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Beneficial Effect of Aqueous Stem Bark Extracts of *Strychnos henningsii* Gilg in Streptozotocin-nicotinamide Induced Type 2 Diabetic Wistar Rats

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Abstract: *Strychnos henningsii* Gilg is commonly used in Southern Africa herbal medicines for the management of diabetes mellitus. The beneficial effect of the aqueous extract of *S. henningsii* at the dose of 125, 250 and 500 mg kg^{-1} was investigated on some biochemical and hematological parameters in diabetic rats after 15 days of experimental periods. Significant decrease of blood glucose levels were found after oral administration of the extract at the dose of 250 mg kg^{-1} (10.03 mmol L^{-1}) followed by 500 mg kg^{-1} (7.60 mmol L^{-1}) and then 125 mg kg $^{-1}$ (6.70 mmol L^{-1}). The extract did not alter the levels of cholesterol, uric acid and kidney body weight ratio. In addition, there was no significant effect on the levels of basophils, monocytes and eosinophil but appreciably increased White Blood Count (WBC), neutrophils and lymphocyte at 500 mg kg^{-1}. Moreover, the level of triglyceride, calcium, urea and liver body weight ratio was drastically reduced at certain doses. Treatment of diabetic rats with these extracts significantly reduced the activities of Aspartate Transaminase (AST) and Alanine Phosphatases (ALP). Whereas the levels of hemoglobin (Hb), Packed Cell Volume (PCV), Mean Concentration Volume (MCV), Red Blood Cell (RBC), Mean Concentration Hemoglobin (MCH), total protein, Alanine Aminotransferase (ALT), albumin and globulin were significantly increased as compared with diabetic rats. The present study indicated potent antidiabetic and antilipidemic properties of *S. henningsii* extract and its capability of regularizing some abnormalities associated with pathophysiologic condition of diabetes mellitus.

Key words: *Strychnos henningsii*, diabetes mellitus, streptozotocin, nicotinamide, biochemical, hematological analysis, clinical significance

INTRODUCTION

Streptozotocin-nicotinamide is a method currently used to induce diabetes in animals that resemble non obese type 2 Diabetes Mellitus (DM) in man (Nakamura *et al*., 2006). The induction of diabetic rats with Streptozotocin (STZ) increases the production of free radicals that damage the pancreatic DNA and thus affect insulin secretion (Oguri *et al*., 2003). This is achieved by depleting Nicotinamide (NAD) which is a substrate of poly ADP ribose synthetase, an enzyme which involved in DNA repair. Pre-treatment of experimental animals with NAD allows minor damage to pancreatic β cell (LeDoux *et al*., 1988). In diabetes, lipid abnormalities, anemia, alteration of liver and kidney functional indices have been implicated as major risk factors to the progression of both microvascular and macrovascular diabetic complications (Mandade and Sreenivas, 2011; Camargo and Gross, 2004). Several, antidiabetic drugs such as biguanide, sulphonylureas along with insulin have been employed for the treatment of this disease. Still, none of these drugs was able to cure the disease without adverse reaction (Anees *et al*., 2007). There is a growing interest in the use of medicinal plants of low side effect for the management of diabetes mellitus especially in countries where access to conventional treatment of DM is inadequate (Rao and Rao, 2001; Pavana *et al*., 2008). Therefore, further scientific investigation is needed to give credence to the traditional usage of these plants in accordance to World Health Organization (http://www.who.org).

Strychnos henningsii Gilg (Loganiaceae) is commonly known as umnono in Zulu language and mostly cultivated in South Africa, Tanzania, Uganda and Kenya (Oyedemi *et al*., 2009). It is a small evergreen tree or shrub with leathery leaves and clean green-reddish stem. It is a tree that grown up to 2-12 month in a dry or moist forest, wooded hillsides, coastal forest and stream banks. The fruit is oblong and brown or orange when ripe. The bark is crown compact with dark green and glossy foliage. In local medicine of South Africa, the decoction or infusions of the stem bark is widely used for the

