CHAPTER 6

ANTIDIABETIC ACTIVITY AND CLINICAL SIGNIFICANCE OF AQUEOUS EXTRACT OF *STRYCHNOS HENNINGSII* GILG BARK IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED DIABETIC RATS

CHAPTER 6

Antidiabetic activity and clinical significance of aqueous extract of *Strychnos henningsii* Gilg bark in streptozotocin-nicotinamide induced diabetic rats

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Antidiabetic activities and clinical significance of aqueous extract of *Strychnos henningsii* Gilg bark in streptozotocin-nicotinamide diabetic rats

S.O. Oyedemi, G. Bradley and A.J. Afolayan

School of Biological Sciences, University of Fort Hare, Alice 5700, South Africa.

Abstract

The aim of this study was to investigate the clinical significance of aqueous bark extract of *Strychnos henningsii* on haematological, hepatic and renal functional indices during diabetes and its resultant effect in blood glucose levels, body weight, feed and water intake. The clinical significance of oral administration of this plant at the doses of 125, 250 and 500 mg/kg body weight was investigated in streptozotocin-nicotinamide induced diabetic rats for 15 days. The administration of the three doses significantly ($P > 0.05$) reduced the level of blood glucose in a dose independently manner while the best result was obtained at 250 mg/kg. The glucose tolerance was reduced significantly to near normal levels after 90 min at certain doses. The weight loss of the diabetic rats after oral administration of plant extracts at doses of 125 and 250 mg/kg was normalized as compared with the normal control rats. Treatment of diabetic rats with the extract did not significantly alter the levels of cholesterol and uric acid. In addition, the extract did not have any significant effect on the levels of basophils, monocytes and eosinophils. Whereas, the level of white blood counts, neutrophils and lymphocyte were remarkably increased at 500 mg/kg. Similarly, the elevated level of triacylglycerol, calcium, urea and liver body weight ratio was drastically reduced at certain doses of the extract. The activities of aspartate aminotransferases and alkaline phosphatase were significantly

lowered in all the three doses. The decreased level of haemoglobin, PCV, MCV, RBC, MCH, total protein, alanine aminotransferases, albumin and globulin in diabetic rats was appreciably increased after oral administration of plant extract at all the three doses. The result showed that *S. henningsii* extract possessed antidiabetic potential and could ameliorate anemic conditions as well as hepatic and renal damage in streptozotocin-nicotinamide induced diabetic rats. It could also prevent various complications in diabetes.

Keywords: *Strychnos henningsii*; diabetes mellitus; streptozotocin; nicotinamide; hypoglycemic.

INTRODUCTION

Streptozotocin- nicotinamide is a method currently used to induce diabetes in animals that resemble non obese type 2 diabetes mellitus in man (Tomonori et al., 2006). The induction of diabetic rats with streptozotocin 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 which is a substrate of poly ADP ribose synthetase, an enzyme which involved in DNA repair. The pretreatment of nicotinamide into diabetic rats allows minor damage to pancreatic beta cell by inhibiting poly ADP ribose synthetase activity and prevents NAD depletion (Le Doux et al., 1988). The administration of streptozotocin- nicotinamide induces diabetes with moderate hyperglycaemia associated with loss of early–phase insulin (Atsuo et al., 2008). This form of diabetes is very common in people over 40 years of age (WHO, 1999).

Recently, there is an emergent interest in the use of herbal remedies due to the undesirable effect associated with the use of oral hypoglycaemic drugs. Hence the consumption of herbal extracts has been increasing rapidly, mostly because of less frequent adverse side effect when compared to western medicine (Hu et al., 2003).

Strychnos henningsii Gilg (Loganiaceae) is one of the plants used in southern Africa as a folk remedy for the management of various diseases (Oyedemi et al., 2009). The decoction or infusion of the bark of *S. henningsii* extract is commonly used by traditional health practitioner in the Eastern Cape of South Africa for the management of diabetes mellitus (Oyedemi et al., 2009). Our previous studies on this plant demonstrated strong antioxidant and free radicals scavenging activity of plant extract both *in vitro* and *in vitro* (Oyedemi et al., 2010). The observation which may be due to the high contents of

phenolics, proanthocyanidins and flavonoids which have been reported to enhance insulin secretion coupled with hypoglycemic properties. The results obtained on sub-acute administration of *S. henningsii* bark extract demonstrated that *S. henningsii* extract is not completely safe as an oral remedy due to the impairment observed on the normal functioning of white blood cells and platelets (Chapter 5).

