Scientific Review
ISSN(e): 2412-2599, ISSN(p): 2413-8835
Vol. 5, Issue. 8, pp: 150-156, 2019
URL: https://arpgweb.com/journal/journal/10
DOI: https://doi.org/10.32861/sr.58.150.156

Academic Research Publishing Group

Original Research **Open Access**

Toxicity Studies of Aqueous-Methanol Extract of *Dennettia tripetala* (Pepper fruit) Fresh Ripe Fruits in Experimental Rats

Salawu K.*
Department of Biochemistry and Forensic Science, Nigeria Police Academy, Wudil, Kano State, Nigeria

Njoku O. U.
Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria

Ogugua V. N.
Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria

Abstract

Traditional medicine still remains the main recourse for a large majority of people for treating health problems in African. Therefore, the aim of this work is to assess the toxicological effect of the fresh ripe fruits using two solvents for extraction. The toxicological evaluation of aqueous-methanol extract of *Dennettia tripetala* fresh ripe fruits at 100, 200 and 400 mg/kg body weight for 14 days on some biochemical parameters in wistar rats was investigated. The extract at all the doses tested show non-significant ($p > 0.05$) increase from the control in ALT, AST, ALP, total protein, albumin, direct bilirubin, creatinine, Na+ and K+, while the level of total bilirubin and urea show significant ($p < 0.05$) increase from the control at 400mg/kg body weight. The levels of SOD, GPx, GST, and GSH in the serum were significantly ($p < 0.05$) decrease in the treated rats at 200 and 400mg/kg body weight, whereas the level of MDA and CAT showed non-significant ($p > 0.05$) increase in all the animals. The results of this finding indicated that the aqueous-methanol extract may not have serious effect on the liver and the kidney at 100 mg/kg b.d., but may be toxic at high doses as observed in the acute toxicity, sub-acute results and antioxidant parameters where it shows a dose-specific effects.

Keywords: *Dennettia tripetala*; Pepper fruit; Fresh ripe fruits; Aqueous-methanol extract; Toxicity; Evaluation.

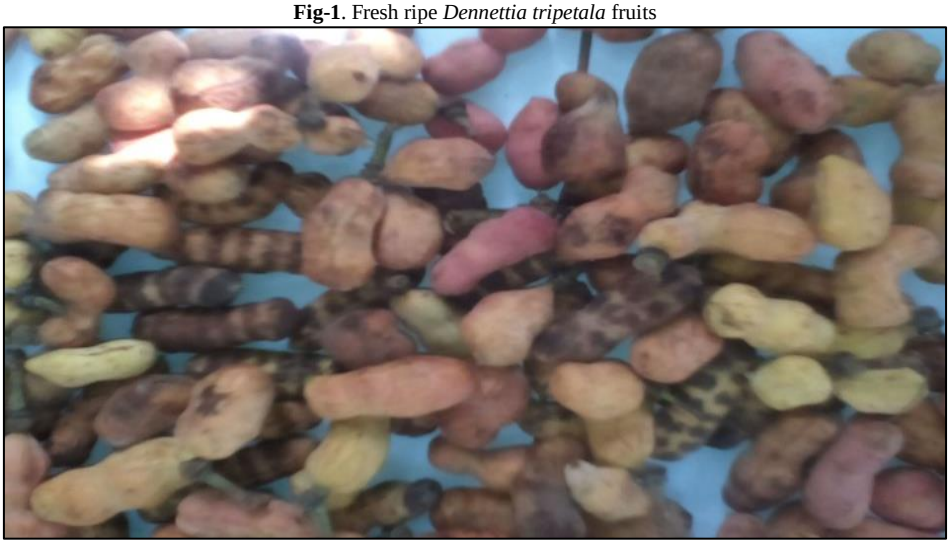
1. Introduction

Herbal remedies have been used in the management of various diseases from time immemorial. These remedies which are commonly self-medicated have no proper dose regimen. Such unformulated use of medicinal plant without recourse to safety may have adverse effects on the biological system [1, 2]. Their uses have increased among the populations of developing countries due to their health benefit, minimal side effects [3, 4], and easy accessibility. Many reports from developed countries have presented cases of acute poisoning of patients admitted to hospitals which resulted into death mainly due to ingestion of toxic medicinal plants [5-8]. The adverse effects of herbs includes allergic reaction, hepatotoxicity [9], nephrotoxicity [10, 11], cardiac toxicity [12], neurotoxicity [13] and even death [8, 14].

