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Antinociceptive and Hypoglycemic Activities of *Scindapsus officinalis* (Roxb.) Schott in Laboratory Animals

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Authors' contributions

This work was carried out in collaboration between all authors. Author NF managed the literature searches, performed the experiments and the statistical analysis and wrote the first draft of the manuscript. Author SUH managed the literature searches and performed the experiments. Author JMAH designed the study, wrote the protocol, and supervised the experiments. All authors read and approved the final manuscript

Research Article

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ABSTRACT

Aims: The fruit of Scindapsus *officinalis* is known as Gajapeepal in Ayurveda. The folk lore claim of *S. officinalis* fruits are antidiabetic, anthelmintic, antidiarrhoeal, carminative, expectorant, tonic, antiprotozoal, anticancer, sharpening hearing, cardiotonic and regulating the bowel and appetite. It is also used in dysentery, asthma, troubles of the throat, bronchitis and for many other medical conditions. Hence the present studies were undertaken to highlight the chemical constituents and pharmacological activities of the fruit.
Study Design: In this study, the ethanol extract of *S. officinalis* (EE0SF) was primarily evaluated through phytochemical screening. The compounds found in the fruit are of pharmacological interest which prompted us to focus the research on its possible analgesic and anti-diabetic activity and whether these effects are of any statistical significance.
Place and Duration of Study: The research experiments were conducted in the Pharmacology laboratory of Department of Pharmacy, North South University, Dhaka, Bangladesh. The studies were carried out during July 2013 to January 2013.
Methodology: Qualitative phytochemical tests for the identification of various chemical

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constituents in the fruit extract were carried out with proper reagents. Analgesic potential of the fruit extract was assessed using acetic acid induced writhing response in Swiss albino mice. In this method, acetic acid is injected intraperitoneally to the experimental animals and the response is contraction of the abdominal muscles and the stretching of the hind limps. The fruit was further subjected to anti-diabetic study through six segment method and was investigated for anti-hyperglycemic effects in Long Evans rats.
Results: Phytochemical analysis of ethanolic extract of *S. officinalis* has indicated the presence of steroid, carbohydrate, flavonoid, alkaloid, tanin, saponin and terpenoid-compounds. The analgesic experiment yielded a significant ($P < 0.05$) reduction in writhing at both 250 and 500 mg/kg body weight dose of extract in a dose dependent manner. The extract, at a dose of 500 mg/kg body weight, caused a significant ($p<0.05$) dose dependent inhibition of sucrose absorption in six different segments of the gut and manifested hypoglycemic effects in rats at four different hours.
Conclusion: In conclusion, these observations provide evidence and possible mechanisms of action for the medicinal properties of fruit of *S.officinalis* claimed in Ayurveda medicine.

Keywords: Analgesic; anti-diabetic; anti-hyperglycemic; EESOF; hypoglycemic effect; phytochemical; Scindapsus officinalis; writhing.

1. INTRODUCTION

During the past decade, traditional systems of medicine have become a topic of global importance. Current estimate suggest that, in many developing countries a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs to mitigate unpleasant sensation occurring in varying degrees of severity as a consequence of injury, tissue-damage, diseases- comprehensively termed pain [1].

Type 1 diabetes is defined by an absolute requirement for administration of exogenous insulin, results from the autoimmune destruction of the insulin-secreting pancreatic β cells. Type 1 diabetes is a severe form associated with ketosis in the untreated state. It arises most commonly in juveniles but occasionally in non-obese adults and elderly. Type 2 or non-insulin-dependent diabetes mellitus is characterized by a relative insulin deficiency due to predominantly an insulin secretory defect with insulin resistance.

In traditional practice medicinal plants are used in many countries to control diabetes mellitus. The hypoglycemic activity of these medicinal plants is being studied. Plant drugs are frequently considered to be less toxic and more free from side effects than synthetic ones

Scindapsus officinalis [Roxb.] Schott (Aracea) is a monocotyledonous medicinal climber (growing along the sub-Himalayan tract in West Bengal, Andhra Pradesh and the Andaman Islands) which has endowed with curative properties against a variety of illness. The folk lore claim of *S.officinalis* fruits are anti-diabetic, anthelmintic, aphrodisiac, galactagogue, stimulant, diaphoretic, antidiarrhoeal, carminative, expectorant, tonic, antiprotozoal, anticancer, sharpening hearing, aphrodisiac, cardio tonic and regulating the bowel and appetite. It is also used in dysentery, asthma, troubles of the throat, rheumatism, asthma, worm infestations, pharyngopathy, helminthiasis and bronchitis [2].

