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Studies on Hypoglycaemic Activity of the Different Extracts of *Spondias mangifera* willd. Root

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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 roots of *Spondias mangifera* (Family: Anacardiaceae) was evaluated for hypoglycemic activity on adult Wistar albino rats at dose levels of 100, 200 and 400 mg/kg p.o. respectively 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 roots in the folklore treatment in diabetes.

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

Introduction:

Diabetes mellitus is the most common endocrine disorder in men and women, and the major public health problem of epidemic proportion[1], once believed to be a diseased of west, is becoming an endemic to modernizing and urbanizing population in our country[2]. Ayurvedic literature reveals that many herbal medicines in different oral formulation have been recommended in madhumeha (diabetes mellitus) and confident claims of cure are on record[3].During the past decade, traditional system of medicines have become a topic of global importance[4]. *Spondias mangifera* Willd. (Family-Anacardiaceae) is a glabrous tree upto 10.5 m high with straight trunk and smooth ash coloured bark having characteristic pleasant smell of wood[5]. In India it is cultivated in Punjab, Maharashtra, Orissa, West Bengal and Assam for the edible fruits[6]. Ethnomedicinally, the bark is used as tonic, refrigerant and for the treatment of articular and muscular rheumatism and in diarrhoea and dysentery[7-9]. The leaves are aromatic, acidic and astringent used for flavouring while its juice is applied in ear ache[8, 10]. The root bark powders have been recommended for regulation of menstruation[11-14] and antibacterial[15], antitumor[16], antipyretic, antispasmodic and antihistamine activities were also reported[17]. The aerial parts are reported to contain daucosterol, β-sitosterol, stigmast-4-en-3-one, cycloartanone 24-methylene and lignoceric acid. Other constituents like β-amyrin and oleanolic acid were also reported from the fruits[18].

The tribes of Ganjam district of Orissa drink the root 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 communication we report the hypoglycemic activity of the root on standard laboratory animal models to provide a scientific support to the folklore claims.

Materials and methods:

Plant Material

The plant material (roots) was collected from the forests of Ganjam 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 / (17)/2009/Tech.II/28] has been kept in our research laboratory for further reference. After authentication, fresh root were collected in bulk, washed, shade dried and pulverized in a mechanical grinder to obtain coarse powder.

Preparation of Extract

The powdered root (500 g) after defatting with petroleum ether (60-80^0 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[19, 20] 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 ± 2^0 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 et al*., 2002[21]. 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 *Mondal et al.,* 2009 was followed[22.] 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 mild 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 eleven 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 to XI -received different extracts at doses of 100, 200 and 400 mg/kg in a similar manner. Blood glucose levels were measured after 1, 2, 4 and 8 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 *Dash et al.,* 2007 was followed[23]. Fasted rats were divided into eleven 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 to XI received the test extract at doses of 100,200 and 400 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, 60,150 and 180 min after glucose administration as per the method described earlier (Table 2).

Alloxan Induced hyperglycaemic rats

The method of *Mondal et al.,* 2009 was followed [22]. 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 blood from the tip of the tail of each rat under mild 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[24].This condition was observed at the end of 48 h after alloxanisation. The animals were segregated into eleven groups of six animals in each. Group-I served as negative control and received vehicle (2 ml/kg p.o.) through oral route.

Table 1: Effect of different extracts of the roots of *S. mangifera* on the blood glucose level in normal rats.

Group	Treatment	Dose (mg/kg)	Fasting	Blood glucose concentration (mg / dl) (normoglycaemic study)			
				Time (h) after treatment			
				1	2	4	8
I	Control	2 ml/kg	96.83± 2.84	97.66± 2.1	98.16±2. 05	97.83±2. 12	98.16±1. 99
II	Glibenclamide	2.5mg/kg	96.5±2 .95	60.83± 2.40** (36.96%)	51±2.12** (47.15%)	49.5±4.6 2** (48.7%)	44.5± 4.85** (53.88%)
III	Chloroform extract	100	97.33± 8.38	95.16± 8.19 (2.22%)	91.33± 8.98 (6.16%)	89.66± 11.19 (7.88%)	92.83± 10.69 (4.62%)
IV		200	96.83± 7.65	93.33± 9.15 (3.61%)	90.16± 9.63 (6.88%)	87.16± 11.02 (9.98%)	91.16± 10.97 (5.85%)
V		400	97.66± 7.82	90.16± 9.63 (7.67%)	85.33± 8.77 (12.62%)	79.5± 8.09 (18.59%)	83.83± 8.28 (14.16%)
VI	Methanol extract	100	99±2.9 2	95.66± 5.28 (3.37%)	93±2.91 (6.06%)	91.66± 8.73 (7.41%)	88.16± 8.68 (10.94%)
VII		200	98.66± 2.21	92.5±10. 35 (6.24%)	84.5± 2.46 (14.35%)	75.66± 3.45* (23.31%)	65.16± 4.4* (33.95%)
VIII		400	98±2.0 4	82.5± 3.6 (15.81%)	67.83± 4.32* (30.78%)	50.5± 4.26** (48.46%)	46.16± 3.42** (52.89%)
IX	Aqueous extract	100	98.16± 8.12	95.5± 7.15 (2.7%)	93.66± 7.86 (4.58%)	83.16± 7.95 (15.28%)	79.83±6. 81 (18.67%)
X		200	98.83± 9.54	94.5± 7.92 (4.38%)	90.83± 8.7 (8.09%)	82.16± 7.24 (16.86%)	74.83± 7.9 (24.28%)
XI		400	97.16± 7.91	90.33± 9.7 (7.02%)	86.83± 9.9 (10.63%)	65±6.07* (33.1%)	63.16± 6.12* (34.99%)

