


ORIGINAL CONTRIBUTION

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Antidiabetic activity of *Neolamarckia cadamba* (Roxb.) Bosser flower extract in alloxan-induced diabetic rats

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Abstract

Background: Various parts i.e., stem bark, leaf, root and flower of *Neolamarckia cadamba* (Roxb.) Bosser has been extensively used by traditional practitioners to treat all types of diabetes. Our present study investigated the antidiabetic property of the extract from its flower and evaluated its traditional use for diabetes.

Results: Phenolic contents were found to be higher in the methanol soluble fraction (MESF) (228.16 ± 0.32 GAE/g of sample) while flavonoids were greater in quantity in the chloroform soluble fraction (CSF) (291 ± 5.99 QE/g of sample). The MESF showed the highest free radical scavenging and α -amylase inhibitory potential with IC_{50} value of 30.52 ± 2.40 and 6.26 ± 0.82 μ g/mL respectively and both the values were promising when compared to the individual standards. Highest reduction of blood glucose level were observed when the *N. cadamba* flower extract (NCFE) were given (not in combination with metformin) orally for 2 weeks. NMCE at 500 mg/kg resulted 60.2% ($p < 0.05$) reduction of blood glucose in the diabetic group, comparable with the effect of standard metformin (68.4%). Gain in body weight were also observed in the test groups indicating an overall improvement of health in the diabetic rats.

Conclusions: The extract of *Neolamarckia cadamba* (Roxb.) flower is rich in phenolic and flavonoid compounds attributing to its potent in vitro antioxidant and α -amylase inhibitory potential. Oral administration of NCFE also resulted in a significant ($p < 0.05$) dose dependent reduction of blood glucose level in diabetic rats, while no synergistic activity was observed when it was given in combination with metformin. These findings imply the possible pharmacological mechanisms behind the antidiabetic property of NCFE could be: inhibition of α -amylase and delaying the absorption of glucose from the intestine, secondly possessing 'metformin like' activity such as increasing cellular uptake and glycolysis of glucose, reducing gluconeogenesis in the liver etc. This study also for the first time provides scientific evidence in favor of the traditional use *N. cadamba* flower against diabetes and demands future study in order to isolate and characterize the bioactive compounds responsible for such bioactivities.

Keywords: Antidiabetic, Antihyperglycemic, *Neolamarckia cadamba*, Kodom, α -Amylase

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Background

Diabetes mellitus is a metabolic disorder resulting in increased blood glucose level in blood, manifested due to either lack of glucose metabolism (decreased insulin secretion) or glucose reuptake (decreased sensitivity towards insulin) [1]. Among the adults (age 20–79 years) with diabetes in the top five South East Asian countries, Bangladesh is in the second position. The number of people with diabetes in Bangladesh was 5.10 million in 2013, which is expected to reach 8.20 million by 2035 [2]. Diabetes is a chronic, incurable disease that requires lifelong management using conventional non-pharmacological (diet, exercise and surgery) and/or pharmacological means (insulin and oral hypoglycemic agents). These conventional approaches are very costly which often leads people in developing countries to seek help in alternative traditional medicines alongside the allopathic treatment [1, 3].

Neolamarckia cadamba (Roxb.) Bosser (family Rubiaceae), locally known as 'Kodom', is widely distributed throughout Bangladesh and in others countries of South Asia [4]. *N. cadamba* is extremely popular among the traditional health practitioners and almost every part of the plant has medicinal values and used to treat ailments such as fever, anemia, and tumor, improve the quality of semen and diabetes etc. [5]. The biological activities that has already been reported include the antidiabetic (stem bark), analgesic (stem bark, leaf), antidiarrheal (flowering top) and hypolipidemic (root) properties etc. [6]. However, no scientific data has yet been published regarding the antidiabetic property of its flower. Therefore our current study aimed to evaluate the antidiabetic potential of NCFE (individually or in combination with metformin) both in vitro and in vivo, validate its safety and efficacy as a traditional medicine and finally propose plausible mechanism(s) contributing to its pharmacological activities.

