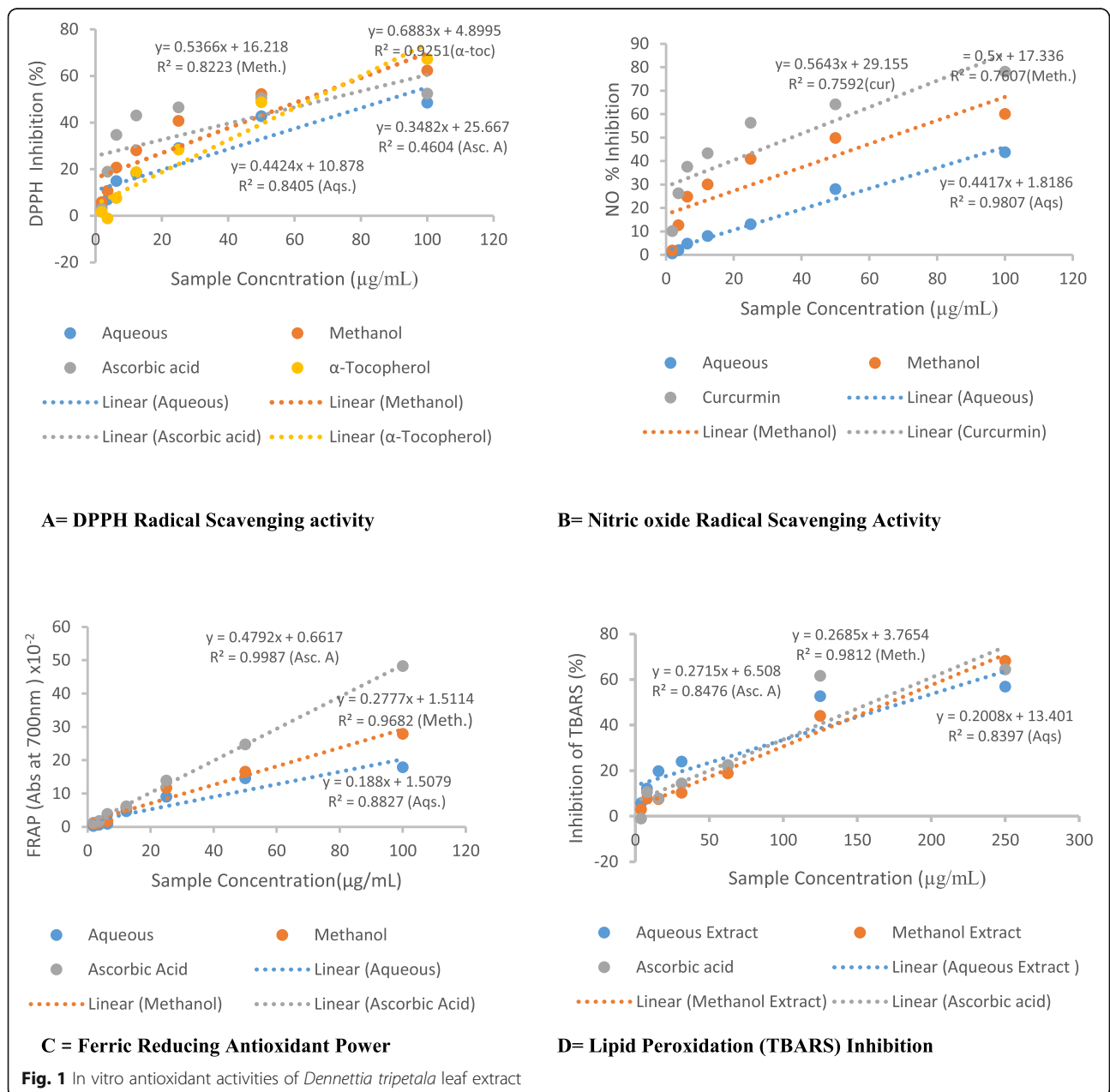
### In vitro antioxidant activity of *Dennettia tripetala* leave extracts

Figure 1 presents the results of the in vitro antioxidant activity of *Dennettia tripetala* leaf extracts. It shows the linear relationship between the antioxidant activities and the concentration of the extracts from which the various IC<sub>50</sub>s and EC<sub>50</sub> was extrapolated. The *Dennettia tripetala* extract had an IC<sub>50</sub> lower than the standard antioxidant vitamins C and E showed (Methanol extract: 62.95  $\mu\text{g/ml}$ ; Aqueous extract: 88.43  $\mu\text{g/ml}$ ; Ascorbic Acid: 69.88  $\mu\text{g/ml}$ ;  $\alpha$ -tocopherol: 65.46  $\mu\text{g/ml}$ ) The trends shown in Fig. 1B for Nitric oxide radical scavenging activity showed that the standard antioxidant

**Table 3** Quantitative phytochemical screening of *Dennettia tripetala* leaves crude sample and extracts

Phytochemical	Crude leaf sample (mg/g)	Aqueous Extract(mg/g)	Methanol Extract(mg/g)
Alkaloids	4.84 ± 0.23	2.04 ± 0.08	3.14 ± 0.30
Saponins	0.38 ± 0.00	0.09 ± 0.00	0.26 ± 0.03
Tannin	3.82 ± 0.26	1.18 ± 0.02	2.86 ± 0.63
Cardiac glycoside	4.18 ± 0.50	2.98 ± 0.04	3.78 ± 0.64
Reducing sugar	3.04 ± 0.37	2.98 ± 0.02	3.13 ± 0.00
Flavonoids	6.09 ± 0.02	4.76 ± 0.66	5.89 ± 0.32
Steroids	0.36 ± 0.02	0.24 ± 0.05	0.11 ± 0.05

