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Evaluation of *In vitro* Antioxidant Activities and *In vivo* Antidiabetic Activity of *Hygrophila schulli* (Buch.-Ham.) Leaf Extract

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Authors' contributions

This work was carried out in collaboration among all authors. This experiment was designed by authors MSA, MAA, MMH and MAR. The plant material was collected, Prepared Extract and performed In-vivo and In-vitro experiments by authors MSA, MAA, MR, MMH, MAR and JSJ. Authors MAA and MAI conducted data analysis and data interpretation. Authors MAA and MMI prepared draft copy of that manuscript. Authors MSA, MAA, MAI, MMH and MAR revised drafted manuscript very carefully. All author read and approved the final draft of the manuscript.

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ABSTRACT

Background: *Hygrophila schulli,* a medicinal herb, has traditionally been used to treat a wide range of ailments such as diarrhoea, dysentery, and cough. The aim of this experiment was to seek the antioxidant and antidiabetic properties of the methanolic extract of *H. schulli* leaf (MEHL) by *in vitro* and *in vivo* study.

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Methods: Methanol was used as a solvent to extract *H. schulli* leaves. *In vitro* antioxidant activity of MEHL was determined by DPPH and ABTS free radical scavenging assay and *in vitro* antidiabetic activity was revealed by α-amylase inhibition assay and α-glucosidase inhibition assay. To explore *in vivo* antidiabetic properties, diabetes was induced in Swiss albno mice by a single intraperitoneal injection of alloxan at a dose of 80 mg/kg body weight. Swiss albino mice were separated into five groups (normal and diabetic) and orally treated with normal pellet diet, water (normal control and diabetic control), glibenclamide (5 mg/kg BW), and MEHL (100 and 200 mg/kg BW).
Results: MEHL showed significant scavenging activity in DPPH and ABTS free radical scavenging assay (IC 50 value is 105.80 µg/mL and 27.47 µg/mL respectively). In the α-amylase inhibition assay, MEHL at a concentration of 120 g/mL inhibited α-amylase activity by 23.13%, furthermore, at 100 g/mL concentration of MEHL, 55.62% inhibition of α-glucosidase activity was detected. Diabetic mice treated with MEHL exhibited a significant decrease in blood glucose levels by 12.66%-33.45% from the 5th to the 21st day in the *in vivo* assay at a concentration of 200 mg/kg body weight. MEHL significantly reduced the activity of serum SGPT and SGOT in diabetic mice as compared to the control group. In diabetic mice, the extract improved TG, TC, LDL, HDL, and VLDL levels as compared to untreated mice.
Conclusion: The current study indicated that *H. schulli* leaf extract is a natural source of antioxidants, has substantial antidiabetic effects and enhances lipid profile markers in diabetic mice and may be utilized as an alternate therapy for diabetes control.

Keywords: Hygrophila schulli (Buch-Ham); antioxidant activity; antidiabetic effect; alloxan; lipid profile.

1. INTRODUCTION

“Nowadays, Diabetes mellitus (DM) is now regarded as one of the world's most prevalent health issues” [1]. “It is a metabolic condition characterized by chronic hyperglycemia and disruptions in carbohydrate, lipid, and protein metabolism caused by abnormalities in insulin production, insulin action, or both” [2]. “According to the International Diabetic Federation (IDF) Atlas 2021, the predicted global pre valence of diabetes in persons aged 20 to 79 years is 537 million people. With a continuous growing tendency, this figure is expected to increase to 643 million by 2030 and 783 million by 2045” [3].

“DM is known to trigger hyperlipidemia through different metabolic derangements, which are found in approximately 40% of people with diabetes, and to weaken antioxidant defense mechanisms through the process of chronic oxidative stress caused by hyperglycemia, which in turn leads to defective insulin gene expression and insulin secretion, as well as increased cell death by apoptosis” [4,5]. “Hyperglycemia-induced metabolic dysfunction may be mediated by reactive oxygen species (ROS) generated in the mitochondrial electron transport chain” [6]. “ROS are physiological metabolites created as a function of respiration in aerobic organisms, but their high levels have been related to the onset of diseases such as cancer, cardiovascular diseases, and diabetes” [7]. “Therefore, the search for antioxidant and antidiabetic compounds from plant sources is an essential tool for combating the condition's broad prevalence. This is because the existing synthetic medications have various downsides ranging from limited efficacy and several side effects such as hypoglycemia, weight gain, as well as chronic tissue damage” [8].

