In vivo Anti-diabetic and Lipid Lowering Activity and In vitro Antimicrobial, Thrombolytic and Cytotoxic Activity of Different Fraction of Methanolic Extract of Solanum melongena

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Abstract

Background: In this study was tried to evaluate the in vitro antimicrobial, thrombolytic with cytotoxic activity and in vivo anti-diabetic and lipid lowering activity of different fraction of methanolic extract of Solanum melongena which is widely used in the folkloric treatment in Bangladesh. The aim of this study was to find out the fundamental mechanisms of the plant extracts which might be helpful in the overall folkloric treatments in Bangladesh.

Results: In anti-diabetic and lipid lowering assay, plant extract reduce glucose level with Urea, protein and total cholesterol level with LDL and HDL were reduced by using plant extract. In thrombolytic assay, positive control streptokinase showed 94.21% lysis of clot and negative control distilled water showed 1.53% of clot. Chloroform soluble fraction (CSF) exhibited highest thrombolytic activity 11.13%. Antimicrobial screening at 400 µg/die revealed antimicrobial activity in opposition to the tested microorganisms and the inhibition zone range was 8 to 13 mm. In brine shrimp lethality, LC50 µg/ml and LC90 µg/ml were obtained for different extracts after 18, 20 and 22 hours gradually. Such as after 18 hours LC50 µg/ml and LC90 µg/ml for distilled crude extract are 2.055 and 5.083, 3.112 and 6.225 for distilled n-hexane extract, 2.421 and 5.369 for distilled ccl3 extract and 4.00 and 6.948 for distilled Ethyl acetate gradually.

Conclusion: Our results yield that crude methanolic extract has better effects than the other in all trials. By reviewing this study, it is very clear that the methanolic extract of Solanum melongena has shown pharmacological effect with its traditional use.

Keywords: Anti-diabetic; Lipid –lowering; Antimicrobial; Thrombolytic; Cytotoxic; Solanum melongena

Introduction

Nature is a complete store-house of remedies [1]. So, we have got different types of drugs from nature in the form of herbs, plants and algae which help to cure the incurable diseases [2]. Herbal remedies have good effects and minimal side effects with relatively low cost in clinical experience. So, interest is growing on Herbal drugs. The biological active compounds of this drug may be unknown, but these are prescribed widely [3].

Epilepsy is a chronic neurological disorder and about 52 million people may affect by Epilepsy in the world [4]. Synthetic drugs are used for epilepsy and inflammation. For this reason, side effects are increasing gradually. At present time we need to keep our concentration on the scientific exploration of herbal drugs having fewer side effects [5].

The researchers are seeking medicine extract from plants sources and trying to use in Ayurveda, Allopathy, in traditional medicine and Homeopathy. Synthetic drugs have side effects and high costly. So medicinal plants are used in the traditional and modern system. Due to the lack of modern medicine and hospital the people of rural area are going to depend on traditional medicine for curing their ailments. Now a day about 70% people are using traditional folk medicine [6]. In our country approximately 80% of the population is living in the village with natural resources [7]. Medicinal plants contain various complex chemical substances of different composition [8]. Medicinal plants provide the raw materials which are helpful for internal pharmaceuticals [9]. During this time the phytochemicals research is very helpful to find out new anti-infective agents from higher plants considering physiological action [10]. We should know about the chemical constituent of plant because knowledge about these may help us to discover the actual valuable remedies [11]. Photochemical and polyphenols (phenolic acid, Hydrolysable tannins and Flavonoids) show antioxidant properties, anti-carcinogenic and anti-mutagenic effects [12]. The bioactive chemical constituents into plants like alkaloids, tannin, flavonoids, phenolic composition etc., are responsible for physiological and biochemical actions in the human body [13,14]. Natural products from plants are also show antitumor and antioxidant activity [15].

