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Hypoglycemic Activities of a Mangrove Plant *Rhizophora apiculata* Blume

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Abstract – The leaves of *Rhizophora apiculata*, a plant belonging to the family *Rhizophoraceae* were collected from the mangrove forest of Sunderbans, West Bengal, India. Alcoholic extract of the leaves of this plant was prepared and hypoglycemic/anti-hyperglycemic activity was studied in fed rats, glucose loaded rats and streptozotocin induced diabetic rats. The results of this study reveal that this plant extract has potential hypoglycemic action.
Keywords – *Rhizophora apiculata*, hypoglycemic, streptozotocin, glucose

Introduction

Rhizophora apiculata Blume is a typical mangrove plant and is distributed abundant by throughout the Gangetic Sunderbans in the state of West Bengal, India (Blasco, 1975; Botanical Survey of India report, 1989). The leaves of *Rhizophora* mainly contain lignin, cellulose and wax (Krishnamurthy, 1974). The plant growth inhibitor *rhizophorine* has been reported to be present in this plant (Kokpol, 1975). Extract of this plant have potential anti-microbial effect (Rojas Hernandez *et al.*, 1989). Recent studies claim inhibitory effect of this plant on human immunodeficiency virus (Premanathan, 1999). A preliminary study has also suggested that, a Mexican variety of *Rhizophora mangle* possesses hypoglycemic action (Alarcon-Aguilara *et al.*, 1998). It has come to our notice that people in the rural areas of Sunderbans, West Bengal (India) use the leaves of *Rhizophora apiculata* crushed with water orally for the treatment of diabetes (Nadkarni, 1982). But its efficacy in experimental models of diabetes has not been substantiated. We have previously published preliminary reports of hypoglycemic activity of this plant (Sur *et al.*, 2001; 2002). This study was therefore undertaken to further investigate into the hypoglycemic/anti-hyperglycemic potential of alcoholic extract of *Rhizophora apiculata* in rats.

Experimental

Plant materials – Fresh leaves of *Rhizophora apiculata* were collected during pre-monsoon season (February-June, 2000) from the Sunderbans, West Bengal. Further identification was done with the kind help of the Botanical Survey of India, West Bengal. A voucher specimen (No. DB/UGC/UCM/003) has been preserved in our laboratory.

Preparation of plant extract – The leaves of *R. apiculata* were washed, air-dried and crushed to make fine powder. The dry powdered leaves of this plant were soaked in 70% ethanol in a percolator for 24 hrs. Thereafter, alcoholic extract was collected and concentrated under reduced pressure. The concentrated extract was then lyophilized and dried extract was obtained.

Phytochemical analysis – Alcoholic extract of the plant was screened for the active phytoconstitutents (Trease and Evans, 1985).

Animals – *In bred* male Wistar rats (150-200g) and male Swiss mice (20-30 g) were used in this study. The animals were kept in colony cages under identical housing conditions, *i.e.*, 12 hrs light : dark cycle, 60-65% humidity and 20-25°C temperature. They were fed with commercial pellet diet made for rat and mouse (Lipton, India) and water *ad libitum*. Food was restricted to the animal overnight before they were used for experimentation. The care and maintenance of the animals were as per the approved guidelines (Committee for the Purpose of Control and Supervision of Experiments on Animals, India). The Institutional Ethics Committee approved the study.

Safety evaluation/Lethality – LD_{50} was determined as described by Ghosh, 1984. The plant extract was administered orally to groups of 10 albino mice in different doses upto 4 g/kg and the animals were observed up to 72 hrs at time

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intervals for mortality.

Selection of doses – The effective doses for blood sugar lowering action of the plant extract were selected to be 250 mg/kg and 500 mg/kg orally, found out by primary screening giving doses at random (Sur *et al.,* 2001). Thereafter, all experiments were done with the above two doses in water.

