EVALUATION OF THE BIOCHEMICAL AND TOXICOLOGICAL PROFILE OF METHANOL EXTRACT OF *DENNETTIA TRIPETALA* (PEPPER FRUIT) FRESH LEAVES ON SOME SELECTED PARAMETERS IN MALE ALBINO RATS

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Received *July 22, 2022;* ***Revised*** *August 13, 2022;* ***Accepted*** *August 31, 2022*

ABSTRACT

The vast majority of Africans still use traditional medicine as the primary means of treatment. The aim of this study was to determine the biochemical and toxicological properties of methanol extract of fresh Dennettia tripetala (pepper fruit) leaves in male albino rats. Twenty healthy male albino rats were divided into four groups of five rats each was used in this study. Group 1 was the control group; groups 2, 3 and 4 received an oral dose of 100, 200, and 400 mg/kg methanol leave extract of D. tripetala daily for 21 days. After 21 days of the administration, the rats were sacrificed, and blood samples were collected through the ocular puncture to assay the biochemical parameters. Animals given the D. tripetala methanol extract at doses up to 1000 mg/kg body weight showed no harm in acute toxicity testing; however, D. tripetala caused a significant derangement in the liver and kidney profile. ALT, AST, ALP, total protein, albumin, direct bilirubin, urea and creatinine levels increased non-significantly (p>0.05) from the control. The activities of the antioxidant parameters showed a non-significant increase at all doses compared to the control. In conclusion, this study has shown that administration of methanol leaves extract of D. tripetala at a dose over 100 mg/kg body weight for an extended period may induce toxicity to the liver and kidney, which could cause hepatic disease and renal dysfunction.

Keywords: Antioxidant, *Dennettia tripetala*, Kidney, Liver, Lipid, Toxicity

INTRODUCTION

The preference for plants over synthetic substances for treating diseases has resulted in extensive international studies into their use as drugs (Edeoga *et al.,* 2005; Ibeh *et al.,* 2020). Nevertheless, the number of plants that have been tested for harmful effects has consequently decreased due to this personal interest in elucidating the therapeutic potentials of plants (Iloanusi *et al.,* 2022). Investigations related to plant efficacies are more likely to report toxicities, and there is a need to include both long- and short-term toxic effects (Dias *et al.,* 2012).

Dennettia tripetala Baker F. 1913 (Magnoliales: Annonaceae) is a hardy indigenous plant of West Tropical Africa (Edeoga *et al.*, 2005). *D. tripetala*, often known as pepper fruit in Nigeria, is a greenish fruit that becomes reddish or pinkish when fully ripe (Egharevba and Idah, 2015). *D. tripetala* possesses therapeutic properties due to certain phytochemicals (Elekwa *et al.*, 2011); because these plants have been discovered as a substantial contributor to the maintenance of good health, their applications have been widely publicized (Ebana *et al.*, 2016). Adedayo *et al.* (2010), revealed that the fruits contain alkaloids, tannins, saponins, flavonoids, terpenoids, steroids, and cardiac glycosides, which differs somewhat from the 2015 study (Egharevba and Idah, 2015). These bioactive chemicals are the foundation for medicinal plants' therapeutic potential (Khadijah, 2015). According to folkloric medicine, *D. tripetala* seed is use to treat cough, fever, diarrhoea, and rheumatism (Akabueze *et al.*, 2016). Locals were also said to make tea from the leaves. Studies on the fruit (seed ripe and unripe) have revealed its anti-diabetic, anti-analgesic, anti-inflammatory, and neuro-pharmacological properties (Dike, 2010).

Several investigations have been conducted to examine the harmful impact of *D. tripetala.* Uvariopsin, an alkaloid found in *D. tripetala*, has been demonstrated to increase bile output and minimize hepatic diseases (Lopez-Martin *et al.,* 2002; Ofem *et al.,* 2004). However, Ofem *et al*. (2004) showed that an ethanolic extract of *D. tripetala* fruits administered at a specific dosage lowers bile production in normal healthy rats. The extract has also been shown to enhance sodium, potassium, and bicarbonate ions in bile while decreasing chloride and unconjugated bilirubin concentration (Ofem *et al.,* 2004). However, there were no evidence-based studies on the kidney and liver toxicity profile of the methanol extract of this plant on any animal model at the time of this research; thus, this study was carried out to analyze the kidney and liver function of the methanol extract of the plant leaves. The findings will help confirm the potential effect of methanol extract of *D. tripetala* leaves on some biochemical parameters of albino rats.

