Original Research Article

Assessing the Antidiabetic properties of Methanolic extract of *Baccaureamotleyana* leaf in Alloxan-induced diabetic mice

Abstract

Background: Medicines made from *Baccaureamotleyana* are used to treat a wide range of illnesses. The current study targeted to investigate the methanolic extract of *Baccaureamotleyana* leaf's antidiabetic qualities.

Results: The leaf extract of *Baccaureamotleyana*at a concentration of 100µg/ml, the α-amylase activity was found to be 37.01% inhibited and the α-glucosidase activity to be 55.05% inhibited in the α-amylase inhibition experiment. The leaf extract considerably ($P < 0.05$) reduced blood glucose levels and improved lipid profile levelssuch as (TG, LDL,TC, VLDL& HDL) in diabetic mice, based on the findings of antidiabetic tests conducted in*in-vivo*. When compared to diabetic animals that were not treated, treatments with the extract of methanol of *Baccaureamotleyana* leaf for 24 days significantly ($P < 0.05$) decreased the effects of the ALT and AST enzymes in the diabetic mice (alloxan induced) also.

Conclusion: According to this study, the leaf extract of *Baccaureamotleyana* has strong antidiabetic effects. Because of this, they may be able to help avoid diabetes mellitus and its complications.

Keywords: *Baccaureamotleyana*, Antidiabetic effects, α-amylase,α-Glucosidase, Alanine transaminase, Aspartate aminotransferase lipid profile.

Introduction

Feedstocks are inexpensive and renewable for that reason phytoextract is a dependable source for discovering new bioactive compounds and developing new drugs. When it comes to drug development, phytobioactive compounds are superior to synthetic compounds because of their profound ability to interact or attach with a wide range of drug targets, such as transporters, signaling molecules, hormones,nucleic acids, enzymes,receptors and ligands [1].Because product related to phytoextract are believed to be less harmful effects and have less adverse effects than synthetic products, there has been an increase in demand for it in recent years as flavoring and agrochemicals,food additives,cosmetics, and perfumes [2]. Therefore, finding plants with potentially advantageous pharmacological effects is an important research objective that will help create new treatments for a range of illnesses.

Diabetes mellitus is a chronic condition that occurs when the body is unable to use insulin properly or the pancreas is not able to produce enough insulin. Consequently, diabetes mellitus is characterized by elevated blood glucose levels [3]. In patients with diabetes mellitus, elevated blood glucose levels have an impact on lipid, protein, and carbohydrate metabolism [4]. Insulin resistance and insufficiency affect numerousmetabolic pathways and enzymes involved in the metabolism od lipid. Diabetes mellitus is defined by elevated levels of triglycerides (TG), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL), and decreased levels of HDL(High-density lipoprotein) [4,5]. Every system in a patient's body is impacted by diabetes mellitus, also known as metabolic illness, including the liver.[6]. There have been prior reports of non-alcoholic liver steatosis,liver cirrhosis, liver cancer, and elevated liver enzymes including ALT and AST in people with diabetes mellitus [7, 8]. Polysaccharides are broken down into monosaccharides and disaccharides by the enzymes α-glucosidase and α-amylase. In patients

with type I and type II diabetes mellitus, the inhibitor of these enzymesare also utilized to reduce blood glucose levels [9]. Many medications are commercially available to treat diabetes mellitus, however long-term usage of these medications can have negative side effects. Thus, researchers are searching for phytomedicines with low levels of toxicity and little to no negative effects [10,11]. Finding new medications that treat diabetes mellitus more effectively and with fewer side effects is therefore crucial [12].

The Phyllanthaceae family includes *Baccaureamotleyana* [13], which is found in Bangladesh and South-East Asia, especially in Indonesia,Malaysia, and Thailand. The plant *Baccaureamotleyana*, also known as Lotkon, is well-known for its fruits and is used as medicine traditionally to treat a variety of conditions, such as ulcers,fevers, blood disorders, and sensations of burning. Another sign of peel extract's of this fruits has possible anti-diabetic effects is its ability to increase liver glycogen levels [14]. This experiment was conducted to determine the antidiabetic effects of extract of methanol of *Baccaureamotleyana* leaf in alloxan induced diabetic rats as well as Verify scientifically the plant's medicinal preparation for diabetes management. From our searching, this is the first experimental report that works on antidiabetic potential of *Baccaureamotleyana* in alloxan induced rats.

