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ANTIHYPERGLYCEMIC EFFECTS OF AQUEOUS LEAF EXTRACT OF *SENNA FISTULA* IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Running title: Antihyperglycemic effect of Senna fistula

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ABSTRACT:
This study investigated antihyperglycemic effects of chronic administration of aqueous leaf extract of Senna fistula in Streptozotocin-induced diabetic rat. Thirty rats were randomly assigned into six groups (A-F). Animals in group A were the control non-diabetic, in group B were diabetic and received distilled water, in group C were diabetic, treated with 2.5 mg/kg body weight of Glibenclamide, while animals in groups D, E and F were diabetic treated with 28.57, 57.14 and 114.28 mg/kg body weight respectively of aqueous leaf extract of Senna fistula for 28 days. At the end of 28 days blood samples were collected for the assay of Insulin, Superoxide Dismutase, Catalase, and Glutathione Peroxidase in serum and liver Glycogen. The result showed that the blood glucose levels of diabetic rats were significantly reduced in the extract and Glibenclamide treated animals when compared with diabetic rats that received distilled water. Similarly, there was a significant increase in serum Insulin level, Superoxide dismutase, Catalase and Glutathione peroxidase activities and liver glycogen in the extract and Glibenclamide treated diabetic groups when compared with diabetic untreated group. The results indicated that oral administration of aqueous extract of Senna fistula has antihyperglycemic effect by stimulating Insulin secretion and activating antioxidant enzymes.

Keywords: Insulin, Superoxide dismutase, Senna fistula, Glibenclamide, Antioxidant

INTRODUCTION:

Diabetes mellitus (DM) is a metabolic disorder caused by several factors. It is characterized by a chronic high level of glucose in the blood due to disorders of protein, carbohydrate and fat, ensuing from defects in insulin secretion, insulin action, or both [1]. The high blood glucose level may occur over a prolonged period and produces the symptoms of polyphagia, polyuria and polydypsia. Untreated, diabetes can lead to many complications [2]. These complications include non-ketotic

hyperosmolar coma and diabetic ketoacidosis which are the acute forms of the complications in Type I DM [3]. Severe long-term complications include damage to the eyes, heart disease, kidney failure, stroke and foot ulcers, which may be due to Type II DM [2].

DM is reaching a pandemic level worldwide and affecting the developing countries of the world much more than the developed countries [4]. In Africa more than 5 million people have been reported to have DM and this figure is expected to rise to 15 million by 2025 [5]. With increased number of people with DM in Africa, the incidence of diabetes complications will also be on the increase equally [6,7]. This may lead to more health care and economic problem, however, with good glycemic control, morbidity and mortality of diabetic patients can be reduced as well as improve their quality of life.

Insulin is the main hormone responsible for controlling the uptake, utilization, and storage of cellular nutrients. Its anabolic action includes stimulation of intracellular utilization and storage of glucose, amino acids and fatty acids while it inhibit breakdown of glycogen, fat and protein mediated through β-adrenoceptor stimulation [8].

Free radicals are highly reactive molecules derived from the metabolism of oxygen; example is reactive oxygen species (ROS) [9]. Some of these free radicals play a positive role in physiological and biochemical processes when present at low/ moderate concentrations. However, over production of these free radicals e.g (ROS) in the body result in oxidative stress [10], causing potential biological damage. The excess ROS can damage cellular proteins, lipids, or DNA, inhibiting their normal function, as a result of this; oxidative stress has been implicated in a numbers of human diseases such as cancer, diabetes, atherosclerosis as well as aging process [10,11]. Inability to remove or destroy excess free radicals (ROS) has been attributed to decrease in endogenous antioxidant enzyme synthesis CAT, SOD, GPx and reduction in non-enzymatic protection (α-tocopherol, ascorbic acid, β carotene and uric acid) [12]. In diabetes, increased oxidative stress is known to be involved in the development and progression of the disease and its complications [13-15]. Therefore, this disease (diabetes) is usually accompanied by increased production of free radicals [14,16,17] or impaired antioxidant defences [18-20]. Studies have shown that a potent scavenger of these free radicals (ROS) may serve as a possible preventive and therapeutic intervention for free radical mediated diseases [21,22].

Traditional medicine has been reported to provide more than 85% of health care in Africa [23]. Similarly in Nigeria, a large segment of the population still rely on medicinal plants and even patronise traditional medicine practitioners for their health care needs [24], and about 46% of people with DM in Nigeria

use herbal remedy in the management of their condition [25].

Several species of plant are on earth [26], of which only small percentages (1-10%) of these are used for food and medicine by human and animals [27]. Medicinal plants have several biologically active compounds such as fat and oil, protein, carbohydrates, enzymes, minerals, vitamins, alkaloids, carotenoids, quinines, terpenoids, flavonoids, sterols, simple phenolic glycosides, tannins, saponins etc. which have medicinal properties.

