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Research Article

Evaluation of antidiabetic potential of ethanolic extract of *Caseria elliptica* in streptozotocin induced diabetic rats

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ABSTRACT

The present study was focussed to evaluate the antidiabetic potential of ethanolic root extract of *Caseria elliptica* (CE) in Streptozotocin (STZ) induced diabetes in rats. In overnight fasted rats OGTT was performed by administration of glucose 2g/kg/p.o and the blood samples were collected periodically for glucose estimation. Diabetes was induced in rats by administration of STZ (40 mg/kg/i.p). Ethanolic root extract of CE (100mg/kg, 200mg/kg and 400mg/kg/p.o) and Glibenclamide (5mg/kg/p.o) were administered to the STZ induced diabetic rats for two weeks. Blood glucose, triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol, and VLDL-cholesterol were estimated from the serum. Tissue liver glycogen content, change in body weight were estimated. The results showed that the extract dose dependently decreased the increased biochemical parameters in rats. Histopathological examinations reveals that the ethanolic extract of CE ameliorated the damaged caused to the pancreatic β-cells in STZ treated rats. From the study it was concluded that ethanolic root extract of CE possess good hypoglycemic and hypolipidemic effect.

Keywords: *Caseria elliptica;* Glibenclamide; Glycogen; Hypoglycemic effect; Diabetes mellitus; Pancreas; Streptozotocin.

INTRODUCTION

Type 2 diabetes mellitus is a complex metabolic disorder affecting all age group people. Approximately it effects 4% of the population worldwide and it will be raised to 5.4% by 2025 (Kim *et al.,* 2006). Diabetes occupies sixth place for cause of death in US (Gambert and Pinkstaff, 2006) and the diabetic cases are largely increasing in India with over 67 million individuals currently diagnosed with disease (IDF, 2014) and expected that huge population are with undiagnosed diabetes mellitus. The morbidity and mortality of diabetes mellitus is due to the clinical manifestations of hyperglycemia and hyperlipidemia (Taskinen, 2002). Though conventional and newer synthetic antidiabetic drugs are available they have considerable side effects like hypoglycaemia, weight gain and acidosis. There is a need in search of potential natural antidiabetic agents from the traditional folk medicines with less tolerable side effects. *Caseria elliptica* root is traditionally used for treating diabetes mellitus in folk medicine. In the present study an attempt was made to scientifically prove the antidiabetic activity of *Caseria elliptica* in streptozotocin induced diabetic model.

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MATERIALS & METHODS

Chemicals

Streptozotocin (STZ), and glibenclamide were purchased from Sigma-Aldrich and all other chemicals used in the experiments were of analytical grade.

Plant collection and authentification

The roots of the plant *Caseria elliptica* was collected from Talakona forest near Tirupathi, A.P in January and dried under shade. The plant was identified and authentified by Dr. K. Madhava chetty, Assistant Professor, S. V. University, Tirupathi.

Preparation of extract

The dried plant material was powdered coarsely and soaked in petroleum ether for 72 hours for defatification, and then extracted with ethanol by continuous hot percolation, using soxhlet apparatus. The obtained extract was concentrated at 40^{0}C by us-ing rotary evaporator.

Preliminary phytochemical studies

The crude extract was subjected for the presence of phytochemicals like alkaloids, glycosides, carbohydrates, sterols, phenolic compounds, tannins, flavonoids, saponins, proteins, and amino acids by using standard procedures (Kokate, 2001).

Animals

Male wistar rats weighing 180-200g were selected for the study. The rats were procured from Sree Venkateswara enterprises, Bangalore, and housed at 22 ± 3 ^{0} C, 45–75% humidity, 12 h light–dark cycle, and fed with standard pellet diet and water ad libitum. They were allowed to acclimatize to the laboratory conditions prior to experimentation. The study was approved by Institutional Animal Ethics Committee (IAEC) of Sree Vidyanikethan Educational Trust, Tirupati. The experimental procedures were performed according to the guidelines of CPCSEA.

Acute toxicity study

The acute toxicity of ethanolic root extract of *Caseria elliptica* (CE) was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). In this study, at 2000 mg/kg dose the extract was found to be safe (OECD, 2002), and it was considered as LD50 (Lethal dose) of the extract.

