Int J Pharm Bio Sci 2014 Jan; 5(1): (P) 646 - 655

Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

PROTECTIVE EFFECT OF *NEWBOULDIA LAEVIS* LEAF EXTRACT AGAINST DYSLIPIDEMIA AND HEPATORENAL DYSFUNCTION IN DIABETIC RATS

***KOLAWOLE O. TIMOTHY[1] AND AKANJI M. ADEWUMI[2]**

[1]Department of Pharmacology and Therapeutics, College of Health Sciences, Ladoke Akintola University of Technology. Ogbomoso, Nigeria
[2]Department of Biochemistry, University of Ilorin, Ilorin, Nigeria

ABSTRACT

Diabetes mellitus has become a global health problem and its prevalence is increasing at an alarming rate. Management of diabetes and its complications with drugs that are easily accessible and have minimal side effects remains a serious challenge among medical scientists. In this study, we investigated the effects of ethanol extract of the leaves of *Newbouldia laevis* on dyslipidemia and hepatorenal dysfunction in diabetic rats. Experimental diabetes was induced in rats by intravenous injection of streptozotocin. Diabetic rats were then treated with graded doses of *Newbouldia laevis* extract for 28 days. Serum analysis showed that there was significant reduction in the serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatinine and urea in extract-treated rats. Serum levels of total cholesterol, triglycerides and low density lipoprotein cholesterol were significantly reduced while serum level of high density lipoprotein cholesterol was raised. The results of this study suggest that extract of the leaves of *Newbouldia laevis* could protect diabetic rats against dyslipidemia and hepatorenal dysfunction.

KEYWORDS: **Diabetes, dyslipidemia, *Newbouldia laevis*, liver, kidney**

KOLAWOLE O. TIMOTHY
Department of Pharmacology and Therapeutics, College of Health Sciences, Ladoke Akintola University of Technology. Ogbomoso, Nigeria

INTRODUCTION

Diabetes mellitus is associated with various metabolic disorders including insulin resistance, hyperglycemia and dyslipidemia. If not controlled, diabetes can result in a host of complications that can affect nearly every organ in the body including the liver and the kidneys[1]. Generally, the damaging effects of diabetes are grouped into acute and chronic complications. Acute complications include diabetic keto acidosis and non-ketotic hyper-osmolar state. While the first is seen primarily in individuals with type 1 diabetes mellitus, the latter is prevalent in individuals with type 2 diabetes mellitus[2]. Chronic complications include microvascular disorders such as retinopathy, neuropathy and nephropathy, and macrovascular complications like coronary artery disease, peripheral vascular disease, and cerebrovascular disease[3]. In diabetes and insulin resistance, there is excessive glucose uptake by insulin-dependent tissues. The influx of glucose promotes the production of oxidants and impairs antioxidant defense system through enzymatic, non-enzymatic and mitochondrial pathways[4]. Many mechanisms about how hyperglycemia causes diabetic complications through these pathways have been reported. These are based on several preclinical studies and clinical trials involving the use of specific inhibitors of these pathways. The mechanisms include activation of protein kinase C isoforms, increased hexosamine pathway, glucose autooxidation, increased advanced glycation end-products (AGEs) formation and increased polyol pathway flux[5]. All these are consequences of accumulation of superoxides in the cells which ultimately results in oxidative damage and tissue injury[6]. Diabetes mellitus is managed by different oral hypoglycemic drugs and insulin. However, the drawbacks associated with these drugs such as insulin resistance and fatty liver have made the search for new and effective remedies necessary. Medicinal plants are widely used to treat diabetes mellitus, especially in developing nations where access to conventional therapeutic interventions is minimal. Therefore medicinal plants are good potential sources of antidiabetic agents. *Newbouldia laevis* (P. Beauv) is a medicinal plant that belongs to Bignoniaceae family. Its common names are 'African Border Tree' and 'Fertility Tree'. In Nigeria, it is known by different indigenous names such as 'Aduruku' (Hausa), 'Ogirisi' (Igbo) and 'Akoko' (Yoruba). In the South West and some other parts of Nigeria, the leaves are soaked in alcohol to treat diabetes mellitus and it has been reported that the extract of the leaves lowered blood glucose level in diabetic rats[7]. However, the effects of this plant on hepatorenal dysfunction and dyslipidemia which are chronic complications of diabetes mellitus have not been scientifically investigated. In this study, we evaluated the effect of leaf extract of *N. laevis* on lipid profile and some indices of hepatic and renal functions in diabetic rats.