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management of DM (Oyedemi *et al*., 2009). It is also used in the eastern part of Africa for the treatment of various ailments including abdominal pain, syphilis, snake bite, gastrointestinal pain, rheumatism, diabetes, malaria and to hasten wound healing in animal (Oyedemi *et al*., 2009). About five compounds have been isolated including indolinic alkaloids, strychnine, brucine, curarine and bitter glycoside (Penelle *et al*., 2000). The plant has also been reported as potential agent in the development of new antinociceptive and antispasmodic drugs due to the presence of retuline-like alkaloids (Penelle *et al*., 2000). Our previous study on the phytochemical constituent revealed the presence of flavonoids, tannins, saponins, proanthocyanidins, phenols and glycosides with significant values (Oyedemi *et al*., 2010a). Strong antioxidant and free radical scavenging activity observed was attributed to the presence of phenolics compounds indicating the ethnotherapeutic usage of this plant for the management of oxidative stress induced diseases. Other studies conducted by Oyedemi *et al*. (2010b) revealed partial safety of sub-acute administration of aqueous stem bark extract of *S. henningsii* (SH) in rats due to negative effect on the normal functioning of white blood cells.

Although, the plant is used traditionally for the management of DM, still need scientific data to give credence to its folkloric usage. A thorough search of the literature reveals that there is no information on the effect of this plant on blood glucose levels and pathophysiologic symptoms in diabetic model. The present study was therefore designed to investigate the effects of the aqueous stem bark extract of SH on some biochemical and hematological parameters in STZ-NAD induced diabetic Wistar rats.

MATERIALS AND METHODS

Sample collection and extract preparation: The stem barks of SH was collected in February, 2009 from a thick forest in Amathole District (Eastern Cape, South Africa). The plant was identified by its vernacular name and later authenticated by Prof. DS. Grierson of Botany Department, University of Fort Hare. Voucher specimen (Sun MED 2009) was deposited at the Giffen Herbarium of the University. The bark material was cut into pieces and air-dried at room temperature in the laboratory. The dried material was then grounded into powder using an electric blender (Waring Products Division, Torrington, USA). About 40 g of the powdered plant material was extracted in 1000 mL of cold distilled water maintained on a mechanical shaker (Stuart Scientific Orbital Shaker, UK) for 48 h. The resulting solution was later filtered using a Buchner funnel and Whatman No.1 filter paper. The filtrate was quickly frozen at -40°C and dried for 48 h using a freeze dryer (Savant Refrigerated vapor Trap, RV T41404, USA) to give a yield of 5.2 g of dry extract. The resulting extract was reconstituted in distilled water to give desired doses of 125, 250 and 500 mg kg^{-1}.

Chemicals: The assay kits for urea, uric acid, calcium, total protein, total bilirubin, albumin, cholesterol, triacylglycerol, alkaline phosphatase, lactate dehydrogenase, gamma glutamyltransferase and alanine as well as aspartate aminotransferases were obtained from Randox Laboratories Limited, Ardmore, Co Antrim, UK. All other reagents used in this study were of analytical grade and were supplied by Merck Chemicals (Pty) Ltd., Bellville, South Africa.

Animals used: Male Wistar albino rats (*Rattus novergicus*) weighing between 125 and 255 g were obtained from the animal house of the Agricultural and Rural Development Research Institute, University of Fort Hare. The animals were kept in aluminum cages and maintained at a controlled temperature of 28°C with a 12 h light-dark cycle and humidity of 45-50%. They were fed with standard rat feed with water *ad libitum* for 15 days but starved 12 h before the start of the experiment. All animal experiments were conducted with NIH guidelines for care and use of laboratory animals which was approved by the animal ethics committee of the University of Fort Hare.

Induction of type 2 DM in animals: The method described by Masiello *et al*. (1998) was adopted for the induction of type 2 diabetes in overnight fasted male Wistar rats.

Animal grouping and extract administration: Thirty-six male rats were separated into six groups of six animals in each group (30 diabetic surviving rats, 6 normal rats). The sex of experimental rats was chosen based on the availability of the animals. All the treatments were given to the animals orally using gavages. Group 1: Normal control rats administered drinking water daily for 15 days; Group 2: Diabetic animals received 0.5 mL of distilled water; Group 3-5: Diabetic rats treated daily with 0.5 mL of 125, 250 and 500 mg kg^{-1} body weight of SH extract, respectively and Group 4: Diabetic animals received 0.5 mL of glibenclamide (0.6 mg kg^{-1} body weight) only. All the animals from each group were sacrificed by ether anesthesia 24 h after their respective 15 daily doses of the extract and distilled water. The serum was prepared by following the method described by Yakubu *et al*. (2005).

