Journal of Medical Sciences

ISSN 1682-4474

science alert

ANSInet
an open access publisher
http://ansinet.com

Research Paper

J. Med. Sci., 13 (8): 700-707
15th November, 2013
DOI: 10.3923/jms.2013.700.707

Hypoglycemic effects of Aqueous and Methanolic Leaf Extracts of *Vitex doniana* on Alloxan Induced Diabetic Albino Rats

[1]Nwogo Ajuka Obasi, [2]Kalu Mong Kalu,
[3]Uchechukwu Okorie and [3]Glory Otuchristian

The hypoglycemic effects of aqueous and methanolic leaf extract of *Vitex doniana* were evaluated in normal and alloxan-induced diabetic rats using standard analytical protocols. The potency of the leaf extract was compared with that of oral anti-diabetic agent (Glibenclamide). A total of 35 albino rats divided into seven groups of five rats each comprising one normal untreated group as animal control, one diabetic untreated group as diabetic control, one normal treated with 750 mg kg^{-1} b.wt. as reference group, three diabetic groups treated with 250, 500 and 750 mg kg^{-1} body weight respectively and one diabetic group treated with 5 mg kg^{-1} Glibenclamide as standard. The result of acute toxicity test obtained indicated lethal dose (LD_{50}) of greater than 5000 mg kg^{-1} extract. Results indicated that administered doses of 250, 500 and 750 mg kg^{-1} body weight of the leaf extract showed significant ($p<0.05$) reduction of blood glucose level, renal function markers (serum creatinine, urea and uric acid) and serum electrolytes (Potassium and sodium ions) in a dose dependent manner in reference and diabetic groups when compared to normal and diabetic control groups, respectively. The results also indicated that administration of the extracts caused a significant ($p<0.05$) increased in the serum total proteins and albumin levels and a significant ($p<0.05$) decrease in the total bilirubin levels in the diabetic rats. These results imply that the leaf extract is relatively safe and could be used in the management of diabetes mellitus and associated complications.

Key words: *Vitex doniana*, serum glucose level, serum electrolytes, renal function markers, diabetes, lethal dose

[1]Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria
[2]Department of Animal and Environmental Biology, Abia State University, Uturu, Nigeria
[3]Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic Unwana, Afikpo, Ebonyi State, Nigeria

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

For further information about this article or if you need reprints, please contact:

N.A. Obasi
Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria

Tel: +234703 336 4146

ANSInet
Asian Network for Scientific Information

INTRODUCTION

The use of plants in ethno-medicine is as old as man (Sofowora, 1993). The study and the use of medicinal properties of plants and plants extracts are called herbalism. It is also known as phytotherapy or botanical medicine (Deepak and Anshu, 2008). Diabetes mellitus 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).The integration of metabolic pathways is highly impaired in Diabetes mellitus and this leads to symptomatic effects in other tissues. The causes of diabetes mellitus varies according to the type and each present symptoms that may reflect complications related to acidosis and related pathologies. WHO (1999) report showed that approximately 140 million people worldwide suffer from diabetes and that the worldwide incidence is 5% in the general population.

Vitex doniana belongs to the family verbenaceae and it is widely distributed in the tropical countries of Africa like Nigeria. The plant commonly called Black plum (English), Dinya (Hausa), Oriri (Yoruba) and Ucha koro (Igbo), is a deciduous ever green tree, usually 4-8 metres high occasionally up to 15 metres with a dense rounded crown. The tree has light grey bark with branches which are not hairy. *Vitex doniana* leaves are broad and long stalked and contain 5-7 leaflets (NNMDA, 2008). The bark of the stem, the leaves and the roots of the plant are used in ethno-medicine for the management and treatment of numerous disorders such as microbial infection, cancer, rheumatism, hypertension and inflammatory diseases (Atawodi, 2005).

There are a number of reports on the chemistry of *Vitex doniana,* these reports described the occurrence of several groups of organic compounds in different parts of the plant collected from various geographical locations. But the importance/biological activities of most of these compounds have not been reported. Atawodi and Ogunbosola (2009) reported that the methanolic and aqueous extracts of the leaf of the plant contained saponin, tannins, flavonoids, anthraquinones, cardiac glycosides and terpenoids. In Nigeria, the leaves of *Vitex doniana* has been used in ethno-medicine for the treatment of various diseases such as ulcer, broad spectrum infection and diabetes mellitus (Atawodi and Ogunbosola 2009; Mohammed *et al*., 2006). Therefore, there is need to access not only the potency of this medicinal plant but also to evaluate the wholesomeness. Thus, the aim of this research is to assess the antidiabetic and other medicinal potentials of *Vitex doniana* leaf extract on normal and alloxan-induced diabetic rats.

