

Antidiabetic and antidyslipidemic potential of *Echinops echinatus* in rat models of type I and type II diabetes

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Abstract: *Echinops echinatus* is traditionally an important plant that finds its extensive use as a diuretic, anti-inflammatory, anti-pyretic, nerve tonic, abortifacient, aphrodisiac, antiasthmatic, and antidiabetic agent. The current study investigates protection against the hyperglycemia and dyslipidemia in alloxan-induced (type I diabetes) and fructose-fed insulin resistance (type II diabetes) models of diabetes treated with aqueous methanolic root extract of *E. echinatus* (Ee.Cr). Albino rats were treated orally with Ee.Cr at doses 100, 300 and 500mg/kg. The fasting blood glucose was measured by glucometer, while standard kits were used to determine the levels of serum total cholesterol, triglycerides and HDL. The administration of Ee.Cr significantly ($P<0.001$) reduced the FBG concentration in a dose-dependent pattern in alloxan-induced and fructose-fed diabetic rats. The Ee.Cr also corrected the dyslipidemia associated with fructose and alloxan-induced diabetes by significantly ($P<0.001$) decreasing the concentration of serum total cholesterol, triglycerides, and LDL and by increasing HDL concentration. Ee.Cr also significantly ($P<0.001$) improved the glucose tolerance in fructose-fed rats. We conclude that Ee.Cr has antidiabetic and antidyslipidemic effects in both insulin-dependent alloxan-induced diabetes and fructose-induced insulin resistance diabetes rat models.

Keywords: Antihyperglycemic, alloxan, antihyperlipidemic, insulin resistance, metabolic syndrome.

INTRODUCTION

Diabetes mellitus is a chronic disease associated with raised blood glucose levels due to absolute insulin deficiency in case of type I diabetes and relative insulin deficiency with or without insulin resistance in type II diabetes. It is associated with extensive malformations in carbohydrate, protein and lipid metabolism and progressively leads to the development of life threatening complications. All the heterogeneous abnormalities grouped under diabetes mellitus share a common feature of chronic hyperglycemia (Heydari *et al.*, 2010). Long standing hyperglycemia can lead to increased circulating levels of advanced glycation end products (AGEs) causing vascular complications that can ultimately lead to diabetic retinopathy, nephropathy, neuropathy and increase the risk of stroke and myocardial infarction (Muhammad *et al.*, 2009). To avoid the synthesis of AGEs and delay vascular complications, maintenance of normal blood glucose levels is crucial. A significant number of adverse effects are associated with the current antidiabetic therapy and there is still a need to search for medicinal plants with therapeutic potential (Yashwant-Kumar *et al.*, 2011).

Echinops echinatus (traditionally known as Untkatalo, Unt-Katara, Brahmadandi, Labh; English: Camel's Thistle) is an important medicinal plant of family Asteraceae. It is used as a diuretic, analgesic, anti-pyretic,

anti-inflammatory, bitter, stomachic, nerve tonic, anthelmintic, aphrodisiac and abortifacient agent (Usmanghani *et al.*, 1997). The plant has shown various pharmacological activities such as antifungal, antibacterial, anti-inflammatory, analgesic, antifertility, antispasmodic, antimalarial, diuretic, ACE inhibitor, and antidiabetic activity (Rai and Mares, 2003; Yadava and Singh, 2006; Patel *et al.*, 2011b; Patel *et al.*, 2011a; Nyman *et al.*, 1998; Bhakuni *et al.*, 1969; Padashetty and Mishra, 2007a; Agrawal *et al.*, 2012).

The root bark powder of *E. echinatus* has been used to treat diabetes (Patel *et al.*, 2011a). Recently, Sarvaiya-Dhara *et al.*, (2015) have shown the antidiabetic and antihyperlipidemic effects of the root bark and aerial parts of *E. echinatus* in alloxan-induced diabetic rats. To explore the antidiabetic and antidyslipidemic effects of the aqueous methanolic root extract of *E. echinatus*, we employed alloxan-induced and fructose-fed insulin resistance models of diabetes. Our data clearly demonstrates that the aqueous methanolic extract from roots of *E. echinatus* protected against hyperglycemia and dyslipidemia induced by alloxan and chronic fructose feeding.

MATERIALS AND METHODS

Preparation of the crude extract

The fresh roots of the plant were harvested from Cholistan, Pakistan and were identified by Mr. Abdul

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Hameed, Botanist at the Cholistan Institute of Desert Studies of the Islamia University of Bahawalpur. The roots were washed, dried in the shade and grounded to coarse powder. A voucher specimen (EE-RT-08-11-28) was submitted in the herbarium at the Faculty of Pharmacy, the Islamia University of Bahawalpur, Pakistan for future reference. The aqueous methanolic crude extract of the roots was prepared as described previously (Jabeen *et al.*, 2009). A thick concentrated paste with reddish dark brown colour and 6% yield was attained. The extract was stored at -20°C after labelling it as Ee.Cr until further use.

Preliminary phytochemical analysis

The qualitative phytochemical analysis for different secondary plant metabolites, such as alkaloids, glycosides, saponins, anthraquinones, flavonoids, coumarins and tannins, was performed as described previously (Gilani *et al.*, 2008).

Experimental animals

Sprague-Dawley albino rats (150-200g) and Swiss albino mice (18-32g) of both sexes, housed in the animal facility of the Pharmacology section at the Faculty of Pharmacy, the Islamia University of Bahawalpur in Pakistan, were used for the study, unless otherwise stated. Animals were exposed to 12:12 hours light and dark periods and maintained at standard temperature (25±2°C) and humidity (50-55%) conditions throughout the study. Animals were allowed to drink water *ad libitum* and were fed on normal diet. Before starting the study, animals were acclimatized to the experimental conditions to minimize stress effects. The local animal ethics committee approved the study protocols and procedures.

Acute toxicity assessment

Mice were randomly divided into four groups with six in each. Three groups received crude extract orally by gavage at doses of 300mg/kg, 1000mg/kg, 3000mg/kg, while a parallel control group received normal saline (10 ml/kg). The mice received food and water *ad libitum*. The animals were observed frequently for any behavioural changes during initial six hours. The lethal effects were assessed after 24h and 48h of extract administration (Gilani *et al.*, 2008).