Report regarding the antidiabetic effect and clinical significance of aqueous extract of *Strychnos henningsii* in diabetic rats is scanty in scientific literature. Therefore, present study was undertaken to investigate the scientific basis for the use of this plant for the management of diabetes mellitus in folkloric medicine. We also assessed the effects of the extract on some haematological and biochemical parameters in streptozotocin-nicotinamide induced diabetic rats.

MATERIALS AND METHODS

Plant material

The bark of *S. henningsii* 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.

Assay Kits and Reagents

The assay kits for the analyses of triacylglycerol and cholesterol were obtained from Randox Laboratories Limited, Ardmore, Co Antrim, UK. All other reagents used

were of analytical grade and were supplied by Merck Chemicals (Pty) Ltd., Bellville, South Africa.

The animals used

Male Wistar rats (*Rattus novergicus*) weighing between 125 and 255g were obtained from the animal house of the Agricultural and Rural Development Research Institute, University of Fort Hare. The animals were maintained at a controlled temperature of 28 ^{o}C with a 12 h light-dark cycle at room temperature and humidity of 45-50 %. The animals were allowed free access to food and water for 15 days. The experiment was approved by the Animal Ethics Committee of the University of Fort Hare.

Preparation of plant extract

The bark material of *S. henningsii* was air-dried at room temperature in the laboratory and later pulverized after drying using an electric blender (Waring Products Division, Torrington, USA). About 60 g of the powdered plant material was extracted in 1 L of cold distilled water maintained on a mechanical shaker (Stuart Scientific Orbital Shaker, UK) for 48 h. The extract was filtered using a Buchner funnel and Whatman No.1 filter paper. The filtrate was quickly frozen at -40 ^{o}C and dried for 48 h using a freeze dryer (Savant Refrigerated vapor Trap, RV T41404, USA) to give a yield of 8.4 g of dry extract. The resulting extract was reconstituted in distilled water to give desired doses (125, 250 and 500 mg/kg) used in this study.

Induction of diabetes in the rats

The method of Pellegrino et al. (1998) was adopted for the induction of type 2 diabetes mellitus in overnight fasted male rats. The animals were induced by a single intraperitoneal injection (i.p) of freshly prepared solution of streptozotocin (60 mg/kg body weight) in 0.1 M citrate buffer (pH 4.5), 15 min after the i.p administration of nicotinamide (110 mg/kg) prepared in normal saline. Diabetes was confirmed in the animals by the elevated plasma glucose levels after 24 h of injection. The rats with diabetes having glycosuria and hyperglycaemia (blood glucose > 8.1 mmol/L) were used for the experiment.

Animal grouping and extract administration

Thirty six male Wistar rats were randomized into six groups of six animals each (30 diabetic surviving rats, 6 normal rats). Group I: normal control rats administered with drinking water daily for 15 days; Group II: diabetic animals received 0.5 ml of distilled water; Group III-V: diabetic rats treated daily with 0.5 ml of 125, 250 and 500mg/kg body weight of *S. henningsii* extract respectively; Group VI: diabetic animals received 0.5 ml of glibenclamide only. No detectable irritation, restlessness, respiratory distress, catalepsy or abnormal locomotion was observed after extract administration at different doses. Blood samples were drawn every fifth days till the end of experimental period. At the end of 15 days, all the animals from each group were sacrificed by ether anesthesia 24 h after their last daily doses of the extract, glibenclamide and distilled water.

Oral glucose tolerance test (OGTT)

Thirty male rats (normal) were fasted for 12 h and assigned randomly into 5 equal groups (n = 6/group). Group I-III: six normal rats were fed orally with 0.5 ml of aqueous bark extract of *S. henningsii* at the doses of 125, 250 and 500 mg/kg body weight respectively using gavage. Group IV: six normal rats were fed orally with 0.5 ml of glibenclamide (0.6 mg/kg) an antidiabetic standard drug and Group V: consisted of six normal rats receiving 0.5 ml of distilled water. Glucose (2 g/kg) was orally administered 30 min prior to the extract and glibenclamide administration and blood was withdrawn from the tail vein at 30, 60 and 90 min (Latha et al., 2004). The fasting plasma glucose level was measured using glucometer (Bayer Health Care, Japan).

