



Hepatoprotective and nephrotoxic effects of methanol leaf extract of *Telfairia occidentalis* (Hook f.) in adult female albino rats (*Rattus norvegicus*)

Chidozie Nwabuisi Okoye¹, John Ikechukwu Ihedioha²,
Onyinyechukwu Ada Agina², Izuchukwu Ochiogu¹, David Ogwu³

¹Department of Veterinary Obstetrics and Reproductive Diseases, University of Nigeria, Nsukka, Enugu State, Nigeria, ²Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Enugu State, Nigeria, ³Department of Veterinary Surgery and Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

Corresponding Author:

Agina Onyinyechukwu Ada, Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria. Phone: +2347039010464, E-mail: onyinye.noel@unn.edu.ng.

Received: 13 Jan 2016

Accepted: 2 Oct 2016

Published: 10 Oct 2016

Keywords:

Telfairia occidentalis,
Rattus norvegicus,
ethnomedicine,
nephrotoxicity

ABSTRACT

The leaf extract of the plant *Telfairia occidentalis*, commonly known as fluted pumpkin, is used as a hematinic by postmenstrual and pregnant women and for the treatment of many different ailments with little consideration of their toxic potentials. **Objective:** This study assessed the effects of the administration of methanol leaf extract of *T. occidentalis* on serum activity of some enzymes and levels of biochemical indices in female albino rats. **Methods:** 15 nulliparous female rats of 12 weeks of age were used for the study. Group A served as untreated control and received only 10 mg/kg distilled water, while Groups B and C received 200 and 800 mg/kg body weight of the methanol leaf extract of *T. occidentalis*, respectively, for 3 weeks. **Results:** Results of the study showed that administration of the extract to the treated groups led to significantly ($P < 0.05$) lower serum activity of alanine aminotransferase on day 14 in Group B rats: Aspartate aminotransferase on day 7 in Group C rats and alkaline phosphatase on day 7 in Group B rats. Significantly ($P < 0.05$) lower serum levels of proteins and cholesterol were recorded for Groups B and C rats, respectively, on day 14. There was significantly ($P < 0.05$) higher serum urea level in the treated groups on days 14 and 21 and significantly ($P < 0.05$) higher creatinine level on day 21. **Conclusion:** It was concluded that administration of methanol extract of *T. occidentalis* as used in the study might lead to membrane stabilizing effects on hepatocytes, depressed hepatocyte synthetic activity, and impaired renal function.

INTRODUCTION

Plants and plant products have been used variously for the treatment of many different ailments with little consideration of their toxic potentials [1]. In Nigeria, *Telfairia occidentalis*, commonly known as fluted pumpkin, is extensively used culinarily and ethnomedically. Leaf extracts of the plant are used as a hematinic by postmenstrual and pregnant women [2,3]. It is also used in the treatment of malaria and convulsion [2,4,5]. The plant leaf is high in vitamins, carotenoids, alkaloids, flavonoids, saponins, oxalates, phenols, glycosides, resins, nitrate, nitrites, and minerals inter alia [5,6]. Nonetheless, the plant leaf extract has been reported by Fasuyi and Nonyerem [7], to contain antinutritive phytochemicals such as phytins, oxalate, tannin,

and cyanide; also the plant root is poisonous [8]. Yet there is an increasing awareness and use of phytomedicine in the treatment of female infertility [9], and this is without actual knowledge of their toxic potentials [1]. Thus, the growing interest in phytomedicine demands information on the toxicity risk assessment of these herbal remedies [1].

Laboratory evaluation of organ-specific serum enzyme activities is useful in clinical diagnosis and toxicity assessment as they are indicative of pathological process or pharmacologic responses to therapeutic interventions [1,10]. The paucity of information on potential hepatotoxicity or nephrotoxicity of *T. occidentalis* (Cucurbitaceae) ethnomedical use by females makes evaluation of the functional status of these organs imperative. This is especially so as these are key organs involved in the regulation

of homeostasis through their wide range of activities which include but not limited to detoxification, biotransformation, protein synthesis, energy metabolism, immune regulation, hormone production and reproduction [11,12].