Hypoglycemic/anti-hyperglycemic studies – Blood sugar lowering effect of alcoholic extract of the plant was evaluated in several experimental models in rats weighing 150-200 g. The extract was given orally at two dose levels, *i.e.,* 250 mg/kg and 500 mg/kg in rats. Glibenclamide (1.5 mg/kg, p.o.) was used as a reference drug. A drop of blood was collected from the tail vein of the rat with proper care at different durations (0-4 hrs). The blood glucose was measured using high precision electronic glucometer, Accutrend® alpha (Roche, Germany). The following experimental models were conducted for establishing the hypoglycemic/anti-hyperglycemic activity of *Rhizophora apiculata.*

Normoglycemic rats – Blood sugar lowering effect of the plant extract was seen, in over-night fasted normal rats. The test drug was given to the rats in previously mentioned dosages and blood glucose was estimated by using glucometer. Standard group received glibenclamide (1.5 mg/kg, p.o.), while control group received only water.

Fed rats – Excess diet fed rats were taken for this study. The blood glucose level was checked first. Thereafter, the rats were divided into 4 groups on the basis of a similar mean blood sugar level. Two doses of the test drug (250 mg/kg and 500 mg/kg) were given orally to two of the four groups. The control group received only water and standard group received glibenclamide (1.5 mg/kg, p.o.). The blood glucose was estimated at regular intervals up to 4 hrs.

Glucose-loaded rats – Glucose solution (50% w/v) was given in the dose of 2.5 g/kg, orally to all the groups (Vogel *et al.*, 1997). In the two test groups same amount of glucose was given orally 5 min after oral administration of the plant extract in two doses as mentioned earlier. Blood was withdrawn from the tip of the tail immediately before, and after 30 min, 1 hr and 2 hrs after administration of the extract. Glibenclamide (1.5 mg/kg, p.o.) was used as a reference drug.

Streptozotocin (STZ)-induced diabetic rats – Male Wistar rats (150-200 g body weight) were injected with 65 mg/kg STZ intravenously (Vogel *et al.,*1997). The animals were checked regularly and maintained properly. The hyperglycemic rats were then divided into four groups for further study. On the day 14, blood glucose was estimated in 18 h fasted rats. Groups of the rats were treated with plant extract (either of the two doses as before) and third group treated with standard, while control rats received only water. Thereafter, blood glucose was estimated at different intervals.

Statistical analysis – Statistical analysis of the data was done using Student's t-test. P value <0.05 was considered statistically significant (Hicks, 1999).

Results

Safety evaluation – The doses of the extract were selected randomly from 0.5 to 4.0 g/kg body weight by oral route. The mortality of mice the treatment with *Rhizophora apiculata* extract in above doses up to 3 days was nil.

Phytochemical analysis – Phytochemical analysis revealed the presence of active phytoconstituents e.g. flavonoids, terpenoids, saponins, tannins, gums, sugars in the alcoholic extract of this plant.

Normoglycemic studies – *Rhizophora apiculata* extract in the dose of 250 mg/kg reduced blood glucose level significantly only at 2 hrs and 3 hrs post treatment compared to the control. Significant reduction in blood glucose level has been observed at 1 hr, 2 hrs and 3hrs when the dose of *Rhizophora apiculata* was increased to 500 mg/kg. Glibenclamide in the dose of 1.5 mg/kg, p.o., showed significant ($p<0.001$) blood glucose lowering action in normal fasting rats (Table 1).

Fed rats – The plant extract in the dose of 250 mg/kg showed significant reduction of blood glucose up to 3 hrs. Further prolongation of time did not show any significant activity when compared to the control group. But, in the dose of 500 mg/kg, it showed reduction in blood sugar up to 4 hrs. Glibenclamide reduced blood sugar concentration

Table 1. Effect of *Rhizophora apiculata* on blood glucose level in normal rats

	Blood glucose (mg/100 ml blood)				
	0 h	1 h	2 h	3 h	4 h
Control	102±4	98±2	96±2	98±2	96±2
R. apiculata (250 mg/kg)	99±2	96±2 (2)	89±2* (7)	88±2** (11)	95±2 (1)
R. apiculata (500 mg/kg)	99±3	87±4* (11)	83±3* (14)	80±3*** (18)	89±4 (7)
Glibenclamide (1.5 mg/kg)	97±3	84±4* (14)	78±3*** (18)	72±1*** (26)	79±4** (18)

n = 6 in each group; Mean ± SEM; the values are compared statistically with respective control by using Student's t-test; * indicate $p < 0.05$, ** indicate $p < 0.01$ and *** indicate $p < 0.001$; parenthesis indicate % change /inhibition in comparison with control.

of fed rats by 23% at 4 hrs (Table 2).