Preparation of serum

The preparation of serum was carried out using the method of Yakubu et al. (2005). The blood samples were collected into clean dry centrifuge tubes. An aliquot (2 ml) of the blood was collected into sample bottles containing EDTA (BD Diagnostics, Pre-analytical Systems, Midrand, USA) for the haematological analysis while another 5 ml was allowed to clot at room temperature for 10 min. This was centrifuged at 1282 g x 5 min using Hermle Bench Top Centrifuge (Model Hermle, Z300, Hamburg, Germany). The sera were later aspirated with Pasteur pipettes into sample bottles and used within 12 h of preparation for the assay of biochemical parameters. The rats were thereafter quickly dissected in the cold; the liver and kidney were excised and transferred into ice-cold 0.25 M sucrose solution. The organs were thereafter freed of fat, blotted with clean tissue paper and then weighed.

Haematological analysis

Haematological parameters includes red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), large unstained cell (LUC), and red cell distribution width (RCDW) as well as white blood cell (WBC) and other related indices such as basophils, monocytes, lymphocytes, neutrophils and eosinophils were analyzed by using Horiba ABX 80 Diagnostics (ABX Pentra Montpellier, France).

Serum biochemical analysis

The levels of alanine and aspartate aminotransferase, alkaline phosphatase, urea, uric acid, total bilirubin, total protein, calcium, albumin, globulin, triacylglycerol and cholesterol in the serum of the animals were determined as described in chapter 5. They were determined spectrophotometrically using assay kits from Randox Laboratories Limited, Ardmore, Co Antrim, UK.

Effect of extract on the weight, feed and water intake of the animals

Feed and water intake were measured everyday at the same hour during the experimental periods while the body weight of the animals were measured before the start and every fifth day throughout the experimental period (15 days).

Statistical analysis

Data were expressed as mean ± SD (standard deviation) of six replicates and were statistically analyzed using one way analysis of variance (ANOVA). Means were separated by the Duncan multiple test using SAS (SAS, 2002). Values were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The earlier studies of *S. henningsii* extract on *in vitro* and *in vivo* antioxidant activities (Chapter 5) showed the prevention of oxidative stress as a result of strong antioxidants and free radical scavenging activities (Oyedemi et al., 2010). In this present study, the results of glucose tolerance test in diabetes, control and normal rats treated with *S. henningsii* extract and glibenclamide after oral administration of glucose at 30, 60 and 90 min are shown in Figure 1. The increased plasma glucose level observed at 30 min was significantly reduced by plant extract at various doses as compared with diabetic control rats. The dose at 250 and 500 mg/kg showed a similar blood glucose lowering capacity while glibenclamide treated rats was comparable to normal control rats. However, the dose of 125 mg/kg did not show strong effect as compared with the control and glibenclamide treated rats. The significant reduction of peak levels of blood sugar level within 90 min manifests the antidiabetogenic potential of *S. henningsii* extract in rat models. This observation could be attributed to antihyperglycemic property of the plant by restoring the delayed insulin response.

