OXFORD

Food Quality and Safety, 2017, 1(3), 211–219
doi:10.1093/fqsafe/fyx019
Research Paper
Advance Access publication 7 August 2017

Research Paper

Toxicological and histopathological effects of *Dennettia tripetala* seed used as grain protectant, food, and medicine

Luke Chinaru Nwosu,*,* Chris Olukayode Adedire,* Emmanuel Oludele Ogunwolu** and Michael Olufemi Ashamo***

*Food Technology Programme, Department of Biology, Federal University of Technology, Akure, Ondo State and **Department of Crop and Environmental Protection, University of Agriculture, Makurdi, Benue State, Nigeria
***Present address: Department of Crop and Soil Science, Faculty of Agriculture, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Rivers State, Nigeria

Correspondence to: Luke Chinaru Nwosu, Department of Crop and Soil Science, Faculty of Agriculture, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Rivers State, Nigeria. E-mail: luke2007ambition@yahoo.com

Received 13 May 2017; Revised 5 June 2017; Editorial Decision 12 June 2017.

Abstract

The study revealed the safety of *Dennettia tripetala* seed on man and the environment. Adult male rats weighing 0.158–0.168 kg housed in standard cages with free access to food and water were used for the experiments. The median lethal dose (LD_{50}) was estimated using revised up and down procedure. The LD_{50} for *D. tripetala* seed extract was 5785 mg/kg and this evoked paralysis in rats for 4 days coupled with discharge from the eyes and eventual death. The least weight gain by the animals administered 75% seed powder of *D. tripetala* (4338.75 mg/kg) was an index of high powder concentration, whereas the weight loss experienced by group V animals is strongly attributed to chemical assault by permethrin designated as a standard insecticide. The high values of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and urea in the groups administered 75% permethrin powder (4338.75 mg/kg) and 75% *D. tripetala* seed powder is an index of liver and kidney injury and dysfunction. The presence of normal serum levels of ALT, AST, alkaline phosphatase, and creatinine; normal liver and kidney structures; and normal weight gains in the animal groups fed basal diet (control) and basal diet plus 25% *D. tripetala* is a strong indication that 25% *D. tripetala* seed powder (1446.25 mg/kg) supplementation is not toxic to the liver and kidney and therefore supports normal organ functions. The LD_{50} recorded strongly indicates that *D. tripetala* has a moderately high safety margin. Supplementation of less than 50% (2892.5 mg/kg) is recommended in the safe use of the plant material as grain protectant, food, and medicine. The botanical insecticide, *D. tripetala*, is safer than the conventional synthetic insecticide, permethrin, on account of the latter showing evidence of kidney damage.

Key words: Safety; botanical insecticide; permethrin; supplementation; kidney damage.

Introduction

Dennettia tripetala G. Baker (Annonaceae) also known as pepper fruit tree (Ikpi and Nku, 2008) is an indigenous spicy medicinal plant in Nigeria, whose seed (whole, powder, or extract) is used as an effective insecticide in grain protection against weevils in particular (Agbakwuru *et al.*, 1978; Adedire, 2001). The seeds are used (in various forms) to protect maize grains in storage. Although farmers, traders, and household-users apply the treatment at varying concentrations (suitable to them), Lale (2002) recommended 2% w/w for all plant products. Furthermore, the seeds are popularly stored and used internationally;

consumed singly; or taken with kola nut, garden egg, or palm wine, particularly during cultural entertainment of guests (Enwere, 1998; Okwu *et al.*, 2005) and traditional ceremonies, such as weddings, festivals, and naming ceremonies. The plant is common in the tropical rainforest region of Nigeria and occasionally found in Savanna areas (Okwu *et al.*, 2005). Some communities in southern Nigeria reportedly utilize the leaves, roots, and fruits for medicinal purpose (Iwu, 1989; Ikpi and Nku, 2008). As masticators and stimulants, *D. tripetala* fruits/seeds produce unique peppery effect when chewed (Keay, 1989; Ikpi and Nku, 2008). *Dennettia tripetala* seeds are applied to diets of pregnant and postpartum women to aid uterine contraction (Achinewhu *et al.*, 1995; Okwu and Morah, 2004). The seed has generally been reported to be used as spice in flavouring and seasoning foods, such as meat, vegetable, soup, and sausage (Ikpi and Nku, 2008). The important nutritive substances of *D. tripetala* fruit/seeds are minerals, vitamins, and fibre, whereas the major phytochemicals are thiamine, riboflavin, niacin, and alkaloids (Okwu and Morah, 2004; Okwu *et al.*, 2005).

From the above background, it is obvious that *D. tripetala* seed serves as a grain protectant, food, and medicine. Food and medicine apart, it is as well necessary to ascertain the safety margin of a grain protectant for the benefit of humans and their environment. There is public advocacy to use plant materials in pest control because they are safe and eco-friendly (Adedire and Lajide, 1999). For *D. tripetala* seed, insecticidal activity has been reported (Agbakwuru *et al.*, 1978; Adedire, 2001). Many members of the family Annonaceae are known to possess various chemical compounds that act as antifeedants, repellents, and growth or development inhibitors against many insect species (Odeyemi *et al.*, 2008). Seed powder and oil extracts of *Dennettia* have been reported to be particularly effective against *Sitophilus zeamais* infestation in stored maize (Adedire, 2001). However, there is belief that plant-derived insecticides are safer than synthetic chemicals such as permethrin because they are of natural origin (Weinzierl and Henn, 1994). This is not always the case (Odeyemi *et al.*, 2008). Indeed, several toxicological and histopathological effects have been associated with plant materials (Odeyemi *et al.*, 2008; Ileke *et al.*, 2014). It was revealed that moderate to high doses of *Dennettia tripetala* fruit extract administered to albino Wistar rats caused haematoxicity to the animals (Ikpi and Nku, 2008). *Momordica charantia* L. at 362.34 mg/0.1 kg of body weight of normal and diabetic albino rats induced a significant decrease in serum glucose levels in both groups. However, the extracts did not show any significant effect in urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (AP) in normal rats, while in diabetic rats, the extracts caused a significant decrease in serum urea, creatinine, ALT, AST, AP, cholesterol, and triglyceride levels (Abd EL Sattar EL Batrans *et al.*, 2006). Odeyemi *et al.* (2008) assessed the effects of leaf extracts of *Tagetes minuta* L. on the serum biochemical indices of albino Wistar rats and revealed that the oil extract caused mild toxicity to the animals and concluded that the effect of the essential oil is dose index selective. Sathya *et al.* (2012) using physical, biochemical, and haematological parameters found that extract of *Acalypha indica* L. exerted no significant signs of toxicity at 100–500 mg/kg body weight of male albino rats. Toxicological and histopathological evaluations of leaf extract of *Paullinia pinnata* L. on albino mice and rats showed that the plant material is well tolerated at the dose of 200 mg/kg body weight but toxic at higher doses, causing liver lesions in some of the animals (Adeyemo-Salami and Makinde, 2013). Ileke *et al.* (2014) using serum biochemical indices observed that 10% concentration of *Astonia boonei* De Wild stem bark powder was toxic to the liver of female albino rats but did not harm the kidney.