“Traditional herbal remedies have an edge in preventing and treating diabetes since they have fewer adverse effects” [9]. “As a result, identifying plants with potentially useful pharmaceutical outcomes is an important research goal that will aid in generating new remedies to cure a number of disorders” [10]. “According to the World Health Organization, nearly 80% of modern civilizations rely on plant-derived substances. Herbs produce bioactive substances, such as antioxidants, which react with free radicals and prevent cell damage and homeostatic disruption” [11]. “Because of the presence of antioxidants, which are often associated with a variety of polyphenol chemicals, medicinal plants play an important role in preventing or managing diseases” [12]. “According to a previous study, plants are one of the main sources of various medications, and nearly 800 plant species possess antidiabetic potential” [13–16]. “Many plants are already being used for the treatment of DM in traditional medicine” [15–17].

“Hygrophila schulli (Buch-Ham) is a medicinal plant that has long been used to cure dropsy jaundice and urinogenital disorders in Indo-China, Nepal, Tropical Africa, and Bangladesh”

[18]. "Previous studies suggest that leaf and root extracts of *H. schulli* have antibacterial properties against diverse bacterial strains" [19]. "Furthermore, the seed extract of *H. schulli* had strong anti-nociceptive and anti-inflammatory effects in a animal model" [20]. This plant also have antitumor [21,22] and hepatoprotective capacity [23]. There is relatively little scientific data on the pharmacological effects of *H. schulli* (Buch.-Ham.) leaf. Hence, this study aimed to assess the *in vitro* antioxidant and antidiabetic and also *in vivo* antidiabetic activity of *H. schulli* (Buch.-Ham.) leaf extract.

2. METHODS

2.1 Chemicals and Reagents

Sodium phosphate, ammonium molybdate, sulphuric acid, ferric chloride ($FeCl_3$), potassium persulfate (K2S2O7), potassium phosphate dibasic, potassium phosphate monobasic, potassium ferricyanide [$K_3Fe(CN)_6$], 2, 2-diphenyl-1-picryl-hydrazyl (DPPH●), 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS●), trichloroacetic acid (TCA), and ascorbic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol was purchased from E-Mark (Germany). The present study employed only analytical-grade chemicals.

2.2 Collection of Plant Materials and Extractions

The plant, *Hygrophila schulli* (Buch.-Ham.) was collected from Motihar, Rajshahi-6205, Bangladesh, in February 2022 and authenticated by the renowned taxonomist (Voucher no. AA109). The leaves were properly washed with water to get rid of any adherent dirt before being shed dried at 25-28°C. The plant components were then crushed into coarse powder using a milling machine (FFC-15, China) and stored at room temperature for future use. About 100 g of dried *H. schulli* leaf powder was placed in a clean round-bottomed bottle, 400 ml of methanol was added, and the mixture was kept for seven days at room temperature with intermittent shaking and stirring. The mixture was filtered using Whatman No. 1 filter paper and the filtrate was collected. Methanol was then evaporated using a rotary evaporator at 39°C to generate methanolic extract of *H. schulli* Leaf (MEHL). The concentrated extract was stored in vials at 4°C for future experimental use.

2.3 Determination of Total Antioxidant Activity

The MEHL's total antioxidant activity was evaluated using the method of Prieto et al [24] with slight modifications. In brief, 0.3 mL of leaf extract at different concentrations was mixed with 3 mL of reaction mixture containing 0.8 M sulphuric acid, 14 mM sodium phosphate, and 0.4% ammonium molybdate and incubated at 95°C for 10 minutes. After the samples had cooled to room temperature, each aqueous solution's absorbance was measured at 695 nm in comparison to a blank. Ascorbic acid was used as standard.

2.4 Determination of Ferric-Reducing Antioxidant Activity

The ferric reducing power of MEHL was measured using Oyaizu et al [25]. Technique, with some minor changes. Different concentrations of 0.25 mL of extract were added with 0.625 mL potassium buffer (0.2 M, pH 6.7) and 0.625 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated for 20 min at 50°C. After incubation, each tube was filled with 0.625 mL of 10% trichloroacetic acid solution, which was then centrifuged for 10 minutes at 3000 rpm. Clear supernatant (1.8 mL) was collected and mixed with an equal amount of deionized water, 0.36 mL of ferric chloride (0.1% w/v) solution was added and absorbance was taken at 700 nm. Here, ascorbic acid was used as the reference standard.

2.5 DPPH Free Radical Scavenging Assay

MEHL's free radical scavenging activity was evaluated against the 2,2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich) radical using the method described by Cheel et al [26]. In brief, 1 mL of MEHL solution in methanol and ascorbic acid were mixed with 3 mL of DPPH solution (0.1 mM) in methanol. The mixture was left to stand for 30 minutes before the absorbance was measured at 517nm. The following formula was used to calculate the free radical scavenging activity of each sample:

$$\text{DPPH Radical scavenging rate (\%)} = [\{A_0 - A\}/ A_0] \times 100$$

Where "A_0" (control) was the absorbance of DPPH blank solution, and "A" was the sample's final absorbance after 30 minutes of incubation.