Materials and Methods

Plant material and extraction

Fresh leaves of *Baccaureamotleyana* were gathered from Rajshahi, Bangladesh's Puthia Nursery, located at Puthia-6260. Awell known taxonomist from the Botany Department at the University of Rajshahi in Bangladesh (Voucher number:AA056) thoroughly recognized the sample. Only Fresh leaf were gathered and thoroughly cleaned. These leaves had dried properly. Following that, it was ground into a powder. 300ml of methanol were used to soak 60g of powdered *BaccaureaMotleyana* leaves in a conical flask. After that, the 500mL conical flask was properly sealed, and the flask was maintained in a rotating shaker for ten days. Following a thorough filtering process using Whatman No. 1 filter paper, the filtrate was gathered. Ultimately, a dry leaf extract was produced by evaporating the methanol.

In-Vitro assays

α-Amylase inhibition activity assay

With minor modifications, the method outlined by Xiao et al. [15] was used to screen for extracts' ability to inhibit α-amylase. A solution of 0.5 mg/ml of α-amylase (from pig pancreas, Sigma-Aldrich, USA) was added to 0.5 ml of 0.02 M Na_3PO_4 buffer (6 mM NaCl; pH = 6.9) containing varying amounts of 0.5 ml leaf extracts. The whole mixture was then incubated for around 10 min at 37°C. After that, 0.5 ml of soluble starch (1% w/v) was mixed to the test tube, and it was incubated at 37°C for 15 min. Later, stopping the enzymatic reaction by adding with 20µl of 1 M HCl, 0.1ml of iodine reagent was added(5 mM Iodine and 5 mM Potassium Iodide). The color of that solution was changed and take final absorbance was at 620 nm. For conducting

this experiment, acarbose was used as a Standard. Using the following formula, the results were expressed as the percentage of inhibition.:

Inhibition activity of α − amylase = $[1-\{(A_1-A_2)/A_0\}]*100$

Where A_2 is the absorbance of the product control (sample without α -amylase solution), A_1 is the absorbance of the test sample, A_0 is the absorbance of the negative control (α -amylase without extract)

α-Glucosidase inhibition assay

The method explained by Schmidt et al. [16] was used to perform the Inhibition of α-glucosidase enzyme activity. In this method, 90µl of 0.1M Na_3PO_4 buffer pH containing 0.02% sodium azide and 10µl of the extract and standard at different concentrations were placed in a 96-well microplate. It was then treated for 10 minutes at 28°C with 80µl of α-glucosidase enzyme (Sigma-Aldrich, USA) solution (2.0 U/ml) in Na_3PO_4 buffer in each well. After the incubation period, 20µl of PNPG (4-Nitrophenyl β-D-glucopyranoside) (0.4 mM, dissolved in Na_3PO_4 buffer) was added to the solution to start the chemical reaction. The rate of 4-Nitrophenyl β-D-glucopyranoside conversion to p-nitrophenol was measured using a Multiscan FC microplate photometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) by measuring the absorbance of the color solution of p-nitrophenol at 405 nm. Acarbose was used as a standard. The following formula was used to measure the proportion of α-glucosidase enzyme inhibition:

α – glucosidase enzyme inhibition activity = {(absorbance of blank solution -absorbance of sample solution)/absorbance of blank solution} *100

Animal care

The Institute of the Biological Sciences (IBSc) regulations of the University of Rajshahi in Bangladesh served as the guidelines for all animal studies. (Memory number: 320/IAMEBBC/IBSc/249(35)). For the aim of acclimatization prior to the experiment, male Swiss albino rats weighing 25–30g were appropriately maintained in a laboratory setting with a temperature of 25±3 ºC and relative humidity of 55ºC±5%. A 12-hour cycle of light and dark was maintained throughout this experiment.

In-Vivo study

Diabetes induction and assessment

Age-matched control rats were given citrate buffer, while overnight-deprived rats got a single intraperitoneal injection of Alloxan (80mg/kg) produced in 0.1M citrate buffer, pH 4.5. After six days of alloxan administration, hyperglycemia was confirmed by drawing blood from the tail vein and measuring plasma glucose levels using a Glucometer (CERA-CHEK 1070, Korea). Mice with blood glucose levels between 15 and 17 mmol/L were selected for this investigation. The following five groups (n = 5) of acclimated, healthy mice were randomly assigned: untreated mice (control), alloxan-induced diabetic control mice group, alloxan-induced diabetic mice treated with standard medicine(glibenclamide tablet, 5.0mg/kg body weight), alloxan-induced diabetic mice treated with extract od methanol of *Baccaureamotleyana* leaf with two dose (200 mg/kg), and (400 mg/kg) group.