MATERIALS AND METHODS

Collection of plant material

Leaves of *Newbouldia laevis* were collected from the premises of College of Health Sciences, Ladoke Akintola University of Technology, Mercyland, Osogbo Campus, Nigeria. The plant sample was identified and authenticated by a taxonomist in Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. A voucher specimen was deposited in the herbarium of the institute (voucher specimen no: FHI 107753).

Extraction procedure

The leaves were thoroughly washed with distilled water to remove soil and other debris that may contaminate the plant sample. The washed sample was then air-dried under shade in the laboratory for 5 days and the dry plant sample was pulverized using an electric grinding machine. The resultant powder sample weighing 500 g was then extracted with 80 % ethanol at 70 ^{0}C by continuous hot percolation using a Soxhlet apparatus. The extraction was carried out for 24 h and the resulting ethanol extract was concentrated at 40 ^{0}C in a rotary evaporator. The solid sample obtained weighed 47.5 g (yield = 9.5 %). The crude ethanol extract (NLet) was kept in an air-tight container and

stored in a refrigerator at 4 ^{0}C until the time of use.

Experimental animals

Wistar rats weighing 180- 200 g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals were housed in polypropylene cages inside a well-ventilated room. A maximum of six animals were kept in one cage. The animals were maintained under standard laboratory conditions of temperature (22 ± 2 ^{0}C), relative humidity (55-65%) and 12 hour light/dark cycle. During the whole experimental period, animals were fed with a standard balanced commercial pellet diet (Ladokun Feeds Ltd. Ibadan, Nigeria.

Ethical consideration

Experimental procedures and protocols used in this study were approved by the Ethics committee of the Ladoke Akintola University of Technology, Nigeria. All procedures were conducted in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals[8] .

Induction of diabetes

Experimental diabetes was induced in rats which had fasted for 12 hr by a single intravenous injection (through the tail vein) of a freshly prepared solution of streptozotocin (STZ) (60 mg/kg b.wt) dissolved in 0.1 M cold citrate buffer[9]. The rats were allowed to drink 5% glucose solution overnight to overcome drug-induced hypoglycemia. Estimation of fasting blood glucose (FBG) was done 72 hours after injection of STZ to confirm induction of diabetes and then on the 7th day to investigate the stability of diabetic condition. Fasting blood glucose was estimated by One Touch® glucometer (Lifescan, Inc. 1995 Milpas, California, USA). Blood sample for the FBG determination was obtained from the tail vein of the rats and those with blood glucose value ≥ 200 mg/dl were selected for the study.

Experimental design

Rats were divided into a group of non-diabetic rats and five groups of STZ – diabetic rats. Each of the six groups consists of 6 rats. Group I = non-diabetic rats (normal control); Group II = STZ – diabetic rats; Group III = diabetic rats treated with NLet (150 mg/kg); Group IV = diabetic rats treated with NLet (300 mg/kg); Group V = diabetic rats treated with NLet (500 mg/kg); Group VI = glibenclamide (5 mg/kg). The extract as well as the standard drug was administered orally twice a day at 8.00 am and 8.00 pm for 28 days using a sterile syringe fitted with a sterile cannula. Rats in groups I and II were treated orally with distilled water for the four weeks. All animals were fasted overnight and blood was collected from the tail vein on Day 0 (before the commencement of treatment) and Day 15. On Day 29, the rats were euthanized under chloroform vapor. The jugular vein was exposed and cut with a sterile scalpel blade, and the rats were bled into specimen bottles. Blood samples were transferred into sterilized centrifuge tubes and allowed to clot at room temperature. The blood samples were centrifuged for 10 minutes at 1500 rpm. The serum obtained was used for the biochemical assays. Lipid profile was analyzed on Day 0, 15 and 29 while markers of hepatic and renal functions were evaluated on Day 29.

Biochemical analysis

Serum total cholesterol (TC), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) and triglycerides (TG) were estimated by commercial kits (Biolabo SA, Maizy, France). Total cholesterol assay is based on CHOD PAP method. This is an enzymatic method described by Allain et al[10]. Serum triglycerides concentration was assayed by GPO method using test-kit based on modified Trinder reaction[11]. HDL-cholesterol assay was based on precipitation method[12].

