

STRYCNOS SPINOSA DECREASES THE BLOOD GLUCOSE AND LIPID LEVELS OF DIABETIC ALBINO RATS

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ABSTRACT

Diabetes mellitus has reached epidemic levels with an estimate of 451 million cases worldwide in 2017. Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin. *Strychnos spinosa* has been used in folk medicine as a remedy for various diseases including diabetes but the exact mechanism of action is still unknown. The present study aimed to evaluate the hypoglycaemic and hypolipidemic effects of the methanolic extract of the *Strychnos spinosa* leaves on alloxan-induced diabetic albino rats. Diabetes mellitus was experimentally induced in rats by intraperitoneal injection of alloxan monohydrate at a dose of 150 mg/kg. The pure extract of *S. spinosa* leaf was given orally once daily for 2 weeks in three graded doses of 150, 300 and 500 mg/kg and glibenclamide was used to treat the positive control group. Following treatments, the glucose level and lipid profile assay were carried out using colorimetric methods. The extracts significantly reduced ($p < 0.05$) the fasting blood glucose, TAG and cholesterol levels of the diabetic and non-diabetic rats. Treatment with the extract also decreased mortalities of the diabetic rats. These findings provides evidence to the increased use of the plant in folk medicine.

Keywords Diabetes, *Strychnos Spinosa*, Alloxan, Glucose, Insulin

INTRODUCTION

Diabetes mellitus is a disorder of carbohydrate, protein and lipid metabolism that affects the physical, social and psychological health of humans (Mohammed *et al.*, 2015). Along with cardiovascular diseases, diabetes contributes significantly to mortalities worldwide. Thus far, over 451 million cases were diagnosed in 2017 and the number of cases is projected to reach 693 million by 2045 (Cho *et al.*, 2018). There are two major types of diabetes – types I and II. Type I diabetes is characterized by complete absence of insulin while type II is as a result of insulin resistance and/or inadequate production of insulin (Jain *et al.*, 2014). Type II diabetes is the most common form of diabetes accounting for over 90% of all cases diagnosed in Western countries and sub-Saharan Africa.

Despite the progress made in the management of the disease, there is still no known cure for diabetes, as such the condition can only be managed. More recently, the use of medicinal herbs in sub-Saharan Africa is gaining momentum. In Nigeria for instance, the boiled extract of *Strychnos spinosa* (*kokiya*) is administered to patients of diabetes to relief the condition. This practice is entirely based on traditional folklore and with little or no scientific data to support its use.

Therefore, the current study was designed to evaluate the antidiabetic properties of the leaves of *S. spinosa*.

MATERIAL AND METHOD

Plant Collection and Preparation

Fresh leaves of *Strychnos spinosa* was obtained from Ahugyuru Kufana, Kajuru Local Government area, Kaduna state. The plant was identified by the resident botanist of the department of Biological Science, Kaduna State University, Mal U S Gallah who assigned the plant the voucher specimen no, 1113. The leaves were shade dried for 4 weeks and pulverized into fine powder.

Extraction of the Plant Material

About 260 g of the pulverized powder of *Strychnos spinosa* was soaked successively in 800 mL each of n-hexane and methanol for 48 h. The extracts were collected by sieving first with muslin cloth and then Whatmann filter paper. The extracts were collected and dried in water bath at 40°C.

Total Polyphenols Assay

Total phenolic content of the extract was estimated using the Folin-Ciocalteu method as described by Ainsworth and Gillespie (2007). Calibration curve of the absorbance values against varying concentration of Gallic acid standard was plotted. The result obtained was expressed as gallic acid equivalent (GAE) per gram of sample (Ainsworth and Gillespie, 2007; Alhakmani *et al.*, 2013).

Total Flavonoid Assay

The total flavonoid content (TFC) were determined using the aluminium chloride colourimetric method. The absorbance of each mixture produced was determined at 510 nm. TFC was determined as mg quercetin equivalent per gram of sample with using a calibration curve of quercetin (Zhishen *et al.*, 1999).

In Vivo Assay

Experimental Animals

Twenty four (24) Albino rats of between 90 to 160 g body weights were purchased from NITR (National Institute of Trypanosome Research) for the study. The rats are of both sexes and housed four (4) per cage in a temperature controlled room (25 to 35°C), with a light/dark cycle of twelve (12) hours. The rats were allowed to acclimatize to the laboratory environment and given free access to the standard rat chow diet (pellets) and tap water. The rats were monitored daily and their cages were cleaned three to four times weekly. Before the commencement of the experiment, the rats were randomly distributed in six different groups.