Acute hypoglycemic effects in normoglycemic rats

Albino male rats weighing 250-350g were used to evaluate the acute hypoglycemic effect of Ee.Cr. Rats were randomized into five groups with six rats in each. Animals were fasted overnight (12h) before administering the extract at 100mg/kg, 300mg/kg and 500mg/kg doses. A standard control group was administered glibenclamide at 5mg/kg and the normal control group received normal saline. A single touch glucometer (Accu-chek Active, Roche, Germany) was used to determine the fasting blood glucose (FBG) concentrations from the rat tail vein blood

at 0h (before Ee.Cr administration) and at 1h, 2h, 4h and 6h after Ee.Cr administration (Yashwant-Kumar *et al.*, 2011). The following experimental design was adopted for treating different groups of animals:

- Group 1: Normal Control (Normal Saline 1ml/kg)
- Group 2: Standard Control (Glibenclamide 5mg/kg)
- Group 3: Ee.Cr 100mg/kg
- Group 4: Ee.Cr 300mg/kg
- Group 5: Ee.Cr 500mg/kg

Alloxan-induced diabetes mellitus

Alloxan monohydrate (Acros Organics, USA) was injected intraperitoneally to 18h fasted rats at the dose of 140mg/kg in normal saline (Tanko *et al.*, 2011; Ashok-Kumar *et al.*, 2012). Pilot studies were done to optimize the alloxan dose. An ice cold normal saline was used for the fresh preparation of alloxan and maintained on ice bath during administration. After six hours of alloxan administration, the animals were supplemented with 20% w/v dextrose solution to mitigate the fatal hypoglycaemia, which occurs due to massive insulin release induced by alloxan (Ahlem *et al.*, 2009). Alloxan injected rats were maintained on 5% glucose supplemented water for next 24h (Yashwant-Kumar *et al.*, 2011). Diabetes was confirmed after 72h of injection by using glucometer and the animals with a fasting blood glucose level ≥200mg/dl were included in the study.

The animals were stabilized until day 7 post alloxan injection and were then randomly selected into six groups for their fasting blood glucose levels assessment (Yashwant-Kumar *et al.*, 2011). After FBG concentration assessment, the animals received different treatments and it was assumed to be the 1st day. Further assessment was done on the 3rd, 5th, 7th and 14th day of the treatment commencement. The treatment protocol is given as below:

- Group 1: Normal Control (Normal Saline 1ml/kg)
- Group 2: Positive Control (Alloxan 140mg/kg + Normal Saline 1ml/kg)
- Group 3: Standard Control (Alloxan 140mg/kg + Glibenclamide 10mg/kg)
- Group 4: Ee.Cr 100mg/kg (Alloxan 140mg/kg + Ee.Cr 100mg/kg)
- Group 5: Ee.Cr 300mg/kg (Alloxan 140mg/kg + Ee.Cr 300mg/kg)
- Group 6: Ee.Cr 500mg/kg (Alloxan 140mg/kg + Ee.Cr 500mg/kg)

High fructose-fed model

Albino rats were randomized into six groups with six rats in each. High fructose-fed rats were administered 60% w/w fructose (BDH, England) in their diet (Ansarullah *et al.*, 2010), while the normal control group received an equivalent amount of vegetable starch in normal chow. Treatment was started after initial FBG concentration

assessment and then further assessments were done on weekly basis until 6th week. The lipid profile assessment was done on the blood withdrawn via cardiac puncture after the animals were sacrificed on completing 6 weeks of treatment with Ee.Cr (Ansarullah *et al.*, 2010).

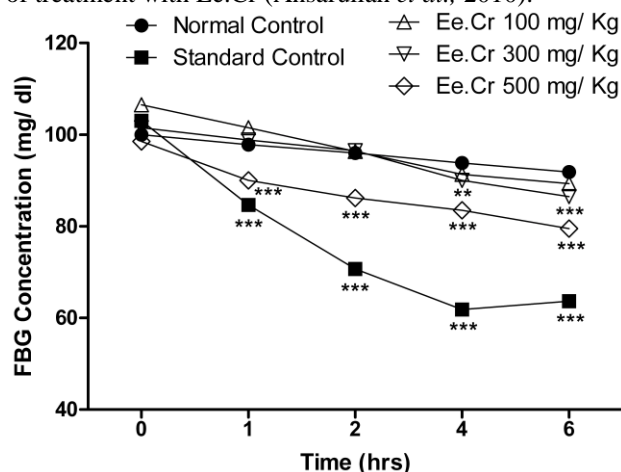


Fig. 1: Acute hypoglycemic effects of different doses of Ee.Cr in normoglycemic rats. (Values are expressed as mean \pm SEM and $n=6$. Two way ANOVA was applied and P values were considered significant as $P<0.05$ (*), $P<0.01$ (**), and $P<0.001$ (***)). Extract-treated groups and standard control were compared to normal control. Normal and Positive Control: Normal Saline 1ml/kg; Standard Control: Glibenclamide 5 mg/kg).

A 30% w/v glucose solution at the dose of 2g/kg was administered orally to overnight fasted rats to determine their glucose tolerance a day before sacrificing (Suwannaphet *et al.*, 2010). The blood glucose measurements were made at 0, 30, 60, and 120 minutes from the rat tail vein using glucometer.

The following treatment protocol was followed:

- Group 1: Normal Control (Normal Diet + Normal Saline 1ml/kg)
- Group 2: Positive Control (High Fructose Diet + Normal Saline 1ml/kg)
- Group 3: Standard Control (High Fructose Diet + Metformin 50mg/kg)
- Group 4: Ee.Cr 100mg/kg (High Fructose Diet + Ee.Cr 100mg/kg)
- Group 5: Ee.Cr 300mg/kg (High Fructose Diet + Ee.Cr 300mg/kg)
- Group 6: Ee.Cr 500mg/kg (High Fructose Diet + Ee.Cr 500mg/kg)

Glucose-induced hyperglycemia

A hyperglycemic state is induced when the animals are administered 10% w/v glucose *ad libitum* for a longer period of time (Midaoui and de-Champlain, 2002). We followed this approach to investigate the antihyperglycemic effects of Ee.Cr in Swiss albino mice (18-30g) of either sex. Mice were randomly assigned to

different treatment groups as described in experimental design below. The normal control mice were given normal drinking water, but other animals received 10% w/v glucose (Merck, Germany) as drinking water *ad libitum*. Animals received different doses of Ee.Cr, standard drug and normal saline intraperitoneally. The FBG concentrations were determined from overnight fasted (12h) mice after 28 days of glucose supplementation with the help of glucometer from tail vein.