Estimation of low density lipoprotein cholesterol was done by using the empirical formula of Friedewald et al[13]. The concentration of LDL- c was calculated as follows: LDL- cholesterol = (Total cholesterol) – (HDL- cholesterol) – (Triglycerides/5). Atherogenic index of (AI) was calculated using the formula: AI = LDL-c / HDL-c[14]. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was estimated by the method of Reitman and

Frankel[15] while alkaline phosphatase (ALP) assay was based on the method of Kind and King[16] using test kits (BioVision Inc, USA). Serum creatinine was estimated by a commercial kit (Vitro Scient Co. Egypt) based on modified kinetic Jaffe reaction[17] and serum urea was also estimated by a commercial kit (Randox Laboratories Ltd, UK).

Statistical analysis

Data obtained from the experiments were expressed as mean ± standard error of mean (SEM) and subjected to one-way analysis of variance (ANOVA) followed by Student's t- test. Results were considered significant at $P < 0.05$. GraphPad Prism version 5.0 for windows was used for the statistical analysis (GraphPad software, San Diego California USA).

RESULTS

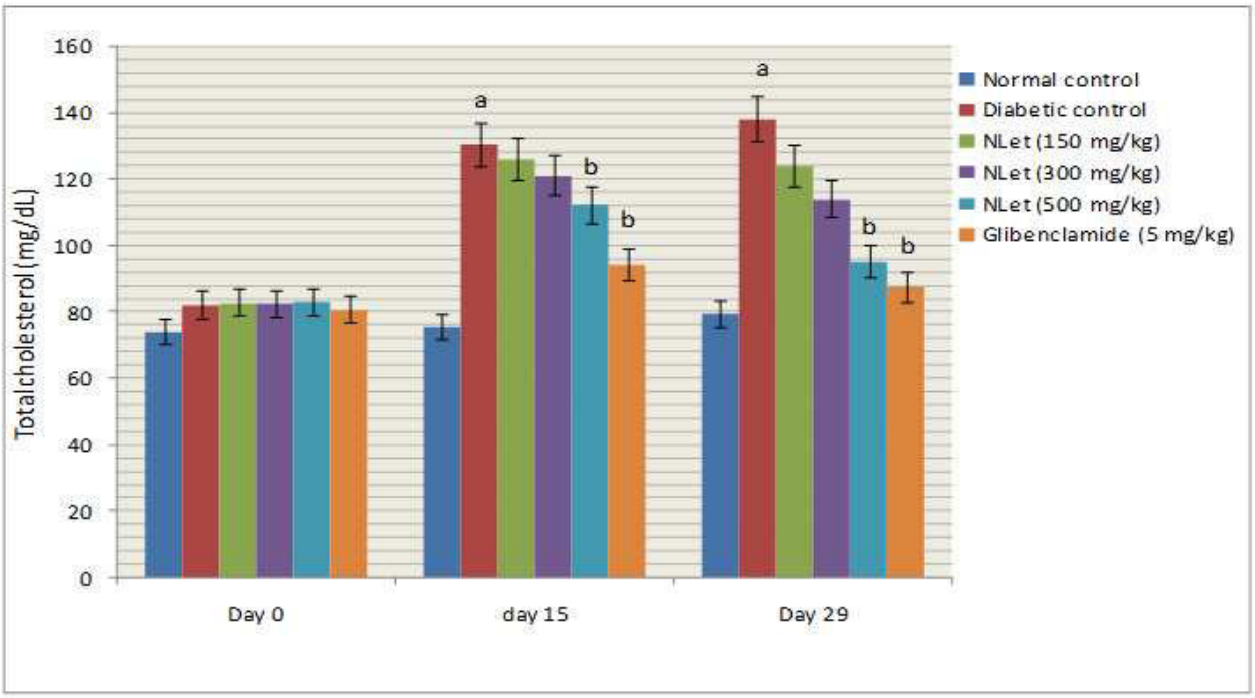
Lipid profile

In diabetic control, serum Total Cholesterol increased by 58.8% and 68.2% on day 15 and day 29 respectively over the initial value on day 0. In the group treated with 500 mg/kg of the extract, there was significant difference ($P < 0.05$) compared to diabetic control group. Total Cholesterol was reduced by 13.9% and 31.1% on day 15 and day 29 respectively. With the administration of glibenclamide, TC was reduced by 27.7% and 36.4% on day 15 and day 29 respectively (Figure 1). There was a significant increase ($P < 0.05$) in serum level of triglycerides in untreated diabetic rats compared with non-diabetic control (normal control). In the diabetic control group, TG increased by 55.7 % and 86.3 % on day 15 and day 29 respectively. Compared with the diabetic control group, serum TG of the group treated with 500 mg/kg NLet was significantly reduced ($P < 0.05$) by 15.5% and 46.9% on day 15 and day 29 respectively. With 300 and 500 mg/kg, there was no significant difference ($P > 0.05$) compared with normal control and the glibenclamide-treated group on day 29 (Figure 4).

