

# Antidiabetic potential of ethanolic leaf extract of *Crataeva magna* in streptozotocin-induced diabetic model

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## Introduction

Diabetes is the group of metabolic abnormalities characterized by chronic hyperglycemia due to a defect in insulin secretion or action or both. It is a defect of glucose metabolism. The WHO classifies DM into insulin-dependent DM (IDDM) or type I, and non-insulin-dependent DM (NIDDM) or type II. Type 1-diabetes where insulin secretion is not sufficient and type-2 diabetes where insulin secretion is normal, but insulin is not able to act on insulin receptors due to the presence of insulin resistance.

## ABSTRACT

**Aim:** In the present study, the ethanolic leaf extracts of *Crataeva magna* (ELECM) at various concentrations were prepared and anti-diabetic activity was evaluated. **Materials and Methods:** Diabetes was induced by single injection of streptozotocin (65 mg/kg, *i.p.*) and various biochemical parameters were assessed using commercial available kits. **Results:** ELECM showed dose-dependent reversal of increase in blood glucose level in diabetic rats. Moreover, ELECM also reversed the abnormal lipid profile in diabetic rats as reflected by decrease level of total cholesterol, triglyceride, very low-density lipoprotein (VLDL), and LDL and increase level of high-density lipoprotein. Moreover, ELECM also showed *in vivo* antioxidant activity by increasing level of antioxidant enzymes such as reduced glutathione (GSH), sodium dismutase, and catalase along with decreasing oxidant markers such as thiobarbituric acid level. **Conclusion:** In the light of the above consideration, the results of the study revealed that ELECM showed promising antidiabetic activity in a dose-dependent manner.

**Keywords:** Antidiabetic activity, antioxidant activity, leaf extract, lipid profile

It is a seriously challenging disease of the 21<sup>st</sup> century, while the prevalence and mortality rate due to this insidious disease continuously rise worldwide. According to the International Diabetes Federation (IDF) report, there are 425 million people living with diabetes within the age group of 20–79 years and this figure is expected to reach 629 million in 2045. In India, around 72.9 million people are suffering from diabetes of age group of 20–79 years who are likely to almost double to reach 134.3 million in 2045.<sup>[1-3]</sup>

The mainstay of non-pharmacological treatment of diabetes is diet and physical activity.<sup>[4]</sup> Other methods of treatment include acupuncture, hydrotherapy, mineral supplementation, and conventional drugs which include exogenous insulin, oral hypoglycemic agents, and transplantation.<sup>[4]</sup> In conventional medical practice, the action of the resultant drugs may have the desired effect; however, the potency and

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efficacy may differ significantly.<sup>[5]</sup> This raises the need for other sources of these inhibitors that have fewer side effects.<sup>[6]</sup> The most obvious choice for these alternatives would be plants with ethnomedical uses in management of diabetes.

Recently, a substantial body of literature provides strong evidence to support the Pharmacological potentials of CM in various pathological conditions. It is one of the best litholytic herbs and most commonly used for the treatment of urolithiasis and crystalluria. Moreover, CM has been explored for its various medicinal properties.<sup>[7]</sup> In this respect, plant extracts are expected to act as a potential strategy to treat diabetes. Thus, the CM plant was selected and extract at various concentrations were evaluated for antidiabetic efficacy in laboratory animals using animal models.

## Materials and Methods

### Plant material

#### *Collection and authentication of CM plant*

Fresh leaves of CM were collected from Tirumala hills, Chittoor district from the state of Andhra Pradesh. The plant materials were taxonomically identified and authenticated by Dr. Madhava Chetty, Asst Professor, Dept. Of Botany, S.V. University, Tirupati Andhra Pradesh, India, and the sample voucher specimen and herbarium have been preserved in the Dept. Of Pharmacognosy, Luqman College of Pharmacy Gulbarga, Karnataka.

### Experimental animals

The Institutional Animal Ethical Committee approved the experimental protocol used in the present study. Age-matched young Wistar rats weighing about 200–250 g were employed in the present study. The animals were housed in the room maintained at approximately  $24 \pm 1^\circ\text{C}$  temperature and humidity of  $55 \pm 5\%$  with 12-h light/dark cycle. Free access to food (standard chow from Ashirwad Industries, Ropar, India) and water was allowed. The animals were acclimatized for at least 3–4 days before the initiation of the experiment and were observed for any sign of disease. The animals were maintained under proper conditions until the termination of the experiment. The animals were sacrificed after a predetermined period of the treatment as per the study design to evaluate various parameters.

### Standard drugs and chemicals

Glucose, total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), high-density lipoproteins (HDL), very LDL (VLDL), and kits were procured from Erba diagnostics Pvt. Ltd.

### Preparation of extract doses for hypoglycemic studies

Oral dose of CM extract at 100–500 mg/kg bwt was prepared in CMC for evaluation of hypoglycemic effect. Doses of the extract were selected on the basis of pilot studies reported in the literature.

