Nigerian Journal of Biochemistry and Molecular Biology 2013; 28(1&2): 22-34

0189-4757/96 $3.0 + 0.00
Printed in Nigeria

Available online at http://www.nsbmb.org/journals.php

NJBMB/003/13

Antidiabetic Activity of Hydromethanolic Leaf Extracts of *Prosopis oblonga* (Benth) in Normal and Alloxan-induced Diabetic Rats

[*1]Akpe, G. C., [1]Hassan, S. W., [1]Abubakar, M. G. and [2]Aliero, A. A.

[1]Department of Biochemistry, Faculty of Science, Usmanu Danfodiyo University, Sokoto, Nigeria.
[2]Botany Unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

ABSTRACT: The hypoglycaemic and toxicological properties of the leaf extract of *Prosopis oblonga*, Benth were evaluated on normoglycaemic and hyperglycaemic rats. The hypoglycaemic activity was evaluated in alloxan monohydrate-induced hyperglycaemic rats (80 mg/kg body weight intraperitoneally). Phytochemical screening of the leaf extracts showed the presence of saponins, tannins, alkaloids, flavonoids, glycosides, saponin-glycosides, cardiac-glycosides, steroids and volatile oils. The potency of the leaf extracts was compared with that of oral hypoglycaemic agent (Glibenclamide). Administered doses of 250, 500 and 750 mg/Kg body weight of the leaf extract to diabetic rats, showed significant ($P < 0.05$) reduction of blood glucose level in a dose dependent manner when compared with the control and the reference drug group. However, in normoglycaemic rats, a significant ($P < 0.05$) reduction in blood glucose was also observed. This showed that the leaf extracts of the plant have a blood sugar lowering effect, though more significantly on diabetic rats. The lethal dosage (LD_{50}) obtained was greater than 5000mg/kg. These results suggest that the leaf extract is relatively safe and could be used in the management of diabetes mellitus.

KEYWORDS: *Prosopis oblonga*, diabetes, alloxan, albino rats, hydromethanolic extract, glibenclamide, toxicity**.**

1.0 Introduction

The use of plants as a source of relief from illness is as old as mankind (Christopherson, 1991). A medicinal plant is any plant which in one or more of its organs, contains substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs (Sofowora, 1993). They have been the source of many important scientific drugs of the modern world. According to Mayers (1982), almost ninety percent of present day medicines are directly or indirectly derived from plants. However, only fifteen percent of these plants have been investigated pharmacologically out of an estimated 250,000 to 500,000 species of higher plants growing on earth (Farnsworth and Bigel, 1977).

Diabetes mellitus is a chronic metabolic disorder resulting from insulin deficiency, characterized by hyperglycaemia, altered metabolism of carbohydrates, protein and lipids, and an increased risk of vascular complication (Barar, 2004). Diabetes arises from a deficient production of insulin by the β-cells of the pancreatic islets which lead to the complete or relative insufficiency of insulin secretion and or insulin action, (Balkau *et al.*, 2000; Murray, 2000). Diabetes has now become an epidemic with a worldwide incidence of 5% in the general population. Approximately, 140 million people worldwide suffer from diabetes (WHO, 1999). Hyperglycaemia is the primary clinical manifestation of diabetes and is thought to contribute to diabetic complications by altering vascular cellular metabolism (Barrett-Conner *et al.*, 1991) and vascular matrix molecules and circulating lipoproteins. It is therefore a major risk factor for the development of cardiovascular disease (Wilson *et al.*, 1998; Andrew, 2000; Chattopadyhay, 2004). Oral hypoglycaemic agents especially biguanides have been commonly employed in the management of diabetes, of especially type II (Bunyapraphatsara *et al.*, 1996). Sulphonylureas e.g Daonil is the most widely used oral

Corresponding Author
Tel.: +2348068318699;
E-mail: agchukunalu@yahoo.com.

hypoglycaemic agents but may have some adverse effects such as exacerbating hyper insulinaemia and ceasing weight gain in patients. The biguanides are weak hypoglycaemics and have limited clinical use (Rang *et al*., 1999). For these reasons therefore, there is a great need to search for acceptable, cheap and safe blood sugar lowering oral hypoglycaemic agents. Herbs and marine sources have been considered the best options (Ogbonnia, 2008).

In Nigeria, varieties of plants have been reported to possess hypoglycaemic activity. These include *Magnifera indica, Mormodica charanitia* and *Tetracera alnifolia* (Onaogbe *et al.,* 1999), as well as *Persea americana, Globaimetula brawi, Treculia african* (Antia *et al.,* 2005; Djomeni *et al.,* 2006; Tanko *et al.,* 2008).

Prosopis oblonga Benth (Hausa name: Kirya), that was selected in this research is traditionally used in the treatment of Diabetes mellitus and belongs to the genus, leguminoseae and fabacae family. It is a multipurpose tree of the inter tropical Africa that grows up to 4-20 m high (Dalziel, 1955). Different parts of the plants are attributed with diverse therapeutic benefits. For example in Ghana, boiled roots serve as poultice for wound cuts (Abbiw, 1990). However, in most part of West Africa, the leaves in particular are used for the treatment of headache, toothache, as well as various other head ailments (Vogt, 1995). Leaves and bark are combined to treat rheumatism, skin diseases, dental caries and fever; eye washes are obtained from the bark (Katende, 1995). In Mali, the leaves and twigs decoction are used to relieve bronchitis, tooth decay, dysentery, malaria and stomach cramps (Hong *et al.,* 1996). Hypoglycaemic activity of the gum extract of *P. oblonga* has also been reported in acetaminophen-induced hepatotoxicity in rats (Ojo *et al.,* 2006).

Despite several claims of hypoglycaemic properties of the leaves of *P. oblonga* by the traditional medical practitioners, to the best of our knowledge, there is no documented literature of the hypoglycaemic activity and toxicological profiles of the leaves of the plant. This research, therefore, evaluates the hypoglycaemic and toxicological properties of the leaf extract of *P. oblonga*

2.0 Materials and Methods

2.1 Collection and Identification of Plant Materials

The fresh leaves of *P. oblonga* (Benth) were collected in May, 2011, at Gwandu village, Gwandu Local government area of Kebbi state, Nigeria. The plant was taxonomically identified and authenticated at the Botany Unit, Usmanu Danfodiyo University, Sokoto. A voucher specimen (No: 026) was deposited at the herbarium of same department for future reference.