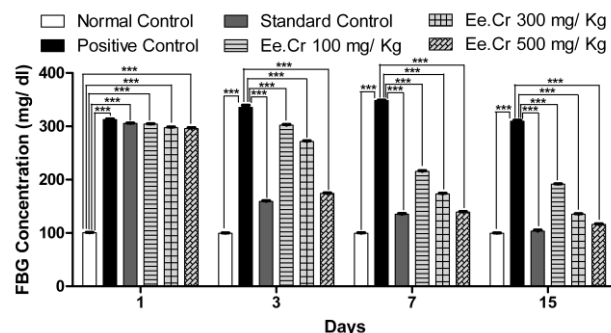


Fig. 2: Effects of chronic administration of different doses of Ee.Cr on hyperglycemia in alloxan-induced diabetic rats. (Values are expressed as mean \pm SEM and $n=6$. Two way ANOVA was applied and P values were considered significant as $P<0.05$ (*), $P<0.01$ (**), and $P<0.001$ (***)). Positive control was compared to normal control, while all extract-treated groups and standard control were compared to positive control. Normal and Positive Control: Normal Saline 1ml/kg; Standard Control: Glibenclamide 10mg/kg).

- Group 1: Normal Control (Normal Saline 1 ml/kg)
- Group 2: Positive Control (10% w/v glucose + Normal Saline 1ml/kg)
- Group 3: Standard Control (10% w/v glucose + Metformin 100mg/kg)
- Group 4: Ee.Cr 100mg/kg (10% w/v glucose + Ee.Cr 100mg/kg)
- Group 5: Ee.Cr 300mg/kg (10% w/v glucose + Ee.Cr 300mg/kg)
- Group 6: Ee.Cr 500mg/kg (10% w/v glucose + Ee.Cr 500mg/kg)

Biochemical analysis

At the end of the study, the rats were intraperitoneally injected ketamine 50mg/kg (Ketasol, Indus Pharma, Pakistan) and diazepam 5mg/kg (Valium, Roche, Pakistan) to anesthetize them for obtaining blood by cardiac puncture. The blood was allowed to clot for 15min and then centrifuged for 15min at 4500rpm to obtain the serum in the centrifuge machine (Hettich Zentrifugen, Germany). Biochemical analysis for serum total cholesterol (TC), triglycerides (TG) and high density lipoproteins (HDL) was performed according to the protocol provided with standard kits (Human Diagnostics,

Wiesbaden, Germany). The respective samples, standards and reagent blanks were run on Merck microlab 300 (Merck, Germany) to obtain the final results. Serum low density lipoprotein (LDL) was estimated indirectly by employing Friedewald's formula as given below:
$$\text{LDL (mg/dl)} = \text{TC} - \text{HDL} - \text{TG}/5$$

Change in body weight

Body weight was measured on a daily basis for each animal in the study. The change in body weight expressed in grams (g) was computed by taking the difference in weight at the start of treatment (Wi) and at the end of treatment (Wf).

STATISTICAL ANALYSIS

The results were expressed as mean \pm SEM. One way and two way ANOVA with Bonferroni post hoc test was employed to demonstrate the level of significance i.e. P value <0.05 was considered significant. The positive controls were compared to normal controls and all other groups were compared to positive controls for statistical significant difference between them. The data was compiled and statistically analyzed with the help of Graph Pad Prism 5.00 (San Diego California, USA).

RESULTS

Preliminary phytochemical analysis and acute toxicity assessment

Ee.Cr tested positive for saponins, tannins, and coumarins (table 1). The extract displayed no toxic effects up to 3g/kg dose.

Acute hypoglycemic effects in normoglycemic rats

The FBG concentration was significantly reduced by glibenclamide 5mg/kg and Ee.Cr at dose 500mg/kg at 1h, 2h, 4h and 6h after treatment, respectively. The FBG concentration was significantly reduced by Ee.Cr at dose 300mg/kg at 4h and 6h after administration, but there was no significant reduction in FBG concentration produced by Ee.Cr at dose 100mg/kg when compared to normal controls (fig. 1).

Alloxan-induced diabetes mellitus

Effects of Ee.Cr on fasting blood glucose concentration

The FBG concentration was significantly raised in all the animals after 72h of alloxan injection and remained elevated until day 1 of the study in comparison to the normal control rats. Glucose assessment at day 3 after commencement of the treatment exhibited significant reduction in FBG concentration in groups treated with glibenclamide 10mg/kg and Ee.Cr at doses 100, 300, and 500mg/kg as compared to positive control (fig. 2). FBG concentration in positive diabetic controls was significantly elevated in comparison to normal controls (fig. 2). A similar effect was seen on the 7th and 15th day

of assessment of FBG concentrations with more pronounced decrease in glycemia with elapsing time as compared to diabetic controls (fig. 2). The hypoglycemic effect of Ee.Cr was dose-dependent and FBG concentration in the Ee.Cr 500mg/kg group was almost similar to normal controls (fig. 2).

Effects of Ee.Cr on total cholesterol, triglycerides, LDL and HDL

Total cholesterol and triglyceride concentrations were significantly raised in positive control rats as compared to normal ones (fig. 3a and 3b). Ee.Cr showed a significant dose-dependent decrease in total cholesterol and triglyceride concentrations as compared to positive control. Ee.Cr at dose 500mg/kg normalized the total cholesterol levels. The serum HDL concentration was significantly decreased in positive controls as compared to normal controls (fig. 3c). Interestingly, chronic administration of Ee.Cr at doses 300 and 500mg/kg significantly increased the serum HDL concentration (fig. 3c). The serum LDL concentration was decreased in a dose-dependent manner with Ee.Cr as compared to positive control, which in turn, showed significantly raised LDL concentration as compared to normal control animals (fig. 3d).

Effects of Ee.Cr on change in body weight

Alloxan-induced diabetes caused significant weight loss due to muscle wasting. Weight loss is a typical clinical observation in type I diabetes due to insulin deficiency, and was similarly observed in the alloxan-induced rat model of type I diabetes (fig. 4). A negative body weight change was found in positive controls in comparison to normal controls. Ee.Cr prevented weight loss in a dose-dependent pattern, while highest weight gain was observed in the glibenclamide 10mg/kg group (fig. 4). These data show that the effects of Ee.Cr on weight gain were intermediate as compared to glibenclamide.

High fructose-fed model

Effects of E. echinatus on fasting blood glucose concentration

Fructose feeding impairs fasting blood glucose levels and does not produce aberrant elevations as observed in alloxan-induced diabetes. At week 1 of assessment, the FBG concentration was significantly raised in positive control rats as compared to normal rats, but only standard control metformin (50mg/kg) and interestingly Ee.Cr 100 mg/kg depicted significant reduction in FBG concentration as compared to positive control (fig. 5). Ee.Cr at doses 300 and 500mg/kg exerted no statistically significant hypoglycemic effects. However, Ee.Cr at dose of 300 and 500mg/kg significantly decreased FBG concentration starting from week 2 until week 6 of assessment. The hypoglycemic action of Ee.Cr at dose 100 mg/kg became significant after 4 weeks of treatment (fig. 5).

