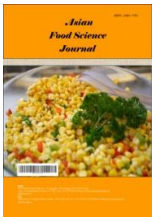

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In Vitro Antioxidant and *In Vivo* Antidiabetic Properties of *Citrus Maxima* Leaf Extracts in Alloxan-Induced Swiss Albino Diabetic Mice

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AI, MNT, MIH and MAI designed the experiment. Authors AI, MNT and MWB collected sample and performed all in vitro and in vivo experiments. Authors AI, MIH and MWB conducted data analysis and data interpretation. Authors AI and MNT prepared draft copy of that manuscript. Authors AI, MNT, MWB, MIH and MAI substantively revised drafted manuscript. All authors read and approved the final draft of the manuscript.

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ABSTRACT

Aims: The current study aimed to explore *in vitro* antioxidant capacity and *in vivo* antidiabetic property of *Citrus maxima* leaf.
Methods: *Citrus maxima* leaf extracts were prepared using methanol (MECML) and ethanol (EECML) in this study. Antioxidant capacity of both extracts was evaluated using total antioxidant capacity (TAC) assay, ferric reducing antioxidant power (FRAP) assay, DPPH free radical scavenging assay, and ABTS free radical scavenging assay. Cytotoxicity of MECML and EECML

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was assessed by brine shrimp lethality bioassay. To explore the *in vivo* antidiabetic property of MECML and EECML, diabetic mellitus (DM) was induced in Swiss albino mice by a single intraperitoneal injection of alloxan. Then diabetic mice were treated with both extracts for 28 days. Effects of both extracts on serum levels of glucose, liver function enzymes, and parameters of lipid profile associated with DM were evaluated.

Results: In TAC and FRAP assays, MECML and EECML represented gradually increased reducing capacity in a dose-dependent manner. In DPPH and ABTS assays, both extracts showed notable free radical scavenging activity with lower IC_{50} values. Additionally, MECML and EECML showed very low toxicity with LC_{50} values of 80.46 and 105.59 µg/mL, respectively in brine shrimp lethality assay. Moreover, both extracts significantly augmented altered levels of serum glucose, parameters of lipid profile, SGPT, SGOT, and C-reactive protein with the treatment of MECML and EECML.

Conclusion: This study suggests *Citrus maxima* leaf possesses significant antioxidant and antidiabetic properties and they might play a potential role to prevent diabetic mellitus and diabetic mellitus associated complications.

Keywords: Citrus maxima; antioxidant activity; antidiabetic activity; cytotoxicity; lipid profile.

ABBREVIATIONS

IC_{50}: The half maximal inhibitory concentration; LC_{50}: Median lethal concentration; ANOVA: Analysis of variance; TC: Total cholesterol; TG: Triglyceride; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein; SGPT: Serum glutamate-pyruvate transaminase; SGOT: Serum glutamic oxaloacetic transaminase; CRP: C Reactive protein; CVDs: Cardiovascular diseases; DPPH: 1,1-diphenyl-2-picrylhydrazyl; ABTS: 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid); DMSO: Dimethyl sulfoxide; SD: Standard deviation; MECML: Methanolic extract of C. maxima leaves; EECML: Ethanolic extract of C. maxima leaves.

1. INTRODUCTION

Diabetic mellitus (DM) is one of the most common diseases which affects millions of people around the world. The number of DM patients is increasing rapidly and it is projected that the number of DM patients throughout the world will be about 600 million by 2035 [1]. With this increasing number of DM patients, death associated DM and other chronic diseases associated with DM is also increasing day by day [2]. However, no treatment has been developed yet which can cure DM.

Diabetic mellitus arises when the pancreas become unable to produce enough insulin or when cells fail to utilize insulin properly produced by pancreas due to oxidative stress and other factors [3,4]. As a consequence, increased blood sugar level is found in DM patients. This prolonged elevated blood glucose level in DM patients affects the metabolism of carbohydrates and lipids [5]. Moreover, the activity of enzymes associated with the metabolism of lipids is also affected by the decreased levels of insulin and insulin resistance [6]. Due to that reason, altered levels of serum triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL are well known characteristics of DM [7,8]. Diabetic mellitus is also known as metabolic disease which affects all system in the body of patients including liver [9]. According to previous reports, DM is associated with several liver diseases such as cirrhosis, fibrosis, non-alcoholic fatty liver, and increased hepatic enzymes including serum glutamate-pyruvate transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) [9,10]. Increased serum levels of C-reactive protein (CRP) is also very common in patients having DM for long time which is a marker of systemic inflammation and emerging risk factor for cardiovascular diseases (CVDs) [11].

The most conventional treatments of DM are lifelong use of insulin or oral hypoglycemic agents, such as sulfonylureas, biguanides, thiazolidinediones, and α-glucosidase inhibitors, but these synthetic drugs have adverse effects such as tingling sensation of hands and feet, lactic acidosis, gastrointestinal disturbance, fluid retention, dizziness, and drowsiness [12-14]. Therefore, many DM patients prefer traditional herbal medicine due to having least adverse effects, less expensive and easy availability [15]. Herbal plants contain several chemical compounds including polyphenols, glycosides,

alkaloids, carotenoids which possess antioxidant and antidiabetic properties [16, 17].

