CHAPTER 5

Effect of administration of aqueous extract of *Hippobromus pauciflorus* leaves in male Wistar rats

CHAPTER 5

Effect of administration of aqueous extract of *Hippobromus pauciflorus* leaves in male Wistar rats

Effect of administration of aqueous extract of *Hippobromus pauciflorus* leaves in male Wistar rats

SC Pendota, MT Yakubu, DS Grierson, and AJ Afolayan*

Department of Botany, University of Fort Hare, Alice 5700, South Africa.

Abstract

The effects of the administration of aqueous extract of *Hippobromus pauciflorus* (L.f.) Radlk (Sapindaceae) leaves at 50, 100 and 200 mg/kg body weight for 14 days on some biochemical parameters of male Wistar rats were investigated. The extract at all the doses tested did not significantly alter the levels of white blood cells, red blood cells, mean corpuscular volume, platelets, neutrophils, monocytes, lymphocytes and large unstained cells. While the levels of haemoglobin, packed cell volume and basophils were increased at specific doses, those of mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and eosinophils were decreased. Again the extract did not significantly alter the computed liver and kidney body weight ratios, sodium, chloride and total protein, through the levels of potassium, inorganic phosphorus, globulin, urea, total and conjugated bilirubin were increased at certain doses. In contrast, the level of albumin and creatinine also decreased at specific doses. While the activities of alkaline phosphatase, gamma glutamyl transferase and alanine aminotransaminase remained unaltered in the serum, that of aspartate aminotransaminase increased only at 200 mg/kg body weight. The atherogenic index as well as the concentrations of cholesterol, high and low-density lipoprotein cholesterol in the serum of the animals was not altered. However, the extract increased the concentration of triacylglycerol. The results suggest that the extract has mild and dose specific haemato-, hepato- and nephrotoxic effects and thus may not be completely safe for oral administration.

Keywords: *Hippobromus pauciflorus*, haematotoxic, hepatotoxic, nephrotoxic, oral remedy

* Corresponding author: AJ Afolayan, Fax: +27 866282295; E-mail: Aafolayan@ufh.ac.za

Introduction

The use of plants for healing purpose is getting increasingly popular as it is believed that botanicals are beneficial and free of side effects (Leonardo *et al*., 2000). With the upsurge in the use of herbal medicines, thorough scientific investigations of these plants are imperative, in order to provide information on their safety or toxicity risk. One of such plants that is widely used in the Eastern Cape of South Africa is *Hippobromus pauciflorus*.

Hippobromus pauciflorus (L.f.) Radlk (Sapindaceae), locally known as Ulathile is a resinous tree that grows up to 5 m in height. It is widely distributed in riverine thickets, along stream banks and at the margins of evergreen forests of South Africa (Pendota *et al*., 2008). The leaves are simple and are arranged in alternate fashion. Several medicinal uses of the plant have been reported. For example, the leaves of *H. pauciflorus* are used by the traditional healers for the treatment of malaria, livestock diseases, dysentery, diarrhoea and conjunctivitis in the Eastern Cape, (Masika and Afolayan, 2003; Clarkson *et al*., 2004; Pendota *et al*., 2008)

To the best of our knowledge and as at the time of carrying out this study, there has not been any previous information on the toxicity of the extract of *Hippobromus pauciflorus* leaves. Therefore, the aim of this study was to investigate the toxic effects of the extract of the plant leaves using Wistar rats as model. The focus of this research was on organ-body weight ratios, haematological parameters and some serum biochemistry.

Materials and Methods

Plant material and authentication

The plant samples were collected in August, 2008 from Sikusthwana village near Alice, in the Eastern Cape. The species was authenticated by Professor DS Grierson of the Department of Botany, University of Fort Hare. A voucher specimen (SC Pendota 01/2008) was deposited at Giffen herbarium of the University.

Experimental animals

Twenty male Wistar rats each weighing between 200 and 230 g were obtained from the Animal House of the Agricultural and Rural Development Research Institute, University of Fort Hare. All the animals were housed in clean metabolic cages placed in well-ventilated house conditions (temperature 23 ± 1˚C; photoperiod: 12 h natural light and 12 h dark: humidity: 45-50%). They were also allowed free access to Balanced Trusty Chunks (Pioneer Foods [Pty] Ltd, Huguenot, South Africa) and tap water. The cleaning of the cages was done daily.