Dennettia tripetala is a member of Annonaceae [15], known as pepper fruits in English and is a well-known Nigerian spicy medicinal plant [16-18]. It is a common ethnomedicinal plant in West Africa, which appears red when ripe and green in an unripe form with a pungent spicy taste (Figure 1) [19]. The different parts of the plant are used in the treatment of fever, cough, anti-emetics, and management of diabetics [20, 21]. The aqueous extract of *Dennettia tripetala* fruit has been reported to have hepato-protective and nephero-protective effect in CCl_4-induced damage [22], while the ethanol extract of the seed has been reported to show anti-ulcer against aspirin-induced ulcer [23]. In developing countries, the toxicity of herbal plants were hardly been reported in hospitals and most of the works done on this plant were on medicinal values and the use of single solvents for extraction. The toxicological effect of the fresh ripe fruits which is the most common used is limited. Therefore, the objective of this work is to evaluate the antioxidants and hepatorenal safety of the fresh ripe fruits using aqueous-methanol as the solvent of extraction.

*Corresponding Author

Fig-1. Fresh ripe *Dennettia tripetala* fruits



2. Materials and Methods

2.1. Collection and Processing of Plant Material

Fresh ripe fruits of *Dennettia tripetala* were purchased from Ibagwa market in Igbo-Eze South Local Government Area of Enugu State, Nigeria. All collections were made between the months of April - May 2015. The fresh ripe fruits sample was authenticated at the Herbarium of the Bioresources Development and Conservation Programme (BDCP), Nsukka, Enugu state, Nigeria. The fresh ripe fruits were washed, drained, and grinded with pestle and mortar to obtained homogenate sample. The homogenate fresh samples were used for this analysis.

2.2. Preparation of Plant Extract

A weighed quantity (600g) of the homogenate sample was macerated at room temperature in 1600ml of aqueous-methanol (1:4) for 48 hours with stirring at regular intervals. The resulting mixture was filtered with watman filter paper 1 and the filtrate was concentrated by evaporating the aqueous-methanol at 60^0C in an oven. The yield of the extract was calculated with respect to the initial weight of the fresh ripe powder fruit taken:

$$\text{Yield (\%)} = \frac{\text{weight of aqueous-methanol extract x 100}}{\textit{weight of fresh ripe powder taken}}$$

2.3. Animals

All the experimental animals used were obtained from the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. The mice and albino rats were kept in wire mesh cages with access to food and water for two weeks during acclimatization in the laboratory. They were maintained on standard animal feeds and clean tap water, before and after daily administration of the aqueous-methanol extract of fresh ripe fruits of *D. tripetala*. The experiment was designed and conducted in accordance with the ethical norms approved by the University Animal Ethics Committee Guidance.

2.4. Acute Toxicity Study

Acute toxicity (LD_{50}) study of the aqueous-methanol extract was carried out according to the method of Lorke [24]. The LD_{50} was used for the selection of a starting dose as well as the determination of LD_{50} of the testing material. Therefore, the acute toxicity was determined using the formular:

$$LD_{50} = \sqrt{(A \times B)}$$

2.5. Sub-Acute Toxicity Study

Twenty (20) male adult of albino rats were selected by stratified randomization and then divided into four groups of five each and treated for 14 days as follows:

Group I (Control group): received distilled water only

Group II: received 100mg/kg of extract

Group III: received 200mg/kg of extract

Group IV: received 400mg/kg of extract

The first day of dosing was taken as day 0 and blood was collected on day 14 and used for biochemical and antioxidant analysis [25].

2.6. Body Weight and Percentage Body Gain

The body weight of each rat was expressed using a calibrated weight balance during the acclimatization period, once before the commencement of dosing, during the period of dosing and on the 14th day before the animals were sacrifice.

2.7. Collection of Blood

Blood samples were collected by the orbital technique. Blood sample for biochemical analysis was collected from the retrobulbar plexus of the medial canthus of the eye to enable outflow of blood into labeled centrifuge tubes, allowed to clot and centrifuged for 15 minutes at 3000 rpm to separate serum and the serum was used for biochemical analysis.