### Oral administration to wistar rats

The required amount of plant extracts was dissolved in minimum amount of CMC and water (preferably 0.5–2 ml) and administered

orally to the rats using a 2 ml syringe with the help of polythene tubing fixed to the tip of a long needle. All plants extract at different doses and standard drugs such as glipizide were administered to diabetic rats after 7 days of injection of streptozotocin (STZ) and their treatment was continued for 4 weeks. All the parameters were assessed at the end of 4 weeks of treatment, that is, 4 weeks of diabetes induction in all groups.

### Induction of diabetes

Diabetes was induced by administration of (STZ; 65 mg/kg, *i.p.*) prepared in fresh citrate buffer (pH 4.5). Determining fasting blood glucose (FBG) level after 72 h of STZ injection confirmed development of diabetes. The rats with FBG level  $\geq 250$  mg/dl were included in the study.

### Experimental design

Experimental animals were divided into seven different groups comprising eight animals each. All plant extracts were evaluated for their antidiabetic effect at dose of 100–500 mg/kg per oral (*p.o.*) and glipizide (4 mg/kg, *p.o.*).

Group I (Untreated normal control rats): Normal control rats received only normal diet and water during the experimental period but without any therapy.

Group II (Plant extract treated normal rats): Normal rats treated with a single dose of aqueous extract of CM orally at a dose of 500 mg/kg bwt daily 1 time.

Group III (Diabetic control rats): Rats of this group were STZ-induced diabetic model and were served as diabetic controls throughout the experimental period but without any therapy.

Group IV (Plant extract treated diabetic rats): Diabetic models of rats treated with a single dose of aqueous extract of CM orally at a dose of 100 mg/kg bwt daily 1 time.

Group V (Plant extract treated diabetic rats): Diabetic models of rats treated with a single dose of aqueous extract of CM orally at a dose of 200 mg/kg bwt daily 1 time

Group VI (Plant extract treated diabetic rats): Diabetic models of rats treated with a single dose of aqueous extract of CM orally at a dose of 500 mg/kg bwt daily 1 time.

Group VII (Glipizide Treated Diabetic Group): The diabetic rats after 1 week of STZ administration were treated with glipizide (4 mg/kg, *p.o.*).

### Assessment of STZ-induced diabetes

#### *Estimation of body weight*

Each animal body weight was measured before induction of STZ. Body weight was measured periodically till the end of study.

### Blood samples for biochemical estimation

Blood samples were withdrawn (under light anesthesia) by retro orbital puncture method in the morning after overnight fasting and analyzed for measurement of blood glucose level, lipid profile (Serum TC, TG, LDL, VLDL, and HDL). Blood was allowed to clot and centrifuged at 4000 rpm for 15 min at 4°C and serum was separated. The serum samples were frozen until analyzing the biochemical parameters. Biochemical estimation was carried out using available laboratory kits of Erba diagnostics Pvt. Ltd.

### Estimation of serum glucose

Blood glucose level was estimated after 72 h of STZ administration to confirm diabetes. FBG level was estimated on 0<sup>th</sup> day, 30<sup>th</sup> day, and 75<sup>th</sup> day.

The glucose concentration was estimated by glucose oxidase peroxidase GOD-POD method using the commercially available kit.<sup>[8]</sup> 1000 µl of working glucose reagent was added to 10 µl of serum, 10 µl of standard glucose (100 mg/dl), and 10 µl of purified water to prepare the test, standard, and blank, respectively. All the test tubes were incubated at room temperature for 30 min. The absorbance of test and standard samples was noted against blank at 505 nm spectrophotometrically (Thermo Double Beam Spectrophotometer, Thermo Electron Corporation, United Kingdom).

### Assessment of blood lipid profile

#### Estimation of serum TC

The TC was estimated by cholesterol oxidase peroxidase CHOD-POD (cholesterol oxidase-peroxidase) method.<sup>[9]</sup> 1000 µl of cholesterol reagent was added to 10 µl of serum, 10 µl of standard cholesterol (200 mg/dl), and 10 µl of purified water to prepare the test, standard, and blank, respectively. All the test tubes were incubated at room temperature for 10 min. The absorbance of test and standard samples was noted against blank at 505 nm spectrophotometrically.

#### Estimation of serum TG

The serum TG was estimated by glycerophosphate oxidase peroxidase GOD-POD method.<sup>[10]</sup> 1000 µl of enzyme reagent was added to 10 µl of serum, 10 µl of standard (200 mg/dl), and 10 µl of purified water to prepare the test, standard, and blank, respectively. All the test tubes were incubated at room temperature for 15 min. The absorbance of test and standard samples was noted against blank at 546 nm spectrophotometrically.

#### Estimation of HDL

The HDL was estimated by cholesterol oxidase peroxidase CHOD-POD method. HDL level in serum was measured by following manufacturer protocol mentioned in kit.<sup>[9]</sup> The overall method for determining HDL can be divided into two steps.

Step 1. 200 µl of serum and 300 µl of precipitating reagent were taken into the centrifuge tube, mixed well and were incubated at room temperature for 5 min and then centrifuged at 3000 rpm for 10 min to get clear supernatant.