Oral glucose tolerance test (OGTT)

In overnight fasted (for 8 h) rats, glucose (2 g/kg b.w.) was orally administered 30 min after administration of the extract CE at various doses (100 mg/kg b.w, 200 mg/kg b.w, 400 mg/kg b.w), Glibenclamide (5 mg/kg b.w), control group (normal saline 10 ml/kg b.w), and blood samples were collected from the tail vein at regular intervals 30, 60, 90 & 120 min (i.e., before and after administration of glucose) to measure the blood glucose levels (Tahara *et al.,* 2011).

Induction of diabetes in rats

Freshly prepared streptozotocin (STZ) at dose of (40 mg/ kg b.w) in 0.1 M citrate buffer (pH = 4.5) was given intraperitoneally to overnight fasted rats to induce diabetes. To avoid hypoglycemia in rats, the water was replaced with 5% glucose solution. The fasting blood glucose is measured 3 days after the vehicle or STZ injection. The rats with the FBG > 250mg /dl were considered diabetic and selected for further pharmacological studies. Then the diabetic animals were treated with plant extract for 2 weeks (Li Zhang, *et al.,* 2010).

Experimental design

All the STZ induced diabetic rats were divided into six groups of 6 rats in each and were treated for 2 weeks as follows. The extract was suspended in 0.5% sodium carboxymethyl cellulose.

Group I: Control (vehicle treated); Group II: Diabetic control (streptozotocin); Group-III: administered with Glibenclamide (5 mg/kg b.w, p.o). Group-IV, V & VI were administered with: CE (100, 200 & 400 mg/kg b.w, p.o) respectively.

Determination of biochemical parameters

At the end of the experiment blood samples were collected from retroorbital plexus and centrifuged at 10,000 rpm at 4^{o}C for 15 min to collect the serum. Blood glucose, TG, TC, HDL-C, SGOT and SGPT levels were determined spectrophotometrically by using commercial kits. Low density lipoprotein (LDL-C) and VLDL-C were calculated by using Friedewald formula (Friedewald *et al.,* 1972).

Liver glycogen estimation

The Liver glycogen was estimated by the method Sam Seifter *et al.,* 1949 by using anthrone reagent.

Histological analysis

At the end of the experiment the pancreatic tissues were isolated and stored in 10% formalin solution. The tissues were sectioned to 4 µm thickness using microtome apparatus and stained with eosin and hematoxylin. The histopathological changes were observed and photographed.

Statistical analysis

All the data were expressed as mean ± SEM of 6 animals. They were analyzed statistically by graphpad prism 5 using Dunnett's t test with P Values significant at $P < 0.05$.

RESULTS

Preliminary phytochemical studies

The phytochemical studies of ethanolic extract of CE revealed the presence of terpenoids, flavanoids, carbohydrates, saponins, tannis, and resins.

Effect of ethanolic extract of CE on OGGT (Oral Glucose Tolerance Test):

OGTT was carried out to test the glucose tolerance in rats. The extract of CE dose dependently decreased the impaired glucose levels, and showed significant reduction ($P < 0.05$) in blood glucose at dose 400mg/kg compared to control and other treated groups (Figure:1). Blood glucose levels were significantly decreased in glibenclamide group compared to other treated groups.

Effect of CE extract on body weight

Figure: 2 shows effect of CE on body weight in diabetic rats. The rats in all the groups does not show any significant variation in their body weight. The body weight of normal control increased significantly from 186.3 ± 3.1g to 202.4 ±2.2 g (Figure-2). The diabetic control rats showed significant decrease in body weight from 177.7±3.7g to 158.7±4.6g. After treatment with CE and glibenclamide, there was slight increase in the body

