Available online on www.ijtpr.com
International Journal of Toxicological and Pharmacological Research 2014; 6(4): 102-106

ISSN: 0975-5160

Research Article

Hypolipidemic and Hematological Effects of Hydromethanolic Extract of the Leaves of *Bridelia micrantha* on Alloxan-Induced Diabetic Rats

Omeh Y.N[1], *Ezeja M.I[2], Onoja S.O[2], Ukattah E.C[1].

[1]*Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, P.M.B 7267 Umuahia Abia State, Nigeria.*
[2]*Department of Veterinary Physiology, Pharmacology and Biochemistry, Michael Okpara University of Agriculture, Umudike, P.M.B 7267 Umuahia Abia State, Nigeria.*

Available Online: Galley proof

ABSTRACT
This study evaluated the hypolipidemic and hematological effects of hydromethanolic extract of the leaves of *Bridelia micrantha* on alloxan-induced diabetic rats. Diabetes was induced in rats by single intraperitoneal administration of 160 mg/kg of alloxan monohydrate. Three doses (125, 250 and 500 mg/kg) of *Bridelia micrantha* extract (BME) were used for the study and the effects compared with a reference drug (glibenclamide, 2mg/kg) and distilled water. The extract and drug were administered to the rats orally for 14 days using gastric gavage. On day 14, blood was collected from the media canthus of the eye of the rats for lipid profile analysis and hematology. Also the rats were weighed on days 0, 7 and 14. *Bridelia micrantha* extract just like the reference drug (glibenclamide 2 mg/kg) caused various levels of significant ($P<0.05$) reduction of the total cholesterol, triglycerides and low density lipoproteins (LDL) and increased the level of high density lipoprotein (HDL) at 125 mg/kg when compared to control group. Very low density lipoprotein (VLDL) was only reduced at 125 mg/kg of BME. The extract also caused non-significant increases in the values of RBC count, PCV and hemoglobin at 125 and 250 mg/kg. The body weights of the distilled water treated rats were decreased on days 7 and 14 while that of the extract and glibenclamide treated rats were significantly ($P<0.05$) increased on days 7 and 14. In conclusion, *Bridelia micrantha* has demonstrated significant hypolipidemic activity and beneficial effect on the hematological parameters of alloxan-induced diabetic rats.

Key words: *Bridelia micrantha*; Hypolipidemic; Glibenclamide; Hematology

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease which has now become an epidemic with a worldwide incidence of about 5% and now kills more than AIDS [1]. It is characterized by a relative or absolute insufficiency of insulin secretion and disturbances in carbohydrate, protein and lipid metabolism [2]. According to international diabetes federation, the number of individuals with diabetes will increase from 240 million in 2007 to about 380 million in 2025 with about 80% of the disease burden occurring in low and middle income countries [3]. Diabetes is associated with a greater risk of mortality from cardiovascular disease (CVD) through artherogenic dyslipidemia characterized by increase in total cholesterol, triglycerides, low density lipoproteins (LDL), very low density lipoproteins (VLDL) and decrease in high density lipoproteins (HDL) particles [4,5]. The prevalence of dyslipidemia in diabetes mellitus is about 95% [6].

Several hematologic abnormalities have been defined in patients with diabetes mellitus [7]. Also it has been suggested that anemia occurrence in diabetes mellitus is due to increased non-enzymatic glycosylation of red blood cells (RBC) membrane proteins which correlates with hyperglycemia[8].

The goal of all treatment strategies for diabetes mellitus is to lower blood glucose concentrations to levels that approximate those representing normal range emphasizing cardiovascular risk reduction with particular reference to hypertension control and correction of dyslipidemia [9].

Several medicinal plants are used as traditional remedies for the treatment of diabetes due to their effectiveness, less side effects and relatively low cost [10]. *Bridelia micrantha* is one of such plants used in Nigerian folkloric medicine for the management of diabetes mellitus.

