



REVELATION OF MECHANISM OF ACTION OF *RHIZOPHORA MUCRONATA* POIR. BARK EXTRACT FOR ITS ANTIDIABETIC ACTIVITY BY GUT PERFUSION AND SIX SEGMENT METHOD IN LONG EVANS RATS

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ABSTRACT

The aqueous bark extract of *Rhizophora mucronata* Poir. is used in Bangladesh as a hypoglycemic aid without any knowledge about its probable mode of action. It is an effort to assess the claimed hypoglycemic property of the crude drug and to get some knowledge about it. The Hypoglycemic effects were investigated in the ethanol extract of bark of *Rhizophora mucronata* Poir on Long Evans rats. Gut perfusion and six segments studies were carried out to assess these activities. In gut-perfusion study the percentage of glucose absorption in control rats vs. rats fed with 500 mg/kg extracts were observed at 5, 10, 15, 20, 25 and 30 minutes and the significant ($p < 0.05$) absorption result was found which were respectively 30.71 vs. 56.34, 36.87 vs. 71.30, 35.87 vs. 62.11, 36.64 vs. 70.44, 36.36 vs. 64.21, 35.24 vs. 56.32. The percentage drug unabsorbed in GIT was better with 500 mg/kg than 250 mg/kg. The six-segment study was performed to assess the amount of sucrose remaining in the GIT at six different positions. The amount of 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 0.1526 vs. 0.1767 which gradually abating with time dependent manner at 60, 180, and 360 minutes in mmol/l. These results suggests *Rhizophora mucronata* bark has significant dose dependant anti-diabetic effects, which significantly suppressed postprandial hyperglycemia after sucrose ingestion and reversibly increases the unabsorbed sucrose content throughout the gut.

Keyword: *Rhizophora mucronata*, Antidiabetic, Gut perfusion, six segment method.

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. It is a wide spread disorder, which has long been in the history of medicine. It has been estimated that 366 million people worldwide, or 8.3% of adults, are estimated to have diabetes in 2011. Prevalence of diabetes in adults worldwide was estimated to be 4.0% in 1995 and will rise to 5.4% by the year 2025. It is higher in developed than in developing countries. The number of adults with diabetes in the world will rise from 135 million in 1995 to 300 million in the year 2025. The major part of this numerical increase will occur in developing countries. There will be a 42% increase, from 51 to 72 million, in the developed countries and a 170% increase, from 84 to 228 million, in the developing countries. Thus, by the year 2025, >75% of people with diabetes will reside in developing countries, as compared with 62% in regions with greatest potential are Asia and Africa¹, where the rate of diabetes could rise to 2–3-fold compared with the present rates (American Diabetes Association 1997). This explosive increase in the prevalence of diabetes and the consequences of its complications and associated disorders represents the greatest health care challenge facing the world today.² Though biguanides and sulfonylureas are valuable in the treatment of diabetes mellitus, their use is restricted by their limited action, pharmacokinetic properties, secondary failure rates and accompanying side effects³. Moreover, these therapies only partially compensate for metabolic derangements seen in diabetes and do not necessarily correct the fundamental biochemical lesion,⁴ as the incidence of diabetes increases rapidly across the globe there is an urgent need to expand the range of effective palliatives available to sufferers.

Nature has been a source of medicinal treatments for thousands of years, and plants-based systems continue to play an essential role in the primary health care of 80% of the world's underdeveloped and developing countries. Biguanides developed from a prototypic plant molecule is an excellent example of anti-diabetic drug development from plants. Thus, it is prudent in the current context to look for new and if possible more efficacious hits from the vast reserves of phytotherapy. Many herbal medicines have been recommended for the treatment of diabetes. On the other hand, as indicated by Marles & Farnsworth (1995),⁵ not all of the plants reported to be useful are entirely safe, and they emphasize the need for carefully planned scientific research to identify those hypoglycemic plants with true therapeutic efficacy and safety.

Renewed attention in alternative medicines and natural therapies has led to a revived interest in the use of traditional plants for the treatment of diabetes. In this regard the screening of plant materials for hypoglycemic properties is important as it might provide a new lead(s) as antidiabetic agent(s). The plant of our choice is *Rhizophora mucronata* (Family: Rhizophoraceae), which 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 used as an astringent and to treat angina, haemorrhaging (extracts from the seedlings in Indochina); According to Wee, in Chinese and Japanese herbal medicine, a decoction of the bark is used to treat diarrhoea and Diabetes. A poultice of the leaves is used to relief armored fish stings.⁶ Old leaves and roots are used during childbirth (Malay). Bark is used to treat blood in the urine and diabetes (Burma).⁷ It has been used as a therapeutic agent for the treatment of diabetes mellitus.⁸ Though the hypoglycemic effect of *Rhizophora mucronata* in experimental animal model has been documented⁹ but the mechanism of action has not yet

been clear. In the present study, this plant was selected to explore the mechanism of action in Long Evans rat.