The plant *C. maxima* are very common in Indian subcontinent regions and belong to Rutaceae family and are being used in traditional medicine [18]. Traditionally its fruits, leaves, roots are used for the treatment of asthma, cough, epilepsy, diarrhea, vomiting and mental aberration [18]. *C. maxima* fruits is also known for having antioxidant and antidiabetic properties [19]. In this study, we have explored *in vitro* antioxidant, cytotoxicity and *in vivo* antidiabetic activity of MECML and EECML in alloxan induced diabetic mice.

2. MATERIALS AND METHODS

2.1 Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), potassium ferricyanide [$K_3Fe(CN)_6$], phosphate buffer, sodium citrate buffer, catechin (CA), ascorbic acid (AA), potassiumpersulfate ($K_2S_2O_7$), aluminum chloride ($AlCl_3$), Trichloro acetic acid (TCA), sodium phosphate, ammonium molybdate, sulfuric acid (H_2SO_4) and $FeCl_3$ were purchased from Sigma Chemical Co.(St. Louis, MO, USA). Methanol, ethanol and diethyl ether were obtained from Merck (Damstadt, Germany). Alloxan was purchased from Sigma-Aldrich (St. Louis, MO, USA). Reagent kit for glucose for the estimation of TC, TG, LDL, VLDL, HDL, CRP, SGPT and SGOT were purchased from Linear chemicals (Barcelona, Spain). Glibenclamide was purchased from Advanced Chemical Industries (ACI) Limited, Bangladesh. All others chemicals were used in analytical grade.

2.2 Collection and Preparation of Samples

Mature *C. maxima* leaves were collected from Muraripur, Damkura, Rajshahi (north-western part of Bangladesh) at March, 2017 and authenticated by the Dr. Mst. Ferdowsi Mahal, Professor, Department of Botany, University of Rajshahi, Bangladesh. All leaves were collected from two *C. maxima* plants aged about 14 years and grown in normal environment of Rajshahi, Bangladesh. The *C. maxima* leaves were washed with water to remove adhering dirt and dried in a well-ventilated room under light (60 watt, Philips). After complete drying, the entire portions were grinded into coarse powder by a grinding machine and stored in an airtight container for further use. Methanol and ethanol were used for the extraction. About 80 gm of the powdered leaves was taken into a clean, round bottomed glass bottle and soaked in 400 mL of solvents. The glass bottle with its content was sealed using cotton plug and aluminum foil and kept 20 days at room temperature with occasional shaking and then filtered using Whatman No. 1 filter paper. After that, both solvents were evaporated using rotary evaporator under reduced pressure at 40 °C, and the residues were stored at -20 °C.

2.3 Determination of Total Antioxidant Capacity (TAC)

Total antioxidant capacity of MECML and EECML were determined by the method of Prieto et al. [20] with some modifications. In short, 0.5 mL of leaf extracts at different concentrations were mixed with reaction mixture (3 mL) containing 0.6 M H_2SO_4, 28 mM sodium phosphate and 1% ammonium molybdate, and incubated at 95 °C for 10 minutes. Then absorbance was taken at 695 nm against blank after cooling at room temperature. CA was used as a standard.

2.4 Determination of Ferric Reducing Antioxidant Power (FRAP)

The reducing capacity was evaluated following the method described by Oyaizu (1986) with some modification [21]. Briefly, 250 µL of samples at different concentrations were mixed with 1.75 mL of 0.2 M phosphate buffer (pH 6.6) and 1 mL of potassium ferricyanide (1%), and that mixture was incubated at 50 °C for 20 min followed by the addition of 1 mL TCA (10%). Then 1 mL of incubated mixture was mixed in a test tube with 1 mL of H_20 and 0.2 mL of $FeCl_3$ (0.1%). The absorbance of that resulting solution was measured at 700 nm after 10 minutes. An increased absorbance of the reaction mixture indicates increased reducing power. AA was used as a standard for comparison.

2.5 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Free Radical Scavenging Assay

2,2-diphenyl-1-picrylhydrazyl free radical scavenging capacity of plant extracts were determined following previously described method [22] with slight modification. Shortly, 0.5 mL of samples at different concentrations was

mixed with 3.5 mL of 0.2 mM methanolic solution of DPPH free radical, and the absorbance was taken at 517 nm after incubation of 30 minutes at room temperature. AA was used as positive control. Radical scavenging activity was calculated by the following formula:

$$\% \text{ Scavenging Activity} = (A_{control} - A_{sample} / A_{control}) \times 100$$

Where, Acontrol = Absorbance of control, Asample = Absorbance of sample.

Then percentages of DPPH radical scavenging activity were plotted against concentrations, and from the graph, IC_{50} value was calculated.