MATERIALS AND METHODS

Sources of plant material: Leaves of *Vitex doniana* were collected from a nearby farm-land in Unwana, Afikpo North, Ebonyi State and authenticated by the Akanu Ibiam Federal Polytechnic Unwana curator, Dr. M.C. Okafor (Taxonomists) of the Department of Science Laboratory Technology.

Preparation of the extract: The leaves were air dried and grinded to powder using electric blender. Exactly 2.0 kg of the powder sample were soaked in 2.0 L of methanol for 48 h at room temperature. Removal of the solvent from the extract under reduced pressure yielded 200 g (10%) of a dark green residue. This residue was put in hexane to remove its hydro-insoluble component. Following filtration through Whatman No. 42 filter paper, the filtrate was again dissolved in ethyl acetate. The final residue obtained (neither soluble in hexane nor ethyl acetate) constitute the methanolic residue extract of *V. doninana.* The purpose of this extraction procedure was to obtain a hydrosoluble organic extract which was closer to aqueous extract normally used traditionally. This extract was solubilized in distilled water prior to administering to the experimental animals.

Experimental animals: Male albino wistar rats of same age group and body weight between 150-200 g were selected for all the experiments. The rats were obtained from the animal facility units of university of Nigeria, Nsukka. They were kept and maintained under standard laboratory conditions of temperature, humidity in a wooden cage, 12 h day, 12 h night cycle and allowed access to rat feed (Grower Pellet of Royal feeds, Enugu) and clean water *ad libitum.*

Acute oral toxicity studies (LD_{50}): The acute oral toxicity study was conducted using the limit doses test of up and down procedure according to organization for economic and cultural development (OECD, 2001; Dixon, 1991). Five groups of rats (one rat in each group) were used for this experiment. They were housed individually after random selection and were allowed to acclimatize to the laboratory conditions for five days. A limit dose of 5,000 mg kg^{-1} body weight of the extract was used. Animals were dosed one at a time and observed at least during the first 24 h and then for another 24 h (observation time 48 h) and thereafter for a total of 14 days. At the expiration of initial 48 h, four additional animals were subsequently dosed and observed as previously described. Animals were observed for signs of acute toxicity morbidity and mortality. The behavioral

changes and other changes observed in the experimental rats were recorded according to OECD (2001) 425 guidelines.

Induction of diabetes: The rats were acclimatized for seven days and diabetes were induced by intraperitoneal administration of aqueous alloxan monohydrate (80 mg kg^{-1}) to a group of overnight fasted rats but were not deprived of water. After three days, the blood glucose level of the animals was checked using a glucometer (a one touch test strip) to ascertain a diabetic state. Depending upon their blood glucose levels, the rats were divided into seven groups with five rats each. Rats were kept for 15 days before the beginning of the experiment to stabilize the diabetic conditions and to allow complications to appear.

Test for diabetic activity: In the experiment, a total of 35 rats (25 diabetic surviving rats, 10 normal rats) were divided into seven groups as shown on Table 1.

Preparation of blood serum and assays: The rats were sacrificed by decapitation after mild chloroform anaesthesia, 24 h after the last treatment. The blood samples obtained were collected using sterilized syringe and needle into test tubes and centrifuged at 3000 rpm for 5 min, the sera collected were used for biochemical analysis.

Biochemical analysis: Serum glucose levels were determined in rats using a method described by Tietz (2000). Creatinine in alkaline solution reacts with picrate to form a coloured complex and this was the basis of creatinine determination according to the method of Henry (1974). Urea was determined by the method of Wybenga *et al.* (1971). Serum total protein was determined spectrophotometrically by the method of Gornall *et al.* (1949). Serum albumin was determined spectrophotometrically using the Bromocresol green (BCG) method of Rodkey (1964). Serum uric acid was determined spectrophotometrically by the method of Morin and Prox (1973). Total serum bilirubin was determined using a modified method outlined by Tietz (2000). Spectrophotometric determination of serum albumin levels was carried out based on a modified method outlined by Tietz (2000). Serum sodium (Na^+) and potassium (K^+) ions concentrations were determined using flame photometric method outlined in Ranjna (1999).