Assay kits

The assay kits for creatinine, urea, calcium, sodium, potassium, chloride, phosphorus, albumin, bilirubin, cholesterol, triacylglycerol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, alkaline phosphatase, gamma glutamyl transferase, alanine and aspartate aminotransferases were obtained from Roche Diagnostic GmbH, Mannhein, Germany. All other reagents used were of analytical grade and were supplied by Merck Chemicals (Pty) Ltd., Bellville, South Africa.

Preparation of extract

The leaves of the plant were air-dried at room temperature for 7 days. The dried material was pulverized with an electric blender. Hundred grams of the powder was extracted in 1000 ml of distilled water for 48 h on an orbital shaker (Stuart Scientific Orbital Shaker, UK). The extract was filtered using a Buchner funnel and Whatman no. 1 filter paper. The resulting filtrate was freeze-dried (Savant Refrigerated Vapour Trap, RV T41404, USA) to give a yield of 12.47 g. This was reconstituted separately in distilled water to give the required doses used in this study.

Animal grouping and administration of extract

Twenty male rats were completely randomized into four groups of five and were orally administered as follows: Group A (control) was administered with 0.5 ml of distilled water while groups B, C and D were given 50, 100 and 200 mg/kg body weight of the extract respectively. The administration was done repeatedly on daily basis for two weeks using metal oropharyngeal cannula. All the rats from each group were sacrificed 24 h after their respective 14 daily doses. The study was carried out following the approval from the Ethical Committee on Animal Use and Care of the University of Fort Hare, South Africa.

Preparation of serum

The procedure described by Yakubu *et al* (2005) was employed in the preparation of the serum. Briefly, under ether anaesthesia, rats were made to bleed through their cut jugular veins which were slightly displaced (to prevent blood contamination by interstitial fluid) into clean, dry centrifuge tubes. An aliquot (2 ml) of the blood was collected into EDTA sample bottles (BD Diagnostics, Preanalytical Systems, Midrand, USA) for the haematological analysis. Another 5 ml of the blood was allowed to clot for 10 min at room temperature and

then centrifuged at 1282 g x 5 min using Hermle Bench Top Centrifuge (Model Hermle, Z300, Hamburg, Germany). The sera were later aspirated with Pasteur pipettes into sample bottles and used within 12 h of preparation for the assay. The rats were thereafter quickly dissected in the cold; the liver and kidney were excised and transferred into ice-cold 0.25 M sucrose solution. The organs were freed of fat, blotted with clean tissue paper and then weighed.

Determination of biochemical parameters

Adopting the method of Tietz *et al* (1994), the levels of sodium, potassium, chloride, inorganic phosphorus, urea, creatinine, total and conjugated bilirubin, albumin, globulin, total protein, alkaline phosphatase, gamma glutamyl transferase, alanine and aspartate aminotransaminases, cholesterol, triacylglycerol, high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol(LDL-C) were determined in the serum using assay kits from Roche Diagnostics on Roche modular (model P800) Mannhein, Germany. The Horiba ABX 80 Diagnostics (ABX pentra Montpellier, France) was used for the determination of haematological parameters: red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), large unstained cell (LUC), red cell distribution width (RCDW), white blood cell (WBC), neutrophils, monocytes, lymphocytes, eosinophils, basophils and platelet.

Statistical analysis

The data obtained were subjected to one way Analysis of Variance (ANOVA) and means were separated by the Duncan Multiple Range Test. Percentage data were transformed to arcsine before analysis. Significant levels were tested at $P < 0.05$.

Results

The effects of the administration of the aqueous extract of *H. Pauciflorus* on the haematological parameters of rats are shown in Table 1. While there was no significant difference in the effects of all the doses on the WBC, RBC, MCV, platelets, neutrophils, monocytes, lymphocytes and LUC, there was dose and parameters specific effects on the other indices. The 100 and 200 mg/kg body weight of the extract increased the levels of Hb and PCV. In contrast, the MCH and MCHC were both decreased by the 200 mg/kg body weight of the extract. While the levels of eosinophil were decreased at 50 and 200 mg/kg body weight, all the doses investigated (50, 100 and 200 mg/kg body weigh) increased the level of basophils.

