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Hypoglycemic activity of the bark of *Spondias pinnata* Linn. Kurz.

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ABSTRACT

Diabetes, the most prevailing metabolic disorder is attracting present research attention towards it. In the present study, the various extracts of the barks of *Spondias pinnata* (Family: Rubiaceae) was evaluated for hypoglycemic activity on adult Wistar albino rats at dose levels of 300 mg/kg p.o. each using normoglycaemic, glucose loaded and alloxan induced hyperglycaemic rats. Glibenclamide (2.5 mg/kg) was used as reference standard for activity comparison. Among the tested extracts, the methanol extract was found to produce promising results that is comparable to that of the reference standard glibenclamide. The preliminary phytochemical examination of the methanol extract revealed presence of flavonoids, tannins, saponins and terpenoids. The present work justifies the use of the bark in the folklore treatment in diabetes.

KEYWORDS: Glibenclamide, Hyperglycaemic, Normoglycaemic, Oral glucose tolerance Test (OGTT), *Spondias pinnata.*

INTRODUCTION

Search for antidiabetic factor in plants remains a potential area of investigation. In accordance with the recommendations of the WHO expert committee on diabetes mellitus, an investigation of antihyperglycaemic agents of plant origin used in traditional medicine seems important. Many herbs and plant products have been shown to have antihyperglycaemic action. *Spondias pinnata* (Linn. f) Kurz (Family: Rubiaceae) is a glabrous tree upto10.5 m high with straight trunk and smooth ash coloured bark having pleasant aromatic smell found in the plains of Orissa, West Bengal and Bihar (1). In Ayurveda, the unripe fruit are believed to destroy "vata", enriches the blood and cures rheumatism. The leaves and bark are aromatic, astringent and useful in preventing vomiting, dysentery and diarrhoea (2, 3). The plant is reported to have anti-tubercular properties (4). The tribes of Mayurbhanj district of Orissa drink the bark paste duly suspended in water in reducing blood sugar in the patients with diabetes mellitus. Studies substantiating its use in diabetes are lacking. In the present paper we report the hypoglycemic activity of the bark on standard laboratory animal models to provide a scientific support to the folklore claims.

MATERIALS AND METHODS

Plant Material

The plant material (barks) was collected from the forests of Mayurbhanj district of Orissa during June 2007 and identified by the taxonomists of the Botanical Survey of India, Shibpur, Howrah. A voucher specimen [Sp. No: CNH/ I-I / (255)/2008Tech.II] has been kept in our research laboratory for further reference. After authentication, fresh barks were collected in bulk, washed, shade dried and pulverized in a mechanical grinder to obtain coarse powder.

Preparation of Extract

The powdered bark (500 g) after defatting with petroleum ether (40-60° C) for 48 h was successively extracted with

chloroform, methanol and water for 48 h in a soxhlet extractor. Following extraction, the liquid extracts were concentrated under vacuum to yield dry extracts. Standard methods (5–6) were used for preliminary phytochemical screening of the different extracts to know the nature of phytoconstituents present within them.

Animals

Swiss albino mice (20–25 g) of either sex were used for acute toxicity study and adult Wistar albino rats (150–200 g) of either sex were used for the antidiabetic evaluation. The animals were kept in standard polypropylene cages at room temperature of 34 ± 2 °C and at 60–65 % relative humidity during the experimental work. The institutional Animal Ethics Committee approved all the experimental protocols

Acute toxicity study

The test was carried out as suggested by *Ganapaty t al.*, 2002 (7). Selected animals were divided into different groups of six in each. The control group received 1% Tween-80 in normal saline (2 ml/kg, p.o.). The other groups separately received 100, 200, 300, 600, 800, 1000, 2000 and 3000 mg/kg of the test extracts respectively in a similar manner. Immediately after dosing, the animals were observed continuously for the first 4 hours for any behavioral changes. They were then kept under observation up to 14 days after drug administration to find out the mortality if any.

Using normoglycaemic rats

The method of *Bhopale et al.*, 2006 (8) was followed. The animals were fasted for 18 h but were allowed free access to water before and throughout the duration of experiment. At the end of the fasting period, taken as zero time (0 h), blood was withdrawn from the tip of the tail of each rat under anaesthesia and the blood glucose was estimated with Senso card blood glucose meter supplied by M/s Avecon health care Pvt. Ltd., Himachal Pradesh. The normal rats were then divided into six groups of six animals each. Group-I served as solvent control and received only vehicle (2 ml/kg) through oral route. Group-II received glibenclamide (2.5 mg/kg). Group-III, IV and V received different extracts at doses of 300 mg/kg in a similar manner. Blood glucose levels were measured after 1, 2, 4 and 6 h of administration of single dose of test samples. The results are depicted in Table 1.