MATERIALS AND METHODS

Sample Collection and Identification: *D. tripetala* fresh leaves were collected from a local farm at Lodu-Ndume, Umuahia, Abia State, Nigeria. They were identified and authenticated by a plant taxonomist in the Department of Plant Science and Biotechnology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, where voucher specimen (PDC/MOUAU/0345) was deposited in the Departmental herbarium.

Sample Preparation and Extraction: The authenticated plant samples were brought to the Department of Biochemistry's laboratory and air-dried for two weeks before being pulverized into a fine powder with a Warring blender (Quilink QBL-20 L40 model). The methanol extract was obtained by soaking 500 g of powdered leaves in 80 % methanol and allowed to ferment for 72 hours with occasional shaking in an air tight container. The mixture was filtered and concentrated using rotary evaporator (Achuba, 2018).

Determination of Percentage Yield of Plant Extract: The percentage yield of the extract was obtained by dividing the weight of the methanol extract by the weight of powdered leaf used. Percentage yield = weight of the powdered leaf used ÷ weight of the extract produced x 100.

Experimental Animals: Thirty six (36) adult male albino rats weighing 110 – 150 g were obtained from the animal house of department of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, Abia state, Nigeria The rats were fed with Vital Feed Growers Marsh containing 20% crude protein and 280 kcal 100^{-1} g metabolizable energy, manufactured by Vital Feed Industries Limited, Nigeria, and had unrestricted access to water in a well-ventilated 12 hour light cycle environment. They were housed in stainless steel cages and given two weeks to acclimate before starting

the studies. The NRC principles and protocols of laboratory animal care (NRC, 2011) were observed throughout the study. All the experimental methods used in this study were examined and approved by the College of Natural Sciences, Michael Okpara University of Agriculture (MOUAU) Research and Ethics Committee.

Acute Toxicity (LD_{50}) Evaluation: Acute oral toxicity (LD_{50}) was performed by the method of Lorke (1983). Three groups of rats each comprising of three rats each were administered with 500, 800 and 1000 mg/kg of herbal formulation by mouth and examined for mortality within 24 hours. Following the results of mortality in each group, another set of three groups of rats were administered higher doses of the test drug, to achieve the least and most toxic value and LD_{50} was calculated by geometric mean of the mortality values. LD_{50} was calculated as: $LD_{50} = [M_0 + M_1] \div 2$, where M_0 = highest dose of test substance that gave no mortality and M_1= lowest dose of test substance that gave mortality.

Phytochemical Composition of *Dennettia tripetala*: The alkaloid, saponin, flavonoid, phenol and tannin contents were adopted from the study of Larayetan *et al.* (2018).

Experimental Design: The experiment was laid down using a complete randomized design (CRD) of four treatments replicated thrice with each replicate having three rats. Group 1 was the control group; groups 2, 3 and 4 received an oral dose of 100, 200 and 400 mg/kg methanol leave extract of *D. tripetala* daily. The test concentrations used were obtained by dividing the LD_{50} (1000 mg/kg) by a factor (10) to get the initial dose (100 mg/kg) that was subsequently doubled (200 and 400 mg/kg). The rats were treated for twenty one days, after which, they were humanely sacrificed under ethyl ether anaesthesia on the 21st day after receiving the extracts.

Somatic Variables Studied

Body weight: The percentage change in body weight was calculated as: Percentage change in weight = Final Weight - Initial Weight ÷ Initial Weight x 100.

Organosomatic index: The ratio of the weight of the liver, kidney and heart in relation to the body weight of rat was calculated separately following the organosomatic index formula as described by Adesina (2017). Organosomatic Index (%) = Organ Weight (g) ÷ Animal Body Weight (g) x 100.