Generally the extract did not significantly alter the computed liver and kidney body weight ratios of the animals. Also it did not produce any significant effect on the levels of sodium, chloride and total protein. There was however, dose specific effect on the other function parameters of the liver and kidney. The 200 mg/kg body weight of the extract increased the concentration of potassium, inorganic phosphorus, globulin as well as total and conjugated bilirubin. In contrast, the level of albumin was decreased by the same dose. Whereas the 50 and 100 mg/kg body weight of the extract increased the serum urea concentration, the 100 and 200 mg/kg body weight decreased the levels of creatinine in the animals. While the activities of alkaline phosphatase, gamma glutamyl transferase and alanine aminotransaminase remained unaltered in the serum, that of aspartate aminotransaminase increased only at 200 mg/kg body weight (Table 2).

The administration of the extract did not produce any significant change in the atherogenic index as well as the concentrations of cholesterol, high-density lipoprotein cholesterol and

low-density lipoprotein cholesterol in the serum of the animals. However, the extract increased the concentration of serum triacylglycerol in the animals (Table 3).

Discussion

Assessment of haematological parameters in rats can be used to determine the extent of deleterious effect of a plant extract on the blood (Yakubu *et al*., 2007). While the aqueous extract of *H. Pauciflorus* had no effect on the WBC, RBC, MCV, platelet, neutrophils, monocytes, lymphocytes and LUC other parameters like Hb, PCV, MCH, MCHC, eosinophils and basophils were affected at specific doses of the extract. This is an indication of the selective effect of the extract on the blood indices. Consequently, the enhanced level of the Hb at 100 and 200 mg/kg body weight of the extract may be due to stimulatory effect on the rate of production over the rate of destruction of the blood corpuscles; this may also account for the increase in PCV of the animals. Since MCHC, MCH and MCV relate to individual red blood cells, the reduction in MCH and MCHC may adversely affect the individual red blood cells at these doses (Adebayo *et al*., 2005). The alterations in the levels of eosinophils and basophils may also suggest an effect on the immune system.

Biochemical evaluation is important because the kidney and liver toxicity has been reported following the use of phytotherapeutic products (Isnard *et al*., 2004; Saad *et al*., 2006). The biochemical indices monitored in the serum such as the electrolytes and other secretory substances of the liver and kidney can be used as 'markers' for assessing the functional capacities of the organs (Yakubu *et al*., 2003). The absence of significant effect on the liver and kidney body weight ratios following the administration of the extracts suggests that the extract did not cause swelling, atrophy or hypertrophy of the organs (Amresh *et al*., 2008).

Albumin, total bilirubin and globulin are mixtures of protein molecules that can be used to measure the integrity of glomeruli and regulation of osmotic pressure (Ganong, 2001). The reduction in the level of serum albumin at 200 mg/kg body weight of the extract may be an indication of diminished synthetic function of the liver, resulting from hepatocellular damage (Woodman, 1996). Bilirubin is an important metabolic product of blood with biological and diagnostic values. The increase in globulin, total and conjugated bilirubin at 200 mg/kg body weight may be an indication of impairment in the liver function capacity (Moudgil and Narang, 1989).

Determination of urea, creatinine and electrolyte concentration in the serum can be used to assess the functional capacity of the kidney. The absence of significant effect of the extract on the serum concentrations of sodium and chloride of the animals suggest that the normal functioning of the organ in relation to these electrolytes were unaffected. However, the increase in the levels of potassium and inorganic phosphorus at 200 mg/kg body weight indicate dose specific effect of the extract. Creatinine, synthesized in the liver, passes into the circulation where it is taken up almost entirely by the skeletal muscles. Its retention in the blood is an evidence of kidney impairment (Wurochekke *et al*., 2008). Therefore, the reduced levels of creatinine in the serum may imply that the extract has interfered with creatinine metabolism. Urea is the main product of protein catabolism. The increase in serum urea level may suggest impairment in the kidney function.

There are many enzymes found in the serum that did not originate from the serum. During tissue damage, some of these enzymes find their way into the serum, probably by leakage (Wills, 1985). Serum enzyme measurements are therefore a valuable tool in clinical diagnosis, providing information on the effect and nature of pathological damage to any tissue. Therefore, the lack of effect on the alkaline phosphatase and gamma glutamyl transferase in the serum suggests that the extract did not cause damage to the plasma

membrane. However, the alterations in the aspartate aminotransaminase at 200 mg/kg body weight suggest dose specific effect of the extract.