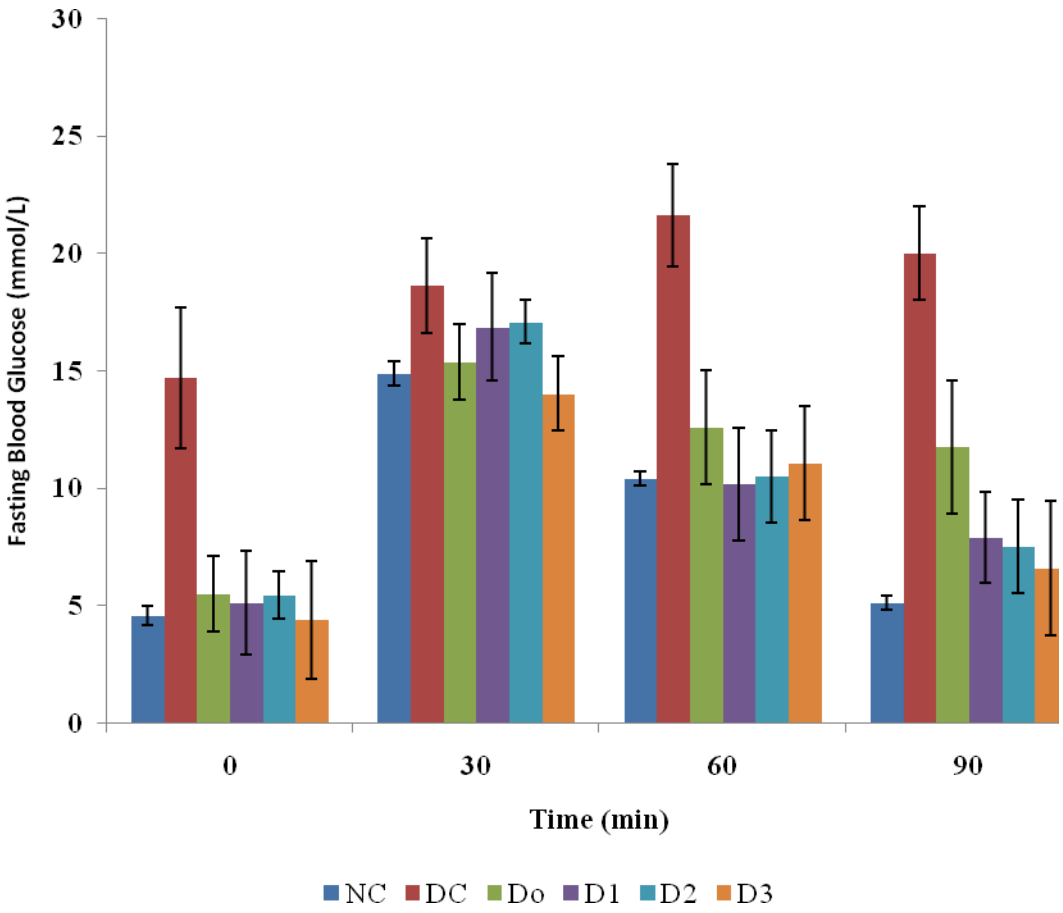

Figure 1: Effect of *S. henningsii* extract on oral glucose tolerance test in rats. The results are expressed as mean ± SD. NC- Normal control; DC- Diabetic control; Do- Normal rats +125 mg/kg SH; D1- Normal rats+250 mg/kg SH; D2- Normal rats + 500 mg/kg SH; D3- Normal rats +glibenclamide (0.6 mg/kg).

A significant decrease in the body weights of diabetic animals was observed 15 days after STZ-NAD treatment when compared with normal control rats. This observation was in agreement with the findings of Sharma et al. (1993). The oral administration of plant extract effectively increased the body weight gain of the animals near that of normal rats but not dose related (Figure 2). The observed result indicated that extract of *S. henningsii* possess the ability to manage glucose properly as well as controlling muscle wasting and induced adipogenesis (Swantson-Flatt et al., 1990).

