In-vivo antidiabetic and antidyslipidemic effects of methanolic leaf extract of *Combretum indicum* in the streptozotocin-induced diabetic rats

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Background
Combretum indicum (locally known as Basantilata) is a notable medicinal plant belonging to the family Combretaceae. Extracts collected from leaves of this plant have activities including antibacterial, antipyretic, and antidiarrheal activities.
Objective
This study was designed to evaluate the crude methanolic leaf extract of *C. indicum* (MLCI) to evaluate its activities in hyperglycemic and dyslipidemic rats.
Materials and methods
In-vivo antidiabetic and antidyslipidemic activities of the extract were studied in streptozotocin-induced diabetic rat models following the standard protocol established earlier. The rats were randomly divided into groups I–V as normal control, diabetic control, metformin, MLCI 250 mg/kg, and MLCI 500 mg/kg body weight, respectively.
Results and conclusion
The in-vivo studies indicated concentration-dependent and significant ($P<0.05$, 0.01, 0.001) reductions of elevated blood glucose, total cholesterol, triglyceride, and low-density lipoprotein-cholesterol levels in the treatment groups compared with diabetes-induced control group. Simultaneously, a significant ($P<0.001$) rise in high-density lipoprotein-cholesterol level was also observed in the study. The results revealed the advantageous roles of the MLCI in the management of diabetes mellitus.

Keywords:
antidyslipidemic, *Combretum indicum*, antidiabetic, pharmacological, streptozotocin

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Introduction

Diabetes mellitus is a common endocrine disorder occurring due to the deficient production or ineffective function of insulin. Due to the spreading capacity and severity of diabetes, it can be termed as an epidemic among people around the world. The rapid progression of this disease is linked to an endocrine imbalance, calorie-rich diet, obesity, severe and continued mental stress, heredity, and so on [1-3]. Apart from modern medicines, there have been many herbal therapeutic options available for the treatment of diabetes for years considering their traditionally claimed hypoglycemic properties. Further, most of these possess antioxidant activities and usually have fewer or no side effects [4-7]. Moreover, earlier studies indicated better affordability provided by herbal medicines and hence, their greater accessibility for many people. That is why, considering the safety and availability of traditional medicines, WHO encourages these therapies to preserve the general health of ordinary people [8].

Combretum indicum (Syn. *Quisqualis indica*, also recognized as Rangoon Creeper) is commonly known as Basantilata in Bangladesh and belongs to the rich Combretaceae family [9,10]. It is widely distributed all over the world, especially in China, India, Philippines, Bangladesh, Myanmar, Malaysia, and in most tropical countries as ornamental plants [11,12]. Nearly, different parts of the plant such as the leaves, flowers, fruits, seeds, and roots are traditionally used in the treatment and management of numerous diseases such as diarrhea, fever, boils, ulcers, helminthiasis, rheumatism, and skin diseases [13-15]. In addition to the traditional use, the presence of many phytoconstituents such as phenolic compounds, flavonoids (quercetin and rutin), tannins, steroids, carbohydrates, protein, alkaloid,

terpenoids (β-sitosterol and lupeol), quisqualic acid, amino acids, saponins, two forms of the cysteine synthase, isoenzyme A and isoenzyme B (enzyme), etc. demonstrates several pharmacological activities of the plant claimed in different literatures [16-19]. Besides, the rapid growth and easy availability of the plant throughout the year has made it more suitable as a potential candidate for herbal medicine [20]. Herbal medicines with potential pharmacological activities and other nutritional values are the most desired choice [21]. However, a number of previously published articles revealed that there is an enormous gap that exists between scientific validation of ethnomedicine and its traditional uses [22].

Hence, the present study was aimed to investigate the antidiabetic potential of *C. indicum* leaf extract, which is not explored yet.

Materials and methods

Collection and identification of plant

Fresh green leaves of *C. indicum* were collected from the Dhaka district of Bangladesh. The plant was identified and authenticated by the National Herbarium of Bangladesh with accession number 45180. The animal study was reviewed and approved by the Institutional Animal Ethics Committee, Department of Pharmacy, Southeast University.