2.6 Determination of ABTS Radical Scavenging Activity

The antioxidant capacity of MECML and EECML were determined in terms of ABTS radical scavenging activity following the method previously described [23]. The ABTS radical was obtained by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate solution, and the mixture was left in the dark at room temperature for 12–16 hours before use. Solution of ABTS radical (stable for 2 days) was diluted with water to obtain an absorbance of 0.70 ± 0.02 at 734 nm. Then ABTS radical solution (3 mL) was added to 1 mL of test sample with various concentrations and mixed vigorously. After 6 minutes, the absorbance was measured at 734 nm. As positive control, AA was used. The ABTS radical scavenging activity of the sample was expressed as;

$$\% \text{ Scavenging Activity} = [(A_{control} - A_{sample}) / A_{control})] \times 100$$

Where, Acontrol is the absorbance of the blank control (ABTS radical solution without test sample) and Asample is the absorbance of the test sample.

2.7 Brine Shrimp Lethality Assay

Brine shrimp lethality bioassay assay was conducted to explore cytotoxic property of MECML and EECML using Brine shrimps (*Artemia salina* Leach) nauplii as test organisms [24]. Brine shrimp eggs were hatched in brine by supplying oxygen for 48 hours. For the experiment, both extracts were dissolved in DMSO at six different concentrations (0, 10, 20, 50, 100, and 200 µg/mL). After that 10 mature nauplii were transferred into glass tube containing 5 mL of brine with leaf extracts at different concentration and incubated for 24 hours. Ampicillin tri-hydrate was used as reference standard and DMSO was used as negative control [25]. After 24 hours of incubation, number of dead nauplii was counted and LC_{50} value was calculated. Sample or compound having LC_{50} value less than 1 mg/mL is considered toxic while a LC_{50} value greater than 1 mg/mL is deemed to be non-toxic [26].

2.8 Animal Care

Swiss albino mice weighing 22-25 gm were purchased from the Animal Resources Branch of the International Centre for Diarrheal Diseases and Research, Bangladesh (icddr,b) for this study. The mice were kept in polypropylene cages in a well ventilated room and maintained under standard laboratory conditions (temperature 25 ± 2 °C; humidity 55 ± 5%) with 12 hours dark/light cycle. All mice were allowed free access to standard diet and water, and were acclimatized to laboratory conditions for one week before starting the experiment. All animal experiments were conducted according to the regulations of the Institute of Biological Sciences (IBSc), University of Rajshahi, Bangladesh (Approval No: 87/320/IAMEBBC/IBSC).

2.9 Induction of Diabetes

Diabetes mellitus was induced in overnight fasting mice by a single intra-peritoneal injection of alloxan (90 mg/kg body weight; BW) in a 0.10 M sodium citrate buffer (pH-4.5). Control mice received an equivalent amount of citrate buffer. The development of DM in mice was confirmed by fasting (16 hours) blood glucose measurement using the tail vein blood after 48 hours of alloxan administration using a portable glucometer. The animals having fasting blood glucose level ≥ 11.0 mmol/L were considered as diabetic and included in this study.

2.10 Experimental Design

The mice were divided into following seven groups (n = 6) and treated for four weeks (ones everyday) as follows:

- Group-I (Normal control): Normal mice feed with standard diet and water.
- Group-II (Diabetic control): Alloxan induced diabetic mice with standard diet and water.
- Group-III (MECML 100 mg/kg BW): The diabetic mice treated with MECML at a dose of 100 mg/kg BW [27].

- Group IV (EECML 100 mg/kg BW): The diabetic mice treated with EECML at a dose of 100 mg/kg BW.
- Group V (MECML 200 mg/kg BW): The diabetic mice treated with MECML at a dose of 200 mg/kg BW.
- Group-VI (EECML 200 mg/kg BW): The diabetic mice treated with EECML at a dose of 200 mg/kg BW.
- Group-VII (Glibenclamide 5 mg/kg BW): The diabetic mice treated by glibenclamide at dose of 5 mg/kg BW [28,29].

2.11 Blood Collection

Blood collection from the tail veins of each mouse was carried out before the start of the treatment and on day 1, 7, 14, 21 and 28 during the treatment using 26 G needle and syringe. At the end of the experiment, mice were sacrificed after overnight fasting by anesthetizing with diethyl ether and blood was collected from the artery. After that, the serum was separated by from blood samples by centrifuging for 10 minutes at 1000 rpm. Thereafter, serum was collected and stored at -80 °C until further experiments.

2.12 Measurement of Blood Parameters

Blood glucose level was measured using a portable glucometer (Accu-Chek Guide Blood Glucose Monitoring System, USA). Parameters of serum lipid profile (TG, TC, HDL, LDL, and VLDL), SGPT, SGOT and CRP were measured using commercially available kits (Linear chemicals, Barcelona, Spain) by an automatic bio-analyzer (Hitachi 7180, Tokyo, Japan).

2.13 Statistical Analysis

GraphPad prism (version 8.0) was used for IC_{50} calculation and all statistical analyses were performed suing MS excel (version 2019) and SPSS software (version 16.0). Data was considered as statistically significant when $P < 0.05$ (one-way analysis of variance; ANOVA followed by Dunnett's t test).

3. RESULTS

3.1 Total Antioxidant Capacity of MECML and EECML

Total antioxidant capacity assay was used in this study to explore the antioxidant potentiality of MECML and EECML. Notable reducing capacity of MECML and EECML compared to CA (standard) was observed. Both extracts showed gradually increased reduction capacity of Mo (VI) to Mo (V) with the increasing concentration of extracts (Fig. 1). However, EECML represented higher reducing capacity compared to MECML (Fig. 1).