2.8. Determination of Biochemical Parameters

The analysis was carried out to determine the serum concentrations of total protein, albumin, creatinine, urea, Na^+, K^+, total and conjugated bilirubin, and activities of liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). The oxidative stress markers such as malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), glutathione (GHS), glutathione peroxidase (GPx), vitamin E and C were analyzed using standard laboratory procedure.

2.9. Statistical Analysis

The data obtained were represented as the mean ± standard deviation. One way analysis of variance (ANOVA), Graph pad instant software (San Diego, USA) was used to compare means across the groups. Mean values with $p < 0.05$ were considered statistically significant.

3. Results

3.1. Percentage Yield

After the extraction of fresh ripe *Dennettia tripetala* fruits using aqueous-methanol solvents in the ratio of 1:4 (v/v), the percentage yield for the crude extract was 15.17% (w/w) (table 1).

Table-1. Percentage yield of aqueous-methanol extract

	Aqueous-methanol extract
Weight of *D. tripetala* fruits (g)	600
Weight of extract after evaporation (g)	91
Percentage yield (%)	15.17

3.2. Acute Toxicity Study

In the acute toxicity of the aqueous-methanol extract, the mice at phase one dosed 10 and 100mg/kg body weight showed no signs of toxicity and no death was recorded, while at 1000 mg/kg body weight a mouse was found dead after 24 hours. In the second phase, there was a sign of toxicity at 1600, 2900 and 5000mg/kg body weight and death was recorded in all the groups (Table 2). The LD_{50} of the extract was calculated to be 1265mg/kg body weight.

Table-2. The median lethal dose of aqueous-methanol extract of fresh ripe fruits of *Dennettia tripetala*

Phase one	**Number of mice**	**Number of death**
10 mg/kg	3	0
100 mg/kg	3	0
1000 mg/kg	3	1
Phase two		
1600 mg/kg	3	2
2900 mg/kg	3	2
5000 mg/kg	3	2

3.3. Percentage Body Weight Gain

The body weight of the rats dosed with extract for 14 days showed a decrease in weight as the concentrations increases when compared with the control group. There was significant ($p < 0.05$) decrease in percentage body weight gain of the treated rats when compared with the control group. The decrease in weight gain was observed in all the treated groups as the concentrations and duration of admistration increases from day 7 to 14 compared with the control group (Table 3).

Table-3. Percentage body weight gain of albino rats administered with aqueous-methanol extract of fresh ripe fruits of *Dennettia tripetala*

Group	**Day 7 weight gain (%)**	**Day 14 weight gain (%)**
Group 1 (control)	26	43
Group 2 (100 mg/kg)	4	8
Group 3 (200 mg/kg)	7	4
Group 4 (300 mg/kg)	2	-5

3.4. Effect of Extract on Liver Function Test (LFT)

The effect of the aqueous-methanol extract on serum aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), alkaline phosphatase (ALP), total protein, total bilirubin, direct bilirubin, and albumin are

shown in table 4. It was observed that repeated daily oral administration of the extract at 100, 200 and 400 mg/kg body weight for the period of 14 days did not show significant ($p > 0.05$) difference in the serum levels of AST, ALT, ALP, total protein, direct bilirubin and albumin of the treated groups compared with the control group, while the total bilirubin of group 4 dosed with 400 mg/kg body weight of the extract showed significant ($p < 0.05$) increase from the control group.

Table-4. Effect of aqueous-methanol extract of fresh ripe fruits of *D. tripetala* on serum LFT