2.6 ABTS Free Radical Scavenging Assay

The ABTS method was used according to Re et al [27] with slight modifications. ABTS was dissolved in distilled water at a final concentration of 7mM and mixed with 2.45mM potassium persulfate and before usage, the mixture was allowed to stand for 12-16 hours in the dark at room temperature. In this experiment, ABTS solution was diluted with distilled water to an absorbance of 0.70±0.02 at 734nm. 1mL of various concentrations of the sample was then mixed with 3.0 mL of ABTS solution and vortexed. Following 6 minutes of incubation, the absorbance was measured at 734 nm. Ascorbic acid was employed as the positive control. The following formula was used to calculate the ABTS free radical scavenging activity of each sample:

ABTS radical scavenging rate (%) = [{A_0 – A}/ A_0] × 100

Where "A_0" (control) was the absorbance of ABTS blank solution, and "A" was the final absorbance of the tested sample.

2.7 α-Amylase Inhibition Assay

Screening for α-amylase inhibition by extract was carried out according to the method described by Wang, Y et al [28] with slight modifications. Various concentrations of 50 µL of extrat were added to 25 µL of α-amylase solution (45 units/mL) containing 0.02 M sodium phosphate buffer (pH 6.9 with 6 mM NaCl) and were mixed and then incubated at 25°C for 10 minutes. Following incubation, each tube received 50 µL of a 1% starch solution in 0.02 M sodium phosphate buffer. The reaction mixtures were further incubated at 25°C for another 10 minutes. Then the reaction was stopped with 50 µL of 3M HCl and subsequently 30 µL of a mixture solution of 5 mM I_2 and 5 mM potassium iodide was added to each test tube as color developing reagent. The absorbance of each test tube was measured at 620 nm and the α-amylase inhibitory activity was calculated using the following formula:

% inhibition of α-amylase = [(A_{cont} – A_{samp}) / A_{cont}] x 100

Where A_{samp} was defined as the absorbance of the sample and A_{cont} was the absorbance of the control.

2.8 α-Glucosidase Inhibition Assay

The α-glucosidase inhibition assay was performed using a method described by Schmidt et al [29] with slight modification. In brief, in a 96-well microtiter microplate, each well was added with 50 µL of extract at various concentrations and 90 µL of 0.1M sodium phosphate buffer (SPB) pH containing 0.02% sodium azide. A solution of 80 µL of α glucosidase (2.0 U/ml) in SPB was then added in each well and the mixture was allowed ro incubate at 28° C for 10 minutes. Acarbose was used as a positive control. After the incubation, 20 µL of pNPG (dissolved in SPB) was mixed into the solution to initiate the reaction. The rate of pNPG conversion to p-nitrophenol was determined by the measurement of absorbance of p-nitrophenol at 405 nm using a Multiscan FC microplate photometer (Thermo Fisher Scientific, Waltham, MA, USA).

The % of alpha-glucosidase inhibition was calculated by using the following formula:

% of α glucosidase inhibition= {(Absorbance of Blank – Absorbance of sample) / Absorbance of blank} × 100

2.9 Animal Care

Swiss albino mice weighing 22-25 g were collected from the Department of Pharmacy, Jahangirnagar University for study. They were acclimated to the animal house (temperature 25 ± 2°C and humidity 55 ± 5% along with 12 h light/ dark cycle), fed with commercial pellets, and had free access to water. To begin the examination, food consumption was withrown before 16-18 hours.

2.10 Induction of Diabetes

Diabetes was induced in overnight fasting mice by a single intraperitoneal injection of alloxan (80 mg/kg body weight) in a 0.1M sodium citrate buffer (pH 4.5). Age-matched control mice were given an equivalent amount of citrate buffer. After alloxan was administered, food and liquid intake were closely monitored regularly. A portable glucometer (CERA-CHEK, Korea) was used to check the mice's tail vein blood 48 hours after receiving alloxan to determine the development of hyperglycemia. Mice with fasting blood glucose levels ≥ 11.0 mmol/L were considered diabetic mice [30].

2.11 Experimental Design

After 1 week of the acclimation period, the mice were randomly divided into five groups. Each group consisted of five rats (n=5), and they were treated for four weeks as follows:

Group-1 (Normal control): Mice were provided a regular pellet diet and water.

Group-2 (Diabetic control): Untreated diabetic mice.

Group-3 (Positive control): Glibenclamide was administered to diabetic mice at a dosage of 5 mg/kg BW [31,32].