Bridelia micrantha belongs to the family Euphorbiaceae. It is commonly called coastal Golden-leaf in English and Ogaofia (boss of the bush) in Igbo language (Nigeria) and the plant is native to Africa. It is a medium to tall tree (up to 20 m), with a dense widely spread crown. The leaves are large, alternate and simple. The tree may be deciduous or evergreen [11,12]. It is found in forests, by rivers, forest edges or open woodlands, savannah and riverine woodland [13].

Herbalists of western Nigeria use the bark for induction of labour in full-term pregnancy and the leaf decoction for management of diabetes, while in Ghana and Ivory Coast, the leaf decoction is given to expel guinea worm and as purgative respectively [13]. In senegal, it is administered in

*Author for correspondence

Table 1: Effect of methanolic extract of *Bridelia micrantha* on the lipid profile of alloxan induced diabetic rats

Treatment	Total Chol. (mg/dl)	Trig (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
D. water (10ml/kg)	60.26±3.14	46.76 ±3.40	11.93 ± 0.26	48.99 ± 3.11	9.35 ± 0.68
Glibenclamide (2mg/kg)	52.59 ±0.64	47.13 ±0.76	42.03±3.33**	21.13 ± 3.71*	9.43 ± 0.15
BME (125mg/kg)	46.24 ±2.94*	19.28 ±3.99*	34.10 ±4.78**	28.29 ± 2.73*	3.86 ± 0.80
BME (250mg/kg)	49.57 ±9.11	38.42±16.35*	10.72 ±4.86	11.16±5.81**	27.68±3.27*
BME (500mg/kg)	55.89±4.48	41.19±15.34	12.87±1.81	24.19±5.60*	28.23±2.49*

**P< 0.05; ** P < 0.01 when compared to negative control (distilled water)*

Table 2: Effect of BME on some hematological indices of alloxan-induced diabetic rats

Treatment	RBC ($x10^{6}$)	PCV (%)	HB (g/dl)	MCV (femtoliter)	MCHC (picogram)
D. water 10 ml/kg	5.79 ± 0.50	35.50 ± 4.16	11.40 ± 0.99	60.75 ± 1.92	32.54 ± 0.99
GlIbenclamide (2 mg/kg)	6.13 ± 0.25	35.00 ± 2.27	12.07 ± 0.50	57.21 ± 3.32	34.92 ± 2.12
BME (125 mg/kg)	6.50 ± 0.13	37.50 ± 2.22	12.80 ± 0.26	57.86 ± 3.79	34.82 ± 2.42
BME (250 mg/kg)	6.00 ± 0.44	36.25 ± 2.92	11.83 ± 0.87	60.34 ± 1.50	32.74 ± 0.83
BME (500 mg/kg)	5.33 ± 0.32	35.67 ± 2.36	10.50 ± 0.63	67.91 ± 6.40	29.99 ± 2.64

No significant difference (P> 0.05) between extract and distilled water treated rats.

various preparations for the treatment of stomach and intestinal problems, sterility and edema. Lin *et al.* [14] evaluated the bark for antidiarrheal properties. The leaf extract has also been reported to have antimicrobial and antiulcer [15], hypoglycemic and antioxidant activities [13].

In this study we evaluated the effects of the plant on lipid profile and some hematological indices of alloxan-induced diabetic rats.

MATERIALS AND METHODS

Plant material: The leaves of *Bridelia micrantha* was collected in June, 2013 from Enugu-Ezike, Igbo-Eze North L.G.A. Enugu state and identified as *Bridelia micrantha* by Mr A. O. Ozioko, a taxonomist with Bioresources Development and Conservation Programme (BDCP), Enugu state, Nigeria. A voucher specimen catalogued MOUAU/CVM/VPP/2013/23 was deposited in the department of Veterinary Physiology, Pharmacology, Biochemistry and Animal Health and Production herbarium for reference purposes.