2.2. Animals

Albino rats of both sexes, weighing between 100-200 g were obtained from the animal facility unit of University of Jos, Jos, Nigeria. They were housed in standard rat cages in the Zoological garden of Usmanu Danfodiyo University, Sokoto. They were allowed to acclimatize for a period of one week with access to clean water and rat feed (Vital Feeds, Jos, Nigeria) *ad libitum*

2.3 Chemicals and Reagents

Assay kits for the determination of biochemical parameters were of Randox Laboratories Co, Northern Ireland, UK. Commercially available Daonil (Glibenclamide) was from May and Baker Ltd, Degenham, England. Other chemicals and Alloxan monohydrate used were obtained from BDH Chemicals, England, Merck, Germany and Sigma Co., USA.

2.4 Preparation of Extract

The plant materials (leaves) were washed, air-dried for 3 days at temperature (30-35^{o}C) to avoid loss of some volatile phytochemicals. The dried materials were pulverized into a dry powder with a pestle and mortar as described by Antia *et al* (2005).

The extraction was carried out using 500 g of the powdered leaves in 500 ml of methanol and water (7:3) at room temperature for 72 hours (with occasional stirring). The extract was then filtered with a clean, white Muslin cloth and

later with sterile cotton wool and Whatman No.1 filter paper respectively. The filtrate was later concentrated using a rotary evaporator at 45ºC and a yield of 22.63% (w/w) was obtained. The residue was reconstituted in 0.9% distilled water at appropriate (desired) concentrations to be used each day of the experiment. The reconstituted extracts as well as the residue were refrigerated at 4ºC.

2.5 Phytochemical Analysis

The phytochemical analysis carried out on the extract includes qualitative tests to identify saponins (saponin glycosides), volatile oils, alkaloids, tannins, glycosides, flavonoids, anthraquinones, steroids and cardiac glycosides using the extract residue. Quantitative test was also carried out to estimate these phytoconstituents using standard procedures as described by Harborne (1973); El-Olemyl *et al* (1994); Trease and Evans, (1999).

2.6 Induction of Diabetes

Diabetes was induced in the albino rats by intraperitoneal administration of alloxan monohydrate (80 mg/kg body weight) for 3 consecutive days (alloxan monohydrate selectively destroys insulin-producing pancreatic β cells). From the third day, the blood glucose level of the animals were checked using a glucometer (a one touch test strips by Randox laboratories Co, Northern Ireland, UK) to ascertain diabetes. Stable hyperglycemia was confirmed on the 5th day and rats with fasting blood glucose levels greater than 200 mg/dl were considered diabetic and used for the study. The animals were maintained on water and pelleted diet at room temperature in cages.

2.7 Animal Grouping

A total of 35 rats (25 diabetic and 10 normal rats) were divided into seven groups (n = 5) as follows:
Group 1: Normal untreated animals (Control)
Group 2: Normal rats orally administered 750 mg/kg body weight of the extract for 5 days
Group 3: Non-treated diabetic rats (Diabetic Control)
Group 4: Diabetic rats administered 250 mg/kg body weight of the extract for 5 days
Group 5: Diabetic rats administered 500 mg/kg body weight of the extract for 5 days
Group 6: Diabetic rats administered 750 mg/kg body weight of the extract for 5 days
Group 7: Diabetic rats administered 5 mg/kg body weight of glibenclamide for 5 days

2.8 Preparation of Serum

The rats were sacrificed by decapitation using mild chloroform anaesthesia, 24 hours after the last treatment (Onaogbe *et al*., 1999). The blood samples obtained were collected using syringe and needle into test tubes and centrifuged at 3000 rpm for 5 minutes. The sera collected were used for biochemical, lipid and electrolyte analyses**.**

2.9 Biochemical Analysis

Blood glucose was determined by adopting the glucose oxidase described by Barham and Trinder (1972), using One Touch Basic Blood Glucose monitoring System (LifeScan Inc.Milipitas, California, U.S.A.). Non-protein nitrogenous compounds were determined as described for urea (Wybenga *et al*., 1971), uric acid (Morin and Prox, 1973) and creatinine (Henry, 1974) was assayed in serum. Lipid profile assayed in the serum of the animals included cholesterol (Trinder, 1969), triglycerides and HDL-C (Burstein *et al*., 1979), and LDL-C (Friedewald *et al*., 1972). The procedures described by Rec (1972) was used for the assay of alkaline phosphatase (ALT) activity while that of Reitman and Frankel (1957) were employed for the determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The total protein (Gornal *et al.,* 1949), albumin (Lee-Rodkly, 1964) and total bilirubin (Jandrassic and Grof, 1938) were determined in the serum while serum sodium (Na^+) and potassium (K^+) ion concentrations were determined using flame photometric method (Ranjna, 1999)

2.10 Acute Oral Toxicity Studies (LD_{50})

The acute oral toxicity study was conducted using the limit dose test of up and down procedure according to Dixon (1991) and the

Organization for Economic and Cultural Development (OECD, 2001) Test guidelines 425. Five groups of rats (one in each group) were used for this experiment. They were housed individually after random selection and allowed to acclimatize to the laboratory conditions for five days. A limit test dose of 5,000 mg/kg body weight of the extract was used. Animals were dosed once at a time and observed at least during the first 30 minutes after dosing and periodically during the first 24 hours, with special attention given during the first 4 hours and then for another 24 hours (observation time 48 hours). This observation continued for a total of 14 days. At the expiration of initial 48 hours, four additional animals were subsequently dosed and observed as previously described. Animals were observed for signs of acute toxicity morbidity and mortality. The behavioural changes and mortality displayed by the experimental rats were recorded accordingly.