Despite the use of *D. tripetala* as grain protectant and its extensive use as food and medicine, the toxicological and histopathological implications of the plant material have been poorly evaluated (Ikpi and Nku, 2008). Therefore, the present study sought to investigate the toxic and histopathological effects of *D. tripetala* seed powder and extract in albino rats.

Materials and Methods

Preparation of D. tripetala seed powder and extract

Air-dried samples of *D. tripetala* seeds were ground with the aid of a mortar and pestle and sieved into fine powder. Two hundred and forty grams of the seed powder were weighed into a round-bottom flask and exhaustively extracted with 2400 ml of 80% methanol. The extraction was by cold maceration at room temperature (25°C–30°C) with intermittent shaking at 2 h intervals for 48 h. Thereafter, the suspension was preliminarily filtered with cotton wool held over the opening of the flask and refiltered using Whatman No 1 filter paper. The filtrate was concentrated in a water bath at 45°C, yielding 23.69 g of extract (9.87% yield), which was stored at 4°C until required for use. Reconstitution was achieved by dissolving 2000 mg of the extract in 2 ml of solvent (0.5 ml of Tween 80 ± 1.5 ml of normal saline) to an appropriate concentration of 1000 mg/ml prior to oral administration. Tween 80 (polysorbate) was used to dissolve the oil extract.

Experimental animals

Thirty healthy young adult male albino rats of age 1.5–2 months were purchased from the Breeding Colony Unit of College of Health Sciences, Benue State University, Makurdi, Nigeria, and used for the experiment. The rats were randomly assigned to five groups of six animals per group in standard cages. The initial weights of the animals ranged from 0.158–0.168 kg each. They were properly maintained in the laboratory at ambient temperature (27.5°C) and relative humidity (72.3%) in accordance with the recommendation of University of Agriculture Makurdi Ethical Committee on the use of animals with free access to food (standard basal diet produced by United Africa Company, Nigeria) and water. They were acclimatized for 2 weeks prior to the commencement of the experiments (Saganuwan, 2012). The experiment was repeated six times.

Determination of median lethal dose

Median lethal dose (LD_{50}) was estimated using revised up-and-down procedure described by Bruce (1985) and Saganuwan (2012). Five healthy male rats were randomly selected (one from each group) without replacement and dosed after an overnight fast. Initial dose of 2000 mg/kg of the extract (0.3 ml in quantity) was administered to the first rat orally and the animal was carefully observed for mortality and other signs of toxicity for a period of 48 h. The first animal survived; therefore, a dose progression using logarithm of 3.2 was used until a dose level of 6, and 585 mg/kg was reached causing paralysis for 4 days and discharge from the eyes and eventual death. The square root of product of geometric mean of the last survived dose and mortality-causing dose was used to estimate the LD_{50}. From the result of the LD_{50}, sublethal doses of *D. tripetala* seed powder used for the dietary toxicity study were calculated applying standard ratios (25%, 50%, and 75%) recommended by Saganuwan (2012). These gave 1446.25, 2892.5, and 4,338.75 mg/kg seed powder sublethal doses, respectively (which all worked out less than 5% weight per body weight of the animals).

Diets for subacute toxicity test with D. tripetala seed powder

The concentrations of the test substances in the dietary toxicity study were maintained at less than 5% (w/w) in the diet (OECD test guidelines 407 and 451). This ensured that the quantities of the test substance in the diet did not interfere with normal nutrition. Feed used was standard basal diet produced by United Africa Company, Nigeria. Rats in group I served as control and they were fed the basal diet. In all, 25%, 50%, and 75% *D. tripetala* seed powder were separately incorporated into basal diet and were used to feed rats in groups II, III, and IV, respectively. Group V rats were included to determine the comparative safety of *D. tripetala* seed (plant-based insecticide) and permethrin (conventional synthetic insecticide) in pest control. Group V rats were fed basal diet plus 75% permethrin. The chemical grade of permethrin used was necessarily pesticide. The albino rats were allowed to feed for 30 days and feed consumption data were taken and analysed. Final weights of the rats were measured at the end of the 30 days prior to blood collection and dissection of the animals following euthanasia.

Collection of blood, liver, and kidney

A blood sample (2.0 ml) was obtained from each rat by intracardiac puncture under ether anaesthesia and put in a non-heparinized sample tube. The blood sample was centrifuged at a 3000 g for 10 min to separate the serum for assay of liver and kidney biochemical indices of the albino rats. The animals were dissected immediately, and liver and kidney of each rat were harvested with the aid of surgical equipment and preserved in 10% formalin for histopathological examination.

Determination of serum biochemical indices

The assay kits for ALT, AST, AP, total protein, urea, and creatinine were products of Agappe Diagnostics, Switzerland. ALT was determined using the method of Wroblewski and LaDue (1956). A 1 ml of the working reagent (containing 500 mmol/L, 1250 U/L LDH, 100 mmol/L Tris buffer, pH 7.5, 15 mmol/L 2-oxoglutarate, and 0.18 mmol/L NADH) was pipetted into labelled test tubes. Test tubes were pre-incubated at 37°C for 5 min. The spectrophotometer was set at zero at 340 nm with distilled water. Then, 100 µl of serum was added to its respective tube, mixed gently, and transferred to a thermo cuvette. Absorbance was read and recorded at 1 min. Incubation continued at the same temperature and absorbance was again recorded at 2 and 3 min. The average absorbance per minute was determined and multiplied by a factor of 1768 for results in U/L.

AST was determined using the same assay procedure described above for ALT except that the working reagent contained 240 mmol/L l-aspartate, 650 U/L MDH (porcine muscle), 650 U/L LDH (rabbit muscle), 80 mmol/L Tris buffer, pH 7.5, 12 mmol/L 2-oxoglutarate, and 0.18 mmol/L NADH.

