

Full Length Research Paper

Assessing plasma glucose and lipid levels, body weight and acute toxicity following oral administration of an aqueous ethanolic extract of *Parinari curatellifolia* Planch, (Chrysobalanaceae) seeds in alloxan-induced diabetes in rats

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The study was aimed at evaluating the safety and hypoglycaemic effects of *Parinari curatellifolia* seeds used in the treatment of diabetes. The plasma glucose level and other biochemical parameters, body weight and liver, heart, renal and acute toxicities were assessed following oral administration of an aqueous ethanol (80%) extract of the seeds in alloxan-induced diabetes in rats. Toxicity of the extract was evaluated in Swiss albino mice by feeding the animals with the graded doses of the extract between 1.0 to 2.0 g/kg body weight orally and observed continuously for the first 4 h and hourly for next 24 h, then 6 hourly for 48 h (72 h, acute toxicity). Diabetes was induced in male and female Wistar rats with alloxan monohydrate (150 mg/kg) dissolved in normal saline and administered intraperitoneally (i.p). The plasma glucose levels of the induced animals were monitored with a glucometer after 72 h. The animals with plasma glucose level >300 mg/dl were classified as diabetic and were included in the study. The diabetic animals were treated with the extract and a reference drug, glibenclamide, respectively for 30 days. Their effects on plasma glucose levels and some biochemical parameters were evaluated at the end of the experiment as indices of their antidiabetic activity. The median acute toxicity value (LD₅₀) of the extract was determined to be 7.27 g/Kg body weight. There was significant reduction ($p < 0.05$) in the plasma glucose and low density lipoprotein (LDL)-cholesterol levels, and significant increase ($p < 0.05$) in high density lipoprotein (HDL)-cholesterol in the treated diabetic groups compared to the control. There was no significant increase in the body weight in the diabetic and normal groups treated with the extract while there was a significant gain in weight for the diabetic rats treated with reference drug. Aspartate aminotransferases (AST) level was not affected in the treated diabetic rats while significant changes in the alanine aminotransferases (ALT) and the creatinine levels were observed in all groups treated with the extract. The LD₅₀ value indicated the drug to be quite safe as a single dose treatment. The results also showed that the extract had good hypoglycemic activity and good effects on cardiovascular risk factors.

Key words: *Parinari curatellifolia* seeds, acute, toxicity, diabetes.

INTRODUCTION

Diabetes has become one of the devastating diseases afflicting health of many people, in recent times, and has

accounted for a high proportion of health problems world wide (Sushruta et al., 2006). It is now recognized as one

of the leading causes of death in the developing countries where the high prevalence of the disease is attributed to improved nutritional status coupled with a gross lack of modern facilities for the early diagnosis of the disease. Diabetes mellitus is described as the common metabolic disorder of carbohydrate and fat metabolism, which is due to absolute or relative lack of insulin and is characterized by hyperglycaemia (Walter, 1977). It is succinctly put by Barnett and O'Gara (2003), as a state of premature cardiovascular death that is associated with chronic hyperglycemia, and may be associated with blindness and renal failure. Diabetes is commonly accompanied by other cardiovascular risk factors such as dyslipidemia, hypertension, prothrombic factors and microvascular problems involving eyes, kidney and peripheral nerves (Grundy et al., 1999; Barnett and O'Gara, 2003). Two main types of diabetes are identified based on their clinical manifestations: type 1 diabetes known as juvenile onset or insulin sensitive diabetes and type 2 diabetes or non insulin dependent diabetes mellitus (NIDDM). The latter is the most prevalent. Type 2 diabetes may have as its underlying metabolic causes, the combined effects of impairment in the insulin-mediated glucose disposal and defective secretion of insulin by the β -cells of the pancreas (Grundy et al., 1999).

Oral hypoglycaemic agents, especially the sulphonylureas and biguanides have been commonly employed in the management of type 2 diabetes. Sulphonylureas are the most widely used oral hypoglycaemic agents but may have some adverse effects such as exacerbating hyperinsulinaemia, thereby causing weight gain in patients (Rastigo, 1977; Egwim, 2005). Biguanides are only weak hypoglycaemics and have limited clinical use (Rastigo, 1977). For these cogent reasons, therefore, there is a great need for a search for an acceptable, cheap and safe blood sugar lowering oral hypoglycaemic agents that would be effective in the management of diabetes and devoid of serious side effects of the currently used oral hypoglycaemic agents. Herbs and marine sources have been considered the best option.

