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Research Article

Antidiabetic and antihyperlipidemic activity of *Premna latifolia* extract in streptozotocin-induced diabetic rats

Neelakanth M. Jeedi and Koti BC*

Department of Pharmacology, KLE College of Pharmacy, Hubballi-580031. A Constituent unit of KLE Academy of Higher Education and Research, Belagavi, Karnataka, India

ABSTRACT

In this research work, we studied the effect of alcoholic extract of plant *Premna latifolia* (leaves) on the enzymes associated with metabolism of carbohydrate and lipid in the rats models with induced diabetes employing Streptozotocin. Administration of alcoholic extract of *Premna latifolia* was made to these diabetic rats at doses of 100 mg/kg and 200 mg/kg once a day for three weeks. Various changes specifically, glucose levels in blood, insulin, total cholesterol content in serum, triglycerides, High Density Lipoprotein levels, Low Density Lipoprotein levels along with variations in the body weight were measured. Numerous enzymes involved in the metabolism of carbohydrate and lipids viz. amylase, lipase, Aldolase, Glucose-6-phosphatase, Phosphogluco isomerase, Fructose-1,6-diphosphatase, and Glucose-6-phosphate dehydrogenase have been evaluated. Further, the histopatholgical changes in pancreas have been studied and reported. This study substantiates that the alcoholic extract of *Premna latifolia* normalizes the blood glucose, insulin, lipid profile by regulating pancreatic and hepatic enzyme levels in diabetic rats. Alcoholic extract of *Premna latifolia* lowers glucose and lipid levels in the blood of diabetic rats.

Keywords: Antidiabetic; *Premna latifolia*; Glucose-6-phosphatase; Insulin; streptozotocin; Pancreatic β-cells.

INTRODUCTION

Diabetes Mellitus (DM) is one among the metabolic disorders contributing majorly for the global health burden. It severely affects the functions of various vital organs like heart, kidneys, eyes, blood vessels and nerves. In 2000, the diabetic incidences were estimated globally in various age groups, evidently the number of people suffering was found to be 171 million and the toll is projected to climb by 366 million till the end of 2030 (Shaw et al., 2010). Every year, there are 1.5 million reported deaths worldwide due to diabetes. As per the report of World Health Organization (WHO), in India alone, nearly about 31 million people have suffered with diabetes in the year 2000 and in the future it is expected to rise up to 79 million by 2030. Presently, 4 to 11.6% of urban and 3% of the rural population above the age of 15 years in India are identified to be diabetic. Type 2 DM is characterized hyperglycemia, due to altered metabolism of carbohydrates, proteins and lipids resulting from defective insulin secretion, insulin action or insulin resistance and pancreatic *β* cell dysfunction. Persistent hyperglycemia can lead to dysfunctioning or failure of various organs. DM is also associated with hypercholesterolemia, an renowned risk factor known to cause coronary and peripheral artery diseases (Barar, 2000). The DM covers a wide range of pathological consequences. Diabetic neuropathy (DN), a major chronic problem in type 1 along with type 2 diabetes, is marked by defect in the functions of both afferent and efferent nerves. Diabetes causes nerve dysfunction, slowing nerve conduction velocity, abnormal thermal perception and degeneration of nerves (HPSCG 2002; Bell et al., 1998). Numbness, tingling of extremities, loss of urinary bladder control, mouth and eyelid drooping, muscle weakness and speech impairment etc. are among the symptoms of DN.

Oral hypoglycemic agents used to treat DM causes different side effects like hematological effects and affects the functions of vital organs like liver and kidney (Manisha et al., 2007). Also, there is no perpetual cure for diabetic neuropathy however, symptomatic treatments have shown limited success. Worldwide now a day, numbers of medicinal plants have been reported and are useful for treating DM. WHO suggests the use of traditional plants for treating DM, as they are effective, nontoxic with little or no side effects, and are also excellent candidates for oral therapy.