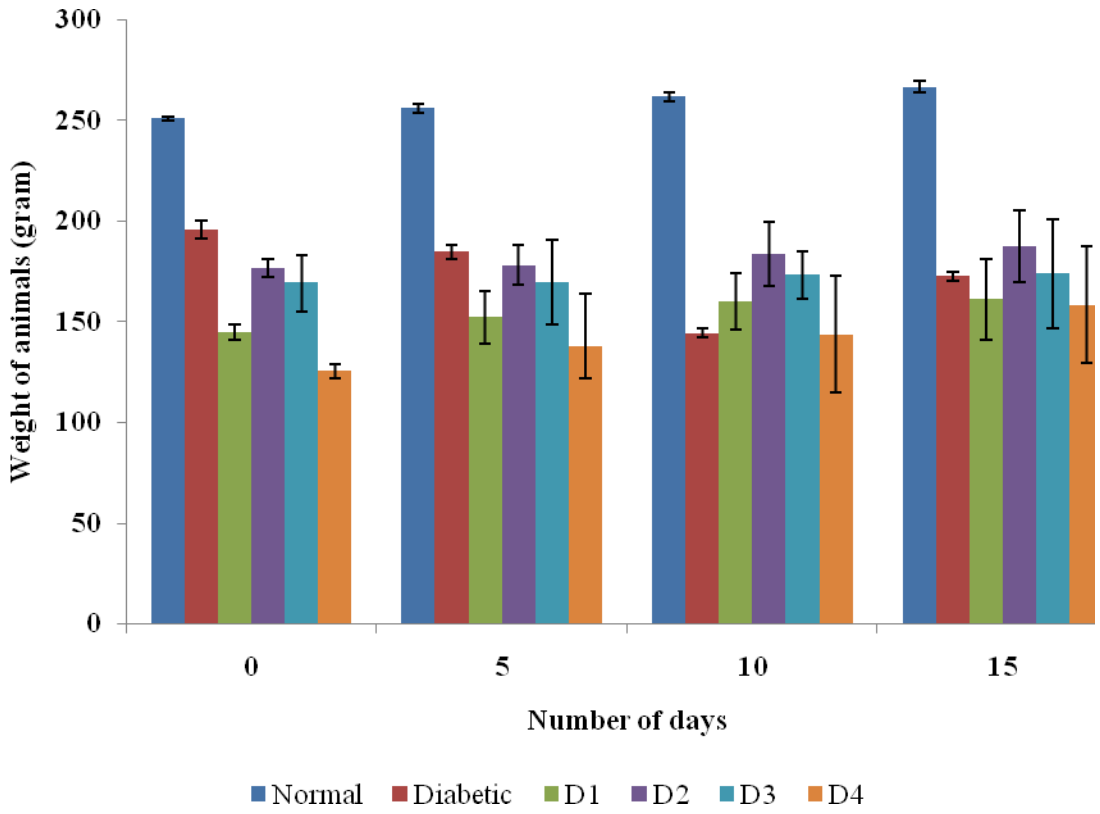

Figure 2: The effect of aqueous extract of *S. henningsii* on the body weight of Wistar rats. Values are mean ± SD of 6 rats in each group. D1 = animals fed with 250 mg/kg body weight of extract, D2 = animals fed with 500 mg/kg body weight of extract, D3 = animals fed with 1000 mg/kg body weight of extract, control = animals receiving only distilled water

Also, the feed and water intake of the diabetic rats were significantly increased in comparison with the normal control rats (Figure 3 and 4 respectively). These symptoms are well known markers of type 2 diabetes in both human and animal models which are direct consequence of insulin deficiency (Shenoy and Ramesh, 2002). The daily administration of plant extract to diabetic rats for 15 days caused a significant decrease in feed and water intake which was an indication of proper glucose utilization in the animals. All three doses of the plant extract improved the feed and water intake of the diabetic rats.