All values are expressed as Mean ± SD of three replicates



curcumin had the least followed by the methanol extract. The IC<sub>50</sub> for *Dennettia tripetala* extracts relative to NO revealed (Methanolic extract: 65.32 µg/ml; Aqs extract: 109.08 µg/ml; Curcumin: 36.94 µg/ml). The Ferric reducing antioxidant power in Fig. 1C also showed similar trend observed for the NO<sup>-</sup> inhibition as the least value of the 50 % effective dose (EC<sub>50</sub>) was observed in the standard antioxidant Vit. C (102.90 µg/ml) and was followed by the methanol extract (174.61 µg/ml) while the aqueous leave extract had the least effective dose (257.94 µg/ml). As in the NO, the lipid peroxidation (TBARS) inhibition assay shown in Fig. 1D also revealed that the standard antioxidant ascorbic acid had a better activity as it had the least IC<sub>50</sub> given at (160.19 µg/ml), this was followed by the methanolic extract which was 172.20 µg/ml) and the aqueous extract which was 182.26 µg/ml). Comparatively to the standard antioxidants, the methanolic extract had more antioxidant potency than the aqueous extract.

#### Effect of *Dennettia tripetala* on weight gain

Figure 2 presents the effect of *Dennettia tripetala* on body weight changes in Rats. It shows a relatively increased weight in rats administered 100 mg/Kg and 200 mg/Kg body weight and relatively reduced weight occasioned by a negative weight gain potential in rats administered 500 mg/Kg bodyweight.

#### Effect of *Dennettia tripetala* on liver function markers

Figure 3 presents the activities of liver function markers in rats administered *Dennettia tripetala* methanolic extract and is indicative that comparative to control, there was a non-significant rise in serum and liver ALT in rats administered 100 and 200 mg/Kg body weight respectively but this increased in rats administered 500 mg/Kg body weight. Similar trends were observed in serum AST for 100 mg/Kg but increase were observed in 200 mg/Kg and 500 mg/Kg respectively. ALP activities

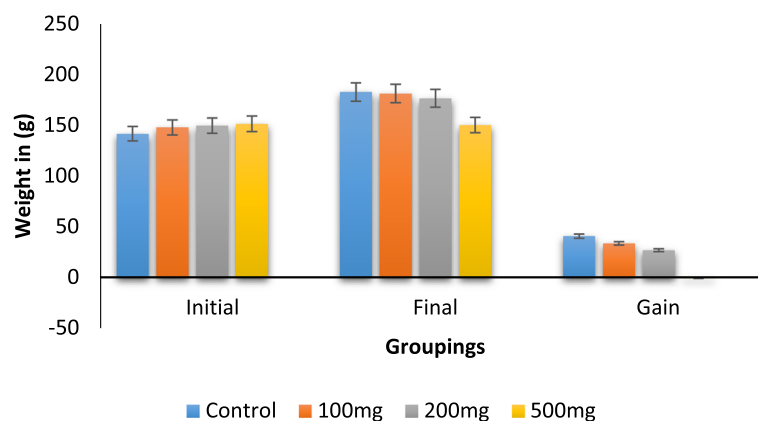
increased in serum for 100 mg/Kg and 500 mg/Kg but not for 200 mg/Kg relative to control while there were observed rise in liver ALP for all test groups relative to control.

Figure 4 presents the data on changes in protein profile of rats following treatment with *Dennettia tripetala* methanolic extract. It shows a non-significant difference across all experimental groups on serum total protein profile while the liver only showed a significant decrease in the 200 mg/Kg group relative to control. The albumin levels on the other hand showed a significant decrease in the serum of the 200 mg/Kg group while all groups showed no significant difference in the liver relative to control.

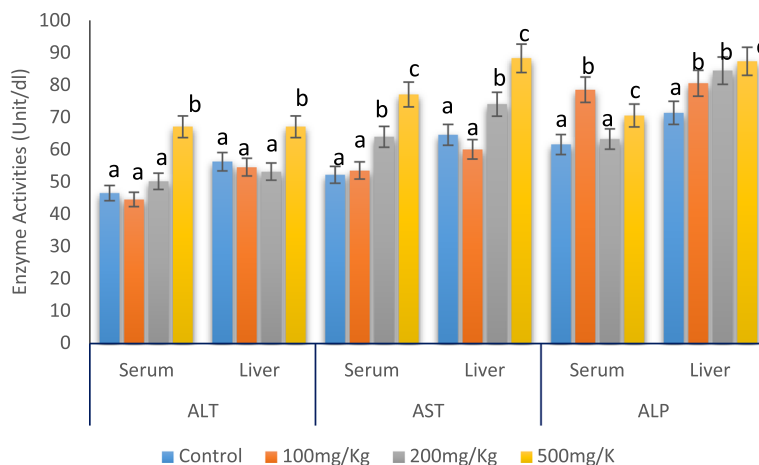
#### Effect of *Dennettia tripetala* on renal function markers

Figure 5 presents the effect of *Dennettia tripetala* methanolic extract on urea and creatinine levels in the serum and kidney. There were observed significant rise in serum urea in the 200 mg/Kg and 500 mg/Kg body weight relative to control while kidney urea was not significantly affected compared to control but on a dose comparison was reduced in the 500 mg/Kg body weight relative to the 100 mg/Kg dose. Serum creatinine levels were observed to reduce significantly in the 100 mg/Kg group but increased significantly in the 200 mg/Kg and 500 mg/Kg group with respect to control. The kidney creatinine decreased significantly in the 200 mg/Kg group and increased in the 500 mg/Kg group compared to control group.