Oral glucose tolerance test (OGTT)

For this experiment, A previously reported technique was used, but with minor changes[26]. All Swiss-albino mice were starved for 16 hours before to the testing. Mice (Groups 4 and 5) were

given oral doses of MEBML 200mg/kg and 400mg/kg Body weight, respectively, and then given an oral glucose solution (1g/kg body weight). Mice in Group 3 received oral glucose delivery (1 g/kg body weight) after being treated with glibenclamide, an antidiabetic medication, at dose of 5mg/kg body weight. In contrast, first group mice did not receive glucose solution.

Afteradministration oral glucose solution, tail vein blood samples were obtained at 0 min, 30 min, 60 min, 90 min, and 120 min. Glucose levels were then measured by vein puncture from tail by using a portable glucometer (Cera-Chek 1070, Korea) [17].

Statistical analysis

The mean ± SEM (standard error of mean) was used to express all values. Dunnett's test was performed after the information was analyzed using ANOVA (one-way analysis of variance); a p-value of less than 0.05 was deemed statistically significant.

Results

α-Amylase and α-glucosidase enzyme inhibition activity

Baccaureamotleyana leaf showed the significant inhibitory activity against α-amylase and α-glucosidase enzymeand the results are presented in (Fig. 1 a, b).

Evaluation of *in-vivo* anti-diabetic activity

Baccaureamotleyana leaf significantly reduced blood glucose levels ($p<0.005$) for the course of the 24-day treatment. In comparison to diabetic control mice, the methanolic extract of *Baccaureamotleyana* leaf reduced the glucose level by 50.92% and 60.64% at eachdoses (200mg/kg and 400mg/kg BW) (Fig. 2a)

Serum transaminase and lipid profile

Glibenclamide (5 mg/kg) and leaf of methanolic extract supplementation (200mg/kg and 400mg/kg) decreased TG,LDL, TC, and VLDL compared to diabetic mice after 24 days. HDL, on the other hand, jumped considerably. Simultaneously, the diabetic control group's serum transaminase levels (ALT and AST) increased dramatically before returning to normal. The information is shown in Figs. 2b and c.

Discussion

The hallmark of diabetes mellitus, a common metabolic disorder defined by elevated level of plasma glucose caused by peripheral insulin resistance or inadequatesynthesis pancreatic insulin, is a high postprandial blood glucose level [18]. By using this technique, the important enzymes α-amylase and α-glucosidase, which are participated in the digestion of starch and glycogen [19], help to regulate postprandial glucose levels. [20]. Inhibiting α-amylase and α-glucosidase, which

reduces postprandial hyperglycemia by delaying glucose intake, is therefore acknowledged as a successful technique for managing type 2 diabetes and related issues [21].The results clearly showed that *Baccaureamotleyana*methanolic leaf extract has significantly inhibited α-amylase and α-glucosidase enzyme activity, suggesting that the leaf of that species may have a stronger ability to suppress elevated blood sugar levels through its expanded reducing capability for the digestion of starch substrates.Using alloxan-induced diabetic mice, the postprandial plasma sugar level suppression capacity of solvent extracts was used to further evaluate the therapeutic efficacy of *Baccaureamotleyana* leaf extract against diabetes (Fig. 2 a). Based on earlier research, we may infer that leaf extracts from *Baccaureamotleyana* may have ability to repaired impaired glucose level homeostasis through an insulin-secreting mechanism or by encouraging β-cell regeneration by lowering oxidative stress andpreventing damage of DNA [22]. Because of the altered metabolism ofcarbohydrates, lipids, and patients with diabetes mellitus often have lower levels of HDL and higher levels of TC,TG, LDL, and VLDL in their serum [23–25]. These abnormalities are connected to the development of cardiovascular diseases in individuals with diabetes mellitus as well as the course of the disease [23]. When compared to diabetic control mice, diabetic mice fed a methanolic extract of leaves had considerably lower blood levels of TC, LDL,TG, and VLDL and higher levels of HDL. These findings indicate potential benefits of utilizing *Baccaureamotleyana* leaves to prevent or reduce lipid metabolism-related DM problems.