Step 2. 1000 µl of cholesterol reagent was added to 100 µl of supernatant (from step 1), 100 µl of HDL cholesterol standard (50 mg/dl), and 100 µl of purified water to prepare the test, standard, and blank, respectively. All the test tubes were incubated at room temperature for 10 min. The absorbance of test and standard samples were noted against blank at 505 nm spectrophotometrically. On addition of the precipitating reagent to the serum, followed by centrifugation, HDL fraction remains in the supernatant while the lipoprotein precipitates out.

#### Serum LDL and VLDL

Serum VLDL and LDL concentrations were calculated according to Friedewald equation.<sup>[11]</sup>

$$\text{LDL cholesterol} = \text{TC} - \text{HDL} - \text{TG}/5.$$

### Assessment of oxidative stress in serum samples

The oxidative and antioxidant parameters in serum samples were assessed by estimating thiobarbituric acid reactive substance (TBARS), glutathione (GSH), catalase (CAT), and sodium dismutase (SOD) (superoxide dismutase) levels.

#### TBARS level in serum

1 ml of 20% trichloroacetic acid was added to 100 µl of serum and 1% TBA reagent (1.0 ml) which were mixed and incubated at 100°C for 30 min. After cooling on ice, samples were centrifuged at 1000 g for 20 min. Serum concentration of TBARS was measured spectrophotometrically at 532 nm. A standard curve using 1, 1, 3, and 3-tertramethoxypropane (1 µM–10 µM) was plotted to calculate the concentration of TBARS.<sup>[12]</sup>

#### Estimation of Serum Reduced GSH

The GSH level was estimated using the methods described by Ellman.<sup>[13]</sup> Ellman's reagent [5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB] is a chemical used for measuring the amount of thiol group. Thiols react with this compound, cleaving the disulfide bond to give 2-nitro-5-thiobenzoate (NTB-), which ionizes to the NTB2- dianion in water at neutral and alkaline pH. This NTB2- ion has a yellow color. The NTB2- is quantified in a spectrophotometer by measuring the absorbance at 412 nm.

100 µl of serum was mixed with 2 ml of 0.3 M disodium hydrogen phosphate buffer (pH 8.4) and 0.4 ml of distilled water. Then, 0.25 ml of 0.001 M freshly prepared DTNB (5,5'-dithiobis [2-nitrobenzoic acid] dissolved in 1% w/v sodium citrate) was added to the reaction mixture, and then incubated for 10-min. The absorbance of the yellow colored complex was noted spectrophotometrically at 412 nm. A standard curve was plotted using the reduced form of GSH (0.1–1 µM), and the results were expressed as mM/g protein.

#### Estimation of superoxide dismutase activity

SOD activity was measured by the method of Misra and Fridovich.<sup>[14]</sup> Auto-oxidation of epinephrine at pH 10.4 was spectrophotometrically measured. In this method, supernatant of the kidney and sciatic nerve tissues was mixed with 0.8 ml of 50 mM glycine buffer, pH 10.4 and

the reaction was started by the addition of 0.02 ml (-)-epinephrine. After 5 min, the absorbance was measured at 480 nm (UV-1800 Spectrophotometer, Shimadzu, Japan). The activity of SOD was expressed as % activity of normal control.

#### Estimation of CAT activity

Serum CAT activity was assayed by the method described earlier.<sup>[15]</sup> The reaction mixture (1.5 ml) contained 1.0 ml of 0.01 mol/l phosphate buffer (pH 7), 0.1 ml of serum, and 0.4 ml of 2 mol/l hydrogen peroxide. The reaction was stopped by the addition of 2 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in a 1:3 ratio). The absorbance was measured at 620 nm using Spectrophotometer (UV-1800, Shimadzu, Japan) and expressed as micromoles of hydrogen peroxide decomposed/min/milligram protein.

### Statistical analysis

Data were presented as mean  $\pm$  S.E.M. For continuous variables, student *t*-test was used to differentiate mean difference. For comparison between more than 2 group, the data were processed by one-way analysis of variance (ANOVA), followed by Dunnett's *post hoc* test. \**P* < 0.05 was considered significant. Statistical analysis was performed using SPSS version 21.

## RESULTS

### Assessment of STZ induced diabetes

#### Effect of ethanolic leaf extracts of *Crataeva magna* (ELECM) on body weight

The effects of STZ administration and ELECM plant on body weight are summarized in Table 1. No difference in the initial body weight was observed in any experimental group. Two-way ANOVA revealed that STZ subjected rats gained less body weight than normal rats. After a period of 28 days of STZ pronounced decrease in body weight was found as compared to normal rats. Higher dose of ELECM and standard drugs significantly prevent the decreased in body weight at 14 and 28 days. Lower treatment did not produce pronounced difference in body weight as compared to diabetic rats.

### Biochemical estimation

#### Blood glucose level

The administration of extracts to normal rats did not produce any significant *per se* effects on various parameters assessed in the present study.