Table 4 presents the effect of *Dennettia tripetala* leaf extract on serum electrolytes. This is indicative of a non-significant change across all experimental groups for K<sup>+</sup> while the only significant rise observed for Na<sup>+</sup> and Ca<sup>+</sup> were for the 500 mg/Kg group. Significant drop in Cl<sup>-</sup> was also observed for 500 mg/Kg group and the other groups remained non-significant compared to control. The bicarbonate ion was observed to have no



**Fig. 2** Effect of *Dennettia tripetalla* Extract on Animal Weight



**Fig. 3** Effect of *Dennettia tripetala* on activities of serum and tissue Liver function markers (ALT, AST, ALP)

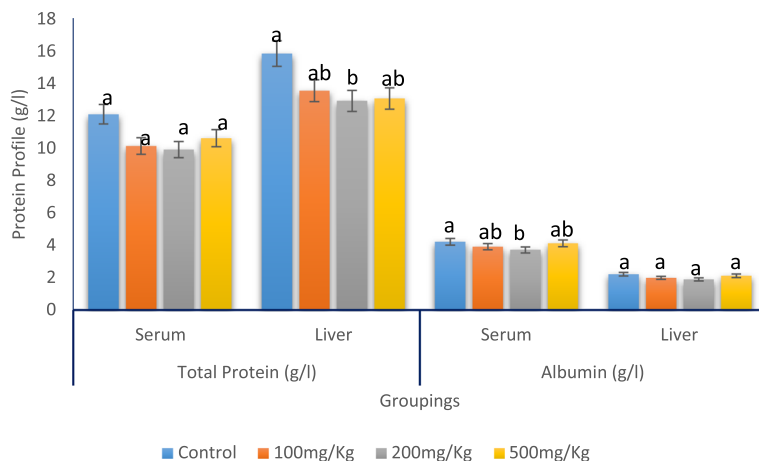
significant change compared to control but reduced in the 100 mg/Kg group compared to 200 and 500 (mg/kg) groups respectively.

Table 5 presents the effects of *Dennettia tripetala* methanolic leaf extract on kidney electrolyte profile and indicates that there was no significant change in K<sup>+</sup> of the 100 mg/Kg and 200 mg/Kg groups compared to control but this was elevated in the 500 mg/Kg group. Likewise, no significant changes were observed in Na<sup>+</sup> in the 100 mg/Kg and 500 mg/Kg while reduction was observed in the 200 mg/Kg relative to control. Ca<sup>+</sup> had no significant change in the 100 mg/Kg group but increased in the 200 mg/Kg and 500 mg/Kg respectively compared to control. The Cl<sup>-</sup> levels in the kidney were reportedly not significantly increased for the 100 mg/Kg group as well as not significantly reduced for the 200 mg/Kg group but was significantly reduced for the 500 mg/Kg group relative to control. Kidney bicarbonate had no significant

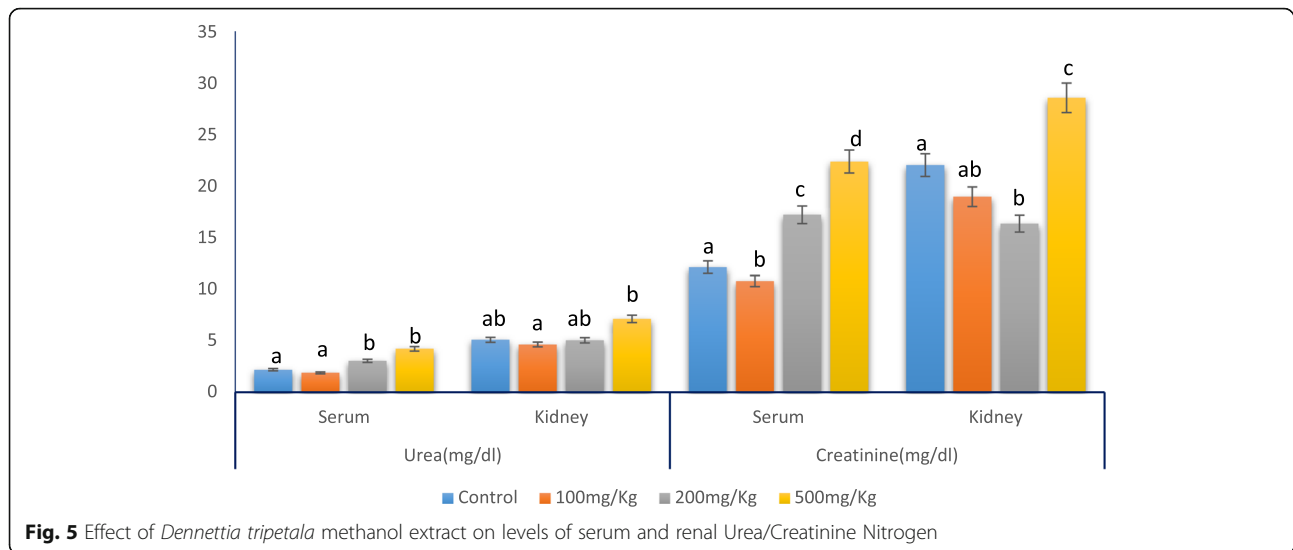
change across groups 100 mg/Kg and 500 mg/Kg but this was significantly reduced in the 200 mg/Kg group compared to control.