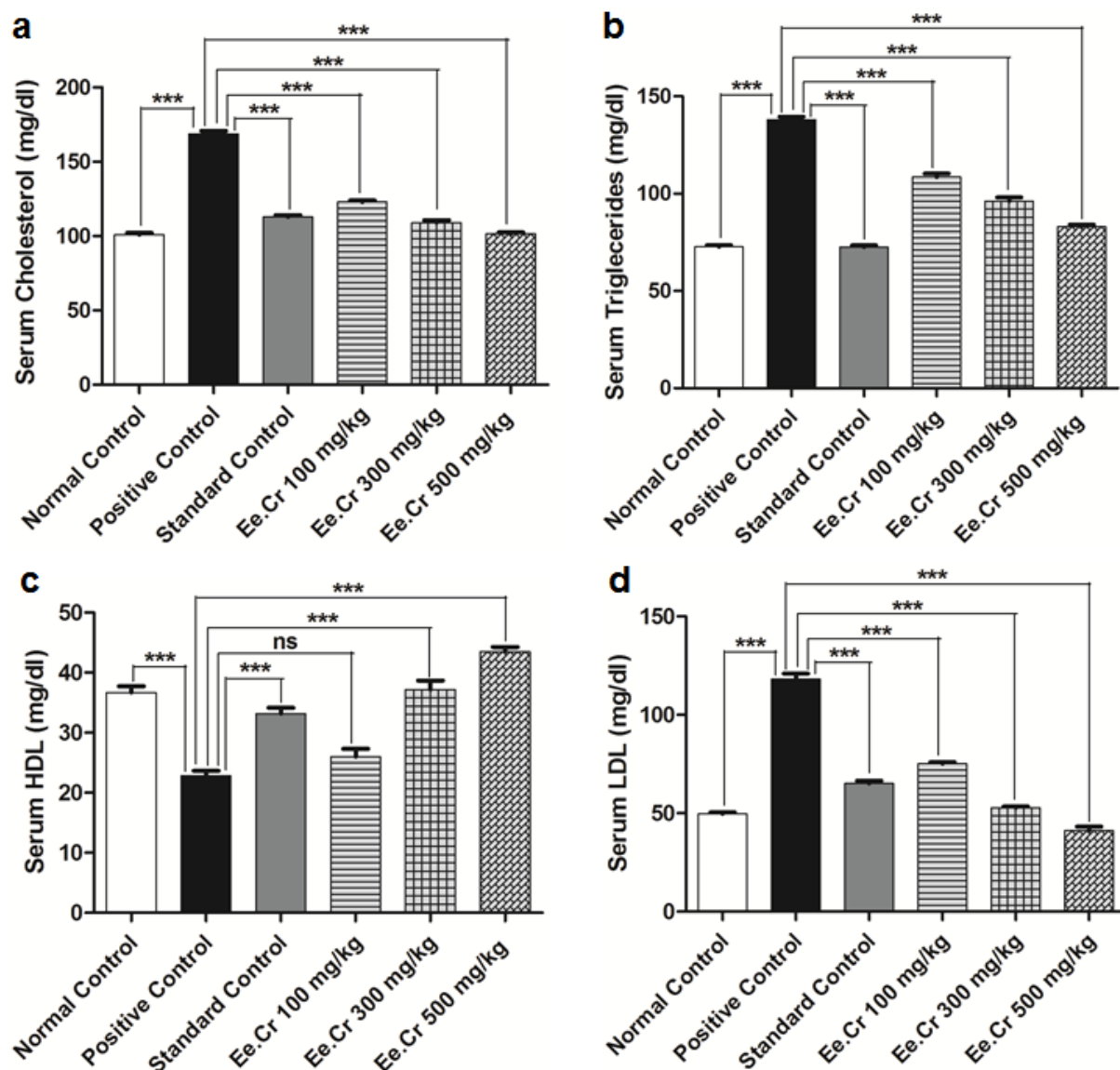


Fig. 3: Effects of chronic administration of different doses of Ee.Cr on lipid profile in alloxan-induced diabetic rats. (Values are expressed as mean \pm SEM and $n = 6$. One way ANOVA was applied and P values were considered significant as $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***). Positive control was compared to normal control, while all extract-treated groups and standard control were compared to positive control. Normal and Positive Control: Normal Saline 1ml/kg; Standard Control: Glibenclamide 10mg/kg).

Effects of Ee.Cr on total cholesterol, triglycerides, LDL and HDL

The serum total cholesterol concentration was significantly increased in fructose-fed positive controls as compared to normal controls (fig. 6a). Administration of Ee.Cr at doses 100, 300 and 500 mg/kg significantly protected against the hypercholesterolemic effects of fructose (fig. 6a). Similarly, serum triglyceride concentrations were elevated in positive controls as compared to normal rats. Ee.Cr at all administered doses prevented the hypertriglyceridemia (fig. 6b). The serum HDL levels were also significantly impaired in fructose-fed rats as compared to normal controls (fig. 6c). Ee.Cr at

doses 100, 300 and 500mg/kg significantly increased HDL levels as compared to positive controls. The effect of Ee.Cr at dose 500mg/kg was almost comparable to standard control metformin (50mg/kg) (fig. 6c). The administration of Ee.Cr at all doses significantly reduced the LDL concentration, which was significantly increased in positive controls as compared to normal chow-fed rats (fig. 6d).

Effects of Ee.Cr on OGTT

Blood glucose concentrations were significantly elevated in positive controls as compared to normal controls at all time points of assessment. Ee.Cr at doses 100, 300, and

500mg/kg significantly improved the glucose tolerance at all time points when compared to fructose-fed rats (fig. 7a). The area under the glucose concentration time curve (AUC) was significantly larger in positive controls as compared to normal rats. The administration of metformin (50mg/kg) and different doses of Ee.Cr significantly reduced the AUC as compared to positive controls (fig. 7b).

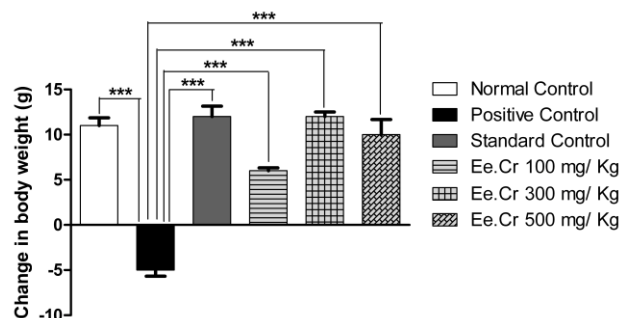


Fig. 4: Effects of chronic administration of different doses of Ee.Cr on change in body weight in alloxan-induced diabetic rats. (Values are expressed as mean \pm SEM and $n=6$. One way ANOVA was applied and P values were considered significant as $P<0.05$ (*), $P<0.01$ (**), and $P<0.001$ (***)). Positive control was compared to normal control, while all extract-treated groups and standard control were compared to positive control. Normal and Positive Control: Normal Saline 1ml/kg; Standard Control: Glibenclamide 10mg/kg).