The objectives of this study were therefore to evaluate the effects of oral administration of methanol leaf extract of *T. occidentalis* on serum alanine aminotransferase (ALT), alkaline phosphatase (AP), aspartate aminotransferase (AST), total bilirubin (TB), albumin (ALB), total proteins (TP), serum urea, creatinine, and cholesterol (CHOL) of female albino (Sprague-Dawley) rats. Among these, the effects on the TB, ALB, and indices of nephrotoxicity, such as serum urea and creatinine, have not been previously reported.

MATERIALS AND METHODS

Plant Material

The plant vines were procured from Opi, Nsukka L.G.A. of Enugu State, Nigeria in June 2012 and identified by a plant taxonomist. The leaves were hand-picked off the vine and then air-dried under shade at room temperature.

Preparation of Extracts

The dried leaves were pulverized and the weight determined. Extraction from the pulverized leaves was by cold maceration method using 80% methanol as described by Okoye *et al.* [3] for 48 h. It was subjected to intermittent shaking, thereafter filtered with Whatman 1 filter paper. The filtrate was concentrated by evaporation in a hot air oven at $\leq 40^{\circ}\text{C}$ to obtain the crude extract. The percentage yield was determined, and the extract was preserved in a refrigerator (4°C) throughout the duration of the study.

Experimental Animals

Adult female Sprague-Dawley albino rats obtained from the Laboratory Animal House of the Department of Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, were used for the study. The rats were kept in groups in stainless steel cages in the Experimental Animal House Unit of the Department of Veterinary Obstetrics and Reproductive Diseases of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, under standard conditions (ambient temperature: $28-32^{\circ}\text{C}$; daylight: Approximately 12 h natural light per day; humidity: 50-60%). Commercial pelleted feed (Vital®, GCOML, Nigeria) containing 14.5% crude protein and 2,500 Kcal/kg metabolizable energy, and potable water were provided *ad-libitum*. The rats were allowed 2 weeks for acclimatization.

Ethical Approval

The housing, handling, and welfare of the rats used for the study were done humanely in accordance with the Ethics and Regulation Guiding the Use of Research Animals as approved by the University of Nigeria, Nsukka.

Acute Toxicity Study

The acute toxicity was determined according to the method of Lorke [13] for mortality and morbidity which include

signs such as anorexia, listlessness, diarrhea, vomiting, seizures, pawing, and shivering. 18 rats were used in two phases. The first phase used nine of the rats, they were divided into three groups of three each and were treated orally with 10, 100, 1000 mg/kg body of the crude extract, respectively. The second phase also used nine rats; they were also divided as above and treated orally with the crude extract at doses of 1600, 2900, and 5000 mg/kg body weight. The extract was first emulsified in Tween 20 (polysorbate 20) which is a polyoxyethylene derivative of sorbitan monolaurate (Sigma-Aldrich Co., USA). The Tween 20 used in emulsifying the extract was 5% of the entire solution thereafter distilled water was added to make up 95% of the solution.

Subacute Toxicity Study

About 15 nulliparous 12 weeks old rats weighing 160 ± 18 g were used for the study. The rats were randomly assigned into 3 groups, Groups A, B, and C of five rats per group. Group A served as untreated control and received only 10 ml/kg body weight of the distilled water, while Groups B and C received 200 and 800 mg/kg body weight of the methanol leaf extract of *T. occidentalis*, respectively, for 21 days. Oral administration of the crude extract was done 24 hourly with the aid of orogastric cannula.

Blood Sample Collection

Blood samples were collected every week. The samples were collected via the ophthalmic venous plexus through the median canthi [14]. The blood samples were dispensed into clean plain glass test tubes and allowed to stand and clot for 30 min at room temperature. Sera for the assays were thereafter harvested by centrifugation. All the serum biochemistry determinations were done immediately on separation of serum from blood clot.

Serum Biochemistry Determination

The serum biochemistry determinations were done using commercial test kits, Quimica Clinica Aplicada (QCA) test kits (QCA, Spain) and a digital colorimeter (Lab-tech, India). The serum ALT and AST activities were determined by the Reitman-Frankel method [15]. The serum AP activity was determined by the phenolphthalein monophosphate method [16,17], while the serum TB was determined by the Jendrassik-Grof method [18]. The serum TP was determined by the direct Biuret method [19] while the serum ALB was determined by the bromocresol green method [20]. The serum urea was determined by the modified Berthelot-Searcy method [21] while the serum creatinine was determined by the modified Jaffe method [22]. The serum CHOL was determined by the enzymatic colorimetric method [23].