Senna fistula belongs to the leguminosae family commonly known as Indian laburnum and locally known by the Yorubas as Aidantoroo. The plant has been used significantly in traditional medicine system for the treatment of many diseases, for example the pulps of the ripe fruit have anti-fungal and a mild pleasant laxative effect [28]. The pods are used in the treatment of blood poisoning and malaria. The decoction of the root is applied to treat ulcer and disinfect wound. Other ethno-medical uses of the plant include anti-dysentery and anti-diarrhoea [29]. The plant is also used in the treatment of DM and skin problem [30]. Its hepato-protective and antioxidant effects have also been evaluated [31].

The use of extracts from medicinal plants for the management of DM has received great attention in recent years. The mechanisms underlying their mode of action need experimental verification. At present only few have been verified why thousands are yet to be. This study investigated antihyperglycemic effects of Senna fistula and its mechanisms of action.

MATERIALS AND METHODS:

Plant material and authentication

Senna fistula leaves was purchased from Itoku market in Abeokuta, Ogun state. The identification of the plant leaves was carried out in the Department of Plant Biology of University of Ilorin, Kwara state, Nigeria, with a voucher specimen (UIH 1020), and a specimen was deposited in the Herbarium of the Department. Fresh leaves of S.fistula were air-dried at room temperature for about 2 weeks. The dried leaves were pulverised using electric blender and kept in a plastic container before the commencement of the study.

Animals:

Male and Female rats of Wistar strain, weight between 120 -130 g, were obtained from the animal holding of the Department of Biochemistry, University of Ilorin. The animals were fed on rats pellet (premier feed limited) and water ad libitum. All animals were maintained under standard laboratory conditions of temperature (22± 20C), humidity (45±5%) and natural photoperiod of about 12h light: dark cycle. All rats were handled in accordance with the guide for the care and use of laboratory animals [32].

Glucometer and Assay kit:

One touch ultra-blood glucose meter kit (lifescan, Inc. Milpitas, USA) and Insulin kit (Monobind Inc. Lake forest, USA) were used for this study.

Drugs and chemicals:

Glibenclamide was a product of HOVID Bhd, Ipoh, Malaysia. Streptozotocin and other chemicals were products of Sigma-Aldrich CHEME GMbH, Steinheim, Germany.

Plant extraction:

The aqueous leaf extract of Senna fistula was prepared using the method described by Yakubu et al., with slight modification [33]. About 158.50g of air-dried and powered leaves of S.fitula was exhaustively extracted with 2 litres of water by maceration for 24 hours, after which it was filtered and the filtrate was evaporated to dryness using water bath regulated at 400C. A dark green extract weighing 43.65g (27.54%) was obtained. The extract was stored in the refrigerator before the commencement of the study.

Animal grouping and drug administration:

Rats of both sexes were randomly assigned into 6 groups of 5 rats each:

In Group A were the control (non-diabetic rats); they received 0.5ml of distilled water. In Group B were the streptozotocin- induced diabetic rats; they received 0.5ml of distilled water (untreated rats). Group C were the diabetic rats treated with 2.5mg/kg body weight (bw) of Glibenclamide. Groups D to E were diabetic rats and treated with different doses of the aqueous extract of S. fistula as follows: 28.57mg/kg bw, 57.14mg/kg bw and 114.28mg/kg bw respectively.

The drug and extracts were administered orally, three times daily for a period of 28 days [34].

Induction of diabetes and determination of blood glucose:

The rats were fasted for 18 hours before induction of diabetes. DM was induced by single intraperitoneal injection of freshly prepared streptozotocin (STZ) (50mg/kg bw) in 0.1M citrate buffer (PH 4.5) [35]. The rats were feed (pellet and 5% dextrose saline) one hour after STZ injection to overcome initial hypoglycemic phase [36]. Five days post STZ injection, blood was collected from the tail vein of each rat and DM was confirmed by glucose oxidase method using one touch ultra-blood glucometer. Rats with blood glucose level higher or equal to 12.00mmol/L were used for the study [33].

The blood glucose level was determined using one touch ultra-blood glucometer (lifeScan USA) before the start of the experiment to ascertain their initial blood glucose, after the induction of diabetes and on the last day (day 28) of the experiment.

Insulin was determined using Accu-Bind ELISA kit (Monobind Lake Forest, USA). The assay was carried out using the procedure recommended by the manufacturer.Glycogen content in the liver was determined by the

method of Van [37]. Superoxide dismutase (SOD) activity was determined according to the spectrophotometric method of Mistra and Fridovich [38]. Glutathione peroxidase activity was measured by the procedure of Flohe and Gunzler [39]. Catalase activity was measured using the method of Aebi [40].