Statistical analysis: All data obtained were expressed as Mean±SEM (standard error of mean). 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 at $p<0.05$. All statistical analysis were carried out using the instat statistic package (instat software, Ver 16.0, Sandiago USA).

RESULTS

Acute oral toxicity studies: Response and effect on the body weight of rats treated for 14 days with 5000 mg kg^{-1} leaf extract of *V. doniana* is shown in Table 2. The results showed that the high doses induced progressive and sustained weight loss from 197, 168, 165, 154 and 187 g at the 0 day to 161, 141, 137, 133 and 158 g, respectively after 14 days of oral administration (Table 2). The results showed that all rats treated with the 5000 mg kg^{-1} limit dose of the leaf extract were hypo-reactive to external stimuli such as touch in the first 30 min to 1 h post administration and subsequently became active and exhibited normal behaviour throughout the 14 days observation period. The limit test does of 5000 mg kg^{-1} did not cause any mortality or any major acute toxicity. The LD_{50} of the leaf extracts using the OECD (2001) guidelines is therefore greater than 5000 mg kg^{-1} because there was no death at all during or after the administration.

Antidiabetic study: Effect of oral administration of *V. doniana* leaf extract and Glibenclamide (the reference drug) on body weights of rats is presented in Table 3. The results indicated significant ($p<0.05$) weight loss in diabetic control rats from 175 to 151 g. Significant ($p<0.05$) weight gain was also observed in normal control (from 193 to 202.4 g), normal control+750 mg kg^{-1}

Table 1: Different group of rats treated with different dose of the extract

Groups	Treatments
1	Normal untreated rats (animal control)
2	Normal rats given only the extract (750 mg kg^{-1} body weighty) in aqueous solution daily by oral administration for 5 consecutive days
3	Diabetic untreated rats (diabetic control)
4	Diabetic rats given extract (250 mg kg^{-1} body weight) in aqueous solution daily by oral administration for 5 consecutive days
5	Diabetic rats given extract (500 mg kg^{-1} body weight) in aqueous solution daily by oral administration for 5 consecutive days
6	Diabetic rats given extract (750 mg kg^{-1} body weight) in aqueous solution by daily oral administration for 5 consecutive days
7	Diabetic rats given daonil (5 mg kg^{-1} body weight) in aqueous solution by daily oral administration for 5 consecutive days

(from 179 g to 182.6 g) and experimental diabetic groups that were administered with the plants extract and Glibenclamide (Table 3).

Table 4 showed the effects of leaf extract of *V. doninaa* on fasting blood glucose in Alloxan-induced diabetic rats. Intraperitoneal administration of Alloxan, induced significant ($p< 0.05$) progressive hyperglycaemia in the treated rats between the 3rd and 5th day of the experiment but diabetic mellitus became fully established in all the rats on the 5th day post-induction. However, the results indicated that daily oral administration of 250-750 mg kg day^{-1} of leaf extract of *V. doniana* to the diabetic rats has induced a significant reduction ($p<0.05$) of blood glucose in a dose-dependent manner (Table 4) when compared to untreated control (Diabetic control). The antidiabetic effect of Glibenclamide was similar but less significantly ($p<0.05$) different when compared to the various doses of the leaf extract. The effects of *Vitex doniana* on serum biochemical is shown in Table 5. There was a significant ($p<0.05$) increase in serum glucose of the untreated diabetic group compare to the control group (Table 5). Administration of the extracts caused a significant ($p<0.05$) increased in the total protein and albumin levels in the diabetic rats. There was a significant ($p<0.05$) increase in total bilirubin levels in diabetic control group over the normal control. Administration of the extracts caused a significant ($p<0.05$) decrease in the billrubin levels in the diabetic rats. The results indicated that the level of renal function markers (serum creatinine, urea and uric acid) and serum electrolytes which increased in diabetic rats were significantly ($p<0.05$) lowered after treatment with the varying doses leaf extracts (Table 6).