**Effect of *Dennettia tripetala* extract on oxidative stress and antioxidant defense system**

Figure 6 presents the results of lipid peroxidation and antioxidant profile of rats following *Dennettia tripetala* methanolic extract administration. The MDA levels were reported to significantly increase in liver tissues across the 200 mg/Kg and 500 mg/Kg group and reduced in the 100 mg/Kg group compared to control. Kidney tissue indicates a non-significant difference for the 100 mg/Kg and significant increase for the 200 mg/Kg and 500 mg/Kg groups. Heart tissues only showed a significant rise in the 500 mg/Kg group and no significant difference in other groups relative to control. Testes indicated no significant change in all experimental groups. SOD



**Fig. 4** Effect of *Dennettia tripetala* on Serum and Tissue Protein Profile



**Fig. 5** Effect of *Dennettia tripetala* methanol extract on levels of serum and renal Urea/Creatinine Nitrogen

activities significantly increased in the liver only for the 500 mg/Kg group relative to control and in the kidney, heart and testes for the 200 mg/Kg and 500 mg/Kg groups respectively with respect to control. Catalase enzyme activities rose significantly across all test groups in the liver compared to control but reduced in the kidney while increasing in the heart and testes for the 200 mg/Kg and 500 mg/Kg respectively compared to control. Kidney, heart and testes GSH levels had no significant changes across all experimental groups while liver GSH was significantly depleted in the 500 mg/Kg group relative to all other groups. Glutathione peroxidase (GPx) enzyme activities were significant for the liver relative to control at the 200 mg/Kg and 500 mg/Kg respectively while the kidney showed no significant change across all groups. Heart GPx activities were only significant at 200 mg/Kg and 100 mg/kg, 200 mg/kg and 500 mg/kg showed significant rise relative to control in the testes. The activities of Glutathione-S-Transferase (GST) showed no significant change across all tissues and all doses of exposure of *Dennettia tripetala* methanolic extract.

Bars in the same organ showing different alphabet superscripts indicates they are significantly different ( $p < 0.05$ ).

#### Effect of *Dennettia tripetala* on liver histology

Liver histology results is indicative of no deleterious observations in control rats and those administered 100 mg/kg and 200 mg/kg as there were very clear hepatic cells (A) and sinusoids emanating from the central vein (B). Rats treated with 500 mg/Kg presents gradual ballooning of the hepatic cells and veins (C) while the rats treated with 1000 mg/Kg shows very visible signs of hepatic necrosis (D) and degeneration of the central vein (E). Findings observed in the liver of experimental animals administered 500 mg/kg and 1000 mg/kg of *Dennettia tripetalla* revealed marked distortion of the liver architecture.

#### Discussions

The importance of plant materials as major sources of active medicinal substances needed for the mitigation of several medical conditions abounds in literature [5, 8, 37, 38]. The present study revealed the presence of several phytochemicals such as alkaloids, saponins, tannins, cardiac glycosides, reducing sugars, flavonoids and steroids in the leaves of *Dennettia tripetalla* extracts via qualitative determinations and was further confirmed using quantitative determinations. These observations

**Table 4** Effects of *Dennettia tripetala* methanol leaf extract on Serum Electrolyte Profile

Groups	K <sup>+</sup> (Meq/L)	Na <sup>+</sup> (Meq/L)	Ca <sup>+</sup> (Meq/L)	Cl <sup>-</sup> (Meq/L)	HCO <sub>3</sub> <sup>-</sup> (Meq/L)
Control	5.07 ± 0.04 <sup>a</sup>	62.44 ± 1.18 <sup>a</sup>	6.27 ± 1.02 <sup>a</sup>	11.16 ± 0.27 <sup>a</sup>	2.08 ± 0.62 <sup>ab</sup>
100 mg/Kg	4.98 ± 0.01 <sup>a</sup>	60.08 ± 0.62 <sup>a</sup>	8.08 ± 2.22 <sup>ab</sup>	11.02 ± 0.04 <sup>a</sup>	1.88 ± 0.06 <sup>a</sup>
200 mg/Kg	5.46 ± 0.06 <sup>a</sup>	58.22 ± 2.44 <sup>a</sup>	6.40 ± 1.01 <sup>a</sup>	10.06 ± 0.11 <sup>a</sup>	2.80 ± 0.77 <sup>b</sup>
500 mg/Kg	5.98 ± 0.06 <sup>a</sup>	68.22 ± 0.66 <sup>b</sup>	9.98 ± 0.06 <sup>b</sup>	8.17 ± 0.085 <sup>b</sup>	2.18 ± 0.76 <sup>ab</sup>