Methods

Collection of plant material and preparation of the crude methanol extract of *N. cadamba*

Fresh flowers of *N. cadamba* were collected during the month of August 2015 from the district of Sirajganj, Bangladesh. The flower sample were authenticated by the Bangladesh National Herbarium, Mirpur, and sample specimen were submitted for future referencing purpose. The collected flowers were then sun dried for several days followed by heating in the oven for a 24 h. period but at low temperature (< 40 °C). The dried fruits were then grounded into coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, State University of Bangladesh. 600 g of the powdered material were measured, equally divided into two parts (300 g each) and later transferred into 2 amber colored reagent bottles both containing 2.0 L of

methanol and chloroform. The containers with their contents were sealed by bottle cap and kept for a period of 15 days accompanied by occasional shaking and stirring. The mixtures were then filtered several times using fresh cotton plug and finally with Whatman No.1 filter paper. The volume of the filtrates were then allowed to evaporate at ambient temperature until approximately 70% solvents were evaporated. The leftover slugs were used as the crude methanol and chloroform extracts respectively [7].

Chemicals used

Folin-Ciocalteu reagent, vincristine sulfate and alloxan were the products of Sigma chemical company, USA. BHT was manufactured by Merck, Germany. Gallic acid was purchased from Wako pure chemicals Ltd., Japan and metformin from Chadwell health essex, England. All the materials were ensured to be of analytical grade.

Animal experiments

Nine weeks old male White-Evans rat (100–120 g) were purchased from Jahangirnagar University (JU), Dhaka, Bangladesh and kept in animal cages under standard environmental conditions (22–25 °C, humidity 60–70%, 12 h light: 12 h dark cycle). During the in vivo bioassay (14 days), all the rats were fed with standard laboratory diet (Purina rat chow) obtained from the JU and water ad libitum. All procedures were performed according to the institutional guidelines for animal experimentation of Department of Pharmacy, State University of Bangladesh. The animals were randomly assigned into group I, II, III, IV, V and VI; 5 rats in each group following the respective treatment protocols for the determination of blood glucose level. Two groups (I and II) were treated as the normal and the diabetic control respectively. Groups III and IV received the crude extract at two different doses. Group V served as the positive control and Group VI received the combined dose of Metformin and NCFE.

Collection of blood and analytical procedure

Blood samples were collected from the tail vein of the rats followed by measurement using the glucose monitoring system purchased from Tyson Bioresearch, Based Industrial Park, Chu-Nan, Taiwan.

Determination of total phenolic and flavonoid content

Total phenolic content of various fractions of NCFE were determined employing the method described by Singleton et al., 1965, involving the Folin-Ciocalteu reagent as the oxidizing agent, gallic acid as the standard and the results expressed as the milligram of gallic acid equivalent (GAE) per gram of plant extract [8]. The total flavonoid content was determined using the well-known aluminum chloride colorimetric method and the results

expressed as the milligram of quercetin equivalent (QE) per gram of plant extract [9].

Determination of antioxidant activity

The widely used method of Brand-William *et. al.*, 1995 were followed in order to assess the antioxidant activity of the soluble fractions [7]. The antioxidant potential was evaluated by the ability of the fractions to neutralize the radicals of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and compared with the standard Tetra-butyl-1-hydroxy-toluene (BHT). The IC_{50} value, concentration of reagent required to neutralize 50% of the oxidant molecule, was calculated using the formula:

$$\text{Percent inhibition} = (1 - A_1/A_0) \times 100\%.$$

Here, A_0 = absorbance of the blank (methanol).

A_1 = absorbance of the sample being analyzed.

In vitro antidiabetic activity

The α -amylase inhibitors are a class of antidiabetic drugs that block the hydrolysis of carbohydrates, decreasing its absorption from the gastrointestinal tract and aiding in controlling the blood glucose level [10]. The *in vitro* α -amylase inhibitory test were performed following the method of Bhandari *et al.*, 2003, using acarbose (ABS) as the reference standard [11].