Determination of Biochemical Parameters

Liver Function Test: Activities of serum aspartate transaminase (AST) and alanine transaminase (ALT) were assayed by the methods of Bergmeyer *et al.* (1986). 0.2 ml of serum with 1 ml of substrate (aspartate and α-ketoglutarate for AST, alanine and α-ketoglutarate for ALT, in phosphate buffer pH 7.4) was incubated for an hour in case of AST and 30 minutes for ALT. 1 ml of DNPH solution was added to arrest the reaction and kept for 20 minutes in room temperature. After incubation 1 ml of 0.4 N NaOH was added and absorbance was read at 540 nm. Activities AST and ALT were expressed as IU/L. Based on the method of King and Armstrong (1934) alkaline phosphates activities (ALP) was assayed using disodium phenyl phosphate as substrate. The colour, developed read at 680 nm after 10 minutes, and the activity of ALP was expressed as IU/L.

Total Protein Estimation: Total protein was assayed using the direct Biuret method of Gornall *et al.* (1949). At alkaline pH value, proteins form a blue coloured complex with copper II ions which was spectrophotometrically measured. 0.02 mL of serum and the standard were mixed with 1 mL reagent in 2 different test tubes. They were stood for 10 minutes at room temperature (25ºC), and their absorbance was read spectrophotometrically at 546 nm wavelength. Calculation: Protein (g/L) =

absorbance of sample ÷ absorbance of standard x 5.

Bilirubin: Total and conjugated bilirubin levels were determined according to the method of Penhaker *et al.* (2013).

Kidney Function Tests

Estimation of serum urea level: This was done following the method of Fawcett and Scott (1960). Urea in serum is hydrolyzed to ammonia in the presence of urease. The NH_3 is then measured spectrophotometrically by Berthelot's reaction. Urea + $H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2$. NH_3 + hypochlorite + phenol $\rightarrow$ indophenol (blue compound). Reagents and samples were pipetted into different test tubes as follows: The reagent 1 (100 μL) was pipetted into test tubes labelled blank, standard and test(s). Later, 10μL of distilled, standard solution and serum samples were dispensed into test tubes labeled blank, standard and test(s). The content was mixed and incubated at 37°C for 10 minutes. Reagent 2 (2.5 mL) and reagent 3 (2.5 mL) were added to all the test tubes, mixed and incubated at 37°C for 15 minutes. The absorbance of the standard and test were read against blank at 546 nm. Urea (mg/dL) = $\frac{Absorbance\ of\ test}{Absorbance\ of\ standard} \times \frac{concentration\ of\ standard}{1}$

Estimation of serum creatinine: Creatinine was measured by an immunoturbidimetric method as described by Henry (1974). At alkaline pH values, creatinine reacts with picric acid to produce a coloured compound, creatinine alkaline picrate, which was spectrophotometrically read at 546 nm.

In vivo **Antioxidant Activities**

Malondialdehyde (MDA) level: Lipid peroxidation was determined spectrophotometrically by measuring the level of lipid peroxidation product, malondialdehyde (MDA), as described by Draper and Hadley (1990). Malondialdehyde reacts with thiobarbituric acid (TBA) to form a red or pink coloured complex which absorbs maximally in acid solution at 532 nm. The serum (50 μL) was deproteinized by adding 1 mL of 14 % trichloroacetic acid and 1 mL of 0.6 % thiobarbituric acid. The mixture was heated in a water bath for 30 minutes to complete the reaction and then cooled on an ice pack for 5 minutes. After centrifugation at 2000 g for 10 minutes, the absorbance of the coloured product (TBARS) was measured at 535 nm with a UV spectrophotometer. The concentration of TBARS was calculated using the molar extinction coefficient of malondialdehyde (1.56 × 105 mol/L/cm) using the formula, A = ΣCL, where A = absorbance, Σ = molar coefficient, C = concentration, and L = path length. The results were expressed in nmol/mg of protein.

Estimation of superoxide dismutase (SOD): Superoxide dismutase (SOD EC 1.15.1.1) activity (IU/L) was determined using the method of Spitz and Oberley (1989). This was based on the principle that the SOD enzyme catalyzes the conversion of two superoxide radicals into the less toxic hydrogen peroxide and molecular oxygen in reactions that inhibit nitro blue tetrazolium (NBT) reduction.