Preparation of plant extract

Collected leaves of the plant were sun-dried for 10 days to avoid the damage of heat-labile components of the leaves, and then the dried leaves were crushed into a coarse powder with a strong suitable blender. Isolation of chemical constituents from the crude plant was done by methanol in a cold extraction process with occasional stirring. After that, the crude extract was filtered with Whatman No. 1 filter paper, and the solvent was vaporized from the extract at room temperature to yield a semisolid extract.

Antidiabetic and antidyslipidemic activities

Experimental animals

Healthy male Long-Evans rats (100–130 g) were purchased from the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR, B). Rats were kept under standard laboratory conditions and fed prepared rodent food and drinking water ad libitum.

Introduction of diabetes in animals

Diabetes was introduced in experimental rats by injecting streptozotocin (STZ) at a dose of 100 mg/kg, body weight. For injection, a single intraperitoneal injection solution of STZ was freshly prepared. After 72 h, the fasting blood glucose level was estimated. Rats having a fasting blood glucose levels of above 7.0 mmol/l were selected for the study [23].

Experimental design

Rats were divided into groups I–V such as normal control, diabetic control, metformin, methanolic leaf extract of *C. indicum* (MLCI) 250 mg/kg and MLCI 500 mg/kg body weight orally, respectively. The group I was nondiabetic and received saline water. Diabetes was induced in groups II–V, whereas group II was treated with saline water, group III received metformin (100 mg/kg, body weight), and groups IV and V were administered orally with the plant extract having doses of 250 mg/kg body weight and 500 mg/kg body weight respectively. All the experiments on rats were performed according to the Institutional Animal Ethics Committee.

Collection and biochemical analysis of blood samples

Fasting blood samples were taken from the tail vein of the rats for estimating blood glucose on days 0, 3, 5, and 7 using a glucose-monitoring machine. After completing the 7 days of treatment, rats were killed and about 3 ml of blood sample was collected from the thoracic artery of the rats, then the blood samples were centrifuged at 4000 rpm for 20 min to obtain serum samples. The serum samples were used to estimate the triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL)-cholesterol, and high-density lipoprotein (HDL)-cholesterol level by a blood analyzer using commercially available wet reagent diagnostic kits (Human GmbH, Wiesbaden, Germany).

Statistical analysis

The results were expressed as mean±SEM and were statistically analyzed with levels of significance set as P value less than 0.05, 0.01, and 0.001. Statistical analysis was performed by analysis of variance followed by Dunnett's test.

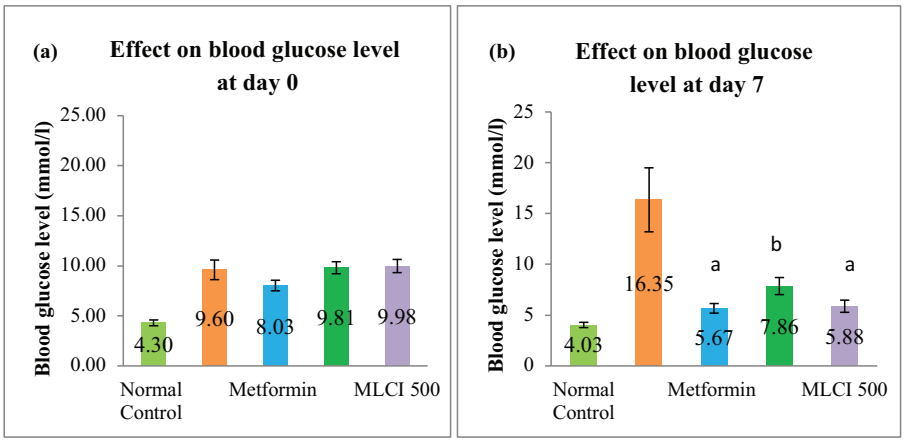
Results

Antidiabetic activity evaluation

Effect of methanolic leaf extract of C. indicum on blood glucose level in streptozotocin-induced diabetic rats

