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STUDIES ON THE HYPOGLYCAEMIC ACTIVITY OF THE BARK OF *SALIX TETRASPERMA* ROXBURGH

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ABSTRACT

Diabetes, the most prevailing metabolic disorder is attracting present research attention towards it. In the present study, the chloroform, methanol and aqueous extracts of the barks of Salix tetrasperma Roxburgh. (Family: Salicaceae) 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, oral glucose tolerance test and alloxan induced hyperglycaemic rats. Glibenclamide (2.5 mg/kg) was used as reference standard for activity comparison. Among the tested extracts, the aqueous extract was found to produce promising results that is comparable to that of the reference standard glibenclamide. The preliminary phytochemical examination of the aqueous extract revealed presence of flavonoids, tannins and saponins. The study established the scientific basis for the utility of this plant in the treatment of diabetes and justifies the use of the bark of the plant for treating diabetes as suggested in folklore remedies.

Keywords: *Salix tetrasperma, Alloxan, Glibenclamide, Hyperglycaemic, Normoglycaemic, Oral glucose tolerance Test (OGTT).*

INTRODUCTION

Search for antidiabetic factor in plants remains a potential area of investigation. Diabetes mellitus is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves. Reports from the World Health Organization (WHO) indicate that diabetes mellitus is one of the major killers of our time, with people in South-east Asia and Western Pacific being most at risk. Plants with various active principles and properties have been used since ancient times by physicians and laymen to treat diabetes. The beneficial multiple activities like manipulating carbohydrate metabolism by various mechanisms, preventing and restoring integrity and function of 6-cells, insulin-releasing activity, improving glucose uptake and utilization and the antioxidant properties present in medicinal plants offer exciting opportunity to develop them into novel therapeutics.

Salix tetrasperma Roxburgh. (Family: Salicaceae), commonly called Indian Willow, It is a medium sized tree of wet and swampy places, shedding the leaves at the end of monsoon season. The bark is rough, with

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deep, vertical fissures and the young shoots leaves are silky[1-4].

The dried leaves are reported to possess cardiotonic and neurotonic activity[5,6]. The leaves and bark of the willow tree have been mentioned in ancient texts from Assyria, Sumer and Egypt[7] as a remedy for aches and fever[8]. The decoction of both leaf and root is used for treating whooping cough in children[9]. The paste of both leaf and root is applied externally in scorpion stings, bug bites, for sores and warts[10]. The decoction of the dried root is taken orally for the treatment of hepatitis[10]. The sap of the stem is used orally by females for treating dysmenorrhea[1]. The hot water extract of the entire plant is used in vaginal cavity to induce abortion in pregnant females and administered rectally to treat local sores in the rectum[1].

The aqueous extract of the stem bark has been reported to increase testosterone level in rats at 500.0 mg/kg, p.o.[11] and also accelerates semen coagulation in rats at a concentration of 2%w/v[12]. A dose of 0.094 mg/kg of aerial parts shows hypothermic activity in mice[13]. Aqueous extract of dried leaf reported to possess cardiotonic activity and the methanol extract of the dried leaf possess reverse transcriptase inhibition effect[14]. Ethanolic extract of the aerial parts is reported to be inactive against Staphylococcus *aureus*, *Bacillus subtilis*, *Salmonella typhosa*, *Escherichia coli*, *Candida albicans*, *Trichophyton mentagrophytes* etc[15, 16].

However, only a few phytochemical have been reported on this plant in the literature like various types of sapogenins such as quinovic acid, salicortin, saligenin, phenolic glycosides and pyrocatechol was isolated from the barks and leaves[17]. The active extract of the bark, called salicin[18] was isolated to its crystalline form. The entire plant is reported contain tannins, triterpenes, viz. β-amyrin, lupeol[5] and chalcinasterol[4], steroids viz. β-sitosterol and stigmasterol[19,20]. Whilst salicortin, saligenin and pyrocatechol can occur in quite large quantities in intact plant material[21,22] free salicylaldehyde seems to occur only in very low concentrations[23]. However, salicylaldehyde may be formed from saligenin[24,25] by the action of an oxidase once plant material is damaged[26-28] both 6-HCH and catechol are potential contact allergens but do not appear to have yet been investigated for such activity in the context of sensitisation to *S. tetrasperma*.

The tribes of Keonjhar district of Orissa drink the bark paste duly suspended in water for the treatment of diabetes mellitus since time immemorial and they claim for its promising activity. The present study was therefore undertaken to establish the scientific basis for the utility of the barks of *S. tetrasperma* in the treatment of diabetes.