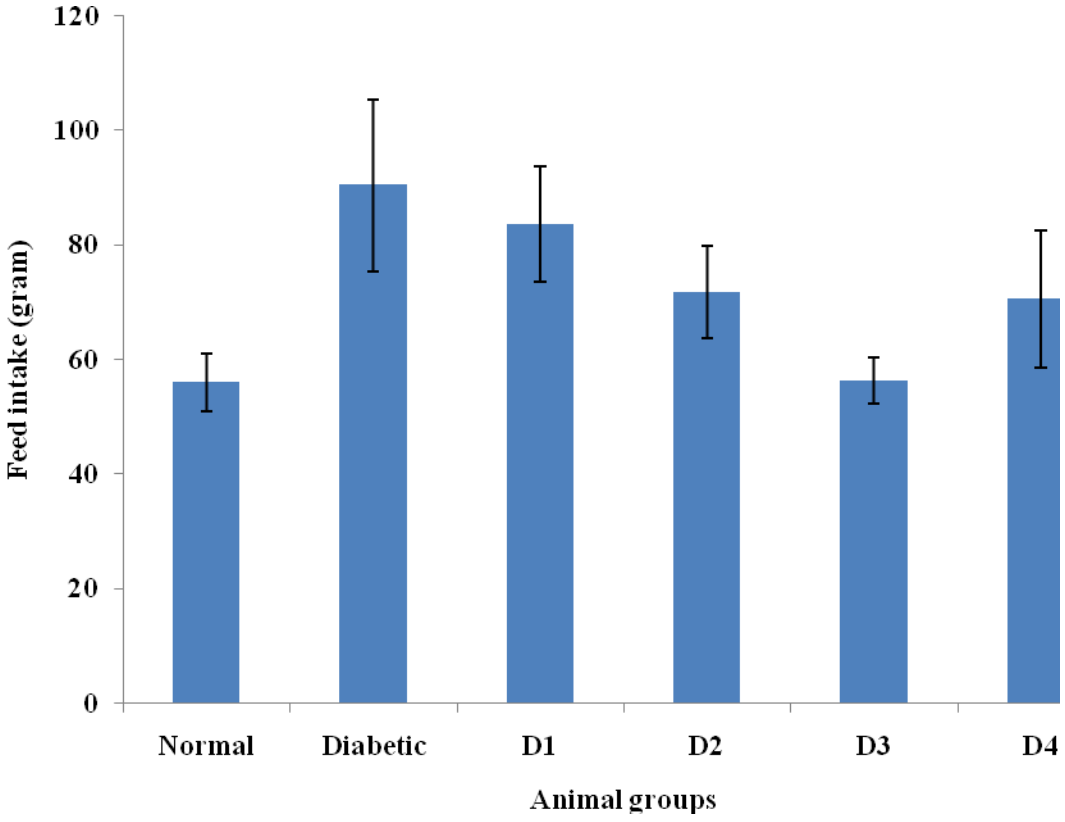

Figure 3: The effect of aqueous extract of *S. henningsii* on the feed intake of diabetic rats. D1 =Diabetic + SH (125 mg/kg), D2 = Diabetic + SH (250 mg/kg), D3 = Diabetic + SH (500 mg/kg) and D4 = Diabetic + glibenclamide (0.6 mg/kg).