Induction of diabetes

The rats were allowed to fast overnight prior to the induction of diabetes, followed by the intra-peritoneal (IP) injection of alloxan

at dose of 120 mg/ kg body of rats. Diabetes was confirmed via the one stop Accu check glucometer. By the 5th day of induction, all rats were confirmed diabetic.

Treatment and Determination of Blood Glucose Level

The animals were randomly distributed into six different groups as shown in Table 1 and treated with the extract and standard drug (glibenclamide) once daily for 2 weeks. Animals from groups I, II and III respectively were administered with the extract at a dose of 150, 300 and 500 mg/kg body weight of rats. Glibenclamide was used as positive control while normal saline was used as negative control (Table 1).

Table 1: Experimental animals' treatment design

| S/No | Groups | Names of Drugs | Dose | No. of Animals | Duration of dosages (days) |
|------|-----------|---------------------------|---------------|----------------|----------------------------|
| 1 | Group I | Positive control | Normal saline | 4 | 11 |
| 2 | Group II | Negative control | Normal saline | 4 | 11 |
| 3 | Group III | Glibenclamide | 5 mg/Kg b.w | 4 | 11 |
| 4 | Group IV | Extract low dose | 150 mg/Kg b.w | 4 | 11 |
| 5 | Group V | Extract intermediate dose | 300 mg/Kg b.w | 4 | 11 |
| 6 | Group VI | Extract high dose | 500 mg/Kg b.w | 4 | 11 |

Kg: kilogram, b.w: body weight

Blood Glucose Assay

About 0.1 mL of blood was collected from lateral tail vein using lance or butterfly needle and blood glucose level was checked by using an Accu chek glucometer (Life Scan Inc, Switzerland) containing glucose assay test strips (Chemelex, South Africa).

Lipid Profile Assay

After 11 days of treatment, the animals were anesthetized with chloroform and blood samples were drawn through cardiac puncture, centrifuged at 4000 rpm for 20 minutes and the sera were separated and stored in the freezer until needed for assay. The level of triglyceride and cholesterol were measured using TAG and total cholesterol assay kit (Chemelex, South Africa) following manufacturer's instruction.

Statistical analysis

One way analysis of variance was used to determine level of significance between groups using SPSS (IBM Inc, USA) version 22. The experiment was carried out in triplicates and values were reported as mean ± standard deviation.

RESULTS AND DISCUSSION

Phenol and flavonoid content of *S. spinosa* leaves

The extraction yield obtained in this study is 14.43 %. The phenolic content of the extract is 32.7% (327.6 ± 0.19 mg gallic acid equivalent per gram of extract) while the flavonoid content is 27.4% (273.5 ± 0.01 mg quercetin equivalent (mgQE/g) per gram of the extract) (Table 2). Phenolic compounds and flavonoid are some of the agents known to contribute to the therapeutic effect of plants (Akowuah *et al.*, 2002).

Table 2: Phenolic and flavonoid contents of the leaves of *S. spinosa*

| Phytochemical | Quantity (%) |
|---------------|--------------|
| Phenolics | 32.8 |
| flavonoid | 27.4 |

Effect of the extract of *S. spinosa* on the glucose level of diabetic and non-diabetic rats

The extract of *S. spinosa* at all doses of administration significantly reduced ($p < 0.05$) the level of glucose as the treatment time progressed from Day 0 to Day 7 (Fig 1). Similarly, the standard drug (glibenclamide) significantly decreased ($p < 0.05$) the glucose level as the treatment time progressed. No significant decrease ($p > 0.05$) was observed in rats treated with normal saline (Fig 1). More so, the lowest dose of extract treatment produced the highest percentage of blood glucose reduction (Fig 1). Plants extracts are known to exert hypoglycemic effect by modulation of the insulin activity, either by stimulating the pancreatic secretion of insulin from the cells of islets of Langerhans or its release from bound insulin, while others act through extra pancreatic mechanisms by inhibition of hepatic glucose production or corrections of insulin resistance (Hu *et al.*, 2003; Xu *et al.*, 2008). We presume that the extract of *S. spinosa* may have acted any of the mechanisms described above. The decreased level of blood glucose following treatment with the extracts further suggests that the extract may have stimulated insulin uptake by the body cells which led to the ultimate decrease after 11 days of treatment.

Effect of the extract of *S. spinosa* on triacylglycerol (TAG) and cholesterol level of diabetic and non-diabetic rats

Treatment with the extract of *S. spinosa* significantly reduced the level of TAG and cholesterol (Fig 2). Interestingly, the lowest dose of extract treatment produced the most significant reduction of TAG and cholesterol level of rats (Fig 2).