Materials and Methods

Plant Material

The plant material (barks) was collected from the forests of Keonjhar district of Orissa during November 2009 and authenticated. The collected barks were washed, dried under shade and powdered in a mixer grinder. The powdered bark (500 g) after defatting with petroleum ether (40-60^0 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[29,30] 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[31]. 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 Normoglycemic Rats

The test was performed as suggested by *Mondal et al.*, 2009[32]. Although free access to water before and throughout the duration of experiment was allowed the acclimatized animals were fasted for 18 h. The end of the fasting period was taken as zero time (0 h), and the collection of blood was done by tail vein method of each rat under mild anesthesia[33]. The blood glucose level was measured 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 in each. Negative control was designated as group I and received vehicle (2 ml/kg) through oral route. Group-II received glibenclamide (2.5 mg/kg p.o.). Then the other groups received 100, 200 and 400 mg/kg, of chloroform, methanol and aqueous extracts. After 1, 2, 4 and 8 h of administration of single dose of test samples blood glucose levels were measured (Table 1).

Oral Glucose Tolerance Test (OGTT) In Rats

The method of *Badole et al.*, 2007 was followed [34]. Fasted rats were divided into eleven groups of six rats each group. Group I served as a control and received only vehicle (2 ml/kg) through oral route. Group-II received glibenclamide (2.5 mg/kg p.o.). Then the other groups received 100, 200 and 400mg/kg of chloroform, methanol and aqueous extracts. 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).

Using Hyperglycemic Rats

The method was performed as suggested by *Mondal et al.*, 2009[32]. The acclimatized animals after fasting for 24 hours with water *ad libitum* and then intraperitoneal injection of a dose of 150 mg/kg of alloxan monohydrate in normal saline was given. The animals were provided standard laboratory diet *ad libitum* after one hour. Under mild anesthesia the blood was withdrawn from the tip of the tail of each rat and the blood glucose level was checked before alloxanisation and 24 h after alloxanisation. The blood glucose level was measured as stated above. Rats having the blood glucose level above 225 mg/dl[35] were selected and grouped into eleven groups consisting of six animals each. This condition was observed at the end of 48 h after alloxanisation. Orally 1% Tween 80 solution (2 ml/kg p.o) was received by the Group-I which served as diabetic control, glibenclamide (2.5 mg/kg) was received by Group-II, chloroform, methanol and aqueous extract at doses of 100, 200 and 400 mg/kg, p.o., (in 1% Tween 80) respectively in a similar manner were received by the other groups. After 1, 2, 4 and 8 hrs of administration of single dose of test samples blood glucose levels were measured (Table 3).

Statistical Analysis

All the results were statistically analysed using one way ANOVA followed by Dunnet's t-test. $^{*}P<0.05$ were considered significant.

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.

Preliminary phytochemical examination of the extracts revealed presence of steroids and sterols, triterpenes, tannins and phenolic compounds, saponins and flavonoids.

The barks of *S. tetrasperma* 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 anti-diabetic activity of *S. tetrasperma* barks 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 alloxan induced diabetic rats at the tested dose levels. In both the models, the activity of the test extracts was found to be in a dose dependant manner.

Reports of the normoglycaemic study (Table 1) reveals that the all extracts exhibited reduction in blood glucose concentration in a dose dependant manner as compared to control, where as the aqueous extract at the dose 400 mg/kg, p.o. there was a significant reduction in blood glucose concentration from 2h and the reference standard glibenclamide (2.5 mg/kg, p.o.) showed reduction in blood glucose concentration in rats after 1 h treatment.

In glucoseloaded animals (Table 2), the aqueous extract at 400 mg/kg, reduced the blood glucose almost to the normal levels. It is possible that the drug may be acting by potentiating the pancreatic secretion or increasing the glucose uptake. 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. tetrasperma* barks 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 aqueous extract was found maximum reduction in blood glucose level at 100, 200 and 400 mg/kg (25.05%, 45.42% and 56.33%) as compare to other extracts and glibenclamide (2.5 mg/kg, p.o.) showed maximum reduction (59.05% decrease blood glucose levels) after 8 h (Table 3).

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. All the extracts of *S. tetrasperma* barks have hypoglycemic activity as it lowers blood glucose level in both normal and diabetic rats.

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

Table 1: Effect of different extracts of the barks of *S. tetrasperma* 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	3	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	2.5 mg/kg	96.5±2.95	60.83±2.40 (36.96%)	51±2.12 (47.15%)	49.5±4.62 (48.7%)	44.5±4.85** (53.88%)
III	Chloroform extract	100	98.66±9.58	96.16±9 (2.53%)	93.66±10.28 (5.06%)	94.26±8.28 (4.45%)	95.3±10.16 (3.40%)
IV		200	97.66±7.82	94.13±10.21 (3.61%)	90.80±7.93 (7.02%)	87.83±9.1 (10.06%)	90.5±10.35 (7.33%)
V		400	98.83±10.01	90.16±9.63 (8.77%)	87.83±11.78 (11.13%)	81.5±9.79 (17.53%)	85.33±10.7 (13.65%)
VI	Methanol extract	100	97.83±8.23	96.5±9.70 (1.35%)	91.16±10.73 (6.81%)	85±11.64 (13.11%)	81.33±12.60 (16.86%)
VII		200	98.83±9.58	93.16±7.14 (5.73%)	88.16±8.93 (10.79%)	83.83±7.9 (15.17%)	75.23±10.99*(23.87%)
VIII		400	97.5±7.84	91.36±10.83 (6.29%)	82.06±8.68 (15.83%)	76.2±12.38 (21.84%)	68.33±9.28* (29.91%)
IX	Aqueous extract	100	99.73±2.84	95.66±2.70 (4.08%)	88.83±2.75 (10.92%)	82.16±3.68 (17.61%)	78.5±2.81 (21.28%)
X		200	98.66±2.21	93.66±2.69 (5.06%)	81.5±2.26 (17.39%)	72.13±4.17 (26.89%)	66.00 ±5.96*(33.10%)
XI		400	98±2.02	78.83±2.31*(19.56%)	69.83±5.48*(28.74%)	52.16±2.70 (46.77%)	47.33±2.77 (51.70%)