Statistical analysis:

All data are expressed as the mean ± Standard error of mean (SEM), the test for significance was done using ANOVA, and Duncan new multiple range test (DMRT). Differences were considered statistically significant at P< 0.05.

RESULTS:

Table 1 depicts the effect of administration of different concentrations of S. fistula extract on blood glucose level of diabetic rats. The diabetic untreated animals had significant high fasting blood glucose (FBG) level. However, the rats treated with different concentrations of Senna fistula extract and Glibenclamide had significant reduction in their fasting blood glucose (FBG) value when compared with diabetic untreated group ($p<0.05$). While there is no significant difference in the fasting blood glucose level of animals treated with different doses of S. fistula and Glibenclamide.

The effects of different concentrations of aqueous extract of Senna fistula on insulin concentration and liver glycogen of diabetic rats are shown in Table 2. There was a significant decrease in insulin level of diabetic untreated animals ($p<0.05$) when compared with the control, extract treated and Glibenclamide treated rats, administration of the extract increased the reduced insulin concentration in diabetic rats which compared favourably with the control and Glibenclamide treated rats. However, there is no significant difference in insulin concentration between the control Glibenclamide and animals treated with different doses of the extract

Similarly, there was a significant decrease in glycogen level of diabetic untreated rats ($p<0.05$) when compared with the control, extract treated and Glibenclamide treated rats, administration of the extract increased the reduced hepatic glycogen concentration in diabetic rats which can be compared with the control and glibenclamide treated rats. There is no significant difference in liver glycogen concentration between glibenclamide and animals treated with different doses of the extract.

Table 3, represents the effect of different concentrations of aqueous extract of S. fistula on catalase, superoxide dismutase and glutathione peroxidase activity of diabetic rats. Catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) showed a significant decrease ($p<0.05$) in their activities in diabetic untreated rats when compared with the control. Administration of different concentrations of aqueous leaf extract of Senna fistula reversed this trend significantly

(p<0.05). However activity of catalase was more in glibenclamide treated group, while, there were no significant difference (p<0.05) in SOD and GPX activities between the glibenclamide and all the extract treated rats.

Table 1: Effect of administration of aqueous leaf extract of S. fistula on fasting blood glucose level of STZ-induced diabetic rats

	Fasting blood glucose (mmol/L)	
Groups/Days	Initial (Post induction)	Final (After 28 days)
Control	4.71±0.45a	4.26±0.38a
Diabetic untreated	23.82±2.56b	30.28±3.52b
Diabetes + Glibenclamide	20.66±2.98b	3.66±0.39a
Diabetes + 28.57 mg/kg bw	33.80±0.00c	6.24±1.14a
Diabetes + 57.14 mg/kg bw	22.22±2.78b	4.76±0.19a
Diabetes + 114.57 mg/kg bw	23.10±2.98b	6.02±0.83a

Values are expressed as Mean ±SEM, n= 5. Mean with different letters are significantly different (p<0.05)

Table 2: Effect of administration of aqueous leaf extract of Senna fistula on insulin and liver glycogen level of streptozotocin-induced diabetic rats

Group/parameters	Insulin (µU/ml)	Glycogen (mg/100g tissue)
Control	10.50±0.63b	6.59±0.25d
Diabetic untreated	5.50±0.87a	1.36±0.14a
Diabetes + glibenclamide	8.75±0.75b	2.39±0.18b
Diabetes + 28.57mg/kg bw	9.50±2.24b	3.23±0.35bc
Diabetes + 57.14mg/kg bw	10.20±0.60b	3.81±0.38c
Diabetes + 114.57mg/kg bw	10.13±1.96b	3.76±0.44c

Values are expressed as Mean ±SEM, n= 5. Mean with different letters are significantly different (p<0.05)

Table 3: Effect of S. fistula leaf extract on antioxidant enzymes of STZ-induced diabetic rats

Group/parameters	Catalase (U/mg protein)	Superoxide dismutase (U/mg protein)	Glutathione peroxidase (U/mg protein)
Control	3.87±0.52c	15.57±3.11c	0.14±0.21c
Diabetic untreated	0.76±0.10a	1.69±0.22a	0.04±0.12a
Diabetes + glibenclamide	7.47±0.83d	8.35±0.17b	0.11±0.14bc
Diabetes + 28.57mg/kg bw	1.90±0.31b	6.16±1.60b	0.08±0.31bc
Diabetes + 57.14mg/kg bw	1.95±0.34b	8.83±2.08b	0.05±0.11b
Diabetes + 114.57mg/kg bw	2.95±0.22bc	5.50±2.42b	0.12±0.25c

