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Study on the Antidiabetic Properties of Methanolic Extract of *Baccaureamotleyana* Leaf in Alloxan-Induced Diabetic Mice

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ABSTRACT

Background: *Baccaureamotleyana* is used as a source of medicine for treating a large number of diseases. The current study aimed to explore In-vitro and In-vivoantidiabetic properties of methanolic extract of *Baccaureamotleyana* leaf.

Results: In α-amylase inhibition assay, 37.01% inhibition in the activity of α-amylase and 55.05% inhibition of α-glucosidase activity was observed at 100µg/ml concentration of the extract. The results of in vivo antidiabetic assays showed that the extract significantly ($P \leq 0.05$) decreased blood glucose levels and ameliorated parameters of lipid profile (TG, TC, LDL, VLDL, and HDL) in diabetic mice. Methanolic extract of *Baccaureamotleyana* leaf treatments for 24 days also reduced the activity of ALT and AST enzymes of diabetic mice significantly ($P \leq 0.05$) compared to that of untreated diabetic mice.

Conclusion: This study suggests that *Baccaureamotleyana* leaf extract possesses significant antidiabetic properties. For that reason, they might play a potential role to prevent diabetic mellitus and diabetic mellitus associated complications.

Keywords: *Baccaureamotleyana*, antidiabetic activity, α-amylase, α-Glucosidase, lipid profile.

Introduction

For identifying novel bioactive chemicals and creating new medications, phytoextract is a reliable source due to the low cost and renewable nature of its feedstocks. Phytobioactive compounds have a profound ability to attach or interact with a broad range of drug targets, including transporters, signaling molecules, nucleic acids, enzymes, hormones, ligands, and receptors, which allow them a superior benefit over synthetic compounds in the development of drug [1]. The demand for phytoextract as flavoring and food additives, agrochemicals, cosmetics and perfumes has risen in current years because products based on phytoextract are thought to be less detrimental and have fewer side effects than synthetic products [2]. Consequently, discovering plant with potentially beneficial pharmacological outcome is a crucial research goal which will aid in developing new remedy in order to treat a variety of diseases.

When the pancreas cannot make enough insulin or the body cannot utilize it correctly, diabetes mellitus, a chronic disease, develops. As a result, increased blood glucose levels are observed in Diabetes mellitus [3]. When the blood glucose level increased that affects carbohydrate, protein, and lipid metabolism in Diabetes mellitus patients [4]. Many enzymes and metabolic pathways involved in lipid metabolism are influenced by insulin shortage and insulin resistance. Lower levels of High Density lipoprotein (HDL) and increased levels of triglycerides (TG), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) are all highly frequent in diabetes mellitus [4,5]. Diabetes mellitus, sometimes referred to as metabolic disease, affects every system in a patient's body, including the liver [6]. In Diabetes mellitus, liver enzymes acts abnormally, such as: Alanine Transaminase (ALT) and Aspartate Transaminase (AST), liver cirrhosis, non-alcoholic liver steatosis, and liver cancer were reported earlier [7,8]. The enzymes α-glucosidase and α-amylase break down polysaccharides into monosaccharides and disaccharides. These enzyme inhibitors are also used to lower blood glucose levels in people with type I and type II diabetes mellitus[9]. There are many drugs commercially available for the treatment of Diabetic mellitus but ln- term use of these drugs occur some adverse effects. Therefore, scientists are looking for phyto-medicines having least or zero adverse effect and low level toxicity [10,11]. Therefore, it is very important to find out new drugs for the treatment of diabetes mellitus with higher efficiency and less adverse effects [12].

Baccaureamotleyana is belong to the Phyllanthaceae family [13] and is widely distributed throughout Bangladesh, and South-East Asia, particularly in Malaysia, Indonesia, and Thailand. *Baccaureamotleyana* called Lotkon, Rambai is a well-known plant for its fruits and is used as a folk medicine to medicate a range of ailments, including respiratory and fevers, ulcers, blood diseases, and burning sensations. The capacity of fruit peel extract to raise

liver glycogen levels is another indication of its potential as an anti-diabetic effect [14]. The present study was conducted to study the antidiabetic activity of Methanolic extract of *Baccaureamotleyana* leaf in alloxan induced rats as well as Scientifically validate the therapeutic preparation of this plant in the control of diabetes. From our knowledge, this is the first report that works on antidiabetic potential of *Baccaureamotleyana* in alloxan induced diabetic rats.