Alterations in the concentration of major lipids like cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglycerides can give useful information on the lipid metabolism as well as predisposition of the animals to atherosclerosis and its associated coronary heart diseases (Yakubu *et al*., (2008). Elevated levels of all lipids except the HDL-C are associated with increased risk of atherosclerosis. The lack of effect by the extract on all the serum lipid parameters investigated in this study except triacylglycerol suggests selective effect on the lipid metabolism of the animals. The increase in the serum triacylglycerol concentration might be due to accelerated lipolysis. This may imply depletion in the store of fatty acids (Yakubu *et* al., 2008).

In conclusion the extract from the leaves of *H. pauciflorus* has brought about selective alterations in the biochemical parameters of male Wistar rats investigated. It is likely that the extract has mild and dose specific haemato-, hepato- and nephrotoxic effects and thus may not be completely safe for oral administration in animals.

Acknowledgement: The authors are grateful to the National Research Foundation of South Africa for supporting this work.

Table 1: Effect of the aqueous extract of *H. pauciflorus* on haematological parameters of rats n=5; mean ± S.D.

	Doses (mg/kg body weight)			
Haematological parameters	Control	50	100	200
WBC ($x10^9 L^{-1}$)	$14.86±1.97^a$	$10.90±2.98^a$	$14.25±3.39^a$	$12.31±1.58^a$
RBC ($x10^{12} L^{-1}$)	$8.66±0.27^a$	$8.46±0.53^a$	$9.25±0.52^a$	$9.24±0.73^a$
Hb ($g Dl^{-1}$)	$15.46±0.35^b$	$15.32±0.37^b$	$16.48±0.68^a$	$15.94±0.46^{ba}$
PCV ($1L^{-1}$)	$0.49±0.02^{ba}$	$0.50±0.01^{ba}$	$0.52±0.01^{aa}$	$0.59±0.12^a$
MCV (fl)	$58.91±1.61^a$	$57.16±2.44^a$	$59.28±2.47^a$	$59.70±1.95^a$
MCH (pg)	$18.26±0.43^{ba}$	$18.08±0.75^{ba}$	$17.84±0.72^{ba}$	$16.54±1.95^b$
MCHC ($g dl^{-1}$)	$31.46±0.47^a$	$30.50±0.32^{ba}$	$31.20±0.53^{ba}$	$27.96± 0.90^b$
Platelet ($x10^9 L^{-1}$)	$896.20± 9.42^a$	$877.4± 8.00^a$	$1090.8±13.95^a$	$1040.8±15.14^a$
Neutrophils (%)	$8.76±0.10^a$	$6.90±0.07^a$	$5.24±0.91^a$	$5.34±0.02^a$
Monocytes (%)	$29.94±2.47^a$	$33.26±1.01^a$	$34.98±2.48^a$	$37.70±1.15^a$
Lymphocytes (%)	$54.46±2.53^a$	$44.18±1.80^a$	$52.28±2.19^a$	$51.06±1.81^a$
LUC (%)	$8.20±0.91^a$	$8.90±0.42^a$	$8.54±0.72^a$	$8.64±0.55^a$
Eosinophils (%)	$2.40±0.18^a$	$1.80±0.18^b$	$2.22±1.32^a$	$0.74±0.31^b$
Basophils (%)	$0.46±0.08^b$	$0.56±0.08^a$	$0.64±0.11^b$	$0.92±0.53^a$

Means with the same superscripts as control across the rows are not significantly different ($p>0.05$).

WBC: White blood cell, RBC: Red blood cell, PCV: Packed cell volume, Hb: Haemoglobin, MCV: Mean corpuscular volume, MCHC: Mean corpuscular haemoglobin concentration, and LUC: Large unstained cell.

Table 2: Effect of the aqueous extract of *H. pauciflorus* on liver and kidney function indices of Wistar rats n=5; mean ± S.D.