Determination of catalase activity: Catalase activity was determined using the method of Sinha (1972). The ultraviolet absorption of hydrogen peroxide can be easily measured at 240 nm. As the hydrogen peroxide is decomposed with catalase, the absorption decreases with time. Hence, catalase activity can be measured. Phosphate buffer (2.5 mL), 2.0 mL of H_2O_2 and 0.5 mL of serum were mixed in a test tube, and 1 mL of this mixture was then added to 2 mL of phosphate buffer. The absorbance was read at 240 nm wavelength after a minute interval.

Statistical Analysis: Data obtained was statistically analyzed using one-way analysis of variance (ANOVA) with Turkey's multiple comparison post hoc tests to compare the level of significance between the test groups. The values of $p<0.05$ were considered significant.

RESULTS

The percentage yield of extract was 11.26 %. The result of the acute toxicity study indicated that the *D. tripetala* leaf extract does not have any acute toxicity since no mortality was recorded at the highest dose of 1000 mg/kg (Table 1).

Table 1: Acute toxicity of methanolic extract of *Dennettia tripetala*

Groups	Concentration (mg/kg)	Mortality
Group 1 (Control)	-	Nil
Group 2	500 mg/kg	Nil
Group 3	800 mg/kg	Nil
Group 4	1000 mg/kg	Nil

The result of the phytochemical assay, revealed the presence of important bioactive compounds such as alkaloid, tannin, flavonoid, saponin, phenolics, terpenoid and steroid (Table 2).

Table 2: Qualitative phytochemical profile of methanolic extract of *Dennettia tripetala*

Name of Phytochemicals	Inference
Tannin	+
Cyanogenic glycosides	+
Saponins	+
Alkaloid	+
Flavonoids	+
Terpenoids	+
Steroids	+

Key: *(+) = Present (Source: Larayetan et al., 2018)*

The effect of *D. tripetala* methanol leave extract on body weight changes in rats indicated that there was progressive weight increases from week one to week three for all the extracts (Figure 1). Weight increase was concentration dependent with 400 mg/Kg body weight having the highest weight.

The values of organosomatic indices of the liver, heart and kidney of rats treated with *D. tripetala* methanol leave extract indicated that the hepatosomatic index (HSI) was highest (3.18 ± 0.16 %) in Group 4 rats treated with 400 mg/kg of the plant extract and least in both the control group (3.05 ± 0.16 %) and Group 1 (3.05 ± 0.03 %) (Table 3).

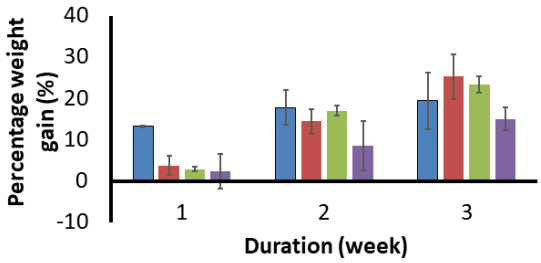

Figure 1: Effect of *Dennettia tripetala* methanol leave extract on the mean body weight changes in the rats. Results are expressed as mean ± Standard Error (n=9). Values are significantly different at p<0.05

However, no significant difference (p>0.05) existed in the HSI values among the rats in the treatment groups. The renosomatic index (RSI) was highest in Group 4 rats (0.70 ± 0.02 %) treated with 400 mg/kg plant extract and least in Group 3 rats (0.59 ± 0.03 %) given 200 mg/kg of the plant extract. However, the values of RSI showed significant differences (p<0.05) among the treatment groups. The cardiosomatic index (CSI) was highest in the control group (0.40 ± 0.00 %) and lowest in Group 2 rats (0.34 ± 0.03 %) fed with 100 mg/kg of the plant extract. However, there was a significant difference (p<0.05) between groups 2 and 3 (Table 3).

Data for the activities of liver function markers in rats administered *D. tripetala* methanol leave extract indicated that there was a significant (p<0.05) rise in serum and liver ALP in rats administered 100, 200 and 400 mg/Kg body weight. Similar trends were observed in a significant increase in serum AST and ALT for 100, 200 and 400 mg/Kg compared to the control (Table 4).

