

Methanol extract of *Solanum violaceum* root possesses antiobesity, hypolipidemic, thrombolytic and membrane stabilizing activity

Sayed Koushik AHAMED^{1,2} Md. Imdadul Huque KHAN¹, Md. Mustahsan BILLAH¹, Mohammad Salim HOSSAIN^{1*}

¹ Department of Pharmacy, Noakhali Science and Technology University, Noakhali-3814, Bangladesh

² Present address: Department of Pharmacy, Comilla University, Comilla, Bangladesh

ABSTRACT: Roots of *Solanum violaceum* (*S. violaceum*) is used traditionally in different therapeutic applications in Bangladesh. Root extract of *S. violaceum* was investigated for total phenolic content (TPC), antiobesity, anti-hyperlipidemic, membrane stability and thrombolytic activity. Total phenolic content (TPC) was determined using folin-ciocalteu method. High fat diet induced obese mice were used for anti obesity and antihyperlipidemic test. Body weight gain, blood total cholesterol, triglyceride level were analyzed. Thrombolytic and membrane stability activity were evaluated by using human erythrocytes. Total phenolic content in root extract of *S. violaceum* was calculated as 51.26 mg gallic acid equivalent GAE/g of dry weight. The extractive supplementation with dose of 200mg/kg and 400mg/kg were capable of lowering the level of triglyceride and total cholesterol significantly ($p < 0.05$) in high fat diet (HFD) induced obese mice in a dose dependent manner. As a membrane stabilizing agent, crude extract was able to inhibit the erythrocyte hemolysis significantly ($p < 0.05$) with a value of $28.02 \pm 5.09\%$ and $32.97 \pm 4.12\%$ respectively for heat and hypotonic solution induced condition. Moreover, $21.56 \pm 2.62\%$ of clot lysis was exhibited by the extract for its thrombolytic activity. The root of *S. violaceum* justifies its potential as anti obese agent. Further research is recommended to find out the active metabolites from this source.

KEYWORDS: Obesity; Antihyperlipidemia; Thrombolysis; Inflammation; *S. violaceum*

1. INTRODUCTION

According to world health organization (WHO) almost 80% of the world population presently uses herbal medicine for their primary health care (1). During the last few decades, advances in the identification of new bioactive compounds from plants have renewed the popularity of herbal medicines (2). In fact, due to belief that 'Green Medicine' is safe, effective and reliable than synthetic drugs, many pharmaceutical companies are interest in plant derived drugs (3). Various pharmaceuticals have been started in preparing various form of medicine using chemical constituents such as the alkaloids, glycosides, flavonoids, tannins, resins and volatile oils obtained as secondary metabolites from medicinal plants (4).

Now a days the prevalence of obesity is dramatically increased around the world. Obesity is considered as a major risk factor for mortality and morbidity associated with others health problems (5). According to WHO reports high cholesterol level in blood is responsible for approximately 56% of cardiovascular disease worldwide and causes 4.4 million deaths each year and hyperlipidemia identified by hypercholesterolemia is the most common cause for this disease. Over the past 30 years, few obesity-treatment drugs have been developed or approved

for which only two drugs are currently available (6). To fight against such type of diseases discovery of new bioactive metabolites is essential. Hence, screening medicinal plants for promising bioactive metabolites to discover novel drug candidate is a necessity (7).

S. violaceum belongs to Solanaceae family which is traditionally used for medicinal treatments. Leaf juice is used to stop vomiting and the seeds are used in the treatment of toothache (8). Fruits are considered anthelmintic, laxative and digestive; useful in pruritus, leucoderma and asthma (9). Traditionally the root is used in bronchitis and asthma. It is also used as a digestive, carminative and astringent to the bowels, fever, etc.

In this present study, we have investigated the root of *S. violaceum* and gone through different biological tests to assure the presence of bioactive metabolites which will lead to find a bioactive novel compound precursor of novel drug.

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Corresponding Author: Mohammad Salim Hossain

E-mail: pharماسalim@yahoo.com

Phone: +8801711200410

ORCID No: 0000-0002-5710-1115

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2. RESULTS

2.1 Total phenolic content in methanolic extract of root of *S. violaceum*

Methanolic crude extract of *S. violaceum* root was subjected for the determination of total phenolic content TPC. It was calculated as gallic acid equivalent (GAE) in the extractive and the value was 51.26 ± 0.13 mg per gm dry weight.