Preparation of the plant extract: Dried and pulverized leaves of *B. micrantha* were extracted by cold maceration method for 48 hours at room temperature using 80% aqueous methanol in a Winchester bottle. The extract was filtered with Whatmann No 1 filter papers. The filtrate was concentrated to dryness in a hot air oven at 40^0C to give a yield of 12.52% w/w. The extract was stored in a refrigerator at 4^0C as *Bridelia micrantha* extract (BME) until time of use.

Experimental animals: Forty male Albino Wistar rats (110-160 g) obtained from the laboratory animal unit of the Department of Veterinary Physiology, Pharmacology Biochemistry and Animal Health and Production, Michael Okpara University of Agriculture, Umudike, Abia State were used for this study. They were housed in wire mesh cages and were fed *ad libitum* with standard commercial pelleted feed (Vital feed®, Nigeria) with free access to clean drinking water. They were kept at normal environmental temperature and natural light/darkness daily cycle. They were maintained in accordance with the recommendation of the *Guide for the care and use of laboratory animals* (DHHS, 1985). They were allowed two weeks to acclimatize before the commencement of the experiment.

Induction of experimental diabetes mellitus: The rats were fasted overnight, weighed using a weighing balance and the fasting blood glucose (normal FBS) level was determined using (ACCU-Check Active®) glucometer test kit with blood collected via a snip on the tail vein. Diabetes was then induced with alloxan monohydrate at the dose of 160 mg/kg body weight injected intraperitoneally. The diabetic state was checked in treated rats by measuring their FBS levels every other day. Diabetes was established on day seven (7) post treatment. Rats with fasting blood glucose levels greater than 7 mMol/L (126 mg/dl) were considered diabetic and used for the study [16].

Experimental procedure: Forty male hyperglycemic rats were randomly divided into five groups (I-V) of eight animals each. The animals were fasted overnight but were allowed free access to water. They were treated as follows: Group I (negative control) received distilled water (10 ml/kg); Group II rats (positive control) received glibenclamide (2 mg/kg) while groups III-V were given graded doses (125, 250 and 500 mg/kg) of BME, respectively. The extract and drug were given orally daily for fourteen (14) days with gastric gavage. At the end of the 14^{th} day, blood was collected from the rats through the media canthus for serum lipid profile analysis and hematology.

Serum lipid profile analysis: Total cholesterol was evaluated using enzymatic colorimetric chod-pap test method[17]; Triglycerides was also determined spectrophotometrically using the method of Tietz [18]; High density lipoproteins (HDL) was evaluated by the method of Grove [19]; Low density lipoprotein (LDL) was determined as the difference between total cholesterol and cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (PVS) in the presence of polyethylene-glycol monomethyl ether [20] and Very low density cholesterol (VLDL) was calculated

Table 3. Effects of BME on the body weights of alloxan-induced hyperglycemic rats

Treatment	Weight (g) ± SEM Diabetic day zero	Day 7	Day 14	% weight gain on day 14
D. Water (10 ml/kg)	111.20 ± 8.85	104.60 ± 8.66	106.50 ± 8.42	-
Glibenclamide (2 mg/kg)	97.00 ± 13.50	126.00 ± 7.00*	151.00 ± 11.55*	35.8
BME (125 mg/kg)	145.20 ± 14.66	155.00 ± 12.41*	158.00 ± 13.5*	8.2
BME (250 mg/kg)	149.60 ± 20.49	159.20 ±19.76*	168.25 ± 14.71*	11.1
BME (500 mg/kg)	116.80 ± 7.14	121.20 ±10.98*	136.75 ± 9.10*	14.6

**P < 0.05 when compared to diabetic day zero*

according to the method of Wilson *et al* [21].