Various pharmacological activities on fruit part of plant (like antioxidant, anti-inflammatory, analgesic, antihistaminic, antibacterial) have been scientifically reported.

Hence, the present studies were undertaken to find out analgesic efficacy and possible anti-diabetic activity of ethanolic extract of *S. officinalis* fruit using- acetic acid induced writhing in mice and six segment method in long Evans rat respectively.

2. MATERIALS AND METHODS

Hot Plate (Model – 35100, UGO BASILE, ITALY), Electronic Balance (Ohous manufacturer, Canada) , Refrigerator(Butterfly Marketing Ltd, LG), Beakers, Petri dishes & glass wrought, Safety rat handling gloves, Mortar & pestle, Hypodermic Syringes, Holder & test tube, Rotary evaporator (Eyela n 1000, Tokyo Rikaki Kai Co. Ltd Rotary vacuum, Japan), Glucose kit, Vortex, Centrifuge, Homogenizer, Screw-cap test tubes, Surgical apparatus (forceps, scissors), Micropipette, Incubator, UV-Spectrophotometer, Sonicator.

2.1 Medicinal Plant (Extract)

Extract was examined in two concentrations of 250 and 500mg/kg body weight of animal.

2.2 Reagent, Control & Positive Control

2.2.1 Phytochemical study

Reagents and chemicals- Wagner Reagent, concentrated HCL, 0.1% Ferric chloride, Molish reagent, conc. H_2SO_4, α-napthol, chloroform.

2.2.2. Analgesic activity

1. Control – Distilled water.
2. Positive control – Diclofenac sodium (Beximco Pharmaceuticals Ltd. Bangladesh) Administered dose – 50mg/kg body weight animal.

2.2.3 Anti-diabetic activity

1. Control- Sucrose solution.
2. Reagents- NaOH(1N), H_2SO_4(2N), Ketamine, Ice-cold saline, 80% ethanol.

2.3 Plant Extraction Method

2.3.1 Collection

The plant sample of *S. officinalis* was collected from an Ayurvedic Institution 'Back to Nature' on June 18, 2012 in the form of fruit shavings. The fruit of the plant was procured and cleansed with water several times to rinse away dirt and undesirable materials.

2.3.2 Drying and grinding

The collected fruit was washed with water, separated from undesirable materials and plant parts, partially dried by fan aeration and then fully dried in the oven at below 40ºC for 2 days. The fully dried fruit was then grinded and coarsely powdered. The freshly dried powdered form weighed 1000g and was stored in the refrigerator at +4ºC for a few days.

2.3.3 Cold extraction (Ethanol extraction)

542g of powdered plant material was then dissolved in 80% ethanol. The alcoholic mixture was sealed in a conical flask and placed on a shaker for 2 days period to receive occasional shaking and stirring. Subsequently, the homogenized mixture was subjected to a coarse filtration by a piece of clean, white cotton material. The primary filtrate underwent a second phase of filtration through whatman filter paper. The filtrate (Ethanol extract) obtained was evaporated by Rotary evaporator (Eyela n 1000, Tokyo Rikaki kai co.ltd, Rotary vacuum, Japan) at 4 to 5 rpm and at 65ºc temperature. It rendered a gummy concentrate of dark brown color, designated as the crude extract of *S. officinalis*. Then the crude extract was dried by freeze drier and preserved at 4ºC. The preserved extract was later subjected to biological screening and pharmacological experiments.

2.4 Phytochemical Analysis

2.4.1 Study design

Qualitative phytochemical tests for the identification of alkaloids, flavonoids, steroids, gum and carbohydrates, reducing sugar, saponins, tannin and terpenoids were carried out for the plant extract by the method described by Harborne and Sazada [3,4]. The freshly prepared extract of *S. officinalis* was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Wagner reagent, flavonoids with the use of conc. HCl, tannins with 0.1% ferric chloride, and saponins with ability to produce suds. Gum was tested using Molish reagents and concentrated sulfuric acid, steroids with sulfuric acid, reducing sugar with the use of ά-napthol and sulfuric acid and terpenoids with chloroform and conc. HCl [5].

2.5 Analgesic Activity

2.5.1 Mice screening

Young Swiss-albino mice aged 4-5 weeks, average weight 20-30 gram were used for these studies. They were kept in standard environmental condition for one week in the animal house of the Department of Pharmacy, North south University, Bangladesh for adaptation after their purchase. The animals were provided with standard laboratory food and tap water

ad libitum and maintained at natural day night cycle. Experimental animals were randomly selected and divided into four groups denoted as group-I, group-II, group-III, group-IV consisting of 6 mice in each group. Individual weighing was done to adjust individual doses.

