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Study the efficacy of *Rhizophora mucornata* Poir. leaves for diabetes therapy in long evans rats

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Abstract

Hypoglycemic effects were investigated in the ethanol extract of leaves of *Rhizophora mucornata* on Long Evans rats. Gut perfusion and six segments studies were carried out to assess these activities. In the gut-perfusion study the percentage of glucose absorption in control rats vs. rats fed with 250 mg/kg extracts were observed at 5, 10, 15, 20, 25 and 30 minutes and the significant ($p<0.05$) absorption result was found at 15 minutes, which was 35.87 vs. 57.29. The percentage of absorption was found better with 250 mg/kg than 500 mg/kg dose level. The six-segment study was performed to assess the amount of sucrose remaining in the GIT at six different positions. The amount sucrose unabsorbed in different GIT segments showed that in control rats vs. rats fed with 500mg/kg extract at 30 minutes in mmol/l was 0.120 vs. 0.135 which were gradually abating with time dependent manner at 60, 180, and 360 minutes in mmol/l. These results suggest that ethanol extract of leaves of *Rhizophora mucornata* has significant dose dependant anti-diabetic effects which may be effective in the treatment of diabetes.

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Introduction

Diabetes mellitus is a clinical syndrome characterized by inappropriate hyperglycemia caused by a relative or absolute deficiency of insulin or by a resistance to the action of insulin at the cellular level (Debra *et. al.*, 1991).It is wide spread disorder, which has long been in the history of medicine. Before the advent of insulin and oral hypoglycaemic drugs the major form of treatment involved the use of the plants. But now from the last two decades there has been a new trend in the preparation and marketing of herbal drugs (Nahar, 1993). Further it has been estimated that in the U.S. 25% of all prescription dispensed from community pharmacies contain plant extracts (Smith and Reynard, 1995).

Diabetes is a major threat to global public health that is rapidly getting worse and biggest impact in on adult of working age in developing countries. There are an estimated 246 million people with diabetes in the world, of whom about 80% reside in developing countries (Sicree *et. al.*, 2006). On the basis of the aetiology, type 1 may be due to immunological destruction of pancreatic β cells resulting in insulin deficiency. Its pathogenesis involves environmental triggers that may activate autoimmune mechanisms in genetically susceptible individuals, leading to progressive loss of pancreatic islet β cells (Harrison and Honeyman, 1999). Many of the acute affects of this disease can be controlled by insulin replacement therapy, but there are long-term adverse effects on blood vessels, nerves and other organ systems. Type 2 DM is associated with both impaired insulin secretion and insulin resistance. Type 2 DM is more prevalent form of the disease and common in individuals over 40 years of age. It is often associated with obesity and hereditary disposition (Zimmet, 1990)

Rhizophora mucronata (Rhizophoraceae), occurs on the coasts of the Indian Ocean and the West-Pacific, is a diverse medicinal plant which has been therapeutically used in the treatment of various diseases. For long year it is reported to be astringent, Asiatic mangrove is a folk remedy for angina, diabetes, diarrhea, *(China, Japan)*, dysentery, hematuria, and hemorrhage. Leaves are poulticed onto armored fish injuries (Watt and Breyer-Brandwijk, 1962). Different communities use it for different purposes as- Indochinese uses the roots for angina and hemorrhage; Malayans use old leaves and/or roots for childbirth; Burmese use the bark for bloody urine; Chinese and Japanese for diarrhea, Indochinese for angina (Perry, 1980).

It has been used as a therapeutic agent for the treatment of diabetes mellitus (Chiranjibi Pattanaik *et al.*, 2008). The hypoglycemic effect of *Rhizophora mucronata* in experimental animal model has been documented (Ramanathan *et al.*, 2008) but the mechanism of action has not yet been clear. In the present study, we tried to establish indigenous system of medicine (herbal therapy) as anti-diabetic drugs instead of chemical drugs. The mode of action of *Rhizophora mucronata* ethanol extract in the treatment of diabetes was investigated.

Materials and methods

Plant Materials and Preparation of test samples

Fresh leaves of *Rhizophora mucronata* were collected from the Botanical garden, Dhaka, Bangladesh. The plant was identified by the Bangladesh National Herbarium, Dhaka and the specimens were stored in there for the further reference (Accession No. DACK-34179).