All values are expressed as Mean ± SD values followed by a different alphabet superscript in the same column indicate a significant difference



**Table 5** Effects of *Dennettia tripetala* methanol leaf extract on Kidney Electrolyte Profile

Groups	K <sup>+</sup> (Meq/L)	Na <sup>+</sup> (Meq/L)	Ca <sup>+</sup> (Meq/L)	Cl <sup>-</sup> (Meq/L)	HCO <sub>3</sub> <sup>-</sup> (Meq/L)
Control	7.27 ± 0.14 <sup>ab</sup>	72.07 ± 0.08 <sup>a</sup>	5.07 ± 0.04 <sup>ab</sup>	9.62 ± 0.70 <sup>ab</sup>	3.81 ± 0.78 <sup>a</sup>
100 mg/Kg	6.82 ± 0.18 <sup>a</sup>	69.08 ± 0.02 <sup>a</sup>	4.98 ± 0.01 <sup>a</sup>	10.20 ± 1.40 <sup>a</sup>	2.99 ± 0.63 <sup>ab</sup>
200 mg/Kg	8.64 ± 0.01 <sup>bc</sup>	58.22 ± 0.02 <sup>b</sup>	7.46 ± 0.06 <sup>b</sup>	8.26 ± 0.01 <sup>bc</sup>	2.88 ± 0.07 <sup>b</sup>
500 mg/Kg	9.82 ± 0.26 <sup>c</sup>	74.22 ± 0.07 <sup>a</sup>	11.11 ± 1.24 <sup>c</sup>	7.98 ± 0.02 <sup>c</sup>	3.08 ± 0.96 <sup>ab</sup>

All values are expressed as Mean ± SD values followed by a different alphabet superscript in the same column indicate a significant difference

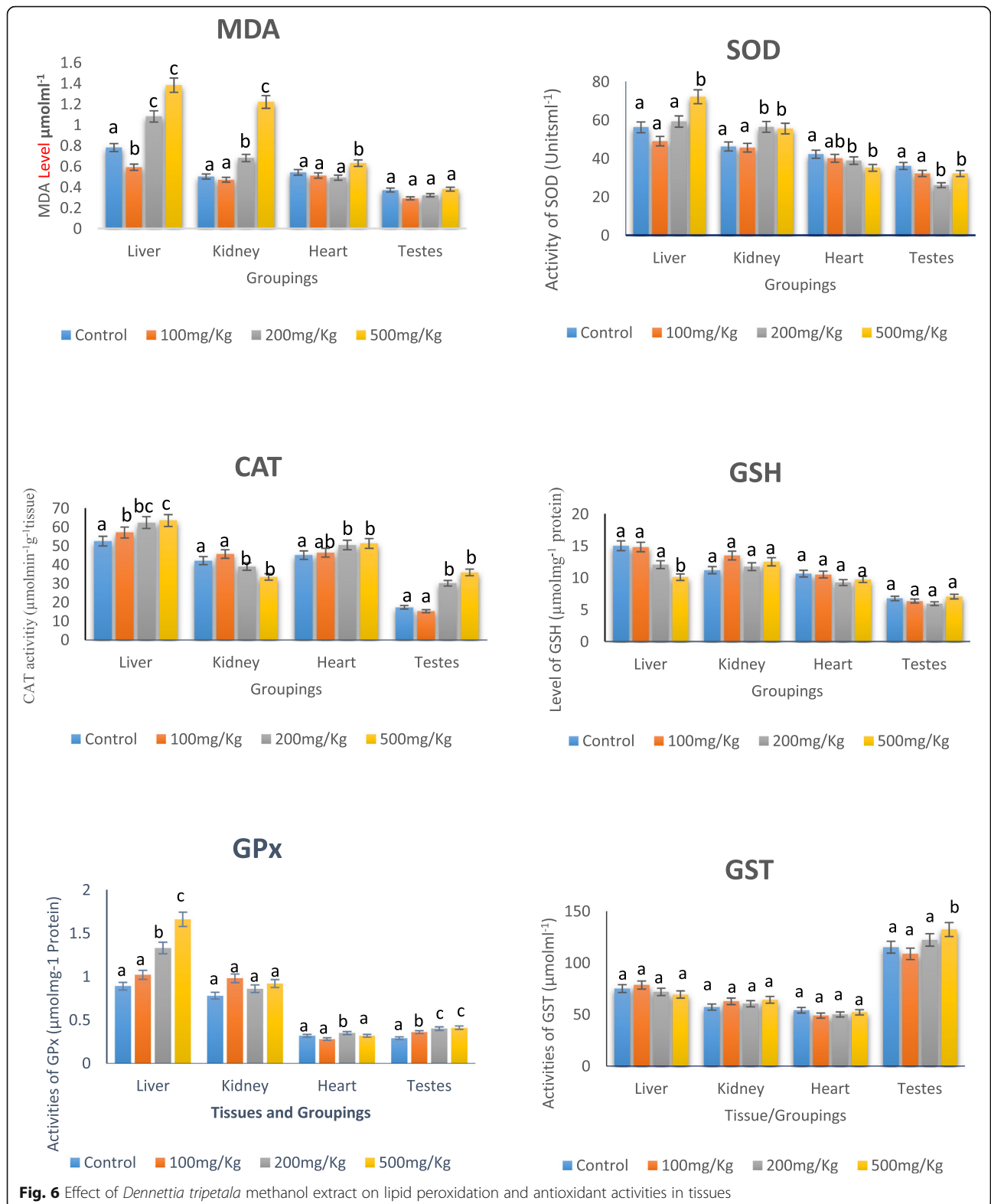
are in tandem with the reports on the fruits of *Dennettia tripetalla* by Omage et al., [39] as well as previous reports [16] on the presence of polyphenols, flavonoids and saponins in the leaf of *Dennettia tripetalla* ethanolic extracts. The medical importance of alkaloids have been reported to include drug synthesis especially analgesics, antibiotics and antispasmodics [16, 40]. Likewise, saponins have been noted to be responsible for the reduction of hypercholesterolemia as well as serving as emulsifiers and expectorants [1, 41, 42]. Saponins have also been previously reported to possess wound healing properties as well as the ability to induce hemolysis while acting as an antifungal agent [43, 44]. The presence of flavonoids and polyphenols in plants often confers on the plant with antioxidant properties and are often the reason while most plants are explored for their medicinal capabilities. Flavonoids and polyphenols are reported to possess anticancer properties and have the ability to reduce inflammation as well as possess antimalarial, antiviral, antibacterial and inhibition of hyper cholesterol synthesis [45–47].

