

Hypoglycaemic effects of *Parkia biglobosa* (Jacq) Benth seed extract in glucose-loaded and NIDDM rats

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ABSTRACT

The antidiabetic potentials of the methanol extract of *Parkia biglobosa* seed (Mimosoideae), its chloroform, hexane, and mother liquor fractions were evaluated in glucose-loaded and alloxan-induced diabetic rats. The methanol extract of the seed exhibited a peak percentage decrease of 64% and 44.1% in blood glucose levels of the glucose-loaded and alloxan-induced diabetic rats respectively. The blood glucose lowering effect of the chloroform fraction (65.7%; $p < 0.05$) was significant and more than that exhibited by the reference drug glibenclamide in the alloxan-induced diabetic rats.

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INTRODUCTION

Parkia biglobosa (Jacq) Benth (Mimosoideae), commonly known as 'African Locust beans', is widely distributed across the Sudan and Guinea savannah ecological zones ranging from Senegal across to Sudan (Hopkins, 1983). *Parkia* species have found use traditionally as foods and medicinal agents. *P. biglobosa* fermented seeds are used in all parts of Nigeria and West Africa for seasoning traditional soups (Ajaiyeoba, 2002).

In folklore medicine, *P. biglobosa* is used by traditional healers in Southwest Nigeria and Senegal for the treatment of Diabetes mellitus (Abo et al., 2008; Die'yea et al., 2008) and in Northern Nigeria, it is used in the treatment of diarrheal infections (Abdulkarim et al., 2005).

Previous biological studies of *P. biglobosa* have reported the antimicrobial activities of the leaf and stem bark (Ajaiyeoba, 2002; Millogo-Kone et al., 2006; 2007; 2008). The aqueous and acetone extract

of *P. biglobosa* raw beans have also demonstrated termiticidal properties (Femi-Ola et al., 2008). Other studies have also reported the possible antidiabetic potentials of the methanol extract of the seeds of *P. biglobosa* in alloxan-induced diabetic rats (Odetola et al., 2006), the hypotensive and/or hypoglycemic effect in guinea pig, rat and rabbit (Kodjo et al., 2006).

With this in mind, this study aimed at investigating the blood glucose lowering effect of the methanol crude extract, n-hexane, chloroform and mother liquor fractions of *P. biglobosa* fermented seeds in oral glucose-loaded and non-insulin dependent diabetic (NIDDM) rats.

MATERIALS AND METHODS

Plant material

Parkia biglobosa fermented seeds were purchased from Bodija market, Ibadan, Oyo State, Nigeria. The plant was authenticated at the Department of Pharmacognosy, Olabisi

Onabanjo University Sagamu with a voucher number FJ051. The plant material was dried and ground into powder.

Phytochemical screening of *P. biglobosa* seed

Phytochemical screening was carried out using methods adopted from Trease and Evans, 1996.

Preparation of methanol extract

100 g dried powdered seed of *P. biglobosa* was macerated in 80% methanol for four days. The extract was filtered and filtrate dried in vacuo. The residue (5.6 g) was stored in dessicator and used for subsequent assays.

Preparation of n-hexane and chloroform fractions of *Parkia biglobosa* fermented seed

Dried crude methanol extract of *P. biglobosa* fermented seed was suspended in MeOH-H₂O (1:9) and partitioned with n-hexane and chloroform successively to yield the n-hexane, chloroform fractions and mother liquor.

Animals

Healthy wistar albino rats weighing between 80 – 250 g were used for the study. The animals were housed in polypropylene cages, maintained under standard conditions. They were fed with standard rat pellet diet (Ladokun feeds, Ibadan) and water *ad libitum*.

Antidiabetic studies

Oral glucose load test

Twenty healthy albino rats fasted overnight (18 h). Group 1 (n=5), group 2 (n=5) and group 3 (n=5) rats were fed 2 g glucose/kg body weight orally (dissolved in water) through a cannula, with a fourth group of rats (n=5) administered water. Immediately after glucose loading, group 1 and 2 rats (n=5) were administered methanol extract (1 g/kg) and glibenclamide (5 mg/kg) respectively, while group 3 rats were administered water only. Blood was withdrawn from the tail of the animals at 0, 1, 2, 3, 4, 5, 6 and 7 hrs. The fasting blood glucose levels were estimated by the O-toluidine methods (Dubowski, 1962; Frings, Ratliff and Dunn, 1970).