Table 2: Response and effect on the body weight of rats treated for 14 days with 5000 mg kg^{-1} leaf extract of *Vitex doniana*

			Body weight (g)			
			Day			Short
Test sequence	Animal identity	Doses (mg kg^{-1})	0	7	14	term outcome
1	01	5000	197	184	161	0
2	02	5000	168	153	141	0
3	03	5000	165	148	137	0
4	04	5000	154	146	133	0
5	05	5000	187	176	158	0

NB: 0 = Mean death

DISCUSSION

Acute toxicity studies: The results of acute toxicity study indicated that the LD_{50} of the leaf extract of *V. doniana* is greater than 5000 mg kg^{-1} body weight. The limit test dose is primarily used in situations where the experimenter has information indicating that the test material is likely to be non-toxic or of low toxicity (OECD, 2001). Thus, the non-lethal effects produced with the high dose of this extract are an indication that the leaf extracts of *V. doniana* is relatively safe on acute oral exposure. It can therefore be concluded that *V. doniana* leaf extracts is non-toxic which is in agreement with Bruce (1987) and American Society for Testing and Materials (1987), that any chemical substance with LD_{50} estimate greater than 3000-5000 mg kg^{-1} (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^{-1} (oral route) as having low acute oral toxicity. This therefore implies that the leaf extract of *V. doniana* is relatively safe.

Antidiatbetic study: The observed significant decrease in the mean body weight in diabetic control rats may be

Table 3: Effect of oral administration of leaf extract of *Vitex doniana* and Glibenclamide on body weight of rats after 28 days

	Weight (g)	
Treatment groups	Before treatment	After treatment
Normal control	193.00±0.15^{a}	202.40±1.04^{a}
Control+750 mg kg^{-1} extract	179.00±1.49^{abc}	182.60±0.93^{b}
Diabetic control	175.00±1.07^{ab}	151.00±1.39^{c}
Diabetic+250 mg kg^{-1} extract	188.00±0.81^{ab}	201.10±0.30^{a}
Diabetic+500 mg kg^{-1} extract	170.00±1.38^{bc}	182.50±0.92^{b}
Diabetic+750 mg kg^{-1} extract	167.00±0.84^{c}	179.00±1.18^{b}
Diabetic+5 mg kg^{-1} Glibenclamide (reference drug)	182.50±1.16^{ab}	194.80±1.04^{ab}

Values are mean±standard error of 3 replications, Means in a column with the same superscripts are not significantly different ($p<0.05$)

Table 4: Effect of 5 days alloxan-induced diabetes and 5 days oral administration of leaf extract of *V. doniana* on fasting blood glucose of rats

	Fasting blood glucose conc. (mg dL^{-1})			
	Before oral administration (day)			After oral administration
Treatment group	0	3	5	day 10
Normal control	66.80± 0.16^{a}	67.90± 1.70^{a}	67.80± 0.12^{a}	67.85±0.65^{b}
Control+750 mg kg^{-1} extract	67.00±0.11^{a}	68.10±53.0^{a}	68.00±0.71^{a}	43.90±1.20^{a}
Diabetic control	66.50±1.00^{a}	168.80±0.25^{b}	251.00±1.02^{bc}	263.60±1.84^{g}
Diabetic+250 mg kg^{-1} extract	66.90±0.12^{a}	170.20±0.18^{bc}	252.50±0.85^{bc}	152.80±1.10^{e}
Diabetic+500 mg kg^{-1} extract	67.05±0.06^{a}	171.30±1.40^{bc}	252.60±1.07^{bc}	125.00±0.86^{d}
Diabetic+750 mg kg^{-1} extract	66.70±1.30^{a}	168.50±1.17^{b}	250.80±1.13^{b}	109.70±1.30^{c}
Diabetic+5 mg kg^{-1} glibenclamide (reference drug)	66.60±0.20^{a}	168.10±1.70^{b}	250.20±1.04^{b}	172.50±1.95^{f}

Values are mean±standard error of 3 replications, Means in a column with the same superscripts are not significantly different ($p<0.05$)

ascribed to the reduction in utilization of food and fluid as well induction of hyperglycemia has been linked to weight loss (Tanko *et al*., 2013; Mahmood *et al*., 2011; Ezekwesili *et al*., 2008). The rats in experimental groups recorded significant ($p<0.05$) weight gains compared to diabetic control groups (Table 3). The *Vitex doniana* leaf extracts may have appetite-and digestion-stimulating properties which may be ultimately to a greater efficiency in the utilization of feed, resulting in enhanced growth. This speculation can be supported with numerous reports which attribute increase in mean body weight of animals fed plant extracts with increase in utilization of food and fluid (Tanko *et al*., 2013; Mahmood *et al*., 2011).