The Results of the in vitro antioxidant properties of the aqueous and methanolic plant leaf extracts (Fig. 1) are indicative that the plant extracts possesses antioxidant properties comparable to standard antioxidants such as curcumin, vitamins C and E. This is owing to their abilities to scavenge DPPH and NO<sup>-</sup> free radicals, possession of good ferric reducing antioxidant power at low concentrations as shown by the IC<sub>50</sub> and EC<sub>50</sub> values and compares favourably with ascorbic acid in the inhibition of lipid peroxidation. These properties were also previously reported in the plants fruit ethanolic and aqueous fruit extract [39]. There is no doubt that these properties may have arisen owing to the high level of phytochemicals earlier reported in this study. Comparatively, the methanol extract possess a better antioxidant property than the aqueous extract. This was exhibited when test indices were compared to standard antioxidant vitamin C, vitamin E and curcumin and may be linked to the fact that methanol had a much more significant capacity to extract the phytochemicals inherent in the plant. This assertion is supported by Onakhurefe et al., [42] who reported that most substances possessing high levels of hydrophobicity will dissolve better in

higher polar compounds. Therefore, the result of this study is indicative that the *Dennettia tripetala* leaf extracts possesses significant high antioxidant properties that are comparable with standard vitamin C and E and the previously reported antioxidant property of the ethanolic and aqueous fruit extracts [39].

Studies have reported the use of *Dennettia tripetala* leaf for the treatment of diarrhea, cough and fever among locals in Nigeria [9, 16, 38] yet no existing study have evaluated the toxicity profile of this leaf. In the current study, we evaluated the toxicity profile of *Dennettia tripetala* by subjecting it to acute and chronic toxicity study. Our result revealed muscle weakness and mood alterations in the experimental animals administered 1500 mg/kg and 2000 mg/kg body weight of *Dennettia tripetala*. It is important to note that the results from the acute toxicity study, though revealed no death of animals at the highest dose of 2000 mg/Kg within 48 h, the purported moody and weakness experienced in rats administered with 1500 mg/Kg and 2000 mg/Kg plant extracts gives an insight into the possible toxicity potentials of the plant leaf. This was corroborated by the elevation of serum ALT and AST activities as well as the reduction in haematological parameters within 48 h at these doses. These observations are in consonance with earlier reports who noted that alterations in these parameters are among the first line of responses to toxicity [30, 48].

Also, the reported death of rats administered 1000 mg/Kg and the eventual reduction in weight by animals administered 500 mg/Kg of the plant extracts following a prolonged period of administration gives further credence to our claims of the possible toxicity potential of the plant leaves. Body weight loss has been previously established as a significant early marker and responses to toxicity potentials of substances especially as it relates to drugs [49, 50]. The observed further significant elevation in liver function enzymes especially as it relates to ALT, AST and ALP at 500 mg/Kg dose relative to control (Fig. 3) may be indicative of a possible compromise on the liver tissue, which may have necessitated the increased leakage of these enzymes into the blood [51, 52]. A look at the serum and hepatic protein profile of rats administered the *Dennettia tripetala* methanolic leaf



extract (Fig. 4) is indicative of a non-alteration of protein synthesis at all doses. The stability of these parameters may also give us an insight into a possible beneficial

contribution of the plant extract to protein synthesis. The ability of plant extracts to significantly contribute to the stability of endoplasmic reticulum and the mediated