Materials and Methods

Plant material and extraction

*Baccaureamotleyana*freshleaves were collected from the Puthia Nursery, Puthia-6260 in Rajshahi, Bangladesh. The sample was thoroughly identified by a taxonomist from the Department of Botany at the University of Rajshahi in Bangladesh (Voucher number- AA056).

Fresh Leaves were collected and washed properly. The leaves was Dried. After that, it grinded to obtain the powder form. In a Conical flask, 60g of Powdered *Baccaureamotleyana* leaves were soaked in 300 ml methanol. The container was then sealed correctly and kept the flask in a rotatory shaker for 10 days. Then the filtrate was collected by properly filtering by Whatman No. 1 filter paper. Finally, the methanol was evaporated and dry extract was obtained.

In-Vitro assays

α-Amylase inhibition activity assay

Screenings for the inhibition of α-amylase by extracts were conducted according to the technique described by Xiao et al. [15] with small modification. Different concentrations of 0.5ml leaf extracts were taken to 0.5ml of 0.02 M sodium phosphate buffer (6 mM sodium chloride; pH = 6.9) containing 0.5 mg/ml of α-amylase (from porcine pancreas, Sigma-Aldrich, USA) solution, and incubated for 10 min at 37ºC. Then the test tube was filled with 0.5ml of soluble starch (1% w/v), which was then incubated for 15 min at 37 °C. The enzymatic reaction was then stopped by adding 20µl of 1 M HCl, and then 0.1 ml of iodine reagent was added. (5 mM I_2 and 5 mM KI). The changing of color was found and the final absorbance was read at 620 nm. Acarbose was used as a Standard. The results were expressed as % inhibition calculated using the formula:

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

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

α-Glucosidase inhibition activity assay

The α-glucosidase inhibition activity was conducted by the technique narrated by Schmidt et al. [16]. In a 96 well microplate, 10 µl at various concentrations of extract and standard and 90 µl of 0.1M sodium phosphate buffer (SPB) pH containing 0.02% sodium azide was taken. After that, 80 µl of α-glucosidase (Sigma-Aldrich, USA) solution (2.0 U/ml) in sodium phosphate buffer were taken in each well, and the mixture was incubated for 10 min at 28°C. Following the incubation, the reaction was started by adding 20µl of PNPG (0.4 mM, dissolved in SPB) to the solution. Using a Multiscan FC microplate photometer, the absorbance of p-nitrophenol at 405 nm was read to assess the rate of PNPG conversion to p-nitrophenol (Thermo Fisher Scientific, Waltham, MA, USA). Acarbose was used as a standard. The following equation was used to determine the proportion of α-glucosidase inhibition:

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

Animal care

All animal experiments were guided according to the regulation of the institute of the biological sciences (IBSc), University of Rajshahi, Bangladesh. (Memo No: 249(35)/320/IAMEBBC/IBSc). Male swiss albino rats 25-30g were properly sustained under the laboratory conditions for Acclimatization purpose before the experiment (temperature of 25±3 ºC with relative humidity 55 ºC±5%). In this experiment, 12h dark and light cycle was maintained.

In-Vivo study

Induction and assessment of Diabetes

A single intraperitoneal injection of Alloxan (80 mg/kg), prepared in 0.1 M citrate buffer, pH 4.5, was given to overnight deprived rats, whereas age-matched control rats simply received citrate buffer. By taking blood from the tail vein and using a Glucometer to measure plasma glucose levels, hyperglycemia was confirmed after 6 days of alloxan administration (CERA-CHEK 1070, Korea), and the mice with 15–17 mmol/L blood glucose level were taken for this experiment. Acclimatized healthy mice were randomly separated into the following five groups (n = 5): untreated (control) group, alloxan-induced diabetic control group, alloxan-induced diabetic mice with standard (glibenclamide, 5.0 mg/kg body weight) treated group, alloxan-induced diabetic mice treated with methanolic extract of *Baccaureamotleyana* leaf (200 mg/kg) group and (400 mg/kg) group.