Glucose-induced hyperglycemia

Experimental animals present with a hyperglycemic state when administered glucose chronically (Midaoui and de-Champlain, 2002). The administration of glucose (10% w/v) in drinking water for 28 days significantly increased the FBG concentration in positive controls as compared to normal mice (fig. 8). Ee.Cr at doses 100, 300, and 500mg/kg significantly prevented hyperglycemia as compared to positive control mice in a dose-dependent manner (fig. 8), confirming the antihyperglycemic effect of *E. echinatus* roots.

DISCUSSION

Diabetes mellitus is a heterogeneous disease with absolute to relative insulin deficiency associated with insulin resistance. Chronic hyperglycemia is a major problem in diabetes mellitus (Heydari *et al.*, 2010). The persistent hyperglycemia leads to the synthesis of advanced glycation end products (AGEs) and consequently, advancement to the microvascular complications over the prolonged course of the disease (Muhammad *et al.*, 2009). Interventions aimed at achieving better blood glucose control with minimum side effects are urgently needed due to increasing incidence of diabetes. In the current study, we investigated the hypoglycemic and

antihyperglycemic effects of the aqueous methanolic extract from roots of *Echinops echinatus* (Ee.Cr) in different animal models of diabetes. Ee.Cr effectively decreased FBG concentration in normoglycemic rats at dose 500mg/kg, but onset of hypoglycemic action in the 300mg/kg group was delayed. The hypoglycemia induced by Ee.Cr 500mg/kg was intermediate in comparison to standard control glibenclamide 5mg/kg (fig. 1), which is well known for causing hypoglycemic coma due to inhibition of compensatory glucagon release (Zammit and Frier, 2005).

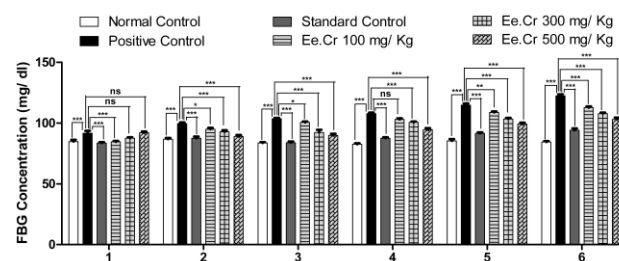


Fig. 5: Effects of chronic administration of different doses of Ee.Cr on FBG concentrations in fructose-fed rats. (Values are expressed as mean \pm SEM and $n=6$. Two way ANOVA was applied and P values were considered significant as $P<0.05$ (*), $P<0.01$ (**), and $P<0.001$ (***)). Positive control was compared to normal control, while all extract-treated groups and standard control were compared to positive control. Normal and Positive Control: Normal Saline 1ml/kg; Standard Control: Metformin 50mg/kg).

Alloxan is known to induce a hyperglycemic state by destroying the pancreatic β cells via redox-mediated mechanisms (Szkudelski, 2001). The increase in FBG concentration in alloxan-injected rats is in agreement with findings of others (Ashok-Kumar *et al.*, 2012). Ee.Cr administration in alloxan-induced diabetic rats prevented hyperglycemia in a dose-dependent pattern and Ee.Cr 500 mg/kg effects were almost comparable to the standard control glibenclamide 10mg/kg (fig. 2). Long standing hyperglycemia in diabetes leads to vascular complications, causing dysfunction, damage and multiple organ failure (Ramachandran *et al.*, 2011). Medicinal plant extracts can serve as valuable antidiabetic agents and can reduce blood glucose levels due to one or more active constituents present in them (Grover *et al.*, 2002). Triterpenoids, tannins and saponins have been reported to contribute towards hypoglycemia (Sharma *et al.*, 2010). The roots of *E. echinatus* contain lupeol and other triterpenoids (Padashetty and Mishra, 2007b). Ee.Cr phytochemical analysis also revealed the presence of tannins and saponins, which may be contributing to the hypoglycemic effects. Our results are in agreement with the findings of Sarvaiya-Dhara *et al.*, (2015).