Data for bilirubin levels indicated that the total bilirubin, direct bilirubin and conjugated bilirubin activities were not significantly (p>0.05) different in all the extract treated groups (100, 200 and 400 mg/kg body weight) compared to the control (Table 5).

The data on changes in total protein, albumin and globulin profile of rats following treatment with *D. tripetala* methanol leave extract showed that there were significant decreases (p<0.05) of total protein in groups treated with 100 and 200 mg/kg body weight extract when compared to the control (Table 6).

Table 3: Effect of *Dennettia tripetala* methanol leave extract on the mean organosomatic index of rats

Treatment	Liver (%)	Heart (%)	Kidney (%)
Group 1 (Control, 0 mg/kg Extract)	3.05 ± 0.16^{a}	0.40 ± 0.00^{c}	0.68 ± 0.03^{b}
Group 2 (100 mg/kg Extract)	3.05 ± 0.03^{a}	0.34 ± 0.03^{a}	0.68 ± 0.05^{b}
Group 3 (200 mg/kg Extract)	3.10 ± 0.08^{ab}	0.37 ± 0.01^{b}	0.59 ± 0.03^{a}
Group 4 (400 mg/kg Extract)	3.18 ± 0.16^{b}	0.35 ± 0.02^{ab}	0.70 ± 0.02^{c}

Means on a column with different superscript letter are significantly different (p<0.05)

Table 4: Effect of *Dennettia tripetala* methanol leave extract on liver function markers (ALP, AST and ALT) of albino rats

Treatment	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
Group 1 (Control, 0 mg/kg Extract)	24.51 ± 0.47^{a}	56.43 ± 1.23^{a}	10.51 ± 0.77^{a}
Group 2 (100 mg/kg Extract)	37.45 ± 0.72^{c}	66.83 ± 1.51^{b}	19.45 ± 0.32^{d}
Group 3 (200 mg/kg Extract)	37.55 ± 0.27^{c}	69.13 ± 2.14^{b}	18.60 ± 0.51^{c}
Group 4 (400 mg/kg Extract)	33.39 ± 0.53^{b}	74.47 ± 2.59^{c}	17.32 ± 0.16^{b}

AST: *Aspartate Transaminase,* ***ALT:*** *Alanine Transaminase,* ***ALP:*** *Alkaline Phosphatase. Means on a column with different superscript letter are significantly different (p<0.05)*

Table 5: Effect of *Dennettia tripetala* methanol leave extract on liver function markers

Treatment	TBIL (mg/dL)	DBIL (mg/dL)	CBIL (mg/dL)
Group 1 (Control, 0 mg/kg Extract)	0.27 ± 0.00^{c}	0.11 ± 0.05^{a}	0.16 ± 0.05^{d}
Group 2 (100 mg/kg Extract)	0.26 ± 0.01^{b}	0.11 ± 0.00^{a}	0.15 ± 0.01^{c}
Group 3 (200 mg/kg Extract)	0.24 ± 0.00^{a}	0.17 ± 0.03^{c}	0.08 ± 0.02^{a}
Group 4 (400 mg/kg Extract)	0.27 ± 0.00^{c}	0.13 ± 0.00^{b}	0.13 ± 0.00^{b}

TBIL: *Total Bilirubin, DBIL: Direct Bilirubin,* ***CBIL:*** *Conjugated Bilirubin. Means on a column with different superscript letter are significantly different (p<0.05)*

Table 6: Effect of *Dennettia tripetala* methanol leave extract on liver function markers (total protein, albumin and globulin)

Treatment	TP (g/dL)	ALB (g/dL)	GLB (g/dL)
Group 1 (Control, 0 mg/kg Extract)	6.17 ± 0.09^{b}	4.22 ± 0.07^{b}	1.95 ± 0.04^{d}
Group 2 (100 mg/kg Extract)	5.75 ± 0.06^{a}	4.02 ± 0.03^{b}	1.72 ± 0.08^{c}
Group 3 (200 mg/kg Extract)	5.74 ± 0.04^{a}	4.16 ± 0.04^{b}	1.58 ± 0.01^{b}
Group 4 (400 mg/kg Extract)	6.09 ± 0.09^{b}	3.95 ± 0.12^{a}	1.14 ± 0.06^{a}