Group-4 (MEHL 100 mg/kg BW): The diabetic mice were given MEHL at a dose of 100 mg/kg BW for 21 days [33].

Group-5 (MEHL 200 mg/kg BW): The diabetic mice were treated with MEHL at a dose of 200 mg/kg BW for 21 days [32]

2.12 Collection of Blood

Blood samples were collected from the tail vein on days 1, 5, 10, 15, and 21 in a fasting state using a 26 G needle and syringe. After an overnight fasting condition, mice were anesthetized with diethyl ether and sacrificed at the end of the experiment's 21-day duration. Then blood was drawn from the artery of the heart. The serum was segregated by allowing blood samples at a temperature of 25 °C for 10 minutes, followed by centrifuging for 20 minutes at 3000 rpm. Following that, the serum was collected and stored in a plastic container at -80°C until further tests were conducted.

2.13 Measurement of Biochemical Parameters

Parameters of serum lipid profile (Total cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein, and very low-density lipoprotein) and level of liver biomarkers (SGPT and SGOT) were evaluated using commercially available kits (Ultracare diagnostic, Egypt). The biochemical parameters were estimated using the Hitachi 7180 automatic analyzer (Hitachi, Tokyo, Japan).

2.14 Statistical Analysis

Statistical analysis was done with one-way analysis of variance (ANOVA) using GraphPad Prism 8. Differences were considered statistically significant at $p < 0.05$.

3. RESULTS

3.1 Total Antioxidant Capacity

Fig. 1 shows the total antioxidant potentials of *H. schulli* leaf extract and ascorbic acid, evaluated from their ability to reduce Mo (VI) to Mo (V) and the formation of a green phosphate/Mo (V) complex at acidic pH. MEHL showed a gradual increase in absorbance with the increasing concentration of extract (Fig. 1). The antioxidant capacity of this extract might be attributed to its chemical composition and phenolic content.

3.2 Ferric Reducing Antioxidant Activity

Fig. 2 shows the reducing capacity of the methanolic extract of *H. schulli* leaf compared to ascorbic acid. “Authors previously discovered a direct relationship between antioxidant activity and the reducing power of specific plant extracts” [34]. The presence of reductones which have been revealed to exert an antioxidant effect by breaking the free radical chain and donating a hydrogen atom is often associated with the presence of reducing characteristics. The maximum ferric-reducing antioxidant capacity was obtained at a concentration of 200 µg/mL.

3.3 DPPH Radical Scavenging Activity

“DPPH is a stable free radical that can be transformed into a stable diamagnetic molecule by accepting an electron or hydrogen radical” [35]. The extract can reduce the radical DPPH to the yellow-colored diphenyl picrylhydrazine. The scavenging effect of MEHL and standard with the DPPH radical at the dose of 25 µg/ml are 96.09% and 47.07% respectively. From Fig. 3 We observed a dose-response relationship in the DPPH radical scavenging activity; the activity increased as the concentration of MEHL increased. The IC_{50} value for ascorbic acid is 25.782 µg/mL whereas for MEHL IC_{50} is 105.80 µg/mL.

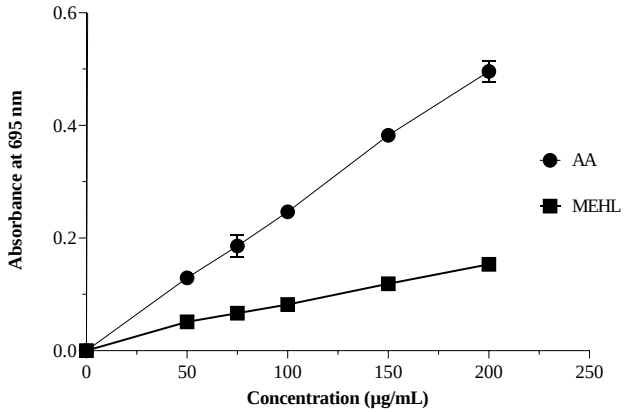

Fig. 1. Total antioxidant capacity of MEHL and ascorbic acid (AA). All data are expressed as mean ± SD (n; number of separate experiments = 3)