The use of herbs and natural product drugs from various plant sources is now of great interest in the management of diabetes mellitus. Several herbs have been reported in folk medicine to be successfully employed in the management of diabetes and have shown effectiveness in non-insulin dependent diabetes (Rastigo, 1977). The seeds of *Parinari curatellifolia* known locally as "Ebere" (by the Yorubas) are commonly used in the Nigerian folk medicine for the treatment diabetes. The seeds are also used in combination with other herbal products by herbalists for the treatment of other diseases.

The aim of this study was to carry out the acute toxicity of the aqueous ethanolic extract of *P. curatellifolia* seeds

in mice and also to evaluate the effects of the extract on the plasma glucose levels, on the cardiovascular risk factors, the body weight and renal toxicity following an oral administration in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Plant material

The *P. curatellifolia* seeds were bought from Mushin market in Lagos suburban and were authenticated at the Department of Botany and Microbiology, University of Lagos, Lagos, and voucher specimen deposited at the Department Herbarium. The seeds (with their coats removed) were dried at an ambient temperature between 35 – 45°C in an oven for four days, and powdered to coarse particles. Five hundred gram of the powder was macerated with ethanol (80%) at room temperature for seven days, with stirring at intervals. After filtration, the solvent was removed under reduced pressure in a rotary evaporator at a temperature below 50°C and dried to a constant weight of 26.45 g (5.29% yield).

Acute toxicity study

The toxicity study was carried out using thirty five (35) male and female Swiss albino mice weighing between 20 – 25 g. The animals were randomly distributed into one control group and six treated groups, containing five animals per group. They were maintained on animal cubes (Feeds Nigeria Ltd), provided with water *ad libitum* and were allowed to acclimatise to the laboratory conditions for seven days before the experiment. After fasting the animals overnight, the control group received 0.3 ml of 5% tween 80 solution orally. The dried extract (8.0 g) was dispersed with 5 ml 5% Tween 80 solution, thoroughly mixed and the volume made up to 10 ml with the Tween solution. Each treated group received orally the doses of the extract as follows: 1.0, 2.50, 5.0, 10.0, 15.0 and 20.0 g/kg. The animals were observed continuously for the first 4 h and then for each hour the next 24 h and at 6 hourly interval for the next 48 h after administering the extract to observe any death or changes in general behaviour and other physiological activities (Shah et al., 1997; Bürger et al., 2005)

Diabetic study

Healthy Wistar adult rats of both sexes weighing 160 \pm 10 g were used. The animals were fed on animal cubes (Feeds Nigeria Ltd) and provided with water *ad libitum*. Diabetes was experimentally induced after fasting animals overnight by administering intraperitoneally (i.p) alloxan monohydrate dissolved in normal saline (150 mg/kg). After 3 days the blood sugar levels were monitored with a glucometer (ACCU-CHEK, Roche Diagnostics) and the rats with plasma glucose >300 mg/dl were classified as diabetic and were included in the study. A total of five groups containing five animals per group were used. Three groups were diabetic while the remaining two groups were used as different controls and were treated daily for 30 days as follows:

- Group 1: Control given only 5% tween 80 (0.5ml/once a day, daily)
- Group 2: Alloxan- induced diabetic rats but not treated
- Group 3: Alloxan- induced diabetic rats treated daily with extract 250 mg/kg body weight
- Group 4: Alloxan- induced diabetic rats treated daily with Glibenclamide 600 μ g/kg b. wt (Mahdi et al., 2003), and
- Group 5: normal rats treated daily with extract 250 mg/kg body weight.

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Table 1. Acute toxicity of the aqueous ethanol extract of *Parinari curatellifolia* seeds in mice.

Number of Mice	Doses of extract g/kg	Number of mice dead	% Cumulative of dead mice
5	0	0	0
5	1.0	0	0
5	2.5	0	0
5	5.0	1	9
5	10.0	2	27
5	15.0	3	55
5	20.0	5	100

Control group received 0.3 ml each of 5% Tween 80 solution.

Table 2. Weight variation of the control, untreated diabetic rats and diabetic rats treated with extract, diabetic rats treated with glibenclamide and normal rats treated with the extract doses for 30 days.