Premna latifolia (Family: Verbinacae): A small tree, growing in the regions of south India and Bengal. As per traditional claims, leaves are used as Antidiabetic (Ayyanar et al., 2008), diuretic and spasmolytic. Stem barks are used as hypoglycemic and other parts of plant *Premna latifolia* have been claimed to be used in fever, fomentation in piles, glandular swelling, musculoskeletal disorder and rheumatism. *Premna latifo-*

* Corresponding Author
Email: bc_koti@yahoo.com
Contact: +91-9945711281 Fax: 0836-2371694
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lia has been reported for anti-inflammatory and antioxidant activity properties. However, beneficial effects in diabetes lack scientific data to substantiate the traditional claim. Therefore, the present research work was carried out to assess antidiabetic and antihyperlipidemic potentials of alcoholic extract of *Premna latifolia* (EEPL) leaves in streptozotocin (STZ) - induced diabetic rats.

MATERIALS AND METHODS

Collection of plant material

Leaves of *Premna latifolia* collected from neighboring places of Udupi. (Dist. Dakshina Kannada, Karnataka). Identification of plant materials was done by Dr. M Jayaraj, Professor Botany Department, Karnataka University at Dharwad and authenticated (voucher specimen RMRC-967) by Dr. H. Hegde, Scientist, RMRC, ICMR, Belagavi. The sample of plant is kept in at that center for the further reference.

Plant extract

Premna latifolia plant leaves are washed using normal tap water and dried in shadow. Size reduced to coarse powder and passed through sieve. For standardization of extraction – 4g of coarse leaves powder was extracted separately with 100 mL of different concentration of ethanol (hydro alcohol) 60%, 70% and 80%. The yield was found to be 4.30%, 5.55% and 4.85% respectively. 70% ethanol gives maximum yield it was taken for bulk extraction. Shade dried coarsely powdered leaves were extracted using 70% of ethanol in soxhlet extractor by hot continuous percolation method, then filtered, concentrated in low pressure by a rotary evaporator maintaining 40 °C and dried completely on hot water bath till no solvent. Dried extract was stored in proper container and used in this study.

Drug solutions

Citrate buffer with pH 4.5 was used to dissolve STZ; this was used to induce diabetes. Freshly prepared 1% Tween 80 solution was used to prepare glibenclamide, EEPL solutions and were used as standard and test drug for the further pharmacological studies.

Preliminary phytochemical screening

Phytochemical investigations is performed (Kokate CK et al., 1994; Harborne JB et al., 2007) to identify the occurrence of steroids, carbohydrates, proteins, amino acids, triterpenoids, alkaloids, tannins, glycosides, flavonoids and phenolic class of compounds in the EEPL leaves.

Chemicals

From Sisco Research Laboratories, Pvt. Ltd. Mumbai, we purchased streptozotocin. Commercial diagnostic kits required to estimate biochemical's like lipid profile, enzymes level etc. were purchased from Erba Diagnostic Pvt. Ltd. The drug Glibenclamide was procured from Elegant Pharma, Hubballi, Karnataka, India. All the reagents procured were of analytical grade from local suppliers at Hubballi, Karnataka, India.

Animals

Wistar albino rats of both sex (150-200g) obtained from Venkateshwara Enterprises, Bengaluru, India. The animals are placed in a room at 22 ± 2 °C with equal hours of natural light and dark cycle. Standard laboratory chow and water *ad libitum* was given to animals. Rats were randomly selected for different experimental groups after seven days of acclimatization. The experimental protocols were reviewed and approved by IAEC (Proposal No.: KLEU's-09-IAEC-HBL-31/Aug 2013) in accordance with CPCSEA norms.

Acute toxicity study

Acute oral toxicity studies were performed by using female *albino* mice (18-25 g) following standard guidelines recommended by Organization for Economic Co-operation and Development (OECD, 423). Doses were prepared using distilled water and tween 80 (1%). The dose of EEPL was administered 2 g/kg body weight and food was refrained for 4 h. Observation of animals was done at 0.5, 1, 2, 3, 4, 24 h and once daily intervals for 14 days.

