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EXPERIMENTAL EVIDENCE FOR THE ANTIDIABETIC ACTIVITY OF *CAJANUS CAJAN* LEAVES IN RATS

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ABSTRACT: *The antidiabetic activity of methanol leaves extract of Cajanus cajan (L.) Millsp. (Fabaceae) was studied in alloxan-diabetic and in oral glucose loaded rats. The acute toxicity and lethality (LD_{50}) and the phytochemical analysis of the extract were also evaluated. The results showed that the extract (400 and 600 mg/kg) significantly ($P<0.05$) reduced fasting blood sugar of alloxan diabetic rats in a dose-related manner, with maximum hypoglycemic effect at 4 – 6 h. The extract (400 and 600 mg/kg) also significantly ($P<0.05$) suppressed the peak postprandial rise in blood glucose of normal rats by 101.8 and 57.40% respectively. Acute toxicity and lethality test of the extract in rats gave an oral LD_{50} greater than 5 g/kg. The findings indicate that the leaves of C. cajan may be beneficial as an antidiabetic therapy.*

KEYWORDS

Cajanus cajan, alloxan, hypoglycemia, postprandial

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INTRODUCTION

Cajanus cajan (*L.*) Millsp. (Fabaceae), commonly known as "Pigeon pea" (English), "Fio-fio" (Igbo - Nigeria), "Guandu" (Brazil), and caja or "Puspo-poroto" (Peru), is an erect woody and annual or short- lived perennial shrub or small tree that is widespread and cultivated throughout the tropics and subtropics. The seeds (pigeon peas) are popular food in developing countries. In Africa, Asia and South America different parts of the plant are used in the management of disorders such as ulcer, diarrhea, pain, diabetes, cough and sores. The plant, often grown as a shade crop is commonly used all over the world for the treatment of diabetes, dysentery, hepatitis, measles, as a febrifuge, and to stabilize menstrual period (1-4). In traditional Chinese medicine, the leaves of pigeon pea have been widely used to relieve pain and kill worms (5), for the treatment of wounds, bedsores and malaria, as well as diet-induced hypercholesterolemia (6-8). Protective effects of the leaf extracts against hypoxic-ischemic brain damage and alcohol-induced liver damage have also been reported (9, 10). The antioxidant activity of the extract of the leaves (11) and the hypoglycemic activity of the seeds have been reported (3).

The isolation of antiplasmodial compounds-betulinic acid (roots), longistylin A and C (leaves) from the plant has been reported (12).

In line with the ethnomedicinal use of the plant in the treatment of diabetes in Nigeria and other parts of the world, we evaluated the antihyperglycemic potentials of *C. cajan* leaf extract in diabetic rats.

MATERIALS AND METHODS

Animals

Adult Swiss albino rats (150 - 250 g) of either sex bred in the Laboratory Animal facility of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka were used for the study. The animals were maintained on standard pellets and water, and allowed 2 weeks for acclimatization before use. All animal experiments were in compliance with the National Institute

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of Health Guide for Care and Use of Laboratory Animals (Pub No. 85 – 23, revised 1985).

Preparation of extract and phytochemical analysis

Fresh leaves of C. *cajan* were collected in Nsukka, Enugu State, Nigeria in April, 2008. The plant was identified and authenticated by Mr. A. Ozioko of the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu State. The leaves were cleaned, shade-dried for 5 days and milled to coarse powder using an electric blender. The leaf powder (700 g) was extracted with methanol by cold maceration for 48 h, with intermittent shaking. The extract was concentrated to dryness in a rotary evaporator (40-50°C) under reduced pressure to yield 85.45 g of the methanol extract (CCM; 12.2% w/w), which was subsequently subjected to phytochemical analysis using standard procedures (13, 14).

Acute toxicity tests

The acute toxicity and lethality (LD_{50}) of the extract was determined in rats using the method described by Lorke (15). Briefly, nine rats randomly divided into three groups (n=3) received oral administrations of 10, 100, and 1000 mg/kg of the extract respectively and were observed for 24 h for death. Since no death was recorded, 1,600, 2,900 and 5,000 mg/kg of the extract were administered to a fresh batch of animals and the number of deaths in 24 h recorded.