Parameters	Control(Group 1)	Group 2	Group 3	Group 4
ALT (IU/L)	13.33 ± 3.79[a]	14.33 ± 1.53[a]	15.67 ± 3.35[a]	16.33 ±2.64[a]
AST (IU/L)	42.67 ± 4.62[a]	41.33 ± 3.51[a]	43.33 ± 0.58[a]	45.00 ± 2.53[a]
ALP (IU/L)	102.12 ±33.58[a]	104.08 ±14.16[a]	104.55 ±11.28[a]	106.04 ±14.12[a]
Total protein (mg/dl)	3.807±1.57[a]	4.35±1.82[a]	3.37±0.22[a]	4.40±0.00[a]
Albumin (g/dl)	4.31±0.30[a]	4.56±1.68[a]	4.12±0.33[a]	4.53±0.31[a]
Total bilirubin (mg/dl)	0.24±0.01[a]	0.24±0.02[a]	0.28±0.00[a]	0.48±0.11[b]
Direct bilirubin (mg/dl)	0.15±0.07[a]	0.15±0.11[a]	0.16±0.07[a]	0.19±0.03[a]

Values are presented as mean ± standard deviation (n = 5), values with the same superscript as control are non-significant ($p > 0.05$) different.

3.5. Effect of Aqueous-Methanol Extract on Kidney Function Test (KFT)

The effect of fresh ripe fruits of *Dennettia tripetala* aqueous-methanol extract on the serum levels of creatinine, urea, Na^+, and K^+ are presented in table 5. The creatinine, Na^+ and K^+ levels in all the treated groups show non-significant ($p > 0.05$) difference from the control, while the urea level in group 4 animals dosed with 400 mg/kg of the extract show significant ($p < 0.05$) difference from the control.

Table-5. Effect of aqueous-methanol extract of fresh ripe fruits of *Dennettia tripetala* on serum KFT

Parameters	Control(Group 1)	Group 2	Group 3	Group 4
Creatinine (mg/dl)	1.43±0.95[a]	1.45±0.38[a]	1.49±1.06[a]	1.50±0.66[a]
Urea (mg/dl)	43.265±9.31[a]	44.170±1.51[a]	46.426±0.00[a]	56.664±0.88[b]
Na^+ (mmol/L)	118.67±43.54[a]	117.33±21.16[a]	119.00±34.04[a]	121.33±19.66[a]
K^+ (mmol/L)	3.03±1.56[a]	3.17±0.63[a]	3.15±0.12[a]	3.18±0.65[a]

Values are presented as mean ± standard deviation (n = 5), values with the same superscript as control are non-significant ($p > 0.05$) different.

3.6. Effect of Aqueous-Methanol Extract on the Oxidative Stress Markers

Table 6 shows the results on the effect of the aqueous-methanol extract of fresh ripe fruits of *D. tripetala* on the level of malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx) and glutathione-S-transferase (GST). The results show that on the administration of the extract for 14 days, there was no significant ($p > 0.05$) difference in the level of MDA and CAT in the treated rats when compared with the control. While the results of SOD, GSH, GPx and GST show significant ($p < 0.05$) decrease in groups 3 and 4, while group 2 show non-significant ($p > 0.05$) difference from the control group.

Table-6. Effect of aqueous-methanol extract of fresh ripe fruits of *Dennettia tripetala* on serum oxidative stress marker

Parameters	Control (Group 1)	Group 2	Group 3	Group 4
MDA (mg/dl)	0.51±0.04[a]	0.52±0.06[a]	0.52±0.07[a]	0.53±0.05[a]
CAT (U/L)	3.83±0.23[a]	3.76±0.82[a]	2.96±0.02[a]	2.96±0.69[a]
SOD (U/L)	83.30±0.95[a]	81.11±0.27[a]	43.80±0.95[b]	42.59±6.52[b]
GSH (U/ml)	43.90±0.50[a]	40.69±5.69[a]	30.60±0.72[b]	27.66±0.49[c]
GPx (U/ml)	24.52±0.86[a]	21.52±0.052[a]	16.13±1.04[b]	16.32±0.90[b]
GSTs (U/ml)	24.44±0.61[a]	22.82±4.83[a]	19.12±3.89[b]	15.06±1.03[b]

Values are presented as mean ± standard deviation (n = 5), values with the same superscript as control are non-significant ($p > 0.05$) different

4. Discussion

Solvent extraction is mostly useful technique for isolation of plant active compounds though the extract yields of the plant materials are highly dependent on the nature of extracting solvent and the solubility of the phytoconstituents in the solvent of choice, due to the presence of different active compounds present in the plant material to be extracted which have varied chemical characteristics and polarities that may or may not be soluble in a particular solvent [26]. The percentage yield of 15.17% (w/w) for the crude extract from this work was a good yield compared to 14.09% obtained using ethanol solvent reported by Ikpi and Nku [17].