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 2: Effect of different extracts of the barks of *S. tetrasperma* on oral glucose tolerance in normal rats

Group	Treatment	Dose (mg/kg)	Fasting	Blood glucose concentration (mg / dl) (oral glucose tolerance study)			
				Post treatment			
				30 min.	60 min.	150 min.	180 min.
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	2.5 mg/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	91.17±3.79	133.16±11.79	127.13±8.1 (4.52%)	125.5±11.78 (5.75%)	120.66±10.86 (9.38%)
IV		200	91.33±8.83	131.16±8.61	124.16±11.85 (5.33%)	120.33±11.21 (8.25%)	118±11.05 (10.03%)
V		400	94.16±8.24	130.5±12.65	122.26±11.56 (6.31%)	117.16±10.1* (10.22%)	110.16±10.44* (15.58%)
VI	Methanol extract	100	92.83±2.75	132.16±10.45	124.33±9.07 (5.92%)	120.66±9.85 (8.70%)	118.16±7.4* (10.59%)
VII		200	91.16±8.73	130.66±8.95	122.16±11.09 (6.50%)	116.5±9.77* (10.83%)	113.33±8.7* (13.26%)
VIII		400	94.34±2.78	129.16±11.25	107.5±8.47* (16.76%)	111.5±8.31* (13.67%)	98.33±7.4* (23.86%)
IX	Aqueous extract	100	90.16±9.63	126.33±11.36	123.33±10.02 (2.37%)	120.83±11.8 (4.35%)	112.16±11.27* (11.21%)
X		200	94.5±3.75	130.66±12.9	122.33±13.1 (6.37%)	108.5±13.01* (16.96%)	98.83±9.41* (24.36%)
XI		400	98.83±10.01	131.5±12.02	106.83±8.13* (18.76%)	92.83±7.09** (29.40%)	80.16±10.63** (39.04%)

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 barks of *S. Tetrasperma* on the blood glucose level in alloxan induce 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	2 ml/kg	239.33±2.2	248.16±1.81	250.5±2.71	255.66±1.9	258.83±2.12
II	Glibenclamide	2.5 mg/kg	240.16±10.2	201±10.11* (16.3%)	155±14.88** (35.45%)	112.66±9.23** (53.08%)	98.33±9.93** (59.05%)
III	Chloroform extract	100	241.16±2.21	240.33±11.44 (0.34%)	238.83±13.6 (0.966%)	230.5±15.36 (4.42%)	228±9.42* (5.45%)
IV		200	237.5±2.01	234.16±13.47 (1.40%)	230.33±13.12 (3.01%)	228.83±13.75 (3.65%)	223.33±14.24 (5.96%)
V		400	236.16±1.75	230.66±14.17 (2.32%)	220.5±14.06 (6.63%)	213.5±13.16 (9.59%)	200.5±18.64* (15.09%)
VI	Methanol extract	100	239.83±11.29	233.33±11.35 (2.71%)	219±13.25 (8.68%)	206.83±11.39* (13.75%)	199.83±14.17* (16.67%)
VII		200	237.5±13.59	228.66±13.21 (3.72%)	202.66±15.02* (14.66%)	199±20.26* (16.21%)	192.83±14.8* (18.8%)
VIII		400	235±13.69	218.66±14.46 (6.95%)	183.5±13.86* (21.91%)	175.83±17.8* (25.17%)	148±22.88** (37.2%)
IX	Aqueous extract	100	236.83±14.84	210.83±16.24* (10.97%)	204.66±15.09* (13.58%)	190.33±16.2* (19.63%)	177.5±22.89* (25.05%)
X		200	234.83±10.16	201±10.11* (14.4%)	186.66±10.15* (20.51%)	158.66±13** (32.43%)	128.16±10.2** (45.42%)
XI		400	235.5±14.73	194.66±14.75* (17.34)	156.33±14.05** (33.61%)	128.5±6.58** (45.43%)	102.83±8.92** (56.33%)

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.

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