Because it absorbs and stores glucose as glycogen, changes it into glucose when needed, and creates glucose from non-carbohydrate sources such amino acid, the liver is an essential organ for regulating blood sugar levels. [26]. Amino acid conversion to keto acids is carried out by the

liver's enzymes ALT and AST, and elevated levels of keto acids may result from liver damage that allows them to seep into the blood. [27]. AST and ALT levels may rise and liver damage may result from diabetes mellitus [28–30]. The AST and ALT levels of the alloxan-induced mice were significantly higher than those of the control animals in this study.(Fig. 2 c). AST and ALT levels in the plasma were considerably lower after 24days of treatment with *Baccaureamotleyana* leaf extract (200mg/kg and 400mg/kg) than in the group of diabetic control mice. According to the study's findings, when diabetic rats were given a methanolic extract of *Baccaureamotleyana* leaf, their plasma levels of the enzymes ALT and AST were reversed to normal levels in comparison to the mean values of the alloxan induced diabetic group. As a result, this study also shows that *Baccaureamotleyana* leaf extract may lower the risk of liver and cardiovascular damage linked to diabetes mellitus.

Conclusion

In conclusion, our study found that *Baccaureamotleyana* leaf methanolic extract has potent antidiabetic effects both *in-vitro* and in animals. Furthermore, the altered levels of TG, TC, LDL, HDL,VLDL, ALT, and AST may be restored in diabetic mice treated with *Baccaureamotleyana* leaf extract. Therefore, our study suggests that *Baccaureamotleyana* leaf may be utilized to prevent and treat diabetes mellitus and its associated conditions. The specific anti-diabetic compounds included in *Baccaureamotleyana* leaves and their mode of action, however, require further investigation.

Abbreviations

ANOVA: Analysis of variance; TC: Total cholesterol; TG: Triglyceride; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low-density lipoprotein; ALT: Alanine Transaminase; AST: Aspartate Transaminase; SD: Standard deviation.

Disclaimer (Artificial intelligence)

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Declarations

All authors have approved this manuscript. The content of this manuscript or any portion thereof has not been published or submitted for publication elsewhere.

Ethics approval and consent to participate

This research work 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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Tables

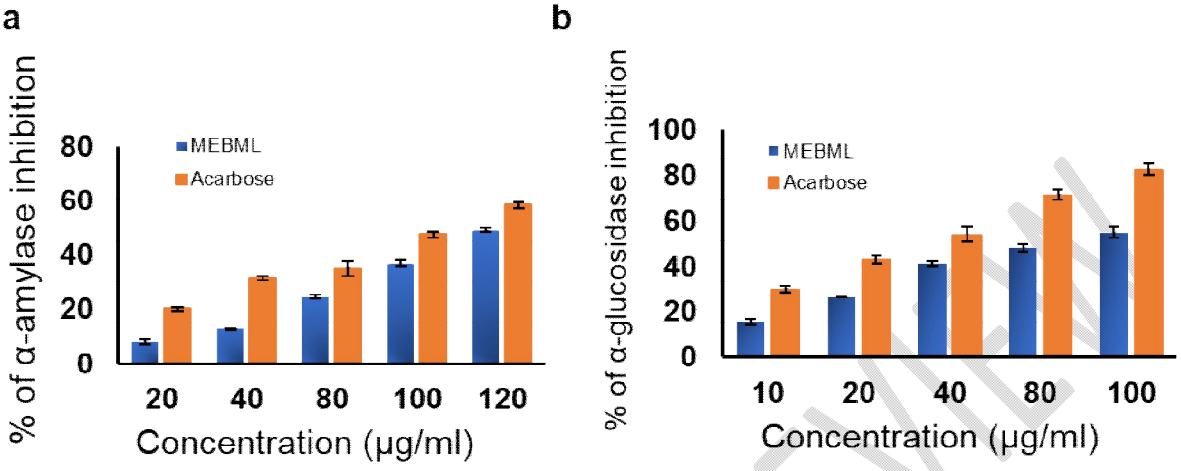
Table 1 Effects of the MEBML on blood glucose levels of diabetic mice in OGTT

Group	Animal Groups	Dose (mg/kg BW)	Blood Glucose level				
			Time after administration of a single dose of Extracts (minutes)				
			0	30	60	90	120
Group 1	Normal	-	4.8±1.2	6.2±1.5	7.3±1.2	5.6±0.7	4.6±1.8
Group 2	Diabetic	-	17.3±1.4	22.8±0.7	24.9±1.4	19.4±1.3	15.6±2.3
Group 3	Positive control	5	7.2±0.8	9.3±0.9	11.4±1.8	9.2±1.3	6.1±0.6
Group 4	MEBML	200	9.2±1.2	14.9±2.8	13.0±1.1	10.2±1.9	9.1±2.6
Group 5	MEBML	400	7.9±1.5	15.9±2.7	15.1±3.1	10.8±2.1	8.1±1.9

All values are expressed as mean ± SD (n; number mice = 5). Here "*" indicates $P \leq 0.05$ compared to diabetic control mice