Induction and assessment of diabetes

STZ (55 mg/kg body weight) was administered by *i.p.* route to overnight fasted animals. Animals were permitted to drink 5% glucose solution to prevent low blood glucose level due to the STZ. Control group animals were injected with the same volume of Citrate buffer of pH 4.5. After 3 days of STZ administration, blood was withdrawn from fasted animals by the retro-orbital plexus using isoflurane anesthetic. Animals of serum glucose level more than 250 mg/dL, were measured as diabetic and incorporated in further study. (Glucose estimation kit - Erba Diagnostics)

Experimental design

Evaluation of extract in STZ- induced diabetic rats:

The animals were divided into following groups:

Group I: Normal control - receive 1% Tween 80 (5 ml/kg., p.o.)
Group II: Diabetic control - receive STZ (55 mg/kg., i.p.).
Group III: Diabetic - treated with Glibenclamide (5 mg/kg., p.o.)
Group IV: Diabetic - treated with EEPL (100 mg/kg., p.o.).
Group V: Diabetic - treated with EEPL (100 mg/kg., p.o.).

The vehicle, glibenclamide and extract solutions were administered to the respective group of animals by oral gavages one time in a day for three weeks. Serum glucose was measured before induction and after induction of diabetes on 3rd, 10th, 17th and 24th day. On the last day the animals were fasted and sacrificed using mild anesthesia. Serum was stored at -20 °C and used

for further investigation (Vats et al., 2003). Effect of EEPL on body weight in STZ-induced diabetic rats was also studied. The pancreas and liver tissues were collected and the same was used for biochemical estimations and histopathological studies. The liver tissue (10% w/v) was homogenized with 0.1 M Tris-HCl buffer of pH 7.4 after washing in cold saline followed by blotting it on filter paper. This tissue homogenate was centrifuged for 15 min, at normal temperature at 3000 rpm. The obtained supernatant was stored at 2 °C for biochemical analysis like lipid profile, insulin level, sugar and fat metabolizing enzymes.

Biochemical analysis

Lipase enzyme was measured by one hour period of hydrolysis method (Roe et al., 1963). Serum insulin in rat was estimated using enzyme-linked immunosorbent assay (ELISA) kit. Assay of glucose-6-phosphatase (G6Pase) enzyme activity was performed as per reported method (Cori et al., 1952). While, fructose-1,6-diphosphatase (F-1,6-DPase) was determined as per established method (Gancedo et al., 1971). Estimation of Phosphoglucose isomerase (PGI) enzyme was performed as illustrated by Horrocks (Horrocks et al., 1963). Aldolase enzyme was estimated by following the method explained by Sibley (Sibley et al., 1949). Glucose-6-phosphate dehydrogenase (G6PDH) was determined by the method of Ellis (Ellis et al., 1961). Animals were sacrificed after blood collection by over dose of anesthesia on the 21st day.

Histopathology

The dissected pancreas was prewashed using ice-cold saline before storing in 10% formalin for tissue characterization. Pancreatic architecture of specimens for each group was examined in detail microscopically.

Statistical analysis

The results were presented as the mean ± S.E.M. The statistical analysis was done by one-way analysis of variance (ANOVA), then subsequently by Tukey's multiple comparison tests. The significance was expressed by p values and if p value is <0.05, the results were regarded as statistically significant.

RESULTS

Phytochemical constituents in EEPL leaves

The phytochemical investigations substantiated the presence of steroids, carbohydrates, alkaloids, tannins, glycosides, flavonoids and phenolic class of compounds in the EEPL leaves.

Effect of EEPL on blood glucose levels in STZ-induced diabetic rats

The effect of EEPL on serum glucose levels in diabetic rats. EEPL was given at doses of 100 and 200 mg/kg for three weeks to treat diabetic rats. It was observed that significant increase ($p<0.001$) in the serum glucose level of diabetic animals compared to the non diabetic animals and further goes on to increase in glucose levels on 10th, 17th and 24th day. The glibenclamide reduced ($p<0.001$) levels of glucose at all time points compared to diabetic animals. Extract treated group animals, EEPL 100 & 200 mg/kg reduced ($p< 0.001$) serum glucose levels on 10th, 17th and 24th day compared to diabetic control. The EEPL reduced blood glucose level dose dependently. On 24th day EEPL 200 mg/kg treated group animal's blood glucose was nearer to normal control, but no substantial difference was observed between the EEPL 200 mg/kg treated animals and glibenclamide treated animals, are depicted in Figure 1