The acute toxicity studies reveals sign of toxicity and incidence of mortality at a dose of 1,600mg/kg body weight and above with calculated LD_{50} of 1,265mg/kg body weight which suggests that the extract is toxic to the mice. This result is in accordance with Oyemitan, *et al.* [20], who earlier reported the estimated LD_{50} values of oil from the plant to be 1,265mg/kg and 775mg/kg for oral and intraperitoneal routes in mice and also reported 2,150mg/kg and 470mg/kg for oral and intraperitoneal in rats respectively. Anaga, *et al.* [15] also reported the LD_{50}

of ethylacetate crude root extract to be 1120mg/kg i.p., while Anosike, *et al.* [23] reported LD_{50} above 5000mg/kg for oral administration of the fruits extract. This indicated that for both aqueous-methanol, ethylacetate extracts and essential oil administered through oral and intraperitoneal route, the LD_{50} is below 2000 mg/kg body weight. While oral route of the ethanol fruit extract reported by Anosike, *et al.* [23] is in contrast to other reports. The type of solvents used for extraction and routes of administration may have aided the toxicity of the compounds. Hilaly, *et al.* [27] reported that the toxic effects observed may be due to yet unidentified additional phytochemicals in plants, which have been isolated by difference in the solvent used.

Change in body weight has been used as an indicator of adverse effects of drugs and chemicals [27, 28], decrease in body weight of the treated rats was observed in percentage body weight gain of the animals treated with the crude extract between day 7 and 14 when compared with the control (Table 3), this indicated that the extract may have a negative effect on the normal growth of the animals. The mild toxic nature of the extract may have led to a loss of appetite and decrease feed intake, resulting in weight loss in the treated animals which may have reduced feed intake. Also, the presence of tannin which can prevent the bioavailability of protein may be the cause of observed decrease in weight gain [29].

Liver toxicity is measured based on activity of ALT, AST, ALP, as well as the levels of total protein, albumin, total bilirubin and direct bilirubin. An elevated level of AST, ALT, and ALP in serum have been reported as an indication of hepatocellular disruption, due to the damage to structural integrity of the liver which is deranged or compromised, leading to leakage of these enzymes from the cytosol into the bloodstream [30, 31], while the bilirubin is an important metabolic product of the blood with biological and diagnostic values [32]. Total protein and albumin can be used as markers for assessing the functional capacities of the liver [32, 33]. The non-significant ($p > 0.05$) difference in all these parameters in rats treated with the extract suggest that the sub-chronic administration of this extract does not seriously affect hepatocyte function in the rats or induce any serious cytotoxic damage to the liver at the tested dose. While the significant ($p < 0.05$) increase in total bilirubin at 400 mg/kg may be due to break up of more hemoglobin present in red blood cells than what the liver can handle. Ikpi and Nku [17], earlier reported that pepper fruits extract have an adverse effect on hematological parameters due to its lowering effect on RBC and WBC. Olson, *et al.* [34], also report that the hematopoietic system is one of the most sensitive targets for toxic compounds. The pharmacological and toxicological activity of most herbal medicines has been linked to the presence of alkaloids, triterpenoids, flavonoids, saponins, steroids, tannins, and other compounds in the herbs [35].

The kidney functioning capacity was assessed by measuring the levels of electrolytes, creatinine, and urea in the serum of the animals. The non-significant ($p > 0.05$) effect of the extract on the serum Na^+, K^+, and creatinine of the animals suggest that the normal functioning of the kidney in relation to these parameters were unaffected. However, since there was no significant reduction in total protein, which may lead to increase in urea a bi-product of protein catabolism; the observed increase in urea level at 400 mg/kg body weight may suggest impaired kidney function of the animals. It may also be an indication of dysfunction at the glomerular and tubular levels of the kidney [32]. This significant ($p < 0.05$) increase in the urea levels of the animal dosed 400mg/kg body weight indicate dose and parameter specific effect of the extract since other parameters were not significantly altered at other dose levels [32]. The aqueous extract of *Dennettia tripetala* fruit has been reported to have a protective effect on the liver and the kidney against CCl_4-induced damage [22].