3.2 Ferric Reducing Antioxidant Power of MECML and EECML

Ferric reducing antioxidant capacity of MECML and EECML observed in this study was represented in Fig. 2). Increasing ferric ion reducing capacity of both extracts on dose dependent manner was found (Fig. 2). In this assay, EECML showed higher ferric ion reducing potentiality compared to MECML at the same concentration. CA was used as a positive control in that experiment.

3.3 DPPH Free Radical Scavenging Activity of MECML and EECML

Concentration dependent free radical scavenging activity of MECML and EECML were observed in this study using DPPH free radical scavenging assay. For comparison, AA was used as positive control. Both extracts of *C. maxima* showed increasing DPPH radical scavenging activity with increasing their concentration. The amount of MECML and EECML extracts required for scavenging 50% of DPPH radical (the half maximal inhibitory concentration; IC_{50}) were 67.81 ± 4.76 µg/mL and 52.67 ± 3.11 µg/mL, respectively. In the DPPH assay, the lower IC_{50} value indicates higher free radical scavenging capacity.

3.4 Scavenging Capacity of MECML and EECML in ABTS Free Radical Assay

In this study, observed ABTS free radical scavenging activity of MECML and EECML was represented in Fig. 4. Gradually increased ABTS radical scavenging capacity of both extracts of *C. maxima* was found in this study. About 50% ABTS free radicals were scavenged by MECML at the concentration of 112.16 ± 5.23 µg/mL and EECML scavenged 50% ABTS free radical at the concentration of 80.12 ± 4.43 µg/mL. Ascorbic acid was used as a standard in this experiment. Compared to MECML, EECML represented higher ABTS radical scavenging capacity in that study.

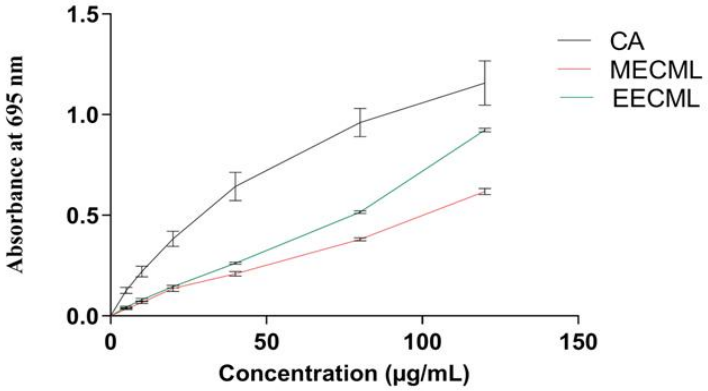

Fig. 1. Total antioxidant capacity of MECML, EECML and CA at different concentrations. All data were expressed as mean ± SD (n = 3)

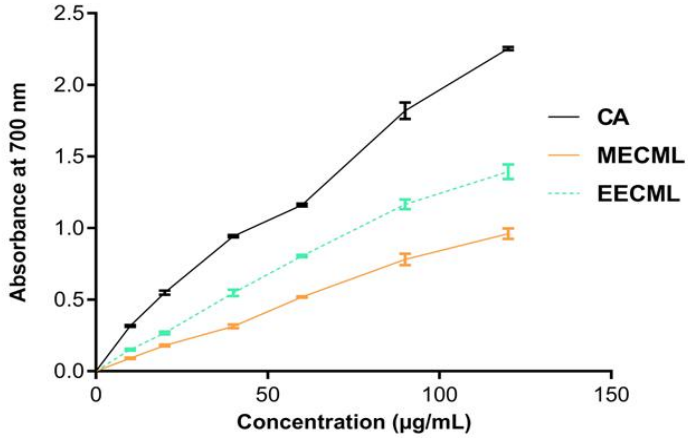

Fig. 2. Ferric ion reducing power of MECML, EECML and CA at different concentrations. All data were expressed as mean ± SD (n = 3)

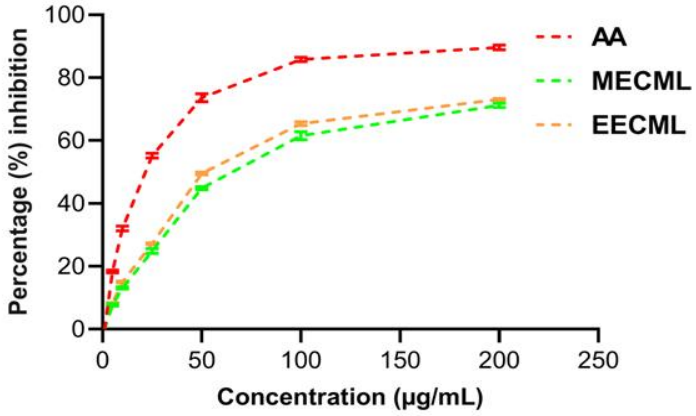

Fig. 3. DPPH free radical scavenging capacity of MECML, EECML and AA at different concentrations. All data were expressed as mean ± SD (n = 3)