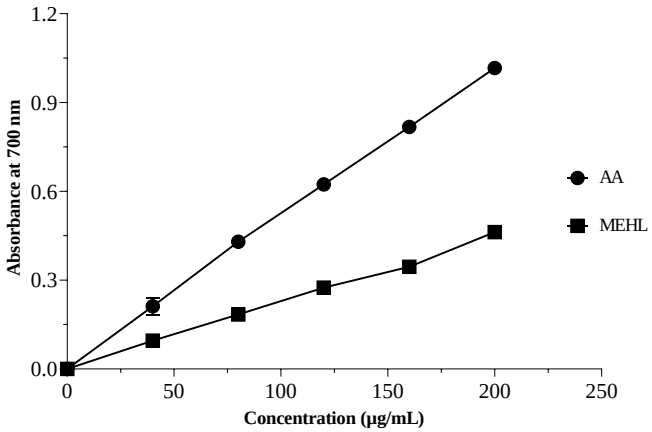

Fig. 2. Ferric-reducing antioxidant activity of MEHL and ascorbic acid. All data are expressed as mean ± SD (n; number of separate experiments = 3)

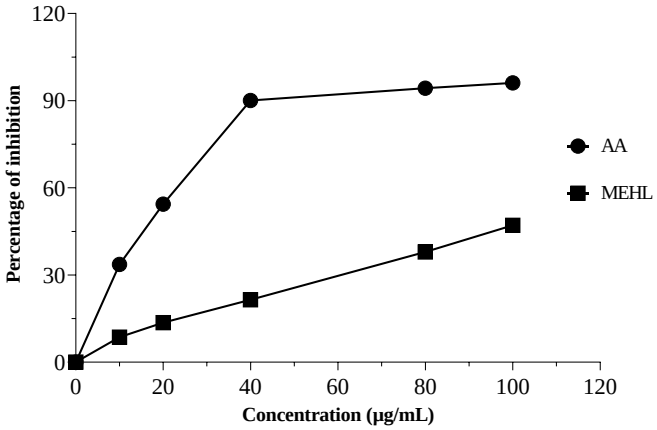

Fig. 3. Scavenging activity of MEHL and ascorbic acid against DPPH radical. All data are expressed as mean ± SD (n; number of separate experiments = 3)

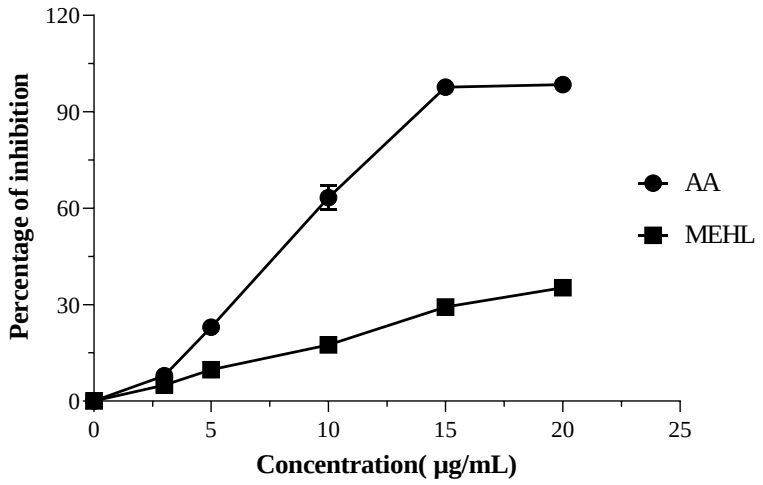

Fig. 4. Scavenging activity of MEHL and ascorbic acid against ABTS radical. All data are expressed as mean ± SD (n; number of separate experiments = 3)

3.4 ABTS Free Radical Scavenging Activity

ABTS• is far more reactive than that of DPPH, and unlike DPPH reactions that require the transfer of a hydrogen atom, ABTS• reactions require the transfer of an electron. In Fig. 4 ascorbic acid revealed 98.45% ABTS• radical scavenging activity at a concentration of 20 µg/mL whereas MEHL Showed 35.271% scavenging activity at the same concentration. The IC_{50} value for ascorbic acid is 9.121 µg/mL on the other hand for MEHL IC_{50} value is 27.47 µg/mL

3.5 α-Amylase Inhibitory Assay

α-amylase inhibitory activity of MEHL is shown in Fig. 5. The assay demonstrated a dose-dependent rise in the percentage of inhibitory activity against α-amylase enzyme. For MEHL, the percent of inhibition of α-amylase activity was 23.13±1.63% at a concentration of 120 µg/mL whereas, at the same concentration, acarbose demonstrated 50.17±2.22% inhibitory activity.

3.6 α-Glucosidase Inhibition Assay

Fig. 6 shows the α-glucosidase inhibitory activity of *H. schulli* leaf extract at various concentrations. The assay demonstrated a dose-dependent increase in inhibitory activity against the α-glucosidase enzyme. The percent of inhibition of leaf at 100 µg/mL was 55.62% and standard acarbose had 80.79% of α-glucosidase inhibition activity at the same concentration. Although the antidiabetic activities of *H. schulli* leaf were relatively low compared to standard acarbose, it might be a good alternative regarding the adverse side effects of synthetic compounds.