The results showed that Rats in Group I (normal control) showed blood glucose levels of  $92.30 \pm 5.21$ ,  $94.80 \pm 1.55$ ,  $91.39 \pm 2.1$ ,  $90.41 \pm 6.21$ , and  $93.12 \pm 8.15$  at 0, 7, 14, 21, and 28 days of experiment. In this normal control group, no change in blood glucose levels is observed at 7, 14, 21, and 28 days of experiment [Table 2].

Rats in Group II showed blood glucose levels of  $91.88 \pm 3.67$ ,  $90.97 \pm 11.64$ ,  $93.34 \pm 7.87$ ,  $87.76 \pm 6.96$ , and  $80.34 \pm 7.09$  at 7, 14, 21, and 28 days of experiment, respectively. In this group, oral administration of ELECM (500 mg/kgbw) was given to normal rats up to 28<sup>th</sup> day. No significant changes in blood glucose levels were observed at 28 day of experiment when compared to Group I.

In Group III, (STZ control) treatment with single dose of STZ at a dose of 65 mg/kgbw after 3 days caused significant increase (*P* < 0.05) in blood glucose levels of rats. Rats showed blood glucose levels of  $94.58 \pm 7.70$ ,  $349.80 \pm 7.81$ ,  $330.45 \pm 8.16$ ,  $336.12 \pm 14.3$ , and  $312.21 \pm 6.32$  at 7, 14, 21, and 28 days of experiment. This group showed significant increase in blood glucose levels at 7, 14, 21, and 28 days of experiment compared to Group I. Nearly 4 time's increase in blood glucose level was observed in STZ control animals.

Rats in Group IV, showed blood glucose levels of  $92.68 \pm 9.78$ ,  $331.19 \pm 7.26$ ,  $311.27 \pm 9.15$ ,  $304.67 \pm 5.44$ , and  $289.84 \pm 6.78$  at 0, 7, 14, 21, and 28 days of experiment, respectively. In this group, oral administration of ELECM (100 mg/kgbw) was given upto 28<sup>th</sup> day. A significant reduction in blood glucose levels was observed at 28 day of experiment when compared to Group III.

Further, a significant decrease (*P* < 0.05) in mean blood glucose level was observed in the hyperglycemic diabetic mice of the Group V treated with ELECM.

**Table 1: Effect of oral administration of ethanolic leaf extracts of *Crataeva magna* on body weight (g) in normal and STZ-induced diabetic rats**

Groups	Treatment	Mean body weight in gram			
		0 <sup>th</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	28 <sup>th</sup> day
Group I (normal control Rats)	Vehicle	227.37 $\pm$ 5.32	242.48 $\pm$ 5.58	256.73 $\pm$ 4.35	270.55 $\pm$ 4.44
Group II (Plant extract per se)	500 mg/kg	231.19 $\pm$ 4.07	239.67 $\pm$ 4.37	260.27 $\pm$ 4.41	275.40 $\pm$ 6.43
Group III (Diabetic control Rats)	STZ only	230.14 $\pm$ 5.94	214.71 $\pm$ 5.17	180.46 $\pm$ 5.55***	154.83 $\pm$ 4.68***
Group IV (Plant extract)	STZ+plant extract (100 mg/kg)	230.55 $\pm$ 6.44	210.43 $\pm$ 4.41	190.18 $\pm$ 7.63	205.37 $\pm$ 0.27
Group V (Plant extract)	STZ+plant extract (200 mg/kg)	229.85 $\pm$ 0.83	218.18 $\pm$ 7.57	210.65 $\pm$ 4.24a	232.78 $\pm$ 3.24a
Group VI (Plant extract)	STZ+plant extract (500 mg/kg)	230.68 $\pm$ 1.24	228.60 $\pm$ 5.16	233.21 $\pm$ 4.67a	242.15 $\pm$ 4.56a

Effect of extract on body weight. Data are mean  $\pm$  SEM; Data were analyzed using one-way ANOVA followed by Tukey's multiple test; \**P* < 0.01 as compared to Vehicle control Group; \*\**P* < 0.05 as compared to diabetic control group. STZ: Streptozotocin

Rats in Group V showed blood glucose levels of  $91.99 \pm 7.21$ ,  $299.87 \pm 10.72$ ,  $265.33 \pm 14.15$ ,  $233.87 \pm 10.87$ , and  $199.72 \pm 12.67$  at 7, 14, 21, and 28 days of experiment, respectively. In this group, significant reduction in blood glucose levels was observed at 14, 21, and 28 days of experiment when compared to Group III.

Rats in Group VI showed blood glucose levels of  $94.35 \pm 11.35$ ,  $277.32 \pm 14.56$ ,  $214.98 \pm 13.56$ ,  $192.55 \pm 16.76$ , and  $151.21 \pm 11.35$  at 7, 14, 21, and 28 days of experiment, respectively. In this group, significant reduction in blood levels was observed at 14, 21, and 28<sup>th</sup> days of experiment when compared to Group II.

