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Antihyperglycemic, Antihyperlipidemic, and Antioxidant Effects of *Eclipta prostrata* L. Aqueous Extract in Streptozotocin-Induced Diabetic Rats

(Kesan Antihiperglisemik, Antihiperlipidemik dan Antioksidan Ekstrak Akueus *Eclipta prostrata* L. pada Tikus Diabetik Aruhan Streptozotocin)

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ABSTRACT

With the rising prevalence of diabetes mellitus around the world, researchers have been searching for a new treatment option that is both more effective and safer than chemotherapy. This study evaluated the antidiabetic activities of aqueous extracts of *Eclipta prostrata* L. in streptozotocin (STZ)-induced diabetic rats. The rats were randomized into six groups: Normal control, STZ-induced diabetic rats (50 mg/kg), STZ+EPE (100 mg/kg), STZ + EPE (200 mg/kg), STZ+glibenclamide (600 µg/kg) and EPE alone (200 mg/kg). The STZ-induced diabetic rats showed significantly ($p<0.05$) elevated glucose, HbA1c, lipid profile, hepatic, and kidney markers, while significantly ($p<0.05$) decreased insulin levels. The changes in activities of carbohydrate metabolizing enzymes such as glucose-6-phosphatase, fructose-1,6–bisphosphatase were significantly ($p<0.05$) increased in diabetic rats, while the activity of glucokinase and glucose-6-phosphate dehydrogenase were significantly ($p<0.05$) reduced. Oral treatment of STZ-induced diabetic rats with *E. prostrata* (100 and 200 mg/kg) and glibenclamide (600 µg/kg) prevented the alteration as mentioned earlier and brought back them to near normalcy. The current findings in experimental diabetic rats suggest that oral treatment with *E. prostrate* ameliorated carbohydrate metabolizing enzymes, showed total cholesterol-lowering effects, improved serum high-density lipoprotein (HDL) cholesterol levels and exhibited intriguing antioxidant activities.

Keywords: Diabetes mellitus; *Eclipta prostrata;* glucose; insulin

ABSTRAK

Dengan peningkatan kelaziman diabetes mellitus di seluruh dunia, penyelidik telah mencari pilihan rawatan baharu yang lebih berkesan dan selamat daripada kemoterapi. Kajian ini menilai aktiviti antidiabetik ekstrak akueus *Eclipta prostrata* L. pada tikus diabetes yang disebabkan oleh streptozotocin (STZ). Tikus telah dibahagikan secara rawak kepada enam kumpulan: Kawalan, tikus diabetes aruhan-STZ (50 mg/kg), STZ+EPE (100 mg/kg), STZ + EPE (200 mg/kg), STZ+glibenclamide (600 µg/ kg) dan EPE sahaja (200 mg/kg). Tikus diabetes aruhan-STZ menunjukkan peningkatan secara signifikan ($p<0.05$) glukosa, HbA1c, profil lipid, penanda hepar dan buah pinggang, manakala secara signifikan ($p<0.05$) menurunkan tahap insulin. Perubahan dalam aktiviti enzim metabolisme karbohidrat seperti glukosa-6-fosfatase, fruktosa-1,6-bisphosphatase meningkat dengan ketara ($p<0.05$) pada tikus diabetes, manakala aktiviti glukokinase dan glukosa-6-fosfat dehidrogenase adalah ketara ($p<0.05$) berkurangan. Rawatan oral tikus diabetes aruhan-STZ dengan *E. prostrata* (100 dan 200 mg/kg) dan glibenclamide (600 µg/kg) menghalang pengubahan seperti yang dinyatakan sebelum ini dan membawanya kembali kepada tahap normal. Penemuan dalam uji kaji tikus diabetik ini menunjukkan bahawa rawatan oral dengan *E. prostrate* memperbaiki enzim metabolisme karbohidrat, menunjukkan kesan penurunan kolesterol secara keseluruhan, meningkatkan paras serum kolesterol lipoprotein berketumpatan tinggi (HDL) dan menunjukkan aktiviti antioksidan yang menarik.

Kata kunci: Diabetes mellitus; *Eclipta prostrata;* glukosa; insulin

Introduction

Diabetes mellitus (DM) is one of the most common chronic diseases. Deficiencies in insulin production and insulin activity are the underlying causes of DM. When a disease is present, structural changes to cellular proteins and lipids occur. This is characterized by high blood glucose levels (Pranata et al. 2021). DM is on the rise due to various factors, including the aging population, increased urbanization, and an increase in economic prosperity. According to the World Health Organization (WHO), nearly half-a-million people are at risk of the disease, with 552 million people worldwide being affected by it in 2019 (Teo et al. 2021). Managing DM is a major problem for people all over the world, and there is no cure yet. Research shows that some drugs are used as hypoglycemic agents (such as biguanides, sulfonylureas, glipizide, glimepiride), α-glucosidase inhibitors, meglitinides, and thiazolidinediones to reduce hyperglycemic conditions by maintaining the glycemic index of blood. But those allopathic hypoglycemic drugs are associated with serious adverse effects like lactic acidosis, fatty liver, and digestion problems (Khan et al. 2021; Omisore et al. 2021). Also, those adverse effects were caused because of poor pharmacodynamics and pharmacokinetics management. Therefore, the search for more effective and safer antidiabetic drugs is an urgent task. Oral hypoglycemic medications are not available or affordable to nearly 80% of adult diabetics, including those who suffer the most debilitating disease complications. As a result, these patients primarily receive treatment with traditional medicines (Stefaniak et al. 2021).

