Original Article

Effect of ethanolic extract of *Cryptolepis sanguinolenta* stem on *in vivo* and *in vitro* glucose absorption and transport: Mechanism of its antidiabetic activity

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ABSTRACT

Objective: Extracts from various morphological parts of *Cryptolepis sanguinolenta* are widely used traditionally in folklore medicine in many parts of the world for the management, control, and/or treatment of a plethora of human ailments, including diabetes mellitus. In order to scientifically appraise some of the ethnomedical uses of *Cryptolepis sanguinolenta*, the present study was undertaken to investigate its influence at varying doses on intestinal glucose absorption and transport in relation to its hypoglycemic and hypolipidemic effects in rat experimental paradigms. **Materials and Methods:** The animals used were divided into four groups. Control animals received 2 ml of distilled water, while treated groups received 50, 150, and 250 mg/kg bw of *Cryptolepis sanguinolenta* extract per oral respectively daily for 21 days. **Results:** *Cryptolepis sanguinolenta* led to a significant decrease in glucose transport and absorption. It also caused significant reductions in plasma glucose, total cholesterol, triglyceride, and LDL cholesterol. Biochemical changes observed were suggestive of dose dependence. Histopathological studies also showed increased sizes of β cells of the pancreas. **Conclusion:** The findings in these normoglycemic laboratory animals suggest that *Cryptolepis sanguinolenta* has hypoglycemic and hypolipidemic activities, possibly by reducing glucose absorption and transport, and enhancing the structural and functional abilities of the β cells. This is the first study to report the effect of *Cryptolepis sanguinolenta* on intestinal glucose absorption. This effect could be attributed to its major bioactive principle, cryptolepine, an indoloquinoline alkaloid. This study thus lends credence to the use of *Cryptolepis sanguinolenta* in the management of diabetes mellitus.

Key words: β cells, *Cryptolepis sanguinolenta*, diabetes, glucose, lipid

Introduction

Diabetes mellitus (DM) is a clinical syndrome characterized by inappropriate hyperglycemia caused by absolute or relative deficiency of insulin or resistance to the action of insulin at the cellular level. DM is one of the most common endocrine and metabolic disorders of the 21st century, and a major threat to healthcare worldwide.[1] DM is associated with alterations in carbohydrate, protein, and lipid metabolism.[2,3] World Health Organization (WHO) suggests that the global population is in the midst of a diabetes epidemic and the number of cases for diabetes that is currently 171 million is predicted to reach 366 million by the year 2030.[4] Majority of patients with diabetes have type 2 or non-insulin-dependent diabetes while the remainder has type 1 or insulin dependent diabetes. Although the two types have distinct pathogenesis, hyperglycemia and various life-threatening complications resulting from long-term hyperglycemia are the most common features.[5] The

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complications of diabetes are associated with elevated levels of plasma triglycerides and cholesterol (due to an increase in plasma concentration of VLDL and LDL). These play significant roles in the accelerated development of arteriosclerotic vascular disease.[6,7] Since the major cause of diabetes is a defect in insulin, it is then required to develop pharmacological agents that could prevent beta-cell destruction or promote beta cell regeneration in the pancreas.

Drugs used in the management of DM are not without side effects. This has led to the continued search for new antidiabetic drugs in the folklore/Ayurvedic medicine with the aim of getting less expensive and safer management options. A plant commonly used for its antidiabetic activity is *Cryptolepis sanguinolenta* containing *cryptolepine*, an indoloquinoline alkaloid.

Cryptolepis sanguinolenta is a thin-stemmed twining and scrambling shrub up to 8 m long, containing yellow-orange juice which becomes red upon drying. It is a member of the family Apocynaceae (subfamily: Periplocoideae). The plant is native to West Africa and is found in countries like Ghana, Cote d'Ivoire, Guinea, Guinea-Bissau, Mali, Nigeria, Senegal, Sierra Leone, Angola, Congo, Uganda, and Cameroon. It is a medicinal plant used by some traditional herbalist in the treatment of fever, urinary, and upper respiratory tract infections.[8] The use of this plant as a medical therapy has increased as it has been proposed that the root and leave extracts have hypotensive, antipyretic, anti-inflammatory, antidiarrhoeal, *in vitro* antibacterial and antimalarial effects.[9] It is commonly called *nibima*, *Kadze*, *gangamau*, or yellow-die root.[8] It is called *paran pupa* in the Yoruba- speaking areas of Nigeria.