Effect of EEPL on lipid profile in diabetic rats

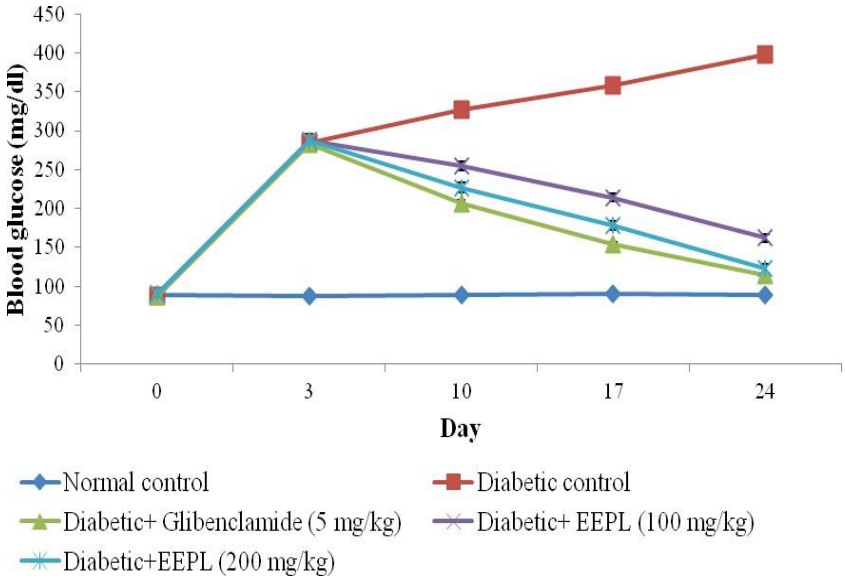
We observed that there was increase ($p<0.001$) in the levels of triglycerides (TG), and Low density lipoprotein (LDL) and reduction ($p<0.001$) in high density lipoprotein (HDL) levels in diabetic animals as compared with normal control. The EEPL at 100 & 200 mg/kg reversed the STZ-induced changes in lipid profile. The effectiveness of EEPL at a dose of 200 mg/kg was noteworthy than compared to 100 mg/kg in correcting the lipid profile. Antihyperlipidemic activity of EEPL (200 mg/kg) was comparable with glibenclamide, a diabetic standard drug. The results are represented in Table 1.

Effect of EEPL on body weight of diabetic rats

Diabetic animals lost their body weight considerably ($p<0.001$) on comparison with normal control. Further, the diabetic animals kept losing their bodyweight constantly during the experiment. Gradual increase in body weight was witnessed ($p<0.001$) in the animals treated with glibenclamide (5 mg/kg) and EEPL (100 and 200 mg/kg) on contrast with diabetic animals. However, there was not much difference in the improvement of body weight of animals treated with glibenclamide and EEPL 100 mg/kg. Results are shown in Fig. 2.

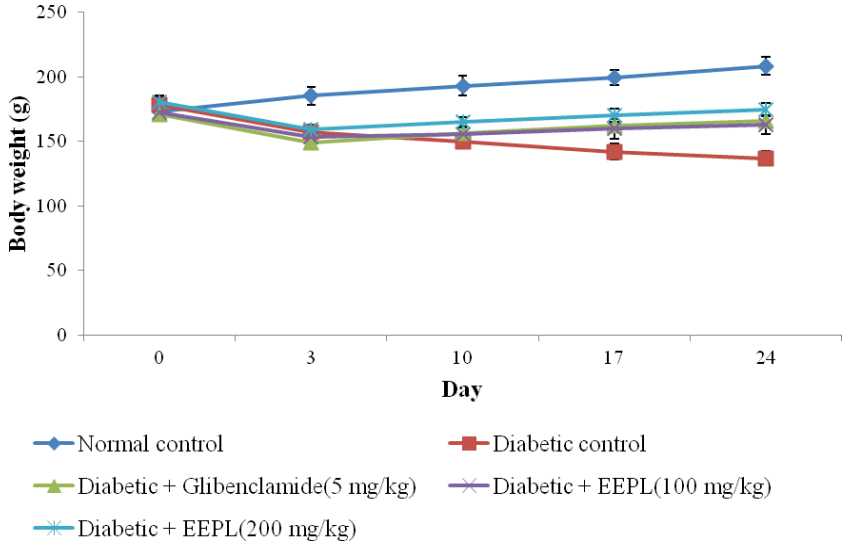
Effect of EEPL on insulin, amylase and lipase levels in diabetic rats