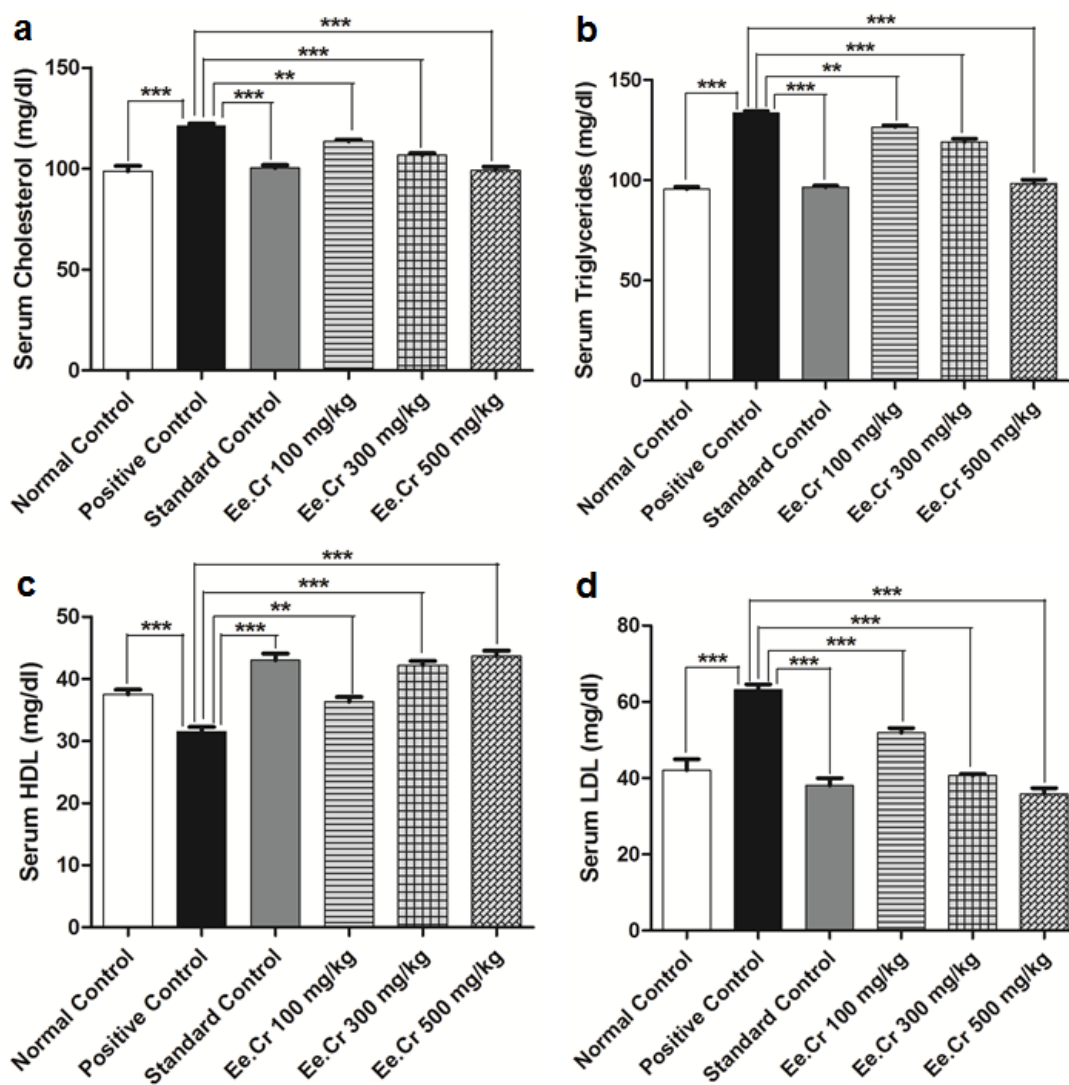


Fig. 6: Effects of chronic administration of different doses of Ee.Cr on lipid profile in fructose-fed rats. (Values are expressed as mean \pm SEM and $n = 6$. One way ANOVA was applied and P values were considered significant as $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***). Positive control was compared to normal control, while all extract-treated groups and standard control were compared to positive control. Normal and Positive Control: Normal Saline 1ml/kg; Standard Control: Metformin 50mg/kg).

Diabetes mellitus associated hyperlipidemia is a well renowned fact (Sharma *et al.*, 1996). Ee.Cr also exerted antidyslipidemic effects by lowering TC, TG, and LDL concentrations and increasing HDL concentrations (fig. 3). Inactivation of lipoprotein lipase results in hypercholesterolemia and hypertriglyceridemia owing to insulin deficiency (Oyedemi *et al.*, 2011). Ee.Cr induced hypocholesterolemia may stem from better control of glycemia in addition to reduced cholesterol absorption or production and lipoprotein metabolism modifications (Slater *et al.*, 1980). The hypocholesterolemic and hypotriglyceridemic effects of Ee.Cr suggest that it may be able to prevent against increased risk of myocardial infarction, coronary heart disease, stroke and atherosclerosis associated with diabetes (Bae *et al.*, 2001).

The dose-dependent decrease in serum LDL may be attributed to better glycemic control and/or may be due to insulinotropic activity of the extract (Mahendran *et al.*, 2011). Coronary heart disease risk is increased with high circulating LDL levels associated with low levels of HDL (Tchobrousky, 1978). Ee.Cr at doses 300 and 500mg/kg effectively raised HDL levels, which may be due to increased extra-hepatic removal and transport of cholesterol to the liver for clearance (Mohammadi and Naik, 2008). The antidyslipidemic effects of Ee.Cr are in agreement with the antidyslipidemic effects of the root bark extract of the *E. echinatus* (Sarvaiya-Dhara *et al.*, 2015).

Insulin deficiency in alloxanized rats leads to increased gluconeogenesis and glycogenolysis with wasting of muscle and loss of tissue proteins, which result in loss of body weight (Swanston-Flatt *et al.*, 1990). The extract protected against weight loss in a dose-dependent manner and also weight gain was favourable as compared to the glibenclamide, which causes excessive weight gain due to its insulin secretagogue action (Haffner *et al.*, 1997).

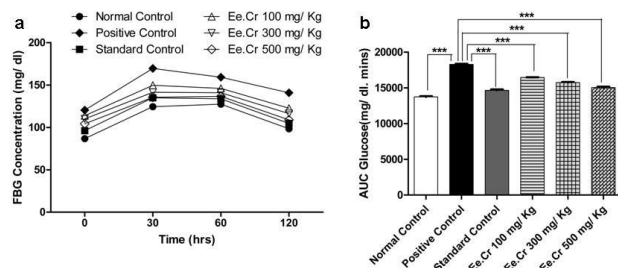


Fig. 7: Effects of different doses of Ee.Cr on OGTT in fructose-fed rats. (Values are expressed as mean \pm SEM and $n = 6$. a. Two way ANOVA and b. One way ANOVA were applied and P values were considered significant as $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***)). Positive control was compared to normal control, while all extract-treated groups and standard control were compared to positive control. Normal and Positive Control: Normal Saline 1ml/kg; Standard Control: Metformin 50mg/kg).

Metabolic syndrome and type II diabetes prevalence has increased dramatically in recent years (Ovalle-Magallanes *et al.*, 2015). Therefore, we investigated the effects of Ee.Cr on FBG and lipid profile in fructose-fed rats that resemble the metabolic syndrome and the insulin resistance state in early type II diabetes. High amounts of fructose in the diet cause insulin resistance manifested by deleterious metabolic effects like hyperinsulinemia, hyperglycemia, glucose intolerance, hypertriglyceridemia and hypertension in rats. The fructose fed rat model mimics the metabolic syndrome (syndrome X) in humans. Fructose-fed rats have shown impaired glucose oxidation in liver, skeletal muscles and adipose tissue due to insulin stimulation (Reuter, 2007). The fructose feeding impaired FBG concentration in agreement with previous findings (Ansarullah *et al.*, 2010) and the administration of Ee.Cr prevented hyperglycemia in a dose-dependent manner. The Ee.Cr antihyperglycemic effect might be due to reduced glucose yield by liver and/or increased peripheral consumption of glucose due to improved insulin sensitivity as observed with standard control metformin (Ansarullah *et al.*, 2010). Ee.Cr also improved glucose tolerance in OGTT conducted in the last week of fructose feeding in a dose-dependent manner. These findings demonstrate that Ee.Cr improves insulin resistance and prevents impaired glucose tolerance, which are common features of syndrome X and early risk factor for developing type II diabetes, (Bacha *et al.*, 2010) thereby increasing the risk of cardiovascular disease (Barr *et al.*, 2009).