Data Analysis

Data generated were subjected to one-way analysis of variance. Variant means were separated by the least significant difference method. A significance was accepted at probability level $P < 0.05$. Final results were presented as means \pm standard deviation.

RESULTS

Acute Toxicity

The percentage yield of the extract was 14.57% of the starting weight of the plant. Acute toxicity test did not show any mortality, morbidity or other obvious signs of discomfort at the doses used. It shows that the plant methanol extract was well tolerated at the maximum dose of 5000 mg/kg body weight.

Subacute Toxicity

The result of the subacute toxicity study showed that there were no significant ($P > 0.05$) variations in the serum ALT activity of the rat groups on days 7 and 21 of treatment, but on day 14 of treatment, the serum ALT activity of rats dosed 200 mg/kg of the crude extract was significantly ($P < 0.05$) lower than that of the control and 800 mg/kg (Table 1). On days 7, 14, and 21, the serum AST activity of rats dosed 800 mg/kg was relatively lower than those of the control and 200 mg/kg, but the difference was only significant ($P < 0.05$) on day 7 of treatment (Table 1). The serum ALP activity of rats treated with 200 mg/kg of the crude extract was significantly ($P < 0.05$) lower than those of control and 800 mg/kg on day 7, but there were no significant ($P > 0.05$) variations among the groups on days 14 and 21 of treatment (Table 1).

There were no significant variations ($P > 0.05$) in the serum TP levels of all the rat groups on day 7 and 21 of treatment, but on day 14 the serum TP levels of rats that received 200 mg/kg of the crude extract was significantly ($P < 0.05$) lower than that of control (Table 2). All through day 7, 14, and 21, there were no significant ($P > 0.05$) variations among the groups in their serum levels of ALB and bilirubin (Table 2). There were no significant ($P > 0.05$) variations among the groups in their serum CHOL levels on days 7 and 21, but on day 14, the serum CHOL levels of rats dosed 800 mg/kg was significantly ($P < 0.05$) lower than that of the control (Table 2).

The serum urea levels did not significantly ($P > 0.05$) vary among the groups on day 7, but on day 14, the serum urea

levels of rats that received 200 and 800 mg/kg of the crude extract were significantly ($P < 0.05$) higher than that of the control, while on day 21, the serum urea levels of rats that received 800 mg/kg alone were significantly ($P < 0.05$) higher than those of the control and 200 mg/kg (Table 3). The serum creatinine levels of rat that were dosed 800 mg/kg of the crude extract were significantly ($P < 0.05$) lower than those of the control and 200 mg/kg on day 7, but on day 14, there were no significant ($P > 0.05$) variation among the groups, while on day 21, the serum creatinine level of rats dosed 800 mg/kg was significantly ($P < 0.05$) higher than that of the control and 200 mg/kg rats (Table 3).

DISCUSSION

Using the method of cold 80% methanol maceration for extraction, it was expected that most of the polar constituents and some nonpolar compounds, normally present in culinary and ethnomedical uses, were extracted from the plant leaves. The results of the acute toxicity tests which showed that the rats tolerated up to 5000 mg/kg body weight without mortality or any obvious signs of toxicity is an indication that the extract is “unlikely to present acute hazard in normal use” [24].

The relatively lower serum activity of ALT, AST, and ALP recorded for the treated rat groups, which was found to be significant for one of the treated groups on day 14 (for ALT) and on day 7 (for AST and ALP) suggests that the extract has cell membrane stabilizing effect that reduced leakage of these enzymes from the cellular (hepatocytes, biliary epithelium, and skeletal myocytes) sources [25,26]. Such cell membrane stabilizing activity has been reported in rats given wood oil of *Cedrus deodara* [27] and also in rats given an ethanolic extract of African garden egg (*Solanum aethiopicum*) [28]. The findings in this study of relatively lower ALT, AST, and ALP in extract-treated rats agree with the reports of Iweala and Obidoa (2009) [29] on enzyme levels in rats given *T. occidentalis* supplemented diets, but contrasted with the reports of Ekpenyong et al. (2012) [30] who reported elevated serum AST activity in rats given aqueous extract of *T. occidentalis*.