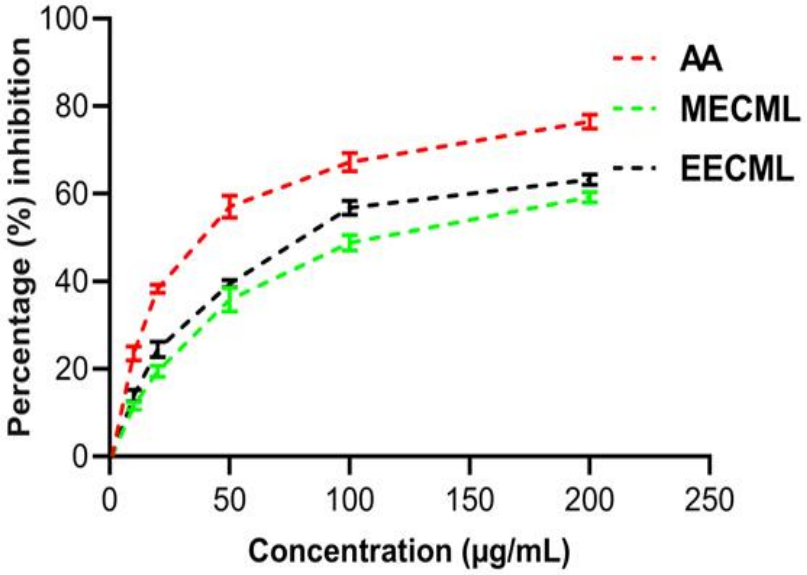

Fig. 4. ABTS free radical scavenging capacity of MECML, EECML and AA at different concentrations. All data were expressed as mean ± SD (n = 3)

3.5 Cytotoxic Effect of MECML and EECML on Brine Shrimp Lethality

The cytotoxic property of MECML and EECML were assayed by brine shrimp lethality bioassay test. In this assay, EECML showed lower cytotoxic property with median lethal concentration (LC_{50}) of 105.59 ± 6.89 µg/mL whereas MECML showed LC_{50} value 80.46 ± 3.33 µg/mL (Fig. 5). In this assay, ampicillin tri-hydrate (AT) was used as positive control which exhibited LC_{50} value 9.22 ± 1.21 µg/mL. Higher LC_{50} value in brine shrimp lethality bioassay indicates lower cytotoxic property of test sample.

3.6 Effect of MECML and EECML on the Blood Glucose Levels in Diabetic Mice

Significant hypoglycemic effects of MECML and EECML in diabetic mice were noted in this study (Fig. 6). A single dose of alloxan (90 mg/kg BW) significantly increased blood glucose levels in mice compared to normal control mice (**p < 0.05). This increased blood glucose levels in diabetic mice were reduced significantly by MECML and EECML treatment compared to that of diabetic control mice (*p < 0.05) at both doses. An antidiabetic drug (glibenclamide) was used in this study at a dose of 5 mg/kg BW as positive control which also decreased blood glucose levels in diabetic mice (Fig. 6).

3.7 Effects of MECML and EECML on the Parameters of Lipid Profile in Diabetic Mice

Effects of MECML and EECML on altered parameters of lipid profile in alloxan induced diabetic mice were explored in this study. Both extracts of *C. maxima* represented promising capability to restore altered levels of lipid profile parameters in diabetic mice (Fig. 7). Significantly increased levels of TC, TG, VLDL, LDL and decreased level of HDL in diabetic mice were found. After 28 days treatments with MECML and EECML, significantly improved serum levels of those parameters in diabetic mice were noted in this study (Fig. 7).

3.8 Effects of MECML and EECML on Serum SGPT, SGOT and CRP Levels in Diabetic Mice

Significant increase in the serum levels of SGPT, SGOT and CRP in diabetic mice were noted in this study (Fig. 8). Twenty-eight days treatment with MECML and EECML at the doses of 100 mg/kg BW and 200 mg/kg BW significantly reduced serum SGPT, SGOT and CRP levels in diabetic mice (Fig. 8). Glibenclamide was used as positive control in this study.

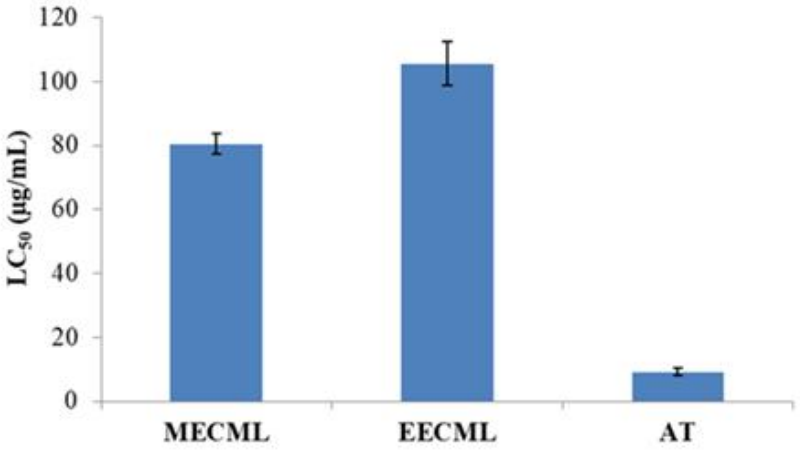

Fig. 5. Cytotoxic effect of MECML, EECML and positive control ampicillin tri-hydrate (AT) on brine shrimp nauplii. All data were expressed as mean ± SD (n = 3)

4. DISCUSSION

The plant *C. maxima* is very common in Bangladesh and other Indian subcontinent, and well known in herbal medicine due to possessing medicinal properties [18]. However, antioxidant and antidiabetic properties of the *C. maxima* leaves are not well known yet. In this study, we have explored the *in vitro* antioxidant property and *in vivo* antidiabetic potentiality of *C. maxima* leaves extracts using methanol and ethanol as solvents.