An aliquot of 0.01 ml of the sample was mixed with 0.5 ml of working reagent (containing 1 mol/l diethanolamine buffer pH 9.8, 0.5 mmol/l $MgCl_2$, and 10 mmol/l *p*-nitrophenylphosphate). The absorbance was then read at 1 min interval for 3 min at 405 nm, and the AP activity was subsequently determined.

The total protein was determined by the direct Biuret method (Gomall, 1949). Twenty microlitres of serum were mixed with 1000 µl of Biuret reagent (6 mmol/L potassium iodide, 21 mmol/L potassium sodium tartarate, 6 mmol/L copper sulphate, and 58 mmol/L sodium hydroxide). Thereafter, the mixture was incubated for 10 min at 37°C and the absorbance taken at 546 nm. Bovine serum albumin was used as the standard protein and the total protein was subsequently calculated using the formula:

$$\text{Total protein concentration (g/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{6}{1}$$

One hundred microlitres of serum were mixed with 1000 µl of the working reagent (60 mmol/L phosphate buffer and 20 KU/L urease). The mixture was incubated for 5 min at 37°. Thereafter, 1000 µl of urea B colour reagent (80 mmol/L sodium salicylate, 4 mmol/L sodium nitroprusside, and 45 mg/dL sodium hypochlorite) were added to the mixture and incubated for 5 min at 37°C. One thousand microlitres of deionized water were added to the mixture and the absorbance of sample and the standard were measured against the reagent blank.

$$\text{Urea concentration (mg/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{40}{1}$$

To determine creatinine, 100 ml of serum (diluted 1/100 with distilled water) was mixed with 1000 µl of the working reagent (8.73 mmol/l picric acid, 300 mmol/l sodium hydroxide, and 25 mmol/ sodium phosphate). The optical density (T_1) was read 60 s after mixing the sample and working reagents. Exactly 60 s after the first reading, a second reading was taken (T_2) for both standard mixture and the sample.

$$\text{Creatinine concentration (mg/dL)} = \frac{(T_2 - T_1) \text{ of sample}}{(T_2 - T_1) \text{ of standard}} \times \frac{2}{1}$$

Histological assessment of liver and kidney of rats fed with *D. tripetala* seed powder and permethrin

The liver and kidney of the albino rats preserved in 10% formalin to prevent decay were used for the histopathological study. The sectioning method described by Akparie (2004) was used for the histological examination. The method has the merit of preserving the relations of cells and tissues to one another. Thin sections (6 µm) of the organs were prepared and examined under a microscope. They were dehydrated in serial alcohol concentration of 50%, 70%, 80%, and 100% for 1½ h each. After dehydration, they were cleared with 100% xylene and were left for 2 h to remove any alcohol and then impregnated in liquid paraffin wax for 2 h for embedding. The embedded organs were sectioned using a microtome and were stained with haematoxylin–eosin (Silva *et al.*, 1999). Excess stain was removed with tap water. After clearing in xylene, Canada balsam was added and cover slips placed on the slides. The preparations were left in the oven at 40°C and then examined under the microscope equipped with a digital camera connected to a computer and photographs were captured.

Statistical analysis

The statistical software was SPSS for Windows® (version 19.0). Consequent upon Levene's test for equality of variances, data were transformed using arcsine transformation (Somta *et al.*, 2008) to stabilize the variance. Data were then subjected to one- way analysis of variance. A more pragmatic multiple comparison test, Tukey's Honestly studentized range, was used to separate significant means from non-significant ones at $\alpha = 0.05$.

Results

Feed intake of experimental rats

Table 1 presents the mean quantity of feed consumed by each rat per day. There were no significant differences in the average feed intakes of control rats fed basal diet (group 1); rats fed with basal diet plus 25%, 50%, and 75 % *D. tripetala* seed powder (groups II,

Table 1. Feed intakes of albino rats used for sub-acute toxicity test (in all cases, df = 4,25).

Treatment	Feed intake (g/rat/day)
Standard basal diet (control)	15.75 ± 0.48[a]
Standard basal diet + 25% *Dennettia tripetala* seed powder	16.14 ± 0.51[a]
Standard basal diet + 50% *D. tripetala* seed powder	16.14 ± 0.31[a]
Standard basal diet + 75% *D. tripetala* seed powder	15.75 ± 0.48[a]
Standard basal diet + 75% permethrin	16.83 ± 0.66[a]
F	0.3
P	>0.05

Data are means ± SEM of six replications. Means in a column followed by the same leter are not significantly different (α = 0.05).

III, and IV, respectively); and rats fed with basal diet supplemented with 75% permethrin insecticide (group V).

Effect of D. tripetala seed powder and permethrin on body weight of albino rats

Data on initial and final body weights of rats as well as weight gain/loss are presented in Table 2. Significant differences were not observed in the body weights of the animal groups at the commencement of the experiment. At the end of the experiment, the final body weight of group V rats alone fed with basal diet plus 75% permethrin differed significantly from others. Weight gain in albino rats fed basal diet plus 75% *D. tripetala* seed powder was significantly lower than rats fed other diets excluding standard basal diet + 75% permethrin. On the latter treatment, rats lost an average of 25% of their body weight after a period of 30 days.

Acute toxicity (LD_{50})

The acute toxicity study showed that the methanolic extract of *D. tripetala* seed powder had a LD_{50} of 5785 mg/kg of rat.

Effects of D. tripetala seed pow*der on some serum biochemical indices of albino rats*

The results on the biochemical parameters of the liver and kidney of albino rats are presented in Table 3. Although alanine aminotransferse increased with increasing dose of *D. tripetala* seed powder, the differences were not statistically significant. In contrast, alanine aminotransferse value for rats fed basal diet plus 75% permethrin was the highest and it differed significantly from other treatments. There were no significant alterations in the level of serum AST in rats fed basal diet and basal diet plus *D. tripetala*; the inclusion of 75% permethrin in rat's diet (group V), however, resulted in a significant increase in the level of AST. Enzyme activity of AP did not vary significantly among treatment diets excluding the standard basal diet plus 75% permethrin. There was no significant difference in the total protein content of all the treatment diets investigated. Normal control rats (group I) had highest level of total protein. Serum urea in rats fed standard basal diet plus 25% *D. tripetala* seed powder was lowest and differed significantly from all treatments excluding control. The concentration of serum creatinine decreased with increasing dose of *D. tripetala*, but this was not a significant observation except between control rats fed basal diet and rats fed basal diet plus 75% *D. tripetala* seed powder.