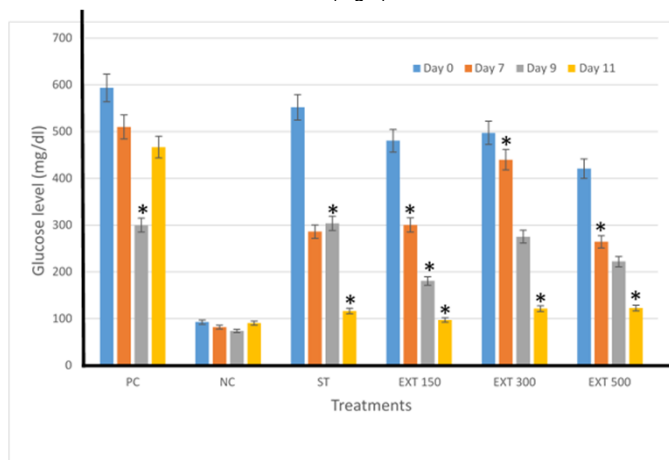


Figure 1: Blood glucose levels of albino rats after treatment with Glibenclamide and extract of *S. spinosa* at 150, 300 and 500 mg/kg body weight. PC – positive control, NC – negative control, ST – standard drug (glibenclamide), EXT 150 – extract (150 mg/kg), EXT 300 – extract (300 mg/kg) and ext 500 – extract (500 mg/kg). Means are ± SD. * signifies statistical significance

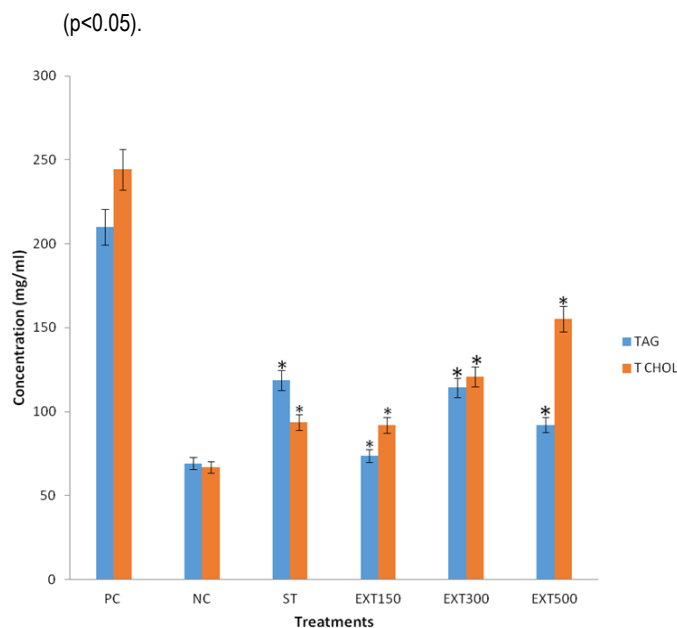


Figure 2: Triacylglyceride (TAG) and total cholesterol (T chol) levels of albino rats after treatment with Glimeclamide and extract of *S. spinosa* at 150, 300 and 500 mg/kg body weight. PC – positive control, NC – negative control, ST – standard drug (glibenclamide), EXT 150 – extract (150 mg/kg), EXT 300 – extract (300 mg/kg) and ext 500 – extract (500 mg/kg). Means are \pm SD. * signifies statistical significance ($p < 0.05$).

In the same vein, the extract significantly reduced the level of TAG and cholesterol in this study. Several plant extracts have been reported to decrease the lipid level of diabetic rats (Gidado *et al.*, 2009; Okoro *et al.*, 2014). Insulin has profound effects on lipid metabolism. The increase in the levels of serum lipids such as cholesterol and triglyceride after inducing diabetes may be due to the fact that under normal circumstances, insulin activates lipoprotein lipase and hydrolyses TAG (Arise *et al.*, 2014). Insulin increases uptake of fatty acids into adipose tissue and increases TAG synthesis (Arise *et al.*, 2014). Moreover, insulin inhibits lipolysis. In case of insulin deficiency, lipolysis is not inhibited but an increased lipolysis which finally leads to hyperlipidaemia. In diabetic condition, the concentration of serum free acids is elevated as a result of free fatty acids outflow from fat deposited, where the balance of the free fatty acids esterification-triglyceride lipolysis cycle is displaced in favour of lipolysis (Arise *et al.*, 2014). Therefore, the extract of *S. spinosa* may have activated insulin and also inhibited the synthesis of TAG and cholesterol. With this, it is evident that the extract of *S. spinosa* contains potent antidiabetic compounds that could be used for the treatment of diabetes.

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