In vivo antidiabetic activity

The *in vivo* antidiabetic potential of the plant extracts were analyzed exclusively as well as in combination with metformin, after inducing diabetes in White-Evans rat by alloxan (120 mg/kg.bd.wt.) as mentioned in the works of Pari *et al.*, 2002 [12]. NCFE at doses 250 and 500 mg/kg.bd.wt. and metformin at 850 mg/kg.bd.wt. were given orally to group III, IV and V respectively for consecutive 14 days. Same procedures were followed for group VI receiving the combined dose of NCFE (250 mg/kg) and metformin (425 mg/kg). Blood glucose level were monitored using glucose monitoring system purchased from Tyson Bioresearch, Based Industrial Park, Chu-Nan, Taiwan.

Data analysis

All the results were recorded in triplicates and results expressed as the mean \pm standard error mean (SEM). In order to determine statistical significance between groups, Dunnett's test and One way ANOVA were performed using SPSS version 25.0 and $p < 0.05$ was considered to be statistically significant.

Results

Percentage yield, total phenolic and flavonoid content

After soaking 300 g of the powdered material, the final weight of the crude fractions of methanol and chloroform extracts were found to be 39.08 g and 25.41 g,

obtaining a percentage yield 13.02% and 8.47%. Among the two extractives, the MESF (228.16 mg of GAE / gm. of dried extract) had the highest phenolic content than the CSF (60.45 mg of GAE / gm. of dried extract). However, the flavonoids were present in higher quantity in the CSF (291 mg of GAE / gm. of dried extract) compared to the MESF (19.33 mg of GAE / gm. of dried extract) (Table 1).

Antioxidant activity

The MESF possessed the highest antioxidant activity among the two fractions with IC_{50} value of 30.52 ± 2.40 μ g/ml when compared to the standard ascorbic acid (16.97 ± 0.41 μ g/ml) (Table 1).

In vitro Antidiabetic activity

The IC_{50} value of MESF, CSF and the standard acarbose in the *in vitro* α -amylase inhibitory assay were 6.26 ± 0.82 , 6.50 ± 0.63 and 5.80 ± 0.77 μ g/ml respectively. Even though MESF possessed slightly higher enzyme inhibitory potential than CSF, both the results were promising and comparable to acarbose (Table 1).

In vivo Antidiabetic activity

The 14 days long oral administration of NCFE in the diabetic rat resulted in a gradual dose dependent decrease in glucose concentration in the blood. The NCFE at 500 mg, resulted in a significant ($p < 0.05$) reduction of blood glucose level by 58.70% when compared to the 68.4% ($p < 0.05$) reduction by the standard metformin. The combined dose of NCFE (250 mg) and metformin (425 mg) also managed to significantly ($p < 0.05$) reduce blood glucose concentration when compared with the diabetic control group but the percentage reduction (57.64%) were lower compared to the individual single dose of NCFE and metformin (Fig. 1). The crude extract alone at two different doses and in combination with metformin also resulted in weight gain in the test animals by 135, 145 and 150 g respectively indicating an overall improvement in the health of the diabetic rats (Fig. 2).

Discussion

The evaluation of the blood glucose level lowering ability of the NCFE has significant implications on its therapeutic use. The preliminary phytochemical screening of this study has indicated the presence of phenolics and flavonoids in NCFE which might be responsible for its high antioxidant property [13]. Furthermore, flavonoids have been attributed to stimulate insulin secretion *in vivo* or possess insulin like effect [14, 15].

The *in vitro* analysis has also revealed for the first time that the partitions of NCFE could inhibit the α -amylase enzyme in the stomach and retard carbohydrate

Table 1 Total phenolic content, total flavonoid content, antioxidant, and α -amylase inhibitory activity of various soluble fractions of the crude extract of *N.cadamba*

Samples/Standards	Total phenolic content (mg of GAE/g of dried extract)	Total flavonoid content (mg of QE/g of dried extract)	Antioxidant activity IC ₅₀ (μ g/ml)	α -amylase inhibition IC ₅₀ (μ g/ml)
MESF	228.16 \pm 0.32	19.33 \pm 0.66	30.52 \pm 2.40	6.26 \pm 0.82
CSF	60.45 \pm 0.29	291 \pm 5.99	147.51 \pm 0.55	6.50 \pm 0.63
VS (std.)	–	–	–	–
ASC (std.)	–	–	16.97 \pm 0.41	–
ABS (std.)	–	–	–	5.80 \pm 0.77