Here, distilled water was given to group I, 50 mg/kg Diclofenac sodium was given to group II, 250 mg/kg and 500mg/kg body weight of the crude extract of *S. officinalis* were given to group III and IV respectively.

2.5.2 Acetic acid induced writhing test in mice

The analgesic activity of the samples was evaluated using acetic acid induced writhing method in mice. In this method, acetic acid is administered intraperitoneally to the experimental animals to create pain sensation. As a positive control, any standard NSAID drug can be used. In the present study Diclofenac sodium was used to serve the purpose. The plant extract was administered orally in two different doses, 250 and 500 mg/kg body weight of animal, to Swiss Albino mice after an overnight fast. Test samples and vehicle were administered orally 30 minutes prior to intraperitoneal administration of 0.7% v/v acetic acid solution (0.1ml/10g) but Diclofenac sodium was administered 15 minutes prior to acetic acid injection. Then the animals were placed in individual beakers for observation. Each mouse of all groups were observed individually for counting the number of writhing they made in 15 minutes commencing just 5 minutes after the intraperitoneal administration of acetic acid solution.

Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly, two half-writhing were taken as one full writhing. The number of writhes in each treated group was compared to that of a control group while Diclofenac sodium (50 mg/kg) was used as a reference substance (positive control).

2.6 Antidiabetic Activity

2.6.1 Experimental animal

Both genders of Long Evans rats (*Rattus norvigicus*) were selected for the present study and acclimatized under standard conditions. Long Evans rats (male and female), weighing 80-200g of either sex were collected from ICDDRB for the study and were kept in standard environmental condition for weeks in the animal house of the Department of Pharmacy, North south University, Bangladesh for adaptation after their purchase. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature of 25.0 ± 2ºC, and 12 hrs light dark cycle). The animals were fed with standard diet (pellets) and had free access to filtered water [6].

2.6.2 Assessment of anti-diabetic activity in different segments of GIT

Plant extract (500mg/kg) along with sucrose solution (2.5g/kg body weight) were administered orally to 24 hours fasted rats. Control group was given equal volume of sucrose only. Ketamine hydrochloride was injected intraperitonially 15 minutes prior to dissection of rats of each hour (30min, 1hr, 2hr & 4hr) to elicit acute anesthetic effect and eventually death. For 30, 60, 180 and 360 minutes respective rats were sacrificed. After sacrificing, the whole GIT was excised into six segments. The segments being – (A) Stomach, (B) Upper 20 cm of small intestine, (C) Middle part of small intestince, (D) Lower

20 cm of small intestine, (E) Caecum and (F) Large intestine. Each segment was then washed with 10 ml of ice cold saline. The solution was then centrifuged for 15 minutes at 3000 rpm. The supernatant was then collected and to this solution, 2N H_2SO_4 (2ml) was added to acidify the solution. These mixtures were then boiled for 2 hours in paraffin oil to hydrolyse the sucrose. After 2 hours, to these mixtures, 1N NaOH was added drop by drop to neutralize the mixture and the pH was set at 6.9-7.

Then the concentration of glucose was obtained by the use of GOD-PAP method and ELISA reader. Blood glucose and the amount of glucose liberated from residual sucrose in the gastrointestinal tract were measured. The gastrointestinal sucrose content was calculated from the amount of liberated glucose [7].

2.6.2.1 Calcution of sucrose from glucose:

Amount of sucrose in certain volume = **C x V x 0.342**
Here, **C=** Conc. Of glucose (mmol/ l).
V= Total volume of solution.

2.7 Acute Toxicity

Oral administration of graded doses (250 & 500mg/kg) of the ethanol extract of *S. officinalis* to rats and mice did not produce any significant changes in behaviour, breathing, cutaneous effects, sensory nervous or systemic responses during the observation period. No mortality was recorded in any group after 24h of administering the extract to the animals.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Phytochemical screening of the ethanolic extract of *S. officinalis* fruit revealed the presence of various bioactive components such as tannins, flavonoids, saponins, gums, steroids, alkaloids, reducing sugar and terpenoids. The result of phytochemical test has been summarized in Table 1.