Indices of hepatic and renal functions

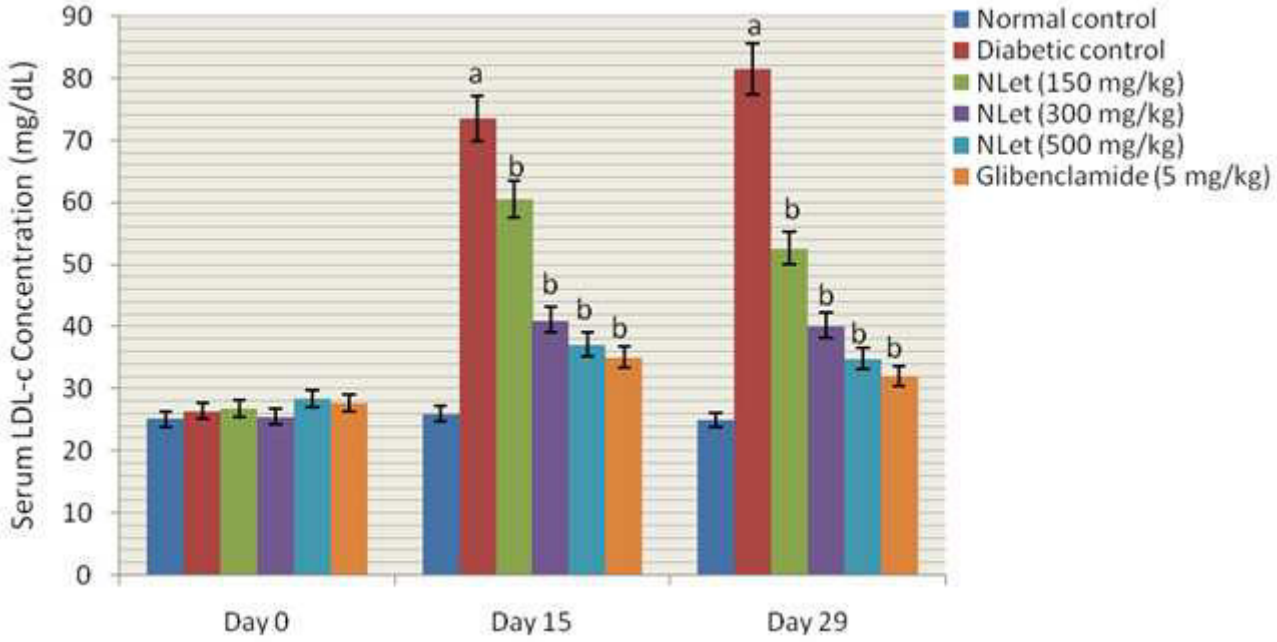
The results of the experiments on the effects of NLet on hepatic and renal function indices in diabetic rats are presented in Table 2. There was significant increase ($P < 0.05$) in the levels AST, ALT, ALP, creatinine and urea in diabetic control group compared to non-diabetic group (normal control). The increase was reversed in NLet-treated and glibenclamide-treated groups. The three doses of the extract caused a significant reduction ($P < 0.05$) in the levels of AST and ALT compared to diabetic control. Significant reduction in ALP, creatinine and urea was also observed with 300 and 500 mg/kg of the extract. There was no significant difference ($P > 0.05$) between glibenclamide-treated rats and those treated with 500 mg/kg of NLet except with regard to urea.

Figure 1
Effect of extract of N. laevis on total cholesterol in diabetic rats.



Values are expressed as mean ± SEM (n = 6). [a]P < 0.05 compared with normal control; [b]P < 0.05 compared with diabetic control.

Figure 2
Effect of extract of N. laevis on low density lipoprotein cholesterol in diabetic rats.