Haematological analysis: After animal anaesthesia with ether, blood samples were collected into sample bottles with EDTA to determine blood cell parameters including Red Blood Cells (RBC), Hemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Large Unstained Cell (LUC) and Red Cell Distribution Width (RCDW). The White Blood Cell (WBC) and other related indices such as basophils, monocytes, lymphocytes, neutrophils and eosinophils were also determined. All blood sample parameters were analyzed using hematology analyzer Coulter Horiba ABX 80 Diagnostics (ABX Pentra Montpellier, France).

Serum biochemical evaluation: Serum samples were collected after centrifugation to determine the levels of Alanine (ALT) and Aspartate Aminotransferase (AST), Alkaline Phosphatases (ALP), urea, uric acid, total bilirubin, total protein, calcium, albumin, globulin, Triacylglycerol (TAG) and cholesterol in the animals by standard methods (Tietz *et al*., 1994). These parameters were estimated spectrophotometrically by using commercial kits obtained from Randox Laboratories Limited, Ardmore, Co Antrim, UK.

Organ/Body weight of animals: The body weights of the animals were measured before the start and after the experimental period (15 days). While the organ body weight ratio was determined after the animals were sacrificed.

Statistical analysis: Data were expressed as Means±SD of five replicates and were statistically analyzed using one way Analysis of Variance (ANOVA). Means were separated by the Duncan multiple test using SAS. Values were considered significant at $p<0.05$.

RESULTS

The intraperitoneal injection of STZ-NAD resulted to significant increase ($p<0.05$) of plasma glucose level in comparison to normal rats as presented in Table 1. The normal rats were euglycemic (5.6- 5.7 mmol L^{-1}) during the course of the study whereas the diabetic control rats were hyperglycemic (18.2-30.1 mmol L^{-1}) throughout the experimental period. After daily oral administration of plant extract or glibenclamide for 15 days, there was a significant reduction of blood glucose level. The blood glucose levels were reduced by 6.7, 10.03 and 7.6 mmol L^{-1} at the doses of 125, 250 and 500 mg kg^{-1}, respectively, while that of glibenclamide treated group was 8.98 mmol L^{-1}. The extract at 250 mg kg^{-1} exhibited an effective reduction of blood glucose than glibenclamide treated group while the dose at 500 mg kg^{-1} displayed a comparative effect.

The beneficial effect of SH extract on red blood cells and its functional indices in diabetic rats is depicted in Table 2. The levels of RBC (4.5×10^{12} L^{-1}) and its differentials was markedly reduced in diabetic rats but increased significantly after oral administration of SH extract in a dose dependent manner. The improvement on these parameters was comparable to that of glibenclamide and the normal control rats.

The significant ($p<0.05$) decrease in the levels of total WBC and other related parameters in diabetic rats was shown in Table 3. The treatment of diabetic rats with SH extracts was parameter and dose selective. The levels of WBC (12.13±4.64), lymphocytes (8.51±4.64) and platelets (821±57.2) were notably increased at 500 mg kg^{-1} followed by 125 and then 250 mg kg^{-1} in comparison with the normal control. All the three dosages of the SH extracts did not improve the levels of basophils, neutrophils, eosinophil and monocytes in comparison with diabetic group.

Table 4 shows the beneficial effect of SH extracts on kidney functional markers such as urea, uric acid and calcium of diabetic rats. The levels of urea, uric acid, calcium were 53.9, 0.4 and 2.6 mM, respectively in diabetic rats but significantly reduced after plant extract treatment. The extracts significantly reduced the concentration of urea but not dose related. The declined level of urea (26.90±0.01) after extract administration at 125 mg kg^{-1} compared favorably well with glibenclamide treated group. Similarly, the level of calcium was markedly reduced at the doses of 250 (1.80±0.01) and 500 mg kg^{-1} (2.00 ±0.05) in comparison with normal and glibenclamide treated groups. Meanwhile, the extracts did not have any significant effect on uric acid at the three dosages investigated in this study.