Hematological study: Packed cell volume (PCV) was estimated using standard technique as described by Coles [22]; Red blood cell (RBC) count was determined using Haemactocrit method; Hemoglobin (Hb) concentration in the blood was determined by Cyanomethaemoglobin method while Mean Corpuscular Volume (MCV) and Mean corpuscular Hemoglobin concentration (MCHC) were also determined using standard methods [23].

Data analysis: Data obtained were analyzed using one-way analysis of variance (ANOVA) and the variant means were separated by least significant difference (LSD) of the different groups. Significance was accepted at the level of $p < 0.05$

RESULTS

Extraction: The yield of the extract was 8.2% w/w dry matter

Effect of *Bridelia micrantha* extract on the lipid profile of alloxan-induced diabetic rats: The result of the effect of the methanolic leaf extract of *Bridelia micrantha* on the lipid profile of alloxan-induced hyperglycemia in rats is presented in Table 1. The result showed that BME caused various levels of significant (*P<0.05*) reduction of the total cholesterol, triglycerides and low density lipoproteins. The reduction was not dose dependent and the optimum activity was observed at 125 mg/kg of the extract. The high density lipoprotein (HDL) was significantly (*P<0.01*) increased by the reference drug (glibenclamide 2 mg/kg) and the extract at 125 mg/kg when compared to the negative control while there was no significant difference between the HDL of the 250 and 500 mg/kg treated rats and the control group. Also, only the 125 mg/kg of BME caused the reduction of very low density lipoprotein (VLDL) while at the doses of 250 and 500 mg/kg the VLDL was increased when compared to negative control.

Effect of *B. micrantha* on the hematological indices of alloxan-induced diabetes in rats: The result of the effect of *Bridelia micrantha* extract on the hematological indices of alloxan-induced hyperglycemia in rats is presented in Table 2. The result showed that the extract caused some marginal increases in the values of RBC count, PCV and hemoglobin at the doses of 125 and 250 mg/kg though the increases were not statistically significant (*P >0.05*). The best effect was observed at the dose of 125 mg/kg of BME. Also there were no significant variations in the MCV and MCHC of both the extract treated and control rats.

Effect of *Bridelia micrantha* extract on body weights of of alloxan-induced diabetes in rats: The result of the effect of BME on the body weights of alloxan-induced hyperglycemic rats is presented in Table 3. From the result, the mean body weights of the distilled water treated rats were decreased on days 7 and 14 while that of the extract and glibenclamide treated rats were significantly (*P <0.05*) increased on days 7 and 14 with the extract causing 8.2%, 11.1% and 14.2% increases in weight gain at the doses of 125, 250 and 500 mg/kg BME, respectively as against 35.8% by glibenclamide (2 mg/kg) on day 14.

DISCUSSION

This study evaluated the effect of *Bridelia micrantha* extract on the serum lipid profile and hematological indices of alloxan-induced hypoglycemia in rats. Alloxan monohydrate is one of the chemicals used to induce experimental diabetes in animals and it has a destructive effect on the β-cells of the pancreas causing reduction in insulin production [24].

This results in decrease in endogenous insulin secretion which paves way for the decreased utilization of glucose by body tissues and consequently elevation of blood glucose level, decreased protein content, increased levels of cholesterol and triglycerides [25]. Also in severe deficiency in insulin there is accelerated lipolysis leading to dyslipidemia [26]. Diabetes-induced hyperlipidemia is attributed to excess mobilization of fat from the adipose tissue due to underutilization of glucose [27].

Lipid abnormalities are a common feature in diabetes mellitus and dyslipidemia make diabetics prone to coronary heart diseases (CHD) and other complications of artherosclerosis [28]. In diabetes there are always elevations of total cholesterol, triglycerides, low density lipoprotein (LDL), very low density lipoproteins (VLDL) and decreases in high density lipoproteins (HDL) [29] as observed in the negative control group in this study.

Administration of BME especially at 125 mg/kg caused significant reduction in the elevated serum levels of total cholesterol, triglycerides, LDL and VLDL and increased the level of HDL of treated rats when compared to negative control group of rats (Table 1). This indicates the protective potential of the extract against diabetic hyperlipidemia which may be beneficial in preventing diabetic complications.