Effect of extract on fasting blood glucose in alloxan-induced diabetic rats

Diabetes was induced by intraperitoneal injection of alloxan monohydrate (100mg/kg; i.p.) (16, 17) to normal healthy Swiss albino rats fasted for 12 h. The animals were housed in cages for 48 h with access to food and water. Subsequently, the 12 hour fasting blood glucose level (FBGL) was determined and rats with blood glucose level (BGL) $\geq$ 250 mg/dl were considered diabetic and used for the study. The diabetic rats were randomly placed into groups (n=6) to receive oral administrations of CCM (400 or 600 mg/kg) suspended in tween 80 (10% v/v). The standard group received glibenclamide (0.5 mg/kg) while the control group received the vehicle (Tween 80, 5 ml/kg). Blood was withdrawn from each animal by tail snipping and blood glucose levels were determined for each animal in each group at fixed intervals 0, ½, 1, 2, 4, 6, and 8 h, using One touch® glucometer kit (Lifescan, Johnson and Johnson Company, Milipitas, CA).

Oral glucose tolerance test in normoglycemic rats

Rats fasted for 16 h but with free access to water were randomly divided into four groups (n = 6) and received oral administrations of CCM (400 or 600 mg/kg) suspended in Tween 80 (10% v/v). The standard group received glibenclamide (0.5 mg/kg) while the control group received the vehicle (Tween 80, 5 ml/kg). Ninety minutes later, the rats were orally fed with glucose (4 g/kg) (18). The blood glucose level of animals in each group was measured before (0) and at 30, 60, 90, 120, 150 and 180 min after glucose load.

Statistical analysis

Data obtained was analyzed using One-Way analysis of variance (ANOVA) and further subjected to LSD post hoc test for multiple comparisons. The results were presented as Mean ± SEM. Differences between means of treatment and control groups were accepted significant at $P<0.05$.

RESULTS

Phytochemical constituents

The phytoconstituents detected in the leaves include saponins, tannins, terpenoids and resins (Table 1).

Acute toxicity and lethality (LD50) test

The LD50 was estimated to be greater than 5000 mg/kg.

Table 1: Phytochemical constituents of *Cajanus cajan* leaves

Phytochemical constituents	CCM
Alkaloids	-
Glycosides	-
Saponins	+
Flavonoids	-
Tannins	+++
Carbohydrates	-
Reducing sugars	++
Proteins	-
Resins	++
Terpenoids	++

+++= Conspicuously present, ++= moderately present, += present, - = absent

Effect of CCM on FBGL in alloxan diabetic rats

The two doses of the extract caused a significant, dose-related and progressive reduction in fasting blood glucose level up to 6 h, compared to control. Also the hypoglycemic effect elicited by CCM (600 mg/kg) was greater than that of glibenclamide up to the 4th hour (Table 2).

Effect of CCM on oral glucose tolerance

Within 30 min of administration of glucose load, there was a progressive increase in the postprandial blood glucose level (BGL) of all the rats which peaked at 60 min. At 60 min, the CCM treated groups (400 and 600 mg/kg) had 78.93% and 123.32% increase in BGL compared to control (180.72%). Hence treatment with CCM suppressed the rise in BGL at 60 min by 101.79% (400 mg/kg) and 57.40% (600 mg/kg). The CCM evoked a progressive, significant and non-dose related decrease in BGL up to 180 min, at which the BGL were close to or below basal levels (Table 3).

DISCUSSION

Besides drugs classically used for the treatment of diabetes mellitus (DM), (insulin, sulphonylureas, biguanides thiazolidinediones, α-glucosidase inhibitors and others), several species of plants are used in various parts of the world to manage diabetes. Also the hypoglycemic and antidiabetic activities of many plants are documented in scientific literature (18-21).

Evaluation of the hypoglycemic activity of CCM in alloxan-induced diabetic and normal hyperglycemic rats showed that the extract reduced blood glucose levels and also suppressed postprandial hyperglycemia in rats.

Oral administration of the extract to diabetic rats elicited significant and dose related hypoglycemic effect, suggesting beneficial effect in DM. Alloxan monohydrate induces diabetes by destruction of the β-cells of Islets of Langerhan's with consequent impairment of insulin secretion leading to hyperglycemia (22-24).