Solanaceae family plants are very important for treatment of primary health care. Many workers in the world have studied about the phytochemical constituents of different plant species in the time being and among them certain authors have tried to report about phytochemical studies of Solanaceae family plants [16-18]. The rest workers have also tried to work on phytochemical constituents by collecting different medicinal plants for example [19-28]. The development of antibiotic resistance gradually increasing by pathogenic microorganisms with synthetic drugs failure and side effects. So, recently 80% of population is going to use medicinal plants for their...
potential antimicrobial activity [29]. There are thousands of species which have antimicrobial activity of plants [30,31]. All plant parts such as (root, stem, flower, fruit etc.) are used as drug extract. So huge study is needed for identifying medicinal properties and should use some medicinal plants against pathogenic microorganisms [32,33]. At past for curing specific diseases different parts of medicinal plants were used [34]. Solanum species belongs to Solanaceae family are so effective plants against pathogenic microorganisms. Antibacterial activity of this family was studied [35-37].

Hyperglycemia is an important factor for progressing of the complications and developing of diabetes mellitus. Diabetes mellitus is treated by insulin and oral administration of hypoglycemic drugs like sulfonylureas and Biguanides. Long-time affecting of the kidney, retina and nervous system is also responsible for diabetes mellitus [38-40]. In the world more than 1250 plant species are used in diabetes phyotherapy which contain anti-hyperglycemic activity [41]. Solanum melongena is a medicinal and food plants. As food plant it is hugely used in our country from previous time. Different studies have found that it has anti-hyperglycemic activity with other physiological actions [42].

Solanum melongena plays an important role in the treatment of typical human diseases from the ancient period of time. Solanum melongena (Eggplant) which is commonly known as melongene. In Southeast Asia, South Africa and South Asia, it is called brinjal [43-46]. This plant is also called eggplant because the fruits of the plant are looked like small white eggs (Eu-Sol: Eggplant history). American, Australian English and sometimes Canadian English it is known as “eggplant” or in British English and Canadian English as “aubergine” cause it bears a same name fruit which has a long history of using as a vegetable through cooking [47]. At present, this plant has an important role in the research sector. Different types of studies have presented different types of physiological actions of Solanum melongena such as, Analgesic activity [48], Antipyretic activity [49], Antioxidant Activity [50,51], Anti-inflammatory Activity [52], Anti-asthmatic Activity [53], Action on Anaphylactic Reactions [54], Hypolipidemic Action [55,56], Spasmogonic Activity [57], Action on the Eye [58], Antiplatelet and Calcium Channel Blocking Activities [59], CNS Depressant Activity [60] and Hypotensive Action [61].

Antifungal property [62] of this plant studied over and there is also found some other properties like Flavonoids isolated from Solanum melongena (brinjal) showed potent antioxidant activity [51]. In vitro and In vivo. Anticancer Activity of the Fruit Peels of Solanum melongena L. against Hepatocellular Carcinoma [63], Cardio protective properties of raw and cooked eggplants and analgesic effect [64]. It was the first time we had tried to study together about in vivo anti-diabetic and lipid lowering activity and in vitro antimicrobial, thrombolytic and cytotoxic activity of different fractions of methanolic extract of Solanum melongena. Solanum melongena was selected because it is available in Bangladesh and used in rural areas for different medicinal treatments.

Methods

Plant material collection and identification

This vegetable plant was collected from Sherpur, Bangladesh. Then an expert botanist of Bangladesh National Herbarium (DACB), Mirpur-1, Dhak1216, Bangladesh was identified and authenticated the leaves of Solanum melongena. The identification No. of this plant was 41102. For the future reference a voucher specimen was submitted at the herbarium.

Chemicals

The chemicals used in this study were purchased from Merck (Darmstadt, Germany) and Sigma Chemical Co. (St. Louis, MO, USA). The chemicals which used were analytical grade.

Extract preparation: The collected plant parts (leaves) were segregated from plants. They were kept in open air for dry for two weeks under shade. A suitable grinder was used for grinding the dried plant’s parts into a coarse powder. An airtight container was used for storing the powder and kept in a cool, dark and dry place for analysis. About 750 gm. of powder material of leaf was taken in a clean glass container and soaked in 3.5 Litre of Methanol. After keeping the content into the container, it was sealed and stored for a period of 14 days with occasional shaking and stirring. The whole mixture was taken in coarse filtration by a piece of clean, white, cotton material. Then it was filtrate through Whatman filter paper. The filtrate (methanol extract) was evaporated in a water bath and under ceiling fan until dried. It changed into greenish black color. The greenish black color extract was entitled as crude extract of methanol.