Values are expressed as Mean ±SEM, n= 5. Mean with different letters are significantly different (p<0.05)

DISCUSSION:

Plants have been a good source of drugs and most available drugs have been obtained directly or indirectly from plant. There are different types of blood glucose lowering drugs, exerting antidiabetic effects through different mechanisms, for example alpha glucosidase act by delaying the intestinal absorption of glucose, also sulphonylurea acts by stimulating insulin secretion while thiazolidinediones act by increasing peripheral uptake of glucose [41]. Anti-hyperglycemic effects of plants are mainly due to their ability to restore and enhance the functions of pancreatic tissue either by causing an increase in insulin output or inhibit intestinal absorption of glucose or reduce hepatic gluconeogenesis [42]. Antidiabetic potential of medicinal plants have been grouped into two; the primary antidiabetic potential which refers to biological activity which have hypoglycemic effects through the actions of insulin producing and insulin responsive cells required for glucose, protein and lipid homeostasis, and the secondary antidiabetic potential involving protection against long term diabetic complications through their antiglycation and antioxidant properties [43]. The significant reduction in the blood glucose level of diabetic rats treated with different doses of S. fistula extract suggests anti-hyperglycemic effect of the plant.

The anti-hyperglycaemic effect of the S. fistula extract may be partly linked to its constituents that have been reported to contain glycosides, flavonoids, terpenoids, Ca, K, Zn, Mn, Mg and Vitamin C [44]. Studies have reported that medicinal plants with anti-hyperglycemic property usually contain flavonoids, tannins, terpenoids and Alkaloids [45,46]

Beta-cells of the pancreas are selectively destroyed by a cytotoxic substance like streptozotocin (STZ) which cause a significant reduction in the synthesis of endogeneous insulin [47]. Glibenclamide on the other hand stimulate the release of insulin from the remaining pancreatic β cells [8]. In STZ-induced diabetic animals, serum insulin level was found to decrease whereas upon administration of the extract, there was a significant increase in serum insulin level in diabetic rats which is comparable to Glibenclamide treated rats. This finding agrees with the study of Einstein et al., [48], where it was documented that methanolic extracts of Cassia fistula bark and leaf caused significant increase in plasma insulin in diabetic rats. Improvement in insulin secretion in extract treated groups signify a pancreatic mode of action of S. fistula, which may be related to the presence of some minerals and secondary metabolites like Zinc, K, Mn, Mg, Terpenoids, Flavonoids, Glycosides and others. This observation is supported by findings that natural products classified into Flavonoids, Terpenoids, Phenolics, Alkaloids etc exhibit antidiabetic potentials through insulinomimetic

activity [42]. Similarly, minerals like K, Ca Zn, Mn Fe etc have been reported to stimulate insulin secretion from the beta cells of the pancreas [49,50]. Therefore it can be deduced that the Senna fistula leaf extract might have acted as an antihyperglycaemic agent by stimulating the release of insulin from the remnant pancreatic β cell [51].

Liver glycogen level is often used to assess antihyperglycaemic activity of any dug. In this study the significant decrease in liver glycogen of the diabetic untreated rats agrees with previous work documenting a decrease in liver glycogen in diabetic rats [52], which may be due to increase in glucose output due to insulin deficiency. The decrease in liver glycogen in diabetic rats may also be linked to decrease in insulin level because glycogen level in the liver is a direct reflection of insulin activity. Insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. Aqueous extract of S. fistula leaves increased the lowered liver glycogen in diabetic rats. The extract might have acted by reactivating glycogen synthase system as a result of increased insulin secretion [53] or due to insulinomimetic effect of the extract leading to increased peripheral glucose uptake [54, 55].

The significant decrease in the level of the activities of CAT, SOD and GPx in STZ-induced diabetic rats may be associated with increased oxidative stress and / or decrease antioxidant defence potentials, because diabetes is usually accompanied by increase production of free radicals [14,16,17], or impaired antioxidant defences [18-20]. The improvement in the activity of CAT, SOD, GPx following treatment with aqueous extract of S. fistula and Glibenclamide agree with studies documenting antioxidant effects of this plant in diabetic rats [56], which indicate the possible mechanisms of the beneficial effects of S. fistula in the treatment of DM. This antioxidant activity may be due to the reduction in the imbalances between ROS production and scavenging enzymes actively in diabetic rats. The extract of S. fistula leaves may act either by directly scavenging reactive oxygen metabolites due to the presence of various antioxidant compounds [57] or by increasing the synthesis of antioxidant.

This study concludes that the significant reduction of the FBG in all the extract treated groups to the value of the control and the reference treated groups signifies anti hyperglycemic property of Senna fistula.

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