A significant increase in serum cholesterol (6.7 mM) and triglycerides (2.2 mM) was observed in diabetic rats induced with STZ-NAD but decreased at certain dosages after administration of SH extracts for 15 days. The treatment of diabetic rats at the dose of 500 mg kg^{-1} significantly reduced the level of cholesterol (4.6 mM) while other dosages did not show any promising effect. The three dosages reflected triglyceride lowering ability. However, the lowest and mid dosages showed a comparative effect with the normal control rats and glibenclamide treated group. Overall, the beneficial effect

Table 1: Effect of oral administration of aqueous extract of *S. henningsii* on plasma glucose level of STZ -NAD induced diabetic rats

	Plasma blood glucose (mmol L^{-1})			
Treatment	0 (day)	5 (day)	10 (day)	15 (day)
Normal control	5.60±0.40	5.40±0.53	5.60±0.30	5.70±0.32
Diabetic control	18.20±0.30	23.45±0.35	28.30±0.40	30.10±0.40
Diabetic+SH (125 mg kg^{-1})	19.33±1.20**	16.35±1.14**	15.53±1.30**	12.56±0.90*
Diabetic+SH (250 mg kg^{-1})	24.30±0.09*	22.20±0.08**	17.57±1.02*	14.27±1.20*
Diabetic+SH (500 mg kg^{-1})	25.30±0.01*	20.20±0.04*	19.28±0.10*	17.70±0.30**
Diabetic+glibenclamide (0.6 mg kg^{-1})	22.50±3.30*	19.30±3.20*	17.78±2.40*	13.52±2.50*

All values are Mean±SD of 6 rats in each group. Significant different at *$p<0.05$ as compared with diabetic control group and significant different at **$p<0.05$ as compared with glibenclamide treated group. values in parenthesis denotes increase or reduction of blood glucose level in mmol L^{-1}: Diabetic (↑11.9); 125mg kg^{-1} (↓6.7); 250 mg kg^{-1} (↓10.03); 500 mg kg^{-1} (↓7.60); Glibenclamide (↓8.98)

Table 2: The effect of aqueous extract of *S. henningsii* bark on red blood cells and the differentials in STZ-NAD induced diabetic rats

	Strychnos henningsii bark extract (mg kg^{-1} body weight)					
Parameters	Control	125	250	500	Glibenclamide	Diabetes
RBC ($\times 10^{12}$ L^{-1})	8.94±0.04^{a}	8.25±0.35^{a}	8.76±1.23^{a}	8.57±0.19^{a}	8.15±0.40^{a}	4.50±0.60^{b}
Hb (g dL^{-1})	15.03±0.06^{ab}	15.80±0.84^{a}	15.90±1.97^{a}	16.03±0.32^{b}	15.33±1.03^{ab}	8.43 ±1.02^{c}
PCV (ll^{-1})	0.50±0.02^{a}	0.49±0.02^{a}	0.49±0.07^{a}	0.48±0.01^{a}	0.48±0.02^{a}	0.26 ±0.03^{b}
MCV (fl)	55.83±1.11^{b}	59.60±2.52^{a}	55.90±0.14^{b}	55.90±1.15^{b}	59.33±1.02^{a}	40.13±0.38^{c}
MCH (pg)	16.93±0.35^{a}	19.13±0.69^{b}	18.15±0.21^{b}	18.70±0.26^{b}	18.80±0.46^{b}	12.20±0.40^{c}
MCHC (g dL^{-1})	30.27±0.82^{a}	32.10±0.66^{b}	32.45±0.35^{b}	33.40±0.35^{b}	31.70±1.25^{ab}	22.23±0.83^{c}
RCDW (%)	13.63±0.90^{a}	14.55±3.13^{b}	16.65±2.47^{c}	15.40±1.77^{b}	15.13±1.20^{b}	9.43±0.55^{d}
LUC (%)	7.57±1.15^{a}	0.05±0.04^{b}	0.04±0.03^{b}	0.13±0.07^{b}	0.24±0.11^{b}	0.02±0.01b

All values are Mean±SD of 6 rats in each group. Test values carrying superscripts different from the control down the column for each red blood and its differentials are significantly different ($p<0.05$) and those with the same superscript as the control are not significantly different

Table 3: The effect of aqueous extract of *S. henningsii* bark on white blood cells and the differentials in STZ-NAD induced type 2 diabetic rats

