

Assay of antidiabetic activity of *Hemidesmus indicus* by gut perfusion and six segment methods on Long Evans rats

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Abstract

The present investigation was designed to assay the antidiabetic effect of ethanol root extract of *Hemidesmus indicus* by gut perfusion and six segment methods on Long Evans rats. In the gut perfusion study the glucose absorption in control rats vs. rats fed with 250 mg/kg and 500 mg/kg extracts were observed at 5, 10, 15, 20, 25 and 30 minutes and the significant ($p < 0.05$) change of intestinal glucose absorption was found throughout the experimental time which was 34.96 vs. 29 vs 37.97, 34.29 vs. 28.04 vs. 37.99, 39.69 vs. 42.85 vs. 38.29, 35.69 vs. 30.32 vs. 36.45, 36.98 vs. 30.44 vs. 35.92 and 34.82 vs. 19.44 vs. 30.77 mmol/L respectively. The change of intestinal glucose absorption was found significant with 250 mg/kg than 500 mg/kg root extract of *Hemidesmus indicus*. The six segment study was performed to assess the amount of glucose remaining in the six different positions of the GIT at 30, 60, 180 and 360 minutes. The data revealed that the 500 mg/kg root extract of *Hemidesmus indicus* had gradually reduced the glucose absorption in GIT compared to control throughout the experimental time. These results strongly suggested that ethanol root extract of *Hemidesmus indicus* has significant dose dependent antidiabetic effect.

KEY WORD: DIABETES MELLITUS, HEMIDESMUS INDICUS, GUT PERFUSION, SIX SEGMENT, LONG EVANS RAT

Introduction

Diabetes mellitus (DM) is becoming a pandemic worldwide. The highest percentages of increases in disease prevalence are likely to be in developing nations, with major increases in the Middle-East, Sub-Saharan Africa, South Asia, and Latin America¹. WHO listed 10 countries to have the highest numbers of people with DM in 2000 and 2030. According to this report, Bangladesh has 3.2 million of DM subjects in 2000 and the number is expected to increase to a staggering 11.1 million by 2030 placing her among the top 10 countries with DM¹. Various extensive screening has been performed on the use of traditional medicinal plants for treating DM in many ethnomedical systems within the Indian subcontinent^{2, 3}. However in Bangladesh the traditional medicinal plants that are used for the treatment of DM have not yet been studied in great detail. Therefore these herbal remedies are important objects of research especially in context of the virtually exploding prevalence of DM in Bangladesh.

Hemidesmus indicus (Hi) (Apocynaceae) commonly referred to as anantamool, nannari is a slender, laticiferous and twining shrub occurs over the greater part of Bangladesh. It is widely recognized in folk medicine and as ingredient in Ayurvedic and Unani preparations against various diseases⁴⁻¹⁴. Different scientific investigations were carried out to explore the antidiabetic effect of the root extract of Hi. 2-Hydroxy 4-methoxy benzoic acid (HMBA) isolated from the roots of Hi to evaluate the anti-diabetic activity on Streptozotocin (STZ) induced diabetic rats. It was found that HMBA could help in controlling DM owing to their hypoglycemic and hypocholesterolemic effects¹⁵. Again, the effect of a single dose of Hi root extract on the blood glucose level in alloxan (150 mg/kg body weight, intraperitoneal) induced diabetic rats was evaluated and found that Hi possesses profound beneficial effects in preventing diabetic related abnormalities by lowering the sugar level as normal¹⁶. Moreover, study also suggested that Hi administration not only reduces blood glucose but also offers protection to diabetes-induced metabolic alterations in rats¹⁷.

The present investigation was designed to explore the mechanism of the hypoglycemic effect of the ethanolic root extract of Hi by gut perfusion and six segment methods on Long Evans rat.