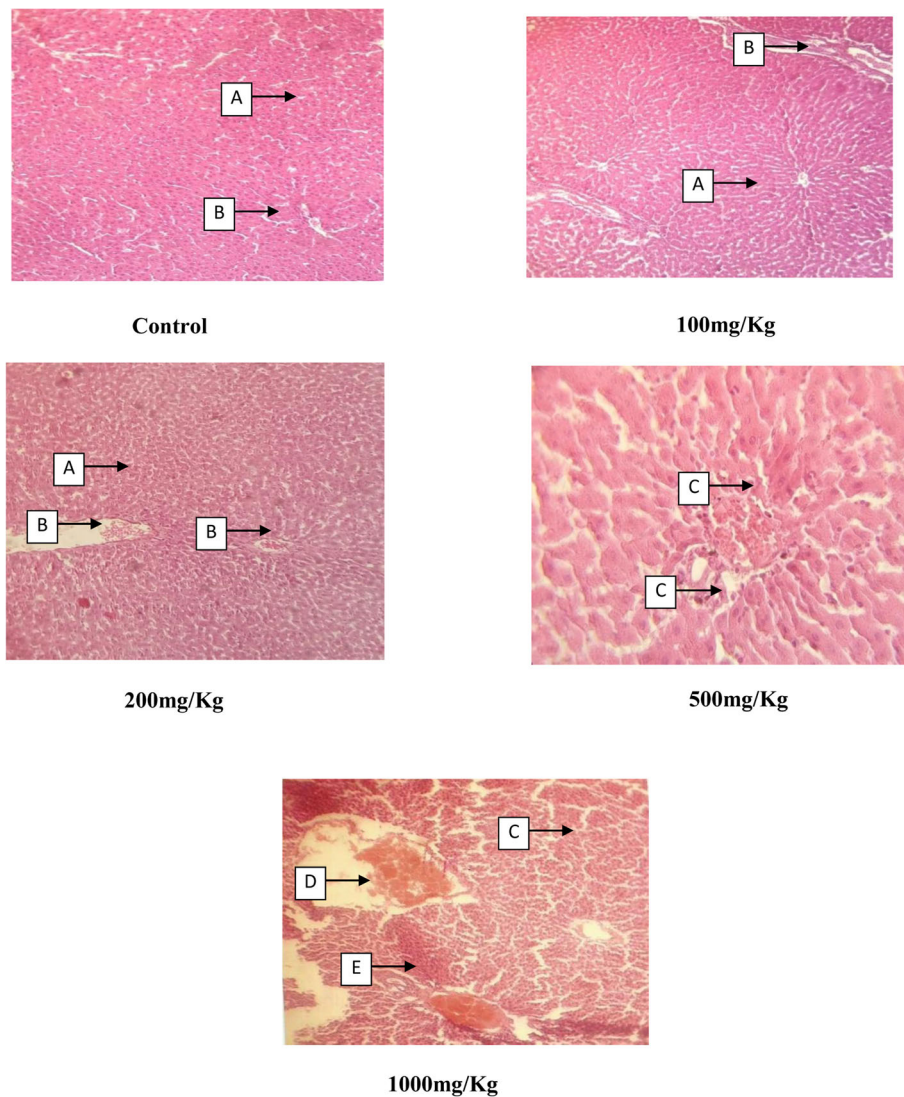
improved hepatic protein synthesis has been previously reported [51, 53]. Also, Omage et al [39] reported that the administration of the ethanolic and aqueous fruit extracts of *Dennettia tripetala* did not alter the serum and hepatic protein profile of rats at a dose up to 1000 mg/Kg body weight. One of the clinical significances of albumin in the blood is that it acts as a transporter for drugs and helps in the prevention of leakage of significant blood vessels [53]. Hence the ability of the *Dennettia tripetala* leaf extract to promote their synthesis may be one of the beneficial effects inherent in the plant which people who use them for treatment of ailments may have continually benefited from.

The kidney is a major organ of metabolic significance owing to their role in the filtration process of toxicants and absorption of essential nutrients and minerals from the blood [18, 54]. The compromise in structure and function of the kidney has a high level of implication in human and animal health. The result in the present study is indicative of a significant elevation of serum urea and creatinine (Fig. 5) at doses of 200 mg/Kg and 500 mg/Kg may also be implicative of a possible renal compromise as their elevation in the serum has been well documented as significant markers of kidney malfunction occasioned by loss of glomeruli filtration [18, 54]. This assertion is also suggestive of the fact that the alleged rise in kidney urea and creatinine is as a result of retention of these toxic substances which ought to be filtered out for excretion so as to promote proper renal function. The electrolytes which are another significant set of indicators for kidney functions were also found to be altered mostly in rats administered the 500 mg/Kg dose of the *Dennettia tripetala* leaf extracts. Although there were no significant alterations in serum potassium ions, a significant rise in serum levels of sodium and calcium as well as reduction in serum chloride ions (Table 4) gives cause for worry. The elevation in serum calcium ions is suggestive of a possible disruption in certain ion pumps and trans-membrane ATPases within the liver and kidney [18, 37, 55]. In another development, changes due to rise in serum sodium levels and reductions in chloride have implications for monitoring heart complications, decreased blood circulations and acid-base abnormalities which contributes significantly to the mal-absorption of these electrolytes within the kidney [56, 57]. It is interesting to note that findings in this study agree with previous studies on the ability of *Aframomium sceptrum* and *Aframomium maligueta* extracts to contribute significantly in the elevation of serum and renal sodium ions in rat species [54, 55]. Comparatively, at the dose of 100 mg/Kg and 200 mg/Kg it was observed that most renal indices were relatively stable when compared to control implying that these doses may have some element of significant beneficial potentials to the

experimental animals. This further brings to fore earlier suggestions [18] that certain plant substances may hold beneficial properties, when administered in the right dose.