Table 1: Serum enzyme activity (means±standard deviation) of rat groups sub chronically administered with methanol leaf extract of *Telfairia occidentalis*

Groups	Day 7	Day 14	Day 21
ALT (IU/L)			
Group A (untreated control)	33.20±5.61	30.53±1.03 ^a	38.67±4.03
Group B (treated with 200 mg/kg b.w. of extract)	30.37±4.38	24.54±2.85 ^b	35.92±3.14
Group C (treated with 800 mg/kg b.w. of extract)	36.09±6.06	28.10±1.73 ^a	37.70±3.23
AST (IU/L)			
Group A (untreated control)	76.08±4.50 ^a	62.42±9.68	66.02±6.62
Group B (treated with 200 mg/kg b.w. of extract)	68.74±4.76 ^{ab}	61.14±4.54	63.54±2.39
Group C (treated with 800 mg/kg b.w. of extract)	60.07±6.56 ^b	57.01±6.70	61.31±4.20
AP (IU/L)			
Group A (untreated control)	91.40±21.09 ^a	149.00±13.53	115.69±33.41
Group B (treated with 200 mg/kg b.w. of extract)	79.90±9.76 ^b	107.40±30.31	111.38±17.96
Group C (treated with 800 mg/kg b.w. of extract)	126.23±18.96 ^a	127.62±23.92	98.00±11.65

^{a,b}Different superscripts within a column indicate significant differences between the means ($P < 0.05$). ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, AP: Alkaline phosphatase

Table 2: Serum total protein, albumin, total bilirubin, and total cholesterol levels (means±standard deviation) of rat groups sub chronically administered with methanol leaf extract of *Telfairia occidentalis*

Groups	Day 7	Day 14	Day 21
Total protein (g/dl)			
Group A (untreated control)	6.76±0.44	7.33±0.27 ^a	7.40±0.90
Group B (treated with 200 mg/kg b.w. of extract)	6.91±0.13	6.55±0.41 ^b	7.95±0.59
Group C (treated with 800 mg/kg b.w. of extract)	7.00±0.40	6.87±0.42 ^{ab}	7.66±0.21
Albumin (g/dl)			
Group A (untreated control)	3.81±0.14	3.25±0.27	3.97±0.07
Group B (treated with 200 mg/kg b.w. of extract)	3.58±0.30	3.47±0.32	4.21±0.61
Group C (treated with 800 mg/kg b.w. of extract)	3.72±0.23	3.42±0.24	4.10±0.43
Total bilirubin (mg/dl)			
Group A (untreated control)	2.25±0.41	2.16±0.43	1.98±0.41
Group B (treated with 200 mg/kg b.w. of extract)	1.94±0.38	2.34±0.27	1.86±0.23
Group C (treated with 800 mg/kg b.w. of extract)	2.09±0.31	2.67±0.24	1.90±0.23
Total cholesterol (mg/dl)			
Group A (untreated control)	110.00±18.18	113.60±8.70 ^a	91.05±9.63
Group B (treated with 200 mg/kg b.w. of extract)	114.54±19.75	111.76±6.50 ^{ab}	92.11±17.22
Group C (treated with 800 mg/kg b.w. of extract)	102.91±16.49	92.80±10.55 ^b	91.93±31.95

^{a,b}Different superscripts within a column indicate significant differences between the means ($P<0.05$)**Table 3:** Serum urea and creatinine levels (means±standard deviation) of rat groups sub chronically administered methanol leaf extract of *Telfairia occidentalis*

Groups	Day 7	Day 14	Day 21
Urea (mg/dl)			
Group A (untreated control)	7.37±2.27	7.88±2.10 ^a	9.61±2.00 ^a
Group B (treated with 200 mg/kg b.w. of extract)	7.19±1.72	11.27±1.52 ^b	9.16±0.88 ^a
Group C (treated with 800 mg/kg b.w. of extract)	5.42±1.63	10.91±1.49 ^b	15.95±2.10 ^b
Creatinine (mg/dl)			
Group A (untreated control)	0.94±0.22 ^a	0.77±0.25	0.67±0.13 ^a
Group B (treated with 200 mg/kg b.w. of extract)	0.92±0.19 ^a	0.88±0.23	0.79±0.19 ^a
Group C (treated with 800 mg/kg b.w. of extract)	0.68±0.13 ^b	0.85±0.19	1.14±0.10 ^b

^{a,b}Different superscripts within a column indicate significant differences between the means ($P<0.05$)

The significantly lower serum protein levels recorded for Group B rats on day 14 seem to be an incidental finding, as the serum protein level of this group was highest among all other groups on day 7 and 21 of treatment. This day 14 finding in this study contrasts with earlier reports that showed that supplementation with or administration of an extract of *T. occidentalis* led to higher levels of serum proteins [29,30]. The lack of significant variations in the serum ALB and bilirubin levels indicate that administration of the extract as used in this study did not significantly affect the ALB synthetic activity of the liver and its ability to excrete bilirubin [31].