Table 1. Result of Phytochemical Screening of Plant Extract

S. officinalis Extract	**Tannins**	**Saponins**	**Flavinoids**	**Gums& Carbohy-drates**	**Alkaloids**	**Reducing Sugars**	**Terpenoids**
80% ethanol	+++	++	++	+++	+++	+++	+++

Symbol (+) indicates presence of phytochemicals

3.2 Analgesic Activity (Acetic acid Induced Writhing)

Table 2 shows the effects of the extract of *S. officinalis* on acetic acid-induced writhing in mice. Both doses of the plant extract showed significant reduction ($p<0.05$) of writhing induced by the acetic acid after oral administration in a dose dependant manner.

Table 2. Analgesic activity for *S. officinalis* using acetic acid induced writhing method

Treatment	Total writhing counts					Mean± SEM	% Inhibition
Control	14	17	20	18	14	16.60±1.16619	
Standard	9	8	6	6	7	7.20±.58310***	**56.62%**
Scindapsus 250 mg/kg	6	8	3	6	9	6.90±1.02956***	**58.00%**
Scindapsus 500 mg/kg	**8**	**7**	**5**	**5**	**9**	**6.75±.67823***	**59.33%**

*Values in the results are expressed as mean ± SEM., Data was analyzed using one-way ANOVA followed by Dunnett's t-test.The results obtained were compared with the vehicle control group. The P values *P<.05, **P< .01 and ***P< .001 were considered as statistically significant.*

After oral administration of two different doses- 250 and 500 mg/kg body weight, the percent inhibition was 58.00% & 59.33% respectively. The reference drug diclofenac sodium was found less potent than the plant extract at all of the dose levels. Data further illustrated in Figs. 1, 2 and 3.