Oral glucose tolerance test (OGTT)

With minor adjustments, a previously documented technique was used for this test [26]. Before the trial, all swiss albino mice were kept starving for 16 hours. Mice (Group 4 and 5) which were orally administrated with MEBML 200 mg/kg and 400 mg/kg BW, respectively, followed by oral administration of the solution of glucose (1 g/kg BW). Mice (Group-3) which were treated with the antidiabetic agent glibenclamide at a dose of 5 mg/kg body weight, followed by oral glucose administration (1 g/kg BW), while the of control group (Group 1) did not receive glucose solution. After that, tail vein blood samples were taken at 0, 30, 60, 90, and 120 minutes after oral glucose delivery, and glucose levels were assessed using a portable glucometer (Cera-Chek 1070, Korea) by vein puncture [17]. (Table 1)

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

Statistical analysis

All values were expressed as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was used for analyzing the data followed by the Dunnett's test, $p<0.05$ was considered as statistically significant.

Results

α-Amylase and α-glucosidase inhibition activity

Baccaureamotleyana leaf showed the inhibitory activity against α-Amylase and α-glucosidase and the results are tabulated in (Fig. 1 a, b).

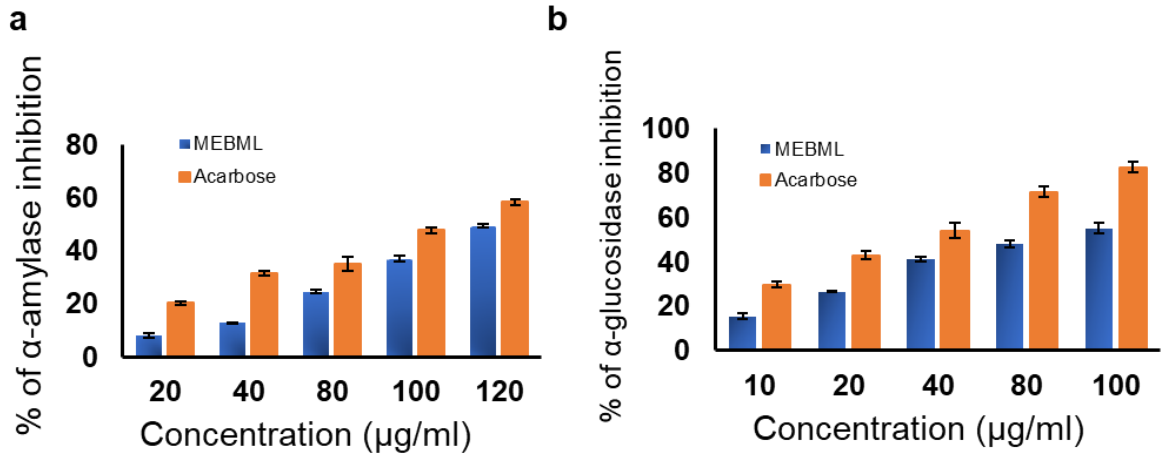

Fig.1α-Amylase and α-glucosidase inhibition activity: (a, b) 6 α-amylase and α-glucosidase inhibition activity of methanolic extract of *Baccaureamotleyana* leaf (MEBML). All data are expressed as mean ± SD (n = 3).

Evaluation of in vivo anti-diabetic activity

In 24 days of treatment, *Baccaureamotleyana* leaves significantly ($p<0.005$) decreased the blood glucose level. The Methanolic extract of *Baccaureamotleyana* leaf at both doses (200 mg/kg and 400 mg/kg BW) lowered the glucose level by 50.92% and 60.64% respectively, compared to diabetic control mice (Fig. 2a)

Serum transaminase and lipid profile

After 24 days of methanolic leaf extract supplementation (200 and 400 mg/kg) and glibenclamide (5mg/kg) TG, TC, LDL, VLDL were reduced as compare to diabetic mice. On the other hand, HDL was significantly increased. At the same time, serum transaminase (ALT and AST) was significantly increased in the diabetic control group and return to the normal level. The data is presented in the Fig 2b, c