3.7 Effects of MEHL on Blood Glucose Level in Alloxan-Induced Swiss Albino

For *in vivo* assay, MEHL was fed orally to diabetic mice to assess the changes in the blood glucose levels. The plant extract significantly changed the blood glucose levels of alloxan-induced diabetic mice (Fig. 7). The Plant extract administered subject demonstrated a reduction of blood glucose level significantly in both concentrations (100mg/kg BW and 200 mg/kg BW). This level of reduction was close to the glibenclamide-administered subject. From the 5th to 21stdays, MEHL at both doses (100 mg/kg and 200 mg/kg body weight) lowered the glucose level by 9.65% - 27.36% and 12.66% - 33.45%, while glibenclamide lowered the blood glucose level by 14.54% - 51.43% compared to the diabetic control group.

3.8 Effects of MEHL on the Parameters of Lipid Profile

Fig. 8 shows the effect of MEHL on serum TC, TG, HDL, LDL, and VLDL levels in the experimental mice. In the experiment, we found a significant increase in the serum level of TC, TG, LDL, and VLDL, and a decreased level of serum HDL in diabetic mice compared to that of normal

control mice. Supplementation of MEHL demonstrated a significant reduction of TC by 10.02% in MEHL 100mg/KG BW and 17.57% in MEHL 200mg/KG BW compared to the DC mice (Fig.8). MEHL 100mg/KG BW and MEHL 200mg/KG BW supplements reduced serum TG 15.78% - 21.02%, LDL 30.37% - 46.16% and VLDL 15.78% - 21.04% compared to the DC mice whereas their is an increase in the serum level of HDL 38.25% - 42.54% compared to the DC mice. Glibenclamide (5 mg/kg body weight) treated mice showed a reduction of TC by 30.34%, TG by 32.52%, LDL by 67.82%, VLDL by 32.52%, and an increase in HDL by 47.02%.

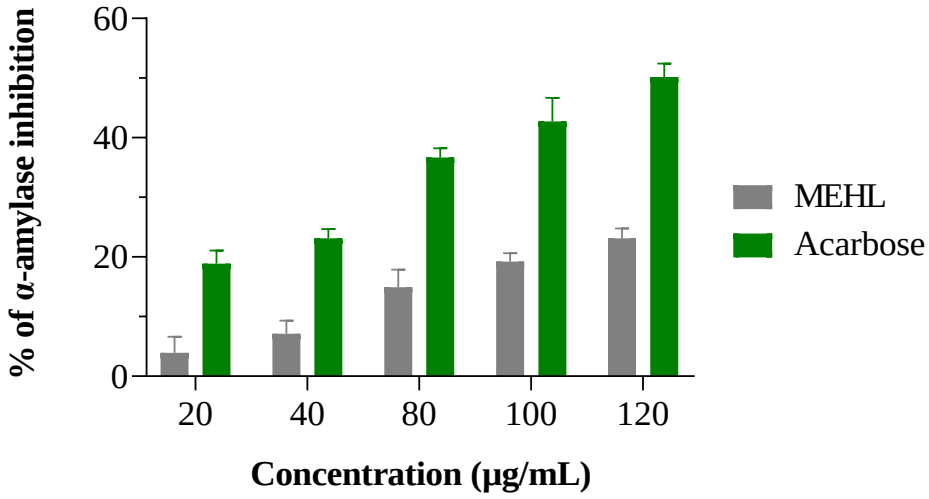

Fig. 5. α-amylase inhibition activity of MEHL. All data are expressed as mean ± SD (n; number of separate experiments = 3)