Substantial decrease ($p<0.001$) in the insulin levels and significant increase ($p<0.001$) in serum amylase and lipase enzyme levels were witnessed in diabetic rats on comparison with the normal rats. Insulin level in diabetic rats was however seen to escalate ($p<0.001$) during daily administration of glibenclamide (5 mg/kg) and EEPL (100 and 200 mg/kg). The levels of amylase and lipase enzymes were seen to diminish ($p<0.001$) in the extract treated groups. The results are expressed in Table 2.



All values are expressed as mean ± S.E.M. (n = 6)

Figure 1: Effect of EEPL on blood glucose levels in diabetic rats



All values are expressed as mean ± S.E.M. (n = 6)

Figure 2: Effect of EEPL on body weight in diabetic rats

Effect of EEPL on G6Pase and F-1,6-DPase in diabetic rats

Levels of G6Pase and F-1,6-DPase enzymes were found to increase ($p<0.001$) in STZ-induced diabetic animals when compared with normal control. Extract (200 mg/kg) and glibenclamide treated groups equally reduced ($p<0.001$) both the enzymes levels to the normal values. However, the EEPL at 100 mg/kg dose was found to be less effective in reducing both the enzyme levels, thus conferring ineffectiveness of EEPL at low dose. The results are further presented in Table 3.

Effect of EEPL on PGI, aldolase and G6PDH in diabetic rats

In the diabetic rats, levels of enzymes namely PGI and G6PDH were found to be reduced ($p<0.001$) whereas, aldolase level increased ($p<0.001$) on comparison with normal rats. In both of the EEPL treated groups, significant increase ($p<0.001$) in PGI and G6PDH levels was observed, whereas the level of aldolase was found to be lesser ($p<0.001$) as shown in Table 4.

Histopathological studies

The normal histological pancreas section showed the architecture of normal acini and group of cells in the islets of Langerhans. There was no damage to islets and no hyperplasia was observed. The diabetic group exhibited the destruction of pancreatic islets. Histopathology of diabetic rats treated with glibenclamide displayed population and size of beta cells restored back to normal. The diabetic rat treated with EEPL (100 mg/kg) showed improvement in size and population of