Materials and Method

Plant material collection and extraction

The plant Hi was collected from Savar, Dhaka, Bangladesh. The plant was taxonomically identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (Accession no: DCAB-35569) and a voucher specimen was also deposited. The collected plants were separated from undesirable materials or plants or plant parts and these were dried by shade drying for twenty days to ensure the active constituents free from decomposition also to avoid any photochemical degradation. The roots were ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced. About 100 gm of powdered material was taken in a clean, flat-bottomed glass container and soaked in 500 ml of 96% ethanol. The container with its contents was sealed and kept for a period of 8 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through whatman filter paper. The filtrate (ethanol extract) obtained was taken into rotary evaporator to evaporate ethanol. Then this filtrate was taken into beaker, the opening of beaker was wrapped by a sheet of aluminum foil to which perforation was done for evaporation of the rest of the ethanol & was kept in dry & cool place for several days & at last evaporation was done under table fan until dried. It rendered concentrate of deep purple type. The concentrate was designated as crude ethanolic extract.

Experimental Animals

The study was conducted with adult male Long-

Evans rats (weighing 110 ± 15 gm). 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 $22 \pm 5^\circ\text{C}$, 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 12 hour 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.

Drugs and Chemicals

Sodium Pentobarbital (Square Pharmaceuticals Ltd.), Ketamin (Incepta Pharma Ltd.), Sucrose solution. NaOH(1N), H_2SO_4 (2N), Ice-cold saline, 80% ethanol

Effects on intestinal glucose absorption

Gut perfusion technique was used to study the effects of Hi extract on intestinal absorption of glucose in rats fasted for 36 hour and anesthetized with sodium pentobarbital (50 mg/kg)¹⁸. The plant extracts were added to a kreb's solution (1.02 CaCl_2 , 7.37 NaCl, 0.20 KCl, 0.065 $\text{NaH}_2\text{PO}_4 \cdot 6\text{H}_2\text{O}$, 0.6 NaHCO_3 gm/L at pH 7.4), supplemented with glucose (54.0 gm/L) and perfused at a perfusion rate of 0.5 ml/min for 30 minute through the duodenum. The perfusate was collected from a catheter set at 40 cm.

Hi 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 gm/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.

Effect on sucrose absorption from gastrointestinal tract

Experiments were carried out on normal rats. Extracts of Hi were fed to the rats by using a syringe (3 ml) 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 hour before receiving a 50% sucrose solution by gavage (2.5 gm/kg body weight) with (for experimental) or without (for control) ethanolic extract of root of Hi (0.5 gm/kg body weight). Blood samples were collected by amputation of the tail tip under mild diethyl ether anesthesia.¹⁹⁻²⁰ Blood samples were collected at 30 minute before sucrose load and at 30, 60, 180 and 360 minute after sucrose administration to determine the glucose level. Finally rats were sacrificed to collect the gastrointestinal tract. The gastrointestinal tract (GIT) was excised and divided into 6 segments: 1. the stomach, 2. the upper 20 cm, 3. middle, 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 H_2SO_4 and centrifuged at 3000 rpm (1000 gm) for 10 minute. The supernatant thus obtained was boiled for 2 hour 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.²¹ Glucose was measured by glucose-oxidase (GOD-PAP) method using commercial kit (Boeringer Mannheim GmbH kit)²².

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 \pm 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

Effects on intestinal glucose absorption

As shown in Table 1, intestinal glucose absorption is less or amount of unabsorbed glucose is maximum in case of 250 mg/kg extract at 30 minute as compared to the extract 500 mg/kg body weight and as well as the control. The efficacy of the 250 mg/kg extract is larger than the respective 500 mg/kg extract. The lowest absorption was at 30 minute which was 19.44 ± 2.84 mmol/L and 30.77 ± 1.49 mmol/L for 250 mg/kg & 500 mg/kg plant extract and the highest absorption was at 15 minute which was 42.85 ± 4.19 mmol/L and 38.29 ± 2.23 mmol/L for 250 mg/kg & 500 mg/kg plant extract. The percent inhibition of glucose absorption is showed in Figure 2.