Decrease in serum activity of SOD, GPx and GST and a decrease in GSH level (oxidative stress biomarkers) and non-significant difference in the levels of MDA and CAT may indicate inhibitory effect on lipid peroxidation and on the enzyme. The reduced levels of both enzymatic and non-enzymatic antioxidants in aqueous-methanol extract might be attributed to some phytochemical constituents present in the extract, which either act as prooxidant, and inhibit the primary and secondary enzymes in the antioxidant cycle or the antioxidants may have acted on the induced oxidative stress generated by the extract. Reports have shown that some flavonoids [36, 37], and other phytochemical constituents [38, 39], can act as pro-oxidants. The preliminary Phytochemical investigation has identify the presence of alkaloids, flavonoids, saponins, steroids, tannins, glycosides, saponin glycosides, terpenes and resins in the aqueous-methanol extract, while Otemenyin, *et al.* [35] have linked the pharmacological and toxicological activity of most herbal medicines to the presence of these phytochemicals. This work is in accordance with work of Mahmoud, *et al.* [40] who shows that *Urtica pilulifera* extracts have an inhibitory effect on the lipid peroxidation by acting as a prooxidant.

5. Conclusion

The results of this finding indicated that the aqueous-methanol extract may have no serious effect on the liver and the kidney at 100 mg/kg b.d., but can be toxic at high doses as observed in the acute toxicity, sub-acute results and the antioxidant parameters where it shows a dose-specific effects.

Recommendation

In order to establish the true toxicity of the aqueous-methanol extract, the period of administration should be extended from 14 to 28 days.

Acknowledgement

We wish to express our gratitude to the Department of Biochemistry, Bayero University Kano and Brain-phosphorylationship Research and Training Centre, Enugu for giving us free access to their laboratories during the cause of carrying out this research work.

References

[1] Aboyade, O. M., Yakubu, M. T., Grierson, D. S., and Afolayan, A. J., 2009. "Studies on the toxicological effect of the aqueous extract of the fresh, dried and boiled berries of solanum aculeastrum dunal in male wistar rats." *Human Experimental Toxicology,* vol. 28, pp. 765-775.

[2] Aboyade, O. M., Yakubu, M. T., Grierson, D. S., and Afolayan, A. J., 2010. "Safety evaluation of aqueous extract of unripe berries of solanum aculeastrum in male wistar rats." *African Journal of Pharmacy and Pharmacology*, vol. 4, pp. 90-97.

[3] Ishii, R., Yoshikawa, H., Minakata, N. T., Komura, K., and Kada, Y., 1984. "Specificity of bio-antimutagens in the plant Kingdom." *Agric. Biol. Chem.*, vol. 48, pp. 2587-2591.

[4] Hoyos, L. S., Au, W. W., Heo, M. Y., Morris, D. L., and Legator, M. S., 1992. "Evaluation of the genotoxic effects of a folk medicine, petriveria alliacea (anamu)." *Mutation Research,* vol. 280, pp. 29-34.

[5] Joubert, P. and Sebata, B., 1982. "The role of prospective epidemiology in the establishment of a toxicology service for a developing community." *South Africa Medical Journal*, vol. 62, pp. 853- 854.

[6] Popat, A., Shear, N. H., Malkiewicz, I., Stewart, M. J., Steenkmap, V., Thomson, S., and Neuman, M. G., 2001. "The toxicity of callilepis laureola, a south african traditional herbal medicine." *Clinical Biochemistry*, vol. 34, pp. 229-236.

[7] Van Wyk, B. E., Van Heerden, F. R., and Van Oudtshoom, B., 2002. *Poisonous plants of South Africa*. Pretoria: Briza Publications.

[8] Assiri, A. S., 2012. "Ricin poisoning causing death after ingestion of herbal medicine." *Annals of Saudi Medicines*, vol. 32, pp. 315-317.

[9] Saad, B., Azaizeh, H., Abu-Hijleh, G., and Said, O., 2006. "Safety, of traditional arab herbal." *Evid Based Complement Alternat Med.,* vol. 3, pp. 433-439.