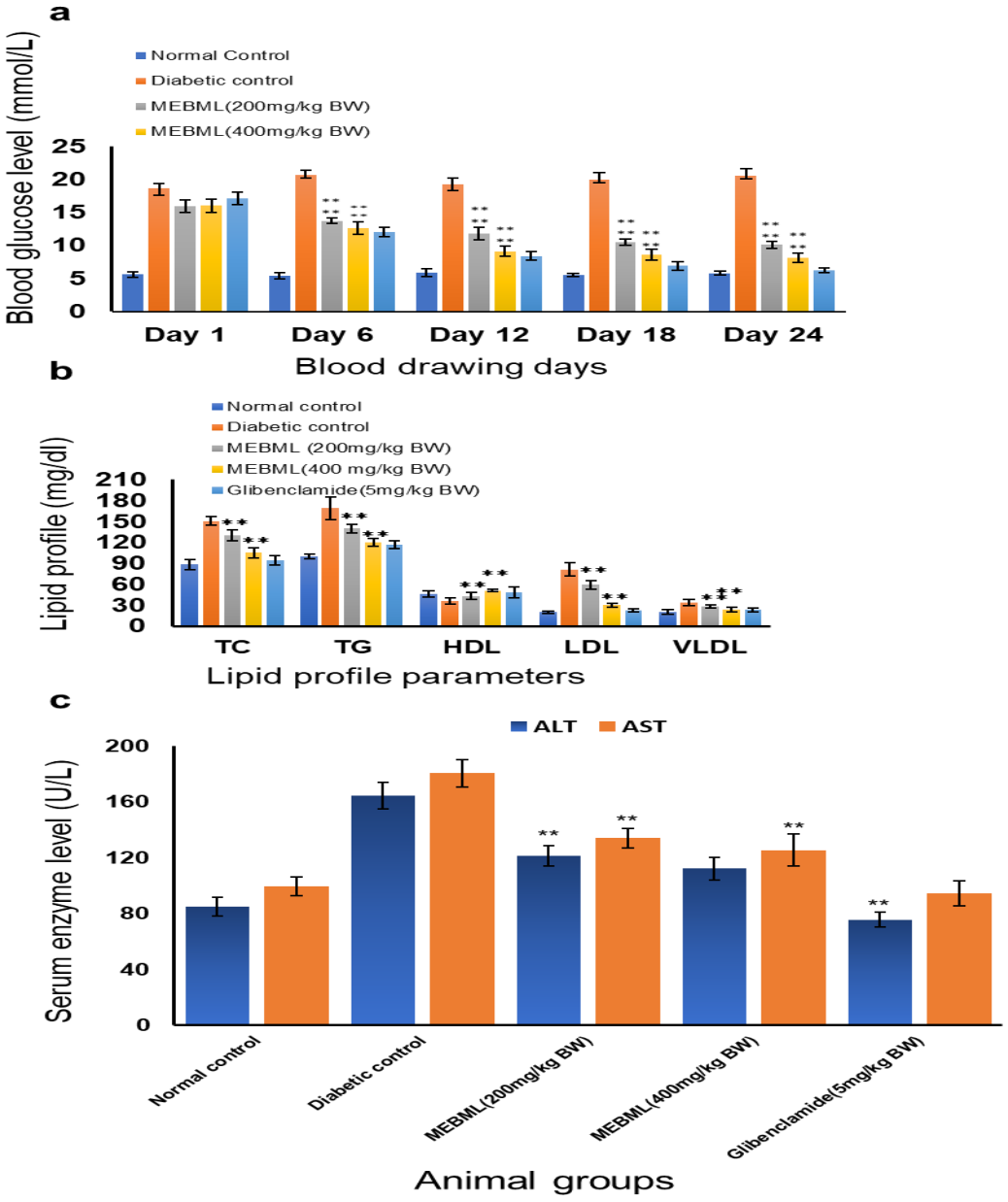

Fig 2: Evaluation of in vivo anti-diabetic activity: (a, b, & c) Effects of Methanolic extract of *Baccaureamotleyana* leaf (MEBML) treatment on blood glucose levels, lipid profile level and ALT, AST level in alloxan-induced diabetic mice. All data are expressed as mean ± SD (n = 5). Here "**" indicates $P \leq 0.05$ vs diabetic control mice.