The collected leaves of *Rhizophora mucronata* were washed with water thoroughly. After washing, the fresh leaves were air dried and then oven dried at 40°C temperature. The dried leaves were then grinded to make powder, which were then screened to get fine powder. The powder was then soaked in 80% ethanol. These suspensions were filtered with thin and clean cloth and then filtered by filter paper. The suspensions were evaporated by Rotary evaporator

(Bibby RE-200, Sterilin Ltd., UK) at 68°C. In this case, 175mbar (to remove ethanol), 72mbar (to remove water) pressure and 160rpm rotation speed were maintained constantly. Finally, freeze-drier (HETOSICC, Heto Lab Equipment, Denmark) was used to get complete extract from the gummy extract and preserved at +4°c.

Experimental Animals

The study was conducted with adult male Long-Evans rats (weighing 110±15g). They were bred at the BIRDEM animal house and in the Pharmacology laboratory of Department of Pharmacy, North South University, maintained at a constant room temperature of 22±5°C, 40-70% humidity conditions and the natural day-night cycle with an *ad libitum* access to food except the day of experimental procedure when animals were used after 12hrs fasting. The influence of circadian rhythms was avoided by starting all experiments at 8.30 a.m.

Effect on sucrose absorption from gastrointestinal tract

Experiments were carried out on normal rats. Extracts of *Rhizophora mucronata* were fed to the rats by using a syringe (3ml) with a metallic tube that was smooth and curved at the end, which led the feed directly to the stomach. Rats were fasted for 12 h before receiving a 50% sucrose solution by gavage (2·5 g/kg body weight) with (for experimental) or without (for control) ethanolic extract of *Rhizophora mucronata* Linn (0·5 g/kg body weight). Blood samples were collected by amputation of the tail tip under mild diethyl ether anesthesia (Mamun et al, 2001). Blood samples were collected at 30 min before sucrose load and at 30, 60, 180 and 360 min after sucrose administration to determine the glucose level. Finally rats were sacrificed to collect the gastrointestinal tract. The gastrointestinal tract was excised and divided into 6 segments: the stomach, the upper 20 cm, middle, and lower 20 cm of the small intestine, the cecum, and the large intestine. Each segment was washed out with ice-cold saline, acidified with H_2SO_4 and centrifuged at 3000 rpm (1000 g) for 10 min. The supernatant thus obtained was boiled for 2 h to hydrolyze the sucrose and then neutralized with NaOH. The blood glucose level and the amount of glucose liberated from residual sucrose in the gastrointestinal tract were measured. Then the gastrointestinal sucrose content was calculated from the amount of liberated glucose (Goto *et al*., 1995). Glucose was measured by glucose-oxidase (GOD-PAP) method.

Effects on intestinal glucose absorption

An intestinal perfusion technique (Swintosky and Pogonowska-Wala, 1982) was used to study the effects of *Rhizophora mucronata* extracts on intestinal absorption of glucose in rats fasted for 36 hours and anesthetized with sodium pentobarbital (50 mg/kg). The plant extracts were added to a kreb's solution (g/L 1.02 $CaCl_2$, 7.37 NaCl, 0.20 KCl, 0.065 $NaH_2PO_4.6H_2O$, 0.6 $NaHCO_3$, pH 7.4), supplemented with glucose (54.0 g/L) and perfused at a perfusion rate of 0.5 mL/min for 30 min through the duodenum. The perfusate was collected from a catheter set at 40 cm. *Rhizophora mucronata* extracts were added to Kreb's solution to a final conc. of 25 mg/mL so that the amount of extract in the perfused intestine is equivalent to the dose of 1.25 g/kg. The control group was perfused only with Kreb's buffer supplemented with glucose. The results were expressed as percentage of absorbed glucose, calculated from the amount of glucose in solution before and after the perfusion.

Biochemical procedure

Serum glucose levels were estimated by glucose oxidase (GOD/POD) method (Sera Pak, USA). The absorbance was measured by microplate ELISA Reader (Bio-Tek EL-340, USA).

