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Anti-diabetic effects of the acetone fraction of *Senna singueana* stem bark in a type 2 diabetes rat model

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Chemical compounds studied in this article:
Streptozotocin (CID 29327)
α-Glucosidase (E.C. 3.2.1.20)
α-Amylase (E.C. 3.2.1.1)
p-Nitrophenyl-α-d-glucopyranoside (pNPG) (CID 11197369)
p-Nitrophenol (CID 980)
Starch (CID 439341)
Dinitrosalicylic acid (DNS) (CID 11873)
Maltose (CID 6255)
Absolute ethanol (CID 702)
Ethyl acetate (CID 8857)
Hydrogen peroxide (CID 784)

ABSTRACT

Ethnopharmacological relevance: *Senna singueana* is currently used in the traditional treatment of diabetes mellitus in Nigeria. The present study examined the anti-diabetic activity of the *Senna singueana* acetone fraction (SSAF) of stem bark in a type 2 diabetes (T2D) rat model.
Materials and methods: Crude ethyl acetate extract of the *Senna singueana* stem bark was fractionated with various solvents and the acetone fraction was selected for *in vivo* studies based on the high α-glucosidase and α-amylase inhibitory activities. In the *in vivo* study, male Sprague-Dawley rats were induced with T2D and treated with the SSAF at 150 and 300 mg/kg body weight. Several T2D-related parameters were measured in the study.
Results: After 4 weeks of intervention, non-fasting blood glucose concentrations were significantly decreased and the glucose tolerance ability was significantly improved in the SSAF treated groups compared to the diabetic control group. Serum insulin concentrations, pancreatic β-cell function (HOMA-β) and liver glycogen were significantly ($P<0.05$) increased while serum alanine transaminase, alkaline phosphatase and urea were significantly decreased in the SSAF treated diabetic rats compared to the diabetic control group. Though insignificantly ($P>0.05$), other T2D-induced abnormalities such as food and fluid intake, body weight, serum lipids, serum fructosamine level and peripheral insulin resistance (HOMA-IR) were also partially ameliorated by the SSAF treatment.
Conclusion: Data of this study suggest that orally administered SSAF could ameliorate most of the T2D-induced abnormalities in a T2D model of rats.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose level resulting from defects in insulin secretion, insulin action or both (ADA, 2005). According to a projection of the International Diabetes Federation (IDF), approximately 366 million people are living with diabetes and this figure is projected to increase to 552 million by the year 2030 (IDF, 2011). Among two major types of diabetes, type 2 is more prevalent than type 1, with more than 90% of the total diabetic patients are suffering from it. Type 2 diabetes (T2D) is a heterogeneous disorder characterized by a progressive decline in insulin action (insulin resistance), followed by the inability of pancreatic β-cells to compensate for insulin resistance (β-cell dysfunction) which leads to hyperglycemia (DeFronzo, 2004).

The control of hyperglycemia is therefore of prime importance to retard the progression of the disease. At present, the use of insulin secretagogues and sensitizers constitute the predominant line of therapy, however, the use of inhibitors of carbohydrate digesting enzymes in order to reduce the intestinal absorption of glucose is also vital as they do not interfere

Abbreviations: ADA, American diabetes association; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; BRU, biomedical resource unit; DBC, diabetic control; DCH, diabetic high dose; DCL, diabetic low dose; DMF, diabetic metformin; DNS, dinitrosalicylic acid; ELISA, enzyme-linked immunosorbent assay; FBG, fasting blood glucose; GC–MS, gas chromatography–mass spectroscopy; HOMA-β, homeostatic model assessment – β-cell function; HOMA-IR, homeostatic model assessment – insulin resistance; IDF, international diabetes federation; NC, normal control; NCT, normal toxicological dose; NFBG, non-fasting blood glucose; OGTT, oral glucose tolerance test; pNPG, p-nitrophenyl-α-d-glucopyranoside; T2D, type 2 diabetes; SSAF, *Senna singueana* acetone fraction; STZ, streptozotocin

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with the carbohydrate metabolism and help to control hyperglycemia in a noninvasive manner (Ghadyale et al., 2012). The α-glucosidase inhibitors are the current class of inhibitors of intestinal carbohydrate absorption which are shown to control postprandial hyperglycemia. However, the leading glucosidase inhibitors, acarbose and miglitol, are often reported to produce diarrhea and other intestinal disturbances, with corresponding bloating, flatulence, cramping and abdominal pain (Fujisawa et al., 2005). Hence, research on diabetes therapy is focused on the search for alternative agents which could decrease postprandial hyperglycemia and other diabetic complications with fewer or no side effects.