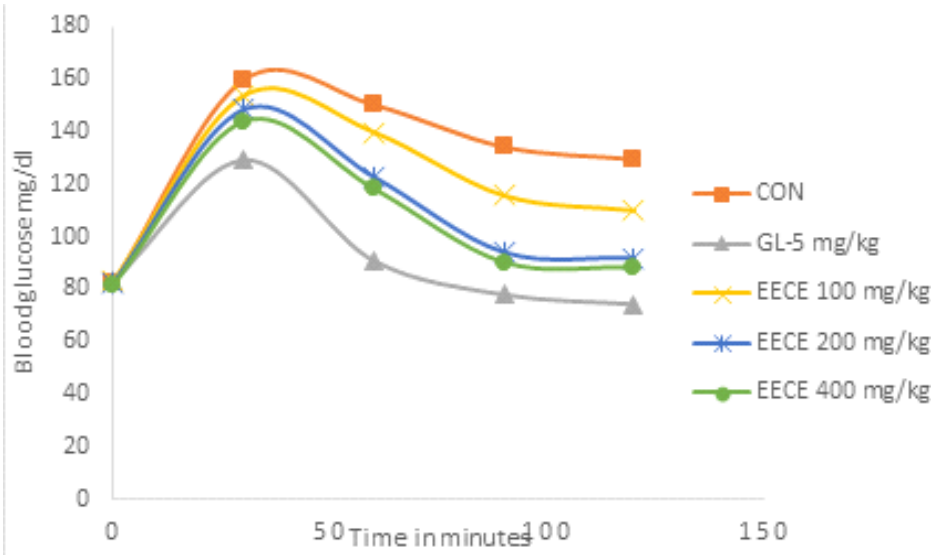

Figure 1: Effect of ethanolic extract of *Caseria elliptica* on oral glucose tolerance test (OGTT) Data expressed as mean ± standard error mean (SEM) of six animals.

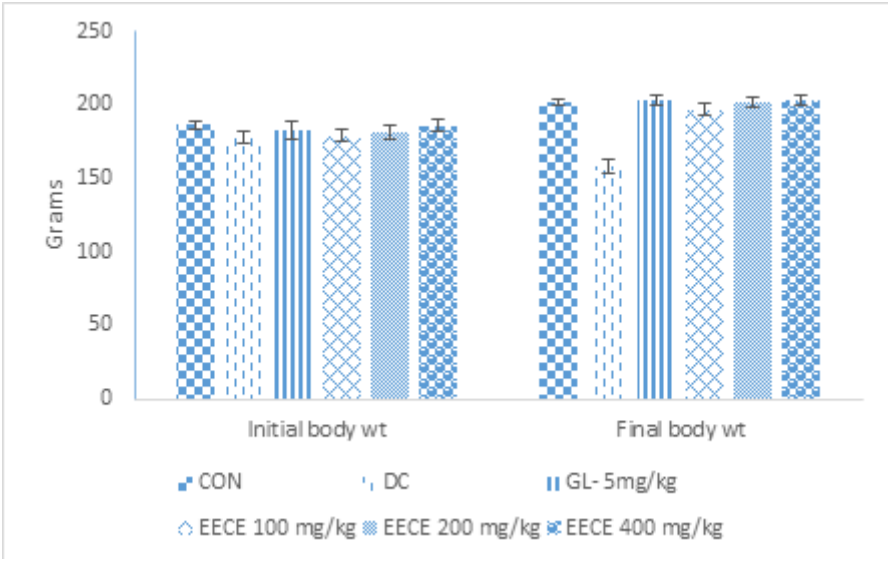

Figure 2: Effect of ethanolic extract of *Caseria elliptica* on body weight in STZ induced diabetic rats. Data expressed as mean ± standard error mean (SEM) of six animals.