Hence, it is not only a noticeable disease from carbohydrate metabolism, but it also causes cardiac arrest, hypertension, kidney damage, and retinopathy due to poor insulin secretion or mutation in GLUT receptor of insulin hormone in the β cells of Langerhans of the pancreas (Daryabor et al. 2020). The liver is a major organ that controls the intermediary metabolism of our body and supplies energy in the form of ATP. But, in the case of diabetic patients, the energy supply of the liver is affected and the liver functions are interrupted by carbohydrate metabolic enzymes (Loria et al. 2013). The hexokinase family includes glucokinase. Gluco-6-phosphatase is the major rate-limiting enzyme and plays a key role in the metabolism of glucose in the liver and pancreas. Glucokinase in the liver and pancreas helps lower blood sugar levels in two ways: glucose-induced activation of the enzyme in pancreatic cells leads to insulin secretion and hepatic glucose uptake and glycogen production (Han et al. 2016). The modulation of carbohydrate enzymes and the maintenance of glucose homeostasis by various medicinal plants is a valuable alternative for the management of diabetes. Therefore, finding natural drugs from medicinal plants with better antidiabetic functions is significant (Ramachandran & Saravanan 2013).

Eclipta prostrata L., syn. *Eclita alba* (L.), is one of the significant medicinal property-rich plants available worldwide. *E. prostrata* is used in managing various communicable and non-communicable diseases such as cough, cold and its related viral hepatitis diseases, diabetes, wounds, and gout in Chinese ethnomedicine (Jahan et al. 2014; Vijayakumar et al. 2020). Chung et al. (2017) reported that *E. prostrata* can be used in the treatment of gastritis and skin infections. Its constituents are responsible for its use as a hair growth stimulator and a medicine to cure jaundice in traditional treatment (Chung et al. 2017). Iqbal et al. (2021) have extensively investigated its effects on the management of nephrotoxicity (Iqbal et al. 2021). Other studies have reported its enhanced anti-oxidative, anti-inflammatory (Tewtrakul et al. 2011), anticancer and antibacterial activities (Yadav et al. 2017). Some research shows that *E. prostrata* reduces blood pressure and cholesterol level (Kim et al. 2008). This study, therefore, seeks to investigate the efficacy of *E. prostrata* plant extract on blood plasma, diabetic parameters, liver and kidney parameters, and its histopathological evaluation study in STZ-induced diabetic rats.

Materials and Methods

Plant material and chemicals

The plant material of *Eclipta prostrata* L. was collected from Hebei, China, and authenticated by the Botanist, Dr. JIA Yu, The Herbarium, Institute of Botany, Academia Sinica, Xiangshan, Beijing 100093, China. The plant material was collected in August 2020 the Qinghai, China (36.6209° N, 101.7801° E). *E. prostrata* is found to well grown in sandy soils. This region is compressed of sandy soils. The average monthly rainfall received in the region was 82.0 mm (3.23 inches) of precipitation during the cultivation. The average daily maximum temperatures were 22.8 °C. This seasonal and soil type is ideal for well-grown and nutritive plant material. Streptomycin and glibenclamide were purchased from Sigma (St. Loius, MO, USA). Rat insulin ELISA kit was purchased from EMD Millipore Corporation (USA). Superoxide

dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) kits were all procured from Nanjing Jiancheng Bioengineering Institute (China). All the other compounds used in this experiment were of analytical grade.

ETHICS

The present study was approved by the Institutional Animal Ethical Committee (IAEC) of Hubei University of Medicine, P.R. China, with the approved ethical accession number 2021887789. As per IAEC approval, the male albino Wistar rats (7-9 weeks old with an average weight of 200 g) were attained from Wuhan Institute of Biological Products Co. (Wuhan, China). Meanwhile, the rats were kept at constant temperature (25 °C) and maintained at 12 h light/12 h dark cycle with a normal diet with water supply through *ad libitum.*

INDUCTION OF DIABETES AND EXPERIMENTAL DESIGN OF STUDY GROUP

For the induction of Type 2 diabetes, 50 mg/kg b. wt of streptozotocin (sodium citrate buffer with pH of 4.5) was intraperitoneally (I.P) injected for five consecutive days. Also, to restrict the drug-induced hypoglycemic condition, the STZ-induced rats were orally administered with 20% of glucose. Then, the STZ-induced rats were allowed to maintain hyperglycemic conditions for a few days. After 72 h of STZ induction, the rats were confirmed their diabetic condition, which exhibited plasma glucose levels above 250 mg/Dl (Babaei-Balderlou & Zare 2012). The rats were categorized into six groups, with each group containing six rats: Group 1: Normal control (With normal chow feed); Group 2: STZ (60 mg/kg) induced DM rats (Negative control); Group 3: STZ + *Eclipta prostrata* L. extract (100 mg/kg); Group 4: STZ + *Eclipta prostrata* L. extract (200 mg/kg); Group 5: STZ + Glibenclamide (600 µg/kg); Group 6: *Eclipta prostrata* L. extract alone (200 mg/kg).

Eclipta prostrata L. leaf material was collected and dried in an incubator for 48 h at 40 °C. Then, the dried material was ground into a fine form (mesh 200) using an electrical grinder. The plant extract was obtained from 100 g of the powder in 500 mL of distilled water using a Soxhlet extractor in a span of 24 h. The solvent was removed using a rotary evaporator, and the extract was then dried using a freeze dryer. The concentrated extract of *E. prostrata* was dissolved in 5% DMSO. The known drug glibenclamide was dissolved in water and orally administered with the help of an intra-gastric tube for 45 days to all the study groups and maintained. At the end of the 45 days of the experimental study, the final body weight was measured and was compared with the initial body weight. Before sacrifice, the animals were kept under overnight fasting and anesthesia was injected through the IM route with the help of ketamine/xylazine (90/10 $mg \cdot kg^{-1}$). The blood samples were collected after sacrificing the animal with an anti-coagulant coated (sodium citrate and sodium fluoride in the 3:1 ratio) tube to assess insulin, plasma glucose, and other biochemical parameters. After the rat was sacrificed, the liver and kidney samples were carefully excised, washed, and homogenized with ice-cold phosphate buffer. Then, the tissue homogenate was centrifuged at 10,000 rpm for 5 min. The supernatant was directly used for various biochemical marker analysis.