Effects of D. tripetala seed powder on the histopathology of albino rat liver

The histopathological examination of the liver showed that rats fed standard basal diet (control) and standard basal diet plus 25% *D. tripetala* (group II) or plus 50% *D. tripetala* (group III) had normal liver structure with good muscles and intact sinusoids (Figure 1). The incorporation of 75% *D. tripetala* or 75% permethrin resulted in haemosiderosis (presence of haemosiderin granules in the liver sinusoids) (Figure 2).

Effects of D. tripetala seed powder on the histopathology of albino rat kidney

Rats fed basal diet as well as those fed basal diet plus 25% *D. tripetala* seed powder had normal kidney structures (Figure 3). Increase in dosage of *D. tripetala* to 50% and 75% resulted in haemorrhage (without necrosis) and congestion of kidney veins (Figure 4). Incorporation of 75% permethrin into standard basal diet caused adherent kidney capsule in addition to haemorrhage without necrosis and vein congestion (Figure 5).

Discussion

The increase in body weight in rats fed standard basal diet (group I) and in rats fed standard basal diet plus *D. tripetala* seed powder (groups II, III, and IV) during the experiment suggests that basal diet and ≤50% *D. tripetala* seed powder supplement will not adversely affect body weight. The loss in weight in rats fed basal diet plus 75% permethrin (group V) can be attributed to the toxic effect of the chemical supplement. Ileke *et al.* (2014) reportedly associated weight loss in albino rats to treatment with petroleum ether extract and powder of *Nigella sativa*. The acute toxicity test of the present study showed that the LD_{50} value of *D. tripetala* seed powder is moderately high. This suggests that the extract has a moderately high safety margin. Ikpi and Nku (2008) in a similar study recorded that the plant material showed a moderately high safety margin.

Furthermore, Odeyemi *et al.* (2008) indicated that assessment of biochemical indices can be used to determine the extent of harmful effect of a foreign compound including plant materials on the blood. Yakubu *et al.* (2007) similarly reported that such parameters can be used to explain blood-related functions as can be influenced by a plant material. However, relationships between serum biochemical indices and liver and kidney functions of albino rats, in particular, have been reported by Ileke *et al.* (2014). Therefore, it is fairly well documented that the evaluations of tissue function parameters play an important role in disease investigation and diagnosis (Yakubu *et al.*, 2003). ALT and AST are important marker enzymes for assessing damage to organs such as liver and kidney. They are released into the serum usually when there is damage to the hepatic membrane due to chemical attack (Oluba *et al.*, 2008). Therefore, serum levels of the enzymes are useful indicators of extent of hepatic damage (Bamisaye *et al.*, 2013). The non-significant effect of ALT in rats fed standard basal diet or standard basal diet plus *D. tripetala* seed powder is an indication that the livers of the animals were not damaged. However, its increase with increase in concentration of the botanical insecticide may suggest organ injury or dysfunction. This is in agreement with the observations of Odutuga *et al.* (2010). The significant alteration in the level of ALT in group V rats fed with basal diet plus 75% permethrin may strongly suggest hepatic damage. The normal values of AST recorded in albino rats fed standard basal diet or standard basal diet plus *D. tripetala* may strongly indicate that

Table 2. Change in body weight of rats fed basal diet, basal diet plus *Dennettia tripetala* seed powder, and basal diet plus permethrin insecticide (in all cases, df = 4,25).

Treatment	Initial weight (g)	Final weight (g)	% weight gain/loss
Standard basal diet (control)	157.50 ± 4.79[a]	171.75 ± 5.27[a]	9.05 ± 0.14[a]
Standard basal diet + 25% *D. tripetala* seed powder	161.38 ± 5.06[a]	175.73 ± 5.63[a]	8.89 ± 0.43[a]
Standard basal diet + 50% *D. tripetala* seed powder	161.38 ± 3.13[a]	175.38 ± 2.77[a]	8.70 ± 0.59[a]
Standard basal diet + 75% *D. tripetala* seed powder	157.50 ± 4.79[a]	167.41 ± 5.14[a]	6.29 ± 0.17[b]
Standard basal diet + 75% permethrin	168.25 ± 6.61[a]	115.70 ± 5.69[b]	–25.35 ± 0.75[c]
F	0.2	2.9	3.0
P	>0.05	0.04	<0.01

Data are means ± SEM of six replications. Means in a column followed by the same letter are not significantly different (α = 0.05).

Table 3. Effect of *Dennettia tripetala* seed powder on some important serum biochemical indices of albino rats (in all cases, df = 4,25).

Treatment	Alanine aminotransferase (U/L)	Aspartate aminotransferase (U/L)	Alkaline phosphatase (U/L)	Total protein (g/dL)	Urea (mg/dL)	Creatinine
Standard basal diet	9.93 ± 1.88[a]	11.70 ± 3.56[a]	55.86 ± 7.92[ab]	3.10 ± 0.78[a]	8.28 ± 12.38[a]	0.73 ± 0.22[a]
Standard basal diet + 25% *D. tripetala* seed powder	17.25 ± 2.41[a]	10.80 ± 1.32[a]	28.96 ± 4.29[a]	1.95 ± 0.60[a]	8.16 ± 3.72[a]	0.72 ± 0.28[a]
Standard basal diet +50% *D. tripetala* seed powder	24.95 ± 6.28[a]	12.06 ± 1.97[a]	23.43 ± 9.85[a]	2.43 ± 1.00[a]	11.00 ± 15.41[a]	0.69 ± 0.09[a]
Standard basal diet + 75% *D. tripetala* seed powder	34.15 ± 6.90[a]	12.15 ± 1.83[a]	27.63 ± 8.66[a]	1.80 ± 0.84[a]	62.80 ± 6.38[b]	0.18 ± 0.07[b]
Standard basal diet + 75% permethrin	82.98 ± 16.11[b]	21.20 ± 3.06[b]	72.30 ± 24.44[b]	2.18 ± 0.98[a]	49.20 ± 6.71[b]	0.15 ± 0.13[b]
F	11.7	2.9	3.0	3.4	4.5	2.8
P	<0.01	< 0.01	< 0.01	0.04	0.02	< 0.01

Data are means ± SEM of six replications. Means in a column followed by the same letter are not significantly different (α = 0.05).

the administration of *D. tripetala* seed powder at supplement concentration of ≤75% did not cause injury to liver and kidney of the albino rats. However, the group V rats that had significantly high level of AST possibly experienced myocardial infarction (Karmen, 1955) and liver toxicity (Ileke *et al.*, 2014).