Gp	Day 1	Day 4	Day 8	Day 12	Day 16	Day 20	Day 24	Day 28	Day 30
1	153.3±6.7	156.6±6.7	158.0±0.50	158.01±0.4	158.02±0.1	158.05±0.2	160.7±8.8	160.7±8.8	161.6±8.3
2	160.4±1.3	158.3±1.1	157.4±2.4	156.40±1.2	153.6±1.7	150.41±1.2	142.3±1.2	140.1±2.3	135.5±2.5
3	155.3±2.5	157.2±1.50*	160.2±1.5*	161.20±3.3	161.7±2.8*	162.2±2.8	162.6±2.2*	163.13±3.3	164.5±1.3*
4	152.5±2.5	130.01±1.07	141.7±2.1	150.03±0.3	155.20±1.2	162.51±1.4	165.20±1.0	167.02±0.2	169.6±0.2
5	153.7±88	146.20±2.3	140.5±3.5	145.02±2.9	141.60±2.2	153.20±2.2	157.02±1.7	162.81±1.7	163.01±17*

Mean ± SEM, n=5, * p<0.05, * p<0.01 vs control group.

Group 1: Control rats received 0.5 mL 5% Tween 80; Group II: diabetic rats without treatment. Group 3: diabetic rats treated with 250 mg extract/kg wt. Group 4: diabetic rats treated with Glibenclamide 600 µg b.wt. Group 5: normal rats treated 250 mg extract /kg wt.

The animals were initially weighed and then weighed every four days from the starting of the treatment. On the 30th day, they were anaesthetised with warm urethane and chloralose (25%:1%v/v) at a dose of 5 ml/ kg and blood obtained via cardiac puncture into heparinized container. The blood was centrifuged within 5 min of collection at 4000 g for 10 min to obtain plasma which was analysed for glucose level, total cholesterol, total triglyceride, HDL-cholesterol levels by precipitation and modified enzymatic procedures from Sigma Diagnostics (Wasan et al., 2001). LDL-cholesterol levels were calculated using Friedwald equation (Crook, 2006). Plasma was analysed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinines by standard enzymatic assay analysis and the plasma protein and glucose contents were determined using enzymatic spectroscopic methods (Hussain and Eshrat, 2002).

Phytochemical evaluation of the crude extracts

Phytochemical screening of the extract was performed for the presence of secondary metabolites using the following reagents and chemicals: alkaloids - with Mayer's and Dragendorff's reagents (Farnsworth, 1966; Harborne, 1998); flavonoids with the use of Mg and HCl (Silva et al., 1993; Houghton and Raman, 1998); tannins with 1% gelatin and 10% NaCl solutions and saponins with ability to produce suds (Houghton and Raman, 1998).

Statistical analysis

Differences in total blood glucose, total plasma cholesterol, total plasma triglyceride, LDL-cholesterol and HDL-cholesterol concentrations, AST, ALT and creatinine levels and body weight for all treated and control rats were determined using an analysis of variance (ANOVA). Significant differences were determined using a

Student's t-test and differences were considered significant if p<0.05. All data are expressed as mean ± standard error of the mean.

RESULTS

In the acute toxicity study (Table 1), all the animals that received 20.0 g/kg b.wt of the extract died within 4 h (100% death) while the animals that received 2.50 g/kg b.wt survived beyond 24 h. LD₅₀ of the extract was calculated to be 7.27 g/kg b.wt.

The effect of the extract on the body weights of the diabetic and normorats and also the effect of the reference drug, glibenclamide, on the diabetic rats are shown in Table 2. There were no significant changes in body weights of the diabetic and normal rats treated with the extract (groups 3 and 5, respectively) compared to the control. There was a significant increase (p<0.05) in weight of the diabetic animals (group 4) treated with glibenclamide, the reference drug. Also observed, was a significant decrease in weight in the non-treated diabetic group when compared with the control.

The effect of the extract and glibenclamide on the organs of the diabetic animals and the effect of the extract only on normal rats are presented in Table 3. The macroscopic examinations of the organs of the animals treated with the extract and glibenclamide did not show any changes in colour compared to the control. The organs of the alloxan-induced diabetic but untreated

Table 3. The effects of the extract and glibenclamide on kidney, heart, liver and brain of the diabetic rats, and also the effect of the extract on normal rats compared with the control.