The use of plants in the treatment of diseases and in particular diabetes mellitus is as old as man (Mohammed *et al*., 2006; Grover *et al*., 2000; Sofowora, 1993). This is because plants have been shown to contain some potent bioactive compounds with antidiabetic properties (Tanko *et al*., 2013; Chakrabarti *et al*., 2003; Onaogbe *et al*., 1999). In this study, diabetes established on the basis of fasting blood glucose concentration in the treated rats on the 5th day of the experiment formed baseline values (Table 4). The results indicated that daily oral administration of reference drug and *Vitex doniana* leaf extracts for 5 consecutive days significantly ($p<0.05$) lowered the fasting blood glucose and in dose dependent manner with the *Vitex doniana* leaf extracts (Table 4). The fasting blood glucose lowering effect of the plant extract was dose dependent and the entire dose showed more potency than Glibenclamide (reference drug). The observed anti-diabetic effect of the *V. doniana* leaf extract is an indication that the extract contain bioactive phytochemicals with potent antidiabetic property. This results compared favourably with the fasting blood glucose lowering effects of *Barleria prionitis* (Dheer and Bhatnagar, 2010), *Withania coagulans* Dunal (Jaiswal *et al*., 2009), *Dorstenia picta* (Florence *et al*., 2007) and *Ceiba pentabdra* (Djomeni *et al*., 2006) among others. 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 may have lowered the concentration of fasting blood glucose level probably by increasing the peripheral utilization of glucose in the diabetic rats (Santaguida *et al*., 2008; Kane *et al*. 2005; Gholap and Kar, 2004). Some bioactive constituents of plants such as flavonoids have been reported to stimulate secretion or possess insulin like effect (Marles and Farnsworth, 1996). This might account for the antidiabetic properties exhibited by this studied plant.

The level of renal function markers (serum creatinine, urea and uric acid) which increased in diabetic rats were significantly reduced after daily oral administration of with leaf extracts (Table 6), revealing that it exhibits potent anti-diabetic activity. Increase in urea level in diabetes may be attributed to enhanced catabolism of both liver and plasma proteins that accompany glyconeogenesis (Khushk *et al*., 2010). Serum creatinine level decreased in the alloxan-induced diabetic rats and this observation compared favourably with reports from similar works (Khushk *et al*., 2010). However, reports have shown that cretininaemia may be attributed to extensive level of muscle breakdown that may result from poorly controlled diabetes mellitus (Gnanong, 2005). So, observation from this study showed that the impairement in the liver functions caused by the induction of the diabetes may be restored by oral administration of the *Vitex doniana* leaf extracts whose bioactive constituents may act by increasing the level of insulin in the rats (Dheer and Bhatnagar, 2010).

Table 5: Effect of leaf extract of *V. doniana* on fasting blood glucose in diabetic rats 5 days before and 5 days after treatment

	Fasting blood glucose conc. (mg dL^{-1})	
Treatment group	Before oral administration	After oral administration
Normal control	67.80±0.12^{a}	67.85±0.65^{b}
Control+750 mg kg^{-1} extract	68.00±0.71^{a}	43.90±1.20^{a}
Diabetic control	251.00±1.02^{bc}	263.60±1.84^{g}
Diabetic+250 mg kg^{-1} extract	252.50±0.85^{bc}	152.80±1.10^{e}
Diabetic+500 mg kg^{-1} extract	252.60±1.07^{bc}	125.00±0.86^{d}
Diabetic+750 mg kg^{-1} extract	250.80±1.13^{b}	109.70±1.30^{c}
Diabetic+5 mg kg^{-1} Glibenclamide (reference drug)	250.20±1.04^{b}	172.50±1.95^{f}

Values are mean±standard error of 3 replications, Means in a column with the same superscripts are not significantly different ($p<0.05$)

Table 6: Effect of leaf extract of *V. doniana* on serum biochemical parameters of control and diabetic rats