Effect on sucrose absorption from gastrointestinal tract

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 different position of gastrointestinal tract are presented in the Table 2. The amount sucrose unabsorbed in GIT showed that in control rats vs. rats fed with 500 mg/kg extract at 30, 60, 180, and 360 minute in mmol/L were 0.120 vs. 0.017, 0.038 vs. 0.065, 0.003 vs. 0.006 and 0.004 vs. 0.002 mmol/L respectively. The data revealed that the extract had gradually reduced the sucrose absorption in GIT. In some of the cases it was found that the sucrose absorption slightly increased for the initial experimental times and after a certain

period, it showed a gradual reduction of absorption.

Discussion

Isolation of pure, pharmacologically active constituents from plants remains a long and tedious process. For this reason, it is necessary to have methods available which eliminate unnecessary separation procedures. Chemical screening is thus performed to allow localization and targeted isolation of new or useful constituents with potential activities. This procedure enables recognition of known metabolites in extracts or at the earliest stages of separation and is thus economically very important.²³⁻²⁴ Previous qualitative phytochemical screening of Hi root extract exhibited the presence of teroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides²⁵.

The flavanoids and tannins have been reported to produce antidiabetic activity²⁶. The present study was undertaken to investigate the antidiabetic activity of Hi root extract in non-diabetic 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 GIT by various mechanisms²⁷. One of the objectives of the study was to investigate whether the hypoglycemic effect is related to the inhibition of glucose absorption in the GIT. From the result we can deduce that the extract of the root of Hi was capable of causing a decrease in the absorption of sucrose solution from the gastrointestinal tract.

This anti-diabetic property can be linked with the ability of the tannins and flavonoids reported in the root extract to inhibit α -glucosidase enzyme. One possible mechanism of the plant extract in inducing hypoglycemia could be by inciting inhibition of α -glucosidase action. The extract retards the digestion and absorption of carbohydrates in the small intestine and hence reduces the increase in blood-glucose concentrations after a carbohydrate load²⁸⁻²⁹.

Another postulation can be made related to the

mechanism of Acarbose. This antidiabetic action may be the results from a competitive, reversible inhibition of pancreatic alpha-amylase enzymes. Pancreatic alpha-amylase hydrolyzes complex starches to oligosaccharides in the lumen of the small intestine. In laboratory animals this enzyme inhibition results in a delayed glucose absorption and a lowering of hyperglycemia. However this hypothesis requires further investigation for validation.

Conclusion

The present investigation showed that ethanol root extract of the Hi significantly ($p < 0.05$) inhibited carbohydrate digestion and absorption in Long Evans rats. The plant was traditionally used in the treatment of DM. The results obtained from both gut perfusion and six-segment method significantly demonstrated more conclusively that the ethanol root extract of the Hi can be effective in diabetic treatment and a noble mechanism of antidiabetic action can be found out. However further research is required to find out a lead to bring this investigation to the next level.

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Dose	5 minute	10 minute	15 minute	20 minute	25 minute	30 minute
Control	34.96 \pm 2.13	34.29 \pm 3.82	39.69 \pm 3.47	35.69 \pm 4.07	36.98 \pm 1.03	34.82 \pm 4.20
Extract 250 mg/kg	29.00 \pm 0.18	28.04 \pm 0.31	42.85 \pm 4.19	30.32 \pm 0.11	30.44 \pm 0.64	19.44 \pm 2.84
Extract 500 mg/kg	37.97 \pm 5.17	37.99 \pm 3.67	38.29 \pm 2.23	36.45 \pm .096	35.92 \pm 0.89	30.77 \pm 1.49

Table 1: Change of intestinal glucose absorption of the extract of *Hemidesmus indicus*
Values are expressed as value \pm Standard Deviation (SD) (n=6)