2.11 Sub Chronic Toxicity Studies

According to OECD testing guidelines 407 (1995) and the method of Wambebe *et al* (1996), the repeated doses for 28 days oral toxicity study was followed. A total of 25 male and female albino rats selected randomly were divided into 5 groups of 5 rats housed in different cages and were allowed a period of 5 days to acclimatize. Animals in group I received equivalent volume (2ml) of distilled water and served as control. Animals in groups 2, 3, 4 and 5 were orally administered the graded doses of the LD_{50} value of 20, 40, 60, and 80% corresponding to the doses of 1000, 2000, 3000 and 4000 mg/kg respectively of the extract of *P. oblonga*. The animals were dosed with the test substance daily for a period of 28 days; the doses were given at similar times each day and adjusted as necessary to maintain a constant dose level in terms of body weight. The body weights of all the animals before and within the days of the treatment were recorded weekly (Days, 1, 7, 14, 21 and 28). Animals were observed at least twice daily for morbidity and mortality. All animals for the experiment after the 28^{th} day were sacrificed by decapitation under mild chloroform anaesthesia on the 29^{th} day and blood samples for biochemical assays were collected into plain vials.

2.12 Statistical Analysis

All data obtained were expressed as mean ± S.E.M. Statistical significance of the results between groups was determined using one-way analysis of variance (ANOVA) followed by Duncan's Post Hoc test to check differences between the individual groups and differences in means were considered to be significant when $P < 0.05$. All statistical analyses were carried out using the Instat Statistical Package (Instat Software, Sandiago USA).

3.0 Results

The extract weighed 37.69 g corresponding to a percentage yield of 15.08% (w/w). Phytochemical screenings showed that the constituents found in this plant were alkaloids, saponins, flavonoids, tannins, volatile oils, glycosides, saponin glycosides and cardiac glycosides (Table 1). Saponins was the most abundant of all the phytochemicals detected (Table 1).

Table 2 depicts the effects of the leaf extract of *P. oblonga* on fasting blood glucose of alloxan-induced diabetic rats. Oral administration of alloxan, induced significant ($P<0.05$) and progressive elevation in the blood glucose in the rats between the 3^{rd} and the 5^{th} day of the experiment but diabetes mellitus became fully established in all the rats on the day 5 post-induction. However, daily oral administration of 250-750 mg/kg/day of the leaf extract of *P. oblonga* to the diabetic rats significantly reduced ($P<0.05$) the elevated blood glucose in a dose-dependent manner (Table 4) when compared to untreated control (Diabetic control). The hypoglycemic effect of glibenclamide was similar but less significantly ($P<0.05$) different when compared to the various doses of the leaf extract.

The effect of oral administration of *P. oblonga* leaf extract and glibenclamide on the body weights of rats is presented in Table 3. There was significant ($P< 0.05$) weight loss in the diabetic control rats. In contrast, there was significant ($p<0.05$) weight gain in both normal control and experimental diabetic groups that were administered the plant extract and glibenclamide.

The effect of the leaf extract of *P. oblonga* on some biochemical constituents of the animals are presented in Tables 4, 5 and 6. Although, the levels of urea, uric acid and creatinine in the serum of diabetic animals, activities of liver marker enzymes (alkaline phosphatase, aspartate transaminase and alanine transaminase) and serum lipid profile (total cholesterol, triglycerides, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol) were significantly altered, administration of the extract reversed these trends towards the control values, but the values dis not compare favourably with their respective controls.

All rats treated with the 5000 mg/kg body weight limit dose of the leaf extract were hyporeactive to external stimuli such as touch in the first 30 minutes to 1 hour post administration and subsequently became active and exhibited normal behaviour throughout the 14 days observation period. The limit test dose of 5000 mg/kg body weight did not cause any mortality or any major acute toxicity. However, this dose induced progressive and sustained weight loss in all the experimental groups (Table 7). The LD_{50} of the leaf extracts using the OECD guidelines is therefore greater than 5000 mg/kg because there was no episode of death of the animals.

No mortality was recorded when varying doses of 1000, 2000, 3000 and 4000 mg/kg body weight of leaf extract of *P. oblonga* were administered orally daily for a period of 28 days (Table 8).

Similarly, on the body weight of the animals, a non significant dose-dependent difference in the weekly mean body weight between control and the animals treated with the extract of *Prosopis oblonga* were observed, except on day 28 where animals treated with the highest dose (4000 mg/kg body weight) produced a significantly lower weight ($P<0.05$) when compared to the control and other treated groups (Table 9).

The results of biochemical studies (aspartate aminotransferase (AST), alanine aminotransferase, (ALT), total bilrubin (TB), total protein (TP) and albumin levels, (Table 10), showed that there were no significant ($P>0.05$) difference between the control and the treated groups, while in serum urea, creatinine, and the K^+ concentrations, a significant ($P<0.05$) reduction was observed in the treated groups when compared with the control group.

4. Discussion

The results of this study indicated that the leaf extract of *P. oblonga* contained saponins, alkaloids, flavonoids, tannins, volatile oils, glycosides, saponin glycosides and cardiac glycosides as major components (Table 1). This is in agreement with the findings of Ojo *et al.*, (2006) and Atawodi and Ogunbosola, (2009) who reported that methanolic extracts of bark and leaves of *P. oblonga* contained alkaloids,

Table 1: Some phytochemical constituents of hydromethanolic leaf extract of *Prosopis oblonga*

Constituents	**Inference**
Alkaloid	0.34 ± 0.03
Saponins	1.56 ± 0.30
Flavonoids	1.25 ± 0.02
Anthraquinones	Not detected
Tannins	+
Volatile oil	+
Glycosides	0.57 ± 0.04
Steroids	Not detected
Saponin glycoside	++
Cardiac glycosides	++

Results are expressed as mean ± SEM

Table 2: Effect of leaf extract of *Prosopis oblonga* on fasting blood glucose in alloxan induced diabetic rats after 5 days of treatment

Treatment Group	Fasting blood glucose concentration (mg/dL)			
	Before Treatment			After treatment
	Day 0	Day 3	Day 5	Day 10
Normal control	65.5 ± 0.78	64.0 ± 2.70	67.00 ± 1.23	66.80 ± 0.76
Control + 750 mg/kg extract	65.3 ± 0.71	68.7 ± 0.73	69.20 ± 0.83	46.60 ± 1.39
Diabetic control	69.8 ± 1.92	143.7 ± 2.34	246.00 ± 3.07	397.20 ± 5.36
Diabetic + 250 mg/kg extract	67.4 ± 0.82	165.4 ± .028	250.80 ± 1.83[a]	147.40 ± 0.88[b]
Diabetic + 500 mg/kg extract	66.2 ± 0.64	155.5 ± 2.0	264.30 ± 3.31[a]	128.30 ± 1.91[b]
Diabetic +750 mg/kg extract	67.8 ± 0.92	134.2 ± 2.30	271. 00 ± 2.51[a]	117.50± 0.85[b]
Diabetic + mg/kg Glibenclamide	60.3 ± 1.20	131.2 ±13.17	238.80 ± 5.14[a]	168.60 ± 5.28[b]

Results are expressed as mean ± SEM (n = 5) a represents significant increase (P<0.05) when compared to diabetic control groups on day 5 while b represents significant decrease. (P<0.05) when compared to diabetic control groups on day 10.