Test animals

For the screening of in vivo anti-diabetic and lipid lowering activity young swiss -albino rats (aged 25–30 days), average weight 25-30 g was used. The Animal Resources Branch of ICDDR, B (International Centre for Diarrheal Disease and Research, Bangladesh) was the source of collecting and kept in suitable condition for 10 days for adaptation. Rodent food and water ad libitum formulated by ICDDB, B were used for their feeding. Throughout the experiments, we took care of all animals during our experiment according to the criteria outlined in the “Guide for the Care and Use of Laboratory Animals”, 8th edition, prepared by the National Academy of Sciences and published by the National Institute of Health (US).

Test Organism

Artemia salina Leach (brine shrimp). The egg of the shrimp was collected from Katabon University Market.

Thrombolytic assay: With minor modifications of Prasad et al. [65] clot lysis activity in vitro of the leaves of Solanum melongena was carried out. In vitro the thrombolytic activity of the Solanum melongena (eggplant) was conducted by using plant extract and distilled water. In a test tube 5 mg of extract was suspended in 10 ml of distilled water and shaken it well. Whole blood (4 ml) was drawn from healthy human volunteer without a history of oral contraceptive or anticoagulant therapy, smoking and then transferred in three different pre-weighed sterilized micro centrifuge tubes. Wait for 5 to 10 min for clot formation in room temperature and after decantation the liquid was removed. Then weight of each test tube and Measure the weight of clot (clot weight = total weight of clot with tube – only weight of tube). To each micro centrifuge tube containing pre-weighed clot, 1 ml of aqueous extract of plant (Solanum melongena) was added separately, 1 ml distilled water is added to clot of tube no. 2, another is for negative control (which is Blank). All the test tube then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation the tubes were kept at lying position for 6 minutes on a tray. At first serum was removed and then liquid was also removed from the tube surface by using the rod of cotton. Each tube was weighed again. Weight of clot lysis was found and calculated by the following way.

Weight of clot lysis = Initial clot weight– resting clot weight. At the end of experiment the weight was measured very minutely, because result may vary for careless weight. So, the balance must be check before weighing.
At last, percentage of clot lysis was calculated by the following equation.

\[
\% \text{ of clot lysis} = \left( \frac{\text{Initial clot weight} - \text{Resting clot weight}}{\text{Initial clot weight}} \right) \times 100
\]

Brine Shrimp Lethality assay: The study was performed according to the Brine shrimp lethality bioassay method Meyer et al. [66] by using Methanol extract of Solanum melongena and Artemia salina Leach (brine shrimp). Stock Solution and Simulated Sea Water were Prepared. After 22 hours hatching the mature nauplii (larve) were taken for bioassay Seven (07) clean test tubes were taken where six (06) of test tube contain different samples concentration respectively 1000 µg/µl, 500 µg/µl, 250 µg/µl, 125 µg/µl, 62.5 µg/µl, 31.25 µg/µl by adding ½(half) portion of stocksolution, 100 µl DMSO solution and 2.5 ml of sea water and one (1) for negative control test. Finally 10 living shrimps were collected by the help of Pasteur pipette and were kept to each of the test tubes. For n-hexane, CCl₃ and ethyl acetate solution prepared by the following above this procedure. After 18 hrs, 20 hrs and 22 hrs the percentage of lethality of brine shrimp nauplii was calculated at each concentration for each sample.

Anti-diabetic and lipid lowering activity: Rats were divided into seven groups containing 5 rats per group. Group I and II received normal diet but Group I served as normal control where Group II consists of alloxan induced rats and serving as diabetic control. Group III consists of alloxan-induced rats by receiving Glibenclamide (synthetic antidiabetic drug) which given 0.5 mg/kg body weight once a day orally for 15 days [67]. While, group IV, V, VI and group VII consist of alloxan induced rats and received concentrated Solanum melongena leaves at a dose of 3 ml/kg body weight and given once a day orally for 15 days each, respectively. 10 mg/ml solution of Alloxan monohydrate was prepared in citrate buffer (0.1 M pH 4.5). Then the solution was kept in ice and administered into the rats within 5 min. Dose was 50 mg/kg body weight intraperitoneally. Glycosuria and hyperglycemia were taken for the experiment from the rats with moderate diabetes after 48 h of alloxan administration.