The significantly lower serum CHOL levels recorded in Group C rats on day 14 suggests that the extract may have exhibited hypocholesterolemic properties, and this is in agreement with reports by Iweala and Obidoa (2009) [29] on rats fed *T. occidentalis* supplemented diets. It further validates

the traditional and folkloric belief that consumption of the vegetable lowers blood CHOL level.

The treatment with the extract led to significantly higher serum level of urea on days 14 and 21 of treatment, and also significantly higher levels of creatinine in all the extract-treated groups on day 21, this finding suggests that treatment, as used in this study, may have led to an adverse or negative effect on renal function as urea and creatinine are known markers of renal function [31,32]. This finding is noteworthy because of the significance of kidney function in overall health; furthermore, urea accumulation is known to negatively affect fertility especially of female individuals. It is, however, thought that this finding may not directly translate to possible adverse effects following routine consumptions of the vegetable in soups which more mimics aqueous extraction as against the methanol extraction method that was used in this study, more so heat

treatment during cooking would inactivate some of the toxic phytochemicals [33].

CONCLUSION

Based on the findings of this study, it was concluded that administration of methanol leaf extract of *T. occidentalis* as used in this study led to significant lowering of serum activity of ALP, AST (on day 7 of treatment with the extract at 200 and 800 mg/kg b.w. of the extract, respectively); ALT and levels of TPs and CHOL (on day 14 of treatment at 200, 200, and 800 mg/kg b.w., respectively) and significant elevation of serum urea (on days 14 and 21) and creatinine (on day 21) at 800 mg/kg b.w. of the extract could be suggestive of enhanced hepatocyte membrane stabilization, reduced protein and CHOL synthetic ability, and possible impairment of renal function in extracted treated rat groups.