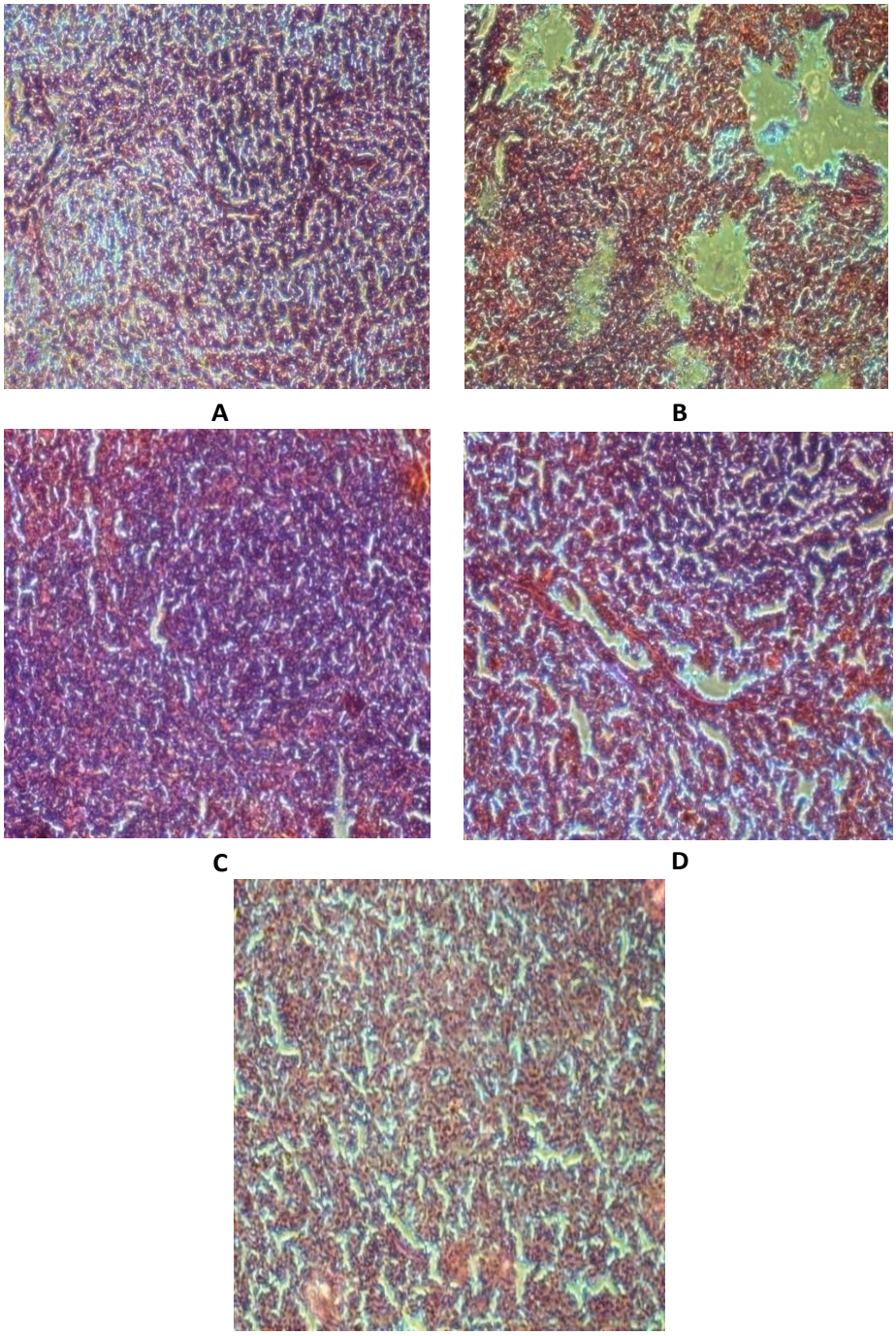


Figure 3: Histopathology of pancreas A: Normal control; B: Diabetic control; C: Diabetic rats treated with Glibenclamide (5 mg/kg); D: Diabetic rat treated with EEPL 100 mg/kg; E: Diabetic rat treated with EEPL 200 mg/kg.

beta cells and lesser damage to islet and enlargement. The EEPL at higher dose restored the architecture of islets to normal level. Population and size of both cells and islets of Langerhans became normal and there was no evidence of damage and enlargement to islet. Refer Fig. 3.

DISCUSSION

This study was planned to assess antidiabetic and also the antihyperlipidemic activities of alcoholic extract of *Premna latifolia* (EEPL) in both normal and diabetic rats. STZ induced diabetes resembles type 2 DM and alters lipid profile as well as carbohydrate and lipid metabolising enzymes level. Hyperglycemic induction by STZ is a result of inhibition of many of the enzymes of DNA synthesis leading to destruction of islets in pancreas (Holemans et al., 1997). STZ also enhances free radical formation and causes defects in antioxidant defense of β-cells of pancreas. Oral administration of EEPL to diabetic rats causes marked reduction in glucose and elevation in insulin levels.