Antibacterial assay: The disc diffusion method was used for antimicrobial assay [68]. By using standard disc diffusion method, the collected fractions from the plant like ethyl acetate, chloroform and n-hexane were tested along with the methanol extracts. We were collected 16 microorganisms from University of Dhaka, Bangladesh. Blank sterile filter paper disc and Standard Kanamycin (30 µg/disc) were used which were represented as positive and negative controls respectively. To prepare fresh cultures Nutrient agar medium (DIFCO) was used. These fresh cultures were used for testing the sensitivity of the organisms. In the agar plates zones were marked. The marked zones were used for placing the standard antibiotic discs, the sample discs and the control discs gently. At 37°C for 24 hours the discs on the plate aerobically were then incubated.

Statistical analysis

One-way ANOVA with Dunnett’s post Hoc test for this experiment was carried out with SPSS 16.0 for Windows* software and the results obtained were compared with the control group. P values <0.001 were considered to be statistically significant.

Results and Discussion

Anti-diabetic and lipid lowering activity

The hot water extracts of Solanum melongena produced significant changes in the alloxan induced diabetic rats (Table 1). This plant reduced glucose level considerably in comparison to treatment of the diabetic rats and the results were comparable with that of Glibenclamide (10 mg/kg). The prolonged treatment also reduced urea, protein and total cholesterol (Tables 1 and 2) level in comparison to diabetic rats.

In alloxan induced diabetic rats the blood glucose data obtained clearly indicate significant antihyper-glycemic effect. That means, the fraction of Solanum melongena may potentiate pancreatic secretion or may reuptake glucose. Hyper-cholesterolemia, hyper-triglyceridemia and hyper-uricemia have been reported to occur in alloxan induced diabetic rats [69]. Increase in glycojen in liver may occurred due to increase or decrease of glycogenolysis. In total protein increase (Table 2) depend on amino acids levels circulating changes, muscle output of amino acid concentrations and hepatic amino acids uptake [70]. Serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) were found to be increased in alloxan-induced diabetic rats. The serum level of urea and liver enzymes came to normal upon treatment with Solanum melongena. The result is shown that trans-aaminase activity is increased in the serum of a diabetic rat which is active in absence of insulin due to the availability of amino acids in the blood [71]. While on the other hand, we also found that the total cholesterol level as increased in diabetic rats was also significantly reduced upon treatment with the fraction of Solanum melongena. Depressed activities of lipogenic and cholesterogen enzymes are responsible for these [72,73].

Thrombolytic activity

As a part of discovery of cardio-protective drugs from natural sources the extractsives of Solanum melongena were assessed for thrombolytic activity and the results are presented in (Table 3 and Figure 1). 100 µl SK was added, a positive control (30,000 I.U.), to the clots and incubated for 90 minutes at 37°C, showed 94.21% lysis of clot. Distilled water which exhibited negligible lysis of clot (1.53%) and was treated as negative control. In this study; the chloroform soluble fraction and CSF= chloroform soluble fraction WSF= water soluble fraction of the Solanum melongena

Antimicrobial activity

The crude extract of Solanum melongena was subjected to antimicrobial screening at 400 µg/disc revealed antimicrobial activity in opposition to the tested microorganisms and the zone of inhibition ranging from 8 to 13 mm was measured (Table 4).