Senna singueana Delile, also known as golden shower, is a member of the family Caesalpiniaceae and commonly used by traditional medical practitioners to treat diabetes mellitus (Etuk et al., 2010) as well as bath for nursing mothers. Preliminary reagent-based phytochemical screening revealed that the methanol extract of the root of the plant contains phenols, saponins, tannins and anthraquinones (Adzu et al., 2003) while the methanol extract of the leaves contains alkaloids, tannins, sterols and terpenes (Ode and Onakpa, 2010). In a recent study, the *in vitro* anti-oxidative potential of the methanolic extract from the leaves was reported (Madubunyi and Ode, 2012). However, in a most recent study, we subjected different solvent crude extracts of the stem bark, root and leaves of the plant to anti-oxidative activity assays using several models and reported that the ethyl acetate extract of the stem bark showed the best anti-oxidative activity among all other extracts (Ibrahim et al., 2013). Subsequently, the phytochemical compounds present in the ethyl acetate extract were identified using GC–MS analysis where it was found to contain phenolic compounds such as 4-propylphenol and resorcinol, sterols such as 6-dehydroestradiol, aromatic esters such as methyl benzoates including some others such as dehydroxylevodopa and 2,3-dihydrobenzofuran (Ibrahim et al., 2013). Along with others, the major compound found in this extract was resorcinol (relative abundance 54.03%) which has been reported to have anti-diabetic activities via enhancing the glucose lowering efficacy of an engineered insulin called acylated insulin degludec (Steensgaard et al., 2013) and by stimulating hepatic glycogen synthesis and storage via leptin and insulin mediated pathways (Aiston and Agius, 1999). Additionally, in another recent study, a plant derived dibenzofuran has been reported to have potent anti-hyperglycemic activity at an oral dose of 20 mg/kg bw/day in a db/db mouse model (Carney et al., 2002). Hence, some of the above-mentioned compounds may have direct or indirect anti-diabetic effects.

Hence, in the present study, the ethyl acetate extract was further fractionated across solvents of different polarity and the acetone fraction derived from it was found to have the highest α-glucosidase and α-amylase inhibitory activities (among other solvent fractions). Subsequently, the acetone fraction was subjected to a detailed anti-diabetic study in an experimentally-induced T2D rat model.

2. Materials and methods

2.1. Chemicals and reagents

Streptozotocin (STZ), powdered *Saccharomyces cerevisiae* α-glucosidase (E.C. 3.2.1.20), powdered porcine pancreatic α-amylase (E.C. 3.2.1.1), p-nitrophenyl-α-d-glucopyranoside (pNPG), and p-nitrophenol were obtained from Sigma-Aldrich through Capital Lab Supplies, New Germany, South Africa. Starch, dinitrosalicylic acid (DNS), maltose, absolute ethanol, ethyl acetate, and hydrogen peroxide reagent were obtained from Merck Chemical Company, Durban, South Africa.

2.2. Plant material

The stem bark of *Senna singueana* was collected in the month of January, 2011 from Zaria, Kaduna state, Nigeria. The plant samples were identified and authenticated by the herbarium unit of the Department of Biological Science, Ahmadu Bello University, Zaria and a voucher specimen number 6863 was deposited. The stem bark was immediately washed and shade-dried for two weeks to constant weights. The dried samples were ground to fine powder using a kitchen blender, and stored individually in air-tight zip-loc polythene bags to transport to the University of KwaZulu-Natal, Westville Campus, Durban, South Africa for subsequent analysis.

2.3. Extraction and solvent-solvent fractionation

Three kilograms (3 kg) of the fine powdered stem bark of the plant was defatted with hexane. The defatted material was extracted with 10 l of ethyl acetate by soaking for 48 h and filtered through Whatmann filter paper (No. 1). The resultant extract was evaporated in vacuum using a rotary evaporator (Buchi Rotavapor II, Buchi, Germany) at 40 °C under reduced pressure to obtain the crude ethyl acetate extract with a yield of 4.04%. Forty grams of the crude ethyl acetate extract of the stem bark was dissolved in 500 ml of distilled water:methanol (9:1) mixture and successively fractionated with hexane (2 × 500 ml), dichloro methane (2 × 500 ml) and acetone (2 × 500 ml). The fractions were evaporated to dryness in vacuum at 40 °C under reduced pressure whereas the remaining aqueous fraction was dried in water bath at 50 °C. The dried fractions were transferred to micro tubes and stored at 4 °C until further analysis.

2.4. α-Glucosidase (E.C. 3.2.1.20) inhibitory activity of the solvent fractions

The α-glucosidase (E.C. 3.2.1.20) inhibitory activity was determined according to the method described by Ademiluyi and Oboh (2013) with slight modifications. Briefly, 250 μl of each fraction or acarbose at different concentrations (30–240 μg/ml) was incubated with 500 μl of 1.0 U/ml α-glucosidase solution in 100 mM phosphate buffer (pH 6.8) at 37 °C for 15 min. Thereafter, 250 μl of pNPG solution (5 mM) in 100 mM phosphate buffer (pH 6.8) was added and the mixture was further incubated at 37 °C for 20 min. The absorbance of the released p-nitrophenol was measured at 405 nm and the inhibitory activity was expressed as percentage of control without inhibitors. The α-glucosidase inhibitory activity was calculated according to the following formula:

$$\text{Inhibitory activity } (\%) = \left(1 - \frac{\text{As}}{\text{Ac}}\right) \times 100$$

where As is the absorbance in the presence of sample and Ac is the absorbance of control.

2.5. α-Amylase (E.C. 3.2.1.1) inhibitory activity of the solvent fractions

The α-amylase (E.C. 3.2.1.1) inhibitory activity was determined according to the method described by Shai et al. (2010) with slight modifications. A volume of 250 μl of each fraction or acarbose at different concentrations (30–240 μg/ml) was incubated with 500 μl of porcine pancreatic amylase (2 U/ml) in 100 mM phosphate buffer (pH 6.8) at 37 °C for 20 min. A 250 μl of 1% starch was dissolved in 100 mM phosphate buffer (pH 6.8) then added to the reaction mixture and incubated at 37 °C for 1 h. One ml of DNS color reagent was then added and boiled for 10 min. The absorbance of the resulting mixture was measured at 540 nm and the inhibitory activity was expressed as percentage of a control without inhibitors. The α-amylase inhibitory activity was calculated using the following