Studies have documented the antidiabetic potentials of *Cryptolepis sanguinolenta*;[10-12] however, there is still a dearth of information in the open scientific literature on the studies that evaluated the effect of the *Cryptolepis sanguinolenta* on intestinal glucose absorption and transport. Therefore, we decided to bridge this gap and provide scientific information on the role of glucose absorption and transport as a mechanism of the antidiabetic activity of *Cryptolepis sanguinolenta*.

Materials and Methods

Plant materials

Stem of *Cryptolepis sanguinolenta* were obtained from Ojurin Akobo- Olorunda road, Oyo, Oyo state, Nigeria. The plant was authenticated by Ugbogu. A, Chukwuma E.C, and Shasanya O.S of the Forest Herbarium, Ibadan, Nigeria, where a voucher specimen (FHI 108847) was deposited. The dried stem was broken into pieces and beaten into smaller sizes using mortar and pestle before being pulverized. The powder formed after pulverization was weighed and stored until required.

Preparation of ethanolic extract of *Cryptolepis Sanguinolenta*

Extract of *cryptolepine sanguinolenta* was prepared as described by Ansah *et al.*,[13] with some modifications. Briefly, 1,226 g of the powdery stem was dissolved in 4.8 l of 65% ethanol. The mixture was dissolved to stand for 48 hours. The extract was filtered using clothen sieve and was evaporated at 40°C. From the viscous solution gotten, a 0.1 M solution of extract was prepared by dissolving 5 ml of viscous solution of extract in 45 ml of distilled water.

Animals

Wistar albino rats weighing 130--170 g were obtained from the Animal House of the Department of Physiology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The rats were housed in standard plastic cages, with 12-hour light--dark cycle. The animals were fed with rat chow and water without restriction. The rats were acclimatized for 2 weeks. They were assigned into four groups.[14] Control rats did not receive any extract. Animals in the test groups (1, 2, and 3) were orally fed using oral cannula once daily with 50 mg/kg, 150 mg/kg, and 250 mg/kg body weight of extract respectively. Administration of the extract was carried out for 21 days.

All animals received humane care in compliance with the institution's guideline and criteria for humane care as outlined in the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

Collection of blood samples

The blood samples used for the glucose test were obtained by pricking the tip of the tail of the rat. The blood samples used for the lipid profile were got via cardiac puncture. The blood was collected into EDTA bottles, spinned with centrifuge for 5 minutes, and the plasma was separated for lipid profile analysis.

Blood glucose analysis

The animals were fasted for a 12-hour period and the initial fasting blood glucose were taken for the four groups on the day one of the experiment using glucometer. The blood taken from the tail tip was placed on designated spot on glucometer strip connected to the glucometer. The final blood glucose of the rats was taken after an about 24-hour fasting period after administering the extract for 21 days.

Intestinal absorption *in vivo*

After an overnight fast, each animal was anesthetized

using urethane (0.6 ml of 25% w/v of urethane per 100 g body weight). The trachea was exposed and cannulated. A midline incision was then made into the abdominal wall. The intestine was gently pushed out without much massage and two intestinal segments (15--20 cm long) were identified for consistency; it was always ensured that the first segment was just distal to the ligament of Trez while the other was just proximal to the ileo--cecal junction. The segments were opened at both ends and washed by syringe irrigation with Kreb's bicarbonate solution the excess fluid being removed by gently forcing air under low pressure through the segment. The unopened end of each segment was then ligated. Each segment was thereafter filled with 4 ml of Kreb's bicarbonate solution containing 5.6 mM glucose and 2 g/l polyethylene glycol (PEG) after which the ends were ligated. The segments were replaced in the abdominal cavity, which was closed with a clamp. The temperature of each animal was maintained with a heating lamp. Each study lasted for an hour, after which the two segments were removed from each animal. Each segment was drained and then weighed. The samples were then analyzed for glucose with the use of glucose strip and glucometer.

