Pharmacognostic Studies and Evaluation of Hypoglycaemic Effects of Leaves of Parinari C Uratellifolia Planch Ex. Berth (Chrysobalanaceae)

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ABSTRACT

Leaves of *Parinari curatellifolia* are used in herbal medicine practice for the treatment of a number of ailments including diabetes mellitus, bronchial infections, leprosy, and other skin diseases as well as hookworm and gonorrhea in different parts of West Africa.

The leaves of *P. curatellifolia* were screened for phytochemical constituents using established methods, and the ethanol extract tested for acute toxicity in mice, LD 50 was calculated. The effect of the extract on blood sugar levels of rats with hyperglycemia induced by glucose or alloxan was investigated. Flavonoids, triterpenoidal saponins glycosides and hydrolysable tannins were identified in the crude drug The LD50 was 1.240 mg/kg. There were no toxic signs at lower doses. The 1000 mg/kg ethanol extract caused significant ($p < 0.05$) hypoglycaemia in the glucose-test and $p < 0.01$ in the alloxan system used. These activities however, were less than the effect of 50 g/kg tolbutamide with the significant level of $p < 0.01$ compared with control. The extract enhanced the activity of tolbutamide when used together in the glucose- induced hyperglycaemia test.

Leaves of *P. curatellifolia* contain bioactive constituents and possess significant activity against glucose- or alloxan - induced hyperglycaemia in rats. Results obtained, provide some scientific basis for the use of the plant in traditional medicine practice for treating diabetes.

Keywords: *Parinari curatellifolia*, phytochemical constituents, LD50, mice, hypoglycaema, rats.

INTRODUCTION

Parinari curatellifolia. has a variety of uses in traditional medicine practice. In West African Countries including Nigeria and the Ivory Coast, the leaves and roots of the plant are used for the treatment of diabetes mellitus, bronchial infections, leprosy and other skin infections as well as for hookworm and gonorrhea.[1] There is resilience of use of traditional medicines across the globe especially in a developing country like Nigeria. As there is still shortage of medial doctors and pharmaceutical products, the majority of the population in the developing countries rely on the Traditional Medicine Practitioners (TMPs) including Herbalists and local medicinal plants for their primary healthcare needs. In view of this fact, there is need for assurance of quality, safety, and the efficacy of medicinal plants and plant products as part of the prerequisites towards the establishment of regulations and registration of herbal medicine products.[1] Current research activities on medicinal plants fundamentally include both the phytochemical and biological screening of the plant material.[2]

In this study, leaves of *P. Curatellifolia* were screened for phytochemical constituents, and the ethanol extract tested for acute toxicity in mice in order to indicate the level of toxicity of the crude drug. The effect of the extract on blood sugar levels of rats in which hyperglycemia was induced by glucose or alloxan was investigated. This is to confirm literature evidence that the plant is used as an anti diabetic drug in traditional medicine practice.[1] Locally, *Parinari curatellifolia* is known as "Rura, Famar rura" by the Hausa[3] and "Idofun, Abo-idofun, Igbudugbudu" Yoruba[4]

MATERIALS AND METHODS

The plant material.

Fresh leaves and stem bark (latter for the purpose of correct identification) of the plant were supplied by the herbalist in Jos. They were identified as *P. curatellifolia* by the Taxonomist at the Federal College of Forestry, Bauchi Road, Jos. Nigeria. Voucher specimen was assigned Pc/PCG.007. The specimen was deposited in the Herbarium of Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City where the study was carried out. The leaves were air-dried and then pulverized in the laboratory wooden mortar and the powder preserved for study in air-tight glass containers.

Phytochemical screening

Phytochemical tests were carried out for the presence of secondary metabolites in the powdered leaf using standard phytochemical techniques [2,5]

Extraction of Plant Material for pharmacological tests

This was done using the Soxhlet Extractor. Five hundred grams of the powdered leaf drug was moistened with ethanol, and allowed to stand for about 20 minutes. It was then packed into the Soxhlet thimble for the extraction. The powder was exhaustively extracted. The extract was concentrated over the water bath, and then dried in an oven at 40°C. The yield was 2.36g.