MESF methanol extract soluble fraction, CSF chloroform soluble fraction, VS vincristine sulfate, ASC ascorbic acid, GAE gallic acid equivalent, QE quercetin equivalent, ABS acarbose, SEM standard error of mean, Std Standard

absorption similar to the antidiabetic drug acarbose. This finding provides pharmacological evidence behind the antidiabetic property of the plant extract and supports the use of this extract as an adjuvant for diabetes management.

Alloxan resulted in the destruction of beta cells in the pancreas [16], lowering insulin production ultimately increasing glycaemia. Oral administration of NCFE alone in the diabetic rats for 2 weeks resulted in the significant decrease blood glucose in a dose dependent manner. This hypoglycemic property of the NCFE could be attributed due to the stimulation of insulin secretion by the remaining beta cells or by increasing the uptake of glucose by increasing the insulin sensitivity. However, since alloxan is a well-known β -cytotoxic agent and the observations that NCFE alone induced glycaemia significantly in the alloxan treated rats lends credence that it has peripheral action similar to that of metformin. Metformin exhibits its glucose level lowering activity by several mechanisms such as: stimulation of glucose uptake

by tissues from blood and subsequent intercellular glycolysis; reduced gluconeogenesis in the liver and kidney etc.; decreasing absorption of glucose from intestine by stimulating enterocytes to convert glucose to lactate and by lowering plasma glucagon level [17]. Therefore, the NCFE could show its glucose lowering ability by any or multiple mechanisms mentioned above. The combined administration of NCFE and metformin did not result in increased effect compared to individual therapy further corroborates the fact that the mechanism of action of NCFE and metformin are similar [1]. Furthermore, if the mechanism of action NCFE and metformin are similar, this additive effect could have other clinical implications. The traditional medicinal use of this plant could complement along with orthodox allopathic medication and result in better management of diabetes by lowering the dose of metformin and its associated side effects. The use of NCFE could also be included as a dietary supplement and can play a significant role in the management of either insulin dependent or non-insulin dependent

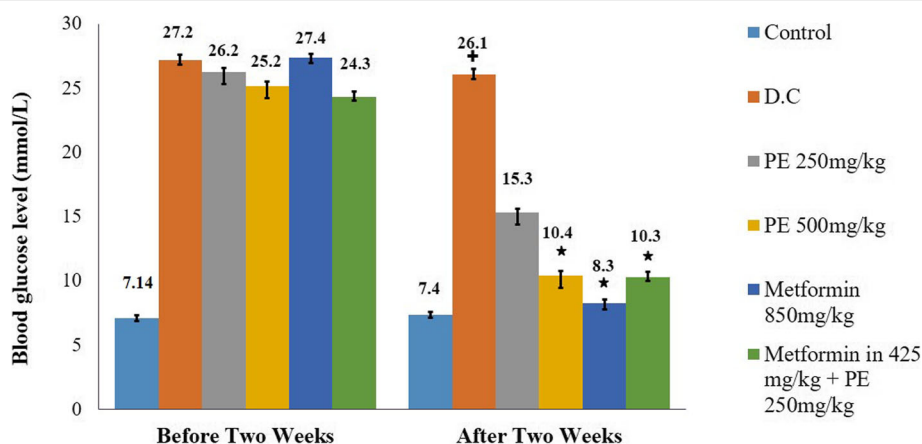


Fig. 1 Effect of the oral administration of *N. cadamba* flower extract on the blood glucose level in normal and diabetic rat. Effect of the oral administration of *N. cadamba* flower extract (given separately at two different doses or in combination with the standard metformin) on the blood glucose level in normal and diabetic rat. Here, DC = Diabetic control, PE = Plant extract. All the values are expressed as mean \pm SEM, sample size ($n = 5$), (*) indicates $p < 0.05$ compared to diabetic control group, (+) indicates $p < 0.05$ compared to normal group