Results expressed as Mean ± SEM from six observations (n=6). *P<0.05, **P<0.01 as compared with control group (One way, ANOVA followed by Dunnet's t-test). Figures in parenthesis denote percentage reduction of blood glucose.

Group-II received glibenclamide (2.5 mg/kg). Group-III to XI received the different extracts at doses of 100, 200 and 400mg/kg in a similar manner. Blood glucose level was estimated at 0, 1, 2, 4 and 8 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.

Table 2: Effect of different extracts of the roots of *S. mangifera* on oral glucose tolerance in normal rats.

Group	Treatment	Dose (mg/kg)	Fasting	Blood glucose concentration (mg / dl) (Oral glucose tolerance study)			
				Time (min) after treatment			
				30	60	150	180
I	Control	2 ml/kg	93.66± 2.69	128.5±10.14	148.66± 12.64	159.83± 13.26	153.33±13.63
II	Glibenclamide	2.5mg/kg	96.83± 2.84	128.16± 7.32	105.16± 9.38* (17.94 %)	91±10.8** (28.99 %)	77.66±10.02** (39.4%)
III	Chloroform extract	100	92.83± 2.75	130.5±12.65	125.16± 8.99 (4.09%)	120.66± 9.04 (7.54%)	117.33±8.85 (10.09%)
IV		200	94.16± 8.24	129.5±10.53	123.33± 8.6 (4.76%)	119.16± 8.31 (7.98%)	116.16±7.55 (10.3%)
V		400	91.33± 8.83	130.83± 12.82	122.33± 9.14 (6.49%)	114.16± 7.71 (12.74 %)	110.33±7.44 (15.66%)
VI	Methanol extract	100	91.17± 3.79	123.5±11.46	117.83± 7.15 (4.59%)	115.33± 6.41 (6.61%)	103±5.84* (16.59%)
VII		200	94.5±3.75	130.66± 12.9	115.16± 7.41* (11.86 %)	106.66± 6.97* (18.36 %)	99.5±5.18** (23.84%)
VIII		400	91.16± 8.73	131.33± 12.17	97.83±7.01* (25.5%)	81.83±5.73** (27.79 %)	79.33±4.27** (39.59%)
IX	Aqueous extract	100	98.83± 10.01	129.16± 11.25	124.5±8.11 (3.6%)	118.83± 7.68 (7.99%)	109.83±6. 41 (14.96%)
X		200	90.16± 9.63	130.66± 8.95	116.66± 6.12* (10.71 %)	110.16± 4.79* (15.68 %)	103±5.01* (21.16%)
XI		400	94.34± 2.78	129.83± 11.08	99.5±6.75* (23.36 %)	94.83±6.86* (26.95 %)	88.16±6.65** (32.09%)

Results expressed as Mean ± SEM from six observations (n=6). *P<0.05, **P<0.01 as compared with control group (One way, ANOVA followed by Dunnet's t-test). Figures in parenthesis denote percentage reduction of blood glucose.

Table 3: Effect of different extracts of the roots of *S. mangifera* on the blood glucose level in alloxan induces diabetic rats.