TP: *Total Protein,* ***ALB:*** *Albumin,* ***GLB:*** *Globulin. Means on a column with different superscript letter are significantly different (p<0.05)*

Furthermore, the result showed a non-significant difference in the group treated with 400 mg/kg body weight extract when compared to the control. The albumin levels, on the other hand, had no significant difference (p>0.05) in the groups treated with 100 and 200 mg/kg body weight extract when compared to the control but showed a significant decrease in the serum of the 400 mg/Kg body weight when compared to the control. While for globulin, the groups treated with 100, 200 and 400 mg/kg body weight extract showed a significant decrease (p<0.05) when compared to the control.

The effect of methanol leaves extract of *D. tripetala* on serum urea and creatinine indicated that there was a significant decrease (p<0.05) in the serum urea of the group treated with 200 mg/kg (19.85 ± 0.08) compared to the control (25.08 ± 0.25) and a non significant increase at dose 400 mg/kg (Table 7). Conversely, serum creatinine showed a non-significant (p<0.05) increase in groups treated with 100 and 400 mg/kg body weight compared to the control.

Data for the activities of antioxidant markers in rats administered *D. tripetala* methanol leave extract indicated that there was significant difference in the activity of SOD among the group treated with 400 mg/kg of the extract when compared to the control group (Table 8). The treated rats exhibited a significantly reduced (p<0.05) catalase enzyme activity at dose of 100 mg/kg compared to the normal control animals.

Table 7: Effect of *Dennettia tripetala* methanol leave extract on kidney function markers (Urea and Creatinine)

Treatment	Urea (mg/dL)	Creatinine (mg/dL)
Group 1 (Control, 0 mg/kg Extract)	25.08 ± 0.25^{b}	0.84 ± 0.08^{a}
Group 2 (100 mg/kg Extract)	25.00 ± 0.88^{b}	1.34 ± 0.05^{d}
Group 3 (200 mg/kg Extract)	19.85 ± 0.08^{a}	0.95 ± 0.06^{b}
Group 4 (400 mg/kg Extract)	25.50 ± 0.30^{b}	1.26 ± 0.05^{c}

Means on a column with different superscript letter are significantly different (p<0.05)

Table 8: Effect of methanol leave extract of *Dennettia tripetala* on antioxidant enzymes (SOD, MDA, GSH and CAT)

Treatment	SOD (IU/g protein)	MDA (nanomole /g protein)	GSH (µg/L)	CAT (U/g protein)
Group 1 (Control, 0 mg/kg Extract)	24.30 ± 0.59^{a}	32.18 ± 2.41^{a}	40.78 ± 9.23^{d}	4.31 ± 0.03^{c}
Group 2 (100 mg/kg Extract)	24.27 ± 0.70^{a}	37.91 ± 0.90^{c}	26.58 ± 1.19^{b}	1.79 ± 0.53^{a}
Group 3 (200 mg/kg Extract)	26.08 ± 0.29^{b}	37.45 ± 2.08^{c}	21.44 ± 5.05^{a}	2.17 ± 0.10^{ab}
Group 4 (400 mg/kg Extract)	27.20 ± 0.98^{c}	36.08 ± 0.74^{b}	27.93 ± 3.15b^{c}	2.63 ± 1.22^{b}

***SOD** = superoxide dismutase, **MDA** = malondialdehyde, **GSH** = glutathione, **CAT** = catalase. Means on a column with different superscript letter are significantly different (p<0.05)*

GSH activity was significantly lowered (p<0.05) in the rats treated with the extracts compared to the control. The level of the oxidative stress marker, MDA was significantly (p<0.05) elevated in the treated rats compared to the normal rats.

DISCUSSION

Dennettia tripetala leaf possesses flavonoids, phenolics, terpenoids and steroids as part of the phytochemical constituents; these bioactive compounds are well known for their hepatoprotective potency (Rehman *et al.,* 2015). Saponin, alkaloids, terpenoids, and flavonoids are well-known antioxidants and have been reported to be useful in managing oxidative stress-mediated diseases (Jin *et al.,* 2011). The acute toxicity of methanol leaf extract of *D. tripetala* in mice recorded no mortality even at a high dose of 1000 mg/kg of the extract; thus the LD_{50} of the leave was above 1000 mg/kg.