Induction of non-insulin dependent diabetes mellitus

NIDDM was induced in thirty-five rats by a single intraperitoneal injection of 60 mg/kg Alloxan monohydrate (Abdel-Barry et al., 1997) (Sigma Aldrich, UK). Hyperglycaemia was confirmed by the elevated glucose level in the blood, determined at 72 hrs after injection.

Experimental design

The diabetic animals were divided into seven groups. Following overnight fast, group 1 diabetic rats (n=5) received methanol extract of *P. biglobosa* (1 g/kg) reconstituted in water, group 2 diabetic rats (n=5) received n-hexane fraction of *P. biglobosa* (1 g/kg) as a suspension, group 3 diabetic rats (n=5) received chloroform fraction of *P. biglobosa* (1g/kg) as a suspension, group 4 diabetic rats (n=5) received mother liquor fraction of *P. biglobosa* (1 g/kg) reconstituted in water, group 5 diabetic rats(n=5) were treated with oral hypoglycaemic agent glibenclamide (5 mg/kg), group 6 diabetic rats (n=5;untreated) received water only(2 ml/kg) and group 7 (n=5) normal (non-diabetic) rats received water only (2 ml/kg). The fasting blood glucose was determined by nipping of the tail tip and measuring the blood glucose with a one touch life scan glucometer (Lifescan, Johnson and Johnson Inc., California) at 0, 2, 4, 6, and 8 hours after extract administration using the glucose-oxidase method.

Statistical analysis

Data are expressed as mean \pm SEM. The significance of the differences between the means of the test and control animals was established by the student t-test.

Activity of the plant in glucose-loaded rats

The administration of *P. biglobosa* methanol extract exhibited a pronounced effect on blood glucose in the glucose-loaded rats at 5 and 6 hrs. A peak hypoglycaemic effect of 34.3% ($p < 0.05$) was observed in the group administered the methanol extract when compared to the untreated glucose-loaded rats and 64% decrease in blood glucose level when compared to 1 hr. The hypoglycaemic effect of the methanol extract at a dose of 1 g/kg was lower than that of the reference drug glibenclamide throughout the study (Table 2).

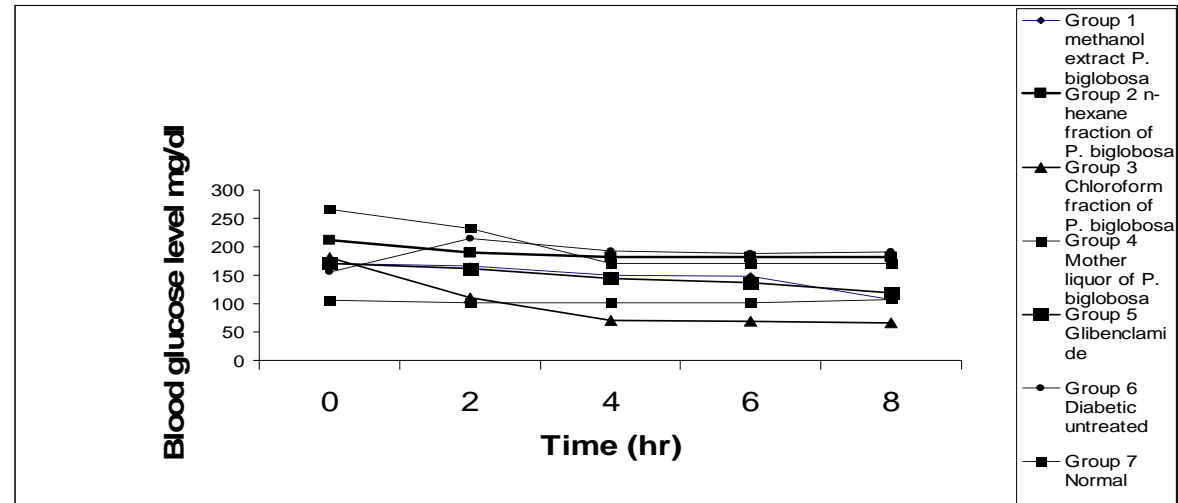


Figure 1: Effect of *Parkia biglobosa* methanol seed extract and fractions on alloxan-induced diabetic rats.