Organ	Group 1	Group 2	Group 3	Group 4	Group 5
Heart (g)	0.69 ± 0.03	0.51 ± 0.23	0.68 ± 0.13	0.72 ± 0.06	0.69 ± 0.23; **
Kidney (g)	0.93 ± 0.10	0.77 ± 0.18	1.31 ± 0.06**	1.39 ± 0.06	1.42 ± 0.24
Liver (g)	5.097 ± 0.70	4.22 ± 0.53	5.14 ± 0.57	5.10 ± 0.63*	4.70 ± 0.43
Brain (g)	1.168 ± 0.14	1.07 ± 0.01*	1.09 ± 0.001	1.11 ± 0.07	1.37 ± 0.02

Mean ± SEM, (n=5) *p<0.05; ** p<0.01 vs control group.

Group 1: Control rats received 0.5 mL 5% Tween 80; Group 2: diabetic rats without treatment. Group 3: diabetic rats treated with 250 mg extract/kg wt. Group 4: diabetic rats treated with glibenclamide 600 µg b.wt. Group 5: normal rats treated 250 mg extract /kg wt.

Table 4. Plasma glucose level and other biochemical profiles of untreated diabetic rats, diabetic but treated with the extract and glibenclamide respectively and the normal rats treated with extract and the control.

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5
Glucose (mg/dl)	98.03 ± 0.6	855.75 ± 20.1	very low	132.95 ± 5.27	75.60 ± 2.45
Cholesterol (mg/dl)	58.46 ± 2.1	456.15 ± 5.2	94.23 ± 1.6	97.12 ± 3.1	18.65 ± 0.54
Triglyceride (mg/dl)	56.87 ± 0.8	197.4 ± 3.6	28.26 ± 0.72	19.57 ± 1.5	28.81 ± 2.1
HDL (mg/dl)	95.32 ± 1.6	35.46 ± 2.9	103.25 ± 2.2	106.52 ± 0.6	54.68 ± 4.1
LDL (mg/dl)	65.30 ± 2.6	322.52 ± 7.40	76.85 ± 0.60	80.83 ± 1.5	60.44 ± 2.2*
Protein (g/dl)	6.89 ± 0.45	3.52 ± 0.05	6.03 ± 0.2*	3.72 ± 0.72	6.17 ± 0.25*
Creatinine (mg/dl)	0.49 ± 0.002	2.95 ± 0.23	0.62 ± 0.06	0.78 ± 0.02	2.47 ± 0.52
AST (I.U/L)	57.18 ± 1.2	107.04 ± 2.1	55.69 ± 2.5 *	53.14 ± 0.02	62.83 ± 0.90
ALT (I.U/L)	24.56 ± 1.5	153.80 ± 4.52	87.60 ± 2.75	105.74 ± 3.53	14.76 ± 1.32

Mean ± SEM, (n=5) *p<0.05; ** p<0.01 vs control group. Control group received 0.5 mL 2% tween 80 solution.

Group 1: Control rats received 0.5 mL 5% Tween 80; Group 2: diabetic rats without treatment. Group 3: diabetic rats treated with 250 mg extract/kg wt. Group 4: diabetic rats treated with glibenclamide 600 µg b.wt. Group 5: normal rats treated 250 mg extract /kg wt.

animals showed some changes compared to the control.

Table 4 summarised the results of the extract and glibenclamide effects on the biochemical parameters. The extract and glibenclamide significantly (p<0.05) reduced the plasma glucose levels of the diabetic rats when compared with the control. The extract proved to have a better lowering effect on the plasma glucose level than glibenclamide. There was an astronomical increase in the plasma glucose level of the alloxan-induced but untreated animals when compared with the control, signifying that they were truly diabetic.

There was a significant increase (p<0.05) in the plasma AST and ALT levels in the untreated diabetic animals, while a significant increase in ALT level was observed in the diabetic animals treated with the extract and glibenclamide, respectively. No significant changes (p>0.05) in AST level were observed in diabetic animals treated with extract or glibenclamide. A significant decrease (p<0.05) in the plasma total cholesterol (TC) level was observed in all the diabetic animals treated with the extract or glibenclamide while a significant increase in TC level was observed in the untreated diabetic rats.

There was also a significant decrease (p<0.05) in both triglyceride (TG) and LDL-cholesterol levels while signifi-

cant increase in HDL-cholesterol levels were observed in all diabetic animals treated with the extract or glibenclamide. The untreated diabetic animals showed a significant increase in both TG and LDL-cholesterol levels and a significant decrease in HDL-cholesterol levels.

There was no significant change observed in the protein and creatinine levels of the diabetic and normal rats treated with the extract compared to control, contrary to significant decrease observed in the untreated diabetic animals and diabetic but treated with glibenclamide.