Diabetes mellitus is a very common disease throughout the world and oxidative stress play a vital role in the development of DM associated complications with its progression [30]. Therefore, complementary supplementation of antioxidants to DM patients is helpful to improve DM associated complications [31]. Plants are a great source of natural antioxidants and many pants rich in antioxidants are being used for the treatment of DM [32-34]. *C. maxima* leaves also contain several bioactive compounds including natural antioxidants [35]. In this study, we have explored notable antioxidative potentiality of MECML and EECML using TAC, FRAP, DPPH and ABTS assays. In all four *in vitro* assays, both extracts showed notable antioxidative capacity. However, EECML showed higher reducing and free radical scavenging capability compared to that of MECML. Bioactive metabolites of *C. maxima* leaf extracts having antioxidant property might be helpful to improve complications of DM associated with oxidative damages. Moreover, many plant are well known for possessing higher antioxidant and cytotoxic properties [36]. To explore cytotoxic property of MECML and EECML, we used brine shrimp lethality bioassay [24]. This assay is very common and widely used for the determination of cytotoxic property of plant extracts as well as synthetic compounds [37]. Both extracts of *C. maxima* leaves represented very low cytotoxic property. Higher LC_{50} values of MECML and EECML observed in this study indicates the non-toxic property of these extracts [26,38].

Hyperglycemia for prolog period is the most common characteristics of DM patients due to the insufficient production of insulin by pancreas or insulin resistance [5]. With the progression of DM mellitus, many other complications arise. Due to insufficient insulin secretion or inability of cells to utilize insulin properly, metabolism of lipids is also affected. As a consequence, increased levels of serum TC, TG, LDL and VLDL, and decreased levels of serum HDL were commonly found in DM patients [8]. Additionally, due to altered metabolism of lipids and carbohydrates, liver damage is also very common in patients having DM for long time [9]. Non-alcoholic liver steatohepatitis, liver cirrhosis, and hepatocellular carcinomas are also common in patients with the progression of DM [9]. As a results, increased activity or levels of