Table 1: Summary of phytochemical screening of *Parkia biglobosa* fermented seed.

Anthraquinones		Tannins	Alkaloids	Cyanogenetic glycosides	Sterols	Saponins	Cardiac glycosides	Flavonoids
Free	Combined							
-	-	++	+	-	+	++	-	-

+++ = Very positive; ++ = Positive; + = Trace; - = absent.

Table 2: Effect of methanol seed extract of *Parkia biglobosa* on oral glucose-loaded test.

Treatment	Time (hour) and mean blood glucose level (mg/dl)							
	0	1	2	3	4	5	6	7
Group 1: <i>P. biglobosa</i> methanol see extract (1 g/kg)	100.0 ± 1.255	263.0 ± 1.061	230.4 ± 0.849 (12.4%)	167.4 ± 0.990 (36.3%)	147.8 ± 0.707 (43.8%)	94.6 ± 0.106* (64.0%)	104.0 ± 0.990* (60.5%)	106.4 ± 1.145 (59.5%)
Group 2: Glibenclamide (5 mg/kg).	81.4 ± 0.127	178.1 ± 0.566	154.0 ± 0.025 (13.5%)	131.8 ± 0.355* (25.9%)	109.5 ± 1.598* (38.5%)	80.4 ± 1.075* (54.9%)	76.4 ± 0.292* (57.1%)	71.9 ± 0.150* (59.6%)
Group 3: Control Diabetic untreated	91.3 ± 1.363	156.3 ± 0.445	154.0 ± 0.294	159.7 ± 0.321	152.9 ± 0.963	144.0 ± 0.113	136.1 ± 0.740	125.8 ± 0.005
Group 4: Normal	100.0 ± 0.141	98.2 ± 0.141	99.0 ± 0.035	99.1 ± 0.033	98.1 ± 0.035	89.5 ± 0.707	90.5 ± 0.532	89.9 ± 0.371

Values are expressed as Mean ± SEM; P<0.05; n= 5; Figures in parenthesis = % decrease in blood glucose level (compared to blood glucose level at 1 hr); * Represents statistical significance vs. control (untreated diabetic group) P<0.05.

Table 3: Effect of *Parkia biglobosa* seed methanol extract, n-hexane, chloroform, mother liquor fractions on alloxan-induced diabetic rats.

Treatment	Time (hour) and mean blood glucose level (mg/dl)				
	0	2	4	6	8
Group 1 : Methanol extract of <i>P. biglobosa</i> seed. (1 g/kg)	170.4 ± 1.200	166.7 ± 0.313 (22.4%)*	150.0 ± 1.249 (22.1%)*	148.1 ± 0.946 (21.6%)*	106.7 ± 1.569 (44.1%)*
Group 2: n-hexane fraction of <i>P. biglobosa</i> seed	212.2 ± 1.204	190.4 ± 0.811 (11.4%)	181.7 ± 1.569 (5.7%)	181.7 ± 1.589 (3.8%)	181.7 ± 1.194 (4.7%)
Group 3 : Chloroform fraction of <i>P. biglobosa</i> seed	180.2 ± 1.146	110.4 ± 1.185 (52.6%)*	70.2 ± 1.2010 (63.6%)*	69.8 ± 1.511 (63.1%)*	65.5 ± 1.284 (65.7%)*
Group 4 : Mother liquor of <i>P. biglobosa</i> seed	246.7 ± 1.205	231.9 ± 1.378	170.3 ± 1.401 11.6%	170.3 ± 1.255 (9.9%)	170.2 ± 1.387 (10.8%)
Group 5 : Glibenclamide (5 mg/kg)	171.1 ± 0.992	162.3 ± 1.215 (24.4%)*	144.4 ± 1.576 (25.0%)*	137.0 ± 0.992 (27.5%)*	118.5 ± 3.139 (37.9%)*
Group 6 : Untreated diabetic	155.6 ± 0.035	214.8 ± 1.005	192.6 ± 0.990	188.9 ± 1.112	190.7 ± 2.970
Group 7: Normal (non-diabetic)	105.5 ± 3.337	101.8 ± 2.903	101.1 ± 0.044	101.1 ± 0.047	106.7 ± 1.213