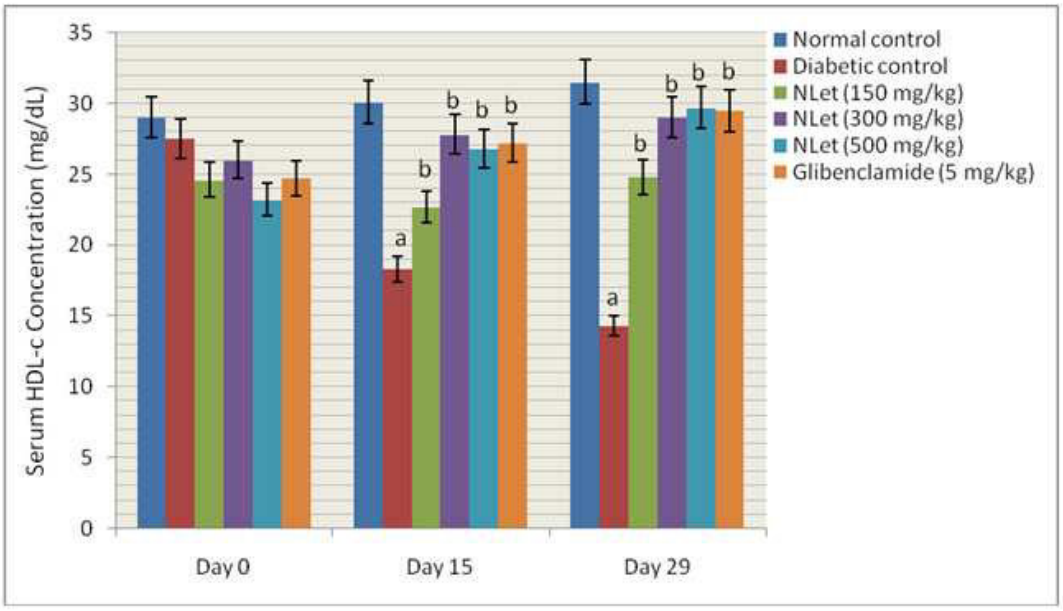
Values are expressed as mean ± SEM (n = 6). [a]P < 0.05 compared with normal control; [b]P < 0.05 compared with diabetic control.

Atherogenic index

There was significant increase ($P < 0.05$) in the atherogenic index (AI) of diabetic rats compared with the normal control. The three doses of the extract significantly reduced ($P < 0.05$) atherogenic index compared to diabetic control and the values were not significantly different ($P > 0.05$) from that of the normal control (Table 1).

Figure 3

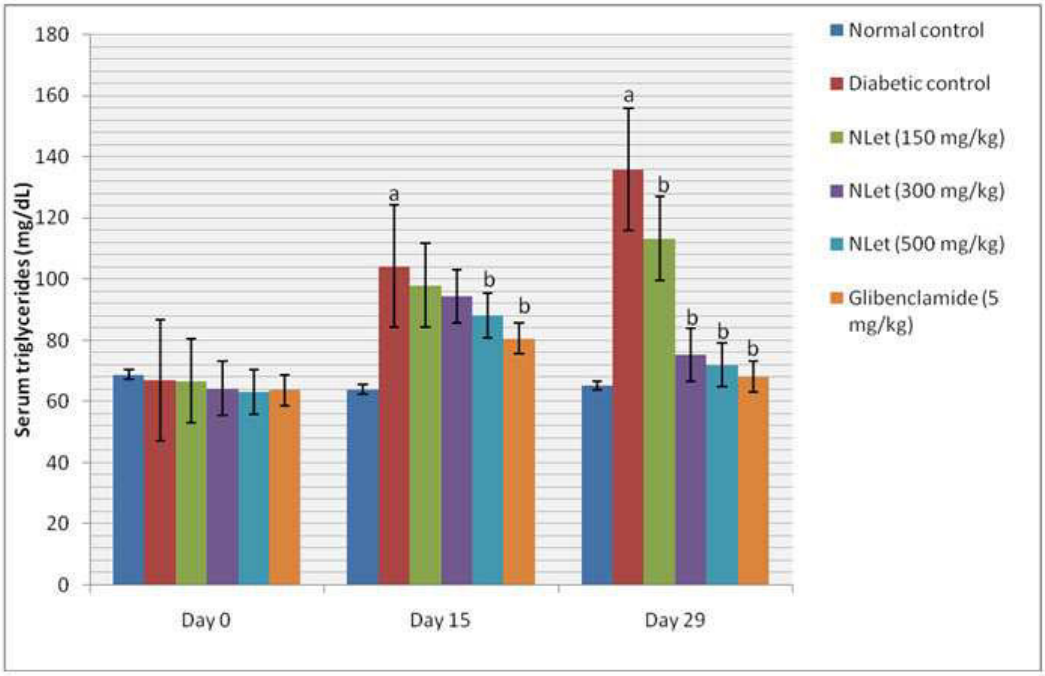
Effects of extract of N. laevis on high density lipoprotein cholesterol (HDL-c) in diabetic rats.