	Strychnos henningsii bark extract (mg kg^{-1} body weight)					
Parameters	Control	125	250	500	Glibenclamide	Diabetes
WBC ($\times 10^{9}$ L^{-1})	17.00±3.20^{a}	10.85±2.03^{bc}	7.72±3.72^{c}	12.13±4.64^{b}	14.98±5.60^{ab}	7.33±0.93^{c}
Neutrophil (%)	5.37±1.17^{a}	0.43±0.01^{b}	0.76±0.30^{b}	1.72±0.30^{b}	1.01±0.16^{b}	0.59±0.51^{b}
Monocytes (%)	17.46±6.11^{a}	0.98±0.41^{b}	1.47±0.01^{b}	1.07±0.41^{b}	3.04±0.64^{c}	4.69±1.00^{d}
Lymphocyte (%)	65.40±6.86^{a}	8.12±2.20^{c}	4.23±3.10^{d}	8.51±3.04^{c}	15.61±5.10^{b}	2.36±0.33^{d}
Eosinophil (%)	3.70±1.18^{b}	1.03±0.22^{a}	1.20±0.60^{a}	1.07±0.60^{a}	1.57±0.32^{a}	1.03±0.78^{a}
Basophils (%)	0.53±0.21^{a}	0.06±0.02^{b}	0.03±0.03^{b}	0.07±0.04^{b}	0.09±0.15^{b}	0.05±0.03^{b}
Platelets ($x10^{9}$)	851.00±78.58^{a}	655.00±68.00^{c}	643.00±20.0^{c}	821.00±57.20^{b}	721.30±55.20^{d}	370.00±31.11^{e}

All values are Mean±SD of 6 rats in each group. Test values carrying superscripts different from the control down the column for each white blood cells and its differentials are significantly different ($p<0.05$) and those with the same superscript as the control are not significantly different ($p>0.05$)

Table 4: Effect of aqueous extract of *S. henningsii* bark on some renal function and lipid profiles parameters in STZ-NAD induced type 2 diabetic rats

Group of animals	Calcium (mM)	Urea (mM)	Uric acid (mM)	Cholesterol (mM)	Triglycerol (mM)
Normal	2.2±0.03	26.9±0.53	0.1±0.02	1.1±0.09	0.8±0.03
Diabetes	2.6±0.01	53.9±0.05	0.4±0.02	6.7±0.02	2.2±0.02
Diabetes+125 mg kg^{-1}	1.1±0.01**	26.9±0.01*	0.7±0.00	6.7±0.01	0.7±0.02*
Diabetes+250 mg kg^{-1}	1.8±0.01*	45.3±0.06**	0.7±0.10	7.4±0.02**	0.7±0.04*
Diabetes+500 mg kg^{-1}	2.0±0.05*	41.1±0.07**	0.6±0.03	4.6±0.05**	1.2±0.05*
Glibenclamide	2.1± 0.01*	24.2±0.02*	0.5±0.03	6.2±0.01	0.5±0.01*

All values are Mean±SD of 6 rats in each group. Significant different at *$p<0.05$ as compared with diabetic control group and significant different at **$p<0.05$ as compared with glibenclamide treated group

Table 5: Effect of aqueous extract of *S. henningsii* bark on some liver functional indices in STZ- NAD induced diabetic rats

		Liver functional indices					
Group of animals	ALP (U L^{-1})	AST (U L^{-1})	ALT (U L^{-1})	Globulin (g L^{-1})	Total protein (g L^{-1})	Albumin (mM)	TotalBilirubin (μM)
Normal	210.0±5.21	165.1±1.91	59.5±3.77	53.3±1.07	69.3±1.78	44.0±0.02	7.5±0.70
Diabetes	257.6±0.01^{a}	185.2±0.00^{a}	65.2±0.00^{b}	11.7±0.03^{a}	55.7±0.02^{a}	16.0±0.71^{a}	44.0±0.05^{a}
125 (mg kg^{-1})	253.5±0.00^{b}	161.5±0.01^{b}	62.8±0.01^{a}	30.8±0.05^{d}	92.1±0.00^{e}	61.3±0.02^{c}	43.2±0.00^{a}
250 (mg kg^{-1})	252.1±0.02^{b}	122.2±0.01^{c}	67.5±0.00^{c}	16.9±0.02^{b}	77.2±0.03^{c}	60.3±0.03^{c}	40.1±0.03^{b}
500 (mg kg^{-1})	249.4±0.02^{bc}	133.5±0.00^{d}	62.6±0.01^{a}	10.0±0.07^{a}	67.3±0.07^{b}	57.3±0.05^{b}	40.3± 0.01^{c}
Glibenclamide	242.5±0.01^{c}	122.2±0.01^{c}	58.7±0.00^{d}	26.6±0.05^{c}	85.3±0.02^{d}	58.7±0.02^{b}	37.7±0.01^{d}