In the present study, all of the experimental diabetic rats, followed by STZ administration, exhibited noticeable increase in their plasma glucose level compared with the control group. However, except in the untreated diabetic control group, the afterward effect of the subsequent 7 days oral administration of MLCI 250, MLCI 500, and metformin in the rest of

Figure 1



Effect of methanolic leaf extract of *Combretum indicum* (MLCI) on blood glucose level at (a) day 0 and (b) day 7 in streptozotocin (STZ)-induced diabetic rats. Values are presented as mean±SEM (n=4), analysis of variance followed by Dunnett test. [a]*P* value less than 0.001, [b]*P* value less than 0.01 when compared with diabetic control; MLCI 500: methanolic leaf extract of *C. indicum* 500 mg/kg body weight and MLCI 250: methanolic leaf extract of *C. indicum* 250 mg/kg body weight.

Table 1 Effect of methanolic leaf extract of *Combretum indicum* on fasting blood glucose levels in streptozotocin-induced diabetic rats

	Fasting blood glucose level (mmol/l) Days of treatment			
Groups	Day 0	Day 3	Day 5	Day 7
Normal control	4.30±0.29	3.90±0.34	3.70±0.21	4.03±0.26
Diabetic control	9.60±0.98	11.28±1.05	13.61±1.90	16.35±3.15
Metformin	8.03±0.53	7.52±0.47[b]	6.65±0.28[a]	5.67± 0.47[a]
MLCI 250	9.81±0.60	9.48±0.58	8.44±0.50[b]	7.86±0.84[b]
MLCI 500	9.98±0.66	8.28±0.40[b]	6.87±0.48[a]	5.88±0.60[a]

Values are presented as mean±SEM (n=4), analysis of variance followed by Dunnett test.
[a]*P* value less than 0.001.
[b]*P* value less than 0.01 when compared with diabetic control; MLCI 500: methanolic leaf extract of *Combretum indicum* 500 mg/kg body weight and MLCI 250: methanolic leaf extract of *Combretum indicum* 250 mg/kg body weight.

the diabetic rats displayed a declining trend in fasting blood glucose levels (Fig. 1). In addition, the MLCI 500 exhibited better hypoglycemic potential than the MLCI 250 throughout the treatment period. Moreover, hypoglycemic activities of all the aforementioned treatment groups were found to be significant (P<0.01, <0.001) compared with the diabetic control group (Table 1). This significant reduction in blood glucose levels manifests the antidiabetogenic potential of *C. indicum* extract in the diabetic rat model.

Effect of methanolic leaf extract of C. indicum on body weight in streptozotocin-induced diabetic rats

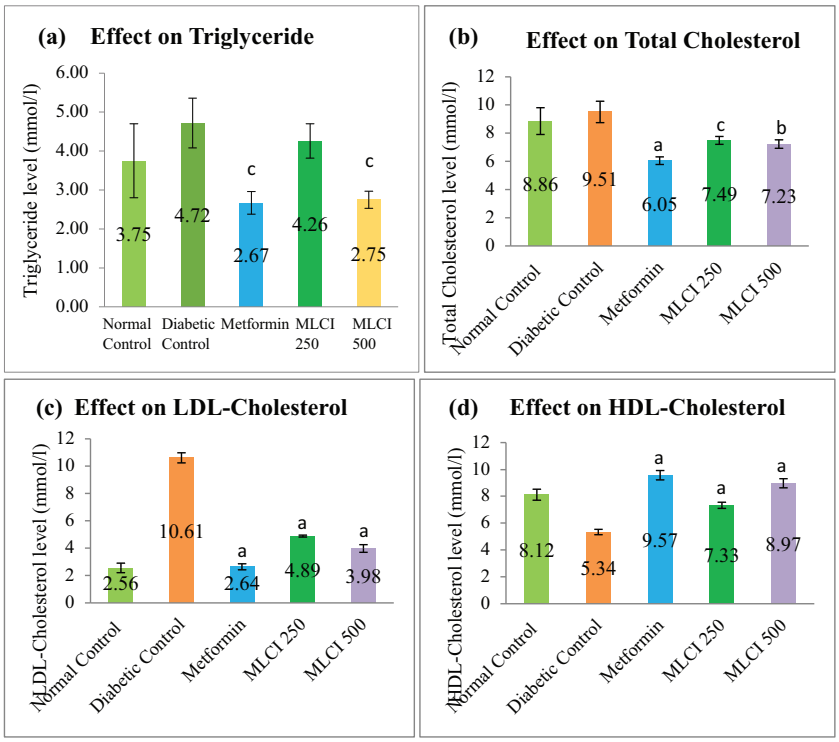
The body weight of all tested groups was recorded before and after treatment for 7 days. Though the STZ-induced diabetes caused a marked loss of body weight in diabetic control groups, the daily administration of Metformin and MLCI test doses increased the weight in other experimental diabetic rats. Meanwhile, the MLCI 500 significantly (P<0.05) elevated the body weight in comparison with the diabetic control group (Table 2). Hence, the observed results suggested an effect of *C. indicum* extract on improving diabetes-induced low body weight in experimental rats.