2.2 Effect of methanolic extract of root of *S. violaceum* on body weight in high fat diet induced obesity

There was no momentous difference in the body weight among the groups of animals at the beginning of the study. Mice fed on high fat diet (HFD) started to gain weight after twelve days of feeding and continued till the end of experimental period ($p < 0.05$) whereas, when extract supplemented with a doses of 200mg/kg and 400mg/kg to HFD, caused the significant ($p < 0.05$) decrease in body weight. Data are presented in Figure 1. Weight gain was preferentially less in the groups supplemented with 400mg/kg than that with 200mg/kg. No remarkable differences in food intake among the groups were noticed.

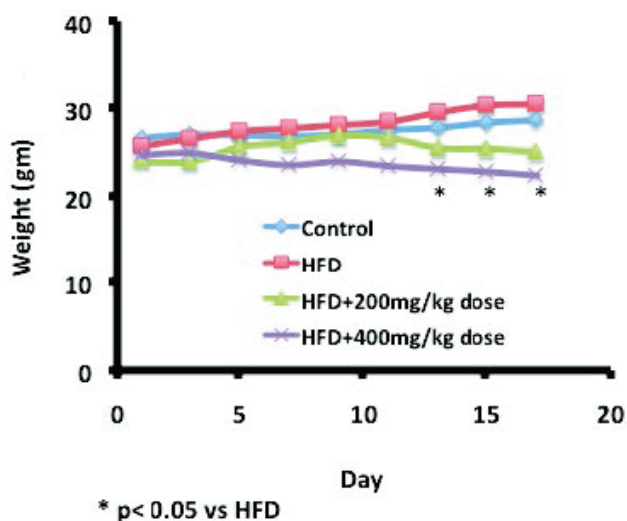


Figure 1. Changes of body weight of different groups

2.3 Effect of methanolic extract of root of *S. violaceum* on hyperlipidemia

Along with the weight gain, HFD group shows significantly elevated level of triglyceride and total cholesterol than that of controlled group as predicted ($p < 0.05$). However, treatment with extractive, reversed the hyperlipidemic effect governed

by high fat diet ($p < 0.05$). Lowering of hyperlipidemia was observed in a dose dependent manner (Figure 2, 3).

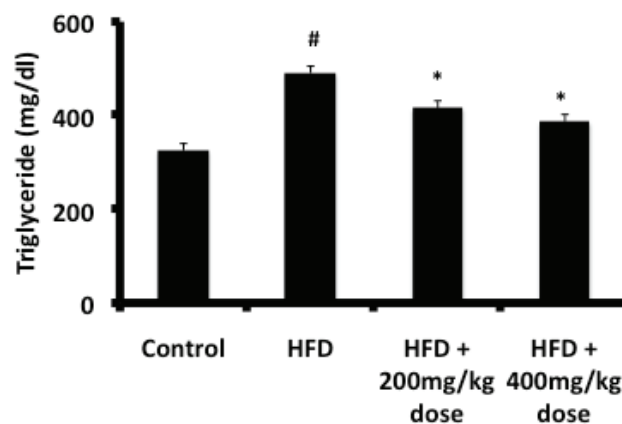


Figure 2. Effect of root of *S. violaceum* on Triglyceride level

$p < 0.05$ vs control

* $p < 0.05$ vs HFD

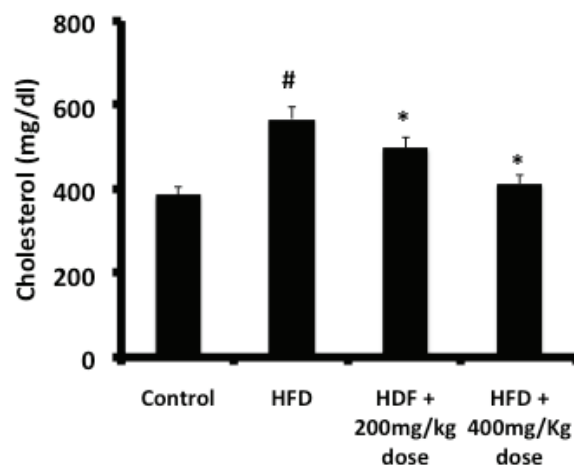


Figure 3. Effect of root of *S. violaceum* on Cholesterol level

$p < 0.05$ vs control

* $p < 0.05$ vs HFD

2.4 Thrombolytic activity of methanolic extract of root of *S. violaceum*

Crude extract was applied to evaluate the thrombolytic activity. The extractive was able to lysis the clot with a value of 21.56% whereas the streptokinase causes about 59.7% clot lysis (Table 1). Although clot lysis activity of the extractive was less than that of standard used but significant degree of clot lysis was observed by the extractive while compared with the control ($p < 0.05$).