Preliminary phytochemical screening (result not tabulated) indicated that the presence of alkaloids, free and bound anthraquinones and polyphenols, saponins were conspicuously absent.

DISCUSSION

Diabetes is now recognized as one of the killer diseases with increasing incidence world-wide over. Oral hypoglycaemic agents especially the sulphonylureas and biguanides have been commonly used for the management of diabetes, especially the type 2, in spite of the associated adverse effects. Attention is now focused on

the use of plants and herbal remedies that would be devoid of serious side effects encountered with sulphonylureas and biguanides as alternatives in the treatment of diabetes. *P. curatellifolia* is one of such medicinal plants with promising activity. Preparations from the seeds are employed locally in the treatment of diabetes.

The median acute toxicity value (LD₅₀) of the aqueous ethanolic extract of the seeds was determined to be 7.27 g/kg body weight. According to Ghosh (1984) and Klassen et al. (1995) *P. curatellifolia* seeds can be classified as being slightly toxic, since the LD₅₀ was found to lie between 5 -15 g/kg.

The treatment with the extract did not decrease the water and food consumption by the animals. Treatment of the diabetic and normal rats with the extract did not produce any significant changes in the body weights of the animals while the diabetic animals treated with glibenclamide showed a significant gain in weight. The extract did not induce weight gains which might lead to obesity when used in treating diabetic subjects. The result, therefore, suggests that the extract lacked the obesity forming tendency, which is one of the serious side effects normally experienced when treating diabetics with sulphonylureas. There were no changes observed in the macroscopic examinations of the organs of the diabetic animals treated with the extract or glibenclamide.

The extract had a remarkable effect on the plasma glucose levels especially on the diabetic rats. It even lowered the plasma glucose level more than glibenclamide. Reducing the blood glucose level minimizes the risk of disease-related complications in people with diabetes. This finding lends support to the use of *P. curatellifolia* seed preparation as a hypoglycaemic agent.

The liver releases alanine aminotransferase (ALT) and an increased plasma concentration is an indicator of liver damage. The liver and heart release aspartate aminotransferase (AST) and ALT, and an elevation in their plasma concentrations indicate liver and heart damage (Wasan et al., 2001; Crook, 2006).

The ALT levels increased in the diabetic animals treated with the extract and glibenclamide while AST levels were unaffected. It implied that the extract and glibenclamide at the doses used did not produce any harmful effects on the heart tissues but did provoke some detrimental effects on liver tissues. There was significant increase ($p < 0.05$) in both the plasma AST and ALT levels in the untreated diabetic animals.

The extract lowered plasma total cholesterol (TC) concentration in the diabetic animals. This strongly suggests the presence hypolipidemic agents in the extract. The extract also decreased both triglyceride (TG) and LDL-cholesterol levels while significantly increased the HDL-cholesterol levels. It could be interpreted that the extract had some beneficial effects on cardiovascular risk factors, which contribute to death of diabetic patient (Barnett and O'Gara, 2003). This observation supported the local use of the seed preparation as a hypoglycaemic agent. The extract did not exert any change in the protein and

creatinine levels of the diabetic and normal rats, contrary to decrease observed in the untreated diabetic animals and diabetic but treated with glibenclamide. An increase in plasma creatinine levels coupled with decrease in the protein levels may be a sign of impaired renal function in the affected animals. The elevation in plasma creatinine concentration indirectly suggests kidney damage, specifically the renal filtration mechanism (Wasan et al., 2001).

Preliminary phytochemical screening indicated the presence of alkaloids, anthraquinones glycosides and polyphenols. Polyphenols such as flavonoids and tannins have been shown to demonstrate numerous health protective properties, which include lowering of blood lipids (Jean, 1999). Furthermore recent reports have suggested that several plant sterols reduce serum cholesterol by the inhibition of intestinal cholesterol absorption (Sushruta et al., 2006). Thus, it could be suggested that the synergistic interaction of polyphenol and tannin contents in the extract would impart hypolipidemic property to the extract, hence the local use of the seeds as an antidiabetic.

Conclusion

The study showed that treatment with *P. curatellifolia* seed extract did not induce weight gain in the animals, which is a major advantage over the serious side effects of some synthetic hypoglycaemic agents. The extract also demonstrated a good hypoglycaemic activity and had desirable effects on the cardiovascular risk factors. It did not show signs of toxic effects on the organs on a long term treatment.

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