Table 2 Effect of methanolic leaf extract of *Combretum indicum* on body weights of streptozotocin-induced diabetic rats

	Body weight (g)	
Groups	Before treatment	After treatment
Normal control	114.14±5.41	130.30±4.18
Diabetic control	125.42±2.56	122.75±2.32
Metformin	117.64±3.74	124.79±3.48
MLCI 250	120.74±2.52	134.33±2.67[c]
MLCI 500	121.81±4.34	135.06±4.16[c]

Values are presented as mean±SEM (n=4), analysis of variance followed by Dunnett test.
[c]*P* value less than 0.05 when compared with diabetic control; MLCI 500: methanolic leaf extract of *Combretum indicum* 500 mg/kg body weight and MLCI 250: methanolic leaf extract of *Combretum indicum* 250 mg/kg body weight.

Figure 2



Effect of methanolic leaf extract of *Combretum indicum* (MLCI) on (a) triglyceride, (b) total cholesterol, (c) LDL-cholesterol, and (d) HDL-cholesterol level in streptozotocin (STZ)-induced diabetic rats. Values are presented as mean±SEM (n=4), analysis of variance followed by Dunnett test. [a]P value less than 0.001, [b]P value less than 0.01, [c]P value less than 0.05 when compared with diabetic control; MLCI 500: methanolic leaf extract of *C. indicum* 500 mg/kg body weight and MLCI 250: methanolic leaf extract of *C. indicum* 250 mg/kg body weight. HDL, high-density lipoprotein-cholesterol; LDL, low-density lipoprotein.

Table 3 Effect of methanolic leaf extract of *Combretum indicum* on lipid profile in streptozotocin-induced diabetic rats

	Lipid profile (mmol/l)			
Groups	TG	TC	LDL-C	HDL-C
Normal control	3.75±0.95	8.86±0.96	2.56±0.35	8.12±0.41
Diabetic control	4.72±0.64	9.51±0.76	10.61±0.38	5.34±0.20
Metformin	2.67±0.30[c]	6.05±0.28[a]	2.64±0.22[a]	9.57±0.35[a]
MLCI 250	4.26±0.44	7.49±0.29[c]	4.89±0.07[a]	7.33±0.23[a]
MLCI 500	2.75±0.22[c]	7.23±0.31[b]	3.98±0.29[a]	8.97±0.35[a]

Values are presented as mean±SEM (n=4), analysis of variance followed by Dunnett test.
HDL, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TC, total cholesterol; TG, triglyceride.
[a]P value less than 0.001.
[b]P value less than 0.01.
[c]P value less than 0.05 when compared with diabetic control; MLCI 500: methanolic leaf extract of *Combretum indicum* 500 mg/kg body weight and MLCI 250: methanolic leaf extract of *Combretum indicum* 250 mg/kg body weight.