In Group VII, treatment with Glipizide (4 mg/kg body weight, 4 weeks) significantly decreased the glucose level in diabetic control rats. Rats in Group VII showed blood glucose levels of  $88.72 \pm 6.14$ ,  $210.18 \pm 6.97$ ,  $177.43 \pm 4.56$ ,  $113.35 \pm 4.35$ , and  $99.63 \pm 7.43$ , at 0, 7, 14, 21, and 28 days of experiment, respectively.

### Effect of ELEFD on serum lipid profile

#### Effect of ELECM on serum TC level

Table 3 showed the effect of plant extract and standard drugs on serum TC level. The increase in serum concentration of TC was noted in diabetic control rats when compared with normal rats. The results showed that the TC levels in normal rats were 95.19 mg/dl. In STZ-induced diabetic rats, the serum TC levels increased to 167.39 mg/dl, while diabetic rats treated with standard drug glipizide decreased

TC levels up to 113.68 mg/dl. ELECM medium and high dose decreased the TC levels up to 132.57 mg/dl and 101.32 mg/dl in dose-dependent manner. However, lower dose did not significantly decrease the TC in diabetic rats. Among the plant extract doses high dose worked effectively and decreased the TC levels in diabetic rats [Table 3].

#### Effect of ELECM on serum TG

Table 3 showed the effect of plant extract and standard drugs on serum TG level. The increase in serum concentration of TG was noted in diabetic control rats when compared with normal rats. The results showed that the TG levels in normal mean of rats were 110.25 mg/dl. STZ-induced diabetic rats have increased level of TG, 178.13 mg/dl. Diabetic rats treated with standard drug glipizide decreased the TG levels up to 123.18 mg/dl. Diabetic rats treated with ELECM medium and high dose decreased the TG levels up to, 148.12 mg/dl and 139.55 mg/dl in dose-dependent manner. However, lower dose did not significantly decrease the TG in diabetic rats.

#### Effect of ELEFD on serum HDL

Table 3 showed the effect of plant extract and standard drugs on serum HDL level. The decrease in serum concentration of HDL was noted in diabetic control rats when compared with normal rats. The results showed that the HDL levels in normal mean of rats were 46.72 mg/dl. STZ-induced diabetic rats have decreased level of HDL, 28.22 mg/dl. Diabetic rats treated with standard drug glipizide increased

**Table 2: Effect of oral administration of ethanolic leaf extracts of *Crataeva magna* on blood glucose in normal and STZ-induced diabetic rats**

Groups	Treatment	Blood glucose level (mg/dl)				
		Day 0	Day 7	Day 14	Day 21	Day 28
(Group-I) Normal	Vehicle	92.30±5.21	94.80±1.55	91.39±2.1	90.41±6.21	93.12±8.15
(Group-II) Plant per se	500 mg/kg	91.88±3.67	90.97±11.64	93.34±7.87	87.76±6.96	80.34±7.09
(Group-III) Diabetic control	STZ only	94.58±7.70	349.80±7.81***	330.45±8.16***	336.12±14.3***	312.21±6.32***
(Group-IV) Plant extract 100 mg/kg	STZ+extract (100 mg/kg)	92.68±9.78	331.19±7.26	311.27±9.15	304.67±5.44	289.84±6.78a
(Group-V) Plant extract 200 mg/kg	STZ+extract (200 mg/kg)	91.99±7.21	299.87±10.72	265.33±14.15a	233.87±10.87aa	199.72±12.67aa
(Group-VI) Plant extract 500 mg/kg	STZ+extract (500 mg/kg)	94.35±11.35	277.32±14.56	214.98±13.56aa	192.55±16.76aaa	151.21±11.35aaa
Standard treatment (Group-VII)	Glipizide (4 mg/kg)	88.72±6.14 <sup>a</sup>	210.18±6.97 <sup>a</sup>	177.43±4.56 <sup>a</sup>	113.35±4.35 <sup>a</sup>	99.63±7.43 <sup>a</sup>

Effect of extract on serum glucose. Data are mean±SEM; Data were analyzed using one-way ANOVA followed by Tukey's multiple test; \*P<0.01 as compared to vehicle control Group; <sup>a</sup>P<0.05 as compared to diabetic control group. STZ: Streptozotocin

**Table 3: Effect of ethanolic leaf extracts of *Crataeva magna* on blood lipid profile in normal and STZ-induced diabetic rats**