Fructose is more lipogenic and leads to hypercholesterolemia and hypertriglyceridemia (Hallfrisch, 1990). It is well known that increased levels of serum TG contribute to insulin resistance due to decrease in insulin receptors (DeFronzo, 2004; Saltiel and Kahn, 2001). Ee.Cr has shown dose-dependent hypocholesterolemic and hypotriglyceridemic effects. High fructose diet administration to rats has shown to decrease levels of HDL and increase LDL concentration (Thirunavukkarasu *et al.*, 2004). Ee.Cr administration increased HDL and reduced LDL concentrations in a dose-dependent pattern. These effects may stem from the ability of the extract to reduce insulin resistance.

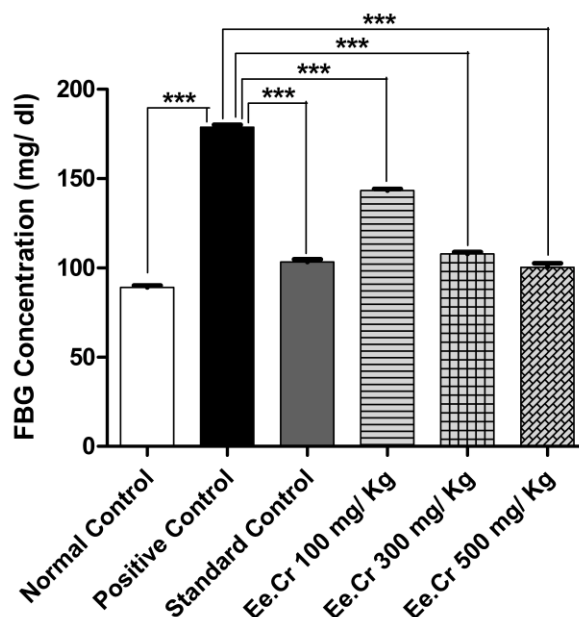


Fig. 8: Effects of different doses of Ee.Cr on FBG concentrations in glucose (10% w/v) fed mice. (Values are expressed as mean \pm SEM and $n = 6$. One way ANOVA was applied and P values were considered significant as $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***)). Positive control was compared to normal control, while all extract-treated groups and standard control were compared to positive control. Normal and Positive Control: Normal Saline 1ml/kg; Standard Control: Metformin 100mg/kg).

Plasma glucose in higher concentrations as observed in diabetes is deleterious to the pancreatic β cells and is often reported to cause glucose toxicity or glucose desensitization (DeFronzo, 2004). Lowering of glucose concentration is associated with increase in insulin sensitivity and insulin release (Kahn, 2003). Ee.Cr administration to glucose-fed mice protected against glucose toxicity by lowering FBG concentration in a dose-dependent manner. Our results demonstrate that Ee.Cr is an effective hypoglycemic and antidyslipidemic agent, however, further investigation is needed to identify which constituents and mechanisms mediate these effects.

CONCLUSION

Aqueous methanolic extract of *Echinops echinatus* roots was an effective hypoglycemic and antihyperglycemic agent in experimental models of type I and type II diabetes. The extract also corrected dyslipidemia associated with alloxan-induced diabetes and fructose feeding. Thus, further studies aimed at isolation of active antidiabetic agents in aqueous methanolic extract of roots and exploring the underlying mechanisms of action are highly encouraged.

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REFERENCES

- Agrawal M, Nahata A and Dixit VK (2012). Protective effects of *Echinops echinatus* on testosterone-induced prostatic hyperplasia in rats. *Eur. J. of Integ. Med.*, **4**: e177-e185.
- Ahlem S, Khaled H, Wafa M, Sofiane B, Mohamed D, Jean-Claude M and Abdelfattah EF (2009). Oral administration of *Eucalyptus globulus* extract reduces the alloxan-induced oxidative stress in rats. *Chem. Biol. Interact.*, **181**: 71-76.
- Ansarullah, Bharucha B, Patel V and Ramachandran AV (2010). *Oreocnide integrifolia* (Gaud.) Miq leaf water extract improves metabolic alterations in high fructose fed insulin resistant and hypertensive rats. *Eur. J. of Integ. Med.*, **2**: 79-87.
- Ashok-Kumar BS, Lakshman K, Jayaveea KN, Sheshadri-Shekar D, Saleemulla K, Thippeswamy BS and Veerapur VP (2012). Antidiabetic, antihyperlipidemic and antioxidant activities of methanolic extract of *Amaranthus viridis* Linn in alloxan induced diabetic rats. *Exp. Toxicol. Pathol.*, **64**: 75-79.
- Bacha F, Lee S, Gungor N and Arslanian SA (2010). From pre-diabetes to type 2 diabetes in obese youth: Pathophysiological characteristics along the spectrum of glucose dysregulation. *Diabetes Care*, **33**: 2225-2231.
- Bae JH, Bassenge E, Kim KB, Kim YN, Kim KS, Lee HJ, Moon KC, Lee MS, Park KY and Schwemmer M (2001). Postprandial hypertriglyceridemia impairs endothelial function by enhanced oxidant stress. *Atherosclerosis*, **155**: 517-523.
- Barr EL, Boyko EJ, Zimmet PZ, Wolfe R, Tonkin AM and Shaw JE (2009). Continuous relationships between non-diabetic hyperglycaemia and both cardiovascular disease and all-cause mortality: The Australian diabetes, obesity, and lifestyle (AusDiab) study. *Diabetologia*, **52**: 415-424.
- Bhakuni DS, Dhar M, Dhar M, Dhawan B and Mehrotra B (1969). Screening of Indian plants for biological activity: Part II. *Indian J. of Exp. Biol.*, **7**: 250-262.
- DeFronzo RA (2004). Pathogenesis of type 2 diabetes mellitus. *Med. Clin. N. Am.*, **88**: 787-835.
- Gilani AH, Jabeen Q, Khan AU and Shah AJ (2008). Gut modulatory, blood pressure lowering, diuretic and sedative activities of cardamom. *J. Ethnopharmacol.*, **115**: 463-472.
- Grover JK, Yadav S and Vats V (2002). Medicinal plants of India with anti-diabetic potential. *J. Ethnopharmacol.*, **81**: 81-100.
- Haffner SM, Hanefeld M, Fischer S, Fucker K and Leonhardt W (1997). Glibenclamide, but not acarbose, increases leptin concentrations parallel to changes in insulin in subjects with NIDDM. *Diabetes Care*, **20**: 1430-1434.
- Hallfrisch J (1990). Metabolic effects of dietary fructose. *FASEB J.*, **4**: 2652-2660.
- Heydari I, Radi V, Razmjou S and Amiri A (2010). Chronic complications of diabetes mellitus in newly diagnosed patients. *Int. J. of Diabetes Mel.*, **2**: 61-63.
- Jabeen Q, Bashir S, Lyoussi B and Gilani AH (2009). Coriander fruit exhibits gut modulatory, blood pressure lowering and diuretic activities. *J. Ethnopharmacol.*, **122**: 123-130.
- Kahn SE (2003). The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of type 2 diabetes. *Diabetologia*, **46**: 3-19.
- Mahendran S, Badami S and Maithili V (2011). Evaluation of antidiabetic effect of embelin from *Embelia ribes* in alloxan induced diabetes in rats. *Biomed. & Prevent. Nut.*, **1**: 25-31.
- Midaoui AE and De-Champlain J (2002). Prevention of hypertension, insulin resistance, and oxidative stress by α -lipoic acid. *Hypertension*, **39**: 303-307.
- Mohammadi J and Naik PR (2008). Evaluation of hypoglycemic effect of *Morus alba* in an animal model. *Indian J. Pharmacol.*, **40**: 15-18.
- Muhammad S, Bierhaus A and Schwaninger M (2009). Reactive oxygen species in diabetes-induced vascular damage, stroke, and Alzheimer's disease. *J. Alzheimers. Dis.*, **16**: 775-785.
- Nyman U, Joshi P, Madsen LB, Pedersen TB, Pinstrup M, Rajasekharan S, George V and Pushpangadan P (1998). Ethnomedical information and in vitro screening for angiotensin-converting enzyme inhibition of plants utilized as traditional medicines in Gujarat, Rajasthan and Kerala (India). *J. Ethnopharmacol.*, **60**: 247-263.
- Ovalle-Magallanes B, Medina-Campos ON, Pedraza-Chaverri J and Mata R (2015). Hypoglycemic and antihyperglycemic effects of phytopreparations and limonoids from *Swietenia humilis*. *Phytochemistry*, **110**: 111-119.