AP is reportedly a maker enzyme for the plasma membrane and endoplasmic reticulum damage and often used to measure the integrity of the plasma membrane (Akanji *et al.*, 1993). The observed reduction in serum AP level in the animal groups supplemented with *D. tripetala* seed powder suggests a possible adverse effect on the transportation of the vital ions on the molecules across the plasma membrane (Akanji *et al.*, 1993; Ileke *et al.*, 2014). It may invariably cause reduction in the phosphate groups available for the phosphorylation of ethanolamine and choline required for the production of important phospholipids such as phosphatidylcholine and phosphatidylethanolamine (Bamisaye *et al.*, 2013). The reduction in serum AP may also imply interference in the syntheses of nuclear acids in the rats fed basal diet plus the botanical insecticide (Ramalingam and Vimaladevi, 2002) and this has implications for effective performance of the testes of the rats. This finding agrees with the report of Ileke *et al.* (2014), who recorded reduction in AP among albino rats administered stem bark powder of *Alstonia boonei*.

The serum total protein evaluated in this study is a useful index of impairment of the functional capacity of the liver and kidney (Odeyemi *et al.*, 2008). Such low level of total proteins in the animal groups with diets supplemented with *D. tripetala* seed powder and permethrin insecticide may be an indication of liver disorder and nephritic damage. Blood urea and creatinine are critical indices that can be used to assess renal function. Urea is the major nitrogen-containing metabolic product of protein breakdown (Odeyemi *et al.*, 2008) and about 90% of it is excreted through the kidney (Ileke *et al.*, 2014; Yakubu *et al.*, 2003). The significant increase in the serum urea content at the highest test dose of 75% *D. tripetala* seed powder (group IV) may be attributed to impairment in the urea cycle (Yakubu *et al.*, 2003). Ileke *et al.* (2014) further inferred that significant increase in urea concentration is an indication of abnormality in the physiological excretion of urea caused by a non-renal factor, plant powder. Since only the highest dose of the plant powder brought about an alteration in urea, it may be logical to infer dose-specific effect. Therefore, the dose-specific effect produced by *D. tripetala* seed powder on this parameter suggests selective toxicity. This is corroborated by Odeyemi *et al.* (2008). Creatinine is a by-product of muscle metabolism (Odeyemi *et al.*, 2008) and will accumulate in the blood of an animal with damaged kidney (Ileke *et al.*, 2014). Chawla (1999) attributed increase in serum creatinine content to glomerular and tubular dysfunction. In the present study, creatinine concentration in the animal groups except the group fed diets supplemented with 75% *D. tripetala* was normal and this indicates normal renal function. Weber *et al.* (2002) indicated that normal range of serum creatinine in rats to be 0.2–0.8 mg/ dL. Therefore, it was unlikely that the animal groups fed basal diet plus 25% and 50% *D. tripetala* seed powder had damaged kidneys. Ileke *et al.* (2014) and Treasure (2003) stressed that serum creatinine is an index of glomerular function.

The result of the present investigation showed that the administration of the botanical insecticide (*D. tripetala*) was not toxic to the liver and kidney of the albino rat on account of ALT, AST, and creatinine appearing in normal concentration in the serum. On the contrary, the supplementation of 75% permethrin insecticide to the

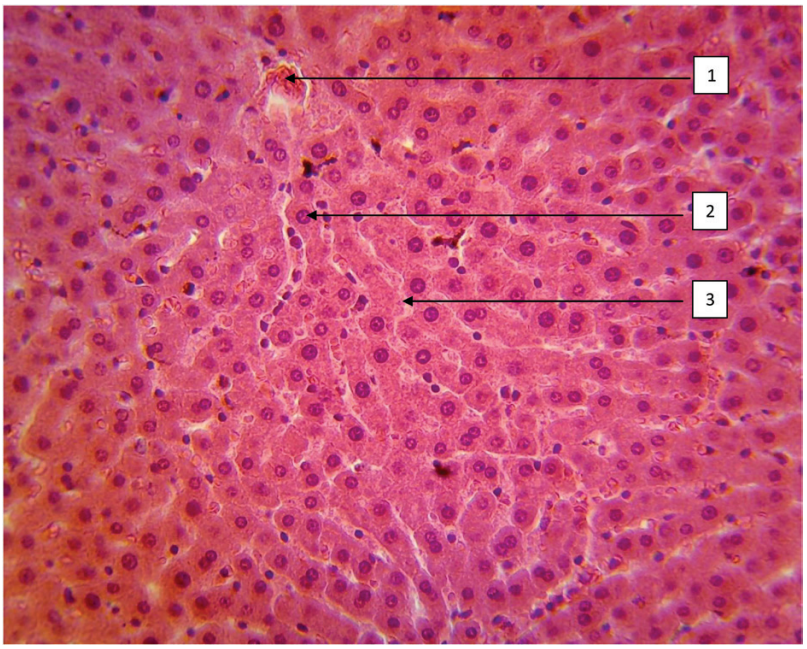

Figure 1. Normal liver structure with good muscles and intact sinusoids in rats fed standard basal diet, standard basal diet + 25% *Dennettia tripetala*, and standard basal diet + 50% *D. tripetala*: arrow 1, central vein; arrow 2, hepatocytes; and arrow 3, sinusoid.

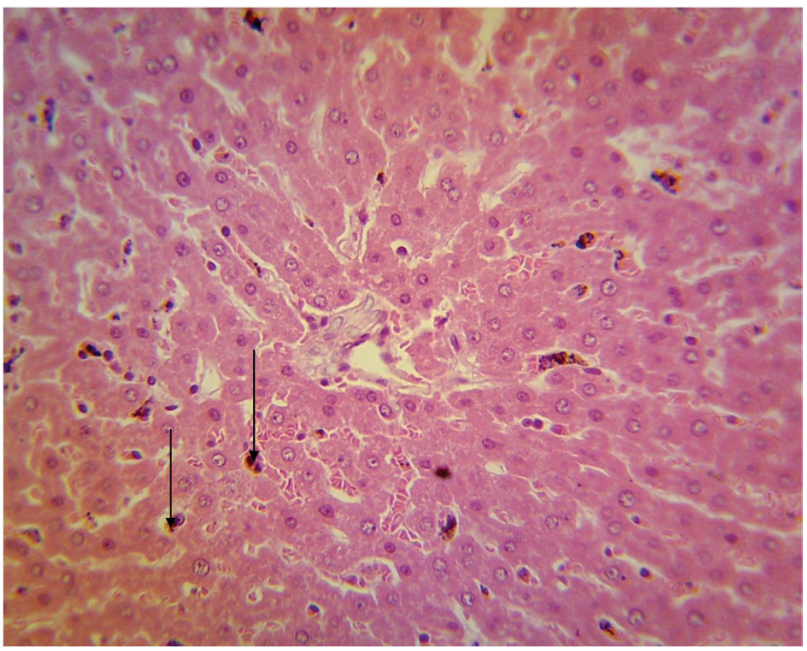

Figure 2. Haemosiderin granules in the liver sinusoids (arrowed) of rats fed standard basal diet + 75% *Dennettia tripetala* seed powder (group IV) and rats fed standard basal diet + 75% permethrin.

diet of the group V animals caused damage to the liver. The inclusion of 75% of the botanical insecticide to rat's diet caused mild toxicity to the kidney on accounts of its significant increase and decrease in serum urea and total protein, respectively.