Groups	Treatment	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	High density lipoproteins	Low density lipoprotein	Very low density lipoprotein
				cholesterol (mg/dl)	cholesterol (mg/dl)	lipoprotein (mg/dl)
(Group-I) Normal	Vehicle	95.19±4.20	110.25±4.72	46.72±4.32	26.42±2.46	22.05±1.36
(Group-II) Plant per se	500 mg/kg	91.32±5.54	106.56±7.67	45.78±5.32	24.23±4.96	21.31±4.67
(Group-III) Diabetic control	STZ only	167.39±6.55***	178.13±3.35***	28.22±5.55***	103.54±5.44***	35.62±1.55***
(Group-IV) Plant extract 100 mg/kg	STZ+extract (100 mg/kg)	150.43±11.54	172.36±11.75	32.54±4.75*	83.42±3.87*	34.47±5.65
(Group-V) Plant extract 200 mg/kg	STZ+extract (200 mg/kg)	132.57±7.56 <sup>a</sup>	148.12±3.89 <sup>ab</sup>	40.03±5.78a	62.92±5.45 <sup>ab</sup>	29.62±2.45 <sup>a</sup>
(Group-VI) Plant extract 500 mg/kg	STZ+extract (500 mg/kg)	101.32±5.72 <sup>aaa</sup>	139.55±6.89 <sup>aa</sup>	42.10±4.14 <sup>aa</sup>	31.31±5.32 <sup>aaa</sup>	27.91±5.55 <sup>aa</sup>
(Group-VII) Standard treatment	Glipizide (4 mg/kg)	113.68±4.22 <sup>aa</sup>	123.18±5.97 <sup>aaa</sup>	44.24±5.56 <sup>aaa</sup>	44.80±4.35 <sup>aaa</sup>	24.63±2.43 <sup>aaa</sup>

Effect of extract on serum lipid. Data are mean±SEM; Data were analyzed using one-way ANOVA followed by Tukey's multiple test; \*P<0.01 as compared to Vehicle control Group; <sup>a</sup>P<0.05 as compared to diabetic control group. STZ: Streptozotocin

the HDL levels up to 44.24 mg/dl. Diabetic rats treated with plant extracts of CM medium and high dose increased the HDL levels up to, 40.03 mg/dl and 42.10 mg/dl in dose-dependent manner. However, lower dose did not significantly increase the HDL in diabetic rats. Among the plant extract doses high dose worked effectively and increased the HDL levels in diabetic rats [Table 3].

#### Effect of ELECM on serum LDL

Table 3 showed the effect of plant extract and standard drugs on serum LDL level. The increase in serum concentration of LDL was noted in diabetic control rats when compared with normal rats. The results showed that the LDL levels in normal mean of rats were 26.42 mg/dl. STZ-induced diabetic rats have increased level of LDL, 103.54 mg/dl. Diabetic rats treated with standard drug glipizide decreased the LDL levels up to 44.80 mg/dl. Diabetic rats treated with ELECM medium and high dose decreased the LDL levels up to, 62.92 mg/dl and 31.31 mg/dl in dose-dependent manner. However, lower dose did not significantly decrease the LDL in diabetic rats. Among the plant extract doses high dose worked effectively and decreased the LDL levels in diabetic rats [Table 3].

#### Effect of ELECM on serum VLDL

Table 3 showed the effect of plant extract and standard drugs on serum VLDL level. The increase in serum concentration of TC was noted in diabetic control rats when compared with normal rats. The results showed that the VLDL levels in normal mean of rats were 22.05 mg/dl. STZ-induced diabetic rats have increased level of VLDL, 35.62 mg/dl. Diabetic rats treated with standard drug glipizide decreased the VLDL levels up to 24.63 mg/dl. Diabetic rats treated with ELECM medium and high dose decreased the VLDL levels up to, 29.62 mg/dl and 27.91 mg/dl in dose-dependent manner. However, lower dose did not significantly decrease the VLDL in diabetic rats among the plant extract doses high dose worked effectively and decreased the VLDL levels in diabetic rats [Table 3].

#### Effect of ELECM on serum oxidative/antioxidant parameters

##### Effect of ELECM on serum TBARS

The levels of oxidative markers detected in serum of normal and diabetic control rats are summarized in Table 4. STZ administration resulted in a profound increase in TBARS levels, as compared with

normal controls. Chronic administration of only higher dose of plant extracts significantly reduced elevated levels of TBARS in serum of diabetic rats in comparison to the levels observed in vehicle treated diabetic control rats. However, plant extract at lower doses (200, 500 mg/kg did not notably influence the TBARS level in STZ rats) [Table 4].

##### Effect of ELECM on serum GSH, SOD, and CAT Levels in diabetic rats

The anti-oxidant enzyme status in serum samples was assessed by measurements of GSH, SOD, and CAT levels. Serum GSH, SOD, and CAT levels were decreased in diabetic rats when compared with normal control group, indicative of impairment in anti-oxidant status. Treatment with plant extract (200 and 500 mg/kg,) and glipizide restored serum GSH, SOD, and CAT levels as compared to OBX control group [Table 4]. Further, lower dose (100 mg/kg, p.o.) did not produce any effect on the anti-oxidant enzyme levels in diabetic rats.