Statistical Analysis

Data from the experiments were analyzed using the Statistical Package for Social Science(SPSS) software for windows version 17 (SPSS Inc., Chicago, Illinois, USA). All the data were expressed as Mean ± SD. Statistical analysis of the results were performed by using one-way analysis of variance (ANOVA) followed by Dunnett's t-test for comparisons. The limit of significance was set at $p<0.05$.

Results

Effect on sucrose absorption from gastrointestinal tract

The six-segment study was performed to assess the amount of sucrose remaining in the GIT at six different positions. The various data for sucrose absorption in the gastrointestinal tract are presented in the Fig. 1. The amount sucrose unabsorbed in different GIT segments showed that in control rats vs. rats fed with 500mg/kg extract at 30, 60, 180, and 360 mins in mmol/l were 0.120 vs. 0.135, 0.038 vs. 0.083, 0.003 vs. 0.004 and 0.004 vs. 0.003 respectively. The data revealed that the extract had glucose absorption and abating tendency. In most of the cases it was found that the glucose absorption increased for the initial experimental times and after a certain period, it showed a gradual reduction of absorption.

Effect on intestinal glucose absorption

As shown in Fig. 2, intestinal glucose absorption in non-diabetic rats was nearly constant during 30 min of perfusion. Addition of *Rhizophora mucronata* to the glucose perfusate resulted an increase in intestinal glucose absorption at 15 min which were gradually decreased from 20 min to end of the experiment. The extract 250mg/kg showed significantly ($p<0.05$) better result than the extract 500mg/kg at 15 min. The percent inhibition of glucose absorption is showed in Fig. 3.

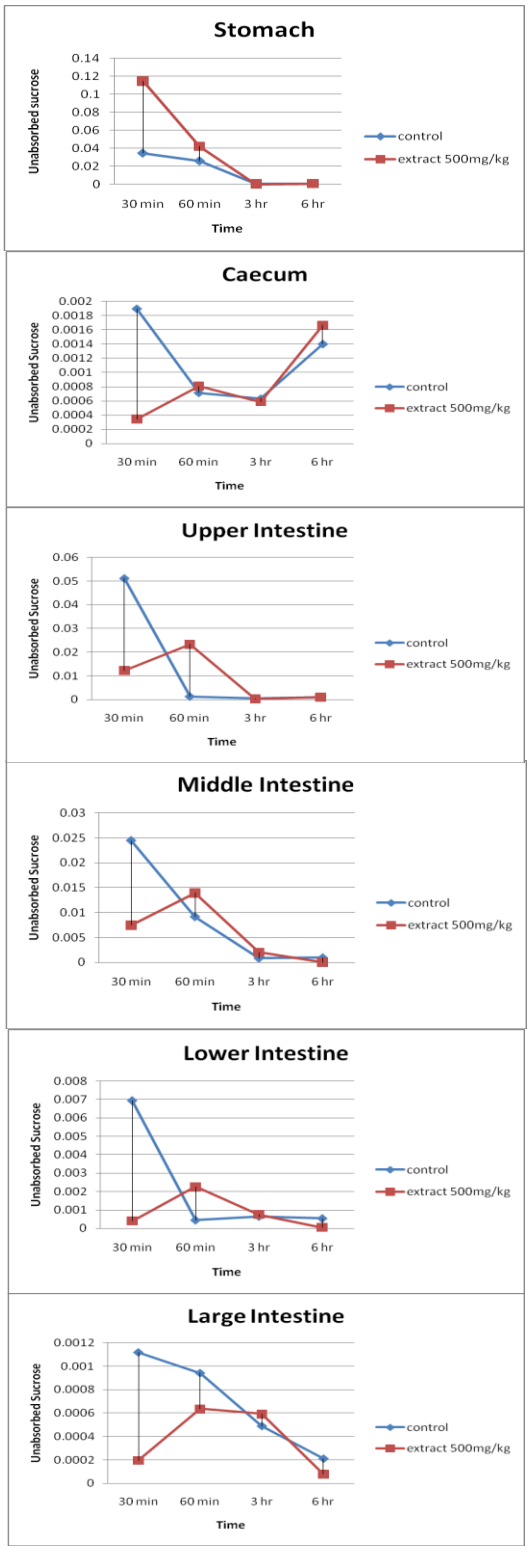


Fig. 1. Graph comparing the total sucrose content in the whole gastrointestinal tract at 30 minutes, 60 minutes, 180minutes, and 360minutes in a group of control rats vs. rats given a gavage with *Rhizophora mucronata* extract.