Glucose-loaded rats – The plant extract in lower dose reduced the sugar level by 23% at 1 hr, while it was lowered by 30% when administered in higher dose in comparison to control. Glibenclamide also showed significant reduction in blood sugar concentration 27% at 1 hr (Table 3).

Streptozotocin (STZ)-induced diabetic rats – The plant extract in the dose of 250 mg/kg showed highest blood sugar lowering action at 4 hrs (22%), while, at higher dose (500 mg/kg) the reduction was 25% at 4 hrs. Similar type of reduction in blood glucose concentration was noted when standard drug was given (Table 4).

Discussion

Diabetes is a chronic disease affecting millions of people worldwide. WHO expert committee has aptly suggested that research should be aimed at investigating the traditional methods of treatment for refractory diseases like diabetes and its complications (WHO report, 1985).

Efforts continue in the field of medicine to find insulin substitutes from synthetic or plant sources for the treatment of diabetes. Active constituents of plants, such as flavonoids, are known to be used for the treatment of diabetes. A thorough investigation of mangroves habitat in India for pharmacological profiles are still lacking. Ethnobotanical survey so far conducted, reveals the use of mangroves in rural areas for medicinal purposes in diseases (Snedaker *et al.,* 1984; Bidhan Chandra Krishi Visva Vidyalaya report, 1991). *Rhizophora apiculata* is reported to be used in diabetes by local people (Nadkarni, 1982), though it has not yet been confirmed.

In the present study, the oral route of administration was preferred as it is simple and physiological. Albino rats were chosen for experiment, because the blood sugar level of rats remain fairly stable during handling, unlike other animals, such as rabbits. Five different test models were used to enhance the sensitivity of the evaluation procedure. These models allowed us to examine the efficacy of the test drug against different backgrounds of blood sugar levels (*i.e.*, euglycemic and hyperglycemic states).

The hypoglycemic action of *Rhizophora apiculata* extract was screened in normal fasting rats (Table 1). The blood glucose level was studied up to 4 hrs in 18 hrs fasting rats.

Table 2. Effect of *Rhizophora apiculata* on blood glucose level in fed rats

	Blood glucose (mg/100 ml blood)				
	0 h	1 h	2 h	3 h	4 h
Control	160±3	175±3	166±2	155±3	143±3
R. apiculata (250 mg/kg)	161±6	165±3* (6)	149±4*** (10)	140±3** (10)	134±3 (6)
R. apiculata (500 mg/kg)	164±5	162±2* (7)	130±5*** (22)	127±3*** (18)	127±4** (11)
Glibenclamide (1.5 mg/kg)	162±8	150±2** (14)	137±4*** (18)	120±4*** (22)	110±5*** (23)

n = 6 in each group; Mean ± SEM; the values are compared statistically with respective control by using Student's t-test; * indicate $p < 0.05$, ** indicate $p < 0.01$ and *** indicate $p < 0.001$; parenthesis indicate % change / inhibition in comparison with control.

Table 3. Effect of *Rhizophora apiculata* on blood glucose level in glucose loaded (2.5 g/kg, 50%w/v, p.o.) rats

	Blood glucose (mg/100 ml blood)			
	0 h	0.5 h	1 h	2 h
Control	99±2	158±6	172±3	140±4
R. apiculata (250 mg/kg)	102±2	125±8** (21)	131±2***(23)	122±3** (13)
R. apiculata (500 mg/kg)	100±3	119±9** (25)	121±3*** (30)	109±2*** (22)
Glibenclamide (1.5 mg/kg)	99±2	117±6*** (27)	125±3***(27)	111±1*** (21)

n = 6 in each group; Mean ± SEM; the values are compared statistically with respective control by using Student's t-test; * indicate $p < 0.05$, ** indicate $p < 0.01$ and *** indicate $p < 0.001$; parenthesis indicate percent change/inhibition in comparison with control.