MATERIALS AND METHODS

Plant Materials and Preparation of test samples: Fresh barks 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 barks of *Rhizophora mucronata* were washed with water thoroughly. After washing, the fresh barks were air dried and then oven dried at 40°C temperature. The dried barks 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 680C. 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. This study was approved by ethics committee of North south university (Approval No: LSAEC-22F-2012) which gave it consent in absolute accordance with the recommendation of the International association of study of pain.

Effect on sucrose absorption from gastrointestinal tract

Experiments were carried out on normal rats. Extracts of *Rhizophora mucronata* Poir 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 bark of *Rhizophora mucronata* Poir (0.5 g/kg body weight). Blood samples were collected by amputation of the tail tip under mild diethyl ether anesthesia.¹⁰⁻¹¹ 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: 1. the stomach, 2. the upper 20 cm, 3. middle, and 4. lower 20 cm of the small intestine, 5. the cesium, and 6. the large intestine. Each segment was washed out with ice-cold saline, acidified with H₂SO₄ 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.¹² Glucose was measured by glucose-oxidase (GOD-PAP) method using commercial kit (Boeringer Mannheim GmbH kit).

Effects on intestinal glucose absorption

An intestinal perfusion technique¹³ 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 (1.02 CaCl₂, 7.37 NaCl, 0.20 KCl, 0.065 NaH₂PO₄.6H₂O, 0.6 NaHCO₃, g/L at 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 unabsorbed 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 micro plate 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 Figure 1. The amount of sucrose unabsorbed in total GIT is shown in Table 1.

The data revealed that the extract had reduced sucrose absorption in GI Tract. In most of the cases it was found that the glucose absorption slightly increased for the initial experimental times and after a certain period, it showed a gradual reduction of absorption. From result we can deduce that the extract of the bark of *Rhizophora mucronata* Poir was capable of causing a decrease in the absorption of sucrose solution from the gastrointestinal tract.

Table 1: Unabsorbed sucrose in Total GIT

| Time | 30 minute | 60 minute | 180 minute | 360 minute |
|------------------|-----------|-----------|------------|------------|
| Control | 0.1526 | 0.0956 | 0.0034 | 0.0028 |
| Extract 500mg/kg | 0.1767 | 0.0039 | 0.1409 | 0.0696 |

Table 2: The amounts of unabsorbed glucose in gut

| Group | 5 minute | 10 minute | 15 minute | 20 minute | 25 minute | 30 minute |
|----------|------------|-------------|------------|------------|-------------|-------------|
| Control | 30.71±2.99 | 36.87± 1.82 | 35.87±0.92 | 36.64±3.47 | 36.36± 2.58 | 35.24±3.54 |
| 250mg/kg | 41.34±4.72 | 23.96±1.12 | 24.25±0.3 | 25.72±0.96 | 26.86±2.58 | 27.21±3.54 |
| 500mg/kg | 56.34±0.28 | 71.30±0.27 | 62.11±0.24 | 70.44±0.23 | 64.21±0.21 | 56.32 ±0.22 |