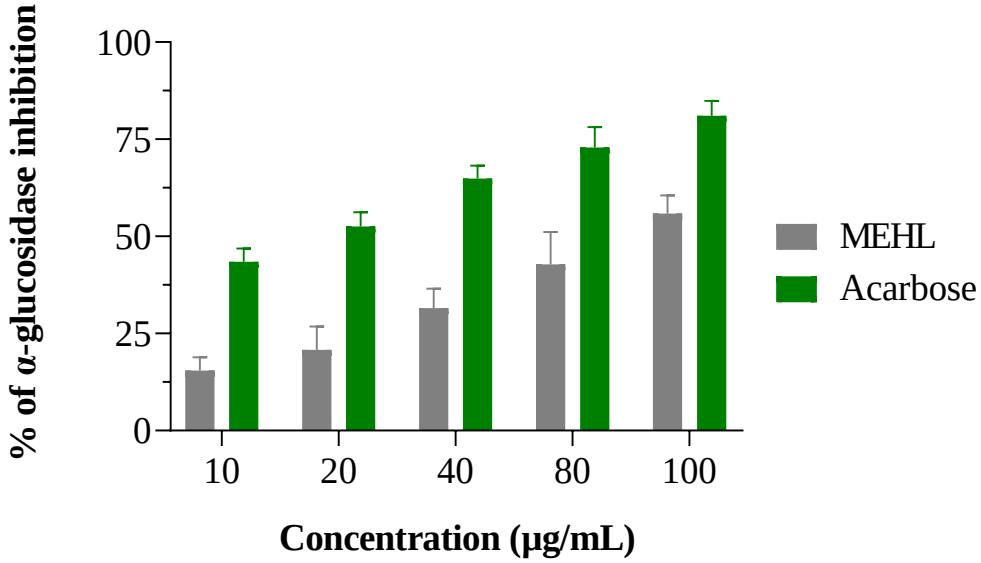

Fig. 6. α-glucosidase inhibition activity of MEHL. All data are expressed as mean ± SD (n; number of separate experiments = 3)

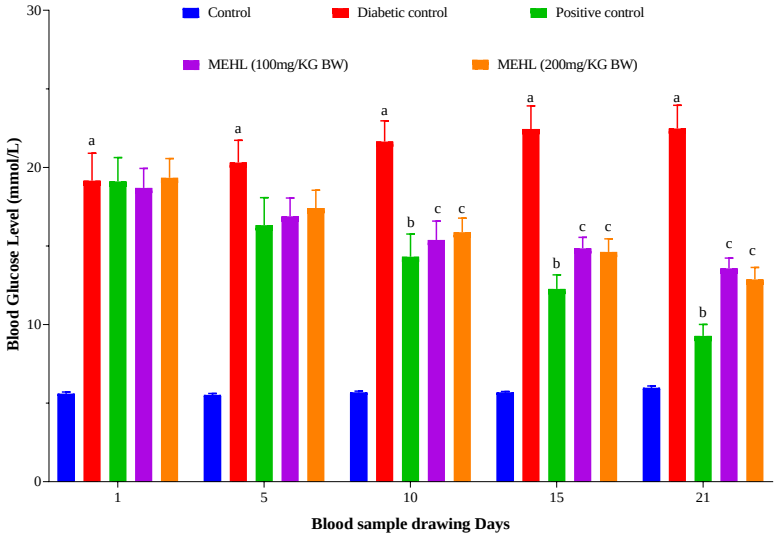

Fig. 7. Change of blood glucose level after methanolic extract of *H. schulli* Leaf (MEHL) treatment in alloxan-induced diabetic mice. All data are expressed as mean ± SEM (n; number of mice = 5). Here "a" indicates p < 0.0001 vs normal control mice, "b" indicates p < 0.001 vs diabetic control mice and "c" indicates p < 0.01 vs diabetic control mice

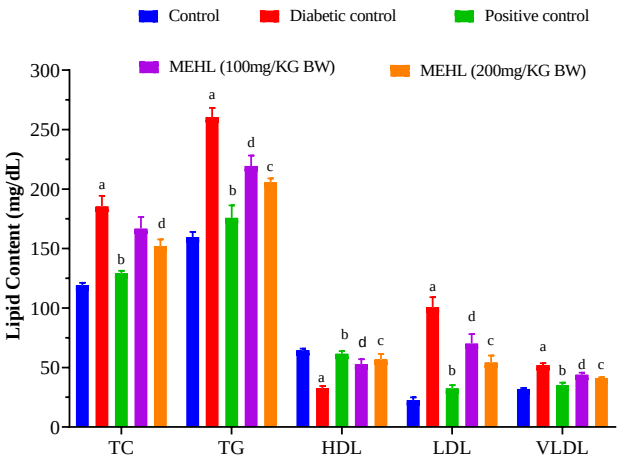

Fig. 8. Effects of Methanol extract of *H. schulli* leaf (MEHL) on lipid profile of diabetic mice after 21 days of treatment. All data are expressed as mean ± SEM (n; number of mice = 5). Here "a" indicates p < 0.0001 vs normal control mice and "b" indicates p < 0.001 vs diabetic control mice, "c" indicates p < 0.01 vs diabetic control mice, "d" indicates p < 0.05 vs diabetic control mice

3.9 Effects of MEHL on Serum SGPT and SGOT

Fig. 9 shows the effects of MEHL supplementation on the activity of serum SGPT and SGOT in diabetic mice. There was a significant (P< 0.001) increase in SGPT and SGOT levels (Fig. 9) in diabetic mice than that of normal control. Compared to diabetic control mice, MEHL decreased the activity of serum SGPT by 16.65% (100 mg/kg BW) and 26.75% (200 mg/kg BW), whereas glibenclamide reduced the activity of serum SGPT by 35.86%. The reduction of SGOT level with the treatment of MEHL was 15.14% (at 100 mg/kg BW) and 19.25% (at 200 mg/kg BW), whereas glibenclamide lowered SGOT activity was 30.85%.