All values are Mean±SD of 6 rats in each group. Test values carrying superscripts different from the control down the column for each liver functional indices are significantly different ($p<0.05$) and those with the same superscript as the control are not significantly different ($p>0.05$)

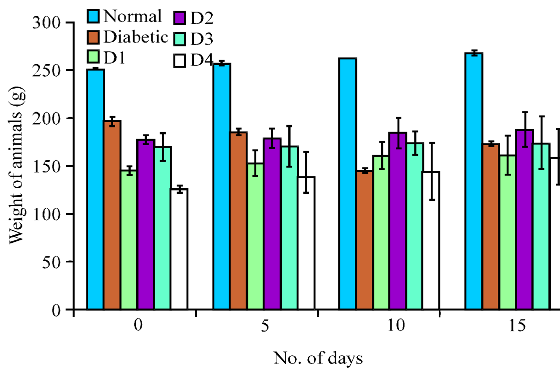

Fig. 1: The effect of aqueous extract of *S. henningsii* on the body weight of Wistar rats over 15 days of treatment. Values are Mean±SD of 5 rats in each group. D1: Animals fed with 125 mg kg^{-1} of extract, D2: Animals fed with 250 mg kg^{-1} of extract, D3: Animals fed with 500 mg kg^{-1} body weight of extract, control: Animals receiving only distilled water

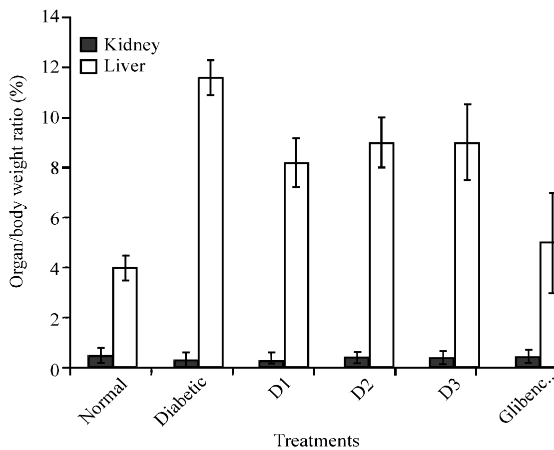

Fig. 2: Organ/body weight ratio over 15 days for different doses of the aqueous extract of *S. henningsii* in STZ-NAD induced diabetic rats. Values are Mean±SD of 5 rats in each group. D1: Animals fed with 125 mg kg^{-1} of extract, D2: Animals fed with 250 mg kg^{-1} of extract, D3: Animals fed with 500 mg kg^{-1} body weight of extract, control: Animals receiving only distilled water.

of plant extract at specific dosages was comparable with the positive control group treated with glibenclamide.

The effect of SH extract on liver functions of diabetic rats is depicted in Table 5. The induction of diabetes in rats significantly ($p<0.05$) reduced the levels of total proteins, globulin and albumin while ALT, AST, ALP and total bilirubin were markedly increased when compared to control animals (Table 5). Treatment of diabetic rats with SH extracts at doses of 250 (40.1±0.03) and 500 mg kg^{-1} (40.3±0.01) remarkably reduced the level of total bilirubin whereas the dosage of 125 mg kg^{-1} (43.2±0.00) exhibited trivial changes on this parameter. Total protein, globulin and albumin levels were significantly ($p<0.05$) increased in diabetic rats after 15 days of treatment with SH extracts or glibenclamide. The increased levels of ALT, AST and ALP were reduced to near normal and compared favorably well with glibenclamide treated group.

Figure 1 shows the continuous reduction of body weight of diabetic rats. Oral administration of plant extract at the three doses significantly improved the body weight (160-185 g) but not dose dependent as compared with normal or glibenclamide treated groups. Similarly, there was a change observed in organ/body weight ratio of diabetic rats (Fig. 2). The plant extract did not have any adverse effect on the kidney but the liver was slightly better (8-9 g) after 15 days treatment with SH extracts.