BIOCHEMICAL PARAMETERS

An ACCU-Check glucose meter was used to analyze the blood samples (Roche, Mannheim, Germany). Glycosylated hemoglobin was measured in a plasma sample prepared as described above using a commercially supplied biological kit on a Chem 5 Plus-V2 Auto-analyser (Erba Mannhein Germany). The carbohydrate metabolizing rate limiting enzyme has a remarkable role in drug-associated metabolic processes in diabetic conditions (MacDonald & Gapinski 1989). On the basis, the carbohydrate metabolism of the rate-limiting enzymes hexokinase, glucose-6-phosphatase and fructose-1,6-bi-phosphatase was assessed by an enzymatic kit method (Nanjing Jiancheng Bioengineering Institute, China) following manufacturer's instructions. Shibayagi's Diabetes Elisa Kit was used to measure the plasma insulin level in control and experimental animals.

ANALYSIS OF DIABETIC ASSOCIATED KIDNEY MARKERS

The urea content in the control and experimental animals in each group was measured by the diacetylmonoxime (DAM) method as described by Wybenga et al. (1971). The principle was based on the formation of a rose purple coloured complex when urea combines with hot acidic DAM in the presence of thiosemicarbazide in acidic medium, which can be detected spectrophotometrically. In addition, TBHBA (2,4,6-tribromide-3-hydroxybenzoic acid) was used to determine uric acid by an enzymatic photometric technique. As described by Henry (1974),

creatinine level was determined using the Jaffe method. The principle was based on the production of a colored complex when creatinine combines with picrate in an alkaline solution. The rate of formation of the colored complex was spectrophotometrically measured.

ESTIMATION OF LIPIDS

Triglycerides, phospholipids, total cholesterol, and high-density lipoprotein HDL-cholesterol (HDL-C) concentrations in serum were measured using commercially available enzymatic kits and an automatic analyzer (Architect c8000 Clinical Chemistry System, USA).

LIVER MARKER ANALYSIS

Liver markers such as serum alanine transaminase (ALT) and serum aspartate transaminase (AST) were assessed by the standard enzymatic UV-kinetic and Reitman-Frankel methods. Briefly, according to the manufacturer's instructions, ALT was determined using a microplate reader by colorimetry at 570 nm. The pyruvate formed a colorless terminal after the amino group of α-ketoglutarate was converted to glutamate by ALT (Hayashi et al. 2003). Aspartate transaminase was determined using a microplate reader with colorimetric detection at 450 nm. The amino group of α-ketoglutarate was converted to glutamate more quickly in this experiment, resulting in a colorless endpoint (Huang et al. 2006).

ANTIOXIDANT ANALYSIS

The activity of SOD was measured spectrophotometrically at 560 nm using a modified method of NADH-phenazinemethosulphate-nitroblue tetrazolium formazan inhibition (Marklund & Marklund 1974). A UV-spectrophotometer was used to evaluate the catalase activity by measuring hydrogen peroxide absorption at 240 nm for 5 min. The enzyme activity was determined from the consumption of H_2O_2 under the same circumstances and molar extinction coefficient (Aebi 1984). Paglia and Valentine method (1967) was used to estimated glutathione peroxidase (GPx). Briefly, 50 mM potassium phosphate buffer, pH 7.0, with 10 U/mL glutathione reductase (GR), 1 M reduced glutathione (GSH), 250 nM nicotinamide adenine dinucleotide phosphate (NADPH) and 2.5% hydrogen peroxide were stirred for 5 min and the responses were measured at 340 nm every 30 s. The analysis of vitamin E (alpha-tocopherol) was performed using a standard HPLC instrument (Catignani & Bieri 1983). Finally, reduced glutathione was processed by Reduced Glutathione Assay Kit (S0053); Beyotime, China) and estimated by colorimetric analysis.

HISTOPATHOLOGY

The liver, pancreas, and kidney were immediately excised and gently rinsed with ice-cold saline solution for histopathological studies. Then, the tissue sample was stored in 10% neutral formalin buffer embedded with paraffin seal tissues, cut into 5-µm thick sections, and stained with hematoxylin and eosin (H and E). Once the tissue sections were fixed with H and E stains, they were viewed under a light microscope (original magnification is ×40).

STATISTICS

All data were presented as the mean ± standard deviation (SD) of sample size (n = 6). One-way analysis of variance (ANOVA, SPSS Version 19) was used to determine the statistical significance and variance. Duncan's multiple range test (DMRT) was used to determine the individual comparisons. The P-values < 0.05 were considered significant.

RESULTS

Table 1 shows the effect of *E. prostrata* extracts (EPE) on glucose and insulin levels in normal and STZ-induced diabetic rats. Totally six groups were selected and experimented. An increase in plasma glucose and insulin level with noticeable significance was found in group 3, 4 and 5 compared to the control group. At the same time, in EPE alone (200 mg/kg) treated rats (Group 6), plasma glucose and insulin levels increased on the first day of the experiment. At the end of the study, they maintained the plasma glucose and insulin equal to the control group. Hence, the dosage of EPE alone (200 mg/kg) was selected for further biochemical and marker studies. The glycosylated haemoglobin (HbA1c) levels in control and treated rats are depicted in Table 1. Reduced HbA1c levels were noted with $p<0.05$ in EPE alone (200 mg/kg) treated diabetic rats than group 4 and 5. Also, the found HbA1c levels were almost like normal control rats of experimental studies.

The levels of regulating enzymes of carbohydrate metabolism are presented in Table 2 as mean ± standard deviation. The enzymes of glucokinase, glucose-6-phosphatase, fructose-1,6 bisphosphatase, and glucose-6-phosphate dehydrogenase were found less in diabetic rats

treated with EPE (200 mg/kg) (Group 3) glibenclamide (Group 4), than control and EPE alone treated groups. Hence, when compared to STZ+EPE treatment, the levels were significant ($p<0.05$) and almost near to those of the normal control rats in EPE alone treatment. Thus, these results prove that EPE alone worked better on diabetic rats.