	Doses (mg/kg body weight)			
Parameters	Control	50	100	200
Liver body weight ratio (%)	4.20±0.13^{a}	4.32±0.86^{a}	4.20±0.11^{a}	4.19±0.36^{a}
Total protein (g L^{-1})	68.00±1.22^{a}	69.40±4.50^{a}	70.60±2.19^{a}	69.80±1.64^{a}
Albumin (mmol L^{-1})	17.80±0.44^{ba}	18.00±1.00^{a}	18.80±1.09^{a}	15.40±1.51^{b}
Globulin (mmol L^{-1})	50.60±1.34^{b}	51.40±3.50^{ba}	51.80±3.03^{ba}	54.40±0.89^{a}
Total bilirubin (mmol L^{-1})	7.40±0.54^{b}	9.80±3.03^{b}	8.40±1.51^{b}	15.40±4.72^{a}
Conjugated bilirubin (umol L^{-1})	2.40±0.54^{b}	3.40±0.54^{b}	3.00±0.02^{b}	6.40±0.88^{b}
Kidney body weight ratio (%)	0.78±0.12^{a}	0.80±0.11^{a}	0.76±0.04^{a}	0.79±0.06^{a}
Sodium (mmol L^{-1})	140.20±1.30^{a}	141.00±1.87^{a}	141.80±0.44^{a}	141.00±1.00^{a}
Potassium (mmol L^{-1})	5.52±0.25^{bc}	5.20±0.18^{c}	5.78±0.32^{ba}	6.18±0.57^{a}
Chloride (mmol L^{-1})	103.80±1.30^{a}	105.40±1.67^{a}	104.00±1.87^{a}	103.40±0.54^{a}
Inorganic phosphorus (mmol L^{-1})	3.06±0.11^{b}	2.86±0.21^{bc}	3.14±0.08^{ba}	3.38±0.21^{a}
Urea (mmol L^{-1})	6.96±0.13^{ba}	8.70±1.39^{bc}	8.50±1.23^{ba}	6.22±0.08^{a}
Creatinine (mmol L^{-1})	44.00±2.34^{a}	44.80±4.76^{a}	34.40±7.70^{b}	37.60±7.23^{ba}
Alkaline phosphatase (U/L)	328.40±12.45^{a}	345.40±7.49^{a}	349.40±6.43^{a}	343.40±4.72^{a}
Gamma glutamyl transferase (U/L)	5.00±0.00^{a}	5.80±1.30^{a}	5.60±1.34^{a}	5.00±0.00^{a}
Alanine aminotransaminase (U/L)	54.80±5.97^{ba}	52.40±3.71^{b}	56.60±8.32^{ba}	53.20±4.00^{a}

Aspartate aminotransaminase (U/L)	209.80±9.71[ba]	200.40±8.39[b]	193.4±14.56[b]	248.00±8.68[a]

Means with the same superscripts as control across the rows are not significantly different (p>0.05)

Table 3: Effect of the aqueous extract of *H. pauciflorus* on serum lipid profile of male rats n=5; mean ± S.D.

	Doses (mg/kg body weight)			
Parameters	Control	50	100	200
Cholesterol (mmol/L)	1.48±0.08[a]	1.44±0.03[a]	1.45±0.01[a]	1.44±0.06[a]
Triacylglycerol (mmol/L)	0.88±0.10[a]	1.16±0.03[b]	1.82±0.09[c]	0.94±0.04[d]
High density lipoprotein cholesterol (mmol/L)	1.14±0.06[a]	1.06±0.05[a]	1.11±0.03[a]	1.08±0.02[a]
Low density lipoprotein cholesterol (mmol/L)	0.71±0.01[a]	0.69±0.03[a]	0.70±0.02[a]	0.71±0.02[a]
Atherogenic index (LDL-C/HDL-C)	0.62	0.65	0.63	0.66

[a-d]Test values for each parameter are significantly different (P<0.05)

References:

1- Adebayo, O.J., Adesokan, A.A., Olatunji, L.A., Buoro, D.O. and Soladoye, A.O. (2005). Effect of ethanolic extract of *Bougainvillea spectabilis* leaves on haematological and serum lipid variables in rats. Biokemistri; 17: 45-50.

2- Amresh, G.R., Singh, P.N. and Rao C.V. (2008). Toxicological screening of traditional medicine Laghupatha (Cissampelos pareira) in experimental animals. Ethanopharmacolo; 116: 454-460.

3- Bent, S., Ko, R. (2004). Commonly used herbal medicines in the United States: a review. The Americ J Medici; 116: 478–485.