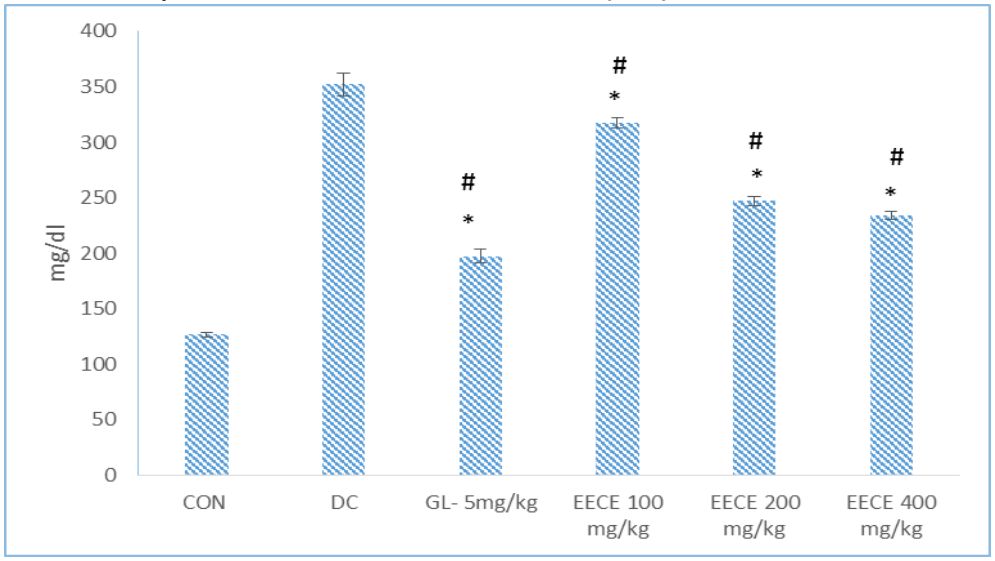

Figure 3: Effect of ethanolic extract of *Caseria elliptica* on blood glucose levels in STZ induced diabetic rats. Data expressed as mean ± standard error mean (SEM) of six animals. Significance is determined by one way ANOVA followed by dunnett's't' test.

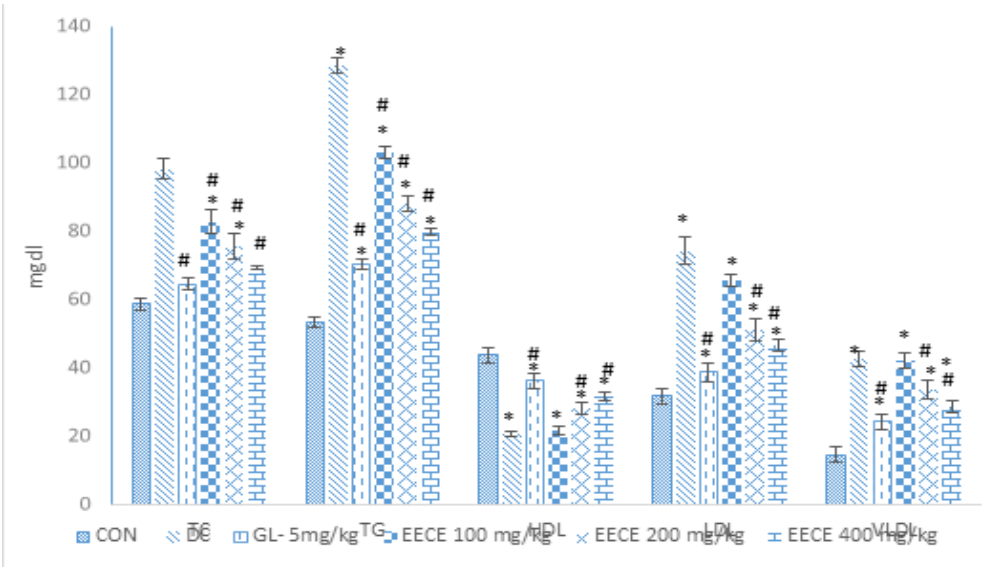

Figure 4: Lipid profile levels in STZ induced diabetic rats treated with EECE. Data expressed as mean ± standard error mean (SEM) of six animals. Significance is determined by one way ANOVA followed by dunnett's't' test.* P < 0.05 vs control group, # P < 0.05 vs