The kidney and liver function conditions were tested by measuring urea, uric acid, creatinine, AST, and ALT (Table 3). An increased activity of AST and ALT was observed in diabetic rats than in the control group (Group 2). The EPE alone (Group 5) treated experimental rats showed uncontrolled AST and ALT activities on day one, whereas at the end of the experiment, they become almost normal like control groups with a $p<0.05$. On the other hand, urea, uric acid, and creatinine were highly excreted in the serum of diabetic rats (Group 2). However, these values were significant ($p<0.05$) in STZ+ EPE treated rats with maintained serum markers (Group 3).

Table 4 lists the important parameters of lipid metabolism, which are significant when compared to diabetic rats. There are increased levels of serum total cholesterol, triglycerides, FFA, phospholipids, LDL, and HDL in diabetic rats (Group 2) than in control. But, in STZ+EPE group (Group 3), the lipid parameters were brought back to normal state after EPE treated experimental groups was significant with $p<0.05$ of DMRT.

Table 5 presents the levels of enzymatic antioxidants (SOD, CAT, GPx) and non-enzymatic antioxidants (vitamin E and reduced glutathione) as mean ± standard deviation. The levels were significantly altered in STZ-treated diabetic rats. Hence, when compared to STZ (Group 2) and EPE (Group 3), the antioxidant levels significantly improved and they were almost near to normal control rats. Again, these results prove that EPE alone worked better in maintaining the antioxidant profile in diabetic rats (Group 5).

Effect of EPE-treatment on diabetic rats, and its histological profile of pancreas studied by H&E staining are depicted in Figure 1. Comparing the histological profile of all the experimental groups, STZ + EPE treated animals show that pancreatic cells overcome diabetes (improved beta cells and its cross-sectional area) like normal cells than in STZ-induced diabetic rats. Figure 2 represents the liver cells, and the cross-sections of the histological profile of STZ-induced diabetic rats. The improved liver after diabetic induces and the EPE treated turns to normal liver cell-like control than STZ induced diabetic rats (Group 3). Also, damage in hepatic cells was found in diabetic induced rats (Group 2), indicating that STZ with EPE-treated (Group 3) is influenced in treating damage. Figure 3 shows that histological profile of kidneys by STZ induced diabetic rats. STZ + EPE treated animals (Group 3) shows that the effect of EPE treated diabetic rats with clear nephrotic histology indicates that our plant extract alone treated nephrotic cells are maintained and avoid damage suggest that better antidiabetic activity.

TABLE 1. Effect of *E. prostrata* extract on HbAC1, insulin and glucose levels in the normal and STZ induced diabetic rats

Groups	HbAC1 (%)	Insulin (µU/mL)	Plasma glucose(mg/dL)	
			Initial day	End day
Control	7.23±1.36^{a}	15.05±1.05^{a}	87.31±7.22^{a}	85.24±6.81^{a}
STZ (60 mg/kg)	15.93±0.92^{e}	6.91±1.20^{e}	255.20±15.64^{b}	301.14±22.84^{e}
STZ + EPE (100 mg/kg)	13.89±1.91^{d}	8.02±0.74^{d}	250.68±18.74^{b}	190.57±17.54^{d}
STZ + EPE (200 mg/kg)	11.36±1.78^{c}	10.09±0.91^{c}	258.57±19.54^{b}	124.85±10.45^{c}
STZ + Glibenclamide (600µg/kg)	8.21±0.25^{b}	13.01±0.86^{b}	252.38±12.56^{b}	110.32±5.43^{b}
EPE alone (200 mg/kg)	7.91±1.52^{a}	15.64±0.52^{a}	257.52±13.94^{b}	89.91±3.60^{a}

The values are given as Mean ± S.D from each group 6 rats. ^{a}EPE alone treated animal showed there is no significant difference compared to the control group. ^{e} STZ alone treated animal were significantly different from the control group at $p < 0.05$. ^{c}Significantly different from the STZ alone treated group at $p <0.05$.
STZ: Streptozotocin; EPE: *E. prostrata* extract

TABLE 2. Effect of *E. prostrata* extract on the activity of carbohydrate metabolic enzymes in the liver of control and STZ induced diabetic rats

Groups	Glucokinase (U*/mg protein)	Glucose 6-phosphatase (Unit#/mg protein)	Fructose 1,6 -bisphosphatase (Unit$/mg protein)	Glucose 6-phosphate dehydrogenase (Unit@/ mIU /mg protein)
Control	0.350 ±0.03[a]	0.230± 0.02[a]	0.425 ± 0.01[a]	4.70 ± 0.35[a]
STZ (60 mg/kg)	0.145 ±0.02[d]	0.450 ±0.04[d]	0.730 ± 0.05[d]	2.10 ± 0.12[d]
STZ + EPE (200 mg/kg)	0.234 ±0.02[c]	0.360 ±0.03[c]	0.554± 0.04[c]	3.06± 0.21[b]
STZ + Glibenclamide (600µg/kg)	0.284 ± 0.02[b]	0.277 ± 0.02[b]	0.501 ± 0.01[b]	2.74± 0.31[c]
EPE alone (200 mg/kg)	0.347± 0.01[a]	0.229± 0.05[a]	0.446 ± 0.02[a]	4.83± 0.26[a]

The values are given as Mean ± S.D from each group 6 rats. [a]EPE alone treated animal showed there is no significant difference compared to the control group. [c] STZ alone treated animal were significantly different from the control group at $p < 0.05$. [c]Significantly different from the STZ alone treated group at $p < 0.05$.
* - mmol of glucose phosphorylated per hour. #- mmol of Pi liberated per minute; $- mmol of Pi liberated per hour. @- mIU/mg of protein.
STZ: Streptozotocin; EPE: *E. prostrata* extract