The extract may have achieved this by blocking cholesterol biosynthesis [30]; binding and reducing the production of bile acids or by increased generation of propionate which has been shown to reduce cholesterol levels [31]. The reduction of the hyperlipidemia by BME also may have been presumably mediated by a control of lipid metabolism [32].

Preliminary phytochemical screening of the plant revealed the presence of flavonoids, alkaloids saponins and tannins

[33]. Many nutritional factors such as saponins and tannins have been reported to contribute to the ability of herbs to improve dyslipidemia [34]. The lipid lowering activity of BME may also be attributed to its phytochemical constituents.

These findings suggest that BME may be effective in ameliorating cardiac disease complications seen in diabetes mellitus

Anemia is a condition indicated by decreased hematological indices (RBC count Hb and PCV) [35]. Also MCV and MCHC are helpful in classifying certain anaemias [36].

It has been suggested that anemia occurrence in diabetes mellitus is due to the increased non-enzymatic glycosylation of RBC membrane proteins which correlates with hyperglyceamia [37]. Also during diabetes the excess glucose present in the blood reacts with hemoglobin to form glycated hemoglobin, so the total hemoglobin level is decreased in alloxan-induced diabetic rats [38].

Following treatment of rats with BME for two weeks, the levels of RBC, HB, PCV and the related hematological indices were appreciably improved especially at 125 mg/kg. This may be due to lowered peroxide levels leading to decreased susceptibility of RBC to hemolysis [39]. This is also an indication that the extract may contain some phytochemicals that can stimulate the formation of erythropoietin which is a glycoprotein hormone that stimulates stem cells in the bone marrow to produce red blood cells [40].

Weight loss is characteristic of poor glycemic control in diabetes mellitus and weight measurement is a valuable tool used in diabetes study to monitor severity and/or response to treatment plan [41]. The observed weight decrease in the untreated rats in this study agrees with earlier reports [42]. Treatment of rats with BME for 2 weeks showed signs of recovery judging from the gain in weight of the treated rats when compared to the untreated control rats.

In conclusion, BME has demonstrated significant hypolilidemic activity and improved hematological indices of alloxan-induced diabetic rats in this study. However, more work is required to isolate the active compound(s) responsible for these activities and to determine the exact mechanism of action.

CONFLICT OF INTEREST STATEMENT

The authors declare that we have no conflict of interests whatsoever.