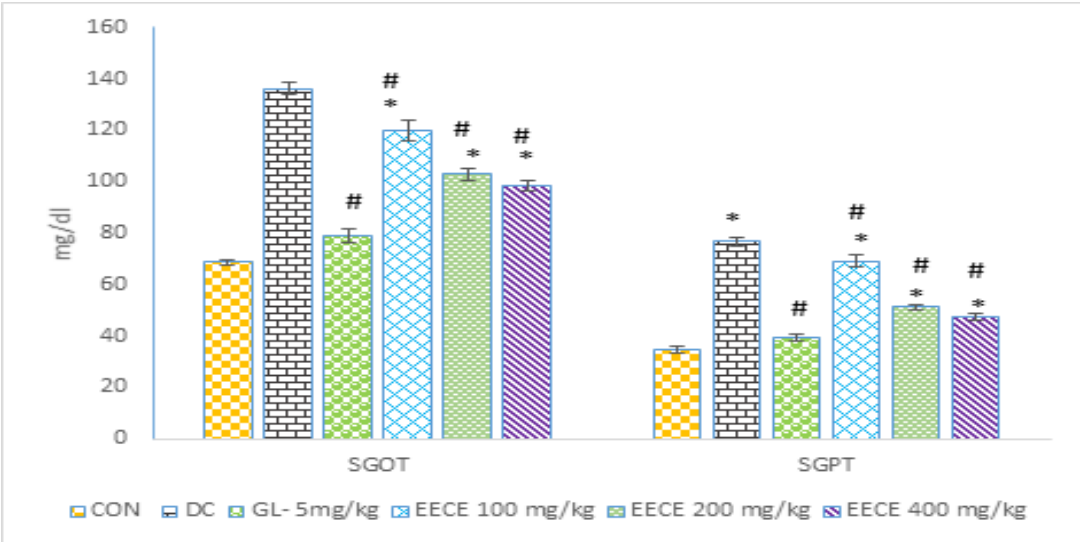

Figure 5: Liver enzyme levels in STZ induced diabetic rats treated with EECE. Data expressed as mean ± standard error mean (SEM) of six animals. Significance is determined by one way ANOVA followed by dunnett's't' test.* P < 0.05 vs control group, # P < 0.05 vs

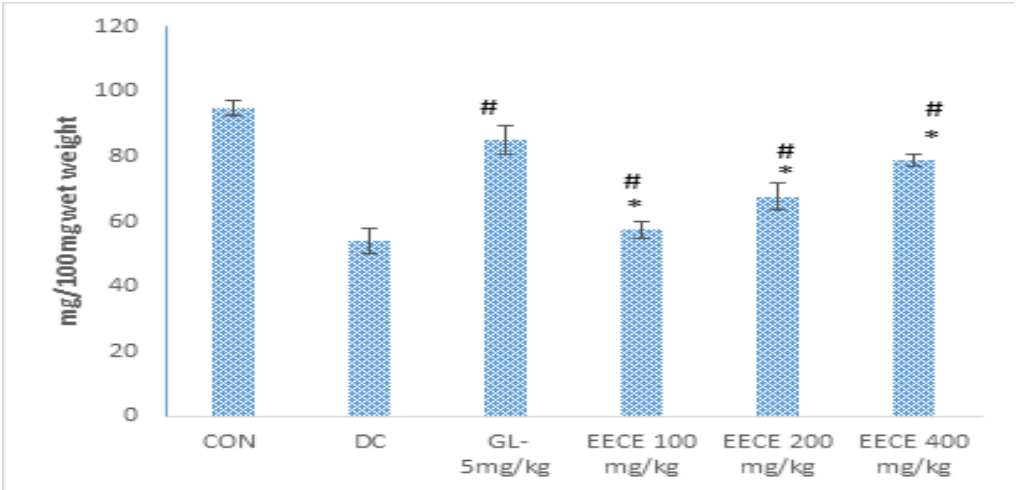

Figure 6: Liver glycogen content in STZ induced diabetic rats treated with EECE. Data expressed as mean ± standard error mean (SEM) of six animals. Significance is determined by one way ANOVA followed by dunnett's't' test.* P < 0.05 vs control group, # P < 0.05 vs diabetic control group.