DISCUSSION

Streptozotocin acts as diabetogenic agent through the destruction of pancreatic β cell which is correlated with a rapid reduction of NAD concentration. NAD is a co-enzyme used as a specific inhibitor of streptozotocin during onset of diabetes mellitus (Grover *et al*., 2000). The prior intraperitoneal injection of NAD into rats was to induce type 2 DM in animals by allowing minor damage to pancreatic β cell. The results obtained from this study after treatments of diabetic rats with SH extracts are very much promising with equivalent effect to that of glibenclamide. The plant extract significantly reduced the blood glucose level at the three doses but not dose related. The dose at 250 mg kg^{-1} showed a potent hypoglycemic activity in comparison to normal or glibenclamide treated group. Similar observation was reported on *Ichnocarpus frutescens* in animals induced with streptozotocin-nicotinamide (Barik *et al*., 2008). Though the probable mechanism of action of this plant was not investigated but could be linked to prevention of death or restoration of partially destroyed pancreatic β cells or increased uptake of glucose (Noor *et al*., 2008). Moreover, the antioxidant and free radical scavenging properties of this plant as shown in our previous work might increase the resistant of β cells to the toxic effect of STZ by activating antioxidant enzymes (Oyedemi *et al*., 2010a). The presence of phenolics compounds as previously reported in our studies may induce insulin secretion in diabetic animal models in accordance to other several studies (Al-Awwadi *et al*., 2004; Arun and Ramesh, 2002).

Hematological parameters have been commonly used in the diagnosis of various diseases and pathological condition of foreign compounds including plant extracts, drugs, dyes and several others on the blood constituents of animals (Oyedemi *et al*., 2010b). In this study, the observed decreased levels of red blood cells, Hb, MCV, MCH, MCHC, RCDW and PCV in diabetic rats gives an indication of anemic condition. The decreased level of Hb concentration has been attributed to impairment on the rate of RBC formation as a result may have negative effect on the oxygen carrying capacity of the animals. This observation corroborated with the report of Baskar *et al*. (2006) but opposed the findings of Junod *et al*. (1969) who reported probable loss of diabetogenic action of STZ due to prior administration of NAD. This observation provided evidence that intraperitoneal injection of nicotinamide into the rats may not completely exterminate the effect of STZ on pancreatic β cells but allow minor damage by repairing poly ADP ribose synthetase (LeDoux *et al*., 1988). The disturbed hematological alteration of red blood cells and its related indices in diabetic animals was improved upon plant extract treatment by preventing hemolysis of erythrocytes caused by lipid peroxidation through its antioxidant property (Oyedemi *et al*., 2010a).

The decreased level of total WBC and its functional indices in STZ induced diabetes concurred with the report of Paul (2002) on the suppression of immune system caused by damaged WBC (Table 3). The reduction of these parameters may have a significant effect against the animals by exposing them to pathogenic infections (Adedapo *et al*., 2007). Oral administration of SH extract for 15 days notably increase the level of WBC, lymphocytes and platelets at the dose of 500 mg kg^{-1}. Meanwhile, there was no significant effect ($p>0.05$) on the levels of monocytes, basophils and eosinophils in comparison to normal and glibenclamide treated groups. This finding was contrary to the report of Oyedemi *et al*. (2011), who found that administration of *A. africana* into diabetic rats induced with STZ had potential to improve the WBC functional indices. The significant increase of lymphocyte levels reflects possible immunomodulatory effects of SH extract in diabetic rats. The data obtained on the white blood counts and platelets indicated that SH extract did not have strong potential to boost the immune and defense system of diabetic animals but could improve the anticoagulation during severe bleeding (Dahlback, 2008). It could be inferred from this study that *S. henningsii* may contain some bioactive compounds with ability to improve the impaired production of WBC by stimulating maturation of committed stem cells responsible for WBC production.