4- Clarkson, C., Vinesh, J.M., Neil, R.C., Olwen, M.G., Pamisha, P., Motlalepula, G.M., Niresh, B., Peter, J.S., Peter, I.F. (2004) *In vitro* antiplasmodial activity of medicinal plants native to ornaturalised in South Africa. J Ethnopharmacol ; 92: 177–191.

5- Eisenberg, D.M., Davis, R.B., and Ettener, S.L. (2008). Trends in alternative medicine use in the United States, 1990–1997: results of a follow-up national survey. The J Americ Medic Associa 1998; 280: 1569–1575.

6- Ganong, W.F. (2001) Review of Medical Physiology. 20th Edition. Lange Medical Books/McGraw-Hill Medical Publishing Division, London; 414-417.

7- Gesler, W.M. (1992) Therapeutic landscapes: medical issues in light of the new cultural geography. Soci Scien and Medici; 34: 735–746.

8- Isnard BC, Deray G, Baumelou A, Le Quintree M, Vanherweghem JL. Herbs and the kidney. Americ J Kidn Disea 2004; 44: 1–11.

9- Leonardo, D.C.L., Franco, A., Gustavo, A.T.L., Luciano, M.A., Lius, F.M.E.S., Gabriele, P.D.S., Isabela, D.M.A., Jose, F.N.N., Israel, F., and Karla, K. (2000). Toxicological evaluation by in vitro and in vivo assays of an aqueous extract prepared from *Echinodorus macrophyllus* leaves. Toxicology Letters; 116: 189–198.

10- Masika, P.J., and Afolayan, A.J. (2003) An Ethnobotanical Study of Plants Used for the treatment of Livestock Diseases in the Eastern Cape Province, South Africa. Pharm Biol; 41: 16–21.

11- Moudgil, K.D. and Narang, B.S. (1989). The liver and the Biliary System. In: Textbook of Biochemistry and Human Biology, 2nd Edn., Talwar, G.P., L.M. Srivastava and K.D. Moudgil (Eds.). Prentice- Hall of India Limited, New Delhi, India; 271-273.

12- Pendota, S.C., Grierson, D.S. and Afolayan, A.J. (2008) An ethanobotanical study of plants used for the treatment of eye infections in the Eastern Cape Province, South Africa. Pak. J. Biolo scien 2008; 11: 2051-2053.

13- Saad, B., Azaizeh, H., Abu-Hijleh, G., Said, S. (2006) Safety of traditional Arab herbal medicine. Evidence-based Complemen and Alterna Medici; 3: 433–439.

14- Talalay, P. and Talalay, P. (2001) The importance of using scientific principles in the development of medicinal agents from plants. Acade Medici; 76: 238–247

15- Tietz, N., Prude, W.E.L. and Sirgard-Anderson, O. (1994). In: T*ietz Textbook of Clinical Chemistry.* ed. Burtis C. A. and Ashwood, E. R; 1354 – 1374. W. B. Saunders Company, London.

16- Wills, D.E. (1985). Biochemical Basis of Medicine. 3rd Edn., John Wright and sons Ltd., Bristol, England; 267-268.

17- Woodman, D.D. (1996). Assessment of hepatotoxicity. In: Evans, G.O. (Ed.), Animal Clinical Chemistry, A Primer for Toxicologists. Taylor & Francis, London; 71–86.

18- Wurochekke, A.U., Anthony, A.E. and Obidah, W. (2008) Biochemical effects on the liver and kidney of rats administered aqueous stem bark extract of *Xemenia Americana.* Afric J Biotechnolo; 7: 2777-2780.

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

20- Yakubu, M.T., Akanji, M.A. and Oladiji, A.T. 2005. Aphrodisiac potentials of aqueous extract of *Fadogia agrestis* (Schweinf. Ex Heim) stem in male albino rats. Asian J. Androl; 7: 399-404.

21- Yakubu, M.T., Akanji, M.A. and Oladiji, A.T. (2007). Haematological evaluation in male albino rats following chronic administration of aqueous extract of *Fadogia agrestis* stem. Pharmacog. Mag; 3: 34-38.

22- Yakubu, M.T., Akanji, M.A. and Oladiji, A.T. (2008). Alterations in serum lipid profile of male rats by oral administration of aqueous extract of *Fadogia agrestis* stem. Research J. Medicinal plant; 2: 66-73.