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Butanol fraction of *Parkia biglobosa* (Jacq.) G. Don leaves enhance pancreatic β-cell functions, stimulates insulin secretion and ameliorates other type 2 diabetes-associated complications in rats

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ABSTRACT

Ethnopharmacological relevance: Ethnopharmacological surveys have reported that *Parkia biglobosa* (Jacq.) G. Don (Leguminosae) is among the plants commonly used in the traditional management of diabetes mellitus in Nigeria and Togo.
Aim of the study: This study investigated the anti-diabetic activity of the butanol fraction of *P. biglobosa* leaves (PBBF) in a type 2 diabetes (T2D) model of rats and a possible bioactive compound in the fraction.
Materials and methods: T2D was induced by feeding rats with a 10% fructose solution *ad libitum* for two weeks followed by an intraperitoneal injection of 40 mg/kg body weight streptozotocin and the animals were orally treated with 150 and 300 mg/kg BW of the PBBF for five days in a week. Another group of rats was non-diabetic but similarly administered with 300 mg/kg BW of the PBBF. Food and fluid intakes, body weight changes and blood glucose levels were monitored during the experiment while other relevant diabetes-associated parameters were measured at the end of the experiment.
Results: The PBBF treatments significantly ($P<0.05$) decreased the blood glucose levels and improved the glucose tolerance ability compared to untreated diabetic rats. Furthermore, the treatments were found to improve pancreatic β cell function (HOMA-β), stimulate insulin secretions, decrease insulin resistance (HOMA-IR), restore liver glycogen, ameliorate serum dyslipidaemia and prevent hepatic and renal damages compared to untreated diabetic rats. Phytochemical analysis of the fraction led to the isolation of lupeol which inhibited α-glucosidase and α-amylase in non-competitive and uncompetitive inhibition patterns respectively.
Conclusion: It was concluded that PBBF possessed remarkable anti-T2D activity which is mediated through modulation of β-cell function and stimulation of insulin secretion and the lower dose (150 mg/kg BW) was found optimum for anti-T2D activity compared to the high dose (300 mg/kg BW) in this study.

1. Introduction

Type 2 diabetes (T2D) is a complex, heterogeneous and polygenic disease characterized mainly by insulin resistance and pancreatic β cell dysfunction (Salas-Salvado et al., 2011). Insulin resistance in the skeletal muscle, liver and adipocytes, followed by progressive failure of pancreatic insulin secretion, is responsible for the metabolic deregulation leading to type 2 diabetes (Aziz and Wheatcroft, 2011). At present, the use of insulin secretagogues and sensitizers as well as of inhibitors of carbohydrate-hydrolyzing enzymes such as α-glucosidase and α-amylase are the commonly exploited approach for the treatment of the disease (Kawamura-Konishi et al., 2012). Although a number of antidiabetic drugs are available for the treatment of T2D, side effects and adverse reactions are of great concern (May et al., 2002; Fujisawa et al., 2005). Recently, many researchers are seeking natural products or dietary interventions to prevent or treat T2D. In this context, African medicinal plants may provide the much needed alternative therapies because of their multiple health benefits (van Wyk, 2008).

Parkia biglobosa (Jacq.) G. Don is also called the African locust bean and belongs to the family Leguminosae which is native to

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Nigeria and other West African countries. It is highly reputed for numerous medicinal values (Millogo-Kone et al., 2008; Gronhaug et al., 2008) and all of the different parts of the plant are used by traditional healers to cure several metabolic or non-metabolic disorders such as hypertension, haemorrhages and dermatosis (Udobi and Onaolapo, 2009; Tokoudagba et al., 2010). The fermented seeds of the plant are also frequently used as natural nutritional condiment with different local names such as 'afitin' in Benin, nététou' in Senegal, and dawadawa in Nigeria (Azokpota et al., 2006). Scientific reports have validated the analgesic and anti-inflammatory (Kouadio et al., 2000), antivenom (Asuzu and Harvey, 2003), antidiarrhoeal (Agunu et al., 2005), antibacterial (Millogo-Kone et al., 2008), vasorelaxant (Tokoudagba et al., 2010) and wound healing (Adetutu et al., 2011) activities. In another study, we reported the anti-oxidative activities of extracts from different parts of the plant as well as identified pyrogallol and phloroglucinol derivatives to be the main phytochemical compounds of the ethanol extract of the leaves (Ibrahim et al., 2013).