The biochemical and toxicological profile of methanol extract of *D. tripetala* (pepper fruit) leaves on some selected parameters in male albino rats indicted that the activities of ALT, AST, ALP, total protein, albumin, total bilirubin, and direct bilirubin were variously affected. A high level of AST, ALT and ALP in serum has been described as an indicator of hepatocellular disruption due to the liver's disturbed or impaired structural integrity, resulting in leakage of these enzymes from the cytosol into the bloodstream (Giannini *et al.*, 2005). On the other hand, bilirubin is a significant blood metabolic product which high activity in the serum is indicative of onset of kidney failure (Li *et al.*, 2021). Total protein and albumin can be utilized as indicators of the liver's functional capacity due to its binding capacity of albumin, including for metals and fatty acids, decreases before the total plasma albumin or bilirubin concentration, or the prothrombin time show abnormalities (Sun *et al.,* 2019). Oettl *et al.* (2013) have studied oxidative albumin damage in patients with chronic liver failure and found that the oxidation state of albumin and the binding capacity of site II in patients with liver failure were significantly impaired. The non-significant (p>0.05) differences in all of these parameters in rats treated with the extract show that administration of this extract did not significantly affect hepatocyte function in rats or cause any substantial cytotoxic damage to the liver at the doses studied. The findings of this study were in agreement with previous studies of Salawu *et al.* (2019) and Ogbonna *et al.* (2020).

Significant increase in creatinine level was obtained in rats administer 200 and 400 mg/kg body weight of methanol leaves extract of *D. tripetala*, respectively, when compared to the control. The increase in creatinine may have resulted from glomerular inflammation and interstitial nephritis (Kodner and Kudrimoti,

2003), though this study did not cover the exact mechanism. In this study, the administration of 200 and 400 mg/kg methanol leave extract of *D. tripetala* may have conferred hypernatraemic effect to the Na+/H+ exchanger instead of Na+/K+ pump (Nwankpa *et al.,* 2018). The membrane-bound aldosterone regulates the absorption of sodium into the cell, while the Na+/K+ pump may have been impaired on the administration of leave extract of *D. tripetala*. This study suggests that the leaf extract of *D. tripetala* may have induced renal damage resulting in impairment of renal function.

A decrease in SOD and GSH level (oxidative stress indicators), and a non-significant difference in MDA and CAT levels might imply an inhibitory impact on lipid peroxidation of the enzyme. Reduced levels of both enzymatic and non-enzymatic antioxidants in the aqueous-methanol extract could be attributed to phytochemical constituents present in the extract, which act as prooxidant and inhibit the primary and secondary enzymes in the antioxidant cycle, or the antioxidants may have acted on the extract's induced oxidative stress. This research supports the findings of Mahmoud *et al.* (2005), who found that *Urtica pilulifera* extracts reduce lipid peroxidation by acting as a prooxidant.

Conclusion: The high values of ALP, AST and ALT recorded in the groups administered with 100, 200 and 400 mg/kg body weight of *D. tripetala* leave extract indicated liver injury or dysfunction. The presence of normal serum levels of the total bilirubin, direct bilirubin and conjugated bilirubin indicated normal liver structures; and reduced levels of total protein, albumin and globulin might be due to the toxic effect of the extract on the liver and therefore may cause adverse effects to man and his environment even at moderately high concentration. Also, the significant changes in the kidney function parameters showed that the leave extract of *D. tripetala* may pose glomerular and tubular dysfunction of the nephron, which indicated that the herbal preparation may contribute to renal failure and should not be taken without proper medical advice and monitoring. However, this study recommends the use of methanol leaves extract of *D. tripetala* at a dose less than 100 mg/kg body weight as administration of methanol leaves extract of *D. tripetala* at a dose over 100 mg/kg body weight for an extended period may induce toxicity to the liver and kidney which may hamper renal dysfunction and liver failure.

ACKNOWLEDGEMENTS

The authors appreciate the Dr Samuel O. Onoja of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State for providing reagents and equipment for this research and also Dr Flora Mukah a plant taxonomist for identifying and authenticating the plant used for this research.

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