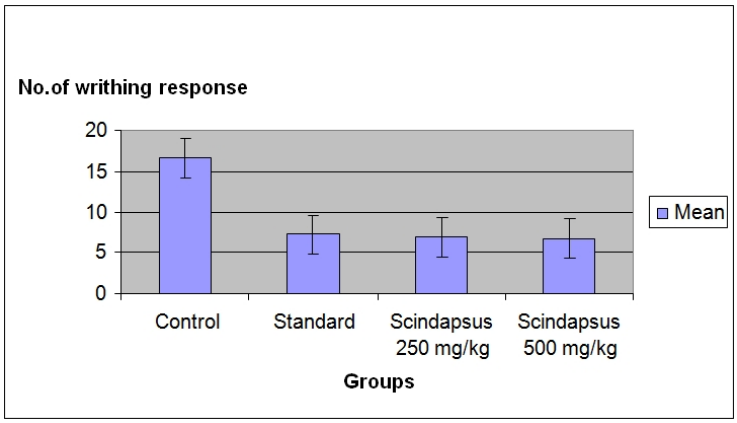

Fig. 1. Analgesic activity of *S. officinalis* in acetic acid induced writhing

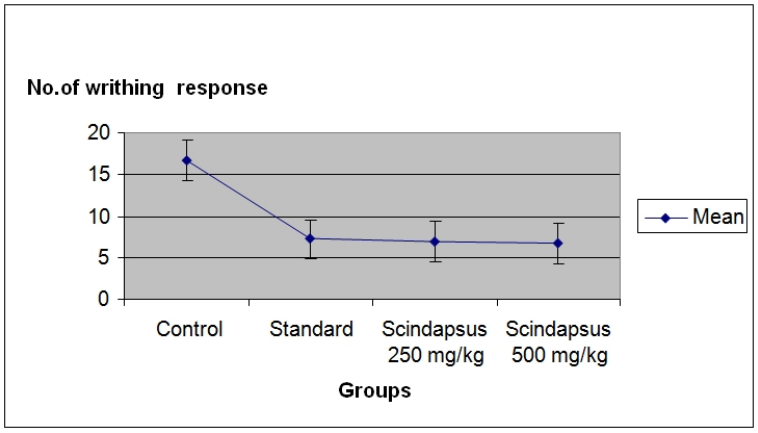

Fig. 2. Analgesic activity of *S. officinalis* in acetic acid induced writhing

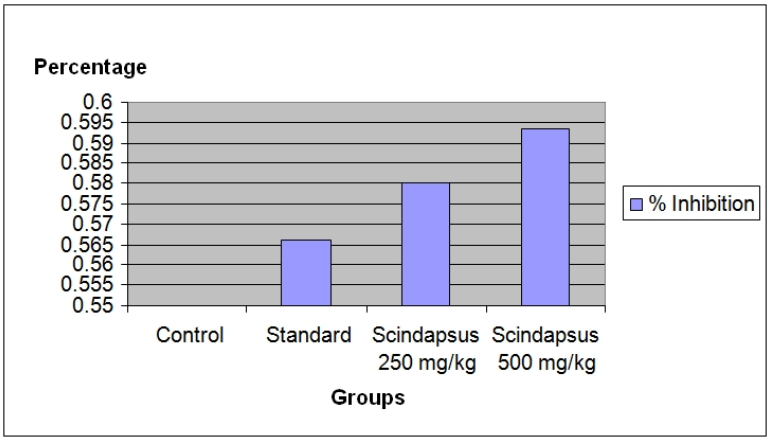

Fig. 3. % Inhibition of *S. officinalis* in acetic acid induced writhing

3.3 Effects on sucrose absorption from gastrointestinal tract

In six segment method, sucrose and extract solution were administered to the model rats, and water and sucrose were administered to the control. Then after 30 minutes, 60 minutes, 120 minutes and 240 minutes the rats were sacrificed to observe the amount of sucrose remaining in the gastrointestinal tract. Results are expressed as (mean value± SD) in mg. Administration of ethanolic extract of *S. officinalis* (0·5 g/kg) with sucrose load in rats increased the residual intestinal sucrose content significantly (P<0·05). The total sucrose content remaining in the gastrointestinal tract was increased in *S. officinalis* extract treated rats compared with normal controls.

From Tables 3-9 and Figs. 4-10 we can deduce that the extract of the fruit of ***S.officinalis*** was able to cause a decrease in the absorption of sucrose solution from the gastrointestinal tract and elicit hypoglycemia.

Table 3. Anti-diabetic activity of *S. officinalis* (500mg) in Upper Intestine

Group	30mins	1hr	2hrs	4hrs
Control	23.19±1.68	17.21±1.17	12.71±1.06	6.28±1.35
Scindapsus500mg	50.68±1.89	37.31±2.31	24.75±1.59	14.86±1.64

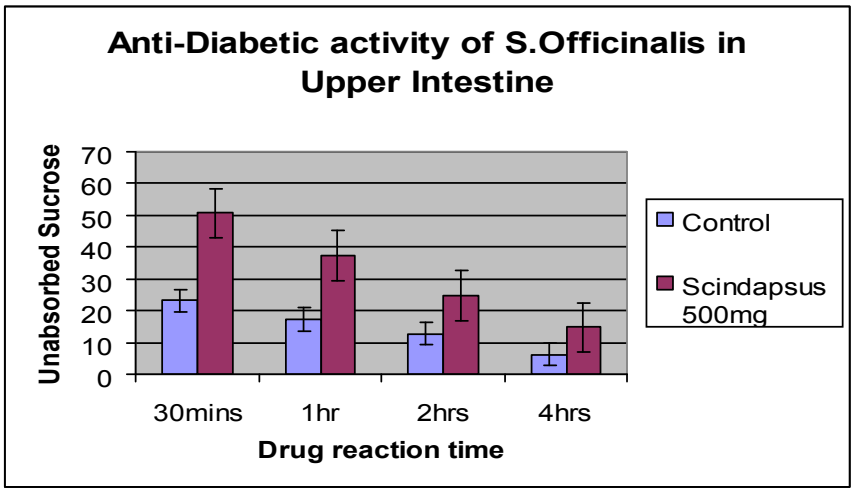

Fig. 4. Anti-diabetic activity of *S. officinalis* in Upper Intestine

Table 4. Anti-diabetic activity of *S. officinalis* (500mg) in Middle Intestine

Group	30mins	1hr	2hrs	4hrs
Control	18.98±.97	13.00±.52	10.02±.70	4.66±.29
Scindapsus500mg	30.81±1.18	18.94±1.08	15.77±1.21	12.12±.71