Intestinal transport *in vitro*

The everted intestinal sac technique was used. After an overnight fast, animals were killed by ether anesthesia. The abdomen was opened and the whole of the small intestine taken out and flushed with Kreb's bicarbonate solution. Polyethylene tubing, closed at one end, was inserted and tied at one end of the intestinal loop; through this, the gut was everted and placed in Kreb's bicarbonate solution. Four segments (5--8 cm) were prepared for eat animal, one of the ends being tied with a thread and the other ends encircled by a ligature of Trez 0.5 ml of Kreb's bicarbonate solution and a bubble of 95% 5% O_2/$C0_2$ mixture was introduced into each sac through a syringe and the loose ligature tied. The sac was then placed in the fest tube containing 10 ml of Kreb's bicarbonate solution kept at 37°C and aerated continuously. After 30 minutes of incubation, the sacs were removed and the contents emptied for the determination of glucose concentration using glucometer and glucometer strips.

Lipid profile analysis

After the experiment period, the animals were killed and blood was taken via cardiac puncture. The blood was collected in EDTA bottles, spinned, and the plasma was collected from the whole blood. The plasma was used for lipid analysis which includes tests for triglyceride (TG), total cholesterol (TC), and high-density lipoprotein (HDL) using a reagent kit from Randox laboratory Ltd., UK. Low-density lipoprotein (LDL) was calculated using Friedewald's equation which is given as:

LDL = TC – HDL – TG/ 50.

Histopathological study

After the 21st day, the animals were killed through cervical dislocation and opened up. The pancreas were collected and preserved in 10% formalin. Tissue processing was carried out by autotechnique. Five micrometer thick sections were prepared and mounted on slides and stained with hematoxylin and eosin (H&E). Stained sections were morphologically evaluated and the microphotograph was taken.

Statistical analysis

Data were expressed as mean ± standard error of mean (n=5). Values for blood glucose before and after treatment were analyzed using a paired t-test, while those for the lipid profile were analyzed using an unpaired t-test to compare each test group and control. Analysis of variance, ANOVA, was used to compare values across all groups. All analyses were done using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA). Differences in mean values were considered significant at $P<0.05$.

Results

There were significant ($P<0.05$) reductions in the blood glucose concentrations in the treated groups [Figure 1]. There were also significant ($P<0.05$) reductions in the fasting plasma levels of TC, TG, and LDL cholesterol in the treated groups. The changes seen were dose related. HDL-cholesterol was significantly increased ($P<0.05$) in a dose dependent manner.

Similarly, intestinal glucose absorption and transport were also significantly ($P<0.05$) reduced in all treated animals in a dose-dependent manner [Figures 2 and 3].

A microscopic study showed an increase in the sizes of Islet of Langerhans and altered β-cells counts [Figure 4].

Discussion

Along with hyperglycemia and abnormalities in serum lipids, diabetes is usually associated with microvascular and macrovascular complications which are the major causes of morbidity and mortality in diabetic individuals.[15] Diabetes can be managed by exercise, diet, and drugs. Hypoglycemic drugs are either too expensive, or possess undesirable side effects. Therefore, the search for more effective and safer hypoglycemic agents from plants and other natural sources has continued to be an area of interest for many researchers.[16] Plants are a major source of drugs

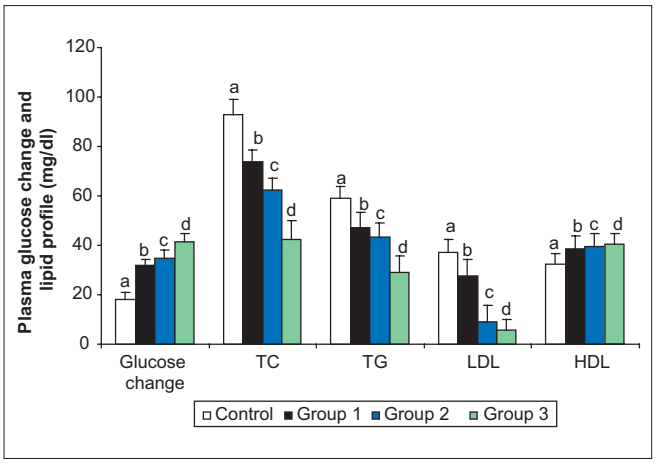

Figure 1: Effects of graded doses of *Cryptolepis sanguinolenta* stem ethanolic extract on plasma glucose and lipid profile in normoglycaemic rats. Bars carrying letter different on each parameter are significantly different at $P<0.05$