Oral glucose tolerance test (OGTT) in rats

The method of *Badole et al.,* 2007 (9) was followed. Fasted rats were divided into six groups of six rats each. Group I served as a control and received only vehicle (2 ml/kg) through oral route. Group-II received glibenclamide (2.5 mg/kg). Group-III, IV and V received the test extract at doses of 300 mg/kg respectively in a similar manner. After 30 min of treatment, rats of all groups were loaded orally with glucose (2 g/kg, p.o). Blood samples were collected before and at 30, 90 and 150 min after glucose administration as per the method described earlier (Table 2).

Alloxan Induced hyperglycaemic rats

The method of *Dash et al.,* 2008 was followed (10). The acclimatized animals were kept fasting for 24 h with water *ad libitum* and injected intraperitoneally a dose of 120 mg/kg of alloxan monohydrate in normal saline. After one hour, the animals were provided standard laboratory diet *ad libitum*. The blood glucose level was checked before alloxanisation and 24 h after alloxanisation by withdrawing

Table 1. Effect of different extracts of the bark of *S. pinnata* on the blood glucose level in normal rats

			Blood glucose concentration (mg / dl) (normoglycaemic study)				
				Time (h) after treatment			
Group	Treatment	Dose	Fasting	1	2	4	6
I	Control	2 ml/kg	96.83 ± 2.48	97.66 ± 2.10	98.16 ± 2.05	97.83 ± 2.12	98.16 ±1.99
II	Glibenclamide	2.5 mg/kg	96.5 ± 2.95	85.00 ± 3.68* (11.91 %)	80.66 ± 2.91** (16.41 %)	66.00 ± 2.58 ** (19.68 %)	50.67 ± 1.66 ** (47.49 %)
III	Chloroform extract	300 mg/kg	99.16 ± 3.33	92.83 ± 2.75 (6.38 %)	83.16 ± 1.92 ** (16.13 %)	77.66 ± 5.23 ** (21.68 %)	73.66 ± 5.71 ** (25.71 %)
IV	Methanol extract	300 mg/kg	98.66 ± 2.21	87.66 ± 3.73 (11.14 %)	81.83 ± 2.31** (17.05 %)	71.67 ± 2.49 ** (27.35 %)	64.16 ± 1.13 ** (34.97 %)
V	Aqueous Extract	300 mg/kg	98.00 ± 2.35	93.66 ± 2.69 (4.42 %)	84.5 ± 2.26 ** (13.77 %)	75.83 ± 4.57 ** (22.62 %)	69.33 ± 5.02 ** (29.25 %)

Values are expressed as mean ± S.E. (n = 6). All columns are significant using ANOVA.;

** P<0.05,*

**P<0.01 when compared to control; Dunnet's t-test. Figures in parenthesis denote percentage reduction of blood glucose.

Table 2. Effect of different extracts of the bark of *S. pinnata* on oral glucose tolerance in normal rats

Group	Treatment	Dose	Blood glucose concentration (mg / dl) (oral glucose tolerance study)			
			Fasting	Post treatment		
				30 min.	90 min.	150 min.
I	Control	2 ml/kg	92.33 ± 2.92	130.33 ± 2.64	136.66 ± 2.15	150.66 ± 5.32
II	Glibenclamide	2.5 mg/kg	91.17 ± 3.79	115.00 ± 9.51	99.00 ± 9.50 ** (13.91 %)	82.33 ± 7.19 ** (28.40 %)
III	Chloroform extract	300 mg/kg	94.34 ± 2.78	140.00 ± 5.87	122.00 ± 10.75 (12.85 %)	100.00 ± 6.22 ** (28.57 %)
IV	Methanol extract	300 mg/kg	93.66 ± 3.65	141.33 ± 5.57	107.00 ± 2.39 * (24.29 %)	87.67 ± 4.02 ** (37.96 %)
V	Aqueous Extract	300 mg/kg	94.5 ± 3.75	141.66 ± 5.68	118.78 ± 4.73 (16.15 %)	95.16±4.19** (32.82 %)

Values are expressed as mean ± S.E. (n = 6). All columns are significant using ANOVA. ;
**P<0.05,*
***P<0.01 when compared to control; Dunnet's t-testFigures in parenthesis denote percentage reduction of blood glucose.*

Table 3. Effect of different extracts of the bark of *S. pinnata* on the blood glucose level in alloxan induce diabetic rats