Pharmacological tests

Animals: Albino mice weighing 25-35g and Sprague Dawley rats of 230-280g body weight of both sexes were purchased from Ambrose Alli University, Ekpoma, Edo State. Nigeria. They were quarantined for ►

four days, being maintained on standard animal pellets and water ad libitum. Before they were used they were denied food for 24 hrs but had water ad libitum.

Acute Toxicity Test: Five groups of five mice per group were treated with 250, 500, 1000, 2000 mg/kg extract or 5 ml/kg normal saline respectively through the oral route through a gastric tube. Animals were well marked for proper identification and placed in multi-compartment cages. They were observed for 48 hours for any signs of toxicity like motor activity, tail erection, startle reaction, abdominal griping, convulsion or any deaths. L.D50 was calculated using the method of Miller and Tainter, (1944)[6].

Effect of extract Glucose-induced hyperglycemia in rats. Basal blood glucose levels (mg/100 ml) were taken at time (0) for thirty rats before drug administration. Tails of animals were washed with warm water and cut to allow collection of 0.5 ml portions blood from the vein, into micro-heparinized tubes. The blood glucose level was determined using the Tinder glucose oxidase method.[7] Each of the thirty rats was first given 2mls of 1.5g glucose solution in normal saline based on rats' body weights to induce hyperglycaemia. After 60 minutes, the blood sugar levels were determined. Twenty eight of the rats, each having blood glucose level above 300mg/100ml were termed diabetic.[8] Twenty five of them were selected on body weight basis for the study. They were shared into five groups (A-E), of five animals per group and were treated as follows: Group A was the control group, received 2ml/kg saline, Group B received 2ml of 1.5g glucose solution based on body weight, Group C was given 500mg/kg extract, Group D was given 50mg/kg tolbutamide and group E was administered with 500 mg/kg extract together with 50mg/kg tolbutamide. At 1st, 2nd and 4th hr after treatment, blood sugar levels were determined as indicated earlier [7] Changes in the levels were calculated from the equation [8and9]

% change in blood glucose level = (Gt - Go x 100) /Go

Where Gt = Value after treatment

Go = Value before treatment with reference to control group.

Effect of extract on alloxan-induced hyperglycemia in rats. Basal blood glucose levels (mg/100 ml) were determined (time 0) for thirty rats before drug administration following the method described [8] 100 mg/kg alloxan (Sigma Chemicals, USA.) was dissolved in 0.15 mol/L citrate buffer at pH 5.0. 2mls each of this was administered to thirty overnight fasted rats through the subcutaneous (sc) route. After 24 hrs blood sugar was determined. Twenty-eight of them with values above 300 mg/100ml blood were selected.[8] Rats were used in groups of 5 animals per group. Group 1 was the control that was given only 2ml/kg of vehicle. Groups 2-4 were given 200mg, 400mg and 1000mg/kg extract respectively. The reference group (E) received 50 mg/kg tolbutamide. These administrations were through the gastric tube.

At 1st, 2nd and 4th hr after treatment, blood sugar levels were determined as idicated earlier [7] Changes in the glucose levels were calculated from the equation [8and9]

Statistical analysis: Student's t-test was applied for the analysis of data. Results are expressed as ± SEM. ($p < 0.05$) was considered significant.

RESULTS.

PHYTOCHEMICAL TESTS.

The plant secondary metabolites detected were flavonoids and triterpenoidal saponins glycosides and hydrolysable tannins.

PHARMACOLOGICAL TESTS:

LD50 of the ethanol extract of the plant material was 1.24g/kg in mice. Two ml of 1.50g/kg glucose induced hyperglycemia in rats as observed from the data of the basal sugar levels of rats and those after glucose administration. The basal blood sugar and the effect of the extract and tolbutamide on blood sugar levels in the glucose-induced hyperglycemia are shown in Table 1. The extract alone at 2 hr. did not produce significant sugar lowering activity compared with activity of tolbutamide alone ($p < 0.05$) or tolbutamide with extract ($p < 0.0$ 1) at the same 2 hr. At 4 hr. extract caused significant activity $p < 0.05$), which was however, less than that of tolbutamide or tolbutamide and extract ($p < 0.01$). (Table 1)

Results of the effects of the extract on alloxan induced hyperglycemia are shown in Table 2. 100mg/kg alloxan induced hyperglycemia in the rats. This confirmed the work of Torres et al [8] that alloxan induces hyperglycemia in rats. 200mg/kg extract did not cause significant hypoglycemic activity. 400 mg/kg showed significant activity from 2h ($p < 0.05$) while 1000mg/kg had the highest activity ($p < 0.01$) compared with control but was however less active than tolbutamide.