Discussion

A high postprandial blood glucose level is the defining characteristic of diabetes mellitus, a metabolic condition defined by higher plasma glucose levels brought on by peripheral insulin resistance or insufficient pancreatic insulin production [18]. The key enzyme named α-amylase and α-glucosidase are involved in the digestion of glycogen and starch [19] and it plays a significant part in controlling the postprandial glucose levels by employing this method. [20]. Therefore, inhibiting α-amylase and α-glucosidase is recognized as an effective strategy for the controlling of T2DM and associated problems, which controls postprandial hyperglycemia through delayed glucose intake [21]. Clearly, From the results indicated that *Baccaureamotleyana* leaf extract has significant α-amylase and α-glucosidase inhibition activity, implying this species might have stronger elevated blood sugar level suppression ability through the expanded retarding capability for starchy substrate digestion.

The therapeutic potential of *Baccaureamotleyana* leaf extract against diabetes was further assessed by the postprandial plasma glucose level suppression potential of solvent extracts through alloxan-induced diabetic mice (Fig. 2 a). According to previous study, we can conclude that *Baccaureamotleyana* leaf extracts may have improved impaired glucose level homeostasis via an insulin-secreting mechanism or by promoting the regeneration of beta cells by reducing oxidative stress and subsequently inhibiting DNA damage[22].

Patients with diabetes mellitus frequently have elevated serum levels of TG, TC, LDL, and VLDL and lower levels of HDL due to the altered metabolism of carbohydrates and lipids[23-25]. These anomalies are linked to the progression of diabetic mellitus and the onset of cardiovascular illnesses in diabetic mellitus patients [23]. In this study, diabetic mice fed with methanolic extract of leaf showed significantly lower levels of blood TC, TG, LDL and VLDL and higher levels of HDL when compared to diabetic control mice. These results show prospective outcomes for using *Baccaureamotleyana* leaf to lessen or avoid complications related to DM in lipid metabolism.

The liver is a crucial organ for controlling blood sugar levels because it absorbs and stores glucose as glycogen, converts it to glucose as necessary, and produces glucose from non-carbohydrate sources such amino acids [26]. The liver's ALT and AST enzymes are in charge of converting amino acids to keto acids, and liver injury that causes keto acids to leak into the blood may cause their levels to rise. [27]. Diabetes mellitus may cause liver damage and increase AST and ALT levels [28-30]. When compared to the control mice in this investigation, the alloxan-induced diabetic mice had considerably higher levels of AST and ALT (Fig. 2 c). After 24 days of treatment with *Baccaureamotleyana* leaf extract (200 and 400 mg/kg) significantly decreased AST and ALT level in the plasma compared to the diabetic control mice group. The finding of this study is that the treatment with methanolic extract of *Baccaureamotleyana* leaf in diabetic rats caused a reversal of this enzyme (ALT, AST) to a normal level in plasma compared to the mean values of the diabetic group. Consequently, this study also reveals the potentiality of *Baccaureamotleyana* leaf extract to reduce DM-associated risk of liver damage and cardiovascular damage.

Conclusion

In summary, this study reported that Methanolic extract of *Baccaureamotleyana* leaf has strong in vitro and in vivo antidiabetic properties. Additionally, the diabetic mice supplemented with *Baccaureamotleyana* leaf extract can restore their altered levels of TG, TC, LDL, VLDL, HDL, ALT, and AST level. As a result, this research raises the possibility that *Baccaureamotleyana* leaf could be used to treat and prevent DM and its related problems. However, additional research should be conducted to examine particular anti-diabetic chemicals found in *Baccaureamotleyana* leaf and their mode of action.

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.

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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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Authors' contributions

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

Competing interests

The authors declare that they have no competing interests.

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