On the other hand, ethnopharmacological surveys revealed that the leaves of *P. biglobosa* are commonly used in the traditional management of diabetes mellitus in northern Nigeria (Etuk et al., 2010) and the central region of Togo (Karou et al., 2011). In a previous study, the fermented seed extract was also reported to possess hypoglycemic activity in an alloxan-induced type 1 diabetes model of rats (Odetola et al., 2006). However, the anti-diabetic effects of the parts of the plant which are actually used for the traditional management of diabetes mellitus have not yet been examined either in humans or experimental animal models of type 2 diabetes.

Hence, the present study was conducted to comprehensively investigate the *in vivo* anti-diabetic activity of the butanol fraction of *P. biglobosa* leaves in a T2D model of rats as well as to isolate a pure α-glucosidase and α-amylase inhibitory compound from the fraction. Additionally, the mechanism of α-glucosidase and α-amylase inhibition by the active compound was determined using the enzyme kinetics approach.

2. Materials and methods

2.1. Plant material

The leaf samples of *P. biglobosa* were freshly collected in the month of January 2011 from Zaria, Kaduna state, Nigeria. The plant was identified and authenticated at the herbarium unit of the Biological Science Department, Ahmadu Bello University, Zaria, Nigeria and a voucher specimen number 3017 was deposited accordingly. The leaves were immediately washed and shade-dried for two weeks to constant weight. The dried samples were ground to a fine powder, and then stored individually in air tight containers for transport to the University of KwaZulu-Natal, Westville campus, South Africa for subsequent analysis.

2.2. Extraction and solvent-partitioned fractionation

Three kilograms (3 kg) of the fine powdered leaves was defatted with hexane. The defatted material was extracted with 10 L of ethanol 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 ethanol extract with a yield of 4.95%. Approximately 40 g of the crude ethanol extract of the leaves was dissolved in 500 mL of distilled water:methanol (9:1) and successively partitioned with hexane (2 × 500 mL), dichloromethane (2 × 500 mL), ethyl acetate (2 × 500 mL) and butanol (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 a water bath at 50 °C. The hexane, dichloromethane, ethyl acetate, butanol and aqueous fractions had yields of 1.6%, 5.1%, 8.2%, 24.8% and 10.7% respectively. The fractions were transferred to micro tubes and stored at 4 °C until further analysis. The butanol fraction (PBBF) displayed the highest *in vitro* α-glucosidase and α-amylase inhibitory effect (data not shown) amongst the fractions and was therefore subjected to the *in vivo* studies below. Furthermore, in order to perform a chemical analysis of the fraction, the PBBF was applied over a silica gel column using a solvent system of dichloromethane with an increasing gradient of methanol to give fifty seven fractions of 15 mL each. Fractions were monitored by thin layer chromatography (TLC) and fractions with similar TLC profiles were pooled together and tested for α-glucosidase and α-amylase inhibitory activity. Fraction 11 however, was found to be a pure α-glucosidase and α-amylase inhibitory compound which was characterized by ^{1}H and ^{13}C NMR. The NMR experiments were performed on a Bruker AvanceIII 400 MHz spectrometer (Bruker BioSpin, Rheinstetten, Germany) at room temperature with all chemical shift (δ) values recorded against the internal standard, tetramethylsilane (TMS). The sample was dissolved in deuterated chloroform ($CDCl_3$, Merck South Africa) and transferred to 5 mm NMR tubes. Chemical shifts for all NMR spectra are reported in parts per million (ppm). The NMR system was controlled by the software TopSpin 2.0.

2.3. α-Glucosidase inhibitory activity of the pure compound

The α-glucosidase inhibitory activity of the pure compound was determined according to the method described by Ademiluyi and Oboh (2013) with slight modifications. Briefly, 250 μL of the compound 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 para-nitrophenylglucopyranose (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 a percentage of a control sample without the inhibitor.

2.4. α-Amylase inhibitory activity of the pure compound

The α-amylase inhibitory activity of the compound was determined according to the method described by Shai et al. (2010) with slight modifications. A volume of 250 μL of the compound 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 dissolved in 100 mM phosphate buffer (pH 6.8) was then added to the reaction mixture and incubated at 37 °C for 1 h. Exactly, 1 mL of dinitrosalicylic acid colour reagent was then added and boiled for 10 min. The absorbance of the resulting mixture was measured at 540 nm and the inhibitory activity expressed as a percentage of a control sample without the inhibitor.

2.5. Mechanism of α-glucosidase and α-amylase inhibitions

The pure compound was subject to kinetic experiments to determine the type of inhibition exerted on α-glucosidase and α-amylase. The experiment was conducted according to the protocols as described above at a constant concentration of the compound (120 μg/mL) with a variable concentration of substrate. For the α-glucosidase inhibition assay, 0.625–5.000 mM of pNPG was used and 0.125–1.000% of starch was used for the α-amylase