Treatment group	Urea (mg dL^{-1})	Uric acid (mg dL^{-1})	Creatinine (mg dL^{-1})	Total protein (mg dL^{-1})	Albumin (g dL^{-1})	Total bilirubin (mg dL^{-1})	Na^{+} (mmol L^{-1})	K^{+} (mmol L^{-1})
Animal control	25.14±0.20^{c}	6.20±0.91^{c}	1.23±0.13^{b}	6.62±0.56^{d}	9.17±0.29^{d}	1.85±0.12^{c}	138.70±0.20^{b}	4.86±0.23^{e}
Control+750 mg kg^{-1} extract	23.10±0.05^{a}	5.43±0.27^{a}	1.19±0.15^{a}	6.88±0.41^{f}	10.26±0.32^{f}	1.68±0.18^{a}	129.35±0.15^{a}	4.05±0.07^{a}
Diabetic control	39.10±0.13^{e}	8.41±1.95^{e}	2.47±0.20^{e}	4.35±0.20^{a}	6.10±0.19^{a}	4.18±0.10^{f}	144.20±0.05^{d}	5.80±0.17^{f}
Diabetic+250 mg kg^{-1} extract	33.62±0.42^{d}	7.00±0.61^{d}	1.89±0.07^{d}	5.77±0.08^{b}	7.15±0.12^{b}	2.96±0.17^{e}	140.50±0.10^{c}	4.50±0.30^{d}
Diabetic+500 mg kg^{-1} extract	28.23±0.22^{d}	6.78±1.48^{cd}	1.31±0.03^{c}	6.65±0.11^{d}	9.18±0.33^{d}	2.05±0.11^{d}	138.30±0.35^{b}	4.32±0.05^{c}
Diabetic+750 mg kg^{-1} extract	24.23±0.13^{b}	5.48±1.48^{b}	1.25±0.03^{b}	6.76±0.10^{e}	10.12±0.23^{e}	1.74±0.18^{b}	131.65±0.23^{a}	4.22±0.12^{b}
Diabetic+5 mg kg^{-1} Glibenclamide (reference drug)	28.59±0.15^{d}	6.90±0.29^{d}	1.34±0.17^{bc}	6.55±0.05^{c}	8.14±0.15^{c}	1.98±0.52^{d}	139.80±0.08^{c}	4.42±0.24^{d}

Values are Mean±standard error of 5 replications (n = 5), Means in a column with the same superscripts is not significantly different ($p<0.05$)

Serum total protein and albumin level also decreased significantly ($p<0.05$) in diabetic control group compared to normal control group (Table 6). This observation may be attributed to numerous effects of hyperglycemia in the alloxan-induced diabetes. Hyperglycemia increases gluconeogenesis and as such leads to excess protein breakdown as well as excess loss of nitrogen resulting in negative nitrogen balance (Murray *et al*., 2000). A decline in total serum protein level in diabetics have been attributed to inhibition of oxidative phosphorylation which leads to decrease in protein synthesis, increase in catabolic processes and reduction in protein absorption (Yassin *et al*., 2004). The results showed that administration of the extracts caused a remarkable increased in the serum total proteins and albumin levels in the diabetic rats (Table 6). These observations may be due to the presence of some compounds which help in provision of a reserved store of protein (Nsirim, 1999). There was an increase in total bilirubin levels in diabetic control group. The results showed that oral administration of the leaf extract significantly ($p<0.05$) lowered the total bilirubin levels in the diabetic rats in a dose dependent manner (Table 6). This observation indicates that hyperglycemia can enhance protein glycation. The increased total bilirubin level is an indication that excess haemoglobin is being destroyed or that there is impairment in the liver function relative to haemoglobin. (Rafighi *et al*., 2011; Andullu and Vardacharyulu, 2001).

From the present study, the level of serum electrolytes (Na^+ and K^+) which increased in diabetic rats were significantly ($p<0.05$) reduced after oral administration of diabetic rats with leaf extracts (Table 6), revealing that it exhibits potent anti-diabetic activity. Electrolytes play an important role in many body processes, such as controlling fluid levels, acid-base balance (pH), nerve conduction and blood clotting and muscle contraction. Electrolyte imbalance resulting from kidney failure, dehydration and fever and vomiting has been suggested as one of the contributing factors toward complications observed in diabetes and other endocrine disorders (Rao, 1992). Diabetes is characterized by increased volume and metabolites excretions via the kidneys, usually in excess of normal thresholds. These usually give rise to derangements in homeostatic balance with respect to electrolytes (Tanko *et al*., 2013).

CONCLUSION

Results of this study showed the safety and wholesomeness of *Vitex doniana* plant leaf extract and that oral administration of the extract exhibited potent hypoglycemic properties with high biochemical and clinical significance. This study therefore, justifies the already traditional practices of using the leaf extract for the treatment of diabetes since it is not only safe as shown in the acute toxicity study conducted but also antidiabetogenic. Further work is necessary to isolate the bioactive constituents in this plant leaf for enhanced phytotherapy.

REFERENCES

American Society for Testing and Materials, 1987. Standard test method for estimating acute oral toxicity in rats. American society for testing and materials E 1163 87, Philadelphia, USA., pp: 55-62.