Values are expressed as Mean ± SEM; P<0.05; n= 5; Figures in parenthesis = % decrease; * Represents statistical significance vs. control (untreated diabetic group) P<0.05.

Activity of the plant in alloxan-induced diabetic rats

In the alloxan diabetic rats, the result of the hypoglycaemic effect of the methanol extract, chloroform, hexane fractions and mother liquor of *P. biglobosa* showed that the chloroform fraction exhibited the most pronounced hypoglycaemic effect ($p < 0.05$). The chloroform fraction maintained a stable hypoglycaemic effect in the alloxan-induced diabetic rats and a peak decrease in blood glucose level of 65.7% at 8 hr was observed. The hypoglycaemic activity of the chloroform fraction at a dose of 1 g/kg was markedly higher than that of the reference drug Glibenclamide (5 mg/kg) in the alloxan-induced diabetic rats. A dose of 1 g/kg of the methanol extract exhibited a similar hypoglycaemic effect as the reference drug at a dose of 5 mg/kg in the alloxan-induced diabetic rats.

RESULTS

Phytochemical

The phytochemical screening of *P. biglobosa* fermented seed revealed the presence of alkaloids, saponins, steroids and tannins (Table 1).

DISCUSSION

The phytochemical screening in this study revealed the presence of alkaloids, saponins, steroids and tannins in the fermented seed. This is in agreement with previous reports of the presence of alkaloids, tannins, cardiac glycoside and steroids in the leaf (Ajaiyeoba, 2002) and presence of sterols/triterpenes, coumarins, flavones, anthraquinones, tannins, anthocyanins and saponins in the stem bark (Millogo – Kone et al., 2006). However, in this study, Cardiac glycosides and anthraquinones were not present in the fermented seeds.

P. biglobosa methanol extract administered to the glucose-loaded diabetic rats exhibited a decrease in the blood glucose levels of the animals throughout the period of the study, with a peak decrease in blood glucose level of 64% at 5 hr. The hypoglycaemic effect of *P. biglobosa* methanol extract is similar to that of glibenclamide. There was no significant difference in the activity of the methanol

extract and glibenclamide at 5 hr, 6 hr and 7 hr (Table 2).

In this study, the methanol extract administered to the alloxan-induced diabetic rats decreased the blood glucose level by 44.1% at 8 hr while in previous study on the possible antidiabetic potentials of *P. biglobosa*, a dose of 6 g/kg of the methanol extract administered to alloxan-induced diabetic rats as a dietary supplement for a period of four weeks, exhibited a decrease in blood glucose level of 64.4% (Odetola et al., 2006). The activity of the methanol extract is similar to that of glibenclamide. This study is in agreement with previous studies and confirms the blood glucose lowering effect of methanol extract of *P. biglobosa* fermented seed.

However, the chloroform fraction exhibited the most pronounced activity with a peak decrease in blood glucose level of 65.7% at 8 hr. The hypoglycaemic activity of the chloroform fraction at a dose of 1 g/kg was markedly higher than that of the reference drug glibenclamide (5 mg/kg) in the alloxan-induced diabetic rats (Table 3).

The exact mechanism of action and group of secondary metabolite(s) responsible for the antidiabetic activity of *P. biglobosa* fermented seed is unknown. Further study is on-going to identify the active compound(s) responsible for the antidiabetic and hypoglycaemic activities in the methanol extract and its chloroform fraction.

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