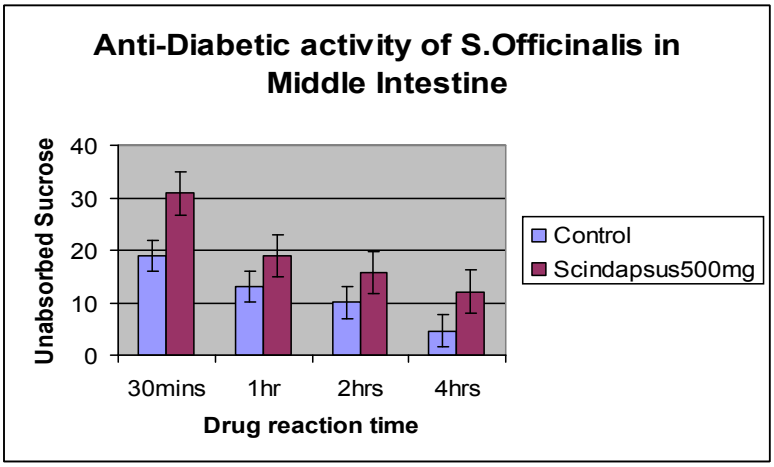

Fig. 5. Anti-diabetic activity of *S. officinalis* in Middle Intestine

Table 5. Anti-diabetic activity of *S. officinalis* (500mg) in Lower Intestine

Group	30mins	1hr	2hrs	4hrs
Control	7.75±.96	16.33±1.18	10.34±.56	5.91±.51
Scindapsus500mg	9.61±.33	19.37±.68	14.64±1.03	10.08±.86

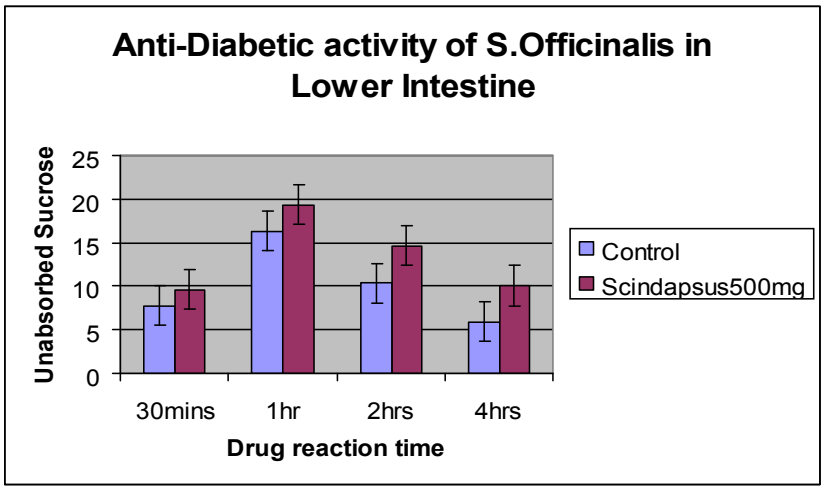

Fig. 6. Anti-diabetic activity of *S. officinalis* in Lower Intestine

Table 6. Anti-diabetic activity of *S. officinalis* (500mg) in Stomach

Group	30mins	1hr	2hrs	4hrs
Control	31.08±1.93	25.53±1.52	15.75±.61	6.32±1.73
Scindapsus500mg	62.46±2.72	47.56±1.30	36.03±1.46	18.54±2.25

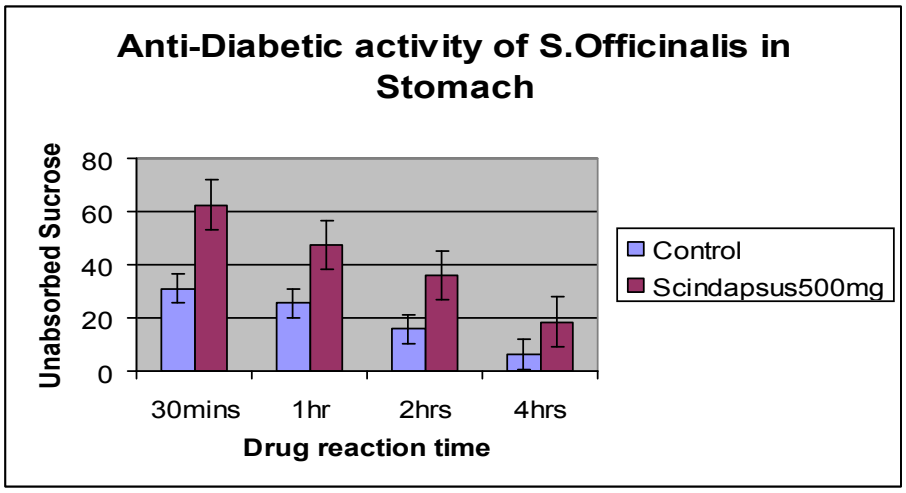

Fig. 7. Anti-diabetic activity of *S. officinalis* in Stomach

Table 7. Anti-diabetic activity of *S. officinalis* (500mg) in Cecum

Group	30mins	1hr	2hrs	4hrs
Control	7.90±.43	20.47±.91	13.91±.99	7.08±.48
Scindapsus500mg	8.77±.63	22.60±1.00	22.29±1.14	9.25±.41