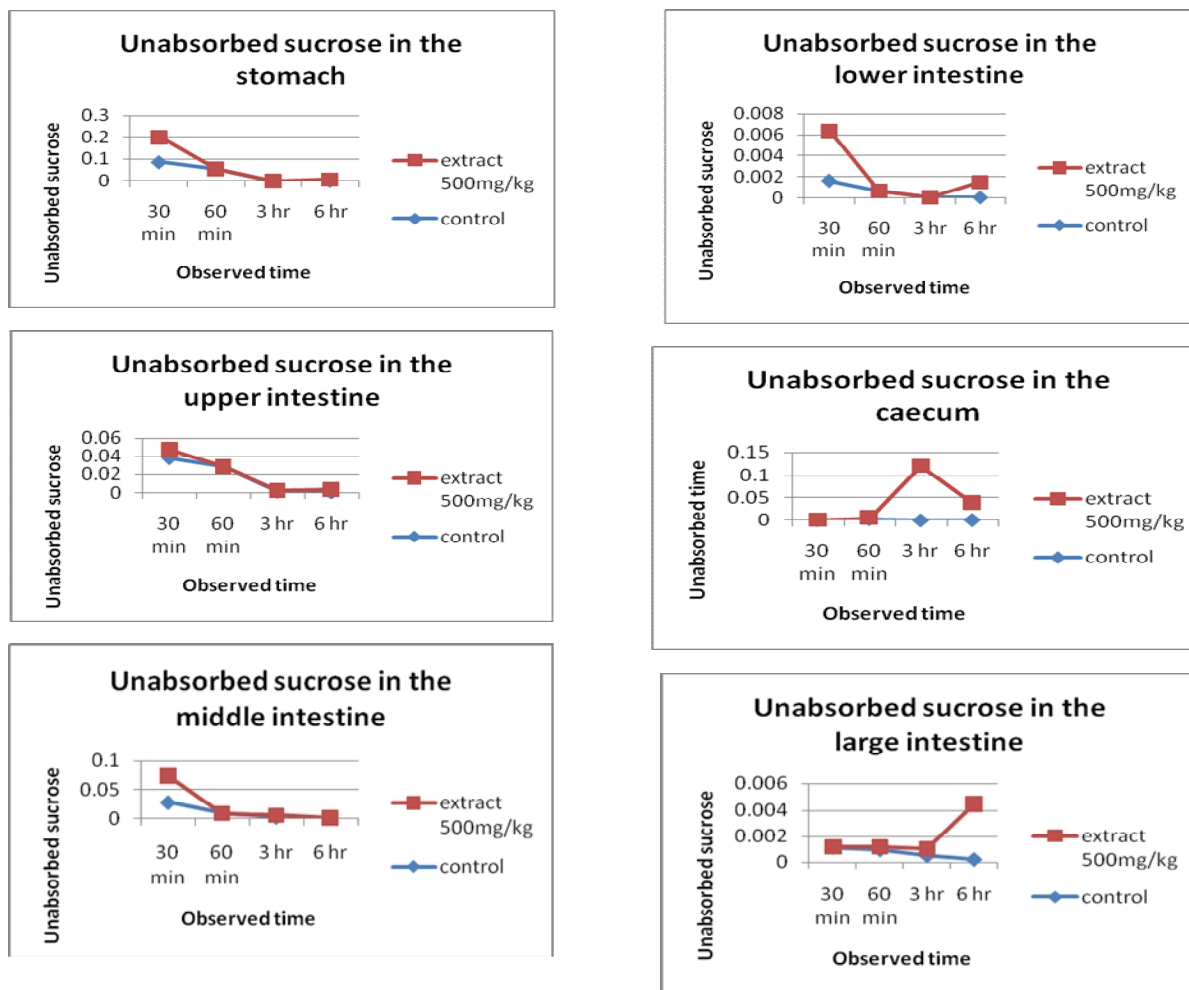


Figure 1: Graph comparing the total sucrose content in the whole gastrointestinal tract at different time interval in a group of control rats vs. rats given a gavage with *Rhizophora mucronata* bark extract

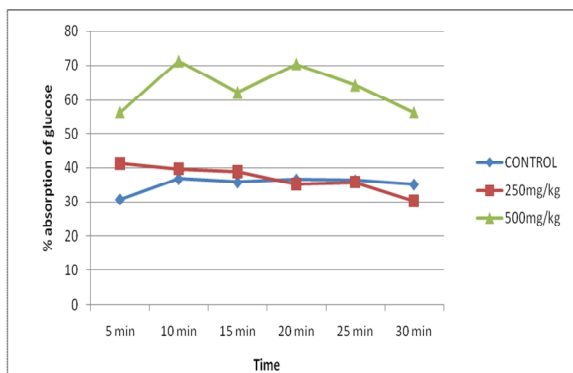


Figure: 2 Percent of unabsorbed glucose in intestine

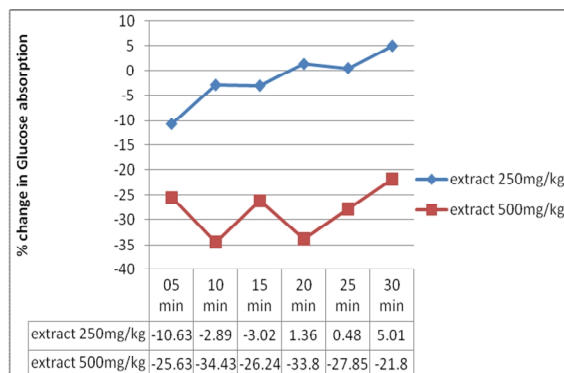


Figure: 3 Inhibition of glucose absorption (%) in intestine

Effect on intestinal glucose absorption

The amounts of unabsorbed glucose in gut are showed in Table 2.

As shown in Figure 2 unabsorbed glucose content (% of absorbed glucose) in non diabetic rat was nearly constant during 30 min of perfusion. Additions of *Rhizophora mucronata* bark extract to the glucose perfusate resulted in increase of unabsorbed glucose content in both 250 mg/ kg and 500 mg /kg extract. In both case glucose absorption gradually increased from 20 minutes. The extract of 500 mg/kg showed significantly ($p<0.005$) better result than extract 250 mg/kg. The percent inhibition of glucose absorption is showed in Figure 3.

DISCUSSION

The present study was undertaken to investigate the antihyperglycemic activity of *Rhizophora mucronata* bark 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 [13]. It may be postulated that the plant extract might stimulate glycogenesis in the liver, which is enhanced by feeding¹⁴. 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.¹⁵ 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. 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 effect¹⁶. Our study confirms this effect as well, because when *Rhizophora mucronata* ethanolic bark 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 *Rhizophora mucronata* decrease glucose absorption for a certain time and after that it shows gradual response which showed that *Rhizophora mucronata* 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 absorption¹⁷.

CONCLUSION

In conclusion, the present study has demonstrated that ethanol extracts of the bark of *Rhizophora mucronata Poir.* showed significant ($p<0.05$) inhibition of carbohydrate digestion and absorption. The plant was traditionally used in the treatment of Diabetes Mellitus, the results obtained from both six-segment method and gut perfusion technique

significantly demonstrates, more conclusively, that the ethanol extract of bark of *Rhizophora mucronata Poir.* can be effective in diabetic treatment. Hopefully this will provide as a lead to carry out further investigation.

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