TABLE 3. Effect of *E. prostrata* extract on serum hepatic and plasma renal markers in the serum of control and STZ induced diabetic rats

Groups	Liver markers		Kidney markers		
	ALT (IU/L)	AST (IU/L)	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)
Control	30.21 ±2.81[a]	80.41 ±7.89[a]	26.54 ±1.54[a]	1.44 ± 0.21[a]	1.01 ± 0.19[a]
STZ (60 mg/kg)	65.24 ± 4.51[d]	120.15 ± 09.54[d]	39.46 ±2.70[d]	2.06 ± 0.12[d]	2.62 ± 1.13[d]
STZ + EPE (200 mg/kg)	48.57 ±2.89[c]	97.75 ± 7.96[c]	31.59 ± 2.51[c]	1.35 ± 0.25[c]	1.94 ±0.23[c]
STZ + Glibenclamide (600µg/kg)	36.95 ± 1.54[b]	91.48 ± 4.37[b]	28.43 ± 1.06[b]	1.26 ± 0.04[b]	1.24 ±0.02[b]
EPE alone (200 mg/kg)	32.41± 1.28[a]	82.71 ± 2.63[a]	25.89 ± 0.94[a]	1.47 ± 0.19[a]	1.04 ±0.11[a]

The values are given as Mean ± S.D from each group 6 rats. [a]EPE alone treated animal showed there is no significant difference compared to the control group. [c] STZ alone treated animal were significantly different from the control group at $p < 0.05$. [c]Significantly different from the STZ alone treated group at $p < 0.05$.
STZ: Streptozotocin; EPE: *E. prostrata* extract

TABLE 4. Effect of *E. prostrata* extract on lipid profile in the plasma of normal and STZ induced diabetic rats

Groups	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	Phospholipids (mg/dl)	Free fatty acids (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)
Control	78.21 ±4.51[a]	61.54 ±5.21[a]	87.19 ± 5.64[a]	59.21 ±5.64[a]	19.18 ±1.53[a]	37.57 ±2.54[a]
STZ (60 mg/kg)	147.72±10.54[d]	164.41 ±12.54[d]	146.24 ± 10.99[d]	109.60 ± 7.64[d]	51.82 ±4.17[d]	25.01 ±1.08[d]
STZ + EPE (200 mg/kg)	117.45 ±8.67[c]	135.24 ± 5.81[c]	108.96 ±8.45[c]	95.04 ± 3.89[c]	42.05±2.64[c]	27.84 ±1.94[c]
STZ + Glibenclamide (600µg/kg)	90.14 ±8.67[b]	86.54 ± 5.81[b]	91.08 ±8.45[b]	65.87 ± 3.89[b]	30.64±2.64[b]	32.17 ±1.94[b]
EPE alone (200 mg/kg)	80.96 ±6.57[a]	64.92 ± 5.48[a]	90.53 ±7.26[a]	62.27 ±4.86[a]	20.75±1.42[a]	38.42 ±2.05[a]

The values are given as Mean ± S.D from each group 6 rats. [a]EPE alone treated animal showed there is no significant difference compared to the control group. [c]STZ alone treated animal were significantly different from the control group at $p < 0.05$. [c]Significantly different from the STZ alone treated group at $p < 0.05$.
STZ: Streptozotocin; EPE: *E. prostrata* extract

TABLE 5. The activities of antioxidants enzyme status in plasma of control and experimental animals in each group

Groups	SOD (U*/mL)	CAT U#/mL	GPx (U§/I)	Vitamin 'E' [mg/dl]	Reduced glutathione (mg/dl)
Control	3.93±0.27[a]	0.65±0.04[a]	142.36±10.57[a]	1.32±0.11[a]	25.17±1.81[a]
STZ (60 mg/kg)	2.38±0.14[c]	0.47±0.03[d]	112.73±15.72[d]	0.97±0.09[b]	17.64±2.04[b]
STZ + EPE (200 mg/kg)	2.86±0.60[d]	0.54±0.04[c]	123.46±13.61[c]	1.27±0.14[a]	20.38±1.61[a]
STZ + Glibenclamide (600µg/kg)	3.05±0.31[b]	0.60±0.02[b]	138.90±14.52[b]	1.21±0.10[c]	23.55±1.07[c]
EPE alone (200 mg/kg)	3.90±0.42[a]	0.67±0.06[a]	143.72±10.93[a]	1.30±0.21[a]	25.03±1.12[a]

Values are means ± S.D. from 6 rats in each group. The values are given as Mean ± S.D from each group 6 rats. [a]EPE alone treated animal showed there is no significant difference compared to the control group. [c]STZ alone treated animal were significantly different from the control group at $p < 0.05$. [c]Significantly different from the STZ alone treated group at $p < 0.05$. STZ: streptozotocin; EPE: *E. prostrata* extract

U* = enzyme concentration required to inhibit the NBT to 50% reduction in one minute; U# = mmole of H_2O_2 consumed/minute; U§ = mg of GSH utilized/minute