The histopathological examination of the liver of the animals fed basal diet, basal diet plus 25% *D. tripetala* seed powder, and basal diet plus 50% of the powder that showed normal liver structure and intact sinusoids implies that basal diet and 25%and 50% of the botanical insecticide do not have deleterious effect on the organ. This observation is in agreement with the previous studies by Akparie (2004) and Ileke *et al.* (2014). The occurrence of haemosiderosis (evidenced from haemosiderin granules in the liver sinusoids) in groups IV and V rats suggests that the supplentation of the basal diet with 75% of the botanical insecticide and 75% of permethrin insecticide both affected adversely the red blood cells of the animals. The adverse effect caused by the high concentration of the plant powder, in particular, corroborates the reports of Akparie (2004) and Ileke *et al.* (2014) that high concentration of some plant material is inimical to animals in general.

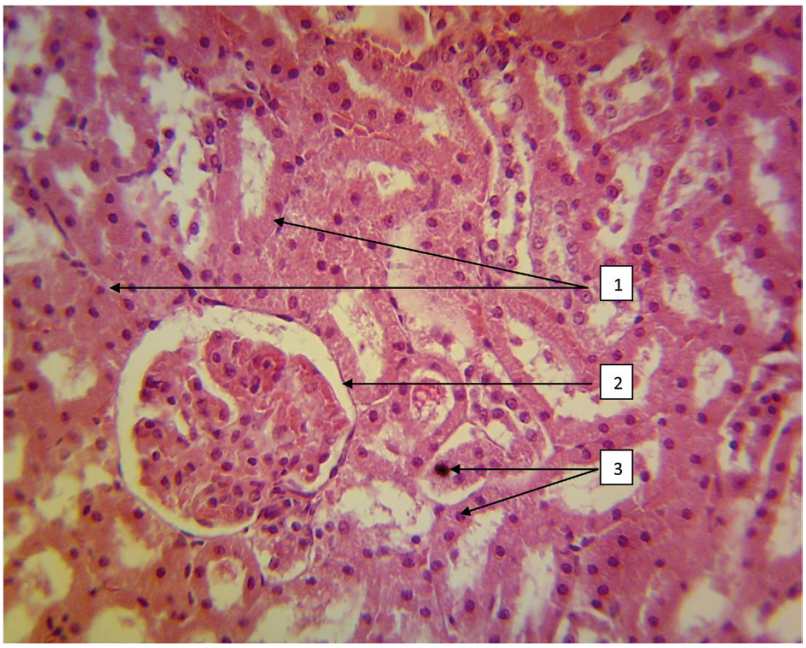

Figure 3. Normal kidney tissues in rats fed standard basal diet and in rats fed standard basal diet + 25 % *Dennettia tripetala* seed powder: arrow 1, connective tissue stroma; arrow 2, glomerular capsule; and arrow 3, papillary ducts.

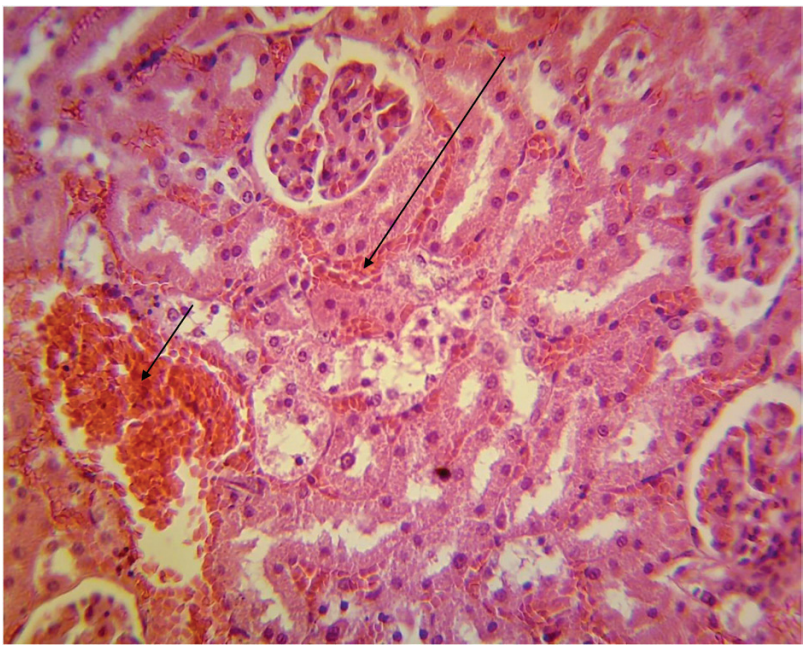

Figure 4. Haemorrhage (indicated by long arrow) and vein congestion (indicated by short arrow) in the kidney of rats fed: (i) standard basal diet + 50% *Dennettia tripetala* seed powder, (ii) standard basal diet + 75% *D. tripetala* seed powder, and (iii) standard basal diet + 75% permethrin.