DISCUSSION

The extract has shown dose-related activity in lowering blood sugar in experimental hyperglycemic rats. The plants secondary metabolites contribute to its bioactivity. Metabolites present in the leaf drug are hydrolysable tannins, flavonoids and triterpenoidal saponins glycosides and tannins. A number of plants containing one or more of the classes of these metabolites identified, have shown activity in lowering blood sugar levels in hyperglycemia cases. Examples of these include flavonoids in roots of Panax ginseng and saponins glycosides in leaves of Azadirachta indica. reported to be useful in the treatment of diabetes mellitus[10]. Tannins have also been reported to possess hypoglycemic activity[11]. Pterocarpus santalinus (Red Sanders) wood extract,[12] Murraya koeigii and Brassica juncea[13] also have hypoglycemia activity.

Taken all together, the hypoglycemic activity of the leaves of *P. curatellifolia* could be due to the flavonoids, saponins and tannins present in the crude drug singularly or collectively. The value of LD 50 shows the plant material is relatively safe especially as herbal medicines are usually administered as dilute extracts. The extract did not show any activity at the 1st and 2nd hr but exhibited significant activity at the 4th hr. (Tables 1 and 2) There was delayed action. It may not be useful in cases of emergency. When the extract was administered along with tolbutamide, there was significant increase in activity (Table 1).

In the alloxan test, the extract showed dose-dependent activity, 200mg/kg had no activity significantly different from that of the control. 400 mg/kg was active ($p < 0.05$) and 1000mg/kg very significantly active.($p < 0.01$). These ▶

results are shown in Table 2.

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Table 1: Effects of ethanol extract on glucose-induced hyperglycemia in rats.

Group/Treatment	0 hr	1 hr	2 hr	4 hr
A Saline (control, No glucose)	73.2 ± 2.8	75.1 ± 3.1	68.9 ± 0.7	74. 8 ± 1.4
B Glucose	74.2 ± 0.50	233.1± 0.9	252.3 ± 2.5	238.4 ± 2.5
C Glucose + Extract	74.6 ± 1.4	228.0 ± 1.3	214.5 ± 3.7	146.6 ± 2.8*
D Glucose + Tolbutamide	73.5 ± 3.5	110. ± 6.5*	107.5 ± 0.3*	88.6 ± 2.1**
E Glucose + Extract + Tolbutamide	75.6 ± 1.6	105.5 ± 2.7**	97.5 ± 0.6**	73.68 ± 0.8**

Basal blood sugar (mg / 100 ml) was 73.7 ± 0.5
Mean ± SEM., N= number of animals per group =5
Saline = 2 ml/kg; Glucose: 2 ml of 1.5 glucose solution given on body weight basis
Extract: 500 mg/kg; Tolbutamide = 50 mg/kg
p <0.0.5, **p < 0.01 in comparison with control. ('p' denotes the level of significance)

Table 2: Effects of the Extract on alloxan induced hyperglycemia in rats.

Treatment	0 hr	1 hr	2 hr	4 hr
Control: 2 ml/kg (Vehicle)	306.7 ± 0.3	307.2 ± 0.1	304.5 ± 0.4	267.5 ± 0.2
Extract 200 mg/kg	315.5 ± 0.5	300.7 ± 0.5	294.5 ± 1.2	260.0± 1.4
Extract 400 mg/kg	312.8 ± 0.6	274.9 ± 0.7	223.9 ± 0.4*	205.8 ± 0.5*
Extract 1000 mg/kg	318.5± 0.8	264.5 ± 3.4	185.9 ± 3.4*	94.6 ± 1.9**
Tolbutamide 5 0 mg/kg	320.2 ± 1.4	170.7 ± 1.5*	125.6 ± 0.9**	75.6 ± 0.8**

Basal blood sugar (mg / 100 ml) for rats before drug administration was 74.3 ± 1.4
Mean ± SEM, N =5
p < 0.0.5, **p < 0.01 in comparison with control ('p' denotes the level of significance)