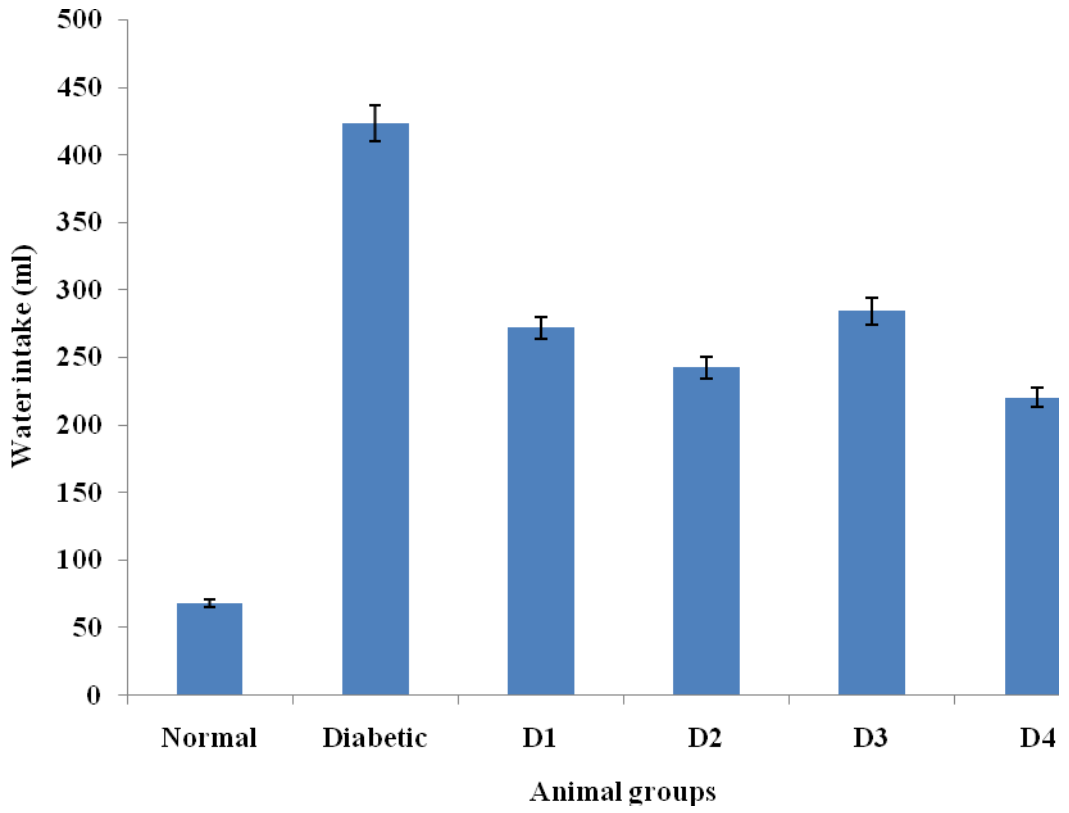

Figure 4: The effect of aqueous extract of *S. henningsii* on the water intake of diabetic rats. D1 = Diabetic + SH (125 mg/kg), D2 = Diabetic + SH (250 mg/kg), D3 = Diabetic + SH (500 mg/kg) and D4 = Diabetic + glibenclamide (0.6 mg/kg).

In this present study, the intraperitoneal injection of streptozotocin-nicotinamide resulted to a significant increase ($P < 0.05$) of plasma glucose level in the diabetic animals than the normal rats (Table 1). The normal rats were euglycemic during the course of the study whereas the diabetic control rats were hyperglycemic throughout the experimental period. The blood glucose in diabetic rats was reduced significantly after oral

administration of aqueous extract of *S. henningsii* in a dose independent manner for 15 days. The reduction of plasma glucose level by the different dosages of the extract was calculated by subtracting the blood glucose level of the final day from the zero day. It was reduced by 6.7, 10.03 and 7.6 mmol/L at the doses of 125, 250 and 500 mg/kg respectively. Meanwhile that of glibenclamide treated group was 8.98 mmol/L. The extract demonstrated hypoglycemic effect throughout the experimental period while the dose at 250 mg/kg showed the best result. The observed result indicated the possibility of increased glucose utilization by the extract owing to the significant reduction of blood glucose (Table 1). This view is supported by the antioxidant activities demonstrated by the plant in our previous study which might increase the resistant of beta cells to the toxic effect of streptozotocin by activating antioxidant mechanism (Yamamoto et al., 1981).

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

Treatment	Plasma blood glucose (mmol/L)			
	0 (day)	5 (day)	10 (day)	15 (day)
Normal control	5.60 ± 0.40	5.40 ± 0.53	5.6 ± 0.30	5.70 ± 0.32
Diabetic control	18.20 ± 0.30[a]	23.45 ± 0.35[a]	28.30 ± 0.40[a]	30.10 ± 0.40[a]
Diabetic+ SH (125 mg/kg)	19.33 ± 1.20[a]	16.35 ± 1.14[b]	15.53 ± 1.30[b]	12.56 ± 0.90[b]
Diabetic+ SH (250 mg/kg)	24.30 ± 0.09[b]	22.20 ± 0.08	17.57 ± 1.02[c]	14.27 ± 1.20[c]
Diabetic+ SH (500 mg/kg)	25.30 ± 0.01[b]	20.20 ± 0.04[c]	19.28 ± 0.10[d]	17.70 ± 0.30[d]
Diabetic+ glibenclamide	22.50 ± 3.30[c]	19.30 ± 3.20[c]	17.78 ± 2.40[c]	13.52 ± 2.50[c]

Values are mean ± SD of 6 rats in each group.

[a-e] Test values carrying superscripts different from the control for each parameter are significantly different ($P < 0.05$).

In our study the level of red blood cells, Hb, MCV, MCH, MCHC, LUC, RCDW and PCV were measured in diabetic rats induced with STZ-NAD to determine the anaemic conditions of the animals. The diabetic animals were severely anaemic while those treated with the plant extract showed no signs of anaemia. The level of red blood cells, Hb, MCV, MCH, MCHC, RCDW and PCV was significantly decreased in diabetic induced rats when compared with non diabetic groups (Table 2). This observation corroborated with the report of Baskar et al. (2006). However, it contradicts the report of Junod et al. (1969) who observed that diabetogenic action of streptozotocin may be lost

due to prior administration of nicotinamide. This observation support that intraperitoneal injection of nicotinamide into the rats may not completely prevent the effect of streptozotocin on pancreatic beta cells but allow minor damage by repairing poly ADP ribose synthetase (Giroix et al., 2002). The disturbed haematological alteration of red blood cells in diabetic animals was improved upon treatment with the plant extract by preventing haemolysis of erythrocytes caused by lipid peroxidation (Ceriello, 2003).