REFERENCES

1. Jerald EE, Joshi SB, Jain DC. Antidiabetic activity of flower buds of *Michehia champaca* (Linn). *Indian J Pharmacol* 2008; 64: 510-571.
2. Rakesh B, Sanjay J, Deep O, Amit J, Girraj ST, Ravi G. Antidiabetic activity of aqueous root extract of *Ichnocarpus fruitescens* in STZ and Nicotinamide-induced Type II diabetes in rats. *Indian Journal of Pharmacology* 2010; 40(1): 19-22.
3. Julianma CN, Chan VM, Weiping J, Takashi K, Chitteranjan SY,Frank BU et al. Diabetes in Asia, epidemiology, risk factors and pathophysiology. *JAMA* 2009; 301(20): 2129-2140.
4. Miller M. The epidemiology of triglycerides as a coronary artery disease factor. *Clin Cardiol* 1999; 22: 111-116.
5. Roberto T, Dodesini AR, Lopere G. Lipid and renal disease. *J Am Soc Nephrol* 2006; 17: Si45-147.
6. Krishna P, Roopakala T, Prasanna KM. Dyslipidemia in type diabetes mellitus in the young. *Int J Diab Dev Ctries* 2005; 25(4): 110-112.
7. Jones RL, Peterson CM. Hematological alterations in diabetes mellitus. *AM J Med* 1981; 70(2): 339-352.
8. Kennedy L, Baynes JW. Non-enzymatic glycosylation and the chronic complication of diabetes: an overview. *Diabetologia* 1984; 24: 93-98.
9. Holman RR, Paul SK, Bethel MA, Mathews DR, Neil, HA. 10 year follow-up of intensive glucose control in Type 2 diabetes. *New England Journal of Medicine* 2008; 359: 1577-1589.
10. Nanu R, Chitme RI, Chanda R. Antidiabetic activity of *Nyctanthes arbortriris. Pharmacogn Mag* 2008; 4: 16-21.
11. Kathriarachi H, Hoffmann P, Samuel R,Wurdack KJ, Chase MW. Molecular phylogenetics of phyllanthaceae inferred from five genes (plastid atpB, matK, 3'ndhF, rbcL, and nuclear PHYC). *Molecular Phylogenetics and Evolution* 2005; 36: 112–134.
12. Ngueyem TA, Brusotti G, Caccialanza G, Vita Finzi P. The genus Bridelia: a phytochemical and ethnopharmacological review. *Journal of Ethnopharmacology* 2009; 124:339-349.
13. Adika OA, Madubunyi II, Asuzu IU. Antidiabetic and antioxidant effects of the methanol extract of *Bridelia micrantha* (Hochst) Baill. (Euphorbiaceae) leaves on alloxan-induced diabetic albino mice. *Comparative Clinical Pathology* 2012; 21(5):945-51.
14. Lin J, Puckree T, Mvelase TP. Antidiarrhoeal Evaluation of Some Medicinal Plants Used by Zulu Traditional Healers. *Journal of Ethnopharmacology* 2002; 79(1):53-56.
15. Adeyemi A, Omonigbehin AE, Stella S, Oluwatosin O, Jumoke S. Antibacterial activity of extract of *Alchornea cordifolia* (Schum and Thonn) Mull. Arg., *Boerhavia diffusa* (L) and *Bridelia micrantha* (Hochst Baill) used in traditional medicine in Nigeria on Helicobacter pylori and four diarrhoeagenic bacterial pathogens. *African Journal of Biotechnology* 2008; 7: 3761-3764.
16. Vinuthan MK, Kumara G, Narayanaswamya M, Veeriab T. Lipid lowering effects of aqueous leaves extract of *Marraya keenigii* (curry leaf) on alloxan-induced male diabetic rats. *Phcog Mag* 2007; 3: 112-115.
17. Allain CC, Poon LS, Chan LS, Richmond CSG, Fu PC. Enzymatic determination of total serum cholesterol. *Clin Chem* 1974; 20: 470-475.
18. Tietz NW. *Clinical guide to laboratory test.* 2nd edition. Philadephia, U.S.A: W.B. Saunders Company; 1990. p. 554-556.