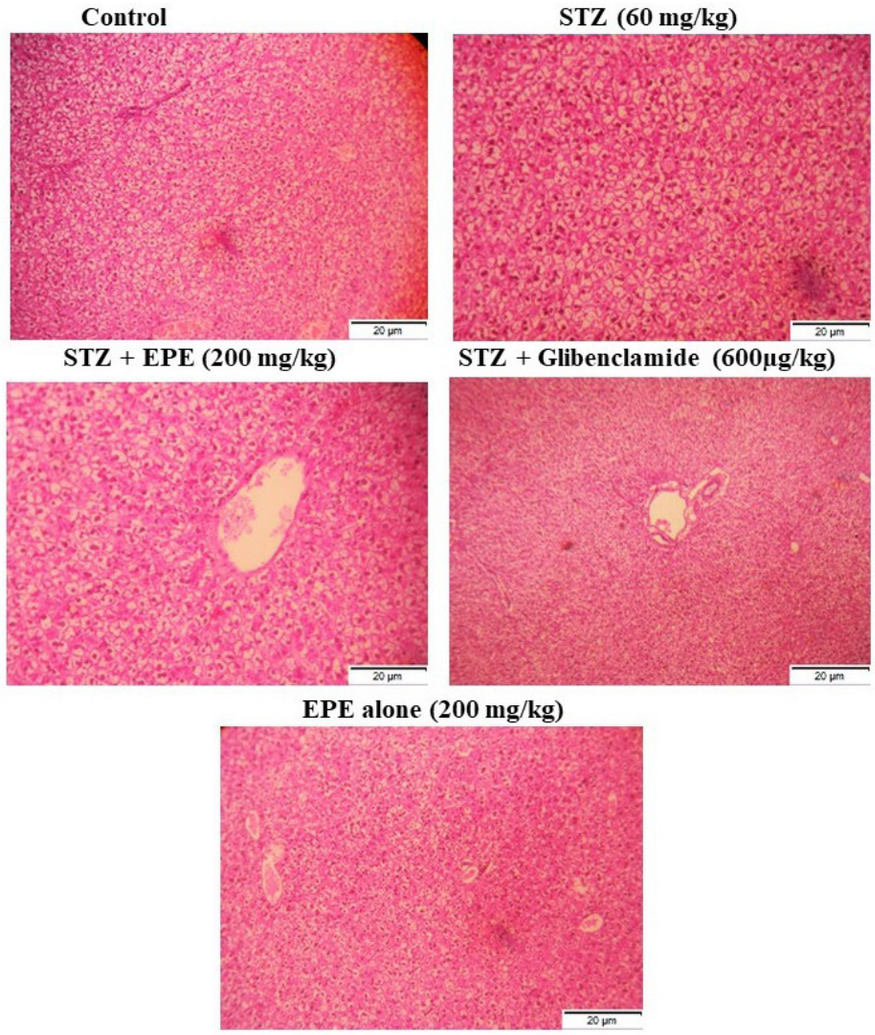

FIGURE 1. Histopathological examination of the diabetic pancreas. The control animals showed normal islet cells. The diabetic group showed shrinkage of islet cells with decrease in islet cells with fibrosis and disarrangement of cells (Black arrow). STZ+EPE (200 mg/kg) with diabetic rats shows mild degenerative changes of pancreatic islet cells (Black arrow). STZ with glibenclamide showed a reduction in adipose tissue and the pancreatic islets within the normal limit (Black arrow)

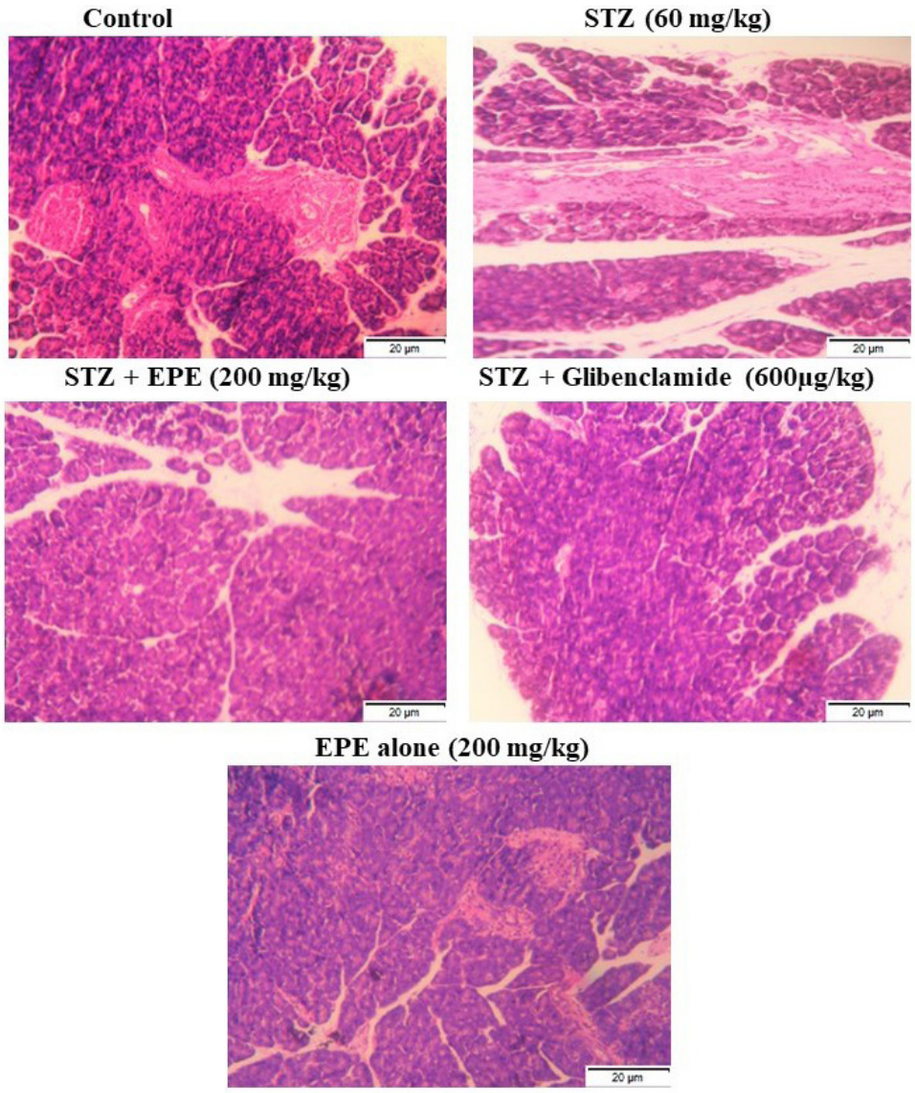

FIGURE 2. The photomicrograph of hematoxylin-eosin staining of liver histopathology of diabetic rats. Control rats showing normal hepatocytes and SZT induced diabetic rats exhibiting fatty change and inflammatory cell infiltrate (Black markerd). Diabetic with EPE (200 mg/kg) treated presenting normal hepatocytes (Black arrow). Glibenclamide treatment rats showing dilated central vein when compared with diabetic rats (Black arrow)