REFERENCES

- Yakubu MT, Adebayo OJ, Egwim EC, Owoyele VB. Increased liver ALP and aminotransferase activities following administration of ethanolic extract of *Khaya senegalensis* stem bark to rats. *Biokemistri* 2005;17:27-32.
- Olaniyan MF, Adeleke A. A study of the effect of pumpkin (*ugu-Telfairia occidentalis*) milk and raw egg mixture in the treatment of anaemic pregnant women in a rural area. *Afr J Tradit Comp Altern Med* 2005; 2005;2:269-73.
- Okoye CN, Ogwu D, Ihedioha JI, Ochiogu IS, Abiaezute CN, Mbegbu EC. Effects of methanolic leaf extract of *Telfairia occidentalis* (Hook f.) on the reproductive indices of female albino rats. *Sci J Vet Adv* 2015;4:25-31.
- Ehiagbonare JE. Conservation studies on *Telfairia occidentalis* Hook f. an indigenous plant used in ethnomedical treatment of anemia in Nigeria. *Afr J Agric Res* 2008;3:74-7.
- Kayode AA, Kayode OT. Some medicinal values of *Telfairia occidentalis*: A review. *Am J Biochem Mol Biol* 2010;1:30-8.
- Oboh G, Nwanna EE, Elusiyan CA. Antioxidant and antimicrobial properties of *Telfairia occidentalis* (Fluted pumpkin) leaf extracts. *J Pharmacol Toxicol* 2006;1:167-75.
- Fasuyi AO, Nonyerem AD. Biochemical, nutritional and haematological implications of *Telfairia occidentalis* leaf meal as protein supplement in broiler starter diets. *Afr J Biotechnol* 2007;6:1055-63.
- Gill LS. Ethnomedical Uses of Plants in Nigeria. Benin, Nigeria: Uniben Press, University of Benin; 1992.
- Ekere SO, Okoye CN, Udoumoh AF. Fertility enhancing effects of methanolic leaf extract of *Dracaena arborea* in albino rats (*Rattus norvegicus*). *Czech J Anim Sci* 2013;58:520-4.
- Gowda S, Desai PB, Kulkarni SS, Hull VV, Math AK, Vernekar SN. Markers of renal function tests. *N Am J Med Sci* 2010;2:170-3.
- Palanivel MG, Rajkapoor B, Kumar RS, Einstein JW, Kumar EP, Kumar MR, et al. Hepatoprotective and antioxidant effect of *Pisonia aculeate* L. against CCL4-induced hepatic damage in rats. *Sci Pharm* 2008;76:203-15.
- Yazdani F, Noori A, Amjad L. Effect of *Artemisia deserti* flowering taps extract on liver in male rats. *Int J Agric Crop Sci* 2013;5:1432-6.
- Lorke D. A new approach to practical acute toxicity testing. *Arch Toxicol* 1983;54:275-87.
- Ihedioha JI, Okafor CC, Ihedioha TE. The haematological profile of the Sprague-Dawley outbred albino rat in Nsukka, Nigeria. *Anim Res Int* 2004;1:125-32.
- Reitman S, Frankel S. A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *Am J Clin Pathol* 1957;28:56-62.
- Klein B, Read PA, Babson AL. Rapid method for the quantitative determination of serum alkaline phosphatase. *Clin Chem* 1960;6:269-75.
- Babson AL, Greeley SJ, Coleman CM, Philips GE. Phenolphthalein monophosphate as a substrate for serum alkaline phosphatase. *Clin Chem* 1966;12:482-90.
- Dumas BT, Perry BW, Sasse EA, Straumfjord JV Jr. Standardization in bilirubin assays: Evaluation of selected methods and stability of bilirubin solutions. *Clin Chem* 1973;19:984-93.
- Lubran MM. The measurement of total serum proteins by the Biuret method. *Ann Clin Lab Sci* 1978;8:106-10.
- Dumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. *Clin Chim Acta* 1971;31:87-96.
- Fawcett JK, Scott JE. A rapid and precise method for the determination of urea. *J Clin Pathol* 1960;13:156-9.
- Blass KG, Thibert RJ, Lam LK. A study of the mechanism of the Jaffé reaction. *Z Klin Chem Klin Biochem* 1974;12:336-43.
- Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. *Clin Chem* 1974;20:470-5.
- WHO. (The World Health Organisation, (WHO) Recommended Classification of Pesticides by Hazards and Guidelines for Classification. 2000-2001. Geneva: WHO; 2001.
- Kaplan LA, Szabo LL, Opherin EK. *Enzymes in Clinical Chemistry: Interpretation and Techniques*. 3rd ed. Philadelphia, PA: Lea and Febiger; 1988. p. 182-184.
- Stockham SL, Scott MA, editors. *Enzymes. Fundamentals of Veterinary Clinical Pathology*. 2nd ed. USA: Blackwell Publishing Iowa; 2008a. p. 639-674.
- Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VI, Saraf MN. Membrane stabilizing activity – A possible mechanism of action for the antiinflammatory activity of *Cedrus deodara* wood oil. *Fitoterapia* 1999;70(3):251-7.
- Anosike CA, Obidoa O, Ezeanyika LU. Membrane stabilization as a mechanism of the antiinflammatory activity of methanol extract of garden egg (*Solanum aethiopicum*). *DARU J Pharm Sci* 2012;20:76-80.
- Iweala EJ, Obidoa CO. Some biochemical, haematological and histological responses to a long term consumption of *Telfairia occidentalis* supplemented diet in rats. *Pak J Nutr* 2009;8:1199-203.
- Ekpenyong CE, Akpan EE, Udoh NS. Phytochemistry and toxicity studies of *Telfairia occidentalis* aqueous leaves extract on liver biochemical indices in wistar rats. *Am J Med Med Sci* 2012;2:103-10.
- Coles EH. *Veterinary Clinical Pathology*. Philadelphia, USA: W. B. Saunders; 1986.
- Stockham SL, Scott MA, editors. *Urinary system. Fundamentals of Veterinary Clinical Pathology*. 2nd ed. Iowa, USA: Blackwell Publishing; 2008b. p. 415-94.
- Olaniyi TO, Odedere MP. The effect of mineral Nitrogen and compost fertilizers on the growth, yield and nutritional values of fluted pumpkin (*Telfairia occidentalis*) in South Western Nigeria. *J Anim Plant Sci* 2009;5:443-9.