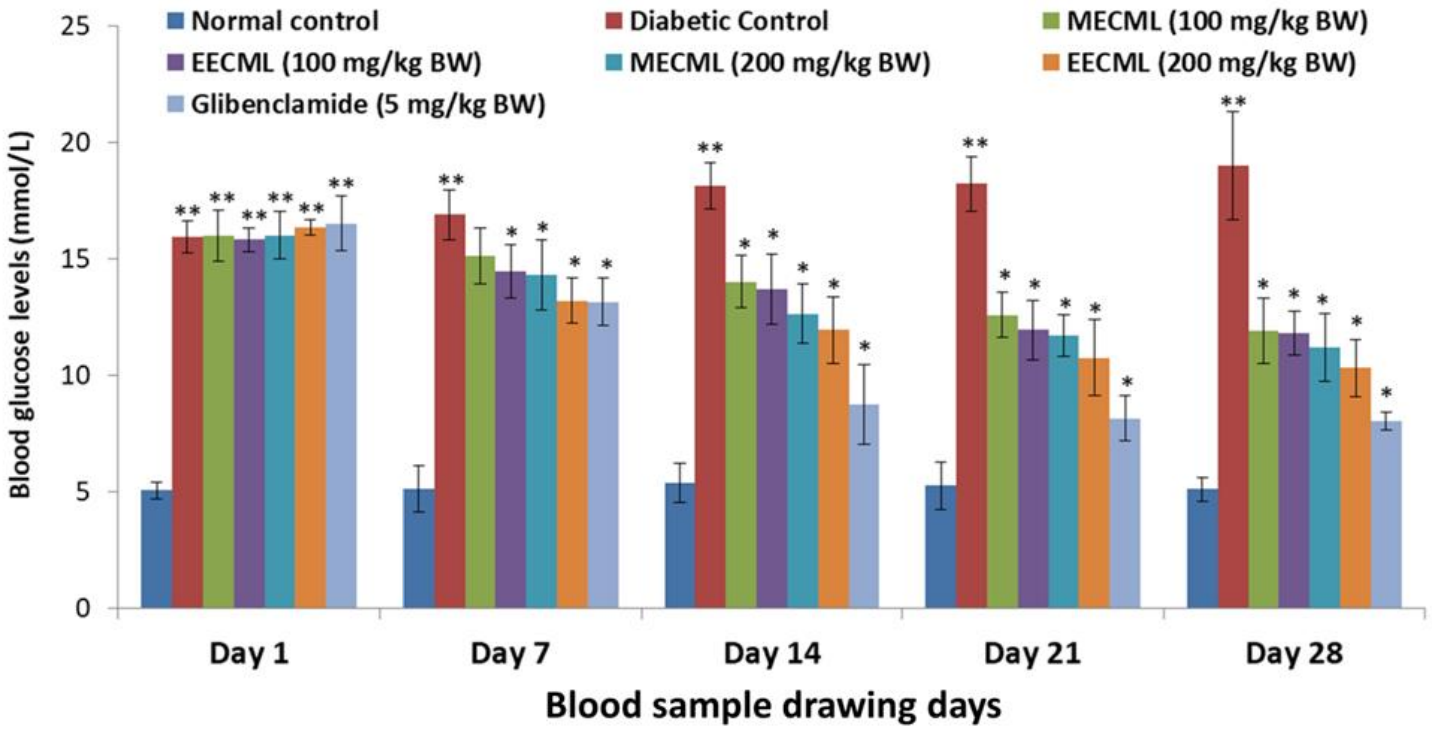

Fig. 6. Effect of MECML and EECML treatment on the blood glucose levels in alloxan induced diabetic mice. Here all values were expressed as mean ± SD (n=6). **$p < 0.05$ *vs* normal control; *$p < 0.05$ *vs* diabetic control

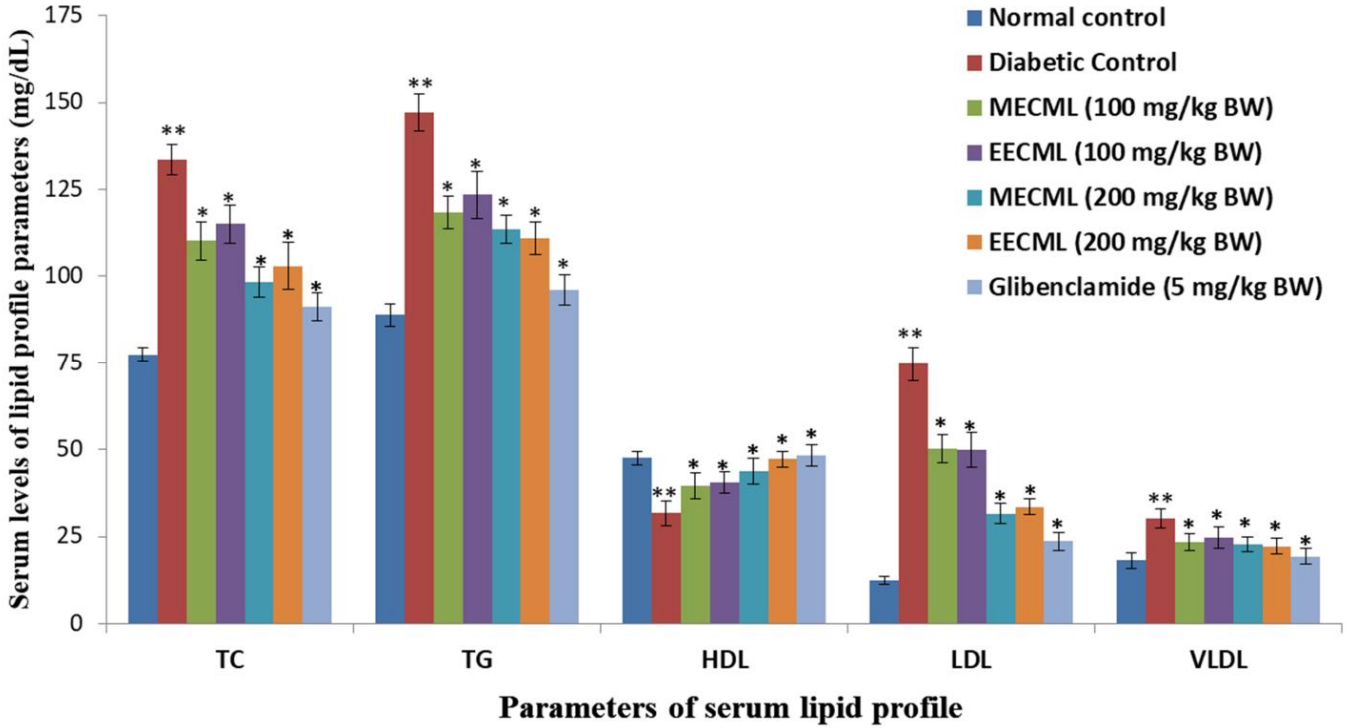

Fig. 7. Effect of 28 days treatment with MECML and EECML on serum lipid profiles in alloxan induced diabetic mice. All values were expressed as mean ± SD (n = 6). **$p < 0.05$ *vs* normal control; *$p < 0.05$ *vs* diabetic control