[10] Colson, C. R. and de Broe, M. E., 2005. "Kidney Injury from alternative medicines." *Adv. Chronic Kidney Disease*, vol. 12, pp. 261-275.

[11] Kwan, T. H., Tong, M. K., Leung, K. T., Lai, C. K., Poon, W. T., and Chan, Y. W., 2006. "Acute renal failure associated with prolonged intake of slimming pills containing anthraquinones." *Hong Kong Medical Journal*, vol. 12, pp. 394-397.

[12] Moritz, F., Compagnan, P., Kaliszczak, I. G., Kaliszczak, Y., Caliskan, V., and Girault, C., 2005. "Severe acute poisoning with homemade aconitum nepellus capsule: Toxicokinetic and clinical data." *Clinical Toxicology,* vol. 43, pp. 873-876.

[13] Ernst, E., 2003. "Serious psychiatric and neurological adverse effects of herbal medicines – a systemic review." *Acta Psychiatr Scand.*, vol. 108, pp. 83-91.

[14] Jensen, W. I. and Allen, J. P., 1981. "Naturally occurring and experimentally induced castor bean (ricinus communis) poisoning in ducks." *Avian Disease*, vol. 5, pp. 184-194.

[15] Anaga, O. A., Shoyinka, O. V. S., and Asuzu, U. I., 2006. "Toxic effects of Dennettia tripetala root extract." *Pharmaceutical Biology*, vol. 44, pp. 451-461.

[16] Okwu, D. E., Morah, F. N. I., and Anam, E. M., 2005. "Isolation and characterization of phenanthrenic alkaloids uvariopsine from dennettia tripetala fruits." *Journal of Medical Aromatic Plant Science*, vol. 27, pp. 496-498.

[17] Ikpi, D. E. and Nku, C. O., 2008. "Effect of ethanolic extract of dennettia tripetala fruits on haemotological parameters in albino wister rats." *Nigeria Journal of Physiological Sciences*, vol. 23, pp. 13-17.

[18] Timothy, C. O. and Okere, C. O., 2008. "Effect of dennettia tripetala (MMIMI) seed intake on the iop of normotensive emmetropic Nigerian Igbos." *JNOA.*, vol. 14, pp. 14-17.

[19] Bassey, U. E., Akpakpan, E. I., Etim, O. E., Akaka, E. U. A., and Ekanemesang, U. M., 2018 "Effect of ethanol extract of ripe fruits of Dennetia tripetala on prostatic and testicular functions of male albino rats." *The Journal of Medical Resaerch*, vol. 4, pp. 89-92.

[20] Oyemitan, I. A., Iwalewa, E. O., Akanmu, M. A., and Olugbade, T. A., 2008. "Antinociceptive and antiinflammatory effects of essential oil of dennettia tripetala, g. Baker (annonaceae) in rodent." *African Journal of Traditional, Complementary and Alternative Medicines*, vol. 5, pp. 355 – 362.

[21] Anaga, O. A. and Asuzu, U. I., 2010. "Antihyperglycaemic properties of the ethyl acetate extract of Dennettia tripetala in diabetic rats." *Journal of Complementary and Integrative Medicine*, vol. 7, pp. 1-11.

[22] Iseghohi, S. O. and Orhue, N. E. J., 2015. "Aqueous extract of Dennettia tripetala (Pepper fruit) protects the liver and kidney against carbon tetrachloride-induced damage in rats." *Niseb Journal*, vol. 15, pp. 106-111. Available: Http://Www.Nisebjournal.Org

[23] Anosike, C. A., Okagu, I. U., and Uchenna, O. K., 2016. "Phytoconstituents, acute toxicity study and protective effect of ethanol extract of Dennettia Tripetala seed against aspirin-induced ulcer in rats." *International Journal of Advanced Science and Research*, vol. 1, pp. 1-6.

[24] Lorke, D., 1983. "A new approach to practical acute toxicity testing." *Archives of Toxicology*, vol. 54, pp. 275-287.

[25] Ekeanyanwu, R. C. and Njoku, O. U., 2014. "Acute and subacute oral toxicity study on the flavonoid-rich fraction of Monodora tenuifolia seed in albino rats." *Asian Pacific Journal of Tropical Biomedicine*, vol. 4, pp. 194-202.