Also studied in this investigation is the antioxidant response mechanisms to a prolonged administration of varied doses of methanol extract of *Dennettia tripetalla* which showed rise in MDA (Fig. 6) at doses of the 200 mg/Kg and 500 mg/Kg in the liver and kidney while heart tissues were only elevated in the 500 mg/Kg group. The evidential rise in lipid peroxidation further substantiates our claim of a possible toxic potential possessed by the plant leaf extract at these doses (200 & 500) mg/Kg as rise in lipid peroxidation is a known marker for breakdown in membrane lipids and increased oxidative stress [48, 58, 59]. Rising lipid peroxidation is often predicated by disruption of the antioxidant defense systems and in some other cases induces the expression of several antioxidant potentials that contributes to the clearance of oxidative radicals arising from peroxidation of lipids [60, 61]. As expected, there were significant rises in the antioxidant enzymes superoxide dismutase and catalase which are known scavengers of the superoxide anion ( $O_2^-$ ) and hydrogen peroxides ( $H_2O_2$ ) converting them to less dangerous species. Although no significant rise were observed in the non-enzymatic antioxidant (GSH) (Fig. 6) across all tissues except in the liver of rats administered 500 mg/Kg body weight, rise in GPx activities at doses of (200 & 500) mg/kg in the liver and testes may be indicative of complementary mechanism to support the clearance of rising peroxides within these tissues. This is simply so because the enzyme GPx together with SOD have been known to work in tandem with the catalase enzyme for clearance of oxidative potentials within tissues [62, 63]. Thus observed rise in these enzymes may have occurred in response to immunity boost of the animals or may have been as a result of the need to rise against the toxic effects of the plants in the animals. These claims and submissions are in agreement with [39] which also reported increased hepatic and renal SOD and catalase in rats administered *Dennettia tripetala* fruit aqueous and ethanol extracts.

In furtherance of our hypothesis, the histopathological analysis of the liver revealed a disruption in the hepatic architecture occasioned by hepatic necrosis for rats administered 1000 mg/Kg body weight of the plant extracts (Fig. 7) after sacrifice on day 18 of the experiment. Also, the gradual ballooning of the hepatic veins and sinusoids in the 500 mg/Kg group (Fig. 7) confirms our claim of the toxic potential of this plant at this dose. The non-presence of necrosis in the rats administered 200 mg/Kg and 100 mg/Kg as compared to control may be indicative that the plant extracts is well tolerated at these doses. Although the exact mechanisms by which this



**Fig. 7** Effect of *Dennettia tripetala* methanol extract on Liver Architecture (H&E  $\times$  100)

plant extract may cause liver toxicity is unknown, it is believed that the liver tissue is prone to drug induced toxicity due to its high capacity in metabolism and the presence of xenobiotics in the liver [64].

### Conclusion

The current study is suggestive that the leaf extract of *Dennettia tripetala* possesses significant phytochemicals of medical interest as well as antioxidant properties comparable to standard antioxidants vitamins C and E and curcumin. However, they stand to possess levels of toxic properties in rats when administered at a dose above 200 mg/Kg over a prolonged period of time and 1500 mg/Kg within a short period of about 48 h hence have the capacity to alter several organ functions related to metabolic stability. At this dose (200 mg/Kg) also, it

could be said to have the ability to induce several indices of antioxidant defense within the system. The implication of this to its ethno-pharmacological application to the treatment of ailments is that its functionality is greatly dependent on a dose response function and is recommended that when used, it should not be used excessively or indiscriminately abused so as not to elicit its toxic properties. It could suffice therefore to say based on our study that the safe dose of *Dennettia tripetala* is at 200 mg/kg body weight for rats.

### Prospects for further studies

The significance of the oxidized and reduced glutathione (GSH/GSSG) ratio in understanding responses to oxidative stress and different health related conditions in mammals has been well established in both in vitro and

in vivo studies [65–68]. Also the use of messenger RNA (mRNA) expression of different tissue antioxidants for confirmatory purposes and a better mechanistic understanding of the trends in antioxidant enzyme activities has been established [69–71]. Although we did not consider this aspect in the current study, there is no doubt that our study being a preliminary study have established the basic data for toxicological understanding of the effects of methanolic leaf extracts of *Dennettia tripetala*. We however hope to consider these factors in follow up studies using this plant so as to further substantiate the claims made by us.

#### Abbreviations

TBARS: Thiobarbituric Acid Reactive Substance; MDA: Malondialdehyde; PUFA: Polyunsaturated Fatty Acid; GSH: Reduced Glutathione; SOD: Superoxide Dismutase; CAT: Catalase; ANOVA: Analysis of Variance; AST: Aspartate amino transferase; ALT: Alanine amino transferase; GST: Glutathione-S-transferase; GPx: Glutathione peroxidase

#### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40816-021-00298-w>.

#### Additional file 1.

#### Authors' contributions

Authors JCM and PCI designed and conceived the study. Authors GRK and PCI carried out the animal care and laboratory analysis under the supervision of JCM. Literature search and data analysis was carried out by authors RNI, JCO and UE. Initial manuscript draft was carried out by authors PCI and JCM. All authors read and approved of the contents in the final draft.

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#### Availability of data and materials

Data used to support the findings of this study are included within the manuscript.

#### Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

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