Table 3: Effect of oral administration of leaf extract of *Prosopis oblonga* on the body weight of rats

	Weight (g)	
Treatment Group	**Before treatment**	**After treatment**
Normal control	185.00 ± 2.92[a]	201.00 ± 4.04[a]
Control + 750 mg/kg extract	168.00 ± 5.49[abc]	174.00 ± 4.93[b]
Diabetic control	176.00 ± 4.77[ab]	149.00 ± 11.39[c]
Diabetic + 250 mg/kg extract	184.00 ± 3.81[ab]	197.00 ± 1.30[a]
Diabetic + 500 mg/kg extract	167.00 ± 6.38[bc]	176.00 ± 6.92[b]
Diabetic + 750 mg/kg extract	158.00 ± 6.84[c]	172.00 ± 6.88[b]
Diabetic + 5 mg/kg Glibenclamide	180.00 ± 6.16[ab]	189.00 ± 1.64[ab]

Values are mean ± standard error of 5 replications (n = 5)
Means in a column with the same superscripts are not significantly different (P>0.05)

Table 4: Effect of leaf extract of *Prosopis oblonga* on serum biochemical parameters of control and diabetic rats

Treatment Group	**Urea (mg/dl)**	**Uric Acid (mg/dl)**	**Creatinine (mg/dl)**
Control	24.85 ± 0.69[g]	6.48 ± 0.26[e]	1.24 ± 0.12[c]
Control + 750 mg/kg extract	30.24 ± 0.95[f]	6.32 ± 0.31[e]	1.34 ± 0.18[c]
Diabetic control	62.34 ± 1.64[a]	18.00 ± 0.43[a]	2.85 ± 0.20[a]
Diabetic + 250 mg/kg extract	43.62 ± 1.07[d]	10.00 ± 0.59[c]	1.24 ± .08[c]
Diabetic + 500 mg/kg extract	47.00 ± 1.08[c]	10.28 ± 0.38[c]	1.78 ± 0.12[b]
Diabetic + 750 mg/kg extract	50.20 ± 0.95[b]	13.46 ± 0.53[b]	1.92 ± 0.03[b]
Diabetic + 5 mg/kg Glibenclamide(Ref drug)	37.21 ± 1.11[e]	8.58 ± 0.34[d]	1.56 ± 0.12[bc]

Values are mean ± standard error of 5 replications (n = 5)
Means in a column with the same superscripts are not significantly different (P>0.05)

Table 5: Effect of leaf extract of *Prosopis oblonga* on liver marker enzymes of control and diabetic rats

Treatment Group	**ALP (IU/L)**	**AST (IU/L)**	**ALT (IU/L)**
Animal control	102.30 ± 0.79^{g}	16.87 ± 0.21^{d}	23.37 ± 0.38^{e}
Control + 750 mg/kg extract	119.59 ± 1.37^{f}	18.20 ± 0.21^{d}	25.16 ± 0.29^{d}
Diabetic control	239.48 ± 2.97^{a}	30.20 ± 0.32^{a}	39.42 ± 0.66^{a}
Diabetic + 250 mg/kg extract	130.46 ± 1.16^{d}	20.00 ± 0.35^{c}	25.12 ± 0.39^{d}
Diabetic + 500 mg/kg extract	138.20 ± 1.71^{c}	20.27 ± 0.52^{c}	27.00 ± 0.80^{c}
Diabetic + 750 mg/kg extract	150.46 ± 0.68^{b}	22.75 ± 0.76^{b}	29.42 ± 0.50^{b}
Diabetic + 5 mg/kg Glibenclamide(Ref)	110.30 ± 2.58^{e}	18.10 ± 0.64^{d}	24.12 ± 0.37^{de}

Values are mean ± standard error of 5 replications (n = 5)
Means in a column with the same superscripts are not significantly different (P>0.05)

Table 6: Effect of leaf extract of *Prosopis oblonga* on lipid profiles of control and diabetic rats

Treatment Group	**Total Cholesterol (mg/dl)**	**Triglyceride (mg/dl)**	**HDL Cholesterol (mg/dl)**	**LDL Cholesterol (mg/dl)**
Control	63.69 ± 1.52^{e}	58.30 ± 0.55^{b}	92.10 ± 0.80^{c}	63.89 ± 0.51^{d}
Control + 750 mg/kg extract	28.65 ± 0.45^{f}	31.42 ± 0.49^{d}	50.46 ± 0.76^{e}	60.28 ± 0.76^{e}
Diabetic control	436.13 ± 7.63^{a}	190.21 ± 1.23^{a}	30.43 ± 0.27^{f}	313.45 ± 1.57^{a}
Diabetic + 250 mg/kg extract	190.26 ± 2.90^{b}	38.00 ± 0.56^{c}	87.64 ± 0.88^{d}	76.09 ± 0.75^{b}
Diabetic + 500 mg/kg extract	126.23 ± 1.27^{c}	20.89 ± 0.47^{f}	96.11 ± 0.26^{b}	70.11 ± 0.89^{c}
Diabetic + 750 mg/kg extract	98.28 ± 3.26^{d}	24.34 ± 0.69^{e}	101.26 ± 0.88^{a}	69.86 ± 1.67^{c}
Diabetic + 5 mg/kg Glibenclamide	90.12 ± 0.86^{d}	20.82 ± 0.58^{f}	100.43 ± 0.61^{a}	70.48 ± 0.51^{c}