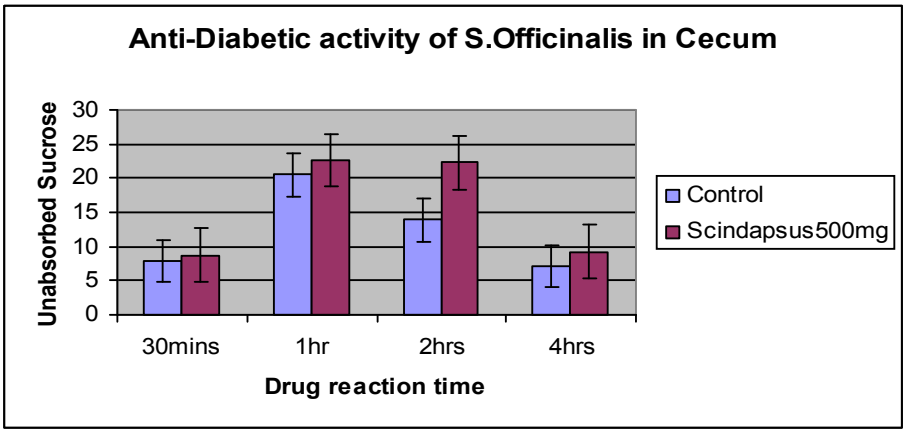

Fig. 8. Anti-diabetic activity of *S. officinalis* in Cecum

Table 8. Anti-diabetic activity of *S. officinalis* (500mg) in Large Intestine

Group	30mins	1hr	2hrs	4hrs
Control	7.74±.963	16.33±1.18	10.33±.560	5.91±.511
Scindapsus500mg	9.60±.334	19.37±.679	14.63±1.03	10.33±.653

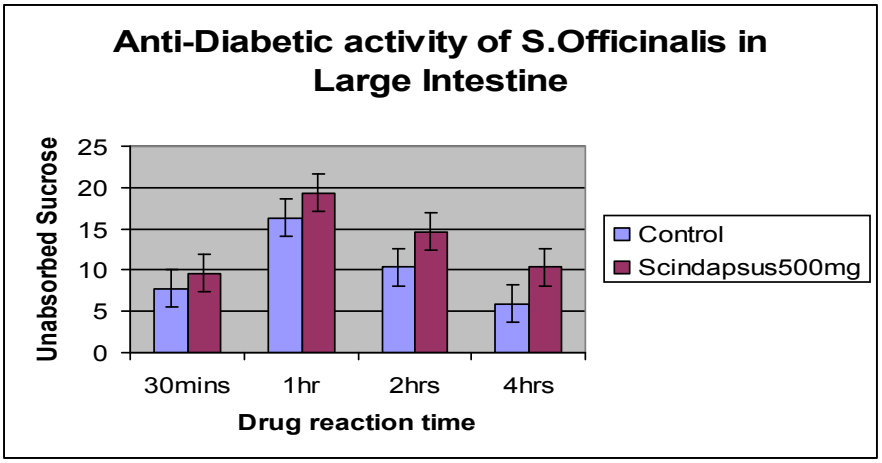

Fig. 9. Anti-diabetic activity of *S. officinalis* in Large Intestine

Table 9. Anti-diabetic activity of *S. officinalis* (500mg) in Total GIT

Group	30mins	1hr	2hrs	4hrs
Control	92.33±2.58	99.81±5.24	75.05±1.81	41.07±6.43
Scindapsus500mg	175.62±9.82**	142.52±10.74**	131.38±3.26***	74.79±2.01***