The abnormal changes observed on the nephropathic markers such as urea and uric acid of diabetic rats reflect the diminish ability of kidneys to filter waste products from the blood as a result of disruption in filtration and electrolyte balance (Atangwho *et al*., 2007). The continued administration of plant extract for 15 days into diabetic rats provide corrective measures on the kidney which is reflected on the drastic reduction of urea and calcium at certain dosages. Similar observation was reported on the aerial parts of *Silybum marianum* (Pakistan plant) against nephropathy in STZ diabetic rats (Vessal *et al*., 2010). Though the histology is not evaluated in this study, however, the data obtained on the kidney body weight ratio suggested that the extract could lessen hypertrophy an indices of nephropathy. This result also implies that SH extract may have the potential to reverse the kidney functional status impaired in diabetic rats and to prevent diabetes related complications as shown in this study.

High levels of lipids such as triglycerides and cholesterol have been observed in diabetes subject due to lack or deficiency of insulin (Anreddy *et al*., 2010). A significant increase in serum cholesterol and triglycerides was observed in diabetic rats induced with STZ-NAD which agrees with the finding of Burcelain *et al.* (1995). This abnormality may therefore be regarded as consequence of increase mobilization of free fatty acids from peripheral fat depots and may have a greater risk for the development of coronary heart diseases (Bopanna *et al*., 1997). In this study, the level of serum cholesterol and triacylglycerol in diabetic rats was significantly reduced at certain doses in comparison with normal or glibenclamide treated groups (Table 4). Karim *et al*. (2011) reported similar results on *A. cepa*, *A. sativum* and *C. roseus* for their successful serum lipid control in diabetic animal model. The lipid lowering ability of this plant justified its glycemic control and thus could be of great advantage for the management of DM and the complications associated with atherosclerosis or coronary heart attack (Ghasi *et al*., 2000).

The data obtained from this study showed the decreased levels of total protein, globulin and albumin in diabetic rats and this is consistent with the result obtained by Bakris (1997) and Tuvemo *et al*. (1997). Similarly, the levels of total bilirubin, ALT, AST and ALP were significantly increased in DM which indicates hepatic dysfunction as reported with other investigators (Irshaid and Mansi, 2009; Al-Attar and Zari, 2007). The increased protein catabolism and urea formation that are seen in diabetic rats could be accountable for the elevation of these tissues transaminases. In this study, administration of SH extracts caused significant ($p<0.05$)

increase in the biosynthesis of total proteins, globulin and albumin as compared with normal group. There was a significant restoration of total bilirubin after SH treatment which indicated the stabilization of plasma membrane and protection of liver cells. In addition, the damage caused by STZ on the liver cells was substantiated by the elevated level of serum ALT, ALP and AST. The increased that might be due to leakages from the cytosol or changes in the permeability of liver membrane. In this investigation, the levels of ALP, ALT and AST were reduced in a dose related manner as compared with diabetic animals thus improving renal and hepatic functions. This observation concurred with the report of Iwalokun *et al.* (2006), who reported hepatoprotective potentials of leaf extracts of *V. amygdalina* in mice.

A significant decrease in the body weights is widely associated with diabetes patient due to loss of appetite which was also confirmed in this study. Similar observation was reported by Safiyeh *et al.* (2007) on STZ induced diabetic rats. The effect of SH extracts on the body weight of diabetic rats was observed after 15 days of treatment. Oral administration of plant extract appreciably increased the body weight gain of the animals near that of normal rats but not dose related (Fig. 1). The observed result signified the ability of SH extract to manage glucose properly as well as control muscle wasting and induced adipogenesis (Swanston-Flatt *et al.*, 1990). The changes in relative organ body weights ratio in the diseased animals are generally established as useful indicators of tissue damage (Fig. 2). The extract elicited slight effect on the liver while there was no significant effect on the kidney which may be due to lack of direct organ toxicity as reported by Oyedemi *et al.* (2010a).

CONCLUSION

In conclusion, the intraperitoneal injection of STZ-NAD induces type 2 diabetes mellitus with instability of some biochemical and hematological parameters. The data obtained from this study specified the reversible of anemic condition by SH extract in diabetic subject. It was further shown that plant extract can ameliorate or prevent secondary complications such as nephropathy and hepatic damage during chronic hyperglycemia. In addition, the plant extract has definitely shown to improve anticoagulant effect during the progression of diabetes. However, the extract did not have any prominent effect on the immune system of the animals. The beneficial effect of aqueous stem bark extract of *S. henningsii* as shown in this study was parameters and dose selective. Further studies are underway to investigate the pharmacological and mechanism of action of this plant in diabetic model.

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