Values are mean ± standard error of 5 replications (n = 5)
Means in a column with the same superscripts are not significantly different (P>0.05)

Table 7: Effect of 5000 mg/kg leaf extract of *Prosopis oblonga* on the body weight and mortality of rats

Test Sequence	Animal Identity	Dose (mg/kg)	Body weight			Short term outcome	Long term outcome
			Day 0	Day 7	Day 14		
1	01	5000	186	177	150	0	0
2	02	5000	150	140	122	0	0
3	03	5000	163	150	129	0	0
4	04	5000	145	136	120	0	0
5	05	5000	178	163	142	0	0

0 = means survival

Table 8: Mean weights of liver and kidney of rats after 28 days treatment with the leaf extract of *Prosopis oblonga*

Group Dose (mg/kg)	Weight of Liver (g)	Weight of Kidney (g)
Control	10.17 ± 0.26 [a]	1.39 ± 0.15 [a]
1000	9.11 ± 0.34 [b]	1.17 ± 0.11 [a]
2000	10.11 ± 0.37 [a]	1.06 ± 0.07 [a]
3000	10.13 ± 0.13 [a]	1.10 ± 0.10 [a]
4000	10.16 ± 0.30 [a]	1.21 ± 0.08 [a]

Values are mean ± standard error of 5 replications (n=5)
Means in a column with the same superscripts are not significantly different (P>0.05)

Table 9: Weekly body weight of rats treated for 28 days with leaf extracts of *Prosopis oblonga*

Group (mg/kg body weight)	Days				
	1	7	14	21	28
Control	186.09 ± 5.63[a]	186.58 ± 5.49[a]	188.90 ± 3.69[a]	189.43 ± 4.04[a]	193.13 ± 2.25[a]
1000	185.18 ± 2.37[a]	185.90 ± 3.00[a]	187.86 ± 1.18[a]	188.97 ± 1.82[ab]	189.00 ± 2.80[a]
2000	186.13 ± 2.86[a]	187.30 ± 3.16[a]	188.30 ± 2.33[a]	188.82 ± 1.16[ab]	189.17 ± 4.83[a]
3000	185.00 ± 2.39[a]	185.62 ± 3.37[ab]	185.72 ± 2.56[a]	185.72 ± 2.37[ab]	187.90 ± 1.28[a]
4000	186.80 ± 2.24[a]	187.74 ± 0.80[a]	185.83 ± 3.35[a]	180.68 ± 2.81[b]	176.54 ± 2.66[b]

Values are mean ± standard error of 5 replications
Means in a column with the same superscripts are not significantly different (P>0.05)

Table 10: Biochemical parameters of rats treated for 28 days with leaf extract of *Prosopis oblonga*

Biochemical Parameters	Group Dose (mg/kg)				
	Control	1000	2000	3000	4000
AST (IU/L)	21.82 ± 0.92[a]	23.14 ± 0.26[a]	22.30 ± 0.40[a]	23.74 ± 1.58[a]	23.46 ± 0.63[a]
ALT (IU/L)	14.46 ± 0.59[a]	15.82 ± 0.80[a]	16.07 ± 1.28[a]	16.53 ± 1.02[a]	17.55 ± 0.98[a]
ALP (IU/L)	62.04 ± 0.92[b]	64.71 ± 1.14[a]	67.41 ± 2.50[a]	66.31 ± 2.51[a]	65.60 ± 1.51[a]
TB (mg/dl)	0.61 ± 0.04[a]	0.67 ± 0.17[a]	0.65 ± 0.02[a]	0.63 ± 0.03[a]	0.69 ± 0.04[a]
TP (g/dl)	5.82 ± 0.56[b]	7.63 ± 0.50[a]	8.00 ± 0.45[a]	6.94 ± 0.46[ab]	6.52 ± 0.34[ab]
Albumin (g/dl)	4.17 ± 0.29[a]	4.42± 0.17[a]	4.34 ± 0.35[a]	3.96 ± 0.58[a]	4.37 ± 0.17[a]
Globulin (g/dl)	1.65 ± 0.12[b]	3.21 ± 0.25 [a]	3.66 ± 0.31[a]	2.98 ± 0.31[a]	2.15 ± 0.13[b]
Urea (mmol/L)	13.67 ± 0.58[a]	10.17 ± 0.30[b]	9.18 ± 0.40[b]	10.16 ± 0.34[b]	9.96 ± 0.51[b]
Creatinine	1.68 ± 0.07[a]	0.97 ± 0.14[b]	1.06 ± 0.10[b]	1.12 ± 0.05[b]	1.49 ± 0.11[a]
Na+ (mmol/L)	128.00 ± 2.00[c]	132.83 ± 1.77[bc]	133.40 ± 2.04[b]	140.25 ± 1.14[a]	142.22 ± 1.58[a]
K+ (mmol/L)	5.80 ± 0.32[a]	4.30 ± 0.15[b]	4.35 ± 0.14[b]	4.82 ± 0.10[b]	4.97 ± 0.28[b]

Values are mean ± standard error of 5 replications (n = 5)
Means in a row with the same superscripts are not significantly different (P>0.05)

saponins, tannins and flavonoids but in different concentrations. The differences in concentration could be attributed to different climatic conditions and soil type (Sofowora, 1993). The phytochemical result is also in agreement with the phytochemical contents of seeds of *P. oblonga* reported by Okide *et al* (2004). Literature has equally implicated the biological activities of alkaloids and flavonoids to include hypoglycaemia (Saidu *et al.,* 2007), hypolipidemia (Oladele *et al.,* 1995), hypoazotemia and hypotension among others (Sudheesh *et al.,* 2005). The presence of these phytoconstituents in *P. oblonga* leaf extract could be responsible for the hypoglycaemic and hypolidaemic properties observed in the present study.