19. Grove TH. Effect of reagent pH on determination of high density lipoprotein cholesterol by precipitation with sodium phosphotungstate-magnesium. *Clin Chem* 1979: 25: 260-264.
20. Bergmenyer HU. *Methods of enzymatic analysis*. 3rd edition. New York: Academic press; 1985. p. 154-160
21. Wilson PW, Abbott RD, Garrison RJ, William PC. Estimation of very low-density lipoprotein cholesterol from data on triglyceride concentration in plasma. *Clin Chem* 1981; 27:2008–2010.
22. Coles EH. Determination of packed cell volume In: *Veterinary Clinical Pathology*. 2nd edition. Philadelphia: W.B. Saunders Co; 1968.p. 17-19.
23. Schalm OW, Jain NC, Carroll EJ. *Veterinary Haematology*. 3rd edition. Philadelphia: Lea and Febieger: 1975. p. 55-77.
24. Szudelski T. The mechanism of alloxan and streptozotocin actions in beta cell of the rats' parcreas. *Physiological Research* 2001; 50: 536-546.
25. Dhanabal SP, Raja MK, Ramanthan M, Suresh P. Hypoglycaemic activity of *Nymphoea stellata* leaves ethanotic extract in alloxan induced diabetic rats. *Fitoterapia* 2007; 78:288-291.
26. Horton NR, Moran LA, Ochs RS, Rawn JD, Scringeour KG. *Principles of biochemistry.* 2nd edition. USA: Pretice Hall Inc: 1996. p. 474-475.
27. Krishinakumar K, Augusti KT, Vijaymmal PL. Hypolipidemic effect of *Salacia oblongata* wall root bark in streptozotocin-induced diabetic rats. *Med Sci* 2000; 28: 65-67.
28. Muragan M, Uma C, Reddy M. Hypoglycaemic and hypolipidemic activity of leaves of *Mucuna pruriens* in alloxan-induced diabetic rats. *Journal of pharmaceutical sciences and technology* 2009; 1 (2): 69-71.
29. ADA .American diabetic association. Standards of medicinal care in diabetes. *Diabetes care* 2007; 30:4-41.
30. Tanabe M, Chen YD, Saito K, Kano Y. Cholesterol biosynthesis inhibitory component from *Zingiber officinale* Roscoe. *Chem Pharm Bull* 1993; 41: 710-713.
31. Spiller GA. *CRC handbook of dietary fibre in human nutrition.*2nd edition. USA: CRC press: 1993. p.36-42.
32. Cho SY, Park TY, Park IM, Choi MS, Lee MU, Joon SM, Yang MK, Kim MJ, Park YB. Alteration of hepatic antioxidant enzyme activities and lipid profile in STZ induced diabetic rats by supplementation of dandetion water extract. *Clin Chem Alta* 2002; 317:109-117.
33. Okoleye BI, Bessong PO, Ndip RN. Preliminary phytochemical *in vitro Helicobacter pylori* activity of extracts of stem bark and leaves of Bridelia micrantha (Hochst Bail). *Molecules* 2011; 16(8): 6193-6205.
34. Nemenibo R. Effect of aqueous extract of *Canavalia ensiformis* seeds on hyperlipidemic and hyperkalemia in alloxan-induced diabetic rats. *Biochemistry* 2003; 15: 7-15.
35. Halim EM, Ali H. Hypoglycaemic, hypolipidemic and antioxidant properties of combination of curcumin from *Cucuma longa* (Linn) and partially parified product from *Abrona aagusta* (Linn) in STZ- induced diabetes. *Indian Journal of Clinical Biochemistry* 1996; 17:33-43.
36. Pratt PW. Laboratory procedures for animal health techniques. 1st ed. Goleta: American Veterinary publications Inc: 1985. p. 56-64
37. Kennedy L, Baynes JW. Non-enzymatic glycosylation and the chronic complication of diabetes: an overview. *Diabetologia* 1984;24: 93-98.
38. Sheela CG, Augusti KT. Antidiabetic effects of S-allyl cryteine sulphoxide isolated from garlic, (*Allium sativum*, Linn). *Indian J Exp Biol* 1992; 30:523-526.
39. Arun GS, Ramesh KG. Improvement of insulin sensivity by perindopril in spontaneously hypertensive and streptozotocin-induced diabetic rats. *Indian J Pharmacol* 2002; 34: 156-164.
40. Ohlsson A, Aher SM. Early erythropoietin for presenting red blood cell transfusion in preterm and/ or low birth weight infants. *Cochrane database Syst Rev* 2006; 3: CD004863.
41. Mayfield J. Diagnosis and classification of diabetes mellitus; new criteria. *American family physician* 1998; 58:1355-1362.
42. Atangwho IJ, Ebong PE, Eyong EU, Erteng MU, Uboh FE. *Vernonia amydalina* Del. A potential prophylactic antidiabetic agent in lipids complication. *Glob J Pure and Applied Sci* 2007; 13(1): 103-106.