Values are expressed as mean ± SEM (n = 6). [a]P < 0.05 compared with normal control; [b]P < 0.05 compared with diabetic control.

Figure 4

Effect of extract of N. laevis on serum triglycerides in diabetic rats.



Values are expressed as mean ± SEM (n = 6). [a]P < 0.05 compared with normal control; [b]P < 0.05 compared with diabetic control.

Table 1
Effect of extract of N. laevis on atherogenic index of diabetic rats

	Day 0	Day 15	Day 29
Normal control	1.11 ± 0.12	1.16 ± 0.20[a]	1.19 ± 0.17[a]
Diabetic control	0.96 ± 0.14	4.02 ± 0.88[b]	5.70 ± 1.05[b]
NLet (150 mg/kg)	1.09 ± 0.18	2.67 ± 0.25[c]	2.13 ± 0.63[c]
NLet (300 mg/kg)	0.98 ± 0.10	1.48 ± 0.31[a]	1.39 ± 0.20[a]
NLet (500 mg/kg)	1.22 ± 0.23	1.39 ± 0.26[a]	1.18 ± 0.19[a]
Glibenclamide (5mg/kg)	1.12 ± 0.13	1.29 ± 0.41[a]	1.09 ± 0.33[a]

Values represent mean ± SEM (n = 6); Means with different superscripts in the same column are significantly different at P < 0.05

Table 2
Effects of extract of N. laevis on serum levels of markers of hepatic and renal functions in diabetic rats

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Creatinine (mg/dL)	Urea (mg/dL)
Normal control	93.6 ± 1.6[a]	67.3 ± 2.9[a]	18.6 ± 2.3[a]	0.5 ± 0.03[a]	26.8 ± 2.3[a]
Diabetic control	172.2± 4.1[b]	138.9 ± 3.8[b]	27.6 ± 2.0[b]	3.6 ± 0.36[b]	53.8 ± 5.4[b]
NLet (150 mg/kg)	144.2 ± 2.7[c]	116.3 ± 6.4[c]	25.4 ± 1.3[b]	3.1 ± 0.55[b]	48.2 ± 2.5[b]
NLet (300 mg/kg)	121.0 ± 3.3[d]	96.4 ± 1.5[d]	20.1 ± 1.6[c]	2.4 ± 0.14[c]	39.1 ± 2.3[c]
NLet (500 mg/kg)	102.5 ± 2.7[e]	82.5 ± 3.9[e]	19.2 ± 1.8[c]	1.2 ± 0.02[d]	35.6 ± 2.9[c]
Glibenclamide (5mg/kg)	98.2 ± 1.6[e]	78.6 ± 3.1[e]	18.6 ± 2.1[c]	0.8 ± 0.18[d]	28.9 ± 3.7[d]

Values are expressed as mean ± SEM (n = 6); Means with different superscript in each column are significantly different (P < 0.05).

DISCUSSION

Defects in insulin action and hyperglycemia associated with diabetes could lead to changes in serum lipoproteins. In poorly controlled diabetes, hyperglyceridemia, reduced HDL cholesterol and increased LDL-c commonly occur[18]. In addition, low density lipoprotein (LDL) is converted to smaller and more atherogenic lipoproteins called small dense LDL[19]. In this study, there was an increase in the levels of total cholesterol, LDL-c and triglycerides in diabetic rats compared with the normal control. This was also accompanied by reduced level of HDL-c. The observed dyslipidemia in the diabetic control group was reversed when the rats were treated with NLet and glibenclamide. The mechanism of dyslipidemia in diabetes mellitus has been explained on the basis of hyperglycemia that results from insulin deficiency. For example hyperglycemia was reported to lower HDL cholesterol level in diabetic patients[20]. The direct relationship of blood glucose level and dyslipidemia has also been confirmed by another study which reported that triglycerides, LDL cholesterol and total serum lipid levels in poorly controlled diabetes were significantly higher than those of the normal control[21]. Hyperglycemia distorts lipoprotein lipase / hepatic lipase ratio resulting in reduced HDL cholesterol level. The increased activity of cholesterol ester transfer protein (CETP) leads to depletion of cholesteryl esters from HDL and this ultimately results in reduced HDL cholesterol[22]. Reduction in lipoprotein lipase (LPL) activity has also been reported to be the cause of increase in triglycerides associated with hyperglycemia since LPL hydrolyzes triglycerides of chylomicrons and very low density lipoproteins [23]. The results of this study agree with the work of Khan[24], who reported that blood glucose level has direct and significant correlation with total cholesterol,