## Discussion

The antidiabetic activity was carried out in STZ-induced diabetes in rat animal model. STZ [2-deoxy-2-(3-(methyl-3-nitrosoureido)-d-glucopyranose)] impaired the glucose oxidation thereby inhibiting insulin synthesis and its secretion as a consequence of  $\beta$ -cells damage in pancreas.<sup>116,171</sup>

The results obtained in treatment group were compared with normal control, diabetic control, and positive control rats treated with glipizide. In accordance to the previous studies, STZ-induced diabetes rats showed high FBG level as compared to control group.<sup>1181</sup> In contrast treatment with plant extract ELECM significantly attenuated the increase FBG, which is consistent with our previous report. Moreover, the plant extracts normalizes the glucose level similar to standard drug and exhibited as a potent antidiabetic effect.

STZ-induced diabetes is also associated with characteristic weight loss and administration of plant extract, improved body weight in diabetic rats. The decreased body weight may be results from the protein metabolism and muscle wasting. On the basis of report the reversed in decrease body weight might be linked to insulin secretion which results in improve glucose level in diabetic animals.

**Table 4: Effect of ethanolic leaf extracts of *Crataeva magna* on oxidant/anti-oxidant profile in normal and STZ-induced diabetic rats**

Groups	Treatment	MDA (nM/mg)	Glutathione ( $\mu$ g/mg)	Catalase ( $\mu$ M/min/mg)	SOD (Unit/min/mg)
(Group-I) Normal	Vehicle	10.50 $\pm$ 0.80	25.77 $\pm$ 3.10	19.13 $\pm$ 1.80	14.47 $\pm$ 1.87
(Group-II) Plant per se	500 mg/kg	11.14 $\pm$ 2.23	24.99 $\pm$ 4.45	20.42 $\pm$ 3.52	15.70 $\pm$ 2.66
(Group-III) Diabetic control	STZ only	21.30 $\pm$ 1.86***	11.67 $\pm$ 2.26***	11.70 $\pm$ 1.75***	5.70 $\pm$ 1.50***
(Group-IV) Plant extract 100mg/kg	STZ+extract (100 mg/kg)	19.98 $\pm$ 0.35	14.56 $\pm$ 3.15	14.20 $\pm$ 2.97	7.12 $\pm$ 2.21**
(Group-V) Plant extract 200mg/kg	STZ+extract (200 mg/kg)	17.53 $\pm$ 2.78	19.53 $\pm$ 0.78aa	16.33 $\pm$ 1.26 <sup>aa</sup>	10.47 $\pm$ 1.99aa
(Group-VI) Plant extract 500 mg/kg	STZ+extract (500 mg/kg)	12.56 $\pm$ 0.15aa	21.98 $\pm$ 0.35aaa	17.91 $\pm$ 1.19 <sup>aaa</sup>	11.87 $\pm$ 1.53aa
(Group-VII) Standard treatment	Glipizide (4 mg/kg)	11.11 $\pm$ 1.67aaa	23.12 $\pm$ 2.37 <sup>aaa</sup>	18.99 $\pm$ 2.55aaa	13.92 $\pm$ 2.09aaa

Effect of extract on oxidant/anti-oxidant profile. Data are mean $\pm$ SEM; Data were analyzed using one-way ANOVA followed by Tukey's multiple test; \* $P$ <0.01 as compared to vehicle control Group; <sup>a</sup> $P$ <0.05 as compared to Diabetic control group. STZ: Streptozotocin

There are large number of studies supported that a positive correlation in hyperglycemia and dyslipidemia.<sup>[19]</sup> Abnormal or increase level of lipids leads to atherosclerosis which may cause the diabetes and diabetes associated complications.<sup>[19]</sup> Alteration in normal lipid profile during DM such as high level of TC, TG, LDL, and VLDL results in the progression of diabetes associated complications.<sup>[19-21]</sup> This leads to mainly complication at level of microvascular and also manifested into cardiovascular disorders. In accordance with the previous reports, in the current study lipid abnormalities such as a significant increase in TC, TG, VLDL, and LDL level was observed along with a remarkable reduction in good lipid such as HDL level in diabetic rats. However, the chronic treatment with ELECM significantly improved the altered lipid profiles. Moreover, the ELECM at higher dose shown quite similar result as like to standard drug in the reduction of increase lipid levels such as TC, TG, LDL, and VLDL indicating equivalent hypolipidemic activity similar to available standard drugs. Moreover, in case of HDL, the highest dose of the extract showed more pronounced effects as compared to standard drug.<sup>[22-24]</sup>

Furthermore, various studies explore the role of several factors such as alteration in antioxidant enzymes, increased oxygen free-radical, GSH metabolism, lipid peroxidation, and non-enzymatic protein glycosylation, involves in the etiology of DM.<sup>[25]</sup> STZ-induced hyperglycemia elevates reactive oxygen species (ROS) generation and reduce the antioxidant defense system resulting in disruption of cellular content and increased lipid peroxidation.<sup>[26]</sup> Moreover, studies reported that disturbed physiological level of oxygen and hydrogen peroxide in diabetes is maintained by antioxidant enzymes.<sup>[26]</sup> Thus, oxidative stress can be diminished through diminution of free radical generation. Antioxidant enzyme such as SOD, CAT, and GSH, provides defense against these free radicals leading to reduced oxidative stress. In our study, SOD, CAT, and GSH were significantly decreased in diabetic rats; however, the level of TBARS increased significantly. The previous studies indicated that the activity of SOD, CAT, and GSH is reduced in serum and tissue homogenate of diabetic rats, whereas level of TBARS increased.<sup>[27]</sup> In this study, chronic treatment with ELECM reversed the altered oxidant/antioxidant system by increasing SOD, CAT as well as GSH levels and reducing TBARS level. Flavonoids and other phenolic compounds are known to reduce oxidative stress, reduce necrosis, and regenerate  $\beta$ -cells. Thus, the presence of these secondary metabolites may prove beneficial for counteracting the diabetes.