| Group     | Treatment                  | Glucose (mg/dl) | Cholesterol (mg/dl) |
|-----------|----------------------------|-----------------|---------------------|
| Group-I   | Control                    | 117.2 ± 1.2     | 122.7 ± 1.3         |
| Group-II  | Diabetic control           | 245.3 ± 1.4     | 236.1 ± 1.3         |
| Group-III | Diabetic+Glibenclamide     | 116.1 ± 1.5     | 121.05 ± 1.7        |
| Group-IV  | Diabetic + MF              | 185.22 ± 1.0**  | 125.78 ± 11.0       |
| Group-V   | Non-diabetic + MF          | 114.2 ± 1.3     | 116.8 ± 1.5         |
| Group-VI  | Diabetic +HSF              | 189.18 ± 0.4**  | 127.1 ± 1.2         |
| Group-VII | Non-diabetic +HSF          | 114.7 ± 1.0     | 118.2 ± 1.2         |
| Group-VIII| Diabetic +CSF              | 188.5 ± 0.7     | 129.7 ± 1.7         |
| Group-IX  | Non-diabetic +CSF          | 117.7 ± 1.0     | 119.3 ± 1.4         |
| Group-X   | Diabetic +EASF             | 184.16 ± 0.4    | 124.1 ± 1.4         |
| Group-XI  | Non-diabetic +EASF         | 113.7 ± 1.0     | 117.2 ± 1.3         |
| Group-XII | Diabetic +WSF              | 180.12 ± 0.4    | 121.1 ± 1.0         |

Note: Values are expressed as Mean ± SEM (n= 5). **: p<0.05 significant compared to diabetic rats.

MF= Methanolic fraction; HSF= n-hexane soluble fraction, EASF= Ethyl acetate soluble fraction and CSF= chloroform soluble fraction WSF= water soluble fraction of the Solanum melongena
In the bioassay, lethality was indicated by methanol extracts by showing the biological activity of the compound which present in the leaves of Solanum melongena. Thiocarboanilide (SK), Kanamycin, methanol, distilled n-hexane extracts, distilled CCl₃ extracts and distilled Ethyl acetate of the leaves of Solanum melongena.

| Sample   | Thrombolytic Activity (% of lysis) |
|----------|-----------------------------------|
| SK       | 94.21%                            |
| Water    | 1.53%                             |
| MF       | 6.11%                             |
| HSF      | 9.71%                             |
| EASF     | 3.98%                             |
| CSF      | 11.13%                            |

Table 3: Thrombolitic activity of different fractions of Solanum melongena.

| Microorganisms | HSF | EASF | CSF | MF          | Kanamycin |
|----------------|-----|------|-----|-------------|-----------|
| Bacillus cereus | 9   | 7    | 8   | 43          |           |
| Bacillus megaterium | 9   | 8    | 7   | 43          |           |
| Bacillus subtilis | 9   | 7    | 12  | 9           | 43        |
| Staphylococcus aureus | 9   | 8    | 7   | 6           | 42        |
| Sarcina lutea | 8   | 7    |     | 8           | 42        |
| Escherichia coli | 9   | 7    |     | 8           | 43        |
| Pseudomonas aeruginosa | 8   | 9    | 7   | 8           | 43        |
| Salmonella paratyphi | 9   | 7    | 12  | 8           | 42        |
| Salmonella typhi | 8   | 8    |     | 9           | 42        |
| Shigellaboydii | 8   | 7    | 7   | 8           | 43        |
| Shigelladyenteriae | 8   | 8    | 13  | 7           | 43        |
| Vibrio mimicus | 9   | 7    |     | 9           | 42        |
| Vibrio parahemolyticus | 8   | 7    |     | 8           | 42        |
| Candida albicans | 9   | 8    | 7   | 7           | 42        |
| Aspergillus niger | 7   | 13   |     | 7           | 42        |
| Saccharomyces cerevisiae | 9   | 7    | 5   | 6           | 42        |

Table 4: Antimicrobial activity of Solanum melongena.

**Brine shrimp lethality bioassay**

In the bioassay, lethality was indicated by methanol extracts by showing the biological activity of the compound which present in the extract. Test samples showed different mortality rate at different concentrations. The LC₅₀ and LC₉₀ value for the extract was obtained from the (Tables 5-7).
Table 6: After 20 hour later result of Brine shrimp lethality bioassay of distilled crude extracts, distilled n-hexane extracts, distilled CCl₃ extracts and distilled Ethyl acetate of the leaves of Solanum melongena.
Conclusion

According to the result, it can be expressed that the methanolic extracts of Solanum melongena have tremendous anti-diabetic, lipid-lowering, anti-microbial, thrombolytic and cytotoxic activities. Therefore, further study may be helpful for better understanding the mechanism of such action scientifically.