Andullu, B. and N.C. Vardacharyulu, 2001. Effect of mulberry leaves on diabetes. Int. J. Diabetes Dev. Countries, 21: 147-151.

Atawodi, S.E., 2005. Comparative *In vitro* trypanocidal activities of petroleum ether, chloroform, methanol and aqueous extracts of some Nigerian savannah plants. Afr. J. Biotechnol., 4: 177-182.

Atawodi, S.E. and F. Ogunbusola, 2009. Evaluation of anti-trypanosomal properties of four extracts of leaves, stem and root barks of *Prosopis africana* in laboratory animals. Biokemistri, 21: 101-108.

Balkau, B., M.A. Charles and E. Eschwege, 2000. Epidemiological discourse on new criteria on diabetes. Mol. Endocrinol., 2: 229-234.

Bruce, R.D., 1987. A confirmatory study of up-and-down method of acute oral toxicological testing. Fundament. Applied Toxicol., 8: 97-100.

Chakrabarti, S., T.K. Biswas, B. Rokeya, L. Ali and M. Mosihuzzaman *et al*., 2003. Advanced studies on the hypoglycemic effect of *Caesalpinia bonducella* F. in type 1 and 2 diabetes in long evans rats. J. Ethnopharmacol., 84: 41-46.

Deepak, A. and S. Anshu, 2008. Indigenous Herbal Medicines: Trial Formulations and Traditional Herbal Practices. Harishkar Publishers Distributor, Jaipur, India, pp: 440-444.

Dheer, R. and P. Bhatnagar, 2010. A study of the antidiabetic activity of *Barleria prionitis* Linn. Ind. J. Pharm., 42: 70-73.

Dixon, W.J., 1991. The up-and-down method. Neurosci. Biohehav. Rev., 15: 47-50.

Djomeni, P.D.D., L. Tedong, E.A. Asongalem, T. Dimo, S.D. Sokeng and K. Pierre, 2006. Hypoglycaemic and antidiabetic effect of root extracts of *Cebia pentandra* in normal and diabetic rats. Afr. J. Trad. Compl. Altern. Med., 3: 129-136.

Ezekwesili, C.N., O. Obidoa and O.F.C. Nwodo, 2008. Effects of ethanol extract of *Acalypha torta* leaves on the lipid profile and serum electrolytes of rabbits. Niger. J. Biochem. Mol. Biol., 23: 15-19.

Florence, N.T., D. Theophile, D.D.P. Desire, V. Bertin and D. Etienne *et al*., 2007. Antidiabetic activities of methanol-derived extract of *Dorstenia picta* twigs in streptozotocin-induced diabetic rats. Asian J. Trad. Med., 2: 140-148.

Gholap, S. and A. Kar, 2004. Hypoglycaemic effects of some plants extracts are possibly mediated through inhibition of corticosteroid concentration. Pharmazie, 59: 876-878.

Gnanong, W.F., 2005. Endocrine Functions of the Pancreas and Regulation of Carbohydrate Metabolism. In: Review of Medical Physiology, Gnanong, W.F. (Ed.). McGraw Hill, New York, USA., pp: 333-355.

Gornall, A.G., C.J. Bardawill and M.M. David, 1949. Determination of serum proteins by means of biuret reaction. J. Biol. Chem., 177: 751-766.

Grover, J.K., S. Yadav and V. Vats, 2000. Medicinal plants in India with anti-diatbetic potential. J. Ethnopharmacol., 81: 81-100.

Henry, R.J., 1974. Determination of Serum Creatinin. In: Clinical Chemistry: Principles and Techniques, Henry, R.J. (Ed.). Harper and Row, London, UK., pp: 525-530.

Jaiswal, D., P.K. Rai and G. Watal, 2009. Antidiabetic effect of *Withania coagulans* in experimental rats. Indian J. Clin. Biochem., 24: 88-93.

Kane, M.P., A. Abu-Baker and R.S. Busch, 2005. The utility of oral diabetes medications in type 2 diabetes of the young. Curr. Diabetes Rev., 1: 83-92.

Khushk, I., M.U. Dahot, S.A. Baloach and M.A. Bhutto, 2010. The evaluation of soybean extracts in alloxan-induced diabetic rabbits. World Applied Sci. J., 8: 22-25.