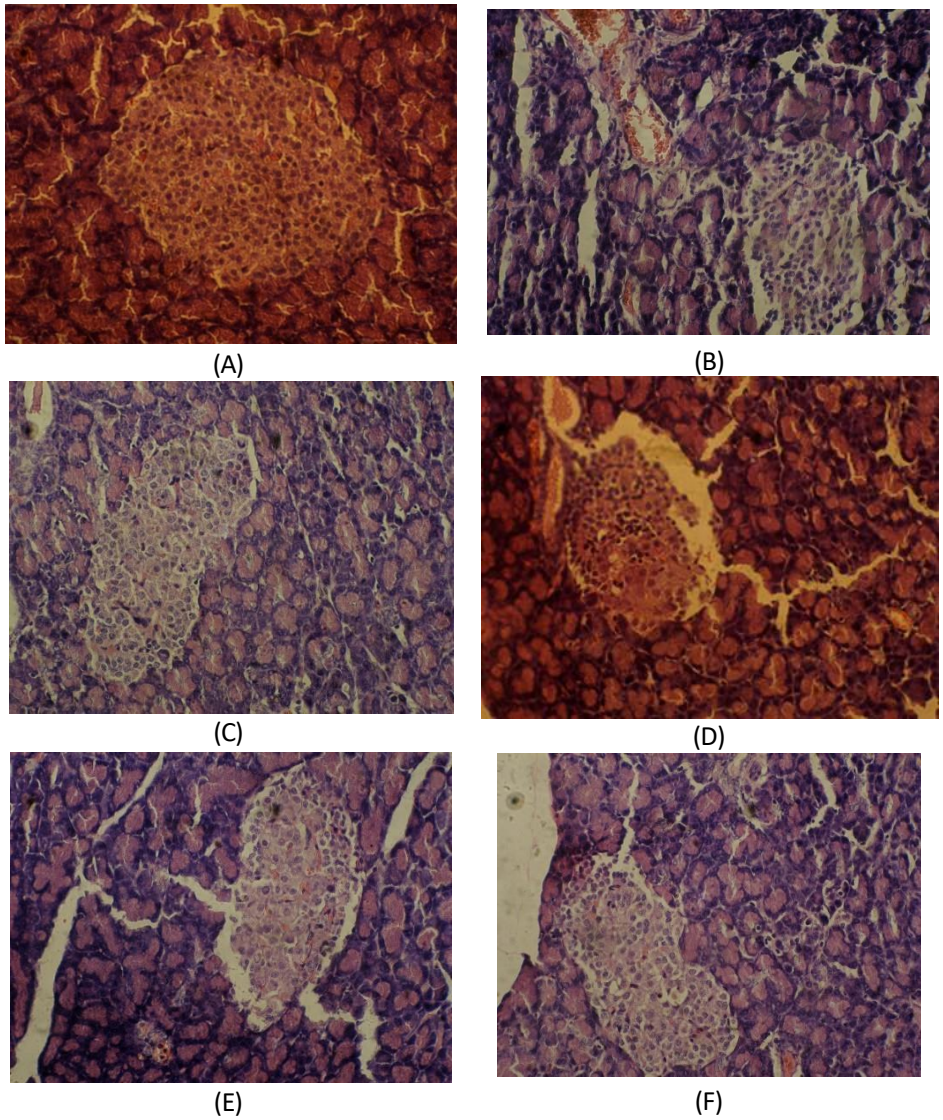

Figure 7: Histopathology of pancreas in rats (control & experimental groups). (A) Control rats with intact β cells of pancreas; (B) STZ control rats with destructive β cells and acinar cells of pancreas; (C) diabetic rats treated with Glibenclamide (5mg/kg) standard shows restored pancreatic cells; (D) diabetic rats treated with EECE 100 mg/kg with mild regeneration of damaged pancreas; (E) diabetic rats treated with EECE 200 mg/kg with moderate restoration pancreatic β cells with fewer vacoulation; (F) diabetic rats treated with EECE 400 mg/kg with increased no. of pancreatic β cells.

weight but with no statistical significance among CE treated groups.

Serum blood glucose levels

Hypoglycaemic effect of extract CE was investigated in normal and diabetic rats. Administration of extract CE for two weeks significantly reduced the hyperglycemia induced by STZ. The extract of CE at dose 400mg/kg b.w, showed significant reduction in blood glucose ($P< 0.05$) compared to other diabetic rats (Figure-3).