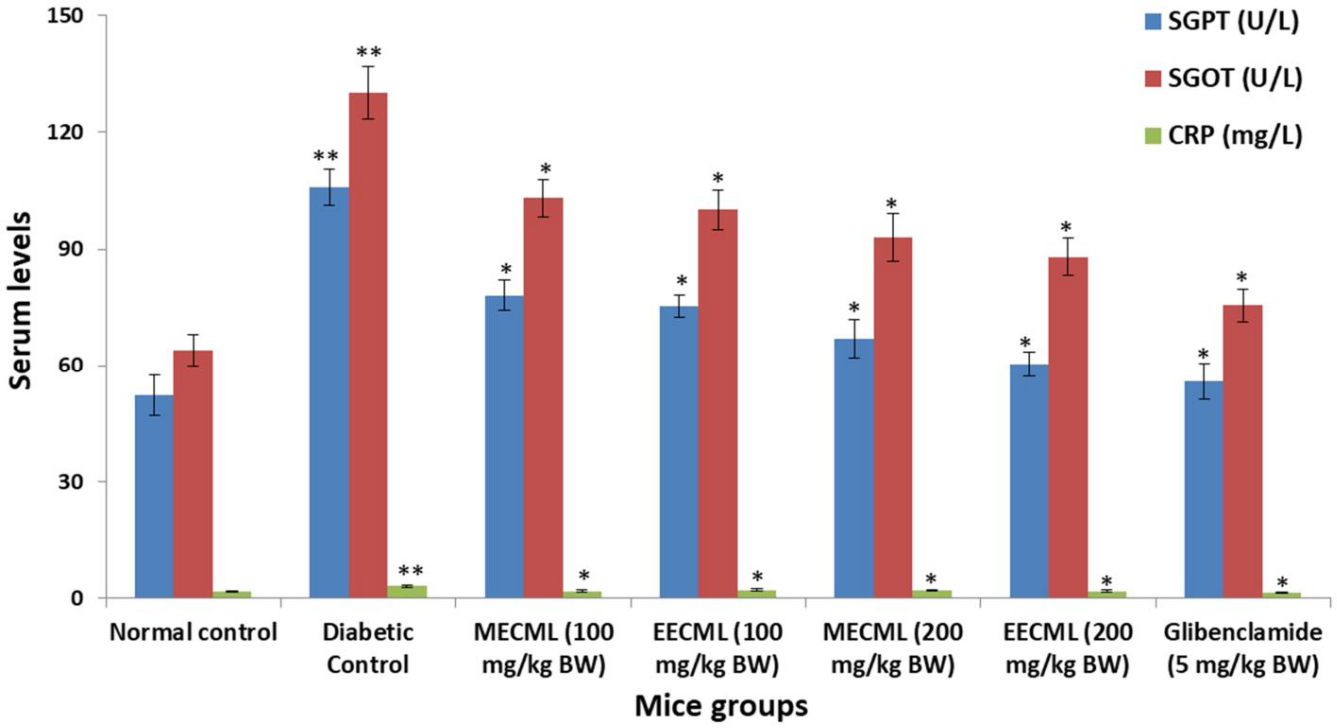

Fig. 8. Effect of MECML and EECML on SGPT, SGOT and CRP levels after 28 days of treatment in diabetic mice. All data were expressed as mean ± SD (n = 6). **$p < 0.05$ compared to the normal control group; *$p < 0.05$ compared with diabetic control group

liver function enzymes such as SGPT and SGOT are prominent in the patients having DM [39]. Development of cardiovascular diseases are also common with the progression of DM [40]. Due to altered lipid and protein metabolism, increased serum levels of CRP often found in DM patients which is the marker of myocardial infarction [41,42]. In this current study, MECML and EECML represented significant hypoglycemic potentiality in alloxan induced diabetic mice. Increased serum levels of TC, TG, LDL and VLDL in diabetic mice were also ameliorated after 28 days of treatment with MECML and EECML. Treatment with both extracts also restored the decreased levels of serum HDL in diabetic mice. Increased levels of SGPT, SGOT and CRP were also observed in alloxan induced diabetic mice compared to that of normal control mice. These increased levels of SGPT, SGOT and CRP were also augmented by MECML and EECML treatment for four weeks. All finding of this study suggest that both MECML and EECML possess higher antioxidative potentiality without having cytotoxic property. Additionally, both extracts have significant hypoglycemic potentiality, and can ameliorate DM associated altered lipid profile in diabetic mice. Moreover, both extracts of *C. maxima* leaves can restore altered levels of liver function enzymes and CRP in diabetic mice which indicate hepatoprotective and cardioprotective potentiality of those extracts. However, further study is required to identify specific plant metabolites present in *C. maxima* leaves possessing antioxidant and antidiabetic properties and their mode of action.

5. CONCLUSION

In this study, *Citrus maxima* leaf extracts represented notable *in vitro* antioxidant and *in vivo* antidiabetic properties in this study. Moreover, *Citrus maxima* leaf extracts also can restore altered lipid profile, levels of liver function enzymes and C-reactive protein in diabetic mice. Therefore, this study suggests antioxidant potentially and possible beneficial effects of *Citrus maxima* leaf extracts for the treatment of diabetes mellitus and diabetes mellitus associated complications in future.

ETHICS APPROVAL

All animal experiments were conducted according to the regulations of the Institute of Biological Sciences (IBSc), University of Rajshahi, Bangladesh (Approval No: 87/320/IAMEBBC/IBSC).

AVAILABILITY OF DATA AND MATERIAL

The corresponding author is entitled to provide the data upon reasonable request.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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