The normal structure of the kidney observed in the animal groups fed basal diet and basal diet plus 25% *D. tripetala* seed powder strongly indicates normal kidney function and suggests that toxicity did not occur at this concentration. Haemorrhagic kidneys culminating in congestion of the veins observed in the animals administered 50% and 75% of the *D. tripetala* powder suggests deleterious effect on the red blood cells. However, from the findings of Ileke *et al.* (2014), it can be inferred that haemorrhage does not always imply kidney damage but sometimes an indicator of presence of a foreign compound. The inference is further supported by the fact that the haemorrhage was without necrosis. Kidney haemorrhage and congestion in the veins and capsule adherence observed in group V animals fed diets supplemented with 75% permethrin suggest that the organ was slightly shrunken due to chemical attack. Comparatively, it can be concluded that permethrin (chemical insecticide) was more toxic to the albino rat kidney than seed powder of *D. tripetala* (botanical insecticide) at the same concentration of 75%. Interestingly, previous studies have shown insecticidal activity of *D. tripetala* seed powder

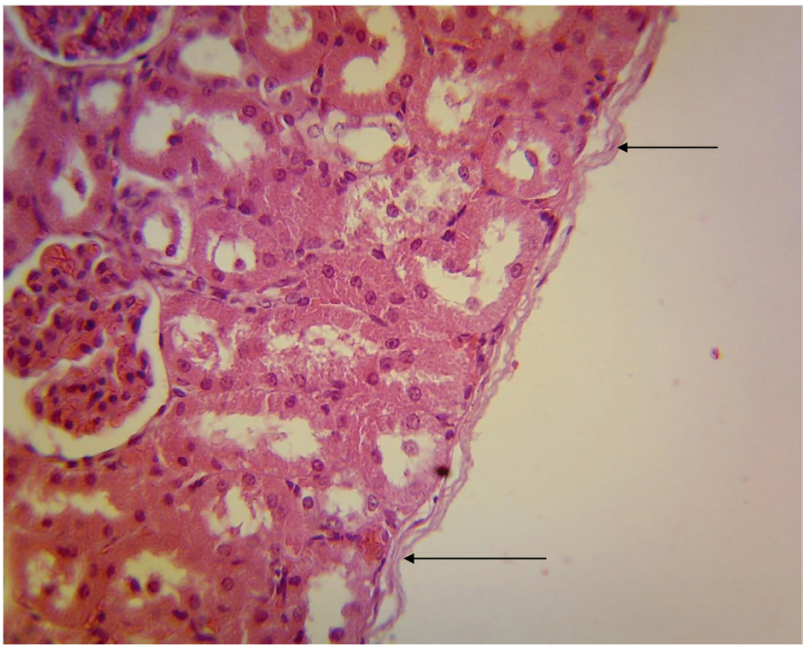

Figure 5. Adherent kidney capsule (arrowed), suggesting shrunken kidney only in rats fed standard basal diet + 75% permethrin.

and extracts against *S. zeamais* infestation (Agbarakwuru *et al*., 1978; Adedire, 2001). Contact and fumigant insecticidal action of *D. tripetala* have been attributed to diverse active constituents, namely, phenols, alkaloids, saponins, tannins, flavonoids, and essential oils (Ejechi and Akpomedaye 2005; Asawalam *et al*., 2006).

Conclusions

The significantly lower weight gain recorded by the animals administered 75% seed powder of *D. tripetala* is an index of high powder concentration. The weight loss experienced by albino rats fed standard basal diet plus 75% permethrin is strongly attributed to chemical assault by permethrin. The value of the LD_{50} obtained in the study strongly indicates that the botanical insecticide has a moderately high safety margin. The high values of ALT, AST and urea recorded in the groups administered 75% permethrin and 75% *D. tripetala* seed powder is an indication of liver and kidney injury or dysfunction. The presence of normal serum levels of ALT, AST, AP, and creatinine; normal liver and kidney structures; and normal weight gains in the animal groups fed basal diet (control) and basal diet plus 25% *D. tripetala* is a strong indication that 25% *D. tripetala* seed powder supplement is undoubtedly not toxic to the liver and kidney and, therefore, supports normal organ function. From the findings of the study, the use of *D. tripetala* seed as food and medicine and in insect pest control will not cause adverse effects to man and his environment even at moderately high concentration. However, the study recommends supplementation of less than 50% (2893 mg/kg body weight) in the safe use of the plant powder. Comparatively, the study concludes that the botanical insecticide, *D. tripetala*, is safer than the conventional synthetic insecticide, permethrin, on account of the latter showing evidence of kidney damage.

Acknowledgements

Examination of prepared organs and result interpretation were done by experts, Dr E.O. Onyekweodiri and Dr Gloria Daniel-Igwe of Department of Veterinary Pathology, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria. This paper is part of the PhD work of the first author in Federal University of Technology Akure, Ondo State, Nigeria. Animal studies were approved by the authors' institutions.

Conflict of interest statement. None declared.

References

AbdEl Sattar, E., El-Gengaihi, S. E., El Shabraw, A. O. (2006). Some toxicological studies of *Momordica charantia* L. on albino rats in normal and alloxan diabetic rats. *Journal of Ethnopharmacology*, 108: 236–242.

Achinewhu, S. G., Ogbonna, C., Hard, A. D. (1995). Chemical composition of indigenous wild herbs, spices, fruits, nuts and leafy vegetables used as food. *Plants Food for Human Nutrition*, 48: 341–348.

Adedire, C. O. (2001). Biology, ecology and control of insect pests of stored cereal grains. In: Ofuya, T. I., Lale, N.E.S. (eds.) *Pests of Stored Cereal and Pulses in Nigeria: Biology, Ecology and Control*. Dave Collins Publications, Akure, Nigeria, pp. 59–94.

Adedire, C. O., Lajide, L. (1999). Toxicity and oviposition deterrency of some plant extracts on cowpea storage bruchid, *Callosobruchus maculatus* Fabricius. *Journal of Plant Diseases and Protection*, 106: 647–653.

Adeyemo-Salami, O. A., Makinde, J. M. (2013). Acute and subacute toxicity studies of the methanol extract of the leaves of *Paullinia pinnata* (Linn.) in Wistar albino mice and rats. *African Journal of Medicine and Medical Sciences*, 42: 81–90.

Agbakwuru, E. O. P., Osisiogu, I. U. W., Ugochukwu, E. N. (1978). Insecticides of Nigerian vegetable origin. Some nitroalkanes as protectants of stored cowpeas and maize against insects pests. *Nigerian Journal of Science*, 12: 493–504.

Akanji, M. A., Olagoke, O. A., Oloyede, O. B. (1993). Effect of chronic consumption of metabisulphite on the integrity of rat cellular system. *Toxicology*, 81: 173–179.

Akparie, S. O. (2004). *General Veterinary Pathology*, 1st edn. Stirling-Horden Publishers (Nig.) Ltd, Oyo, Nigeria, 136 pp.

Asawalam, E. F., Emosairue, S. O., Ekeleme, F., Wokocha, R. C. (2006). Insecticidal effects of powdered parts of eight Nigerian plant species against maize weevil *Sitophilus zeamais* Mostchulsky (Coleoptera: Curculionidae). *Nigerian Agricultural Journal*, 37: 106–113.