Antidyslipidemic activity evaluation

Effect methanolic leaf extract of C. indicum on lipid profile in streptozotocin-induced diabetic rats

After 7 days of treatment, MLCI and metformin administration caused a significant (P<0.001, 0.01, 0.05) dose-dependent reduction in TG, TC, and LDL-cholesterol levels in treatment groups when compared with the diabetic control group (Fig. 2). Yet, the HDL-cholesterol level noticeably elevated (P<0.001) in diabetic rats of the aforementioned treatment groups simultaneously (Table 3). Hence, the result obtained from this study may be attributed to the antidyslipidemic potential of *C. indicum* extract in the diabetic rat model.

Discussion

The STZ-induced diabetes model is widely known for in-vivo antidiabetic studies. Several investigations have shown that the insulin-secreting pancreatic beta cells destroyed by streptozotocin leads to the diabetogenic effect [24]. Usually, the single dose (70–250 mg/kg, body weight) and multiple lower doses of STZ cause the entire and partial impairment of pancreatic beta

cells, respectively. Such destruction of beta cells consequently triggers the inflammation that causes insulin deficiency [25,26]. In this study, the diabetogenic dose of STZ (100 mg/kg body weight IP) produced diabetes mellitus in experimental animals and continuously increased the blood glucose concentration in the diabetic control group throughout the subsequent 7-day study period. The elevated blood glucose level was subjected to STZ-induced abnormalities in pancreatic beta cells and was also quite similar to a previously reported study on STZ-induced diabetic model [27,28]. However, the administration of MLCI in experimental diabetic rats showed significant ($P<0.001$, 0.01) reduction in hyperglycemia within 7 days compared with the diabetic control group in a dose-dependent manner. MLCI 500 showed better hypoglycemic activity than MLCI 250.

Furthermore, experimental rats of the diabetic control group also showed characteristic hyperlipidemia and reduced HDL-cholesterol levels compared with normal control. Hyperlipidemia, a secondary complication of diabetes, may be occurred due the enhanced activity of hormone-sensitive lipases [29,30]. But both the extracts significantly decreased ($P<0.05$, 0.01, 0.001) the TG, TC, and LDL-cholesterol levels and increased ($P<0.001$) the HDL-cholesterol level in comparison with the diabetes-induced control group. The antidyslipidemic effects were found in a dose-dependent manner as well.

In the current study, loss of body weight was essentially found as another diabetes-induced complication, in agreement with the earlier reported studies on diabetes [31]. MLCI 500 and MLCI 250 significantly ($P<0.05$) improved body weight throughout the study period. Moreover, several phytochemical screenings reported on the extract showed the presence of phytoconstituents such as tannins, terpenoids, flavonoids, phenols, glycosides, sterols, saponins, and alkaloids in it. These compounds are known to have a wide range of pharmacological activities such as antioxidant, antimicrobial, anti-inflammatory, hypoglycemic, hypolipidemic effects, and so on [17,19,20,32] Hence, the presence of such compounds might contribute to the hypoglycemic and antidyslipidemic effects of the extract as observed in the present study.

Conclusion

The results indicated a significant controlling effect of MLCI in hyperglycemia and dyslipidemia of STZ-induced diabetic rats. Moreover, the easy availability of the plant will be of greater value from the economic point of view. Therefore, in light of the present study, the use of MLCI in the treatment of diabetes mellitus and its associated complications stands confirmed. Hence, it seems that the extract could be considered as a potential natural aid in the treatment of diabetic patients. However, the plant should be investigated more meticulously for prospective drug development in future.

Authors contribution: S.M., I.J.B., and M.R.R. participated in research design. S.M., H.M.A.H., and Z.A. conducted experiments. S.M., H.M.A.H., and I.J.B. performed data analysis. S.M., H.M.A.H., M.R.R., and Z.A. contributed to the writing of the manuscript.

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Conflict of interest
There are no conflicts of interest.

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