DISCUSSION

In recent years, the world is struggling with the management of DM. Research shows that managing people with diabetes is challenging, especially if they are in their 50's. Patients nowadays prefer natural medication/organic foods to allopathic medicine to avoid side effects (Luna & Feinglos 2001). Hence, the present study aims to formulate a diabetic management solution from *Eclipta prostrata* L. plant through an animal model. Streptozotocin (50 mg/kg) was injected to the experimental rats intraperitoneally to damage the beta cells of the pancreas, which in turn would reduce the insulin secretion. The reduced insulin secretion was confirmed with plasma glucose levels in type II diabetes (Wang-Fischer & Garyantes 2018). In the present study, STZ-injected rats showed a high plasma

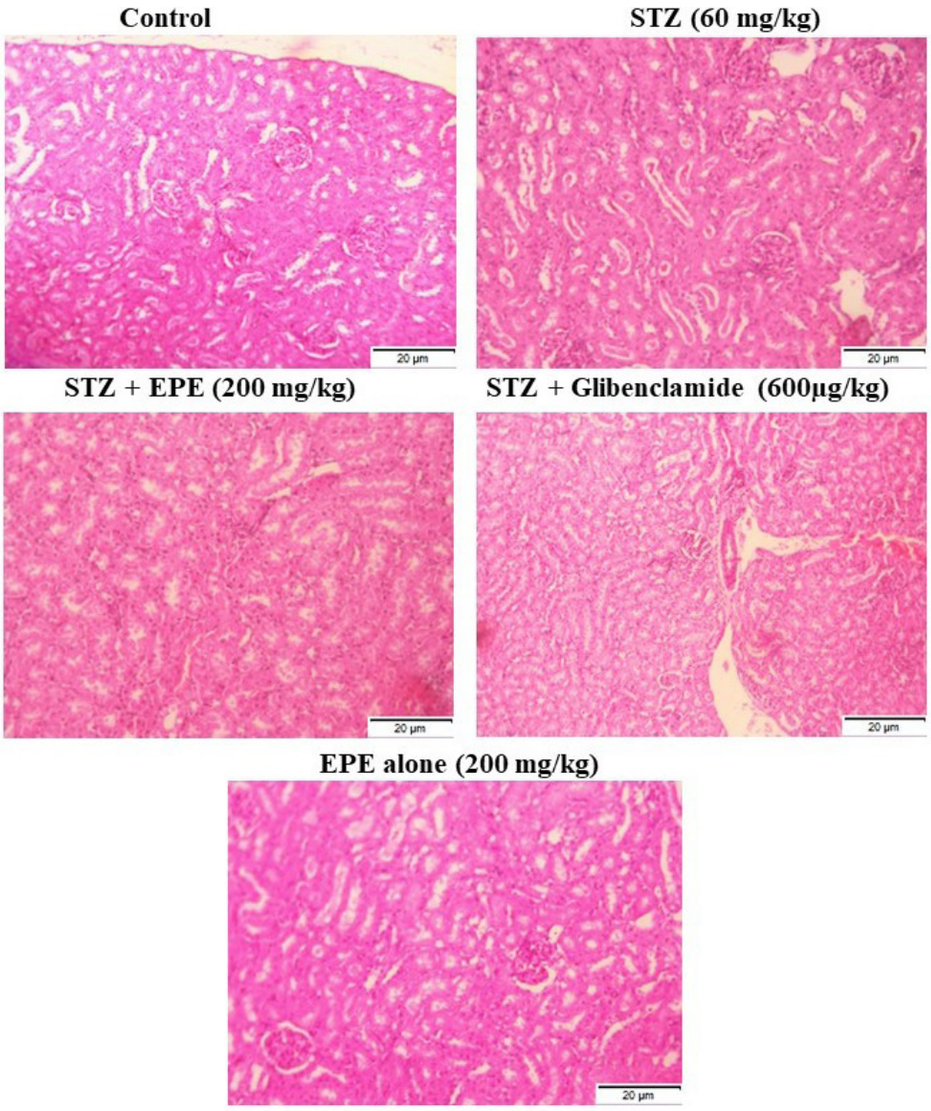

FIGURE 3. Histopathological of hematoxylin-eosin staining of kidney of *E. prostrata* and diabetic rats. Control rats showing architecture with glomeruli and normal tubular cells. Renal of diabetic rats exhibiting damaged glomeruli and fatty infiltration tubules (Black marked). Diabetic rats treated with EPE (200 mg/kg) decrease the cell necrosis with partial inflammation cells (Black arrow). Glibenclamide treated animald showed improved glomeruli and tubules with lack of renal inflammatory cells (Black arrow)

glucose and low insulin level. But in EPE-treated rats, the plasma glucose was reduced in comparison with the diabetic condition. The statistical studies show that pancreatic beta secretion is brought back to normalcy by the medicinal plant under investigation. Arresting the movement of ATase/K+ channels in the pancreas will affect the beta cells, degeneration was done by our like control drug glibenclamide function. Also, triterpenoids possessing significant antioxidant and anticancer activities have been proved to inhibits sensitive ATPase/K+ channels while regulating plasma glucose (Kemboi et al. 2020). Hence, *E. prostrata* plant, rich in triterpenoids, worked on STZ-induced experimental rats.