Group	Treatment	Dose	Blood glucose concentration (mg / dl)				
			0 hour	1 hour	2 hour	4 hour	6 hour
I	Control	2 ml/kg	234.00 ± 8.21	239.19 ± 6.61	246.55 ± 6.17	252.5 ± 5.65	255.66 ± 4.33
II	Glibenclamide	2.5 mg/kg	232.33 ± 4.06	205.66 ± 7.84* (11.47 %)	178.5 ± 3.79 ** (23.16 %)	122.83 ± 6.27** (47.13 %)	83.00 ± 7.30** (64.27 %)
III	Chloroform extract	300 mg/kg	236.67 ± 8.90	232.16 ± 8.98 (1.90 %)	222.66 ± 7.91 (5.91 %)	172.83 ± 14.5** (26.97 %)	135.33 ± 8.51** (42.81 %)
IV	Methanol extract	300 mg/kg	241.33 ± 7.75	230.16 ± 6.21 (4.62 %)	204.00 ± 5.06 ** (15.13 %)	161.00 ± 6.92** (33.28 %)	89.00 ± 6.39** (63.12 %)
V	Aqueous Extract	300 mg/kg	245.16 ± 5.50	242.00 ± 6.08 (1.28 %)	209.33 ± 6.52** (14.61 %)	164.33 ± 18.49** (32.97 %)	103.00 ± 6.52** (57.98 %)

Values are expressed as mean ± S.E. (n = 6). All columns are significant using ANOVA ;
** P<0.05,*
***P<0.01 when compared to control; Dunnet's t-test .Figures in parenthesis denote percentage reduction of blood glucose.*

blood from the tip of the tail of each rat under anaesthesia. The blood glucose level was measured as above. Animals were considered diabetic when the blood glucose level was raised beyond 225 mg/dl as suggested by *Edwin et al*, 2007(11). This condition was observed at the end of 48 h after alloxanisation. The animals were segregated into six groups of six animals in each. Group-I served as negative control and received vehicle (2 ml/kg p.o.) through oral route. Group-II received glibenclamide (2.5 mg/kg). Group-III, IV and V received the different extracts at doses of 300 mg/kg in a similar manner. Blood glucose level was estimated at 0, 1, 2, 4 and 6 h respectively after administration of single dose of test samples (Table 3).

Statistical analysis

The data obtained in the studies were subjected to one way of analysis of variance (ANOVA) for determining the significant difference. The inter group significance was analyzed using Dunnet's-t test. A P-value<0.05 were considered to be significant. All the values were expressed as mean ± SEM.

RESULTS AND DISCUSSION

In acute toxicity study, it was found that the chloroform and methanol extract induced sedation, diuresis and purgation at all tested doses. However, there was no mortality in any of the extracts at tested doses till the end of 14 days of observation.

Reports of the normoglycaemic study (Table 1) reveals that the all extracts exhibited significant reduction in blood glucose concentration as compare to control. It was observed that chloroform, methanol and aqueous extracts reduced 25.71%, 34.97% and 29.25% blood glucose levels respectively where as glibenclamide showed 47.49% in rats after 6h treatment.

The effect of different extracts on glucose tolerance test in normal rats is shown in Table 2. At 30 min after glucose administration the peak of blood glucose level increased rapidly from the fasting value and then subsequently decreased. All the tested extracts (300 mg/kg, p.o.) exhibited significant hypoglycaemic effect but glibenclamide and methanol extract significantly depressed the peak of blood glucose level at 90 min after glucose loading.

In antihyperglycaemic study (Table 3), the rise in the blood glucose level was observed after 24 h of alloxanization to the animals. Single administration (300 mg/kg) of the different extracts of bark of *S. pinnata* in diabetic rats showed significant reduction in blood glucose level, where as methanol extract was found maximum reduction in blood glucose level (63.12%)

as compare to other extracts under test at the end of 6 h. The results of the methanol extract are comparable to that of the reference standard glibenclamide. The preliminary phytochemical examination of the methanol extract revealed presence of flavonoids, tannins, saponins and terpenoids.

The exact biological active constitutent(s) responsible for the said effect are neither reported nor the exact mode of action of the hypoglycaemic activity was reported earlier, with the lone observation that it is used in folklore diabetic treatments. Preliminary phytochemical examination of the different extracts revealed presence of steroids and sterols, tannins and phenolic compounds, saponins, terpenoids and flavonoids. All the extracts of the bark of *S. pinnata* has hypoglycemic activity as it lowers blood glucose level in both normal and diabetic rats.

CONCLUSION

The results of the present study justify the use of the barks of the plant for treating diabetes as suggested in the folklore remedies.

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