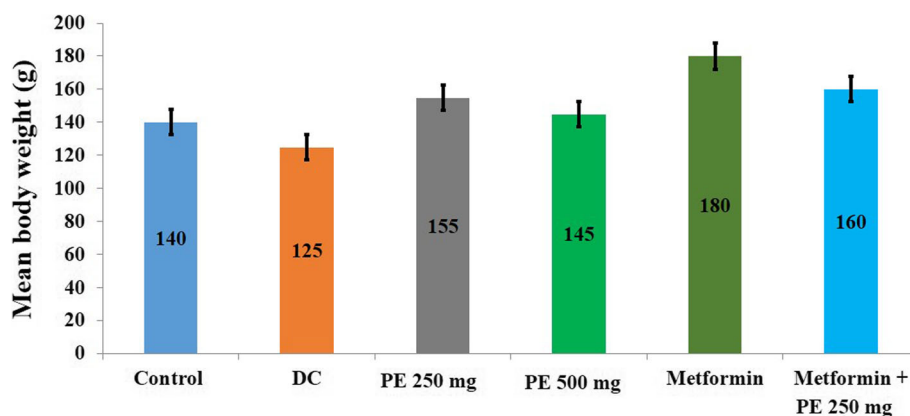


Fig. 2 Effect of the oral administration of *N. cadamba* flower extract on the mean body weight in normal and diabetic rat. Effect of the oral administration of *N. cadamba* flower extract (given separately at two different doses or in combination with the standard metformin) on the mean body weight in normal and diabetic rat. Here, DC = Diabetic control, PE = Plant extract. All the values are expressed as mean \pm SEM, sample size (n = 5), (*) indicates $p < 0.05$ compared to diabetic control group, (+) indicates $p < 0.05$ compared to normal group

diabetes. There are evidences in past studies where a plant had been suggested to be included in the diet for diabetes management alongside allopathic therapy [18].

Conclusions

Even though various authors have already confirmed that the crude extracts of the leaf, stem bark and root of *N. cadamba* possess in vivo antidiabetic and other activities, our present study, for the first time, confirms the antidiabetic potential of extract from its flower and thereby supporting the traditional use of NCFE for treating diabetes. In terms of elucidating the plausible mechanism behind the antidiabetic activity of NCFE, the in vitro bioassay confirms that this crude extract has α -amylase inhibitory potential. Furthermore, comparing the antidiabetic activity of NCFE with metformin it could be deduced that the in vivo antidiabetic activity by the NCFE is exerted by mechanisms similar by which metformin works and both targets the same molecular pathway. Moreover, the NCFE did not show any antihyperglycemic activity in normal animal group which indicates that the high safety profile and low side effects have aided its traditional use. This study should further stimulate the urge to continue experiments in order to isolate the phytoconstituents responsible for the antidiabetic activity of NCFE and also to confirm its mechanism of action.

Abbreviations

NCFE: *Neolamarckia cadamba* flower extract; JU: Jahangirnagar University; DPPH: 2,2-diphenyl-1-picrylhydrazyl; MESF: Methanol extract soluble fraction; CSF: Chloroform soluble fraction; DC: Diabetic control; SEM: Standard error mean; PE: Plant extract; ASC: Ascorbic acid; ASA: Acetyl salicylic acid; VS: Vincristine sulfate; IC: Inhibitory concentration; LC: Lethal concentration; GAE: Gallic acid equivalent; QE: Quercetin equivalent; BHT: Tetra-butyl-1-hydroxytoluene; ABS: Acarbose; Std: Standard

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Authors' contributions

SM and YB designed the study. SM, YB and LN carried out the experiments. SM, YB, MSI and LN analyzed and discussed the data. TA and LN were the major contributors in writing this manuscript. MAR and MMRS had been involved in drafting the manuscript and revising it critically for important intellectual content. All authors read, agreed to be accountable for all aspects of the work and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Ethics approval and consent to participate

The experimental procedures on laboratory animals were approved by the Animal Ethical Committee of Pharmacy Department, State University of Bangladesh, Bangladesh.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests in this section.

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