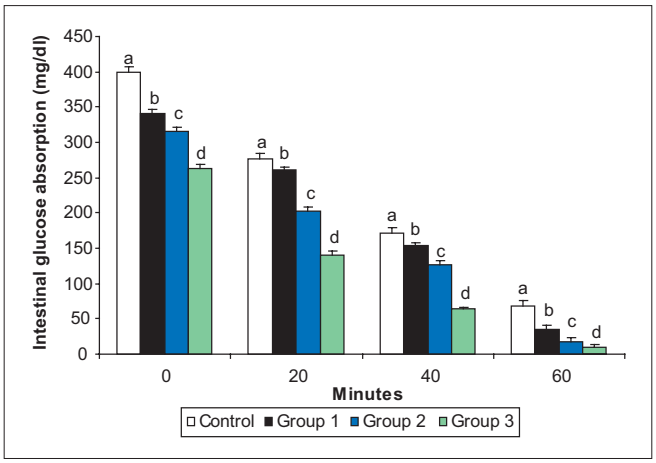

Figure 2: Effect of graded doses of *Cryptolepis sanguinolenta* stem ethanolic extract on intestinal glucose absorption (*in vivo*) in normoglycaemic rats. Bars carrying different letters on each minute are significantly different at $P<0.05$

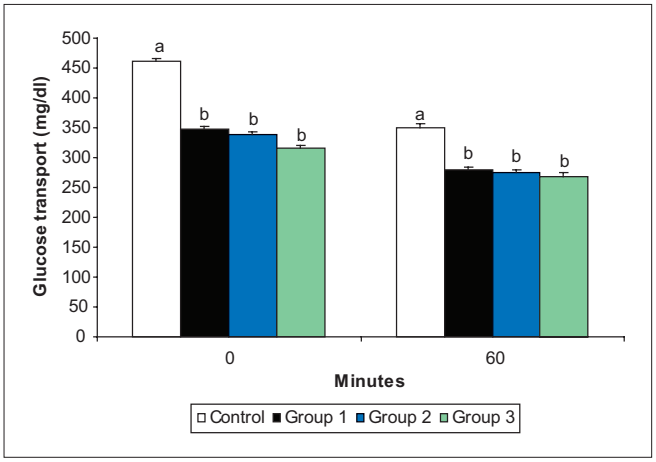

Figure 3: Effect of graded doses of *Cryptolepis sanguinolenta* stem ethanolic extract on glucose transport (*in vitro*) in normoglycaemic rats. Bars carrying different letters on each minute are significantly different at $P<0.05$

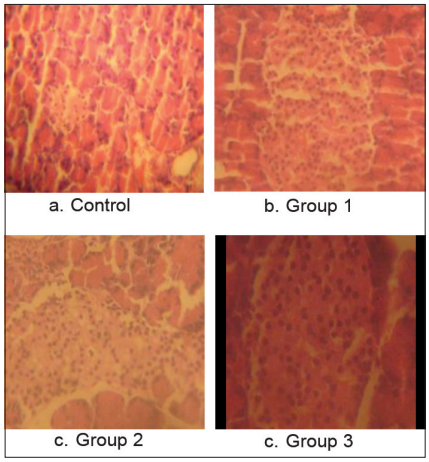

Figure 4: Histograph of the pancreas showing the Islet of Langerhans of the rats. (a) The slide above shows the normal size of the islet of Langerhans and normal count of the β-cells. (b) There are increased sizes of the Islet of Langerhans and the number of β-cells equally increased. The number of β-cells seen here is more than 200. (c) The sizes of the islet of Langerhans are increased. The number of β-cells is much but the count is reduced compared to the count seen in the control group. (d) The Langerhans are enlarged compared to other groups and the β-cells count has been reduced to about 28 counts. The proportion of β-cells count to the size is reduced