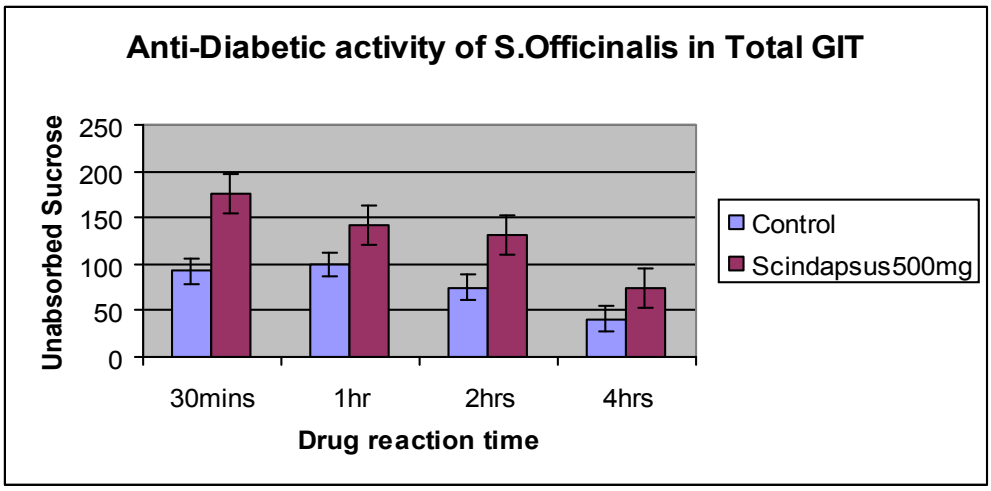

Fig. 10. Anti-diabetic activity of *S. officinalis* in Total GIT

3.4 Statistical Analysis

All the results were expressed as Mean ± Standard deviation (SD). Data was analyzed using one-way ANOVA followed by Dunnett's t-test. The results obtained were compared with the vehicle control group. The P values P<.05, P<.01 and P<.001 were considered as statistically significant. The confidence interval is 95%.All the statistical tests were carried out using SPSS statistical software.

4. 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 [8,9]

Preliminary qualitative phytochemical screening of *S. officinalis* fruit extract exhibited the presence of alkaloids, carbohydrates and gums, flavonoids, reducing sugars, saponin and terpenoids. Therefore it is assumed that these compounds may be responsible for the observed analgesic activity. Flavonoids were reported to have a role in analgesic activity primarily by targeting prostaglandins [10,11]. There are also reports on the role of tannins in anti-nociceptive activity [12]. Besides, alkaloids are well known for their ability to inhibit pain perception [13]. Flavonoids and other phenolic compounds of plant origin have been reported as antioxidants and as scavengers of free radicals. Antioxidants can also exert anti-inflammatory effects [14]. The flavanoids and tannins have been reported to produce anti-diabetic activity [15].

Acetic acid induced writhing in mice attributed visceral pain finds much attention of screening analgesic drugs [16]. The ethanol extract of the *S. officinalis* plant showed significant analgesic action ($p<0.01$ and $p<0.001$) compared to the reference drug diclofenac sodium

against acetic acid induced pain in mice at two dose levels i.e. 250 & 500 mg/kg body weight. Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting release of free arachidonic acid from tissue phospholipid [17] via cyclooxygenase (COX), and prostaglandin biosynthesis [18]. In other words, the acetic acid induced writhing has been associated with increased level of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products [19]. The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability. The acetic acid induced writhing method was found effective to evaluate peripherally active analgesics. The agent reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition. The significant pain reduction of the plant extract might be due to the presence of analgesic principles acting with the prostaglandin pathways.

The present study was undertaken to investigate the hypo-/antihyperglycemic activity of *S.officinalis* 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 GI by various mechanisms [20]. One of the objectives of the study was to investigate whether the hypoglycemic effect is related to the inhibition of glucose absorption in the GI. From the result we can deduce that the extract of the fruit of *S.officinalis* 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 polyphenolic tannins and flavonoids (present in the fruit 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 load [21,22].However this hypothesis requires further investigation for validation. Beside small intestine, the fruit extract caused increased sucrose retention in stomach, large intestine and cecum eliciting significant decrease in disaccharide absorption all throughout the gastrointestinal tract. The ethanolic extract showed significant dose dependent inhibition of carbohydrate absorption in sucrose fed rats compared with the controls.

5. CONCLUSION

It has been reported that a number of flavonoids possess anti-inflammatory and analgesic activities. Flavonoids are known to inhibit the enzyme prostaglandin synthetase, more specifically the endoperoxidase. Prostaglandins are involved in the pain perception and inhibition of their synthesis might be possible reason for the analgesic activity of the ethanolic extract of the fruit [23,24].

Since glucose lowering effect of *S. officinalis* was clearly evident from previous study reports and claims, glucose absorption inhibition could have been a possible mechanism responsible for the hypoglycemic effect [25,26]. Our study confirms this effect, because when ethanolic extract of *S. officinalis* was given along with sucrose solution, it significantly increased sucrose retention in the gut compared to only sucrose solution fed control group of rats.

In conclusion, the present study demonstrated that the ehtanolic extract of *S. officinalis* elicited significant pain reduction and inhibition of carbohydrate digestion and absorption, which has resulted in the well-known analgesic and hypoglycemic effects of the fruit extract.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee of North South University, Dhaka, Bangladesh.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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