Table 1. Thrombolytic activity in terms of % of clot lyses by standard and *S.violaceum* crude extract

Test samples	% clot lyses (Thrombolytic activity)
Streptokinase (Standard)	59.7*
Methanol extract	21.56*
control	3.06

* P < 0.05 vs control

2.5 Membrane stabilizing activity of methanolic extract of root of *S. violaceum*

Membrane stabilizing activity as function of anti-inflammatory response by the extractive was documented using human erythrocyte as a model. Haemolysis of erythrocyte was induced by either hypotonic solution or heat. Inhibition of haemolysis by the extractive was calculated and the data was presented in Table 2. Methanolic extract showed significant ($p < 0.05$) inhibition of haemolysis in both the cases compared with control. Extractive was more effective to inhibit the hypotonic solution induced haemolysis than that caused by heat.

Table 2. Erythrocyte membrane stabilization in terms of % inhibition of haemolysis (in hypotonic solution – and heat-induced conditions) by standard and *S.violaceum* crude extract

Test samples	% Inhibition of Haemolysis (Erythrocyte membrane stabilization)	
	Hypotonic solution (50 mM) induced	Heat-induced
Acetyl salicylic acid (Standard)	70.99*	75.81*
Methanol extract	25.7*	28.02*

* P < 0.05 vs control

3. DISCUSSION

Plants from Solanaceae family have been documented for anti-diabetic, anti-ulcerogenic properties (10, 11). Here methanolic extract of roots of *S. violaceum*, a plant from Solanaceae has been studied for the antiobesity, antihyperlipidemic, and other related activities. Although lethal dose of this test-extract was not determined in this experiment, but we did not observe any death of experimental animal with the doses applied for the study.

Obesity is a major risk factor for mortality and morbidity associated with other health problems (5). HFD induced model for obesity has well been studied previously as it mimic the basic adipogenic episodes in human (12). Body

weight gain is an indicator for the development of obesity, leading to other metabolic disorders (13). In this study, we found the significant weight gain by the mice feed with HFD, compared with normal diet feed group, thus it confirms the obese status. Treatment of HFD mice with extract of *S. violaceum* at 200mg/kg and 400mg/kg significantly reduced the body weight when compared with HFD mice. This result suggests that the test-extract supplementation has remarkable capability to prevent body weight gain. We assumed this loss of weight gain might be associated with inhibition of food intake by the treated mice but interestingly we did not observed any remarkable changes in the rate of food intake by the animals even in any group.

Furthermore, hyperlipidemia, characterized by hypertriglycemia and hypercholesterolemia, is another parameter to study the pathogenesis of obesity. In this study, HFD mice showed a considerable elevation in triglyceride and total cholesterol compared with normal diet, while the treatment with extract in HFD mice reduced the triglyceride and total cholesterol level significantly, confirming further, the ability of the extract to ameliorate the development of obesity and other related complications. The anti-hyperlipidemic activity of the crude extract was dose dependent, i.e dose at 400mg/kg was more effective than 200mg/kg to reduce the accumulation of triglycerides and total cholesterol. It is evidenced that, drug like simvastatin, showing the ability to lower the cholesterol level (14) are used to treat obesity mediated cardiac complication. Although it is not clear, how does this extract lower the lipid accumulation in HFD mice but several lines of evidence has been reported that the natural products may decrease the lipid absorption by inhibiting the activity of pancreatic lipase (15). Orlistat an antiobese drug is a derivative of naturally occurring lipase inhibitor (16). Furthermore, polyphenols present in plant extract shows a significant inhibitory activity against pancreatic lipase (17-20). Moreover, polyphenols are also associated with apoptosis of adipocytes (21, 22) and inhibit the differentiation of 3T3-L1 cells by arresting the cell cycle at G1 phase (23). The phenolic content present in this extract may play a role in antiobese functions of the plant extract either by any mechanism stated above. Moreover, besides phenolic compounds, *S. violaceum* contains many other phytochemicals like alkaloids, glycosides and others (24), these bioactive metabolites might also contributed to the attenuation of HFD induced obesity by regulating the synthesis and metabolic pathway of fatty acid and cholesterol. To confirm the exact mechanism further investigation is warrant.