[26] Okoronkwo, N. E., Ajomiwe, M. O., Chioma, A., and Ike-Amadi, C. A., 2012. "Comparative study of fatty acid composition of dennettia tripetala leaves extracted with different solvents." *Chemistry Journal*, vol. 1, pp. 35-40.

[27] Hilaly, J. E., Israili, H. Z., and Lyoussi, B., 2004. "Acute and chronic toxicological studies of Ajuga iva in experimental animals." *Journal of Ethnopharmacology,* vol. 91, pp. 43-50.

[28] Yacine, B., Noureddine, B., and Mustapha, T., 2013. "Acute and sub-chronic toxicity study of nigella damascene methanolic seed extract in mice." *International Journal of Pharm and Biosciences,* vol. 4, pp. 413-419.

[29] Alagbaoso, S. O., Nwosu, J. N., Njoku, N. E., Ojukwu, M., Okafor, D. C., and Eluchie, C. N., 2015. "Grouth performance and haematology of albino rats fed varying inclusion of autoclaved Canavalia plagiosperma piper seed meal based-diets." *American Journal of Food Nutrition,* vol. 5, pp. 35-48.

[30] Vermuelen, N. P. E., Bessems, J. G. M., and Van De Straat, R., 1992. "Molecular aspects of paracetamol-induced hepatotoxicity and its mechanism–based prevention." *Drug Metals Development,* vol. 24, pp. 367-407.

[31] Ighodaro, O. M. and Omole, J. O., 2010. "Effects of Cajanus cajan aqueous leaf extract on serum amino transferase, alkaline phosphatase and electrolytes concentrations of normal wistar rats." *Animal Research International,* vol. 7, pp. 1304-1308.

[32] Pendota, S. C., Yakubu, M. T., S., G. D., and Afolayan, A. J., 2010. "Effect of administration of aqueous extract of Hippobromus pauciflorus leaves in male wistar rats." *African Journal of Traditional, Complementary and Alternative Medicines,* vol. 7, pp. 40-46.

[33] Jesse, B., 1982. *Animal anatomy and physiology*. Reston, USA: Reston Publishing Company. Inc., p. 521.

[34] Olson, H., Betton, G., Robinson, D., Thomas, K., Monro, A., and Kolaga, G., 2000. "Concordance of toxicity of pharmaceuticals in humans and in animals." *Reg. Toxicol Pharmacol,* vol. 32, pp. 56-67.

[35] Otemenyin, S. O., Olorunfemi, P. O., Audu, O. M., and Sabo, S., 2013. "Sub-acute toxicity study of herbal blood tonic (Hamebuild) in albino rats." *Asian Journal of Pharmaceutical and Clinical Research,* vol. 6, pp. 78-83.

[36] Metodieva, D., Jaiswal, A. K., Cenas, N., Dickancaite, E., and J., S.-A., 1999. "Quercetin may act as a cytotoxic prooxidant after its metabolic activation to semiquinone and quinoidal product." *Free Radical Biology and Medicine,* vol. 26, pp. 107-16.

[37] Halliwell, B., 2008. "Are polyphenols antioxidants or prooxidants? What do we learn from cell culture and in vivo studies." *Archives of Biochemistry and Biophysics,* vol. 476, pp. 107-112.

[38] Ewertowska, M., Jodynis-Liebert, J., Kujawska, M., Adamska, T., Matławska, I., and Szaufer-Hajdrych, M., 2009. "Effect of Aquilegia vulgaris (L.) Ethyl ether extract on liver antioxidant defense system in rats." *International Journal of Occupational Medicine and Environmental Health,* vol. 22, pp. 115-123.

[39] Martín-Cordero, C., León-González, A. J., Calderón-Montaño, J. M., Burgos-Morón, E., and López-Lázaro, M., 2012. "Pro-oxidant natural products as anticancer agents." *Current Drug Targets,* vol. 13, pp. 1006-1028.

[40] Mahmoud, A. H., Motawa, H. M., Wahba, H. E., and Ebrahim, A. Y., 2006. "Study of some antioxidant parameters in mice livers affected with urtica pilulifera extracts." *Asian Journal of Biochemistry,* vol. 1, pp. 67-74.