Bamisaye, F. A., Odutuga, A. A., Minari, J. B., Dairo, J. O., Oluba, O. M., Babatola, L.J. (2013). Evaluation of hypoglycemic and toxicological effects of leaf extracts

of *Morinda lucida* in hyperglycemic albino rats. *International Research Journal of Biochemistry and Bioinformatics*, 3: 37–43.

Bruce, R. D. (1985). An up-and-down procedure for acute toxicity testing. *Fundamental Applied Toxicology*, 5: 151–157.

Chawla, R. (1999). Kidney function tests. In: *Practical Clinical Biochemistry, Methods and Interpretation*. 2nd edn. Jaypee Brothers Medical Publishers (P) Ltd., New Delhi, India. pp. 90–92.

Ejechi, B. O., Akpomedaye, D. E. (2005). Activity of essential oil and phenolic acid extracts of pepper fruit (*D. tripetala*) against some food borne micro organism. *African Journal of Biotechnology*, 4: 258–261.

Enwere, N. J. (1998). *Foods of Plant Origin*. Afro-Orbis Publications Ltd., University of Nigeria, Nsukka, pp. 169–180.

Gomall, A. J. (1949). *Biological Chemistry*. 177 C, Practical Manual, Agappe, Diagnostics, Switzerland. 751 pp.

Ikpi, D. E., Nku, O. (2008). Effect of ethanolic extract of *Dennettia tripetala* fruit on haematological parameters in albino wistar rats. *Nigerian Journal of Physiological Sciences*, 23: 13–17.

Ileke, K. D., Odeyemi, O. O., Ashamo, M. O., Oboh, G. (2014). Toxicological and histopathological effects of cheesewood, *Alstonia boonei* de wild, stem bark powder used as cowpea protectant against cowpea bruchid, *Callosobruchus maculatus* (Fab.) [Coleoptera: Chrysomelidae] on albino rats. *Ife Journal of Science*, 16: 23–33.

Iwu, M. M. (1989). *Food for Medicine in Dietary Plants and Masticators as Sources of Biologically Active Substances*. University of Ife Press, Ile-Ife, Nigeria, pp. 303–310.

Karmen, A. (1955). A note on the spectrophotometric assay of glutamic oxol-acetic transaminase in human blood. *Journal of Clinical Investment*, 34: 13–131.

Keay, R. W. J. (1989). *Trees of Nigeria*. Clarendon Press, Oxford, UK, pp. 19–30.

Lale, N. E. S. (2002). *Stored-Product Entomology and Acarology in Tropical Africa*. Mole Publications Ltd., Maiduguri, Nigeria, 204 pp.

Munyiri, S. W., Mugo, S. N., Otim, M., Mwololo, J. K., Okori, P. (2013). Mechanisms and sources of resistance in tropical maize inbred lines to *Chilo partellus* stem borers. *Journal of Agricultural Sciences*, 5: 51–60.

Odeyemi, O. O., Masika, P., Afolayan, A. J. (2008). A review of the use of phytochemicals for insect pest control. *African Plant Protection*, 14: 1–7.

Odutuga, A. A., Dairo, J. O., Minari, J. B., Bamisaye, F. A. (2010). Anti-diabetic effect of *Morinda lucida* stem bark extracts on alloxan-induced diabetic rats. *Research Journal of Pharmacology*, 4: 78–82.

Okwu, D. E., Morah, F. N. I. (2004). Mineral and nutritive value of *Dennettia tripetala* fruits. *Fruits*, 59: 437–442.

Okwu, D. E., Morah, F. N. I., Anam, E. M. (2005). Isolation and characterization of phenanthrenic alkaloid uvariopsine from *Dennettia tripetala* fruits. *Journal of Medicinal and Aromatic Plant Science,* 27: 496–498.

Oluba, O. M., Adeyemi, O., Ojeih, G. C., Adebisi, K. E., Isiosio, I. O., Aboluwoye, C. O. (2008). Effect of dietary cholesterol on some serum enzymes. *Journal of Medical Sciences*, 3: 390–394.

Ramalingam, V., Vimaladevi, V. (2002). Effect of mercuric chloride on membrane-bound enzymes in rat testis. *Asian Journal of Andrology*, 4: 309–311.

Saganuwan, A. S. (2012). *Principles of Pharmacological Calculations*. Ahmadu Bello University Press Ltd, Samaru, Zaria, Nigeria, 529 pp.

Sathya, M. K., Kokilavani, R., Anantateepa, K. S. (2012). Acute and subacute toxicity studies of ethanolic extract of *Acalypha indica* Linn in male Wistar albino rats. *Asian Journal of Pharmaceutical and Clinical Research*, 5: 97–100.

Silva, A. M. *et al.* (1999). Protective effect of bifidus milk on the experimental infection with *Salmonella enteritidis* subsp. *trphimurium* in conventional and gnotobiotic mice. *Journal of Applied Microbiology*, 86: 331–336.

Somta, C., Somta, P., Tomooka, N., Ooi, P. A. C., Vaughan, D. A., Srinives, P. (2008). Characterization of new sources of munbean (*Vigna radiate* (L.) Wilczek) resistance to bruchids, *Callosobruchus* spp. (Coleoptera: Bruchidae). *Journal of Stored Products Research*, 44: 316–321.

Treasure, J. (2003). Urtica semen reduces serum creatinine levels. *Journal of American Herbal Guild*, 4: 22–25.

Weber, D. K., Danielson, K., Wright, S., Foley, J. E. (2002). Hematology and serum biochemistry values of dusky-footed wood rat (neotoma fuscipes). *Journal of Wildlife Diseases*, 38: 576–582.

Weinzierl, R., Henn, T. (1994). Botanical insecticides and insecticidal soaps. In: Leslie, A.R. (ed.) *Handbook of Integrated Pest Management for Turf and Ornamentals*. Lewis Publisher, Boca Raton, FL, pp. 542–555.

Wroblewski, F., LaDue, J. S. (1956). Biology and medicine. *Proceedings of the Society of Experimental Biology and Medicine*, 91: 569–569.

Yakubu, M. T., Akanji, M. A., Oladiji, A. T. (2007). Haematological evaluation in male albino rats following chronic administration of aqueous extract of *Fadogia agrestis* stem. *Pharmacology Magazine*, 3: 34–38.

Yakubu, M. T., Bilbis, L. S., Lawal, M., Akanji, M. A. (2003). Evaluation of selected parameters of rat liver and kidney function following repeated administration of yohimbine. *Biochemistry*, 15: 50–56.