The results of acute toxicity study indicated that the LD_{50} of the leaf extract of *P. oblonga* was greater than 5000 mg/kg body weight. The limit test dose is primarily used in situations where there is information indicating that the test material is likely to be non-toxic or of low toxicity (OECD, 2000). Thus, the lack of associated lethality with the high dose of this extract is an indication that the leaf extract of *P. oblonga* is relatively safe on acute oral exposure. These findings also agree with the LD_{50} value of 5000 mg/kg obtained in gum extract of *P. oblonga* (Okide *et al.,* 2004). From the result, there was no mortality at 5000 mg/kg body weight and this indicates that the leaf extracts of *P. oblonga* is non toxic. This is in agreement with Bruce (1985, 1987) and American Society for Testing and Materials (1987), that any chemical substance with LD_{50} estimate greater than 3000-5000 mg/kg (oral route) could be considered of low toxicity and safe. OECD (2001) also recommended the use of limit test dose with LD_{50} greater than 5000 mg/kg (oral route) as having low acute oral toxicity. This therefore implies that the leaf extract of *P. oblonga* is relatively safe.

In the sub-chronic study, the leaf extract of *P. oblonga* had no effect on the weight of the liver and kidney of the animals. This indicates that the organs weights of the treated rats were comparable with the normal rats. Also, the decrease in the body weight of the rats treated with 400 mg/kg body weight for 28 days suggest that the extract at a dose up to 4000 mg/kg body weight and above may cause lethargy, loss of appetite and anorexia. The reason for the absence of an effect on the body weight does at moderate doses of the extract is not clear, but could possibly be due to the different active phytochemicals present in the extract exhibiting dose specific effects. Since tannins have been reported to be responsible for most behavioural toxicities induced by medicinal plants (Muyibi *et al.,* 2000), this phytochemical and other biological principles may be responsible for the observed weight loss in the rats. However, at lower doses of the plant extracts, these behavioural toxicities were absent.

Plasma activities of liver biomarker enzymes, AST and ALT which when elevated are diagnostic of hepatic damage. Again, the renal function markers (serum creatinine and urea) which were increased in diabetic rats were inhibited after treatment with leaf extracts. It therefore implies that the extract at the doses used did not produce any harmful effects on the heart and liver tissues, revealing its potent antidiabetic activity. The findings are in agreement with the report by Saidu *et al* (2007), who observed significant reduction in plasma AST, ALP ALT activities of alloxan- induced diabetic rats. It is also in agreement with Shrabana *et al* (2003), who observed decreased plasma levels of creatinine, urea and uric acid by *Caesalpina bonducella* extracts in alloxan-induced diabetic rats.

Traditionally, various *in vivo* models (e.g. diazoxide, alloxan or streptozotocin-induced diabetic rats) are used in evaluating medicinal plants acclaimed to have hypoglycaemic potentials. This dose of 80 mg/kg body weight of alloxan established diabetes mellitus in the treated rats 5 days post-induction in the present study. Diabetes was fully established as evidenced by the significant elevation in the fasting blood glucose. The fasting blood glucose lowering effect of the plant extract was dose related and all the doses showed more potency than glibenclamide. This may suggest that the various phytochemicals such as flavonoids and alkaloids which are attributed with antidiabetic potential are acting at the doses used in the present study. Furthermore, solanine, an alkaloid found in *S. gilo* fruit (Rama and Narasimham, 1993) has been associated with improvement in the symptoms of diabetes mellitus (Gupta and Seth, 1962; Chatterjee,

1963). Marles and Farnsworth, (1996) have also reported flavonoids to stimulate secretion or possess an insulin like effect. The presence of alkaloids and flavonoids in high concentration in the leaf extracts of *P. oblonga* may also be responsible for the oral hypoglycaemic effects in the present study.

The hypoglycaemic effect of the *P. oblonga* leaf extract is an indication that the extract contains active principle(s) with potent hypoglycaemic property. In normal rats the extract could be acting via increased insulin secretion or increased peripheral utilization of glucose but in the *in vivo* type II diabetes model created in this study, the leaf extract lowered hyperglycaemia probably by increasing the peripheral utilization of glucose in the diabetic rats. Result of this study is in consonance with that given for gum extract of *P. oblonga* seeds in normoglycaemic and hyperglycaemic rats by Okide *et al* (2004).

Abnormalities in lipid profiles are one of the most common complications in diabetes mellitus. High levels of total cholesterol and more importantly LDL-cholesterol in blood are major cardiovascular risk factors (Okoli, 2010). Insulin deficiency causes an increase in free fatty acid mobilization from adipose tissues which results in increased production of cholesterol rich LDL particle and dyslipidaemia (Edwin *et al*., 2006). Several studies propose that most of the drugs that decrease total cholesterol also decrease HDL cholesterol. From this point of view, it is encouraging that *P. oblonga* extracts decreased the elevated TC levels after 5 days of treatment and also increased the HDL level which is commonly considered good cholesterol. An increase in triglyceride may be due to the lack of insulin under diabetic condition while insulin activates the lipoprotein lipase and hydrolyses TG under normal condition. In the present study also, treatment with the leaf extracts of *P. oblonga* effectively reduced triglycerides possibly by decreasing the non-esterified fatty acids.

The elevation of liver biomarker enzymes such as AST, ALT, and ALP in diabetic rats indicates hepatic damage (Rathod *et al.,* 2009). Rats treated with *P. oblonga* leaf extracts showed its ability to restore the normal functional status of the damaged liver.

From the present study, the levels of renal function markers such as serum creatinine, urea and uric acid which were increased in diabetic rats were reduced after treatment with leaf extracts, revealing that it exhibits potent antidiabetic activity.

Administration of alloxan causes decrease in glycogen content due to enhanced glycogenolysis, which is due to insulin deficiency (Dheer and Bhatnagar, 2010). So the normal capacity of the liver to synthesize glycogen is impaired. An increase in the liver glycogen following the administration of the leaf extract of *P. oblonga* may be due to an increase in the level of insulin.

In conclusion, this study showed that the leaf extract of *Prosopis oblonga* has potent oral hypoglycemic property and is safe thus justifying its ancestral use in the management of suspected type II diabetic patients. However, based on the findings of this study, we suggest for further studies, that the active antidiabetic biological constituent(s) of the leaf extract of *Prosopis oblonga* should be fractionated, isolated and characterized using chromatographic techniques and the mechanism of the hypoglycaemic activity of the plant elucidated.