Obesity is also considered as risk factor for the development of arterial thrombosis and venous thromboembolism (25). In developed countries, thromboembolic disorders such as pulmonary emboli, deep vein thrombosis, heart attacks, and strokes are the main causes of mortality and morbidity (26). Again, the first generation thrombolytic agents such as streptokinase, urokinase show relatively weak substrate specificity and can cause side effects of systemic

fibrinolysis and associated bleeding complications (27). Hence, to develop improved recombinant variants of these drugs, attempts are underway (28). Considering these, we studied the thrombolytic activity of the crude extract. Crude methanolic extract of *S. violaceum* showed mild thrombolytic activity compared with streptokinase. This finding may drive the scientist to consider the *S. violaceum* root as a target to develop the drug at the intersection of obesity and thrombolysis.

Inflammation is a complex biological response of vascular tissues to harmful stimuli. Inflammatory mediators like prostaglandin, is associated with the adipogenesis procedure and treatment with COX inhibitors significantly reduced the fat accumulation in 3T3-L1 cells with attenuated synthesis of prostaglandin (29). Taking this in mind, we examined the anti-inflammatory potentials of the crude extract by assessing the membrane stabilizing activity using acetyl salicylic acid as a positive control. Membrane stabilization is well studied as a mechanism of anti-inflammatory response (30). Our data revealed that the methanolic extract of *S. violaceum* has significant inhibition capacity of erythrocyte haemolysis in hypotonic solution and heat-induced condition. As erythrocyte cell membrane is similar to lysosomal membrane components, it is extrapolated that the drugs which stabilizes erythrocyte membrane, also can stabilizes lysosomal membrane (31) and thus interfere with the release and or action of mediators responsible for inflammation like histamine, serotonin, prostaglandins, etc. (30). It is reported that the extract from plants of Solanaceae family poses the membrane stabilizing and anti-inflammatory activities (32). Thus, our test-extract may be a potent inhibitor to release the inflammatory mediators and attenuates the inflammatory responses in obese condition.

4. CONCLUSION

In conclusion, despite of the unknown mechanism, our data suggests that, root extract of *S. violaceum* poses a good potentiality to attenuate the obesity and obesity related complications. Thus finding out the bioactive metabolites from this source may quench the thirst of new drugs for the treatment of obesity and related disorders.

5. MATERIALS AND METHODS

5.1 General experimental procedure

Rotary evaporator (Büchi 011, USA) was used to concentrate extract. Cholesterol and Triglycerides liquicolor (Origin GmbH, Germany, Centonic Germany), Colorimeter (AP-101, APEL, Japan), Glucometer (Model No – Sensocard, Hungary) and UV-spectrophotometer (Shimatzu, Japan) were used for different experiments. All chemicals used for experiment were of analytical grade.

5.2 Plant material

The Root of *S. violaceum* was collected from Sonapur, Noakhali, Bangladesh in March, 2013. The plant sample was identified and authenticated by Bangladesh National Herbarium, Mirpur, Dhaka (Accession number: 37751).

5.3 Preparation of extracts

The washed and shade dried roots of the plants were ground into fine powder by blending machine. The crude powdered materials (800mg) were cold extracted and macerated with methanol at room temperature for 7 days. The methanol extract thus obtained was filtrated and evaporated to dryness with a rotary evaporator to get semi-solid extract.

5.4 Experimental Animals

Twenty healthy *Swiss Albino* mice (Sex: Male) were collected from Jahangir Nagar University, Dhaka, Bangladesh and were kept in dry polypropylene cages with 12 hours light dark cycle at 25±2°C and 55±10% relative humidity. Throughout the whole experiments, all mice were given human care according to the criteria mentioned in the 'Guide for the Care and Use of Laboratory Animals', which was prepared by the National Academy of Sciences and published by the National Institute of Health (US) and the permission was taken from the concern institute.

5.5 Total phenolic content

Total phenolic content of root of *S. violaceum* was estimated as described by Singleton and Rossi (33) and modified by Kim et al (34). Briefly, 0.5 ml of extract (1.0 mg/ml, triplicates) were introduced into test tubes; 2.5 ml of Folin-Ciocalteu's reagent and 2.0 ml of sodium carbonate (7.5% w/v) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 760 nm was measured (Systronics UV-vis spectrophotometer). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram dry material.

5.6 Anti obesity test

Extractive of the *S. violaceum* was dispersed in 0.9% saline water and 1-2 drops of tween 80 was added to facilitate the administration of extractives orally.

The *Swiss Albino* mice were divided into four separate groups of 5 mice in each. Group 1 was considered as control group and was administrated normal diet, Group 2 was considered as obese and was administered high fat diet (regular diet supplemented with 20% fat). Group-3 and 4 both were administrated high fat diet along with 200mg/kg/day of extract and 400 mg/kg/day of extract, respectively.