Effect of CE extract on lipid profile

The effect of CE root extract on lipid profile in STZ induced diabetic rats is shown in the Figureure-4. In the present study administration of STZ significantly increased the TC, TG, LDL-C, VLDL-C levels and decreased the HDL-C levels compared to control group. After administration of the extract CE for two weeks, the extract reduced the significant rise in the levels of parameters mentioned above and increased the levels of HDL-C in diabetic rats. Glibenclamide treated group showed marked decrease in the lipid profile levels with increase in HDL-C levels. The extract of CE at dose 400mg/kg showed significant hypolipidemic effect compared to other treated groups.

Effect of CE extract on SGOT & SGPT in serum

SGOT and SGPT levels were significantly increased in diabetic rats compared to control group. The rise in these levels were significantly ameliorated in CE treated groups dose dependently (Figure-5). Glibenclamide treated group markedly decreased these levels. The extract at dose 400mg/kg reduced the levels of liver enzymes significantly ($P<0.05$) compared to diabetic control and other groups.

Effect of CE extract on liver glycogen

The liver glycogen content was significantly lowered in diabetic rats when compared to other groups. After treatment with the extract CE liver glycogen levels were significantly increased compared diabetic control (Figure-6).

DISCUSSION

In our study, we examined the protective effect of ethanolic root extract of CE against pancreatic β-cell damage in streptozotocin induced diabetic rats. Type 2 diabetes mellitus is metabolic disorder with impaired insulin secretion. Hyperglycemia is the clinical manifestation of this disease and is responsible for the development complications in later stage. In the study diabetes was induced to rats by administration of streptozotocin. Several studies revealed that streptozotocin-induced diabetic rats showed increased blood glucose levels and decreased insulin secretion. These changes were due to damage induced by streptozotocin. Free radicals generated by STZ causes DNA fragmentation and pancreatic β-cell damage (Bolaffi et al., 1987; Nukatsuka et al., 1990; Riad et al., 2007; Fukudome et al., 2008; Takasu et al., 1991).

In our study, the ethanolic extract of *Caseria elliptica (*400mg/kg) significantly decreased the glucose concentration after oral glucose loading similar to glibenclamide, which shows that *Caseria elliptica* possess antidiabetic activity. In STZ induced diabetic rats the blood glucose levels were elevated and it was significantly lowered after administration of ethanolic extract of *Caseria elliptica*. The other abnormalities of diabetes mellitus is hyperlipidemia with increased lipid profile, characterized by high total cholesterol, total triglycerides, LDL-C, VLDL-C and decreased HDL-C levels which were observed in STZ induced diabetic rats (Merzouk et al., 2000). Our study showed that the ethanolic extract of CE not only lowered the blood glucose levels but also significantly decreased the levels of total cholesterol, triglycerides, LDL-C, VLDL-C, and increased HDL-C levels. Several studies have reported that diabetes alters the levels of liver enzymes. The present study revealed that STZ treated rats showed increased levels of liver enzymes which were reduced in extract treated groups. Histopathological studies of pancreas in diabetic rats showed degeneration of β-cells. After administration of *Caseria elliptica* there is regeneration pancreatic β-cell in diabetic rats and it confirms that *Caseria elliptica* has protective effect from STZ induced pancreatic damage. The possible mechanism of our ethanolic extract of CE might be due to (i) increase in glucose utilization by tissues, (ii) uptake of glucose might be increased in liver or (iii) stimulating the insulin secretion from β-cells and also may be due its antioxidant property. This was supported from our study that the liver glycogen levels were decreased in STZ induced diabetic rats and after treatment with ethanolic extract of *Caseria elliptica* the liver glycogen levels were significantly increased. However, the exact molecular mechanism by which *Caseria elliptica* generates its protective effect on β cells has to be determined.

CONCLUSION

The study suggests that *Caseria elliptica* showed protective effect against STZ induced diabetes in rats and reversed the changes in blood glucose, body weight, liver glycogen, and other biochemical parameters. This study confirms that *Caseria elliptica* possess excellent antidiabetic and antihyperlipidemic activity. However further studies are needed to evaluate the exact molecular mechanism of *Caseria elliptica.*

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