Competing Interests

The authors declare that they have no competing interests.

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Authors’ Contributions

KJ and MA carried out the collection of plants extraction process and conducting the experiments. KJ and MA have equal contribution of this study. MFRM and MSI* carried out conception and design of the study, analysis and interpretation of data. All authors read and approved the final manuscript.

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| Conc. of Extract µg/ml | No of Brine shrimp inserted | No of Alive Brine Shrimp | No of death Brine shrimp | % Mortality | Log Conc. µg/ml | LC 50 µg/ml | LC 90 µg/ml |
|------------------------|-----------------------------|-------------------------|--------------------------|-------------|----------------|-------------|-------------|
| Distilled crude extracts |                            |                         |                          |             |                |             |             |
| 0(blank)               | 10                          | 9                       | 1                        | 10          |                |             |             |
| 1000                   | 10                          | 9                       | 1                        | 10          |                |             |             |
| 500                    | 10                          | 0                       | 10                       | 100         | 3              |             |             |
| 250                    | 10                          | 0                       | 10                       | 100         | 2.69           |             |             |
| 125                    | 10                          | 1                       | 9                        | 90          | 2.39           | 1.251       | 4.753       |
| 62.5                   | 10                          | 1                       | 9                        | 90          | 1.79           |             |             |
| 31.25                  | 10                          | 2                       | 8                        | 80          | 1.49           |             |             |
| Distilled n-hexane extracts |                            |                         |                          |             |                |             |             |
| 0(blank)               | 10                          | 9                       | 1                        | 10          |                |             |             |
| 1000                   | 10                          | 0                       | 10                       | 100         | 3              |             |             |
| 500                    | 10                          | 0                       | 10                       | 100         | 2.69           |             |             |
| 250                    | 10                          | 0                       | 10                       | 100         | 2.39           | 2.488       | 5.515       |
| 125                    | 10                          | 2                       | 8                        | 80          | 2.09           |             |             |
| 62.5                   | 10                          | 3                       | 7                        | 70          | 2.79           |             |             |
| 31.25                  | 10                          | 5                       | 5                        | 50          | 1.49           |             |             |
| Distilled CCl 3 extracts |                            |                         |                          |             |                |             |             |
| 0(blank)               | 10                          | 9                       | 1                        | 10          |                |             |             |
| 1000                   | 10                          | 0                       | 10                       | 100         | 3              |             |             |
| 500                    | 10                          | 0                       | 10                       | 100         | 2.69           |             |             |
| 250                    | 10                          | 2                       | 8                        | 80          | 2.39           | 2.771       | 5.645       |
| 125                    | 10                          | 3                       | 7                        | 70          | 2.09           |             |             |
| 62.5                   | 10                          | 4                       | 6                        | 60          | 2.79           |             |             |
| 31.25                  | 10                          | 5                       | 5                        | 50          | 1.49           |             |             |
| Distilled Ethyl acetate |                            |                         |                          |             |                |             |             |
| 0(blank)               | 10                          | 9                       | 1                        | 10          |                |             |             |
| 1000                   | 10                          | 0                       | 10                       | 100         | 3              |             |             |
| 500                    | 10                          | 0                       | 10                       | 100         | 2.69           |             |             |
| 250                    | 10                          | 3                       | 7                        | 70          | 2.39           | 3.901       | 6.702       |
| 125                    | 10                          | 5                       | 5                        | 50          | 2.09           |             |             |
| 62.5                   | 10                          | 6                       | 4                        | 40          | 2.79           |             |             |
| 31.25                  | 10                          | 8                       | 2                        | 20          | 1.49           |             |             |

Table 7: After 22 hour later result of Brine shrimp lethality bioassay of distilled crude extracts, distilled n-hexane extracts, distilled CCl 3 extracts and distilled Ethyl acetate of the leaves of Solanum melongena.
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