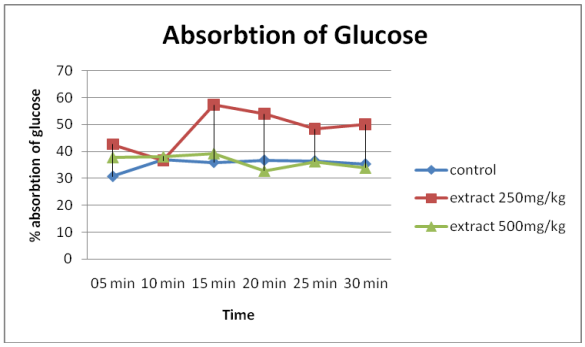
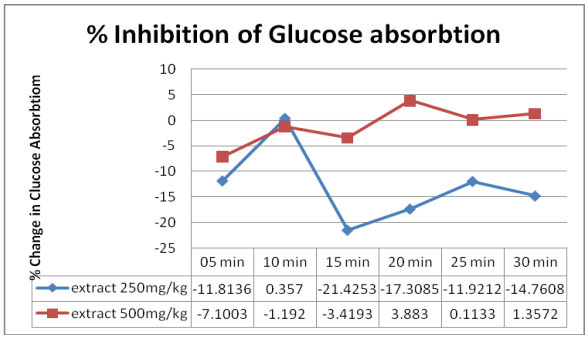


Fig. 2. Intestinal glucose absorption (%).



	05 min	10 min	15 min	20 min	25 min	30 min
extract 250mg/kg	-11.8136	0.357	-21.4253	-17.3085	-11.9212	-14.7608
extract 500mg/kg	-7.1003	-1.192	-3.4193	3.883	0.1133	1.3572

Fig. 3. Inhibition of glucose absorption (%) in the intestine.

Discussion

The present study was undertaken to investigate the hypo-/antihyperglycemic activity of *Rhizophora mucronata* extracts in nondiabetic rats. Hypoglycemic activity that is found when given with a simultaneous glucose load in diabetic rats indicates that the extracts may interfere with the intestinal glucose absorption in the gut by various mechanisms (Nahar *et al*., 2000; Vinik and Wing, 1990; Lempcke, 1987). It may be postulated that the plant extract might stimulate glycogenesis in the liver, which is enhanced by feeding (Creutzfeld *et al*., 1979). One of the objectives of the present study was to investigate whether the hypoglycemic effect is related to the inhibition of glucose absorption in the gut. This was investigated in gut perfusion experiment where the ethanol extracts showed sudden increase and then a gradual decrease in glucose absorption. Aderibigbe *et al*. (2001) claimed that hypoglycemic effect of some plant extracts were compatible with chlorpropamide (oral hypoglycemic agents) and the action may be parts due to an intestinal reduction of the absorption of glucose.

Rhizophora mucronata has been using as an antidiabetic agent for a long time. Efficacy of this plant in the treatment of diabetes has been studied in details but the mechanism of action has not yet been clear. Since glucose lowering effect of some plants was clearly evident from previous study reports, glucose absorption inhibition could have been a possible mechanism responsible for the hypoglycemic effect (Lembcke, 1987). Our study confirms this effect as well, because when *R. mucornata* ethanolic extract was given along with sucrose solution, it significantly increased sucrose retention in the gut compared with only the sucrose solution in control group of rats. Further the extract also showed significant reduction in glucose absorption in the gut during *in situ* perfusion of small intestine. In both of the cases, it was found that the extract of *R. mucornata* increased glucose absorption for a certain time and after that it decreased which showed that *R. mucornata* ethanol extract has the potency to inhibit glucotoxicity. Similar *in vitro* studies carried out with high concentrations of metformin also showed such inhibition of glucose absorption (Caspary & Creutzfeld, 1971)

In conclusion, the present study demonstrated that the ethanol extracts of *Rhizophora mucronata* showed significant ($p<0.05$) inhibition of carbohydrate digestion and absorption, which has resulted in the well known hypoglycemic effects of *Rhizophora mucronata*.

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