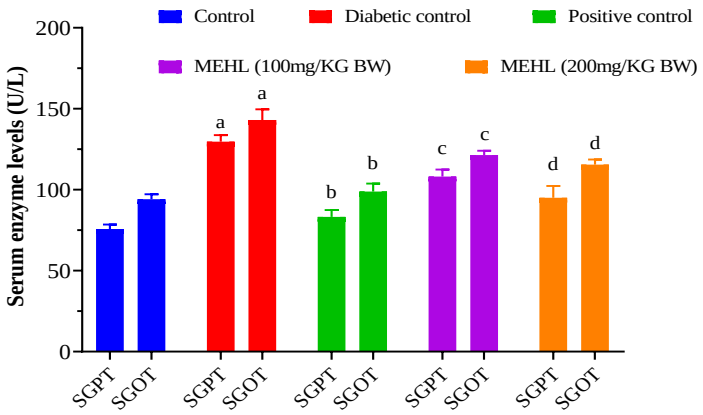

Fig. 9. Effect of *H. schulli* leaf (MEHL) on serum SGPT and SGOT in diabetic mice after 21 days of treatment. All data are expressed as mean ± SEM (n; number of mice = 5). Here "a" indicates p < 0.0001 vs normal control mice, "b" indicates p < 0.001 vs diabetic control mice and "c" indicates p < 0.05 vs diabetic control mice, "d" indicates p < 0.01 vs diabetic control mice

4. DISCUSSION

"Diabetes mellitus, one of the most common chronic diseases, is connected with hyperlipidemia and comorbidities such as obesity and hypertension" [36]. "Many new bioactive phytochemicals derived from plants with hypoglycemic and antihyperglycemic activities demonstrate the same antidiabetic action and are often even more effective than existing oral hypoglycemic medications" [37,38]. Therefore, identifying new antidiabetic molecules with fewer side effects is particularly crucial. The current study focused on the antidiabetic and antioxidant properties of a methanolic extract of *H. schulli* leaf in alloxan-induced diabetic mice, both *in vitro* and *in vivo*.

"All of the data in this study demonstrated that MEHL has significant antioxidant capacity and has antidiabetic and antioxidant effects both in vitro and in vivo. The most common feature of diabetes is elevated blood glucose levels, resulting from a lack of insulin or insulin resistance" (2). Orally Treatment of MEHL reduced blood glucose levels dramatically in diabetic mice compared to that of diabetic control mice and the level of blood glucose level reduction by MEHL is almost close to glibenclamide-treated mice. These findings were similar with distinct findings for MEHL components in some prior investigations [39, 40]; therefore our present study supports the results of the previous studies.

"Hyperlipidemia is common in DM because DM affects lipid metabolism. In DM elevated levels of TG, TC, LDL, and VLDL and decreased level of HDL is found, these anomalies lead to the development of cardiovascular diseases also" [41,42].

This study found significantly lower levels of serum TC, TG, LDL, and VLDL, as well as higher levels of HDL, in MEHL-treated diabetic mice compared to diabetic control animals. These data represent possible potential effects of MEHL to reduce or prevent DM-associated complications in lipid metabolism.

"Chronic DM also causes liver damage by altering the metabolism of lipids, carbohydrates, and proteins, which can develop to non-alcoholic steatohepatitis, liver cirrhosis, and finally hepatocellular carcinomas, boosting oxidative stress and inflammatory response" (43). In diabetic patients, liver damage leads to increased activity of liver function enzymes such as SGPT and SGOT. MEHL treatment for 21 days significantly reduced SGPT and SGOT levels in diabetic mice as compared to diabetic control mice.

5. CONCLUSION

This research work suggests the effects of MEHL in controlling the abnormalities of diabetes as well as alleviating the development of cardiovascular diseases. Moreover, MEHL eases liver damage from chronic diabetes. Therefore, our study reveals that MEHL has antioxidant potential and may have favorable benefits for the treatment of diabetes mellitus and diabetes-related problems in the future.

ETHICAL APPROVAL

The experimental protocol was approved by the Institutional Animal, Medical Ethics, Bio-Safety and Bio-Security Committee (IAMEBBC) for Experimentations on Animal, Human, Microbes, and Living Natural Sources, Memo No: 249(35)/320/IAMEBBC/IBSc. Institute of Biological Sciences, University of Rajshahi, Bangladesh.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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