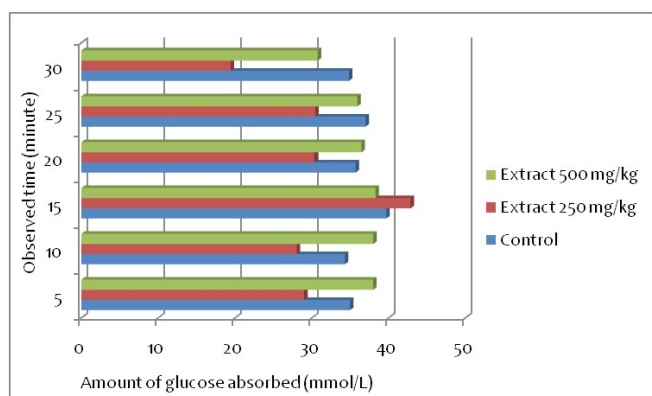


Figure 1: Change of intestinal glucose absorption

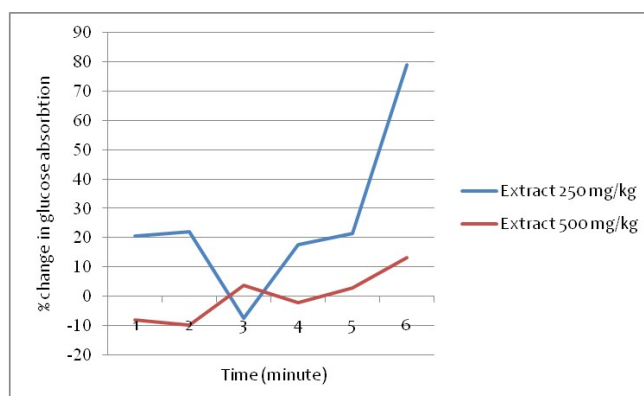


Figure 2: Inhibition of glucose absorption (%) in intestine

Dose	30 minute	60 minute	3 hour	6 hour
Unabsorbed sucrose in the stomach				
Control	0.03458±0.0004	0.02592±0.0001	0.000133722±0.0002	0.00044±0.0002
Extract (500 mg/kg)	0.00132±0.0001	0.04144±0.0002	0.00036936±0.0001	0.00195±0.0001
Unabsorbed sucrose in the upper 20 cm intestine				
Control	0.051059±0.0003	0.00125±0.0001	0.000440154±0.0001	0.00081±0.0001
Extract (500 mg/kg)	0.00076±0.0001	0.01105±0.0001	0.00048564±0.0001	0.0054±0.0001
Unabsorbed sucrose in the middle intestine				
Control	0.02444±0.0001	0.00914±0.0001	0.00081396±0.0001	0.0009±0.0001
Extract (500 mg/kg)	0.00479±0.0001	0.00127±0.0001	0.00208791±0.0001	0.00339±0.0001
Unabsorbed sucrose in the lower 20 cm intestine				
Control	0.00694±0.0001	0.00044±0.0001	0.000641592±0.0001	0.00053±0.0001
Extract (500 mg/kg)	0.00049±0.0001	0.00049±0.0001	0.000613758±0.0001	0.00117±0.0001
Unabsorbed sucrose in the cecum				
Control	0.00189±0.0001	0.00071±0.0001	0.000632016±0.0001	0.0014±0.0001
Extract (500 mg/kg)	0.00695±0.0001	0.00161±0.0001	0.001838592±0.0001	0.00109±0.0001
Unabsorbed sucrose in the large intestine				
Control	0.00112±0.0001	0.00094±0.0001	0.000488376±0.0001	0.00021±0.0001
Extract (500 mg/kg)	0.00065±0.0001	0.00141±0.0001	0.000500346±0.0001	0.00047±0.0001
Total unabsorbed sucrose in the GIT				
Control	0.120031±0.0001	0.038409±0.0001	0.00314982±0.0003	0.00429±0.0003
Extract (500 mg/kg)	0.017954±0.0001	0.065182±0.0004	0.006495606±0.0001	0.00291±0.0001

Table 2: Amount (mmol/L) of unabsorbed sucrose in different parts of the intestine
 Values are expressed as value ± Standard Deviation (SD) (n=4)