Table 4. Effect of *Rhizophora apiculata* extract on blood glucose level in streptozotocin (65 mg/kg, i.v.) induced diabetic rats

	Blood glucose (mg/100 ml blood)				
	0 h	1 h	2 h	3 h	4 h
Control	406±7	409±7	410±6	408±7	405±7
R. apiculata (250 mg/kg)	406±7	389±5* (5)	366±7*** (11)	336±5*** (17)	318±6*** (22)
R. apiculata (500 mg/kg)	409±6	387±4* (5)	349±11*** (15)	319±8*** (22)	302±5*** (25)
Glibenclamide (1.5 mg/kg)	403±7	355±4** (13)	326±9*** (20)	305±10*** (25)	296±6*** (27)

n = 6 in each group; Mean ± SEM; the values are compared statistically with respective control by using Student's t test; * indicate $p < 0.05$, ** indicate $p < 0.01$ and ** indicate $p < 0.001$; parenthesis indicate percent change/inhibition in comparison with control.

The blood glucose lowering action was noted at hourly intervals. *Rhizophora apiculata* showed maximum reduction of blood glucose level after 3 hrs. This findings indicate that the plant extract has blood sugar reducing action in normal conditions.

In fed rats, when the blood glucose level was high, *Rhizophora apiculata* showed significant anti-hyperglycemic potentiality (Table 2). *Rhizophora apiculata* showed maximum reduction in blood glucose level in fed condition after 2 hrs interval. These findings support the hypothesis that *Rhizophora apiculata* has probable anti-hyperglycemic function.

Glucose loaded model indicates the kinetic functional changes induced by any anti-diabetic drug. The blood sugar lowering action of the plant extract showed statistical significance when it was studied at both the doses (Table 3). The results indicate that the maximum reduction in the blood glucose level was at 1 hr.

Streptozotocin (STZ) induced diabetes in laboratory animals has become a valuable tool in diabetes research. In rodents, STZ is cytotoxic to β-cells of islets of Langerhan. Severity and onset of diabetic symptoms depend on the dose of STZ (Vogel *et al.*,1997). The present study indicated that after intravenous injection of STZ (65 mg/kg) in rats, the blood glucose level was nearly 400 mg/dl after 14 days. The enhancement was more than 3 times and statistically highly significant. Actually, STZ alkylates DNA, resulting in the cessation of functions of β-cell, eventually leading to cell death and diabetes. β-cells are most sensitive to STZ, presumably because of a glucose moiety in its structure (Yammoto *et al.*, 1981). It has been suggested that STZ breaks nuclear DNA strands of β-cells by generating free-radical oxygen (Uchigata *et al.*,1982). Wilson and co-workers (1988) had proposed that STZ leads to alkylation of glycolytic and mitochondrial enzymes, necessary for the generation of ATP. But, pretreatment with *Rhizophora apiculata* extract resulted in reduction in the blood sugar level. The maximal reduction in blood glucose level was observed after 4 hrs in *Rhizophora apiculata* extract treated rats. This finding was similar to standard glibenclamide treated rats. This clearly indicated that *Rhizophora apiculata* extract has potential hypoglycemic action in STZ induced diabetic rats.

The extract was also evaluated for biological toxicity. The starting point of these tests is lethal dose selection in animals. In the present study, lethal dose selection was done in mice. The results revealed that the lethal dose of the extract was higher than 4 g/kg body weight, which meant it is practically non-toxic in the doses it is being used.

The pharmacological actions and medicinal properties of the plants are largely dependent on the activities of active components present in it. *Rhizophora apiculata* extracts contains flavonoids, terpenoids, saponins, tannins, gums, sugars. These organic substances, particularly, flavonoids may be responsible for its hypoglycemic action (Choi *et. al.*,1991). Other reports support that *Rhizophora apiculata* contains lignin, cellulose and wax (Krishnamurthy, 1974).

It is therefore, now unequivocally established that the test material *i.e.*, alcoholic extract of *Rhizophora apiculata* has marked hypoglycemic action.

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