## Conclusion

In this study, the anti-diabetic activity of ELECM was carried out. The result of this study concluded that anti-diabetic activities of ELECM were explored in a dose dependent manner.

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## References

- World Health Organization. Global Health Estimates 2016: Deaths by Cause, Age, Sex, and Country, 2000-2012. Geneva: World Health Organization; 2014.
- International Diabetes Federation. IDF Diabetes Atlas. 6<sup>th</sup> ed. Brussels, Belgium: International Diabetes Federation; 2013.
- Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004;27:1047-53.
- World Health Organization. (2003). Laboratory Diagnosis and Monitoring of Diabetes Mellitus. Geneva: World Health Organization. Available from: <https://www.who.int/iris/handle/10665/42642>. [Last accessed on 2021 Mar 13].
- Singh SK. Commentary on "consensus recommendations on sulfonylurea and sulfonylurea combinations in the management of Type 2 diabetes mellitus: International Task Force. *Indian J Endocrinol Metab* 2018;22:158-9.
- Greenspan F, Gardner D. Basic and Clinical Endocrinology. 6<sup>th</sup> ed. New York: McGraw-Hill; 2001. p. 295-361.
- Gagandeep M, Kalidhar S. Chemical constituents of *Crataeva nurvala* (Buchham) leaves. *Int J Pharm Sci* 2006;68:804-6.
- Kinoshita T, Hiraga Y, Nakamura N, Kitajo A, Iinuma F. Determination of glucose in blood using glucose oxidase-peroxidase system and 8-hydroxyquinoline-p-anisidine. *Chem Pharm Bull (Tokyo)* 1969;27:568-70.
- Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. *Clin Chem* 1974;20:470-5.
- Werner M, Gabrielson DG, Eastman J. Ultramicro determination of serum triglycerides by bioluminescent assay. *Clin Chem* 1981;27:268-71.
- Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499-502.
- Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;95:351-8.
- Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959;82:70-7.
- Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem* 1972;247:3170-5.
- Sinha AK. Colorimetric assay of catalase. *Anal Biochem* 1972;47:389-94.
- Skowera A, Ellis RJ, Varela-Calviño R, Arif S, Huang GC, Van-Krinks C, et al. CTLs are targeted to kill beta cells in patients with type 1 diabetes through recognition of a glucose-regulated preproinsulin epitope. *J Clin Invest* 2008;118:3390-402.
- Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol Res* 2001;50:537-46.
- Madhavan V, Joshi R, Murali A, Yoganarasimhan SN. Antidiabetic activity of *Curculigo orchioides* root tuber. *Pharm Biol* 2007;45:18-21.
- Thomas M, Gunnam R, Reddy GN. Evaluation of anti-hyperlipidemic effect of *Bacopa monnieri* linn. in atherogenic diet-induced hyperlipidemic rats. *Int J Adv Pharm Sci* 2014;5:2362-7.
- Lönn ME, Dennis JM, Stocker R. Actions of "antioxidants" in the protection against atherosclerosis. *Free Radic Biol Med* 2012;53:863-84.
- Thomas S, Karalliedde J. Diabetic nephropathy. *Medicine* 2015;43:20-5.
- Song Y, Manson JE, Tinker L, Rifai N, Cook NR, Hu FB, et al. Circulating levels of endothelial adhesion molecules and risk of diabetes in an ethnically diverse cohort of women. *Diabetes* 2007;56:1898-904.
- Mooradian AD. Dyslipidemia in type 2 diabetes mellitus. *Nat Clin Pract Endocrinol Metab* 2009;5:150-9.
- Nogueira JP, Britesc FD. Role of enterocytes in dyslipidemia of insulin-resistant states. *Endocrinol Nutr* 2013;60:179-89.
- Yazdanparast R, Ardestani A, Jamshidi S. Experimental diabetes treated with *Achillea santolina*: Effect on pancreatic oxidative parameters. *J Ethnopharmacol* 2007;112:13-8.
- Cameron NE, Cotter MA. Potential therapeutic approaches to the treatment or prevention of diabetic neuropathy: Evidence from experimental studies. *Diabet Med* 1993;10:593-605.
- Kishore L, Kaur N, Singh R. Effect of kaempferol isolated from seeds of *Eruca sativa* on changes of pain sensitivity in streptozotocin-induced diabetic neuropathy. *Inflammopharmacology* 2018;26:993-1003.