- Oyedemi S, Yakubu M and Afolayan A (2011). Antidiabetic activities of aqueous leaves extract of *Leonotis leonurus* in streptozotocin induced diabetic rats. *J. Med. Plants Res.*, **5**: 119-125.
- Padashetty S and Mishra S (2007a). An HPTLC method for the evaluation of two medicinal plants commercially available in the Indian market under the common trade name Brahmadandi. *Chromatographia*, **66**: 447-449.
- Padashetty SA and Mishra SH (2007b). Effect of terpenoidal fraction of *Echinops echinatus* roots on reproductive parameters of male rats. *J. Nat. Med.*, **61**: 452-457.
- Patel AJ, Patel NM, Patel AA, Patel J and Patel S (2011a). Comparative analgesic activity of root and aerial part methanolic extracts of *Echinops echinatus* Roxb. *Int. J. Pharm. Inno.*, **1**: 23-29.
- Patel AJ, Patel NM, Patel AA, Patel J and Patel S (2011b). Comparative diuretic activity of root and aerial part methanolic extracts of *Echinops echinatus* Roxb. *Der Pharmacia Lettre*, **3**: 168-172.
- Rai K and Mares D (2003). Plant-Derived Antimycotics: Current Trends and Future Prospects, Taylor & Francis.
- Ramachandran S, Asokkumar K, Uma-Maheswari M, Ravi TK, Sivashanmugam AT, Saravanan S, Rajasekaran A and Dharman J (2011). Investigation of antidiabetic, antihyperlipidemic and in vivo antioxidant properties of *Sphaeranthus indicus* Linn. in type 1 diabetic rats: An identification of possible biomarkers. *Evid. Based Compl. Alt. Med.*, pp.1-8.
- Reuter TY (2007). Diet-induced models for obesity and type 2 diabetes. *Drug Discov. Today: Disease Models*, **4**: 3-8.
- Saltiel AR and Kahn CR (2001). Insulin signalling and the regulation of glucose and lipid metabolism. *Nature*, **414**: 799-806.
- Sarvaiya-Dhara D, Shah KK and Patel NM (2015). Antidiabetic activity of roots and aerial parts of *Echinops echinatus* Roxb. in alloxan induced diabetic rats. *Planta Activa*, **1**: 8-11.
- Sharma RD, Sarkar A, Hazra DK, Misra B, Singh JB, Maheshwari BB and Sharma SK (1996). Hypolipidaemic effect of Fenugreek seeds: A chronic study in non-insulin dependent diabetic patients. *Phytother. Res.*, **10**: 332-334.
- Sharma VK, Kumar S, Patel HJ and Hugar S (2010). Hypoglycemic activity of *Ficus glomerata* in alloxan induced diabetic rats. *Int. J. Pharm. Sci. Rev. and Res.*, **1**: 18-22.
- Slater HR, Packard CJ, Bicker S and Shepherd J (1980). Effects of cholestyramine on receptor-mediated plasma clearance and tissue uptake of human low density lipoproteins in the rabbit. *J. Biol. Chem.*, **255**: 10210-10213.
- Suwannaphet W, Meeprom A, Yibchok-Anun S and Adisakwattana S (2010). Preventive effect of grape seed extract against high-fructose diet-induced insulin resistance and oxidative stress in rats. *Food Chem. Toxicol.*, **48**: 1853-1857.
- Swanston-Flatt SK, Day C, Bailey CJ and Flatt PR (1990). Traditional plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice. *Diabetologia*, **33**: 462-464.
- Szkudelski T (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol. Res.*, **50**: 537-546.
- Tanko Y, Mabrouk M, Adelaiye A, Fatihu M and Musa K (2011). Anti-diabetic and some haematological effects of ethylacetate and n-butanol fractions of *Indigofera pulchra* extract on alloxan-induced diabetic Wistar rats. *J. Diabetes Endocrinol.*, **2**: 1-7.
- Tchobroutsky G (1978). Relation of diabetic control to development of microvascular complications. *Diabetologia*, **15**: 143-152.
- Thirunavukkarasu V, Nandhini A and Anuradha C (2004). Effect of α -lipoic acid on lipid profile in rats fed a high-fructose diet. *J. Diabetes Res.*, **5**: 195-200.
- Usmanghani K, Saeed A and Alam MT (1997). Indusynic medicine: traditional medicine of herbal, animal, and mineral origin in Pakistan, Karachi.
- Yadava RN and Singh SK (2006). New anti-inflammatory active flavanone glycoside from the *Echinops echinatus* Roxb. *Indian J. Chem.*, **45**: 1004-1008.
- Yashwant-Kumar A, Nandakumar K, Handral M, Talwar S and Dhayabaran D (2011). Hypoglycaemic and anti-diabetic activity of stem bark extracts *Erythrina indica* in normal and alloxan-induced diabetic rats. *Saudi Pharm. J.*, **19**: 35-42.
- Zammit NN and Frier BM (2005). Hypoglycemia in type 2 diabetes: Pathophysiology, frequency and effects of different treatment modalities. *Diabetes Care*, **28**: 2948-2961.