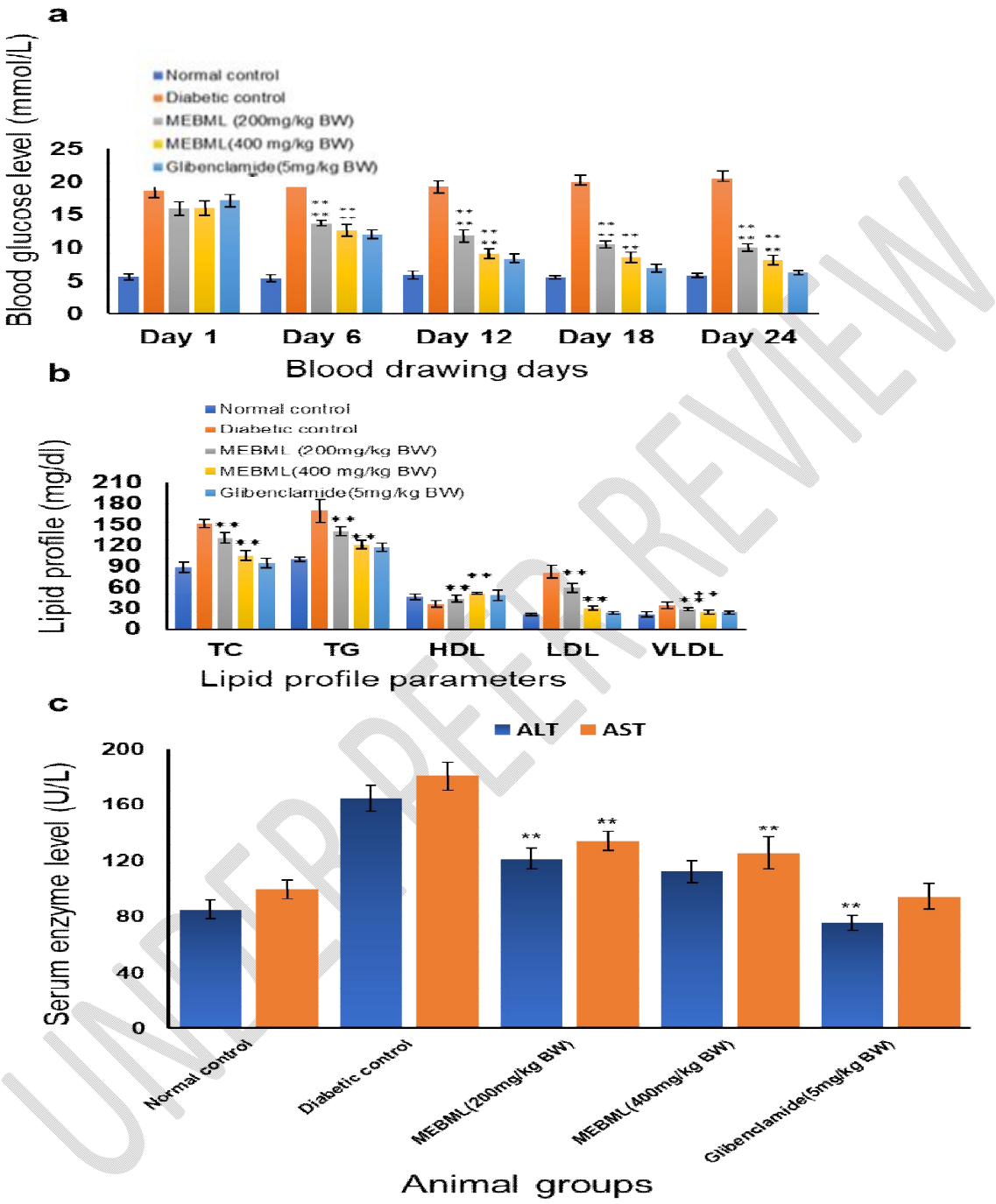
Figures and legends

Fig.1



α-Amylase and α-glucosidase inhibition activity: (a, b) 6 α-amylase and α-glucosidase inhibition activity of methanolic extract of *Baccaureamotleyana* leaf (MEBML). All data are expressed as mean ± SD (n = 3).

Fig 2



Evaluation of *in-vivo* anti-diabetic activity: (a, b, & c) Effects of Methanolic extract of *Baccaureamotleyana* leaf (MEBML) treatment on blood glucose levels, lipid profile level and ALT, AST level in alloxan-induced diabetic mice. All data are expressed as mean ± SD (n = 5). Here "**" indicates $P \leq 0.05$ vs diabetic control mice.