Acknowledgement

The Authors are thankful to Department of Biochemistry, Usmanu Danfodiyo University, Nigeria, for providing research facilities to undertake this study.

References

Abbiw, D. (1990). *Useful plants of Ghana*. Intermediate Technology Publication and the Royal Botanical Bardins Kew. pp. 367- 89.

American Society for Testing and Materials (1987).Standard test method for estimating Acute Oral Toxicity in Rats. American Society for Testing and Materials E. 116387, Philadelphia U.S.A.

Antia, B. S., Okokon, J. E. and Okon, P. A. (2005). Hypoglacaemic activities of aqueous extract of *Persea Americana* Mill. Journal of Pharmacology. 37: 325- 326.

Andrew, J. K. (2000). *Diabetes.* Churchill Livingstone New-York. pp. 365

Atawodi, S. E. and Ogunbosola, F. (2009). Evaluation of antitrypanasomal properties of

extracts of leaves, stem and root barks of *Prosopis africana* in laboratory animals. Biokemistri. 21(2):101-108.

Balkau, B., Charles, M. A. and Eschawege, E. (2000). Epidemiological discourse on new criteria on diabetes. Molecular Endocrinology 2: 229-234.

Barar, F. S. K. (2004). *Essentials of Pharcotherapeutics*. 3rd ed. S Chand and Company Ltd. New Delhi. pp 279-298.

Barham, D. and Trinder, D. (1972). An improved colour reagent for the determination of blood glucose by the oxidase system. The Analyst **97**: 142 - 146

Barrette-Conner, E. L., Cohn, B. A., Wingard D. L. and Edestein, S. L. (1991). Why is diabetes mellitus a stronger risk factor for Fatal Ischaemic Heart Disease in Women than in men? The Ramcho Bernado Study. Journal of American Medical Association 265: 627-631.

Bruce, R. D. (1985). An up- and – down procedure for acute Toxicity Testing. Fundam. Applied Toxicology 5: 151-157.

Bruce, R. D. (1987). A confimatory study of up- and – down method of Acute Oral Toxicity Testing. Fundamental Applied Toxicology. 8: 97-100.

Burstein, M., Scholnick, A., Morin, R. (1979). Rapid method for iIsolation of lipoproteins from human serum by precipation with Polyanions. Journal of Lipid Research 11:583-595.

Bunyapraphatsara, N., Yongchaiyudha, S., Rungpitarangsi, V. and Chokechaijaroenporn, O. (1996). Antidiabetic activity of *Aloe vera* L. juice. II. Clinical trial in diabetes mellitus patients in combination with glibenclamide. Phytomedicine 3: 245-248.

Chattopardyhay, R. B. (2004). Effect of *Azadiracta indica* leaf extract on serum lipid profile change in normal and streptozotocin-induced diabetic rats. African Journal of Biomedical Research 8: 101-104.

Christopherson, B. C. (1991). *Traditional Medicine. A Potential Resource Exploitation of Natural Products*. The H-C Qrte Institute, Copenhagen. pp. 8- 12.

Dheer, R. and Bhatnagar, P. (2010). A Study of the antidiabetic activity of *Barleria prionitis* Linn. Indian Journal of Pharmacology 42: 70- 73.

Dixon, W. J. (1991). Staricase Bioassay. The up- and - down method. Neuroscience & Behaviour Review 15: 47-50.

Djomeni, D. D. Teddong, L. Asongalem, E. A., Dino T., Soken, S. D. and Kamtchouing, P. (2006). Hypoglycameic and anti-diabetic effects of root extract of *Ceiba pentabdra* in normal and diabetic rats. African Journal of Traditional, Complementary and Alternative Medicine 3 (1): 129-136.

Edwin, E. S., Heeja, E., Gupta, V. B. and Jain, D. C. (2006). Fight diabetes the herbal way. Express Pharmaceutical Review. 1:41- 42.

El-Olemyl, M. M., Al-Muhtadi, F. J. and Afifi, A. A. (1994). *Experimental Phytochemistry*. A Laboratory Manual. College of Pharmacy, King Saud University Pres UK, pp: 1-34.

Farnsworth, N. R. and Bingel, A. S. (1977). In: Wager, H and Wolf P. (eds.) *New Natural Products and Plant Drugs with Pharmacological, Biological and Therapeutical Activity*. Springer – Verlage, New York. pp. 678- 684.

Friedewald, W., Levy, R. and Fredrickson, D. (1972). Estimation of the concentration of low density lipoprotein. cholesterol in plasma without use of preparative ultracentrifuge. Clinical Chemistry. 18: 499-509.

Gornal, A. G., Bardwill, G. S. and David, M. (1949). Determination of serum proteins by means of Biuret reactions. Journal of Biological Chemistry. 177: 751-766.

Gupta, S. A. and Seth, C. B. (1962). Effects of *Momordica charantia* on glucose tolerance on albino rats. Indian Journal of Physiology and Pharmacology. 7: 240-244.

Harborne, J. B. (1973). *Phytochemical methods. A Guide to Modern Techniques of Plant Analysis* 3rd edition. Chapman and Hall, London. pp. 475.

Henry, R. J. (1974). Determination of serum creatinine. In: *Clinical Chemistry:* Harper and Row, pp. 525.

Hong, T. D., Linington, S. and Ellis, R. H. (1996). *Seed storage Behaviour*. A Compendium handbooks for gene banks, No. 41 PGRI.

Katende, A. B. (1995). *Useful trees and shrubs for Uganda*, Identification propagation and management for Agricultural and personal communities Regional Soil Conservation Unit (RSLU) Swedish International Development Authority (SIDA). pp. 145.

Lee-Rodkly, F. (1964). Binding of bromocresol green by human serum albumin. Archives of Biochemistry and Biophysics 108:510-513.

Marles, R. J. and Farnsworth, N. (1996). Antidiabetic plants and their active constituents: an update. Prot. Journal of Botany & Medicine. 1:135.

Mayers, N. (1982). *Readers Digest*. Vol 121, No 223. Readers Digest Association Inc. USA. pp. 24-128.

Morin, L. G. and Prox, J. (1973). Quantitative determination of uric acid in serum. American Journal of Clinical Pathology 60(5): 690-694.