Table 2: Effect of extract on FBGL of alloxan induced diabetic rats

Treatment	Dose (mg/kg)	Blood glucose concentration (mg/dl)						
		0 h	0.5 h	1 h	2 h	4 h	6 h	8 h
CCM	400	398.2 ± 27.4	371.4 ± 28.8 (6.70%)	322.8 ± 22.9 (12.2%)	270.2 ± 27.2* (32.1%)	155.6 ± 40.1* (60.9%)	129.0 ± 11.9* (67.6%)	171.6 ± 13.8* (56.9%)
	600	364.0 ± 29.1	276.0 ± 20.4 (24.1%)	221.8 ± 20.2 (39.0%)	167.0 ± 20.0* (54.1%)	70.20 ± 6.85* (80.7%)	104.2 ± 8.78* (71.3%)	166.8 ± 10.1* (54.1%)
Glibenclamide	0.5	451.8 ± 38.2	360.8 ± 29.4 (20.1%)	318.6 ± 28.9 (29.4%)	257.4 ± 26.9* (43.0%)	157.2 ± 24.9* (65.2%)	129.4 ± 26.6* (71.3%)	33.0 ± 5.39* (92.6%)
Control	-	369.0 ± 28.8	378.8 ± 28.6 (-2.6%)	398.8 ± 30.6 (-8.0%)	416.0 ± 31.5 (-12.7%)	428.2 ± 30.3 (-16.0%)	447.2 ± 37.8 (-21.0%)	478.0 ± 43.7 (-29.5%)

n=6; *$P \leq 0.05$ compared to control; Values in parenthesis represent change (%) in blood glucose level calculated relative to 0 hr.

Table 3: Effect of extract on oral glucose tolerance in normoglycemic rats

Treatment	Dose (mg/kg)	Blood glucose concentration (mg/dl)					
		0 min	30 min	60 min	90 min	120 min	180 min
CCM	400	71.20 ± 8.43	126.8 ± 12.5* (78.09%)	127.4 ± 10.1 (78.93%)	106.2 ± 8.30 (49.16%)	72.80 ± 6.00* (2.25%)	70.20 ± 5.41 (1.40%)
	600	62.60 ± 4.99	127.6 ± 7.58* (103.83%)	139.8 ± 4.78 (123.32%)	84.20 ± 4.39 (34.50%)	72.20 ± 1.46* (15.34%)	68.0 ± 2.12 (8.63%)
Glibenclamide	0.5	74.40 ± 11.1	87.80 ± 5.06* (18.01%)	101.0 ± 9.18 (35.75%)	43.80 ± 7.24 (41.13%)	40.00 ± 4.83* (46.24%)	32.20 ± 6.16 (56.72%)
Control	5 ml/kg	66.40 ± 11.1	184.2 ± 28.7 (177.41%)	186.4 ± 30.1 (180.72%)	118.2 ± 22.2 (78.01%)	92.00 ± 7.53 (38.55%)	82.60 ± 7.29 (24.40%)

n=6; *$P \leq 0.05$ compared to control; Values in parenthesis represent change (%) in blood glucose level calculated relative to 0 min.

Hence, the extract may act through mechanism(s) that enhance/boost insulin secretion. In addition, effective and sustained reduction in blood glucose levels of treated diabetic rats by the extract indicates that it may be useful in overt cases of DM.

The extract also suppressed the rise in blood glucose level after a heavy glucose meal with maximum suppression at 60 min, the time of peak postprandial hyperglycemia; this indicates an ability to suppress and control postprandial hyperglycemia. Chronic hyperglycemia, a risk factor for the development of life threatening and other complications of DM, is constantly fuelled by postprandial elevation of blood glucose. Hence, control of postprandial hyperglycemia is of great importance due to its strong association with the risk of micro and macro-vascular complications and death (25, 26). Interestingly, in addition to hypoglycemic effect, the extract may also suppress postprandial rise in blood glucose level both of which are indices of effective glycemic control.

The glucose lowering effect observed in the extract may be attributable to the presence of saponins, terpenoids, resins and tannins. Tannins isolated from some antidiabetic medicinal plants have been found to stimulate secretion or possess an insulin like-effect (27). In addition, terpenoids like clerodane-type diterpenes from the stem bark of *Croton cajucara* have been shown to possess hypoglycemic properties (28, 29). The LD_{50} estimated to be greater than 5000 mg/kg, indicates that the extract is relatively safe.

In conclusion, the results of the study demonstrate the potentials of the leaves of C. *cajan* as anti-diabetic therapy, which may be attributable to hypoglycemic activity and an improvement of post prandial hyperglycemia in diabetes mellitus.

CONFLICT OF INTEREST: None

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