triglycerides and LDL-c and inverse correlation with HDL-c. Blood glucose lowering effect of *N. laevis* has been reported[7]. Therefore, serum levels of total cholesterol, triglycerides and LDL-c were significantly reduced while HDL-c level was raised as a result of decrease in blood glucose. The elevation of HDL-c is beneficial and atheroprotective because it has the ability to remove cholesterol from peripheral tissues and returns it to the liver, a process known as reversed cholesterol transport. HDL is also associated with anti-inflammatory[25], antioxidative[26] and anti-apoptotic[27] properties, all of which contribute to the beneficial effects of HDL. These are probably the mechanisms through which NLet protected diabetic rats against dyslipidemia and cardiovascular risk as reflected by atherogenic index. An increase in the serum levels of creatinine and urea is a sensitive indicator of kidney injury while the release of intracellular transaminase enzymes and alkaline phosphatase into the circulation is an indicator of hepatocellular damage[28]. Therefore the significant increase in the serum levels of AST and ALT observed in diabetic rats in this study is an indicator of liver injury. Likewise renal dysfunction was established by the elevation in serum levels of creatinine and urea. The significant increase in serum level of ALP observed in the diabetic rats also suggests that there was tissue damage in the rats[29]. Elevated activity of this enzyme is indicative of cellular leakage and loss of functional integrity of the cell membrane[30]. Damage to kidney, liver, small intestine and bone may increase the serum level of ALP[31]. Treatment of the diabetic rats with NLet and glibenclamide significantly reduced the levels of these enzymes. This is an indication that NLet could ameliorate the hepatic and renal damage associated with STZ-induced diabetes. It is also interesting to know that ethanol extract of *N. laevis* has low toxicity profile as previously reported[32]

CONCLUSION

From the results of this study, it can be concluded that ethanol extract of the leaves of *N. laevis* has protective effect against dyslipidemia and hepatorenal dysfunction associated with diabetes mellitus.

ACKNOWLEDGEMENT

The authors appreciate the technical assistance provided by Mr A. Omodara of the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

REFERENCES

1. BarathManiKanth S, Kalishwaralal K, Sriram M, Pandian SRK, Youn H, Eom S, Gurunathan S, Anti-oxidant effect of gold nanoparticles restrains hyperglycemic conditions in diabetic mice. Journal of Nanobiotechnology, 8:16 (2010)
2. Tripathi BK, Srivastava AK, Diabetes mellitus: complications and therapeutics. Med Sci Monit, 12 (7): 130-147 (2006)
3. Fong DS, Aiello LP, Ferris FL, Klein R, Diabetic retinopathy. Diabetes Care, 27: 2540-2553 (2004)
4. King, GL., and Loeken MR, Hyperglycemia-induced oxidative stress in diabetic complication. Histochem. Cell Biol, 122: 333–338 (2004)
5. Mohora M, Virgolici B, Coman A, Muscurel C, Gaman L, Gruia V, Greabu M, Diabetic foot patients with and without retinopathy and plasma oxidative stress. Rom J Intern Med, 1: 45–51 (2007)
6. Kaleem M, Asif M, Ahmed OU, Bano B, Antidiabetic and antioxidant activity of *Annona squamosa* extract in streptozotocin-induced diabetic rats. Singapore Med J, 47(8): 670-675 (2006)
7. Owolabi OJ, Amaechina FC, Okoro M, Effect of ethanol leaf extract of *Newbouldia laevis* on blood glucose levels in diabetic rats. Tropical J Pharm Res, 10 (3): 249-254 (2011)