Murray, M. (2000). *Encyclopedia of National Medicine* 2nd Edition. Prima Health Publishing Rockling, USA. pp. 401.

OECD. (1995). Repeated Dose, 28 days oral toxicity studies in rodents. In: Guidance document for the development of OECD guidelines for testing of chemicals. Ornamental monographs No 76.

OECD. (2000). Guidance Document on Acute Oral Toxicity Environmental Health and Safety Monograph on Testing and Assessment. No 24.

OECD. (2001). Guidelines for testing chemical, Acute Oral toxicities up and down procedure 425: 1-26.

Ogbonnia, S. O., Odimegwu, J. I. and Enwuru, V. N. (2008). Evaluation of hypoglycaemic and hypolidaemic effects of aqueous ethanolic extracts of *Treculia africana*, Decne and *Bryophyllum pinnatum* Lan and their mixture on streptozotocin-induced diabetic rats. African Journal of Biotechnology. 7(6): 2535-2539.

Ojo, O. O., Nadro, M. S. and Tella, I. O. (2006). Protection of rats by extracts of some common Nigerian trees against acetaminophen-induced hepatoxicity. African Journal of Biotechnology. 9: 755-760.

Okide, G. B., Udoh, U. E. and Ezugu, C. O. (2004). Hypoglycaemic activity of the *Prosopis africana* seed. Journal of Tropical Medicine, Plants **4** (1): 51-56.

Okoli, C. O., Ibiam, A. F., Ezike, A. C., Akah, P. A. and Okoye, Z. C. (2010). Evaluation of anti-diabetic potentials of *Phyllanthus nitiri* in alloxan diabetic rats. African Journal of Biotechnology. 9: 245-259.

Oladele, S. B., Ayo, J. O. and Adaudi, A. O. (1995). Medicinal and physiological properties of flavonoids, coumarins and anthraquinones of plant origin. West African Journal of Pharmacology and drug Research. 11: 134-144.

Onaogbe, I. O., Ebhota, A. O., Udegbe, H. C., Omondia, E., Edeni, D. and Egengbo, S. O. (1999). Assessment of some medicinal plant for hypoglycaemic activities in rats and rabbits. Bioscience Research Communication 11(12): 795-807.

Rama, M. V. and Narasimham, P. (1993). Fruits of the *Solanacea* in encyclopedia of food science. Food Technology and Nutrition 6: 3677- 3682.

Rang, M., Daniel, S. F. and Robert, S. B. (1999). Oral antidiabetic therapies for type 2 diabetes. American Alternative Medicine 137: 262-296.

Ranjna, C. (1999). *Practical Clinical Biochemistry Methods and Interpretation* 2nd edition. Academy Press. pp. 200- 240.

Rathod, N. R., Raghuveer, I., Chotine, H. R. and Chanda, R. (2009). Free radical scavenging activity of *Calotropis gigamtea* on streptozoticin induced diabetic rats. Indian Journal of Pharmaceutical Science 71: 615-621.

Reitman, S. and Frankel, S. (1957). Colorimetric method for the determination of serum transaminases. American Journal of Clinical Pathology 28: 56-61.

Rec, G. S. C. C. (1972). Optimised standard colorimetric methods (Serum alkaline phosphatase). Journal Clinical Chemistry and Biochemistry 10: 182.

Saidu. Y., Lawal, M., Isezuo, S. A., Shehu, R. A., Sahabi, D. M. and Bilbis, L. S. (2007). Partial purification and elucidation of mechanism of hypoglycaemic agents of aqueous leaf extract of *Albizza chevalieri* Harms (Leguminosae). Journal of Pharmacology and Toxicology 2(6): 513-523.

Shrabana, C., Tuhin, K. B., Begum, R., Liaquat, A., Mosihuzzamam, M., Nilufer, N., Azad, K. A. and Biwapat M. (2003). Advanced studies on the hypoglycaemic effect of *Caesalpina bonducella* F in type 1 and 2 diabetes in long Evans rats. Journal of Ethnopharmacology **84:** 41-46.

Sofowora, E. A. (1993). *Medicinal Plants and Traditional Medicine in Africa.* John Willey and Sons, New York. pp. 93- 106.

Sudheesh, S., Manital, V. B. and Vijayalakshmi, N. R. (2005). Potential health promoting effects of flavonoids: A comparative study on hypolipidaemic and hypoglcaemic activities. In: Abstract of Posters, final programme and book of abstract, 53rd Annual meeting of the society medical plant research (GA) and joint congress with the Italian society of photochemistry (SIF), Florence Italy. pp. 179.

Tanko, Y., Okasha, M. A., Magaji, G. M., Yerima, M., Yaro, A. H., Saleh, M. I. A. and Mohammed, A. (2008). Anitdiabetic properties of *Secureinega virosa* (Euphorbiaceae) leaf extract. African Journal of Biotechnology 7(1): 22-24.

Trinder, P. (1969). Determination of total cholesterol. Annals of Clinical Biochemistry 6: 24-27.

Trease, G. E. and Evans, W. C. (1999). *Textbook of Pharmacognosy*, 14th ed. W. B. Saunders Ltd. London. pp. 341- 344.

Vogt, K. (1995). *A Field Guide to the Identification Propagation and Uses of Common Trees and Shrubs of Dry Land.* SUDAN. SOS (Sahel of Savannan). pp. 67-80.

Wambebe, K.., Gamaniel, J. E., Awodogun, A. C. and Okogun, J. I. (1996). Acute and short term toxicity of Niprissan in rats: A Biochemical Study. Journal of Pharmaceutical Research and Development 1: 36- 49.

WHO. (1999). Diabetes mellitus fact sheet Number 138 and 236 Geneva.

Wilson, P. W., Agostino, R. B., Levy, D., Belanger, A. M., Silvershatz, H. and Kannel, W. B. (1998). Prediction of Coronary Heart Disease. American Journal of Kidney Disease 32: 589-595.

Wybenga, D. R. D., Glorgia, J. M. and Pileggi, V. J. (1971). Determination of serum urea by diacetyl monoxime method. Clincial Chemistry 17: 891-895.