Numerous *in vivo* and human clinical trials state that middle-aged people should maintain their insulin level to sustain a homeostatic state as well as the amphibolic role of TCA cycle for a continuous supply of glucose by both liver and muscle gluconeogenesis and glycogenolysis (Nilsson et al. 2015). Also, few reports state that a decrease in carbohydrate metabolic regulatory enzymes, and its negative association with tissue proteins might influence a person's body weight (Hall et al. 2012; Home 2015). Hence, insulin influences the body weight (BMI) of diabetic patients. Some of the preceding claims that lack of ATP supply to tissue due to poor ATP production by carbohydrate metabolism might be a reason for poor structural protein and its synthesis (Canfora et al. 2015). The results of the present study show that the STZ-induced diabetic rats showed better reversal of glucose levels after the oral administration of EPE compared with the control drug. The results of the present investigation agree well with a previous report (Jaiswal et al. 2017; Sasidharan et al. 2011). Moreover, the insulin hormone controls protein metabolism by stimulating protein synthesis and delays protein catabolism. But lower insulin production reflects reduced structural protein synthesis with higher protein degradation. As a result, unconditional glycation, which includes haemoglobin, albumin, LDL, fibronectin, and collagen, lead to unconditional diabetes (Dardevet et al. 1998). Murray et al. (2006) have reported that low insulin levels damage the red blood cell protein called HbA1c (glycosylated hemoglobin). Therefore, HbA1c is the final validating marker that will declare whether a patient belongs to the diabetic or non-diabetic category. In the present study group, the HbA1c levels of EPE-treated STZ–induced diabetic rats (Group 6) were observed to be normal like control compared to any other experimental group.

Glucokinase (GK) is an essential irreversible rate-limiting enzyme in glycolysis and glycogen metabolism. The absence or impaired function and results in diminished glucose oxidation by glycolysis leads to hyperglycemia with reduced ATP synthesis by carbohydrate metabolism (Matschinsky & Wilson 2019). Hence, this will make a way to non-enzymatic glycation of glucose as glucose-6-phosphate, which is catalyzed by phosphoglucoisomerase as an alternative pathway of glyoxalate cycle that synthesizes low ATP by direct glycolysis. The release of GK was lower in diabetic rats than oral administered EPE group rats.

When the glucose levels are low, glycogen metabolism and gluconeogenesis balance the supply of glucose. However, in diabetic conditions, poor insulin enhances the G-6-Pase and F-1,6-BPase. G6Pase and F1,6BPase are also crucial regulating enzymes in glycolysis and enhance the glucose level in the diabetic state. Hence, both the enzymes were found with poor recognition of insulin and released highly in diabetic rats. The drug-treated experimental rats show high enzymes in the initial days and at the end of medication they are brought to an average level like in control. This observation also conforms to previously published results (Rahman et al. 2011). Another essential enzyme of carbohydrate metabolism is glucose-6 phosphate-dehydrogenase (G6PDH), from HMP shunt as an alternative pathway of the TCA cycle (Bakar et al. 2015). Our study shows that increased G6PDH was found in the EPE-treated experimental group than in diabetic rats.

A recent report shows that poor insulin secretion enhances the transaminase enzymes of both AST and ALT, which will trigger the protein catabolism directed gluconeogenesis resulting in a hyperglycemic condition in both alcoholic and diabetic condition (Manimegalai et al. 2020). This confirms that liver markers can also be influenced by poor insulin secretion, which would become worse in diabetic cases. In the present study, EPE-administered experimental groups initially showed an increased liver marker compared to the STZ induced-diabetic rats and at the end of treatment, the level was maintained as shown in the histopathology of liver cells. In an uncontrolled diabetic condition, the excess of glucose molecules will be accumulated in the glomerulus, and the excretion mechanism of the kidney becomes worse (Mora-Fernández et al. 2014).

In the present study, STZ-induced diabetic rats showed higher levels of urea, uric acid, and creatinine than normal levels, denoting diabetes was in uncontrolled than control. But, in group 6, the levels are maintained, which was confirmed by comparing normal control with the drug control group. This again proved that oral administration of EPE is capable of protecting kidney from an excessive load of excretion and its damage. After protein and carbohydrate metabolism, lipid and its classes play a significant role in controlling diabetes. Low insulin secretion immediately impairs degradation of triglycerides and phospholipids through gluconeogenesis leading to hyperglycemic condition (Czech 2017). The present study estimated TC, PL, TG, and LDL and HDL in diabetic rats. Although oral administered EPE experimental groups have high HDL, other lipid parameters were found almost the same as standard control drug group. Once again, lipid

parameters confirm the action of oral administration of EPE in regulating diabetes in rats. Enzymatic and non-enzymatic antioxidants serve in producing cells and tissues from cellular injuries caused by imbalanced free radicals. After that, free radicals, homeostatic fluid balance, and molecular equivalents play a significant role in maintaining cell membranes from electrical shock. Not only protecting membranes, supplying reducing equivalents to balance the coenzymes during ATP production by TCA cycle and HMP shunt (Lobo et al. 2010). Superoxide dismutase, catalase, α-tocopherol, and reduced glutathione were lower in diabetic rats than in EPE-administered experimental rats. Finally, EPE administration shows a better effect on maintaining the homeostat and increased antioxidant activity.

Conclusion

Our study claims that treatment of STZ-induced diabetic rats by oral administration of *E. prostrata* was effective in the management of diabetes. This was evidenced by triggered insulin secretion from degenerated beta cells resulting in controlled plasma glucose level with kidney liver markers and scavenging mechanisms. These results clearly indicate that the aqueous extracts of *E. prostrata* has a high antidiabetic potential along with significant hypoglycemic and hypolipidemic effects and may be applicable in the pharmaceutical industry. However, further studies are necessary to explore the anti-diabetic constituents in the plant extracts.

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