During the experimental period, mice weight was recorded in every two days. After 18 days the animals were killed by decapitation, and blood was collected for analysis.

5.7 Hypolipidemic activity

The total serum cholesterol was estimated by enzymatic end point (CHOD-PAP) method (35) whereas triglyceride was estimated by enzymatic colorimetric GPO-PAP method (36) using commercial analytical kit as per manufacturer instructions. Cholesterol kit (CAT # CH 200, Randox Laboratories, UK) Triglyceride kit (CAT # CS 611, Crescent Diagnostics, Soudia Arabia) respectively.

5.8 Thrombolytic activity

In vitro thrombolytic activity was evaluated by the method developed by Prasad et al. (37) by using Lyophilized Alteplase (Streptokinase) as a positive control and distilled water as a negative control, respectively. Method described briefly as, venous blood (5 ml) was drawn from healthy volunteers, and transferred in pre-weighed sterile micro tubes to form clots and incubated at 37°C for 45 minutes. After the clot formation, the serum was removed without disturbing the clot and tubes were re-weighted to get the clot weight (Clot weight = weight of the tube containing clot – weight of the tube alone). Crude methanolic extractive, streptokinase (100 ml), when applicable, was added to the tubes containing the clot and the tubes were incubated for 90 minutes at 37°C to observe the clot lysis. After the incubation, released fluid was removed and the tubes were weighted again to estimate the difference in weight after clot disruption. Differences obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis as below

$$\% \text{ of clot lysis} = (\text{Weight of released clot} / \text{clot weight}) \times 100$$

5.9 Membrane stabilizing activity

The membrane stabilizing activity using in hypotonic and heat-induced conditions was determined following the method developed by Omale et al. (31) with slight modification. Briefly described as

Erythrocyte suspension: Whole blood from male human volunteers was collected under standard condition. EDTA was used to prevent clotting. The blood was washed three times with isotonic solution (154 mM NaCl in 10 mM sodium phosphate buffer, pH 7.4) through centrifugation for 10 min at 3000 rpm. Thus the suspension finally collected was considered as stock erythrocyte (RBC) suspension.

Hypotonic solution induced haemolysis: Extractive (1.0 mg/ml) or acetyl salicylic acid (0.1 mg/ml) was added to the erythrocyte (RBC) suspension (0.5 ml) in 5 ml hypotonic solution (50 mM NaCl in 10 mM sodium phosphate buffer,

pH 7.4) and the mixtures were incubated for 10 min at room temperature and then centrifuged for 10 min at 3000 rpm and. The absorbance of supernatant was measured at 540 nm using UV-spectrophotometer. The percent inhibition of haemolysis was calculated as below

$$\% \text{ inhibition of haemolysis} = 100 \times \{ (OD1 - OD2) / OD1 \}$$

OD1= Absorbance of supernatant without extractive, and
OD2= Absorbance of supernatant with extractive

Heat-induce haemolysis: Aliquots (5 ml) of the isotonic buffer, containing 1.0 mg/ml of different extracts of the plant were put into two duplicate sets of centrifuge tubes The vehicle, in the same amount, was added to another tube as control. Erythrocyte suspension (30 µL) was added to each tube and mixed gently by inversion. One pair of the tubes was incubated at 54 °C for 20 min in a water bath. The other pair was maintained at 0-5°C in an ice bath. The reaction mixture was centrifuged for 3 min at 1300 g and the absorbance of the supernatant was measured at 540 nm using UV spectrometer. The percentage inhibition or acceleration of hemolysis in tests and was calculated using the following equation:

$$\% \text{ inhibition of hemolysis} = 100 \times \{ (OD2 - OD1) / (OD3 - OD1) \}$$

Where, OD1 = test sample unheated, OD2 = test sample heated and, OD3 = control sample heated

5.10 Statistical analysis

The data were expressed as Mean ± SEM. The statistical analysis was carried out using t-test. A p value ≤ 0.05 was considered as statistically significant.

Ethical approval: Ethical consent was taken from the institute for this experiment (1103MS 101)

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Authorship statement

Concept – MSH, Design-SKA, MSH, Supervision-MSH, Resources – MSH, Materials-MSH, Data collection/processing-SKA, Analysis/interpretation – SKA, MIHK, MMB, Literature Search-SKA, MIHK, MMB, Manuscript writing-SKA, MIHK, MSH, Critical review – SKA, MIHK, MMB, MSH

Conflict of Interest

None

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