and many currently available drugs have been directly or indirectly obtained from botanicals.[17] Phytochemical screening helps to reveal the chemical constituents of the plant extract and the one that predominates over the others. It is also used to search for bioactive agents for starting products used in the synthesis of some useful drugs.[18] Results from the phytochemical analyses of *Cryptolepis sanguinolenta* have shown the presence of *Cryptolepine*, an indoloquinoline alkaloid, as its major alkaloids.[19-22] Crude extracts of *Cryptolepis sanguinolenta* and their fractions, as well as indoquinoline alkaloids isolated from the plant, have been shown to have antidiabetic activity.[10,11,13,23] Various studies have linked its antidiabetic activity to its effects on pancreas and glucose transport, but none has associated it with glucose intestinal absorption. This study thus documents the influence of *Cryptolepis sanguinolenta* on intestinal glucose absorption and transport in relation to its antihyperglycemic potentials.

In the present study, we noticed dose-related reductions in plasma glucose in *Cryptolepis sanguinolenta*-treated normoglycemic rats. Similarly, the extract reduced total cholesterol, triglyceride, and LDL cholesterol in treated rats when compared to the control. Lipids play an important role in the pathogenesis of diabetes mellitus. This is in consistence with previous studies.[10,11,13,23] The level of serum lipids is usually raised in diabetes, and such an elevation represents a risk factor for coronary heart

diseases.[24] The alterations in plasma glucose and lipid profile seen in treated animals could be beneficial in preventing diabetic complications as well as in improving lipid metabolism in diabetics.

The findings of this experimental animal study also showed that *Cryptolepis sanguinolenta* significantly depressed intestinal glucose absorption and transport in the normoglycemic rats in a dose-related manner. The depression of glucose absorption and transport observed may account for the antihyperglycemic effect of the botanical with resultant enhancement of lipid profile. This is in tandem with previous study[10,11] that reported that *Cryptolepis sanguinolenta* reduced glucose transport and caused hypoglycemia.

Observations in this study also showed that *Cryptolepis sanguinolenta* treatment led to increased β cell sizes. This probably enhanced insulin activities. The fundamental mechanism underlying hyperglycemia involves overproduction (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues.[25] The hypertrophied β cells might be responsible for enhanced insulin production and activity with resultant hypoglycemia. The reduction in the blood glucose levels of the treated animals confirms the hypoglycemic effect of *Cryptolepis sanguinolenta* stem extract. The findings in this study revealed that the extract acted via several mechanisms such as slowing down the absorption and transport of glucose from the guts, and increasing insulin production by the pancreas from possibly the hypertrophied β cells. These mechanisms of action corroborates other mechanisms previously reported such as increase glucose uptake by 3T3-L1 cells,[10-12] and enhancement of insulin-mediated glucose disposal.[12]

The hypoglycemic activity of the extract seen in the present study could be attributed to its alkaloid constituents. Alkaloids have been reported to improve insulin resistance in mice and high fat-fed rats. They can activate AMP-activated protein kinase in 3T3-L1 adipocytes and L6 myotubes and facilitate GLUT4 translocation in L6 myotubes in a phosphatidylinositol 3-kinase-independent manner.[26] They have also been reported to promote glucose uptake in HepG2 and 3T3-L1 cells independent of insulin action.[27,28] In addition, it effectively inhibits sucrase and maltase activities to the same extent as acarbose does in Caco-2 intestinal cells and possibly inhibit α-glucosidase activities to reduce glucose absorption.[29] It has also been documented to significantly reduce serum IL-6 with insulin sensitivity improvement.[30-32] This study provides a new mechanism of action of the hypoglycemic activity of alkaloids-containing *Cryptolepis sanguinolenta* stem extract.

Conclusion

Based on our findings, we lend credence to the use of *Cryptolepis sanguinolenta* in the management of diabetes mellitus. The hypoglycemic activity of *Cryptolepis sanguinolenta* is associated with its influence to reduce intestinal glucose absorption and transport. It is also related to its hypertrophic effect on β cells.

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