Mahmood, S., A. Talat, S. Karim, R. Khurshid and A. Zia, 2011. Effect of cinnamon extract on blood glucose level and lipid profile in alloxan induced diabetic rats. Pak. J. Physiol., 7: 13-16.

Marles, R.J. and N. Farnsworth, 1996. Antidiatic Plants and their active constituents an update. Prot. J. Bot. Med., 1: 135-139.

Mohammed, B., Z. Adderrahim, M. Hassane, T. Abdulhafid and L. Abdulkhahaleg, 2006. Medical plants with potential antidiabetic activity. A review of ten year herbal medicine research (1999-2009). Int. J. Diabetes Metab., 14: 1-25.

Morin, L.G. and J. Prox, 1973. Quantitative determination of uric acid in serum. Am. J. Clin. Pathol., 60: 690-694.

Murray, M., 2000. Encyclopedia of National Medicine. 2nd Edn., Prima Health Publishing, Rocking, USA., Pages: 401.

Murray, R.K., D.K. Granner, P.A. Mayes and V.W. Rodwell, 2000. Harper's Biochemistry. 25th Edn., Appleton and Lange, Philadelphia, pp: 179-203, 621-630.

NNMDA, 2008. Medicinal Plants of Nigeria, South-East Zone. Vol. 1, Nigeria Natural Medicine Development Agency, Federal Ministry of Science and Technology, Lagos, Nigeria, pp: 8-159.

Nsirim, N., 1999. Clinical Biochemistry. 1st Edn., Longman Nigeria Plc., Nigeria, pp: 74-86.

OECD, 2001. Guidelines for testing chemical, Acute Oral toxicities up and down produce. OECD Reports 425, pp:1-26.

Onaogbe, I.O., A.O. Ebhota, H.C. Udegbe, E. Omondia, D. Edeni and S.O. Egengbo, 1999. Assessment of some medicinal plant for hypoglycemic activities in rats and rabbits. Biosci. Res. Commun., 11: 795-807.

Rafighi, Z., S. Arab, R.M. Yusof and A. Shiva, 2011. The effect of vitamin C and E on lipid profile in people with type 2 diabetes mellitus. Global J. Health Sci., 3: 69-74.

Ranjna, C., 1999. Practical Clinical Biochemistry: Methods and Interpretations. 2nd Edn., Academy Press, London, UK., pp: 200-240.

Rao, G.M., 1992. Serum electrolytes and Osmolarity in Diabetes mellitus. Indian J. Med. Sci., 46: 301-303.

Rodkey, F.L., 1964. Binding of bromocresol green by human serum albumin. Arch. Biochem. Biophys., 108: 510-513.

Santaguida, P.L., C. Bilion, D. Hunt, K. Morrison and H. Geistein *et al*., 2008. Diagnosis, prognosis and treatment of impaired glucose tolerance and impaired fasting glucose. Summary of Evidence Report/Technology Assessment No. 128, Agency for Healthcare Research and Quality, pp: 80-94.

Sofowora, A., 1993. Medicinal Plants and Traditional Medicine in Africa. 2nd Edn., Spectrum Books, Nigeria, ISBN-13: 9789782462190, pp: 142-144.

Tanko, Y., N.H. Sada, K.A. Mohammed, Jimoh, M. Yerima and A. Mohammed, 2013. Effect of ethanolic extract of *Caralluma daizielii* on serum electrolytes levels on fructose-induced diabetes in Wistar rats. Ann. Biol. Res., 4: 157-161.

Tietz, N.W., 2000. Fundermental of Clinical Chemistry. W.B. Saunders Company, London pp: 1020-1038.

WHO, 1999. Definition, diagnosis and classification of diabetes meilitus and its complication. Report of WHO Consultation (part 1): Diagnosis and Classification of Diabetes Mellitus. WHO/NCD/NCS/99.2. World Health organization, Department of Noncommunicable Disease Survillance, Genera, pp: 1-27.

World Health Organization, 1980. Second Report of the WHO Expert Committee on Diabetes Mellitus. World Health Organization, Geneva.

Wybenga, D.R.D., J. Glorgio and V.J. Pileggi, 1971. Determination of serum urea by *Diacetyl monoxime* Method. J. Clin. Chem., 17: 891-895.

Yassin, M.M., A.R.A. Ashour and N.R. Elyazji, 2004. Alterations in body weight, protein profile, non-protein nitrogen constituents and kidney structure in diabetic rats under glibenclamide treatment. J. Islamic Univ. Gaza, 12: P37-P54.