8. National Institute of Health, Guide for the use of laboratory animals. DHHS, PHS, NIH Publication No. 85- 23 Revised (1985)
9. Chen H, Brahmbhatt S, Gupta A, Sharma AC, Duration of streptozotocin-induced diabetes differentially affects p38-mitogen-activated protein kinase (MAPK) phosphorylation in renal and vascular dysfunction. Cardiovascular Diabetology, 4:3 (2005)
10. Allain CC, Poon LS, Chon CSG, Richmond W, Fu PC, Enzymatic determination of total serum cholesterol. Clin Chem, 20: 470-475 (1974)
11. Fossati P and Prencipe L, Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem, 28(10): 2077-2080 (1982)
12. Virella-Lopes MFL, Stone PG, Colwel JA, Serum High Density Lipoprotein in diabetic patients. Diabetologia, 13: 285-291 (1977)
13. Friedewald WT, Levy RI, Fredickson DS, Estimation of the concentration of low density lipoprotein cholesterol in plasma without the use of preparative ultracentrifugation. Clin Chem, 18: 499-502 (1972)
14. Abbott RD, Wilson PW, Kannel WB, Castelli WP, High density lipoprotein cholesterol, total cholesterol screening and myocardial infarction. The Framingham Study. Arterosclerosis, 8: 207-211 (1988)
15. Reitman S and Frankel S, A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol, 28: 56-63 (1957)
16. Kind PRN and King EJ, Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J Clin Pathol, 7: 322-326 (1954)
17. Henry RJ, Cannon DC, Winkelman JW, Clinical Chemistry, Principles and Techniques. 2nd ed., Harper and Row, p. 525 (1974)
18. Ginsberg HN**,** Diabetic dyslipidemia: basic mechanisms underlying the common hypertriglyceridemia and low HDL cholesterol levels. Diabetes, 45(Suppl 3): S27-S30 (1996)
19. Krauss RM**,** Heterogeneity of plasma low-density lipoproteins and atherosclerosis risk. Curr Opin Lipidol, 5: 339 -349 (1994)
20. Siraj ES, Seyoum B, Saenz C, Abdulkadir J, Lipid and lipoprotein profiles in Ethiopian patients with diabetes mellitus. Metabolism, 55(6):706-710 (2007)
21. Mohammadi H, Abdelouahed EM, Hassar M, Bouchrif B, Qarbal B, Dahbi F, Hilal L, Ghalim N, Glycemic control, HbA1c, and lipid profile in children with type 1 diabetes mellitus. Eur J Sci Res, 29(2): 289-294 (2009)
22. Mooradian AD, Dyslipidemia in type 2 diabetes mellitus. Nat Clin Pract Endocrinol Metab, 5: 150-159 (2009)
23. Nikkila EA and Taskinen MR, Lipoprotein lipase of adipose tissue and skeletal muscle in human obesity: response to glucose and to semi- starvation. Metabolism, 30(8): 810-817 (1981)
24. Khan AH, Clinical significance of HbA1c as a marker of circulating lipids in male and female type 2 diabetic patients. Acta Diabetol, 44(4):193-200 (2007)
25. Nicholls SJ, Dusting GJ, Cutri B, Bao S, Drummond GR, Rye KA, Barter PJ, Reconstituted high-density lipoproteins inhibit the acute pro-oxidant and proinflammatory vascular changes induced by a periarterial collar in normocholesterolemic rabbits. Circulation, 111(12): 1543- 1550 (2005)
26. Hansel B, Gira P, Nobecourt E, Chantepie S, Bruckert E, Chapman MJ, Kontush A, Metabolic syndrome is associated with elevated oxidative stress and dysfunctional dense high-density lipoprotein particles displaying impaired antioxidative activity. J Clin Endocrinol Metab, 89(10): 4963-4971 (2004)
27. De Souza JA, Vindis C, Hansel B, Negre-Salvayre A, Therond B, Serrano CV. Jr, Chantepie S, Salvayre R, Bruckert E, Chapman MJ, Kontush A, Metabolic syndrome features small, apolipoprotein A-I-poor, triglyceride-rich HDL3 particles with

defective anti-apoptotic activity. Atherosclerosis, 197(1): 84-94 (2008)
28. Saeed MK, Deng Y, Dai R, Attenuation of biochemical parameters in streptozotocin-induced diabetic rats by oral administration of extracts and fraction of *Cephalotaxus sinensis*. J Clin Biochem Nutr, 42: 21-28 (2008)
29. Emmanuel S, Rani MS, Sreekanth MR, Antidiabetic activity of *Cassia occidentalis* Linn. in streptozotocin-induced diabetic rats: A dose dependent study. Int J Pharm. Bio. Sci, 1(4): B14-B25 (2010)
30. Akanji MA, Olagoke OA, Oloyede OB, Effect of chronic consumption of metabisulphite on the integrity of rat liver cellular system. Toxicology, 81: 173-179 (1993)
31. Adedapo AA, Abatan MO, Olorunsogo OO, Effects of some plants of spurge family on the haematological and biochemical parameters of rats. Vet Archiv, 77: 29-38 (2007).
32. Kolawole OT, Akanji MA, Akiibinu MO. Toxicological assessment of ethanolic extract of the leaves of *Newbouldia laevis* (P. Beauv). American Journal of Medicine and Medical Sciences, 3(4):74-80 (2013)