Group	Treatment	Dose (mg/kg)	Fasting	Blood glucose concentration (mg / dl) (Hypoglycemic study)			
				Time (h) after treatment			
				1	2	4	8
I	Control	2ml/kg	239.33±2.2	248.16±1.81	250.5±2.71	255.66±1.9	258.83±2.12
II	Glibenclamide	2.5mg/kg	240.16±10.2	201±10.11* (16.30%)	155±14.88** (35.45%)	112.66±9.23** (53.08%)	88.33±9.93** (63.22%)
III	Chloroform extract	100	239.83±9.88	231.33±11.35 (3.54%)	226.83±12.33 (5.42%)	221.5±14.41 (7.64%)	217.5±10.83 (9.31%)
IV		200	237.5±11.56	228.66±13.21 (3.72%)	223.33±14.1 (5.96%)	216±13.02 (9.05%)	211.66±13.68 (10.88%)
V		400	235±12.2	224.66±14.46 (4.4%)	216.83±14.13 (7.73%)	207.33±15.86 (11.77%)	200.33±9.26 (14.75%)
VI	Methanol extract	100	236.83±13.11	227.16±14.22 (4.08%)	212.5±9.66* (10.27%)	207.83±9.65* (12.24%)	199.83±11.1* (15.62%)
VII		200	234.83±14.62	211.66±9.59* (9.86%)	190±16.01* (19.09%)	182±16.01* (22.49%)	143.83±6.05** (38.75%)
VIII		400	235.5±23.13	197.5±10.68* (17.05%)	166.66±14** (29.23%)	132.16±8.89** (43.88%)	98±9.85** (58.38%)
IX	Aqueous extract	100	238.66±10.05	235.66±11.89 (1.25%)	229.5±12.93 (3.83%)	206.83±12.1* (13.33%)	202.66±13.68* (15.08%)
X		200	236.83±11.33	227.33±7.4 (4.01%)	199.66±12.3* (15.69%)	198.5±12.27* (16.18%)	190.16±15.64* (19.7%)
XI		400	236.33±15.65	199.16±14.37* (15.72%)	194.5±14.59* (17.69%)	188.16±14.59* (20.38%)	156.16±16.41** (33.92%)

Results expressed as Mean ± SEM from six observations (n=6). *P<0.05, **P<0.01 as compared with control group (One way, ANOVA followed by Dunnet's t-test). Figures in parenthesis denote percentage reduction of blood glucose.

All the values were expressed as mean ± SEM.

Results and discussion:

The results of the preliminary phytochemical screening of different extracts revealed presence of steroids, terpenoids, flavonoids, tannins, saponins and sugars in the test extracts.

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.

The roots of *S. mangifera* have been used by the local tribes for the treatment of diabetes mellitus since time immemorial and they claim for its promising activity. Results of antidiabetic activity of *S. mangifera* roots extract established the scientific basis for the utility of this plant in the treatment of diabetes. The test extract has shown significant reduction in blood glucose levels in both normal and oral glucose tolerance in normal rats and alloxan induced diabetic's rats at the tested dose levels. In all the models, the activity of the extract was found to be in a dose dependant manner (Table-1 to 3).

Reports of the normoglycaemic study (Table 1) reveals that the methanol extract of *S. mangifera* exhibited reduction in blood glucose concentration in a dose dependant manner as compare to control and at the dose 400 mg/kg significant reduction in blood glucose concentration from 2 h respectively where as glibenclamide showed reduction in blood glucose concentration in rats after 1 h treatment but the aqueous extract at the dose 400 mg/kg significant reduction in blood glucose concentration from 4 h respectively, where as choloroform extract did not show any promising effect throughout the experiment.

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, but the glibenclamide (2.5 mg/kg, p.o.), methanol and aqueous extract significantly depressed the peak of blood glucose level at the tested dose level (200 and 400 mg/kg, p.o.) from 60 min after glucose loading, where as choloroform extract did not elicit any promising activity.

In antihyperglycaemic study, the rise in the blood glucose level was observed after 24 h of alloxanization to the animals. Single administration of the different extracts of *S. mangifera* roots at the tested dose level (100, 200 and 400 mg/kg, p.o.) in diabetic rats showed significant reduction in blood glucose level. Where as the methanol extract exhibit maximum reduction in blood glucose level at all tested doses through out the experiment as compare with other extracts. Glibenclamide (2.5 mg/kg, p.o.) showed maximum reduction (63.22% decrease blood glucose levels) after 8 h, where as methanol extract (400 mg/kg, p.o.) reduces 58.38% blood glucose levels.

Conclusion:

The comparable effect of the extract with glibenclamide may suggest similar mode of action, since alloxan permanently destroys the pancreatic β-cells and the extract lowered blood sugar level in alloxanized rats, indicating that the extract possesses extrapancreatic effects[22]. The exact biological active constitutent(s) responsible for the said effect are neither reported nor was the exact mode of action of the hypoglycaemic activity reported earlier, with the lone observation that it is used in folklore diabetic treatments. The present work justifies the use of the roots of *S. mangifera* in the folklore treatment in diabetes.

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