Table 1: Effect of EEPL on serum lipids in diabetic rats

Group	Total Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL-Cholesterol (mg/dL)	LDL (mg/dL)
Normal control	83.47±0.38	86.54±0.4117	29.68±0.3816	32.11±0.4647
Diabetic control	162.7±0.53^{c}	132.6±0.3953^{c}	17.67±0.3918^{c}	149.5±0.8466^{c}
Diabetic + Glibenclamide (5 mg/kg)	87.65±0.67^{z}	98.17±0.6911^{z}	25.80±0.5293^{z}	49.81±0.4293^{z}
Diabetic + EEPL (100 mg/kg)	123.7±1.9^{z}	117.4±1.246^{z}	19.42±0.897^{y}	93.4±2.567^{z}
Diabetic + EEPL (200 mg/kg)	98.54±2.32^{z}	106.17±1.875^{z}	21.85±0.854^{z}	67.2±1.568^{z}

All values are expressed as mean ± S.E.M. (n = 6); ^{c}p< 0.001 when compared to normal control; ^{y}p < 0.01, ^{z}p< 0.001 when compared to diabetic control.

Table 2: Effect of EEPL on insulin, amylase and lipase levels in diabetic rats

Group	Insulin (µU/mL)	Amylase (U/L)	Lipase (U/L)
Normal control	16.32±0.3584	46.4±2.241	8.942±0.567
Diabetic control	6.37±0.1699^{c}	127.6±3.924^{c}	37.85±3.236^{c}
Diabetic + Glibenclamide (5 mg/kg)	13.26±0.3709^{z}	63.2±2.798^{z}	16.24±2.879^{z}
Diabetic + EEPL (100 mg/kg)	8.54±0.521^{z}	93.5±2.367	28.24±1.547^{z}
Diabetic + EEPL (200 mg/kg)	10.87±0.879^{z}	72.5±2.352	23.75±2.938^{z}

All values are expressed as mean ± S.E.M. (n = 6); ^{c}p< 0.001 when compared to normal control; ^{z}p< 0.001 when compared diabetic control

Table 3: Effect of EEPL on glucose-6-phosphatase and fructose-1,6-diphosphatase in diabetic rats

Group	Glucose-6- phosphatase (U^{a}/mg protein)	Fructose-1,6-diphosphatase (U^{b}/mg protein)
Normal control	0.1587±0.01854	0.3754±0.01854
Diabetic control	0.2813±0.01788^{c}	0.6014±0.01764^{c}
Diabetic + Glibenclamide (5 mg/kg)	0.1837±0.0203^{z}	0.3947±0.0029^{z}
Diabetic + EEPL (100 mg/kg).	0.2471±0.01624^{z}	0.5164±0.01758^{z}
Diabetic + EEPL (200 mg/kg)	0.2158±0.09785^{z}	0.4628±0.01536^{z}

All values are expressed as mean ± S.E.M. (n = 6); a = nmoles of phosphorous produced/min/mg protein; b = nmoles of phosphorous produced/min/mg protein; ^{c}p< 0.001 when compared to normal control; ^{z}p< 0.001 when compared to diabetic control.

Table 4: Effect of EEPL on phosphogluco isomerase, aldolase and glucose-6-phosphate dehydrogenase in diabetic rats

Group	Phosphogluco isomerase (U^{d}/mg protein)	Aldolase (U^{e}/mg protein)	Glucose-6-phosphate dehydrogenase (U^{f}/mg protein)
Normal control	49.76±1.652	0.1558±0.0087	3.452±0.3542
Diabetic control	24.52±1.895^{c}	0.2617±0.0063^{c}	1.264±0.2756^{c}
Diabetic + Glibenclamide (5 mg/kg)	30.54±2.137^{z}	0.1857±0.0094^{z}	1.956±0.1856^{z}
Diabetic + EEPL (100 mg/kg)	37.82±1.487^{z}	0.2473±0.0052^{z}	2.457±0.3482^{z}
Diabetic + EEPL (200 mg/kg)	34.95±1.524^{z}	0.2168±0.0067^{z}	2.365±0.2461^{z}

All values are expressed as mean ± S.E.M. (n = 6); d = nmoles of fructose produced/ min/mg protein; e = µmoles of glyceraldehydes; produced/min/mg protein; f = nmoles of NADPH produced/min/mg protein; ^{c}p< 0.001 when compared to normal control; ^{z}p< 0.001 when compared to diabetic control.

The EEPL 200 mg/kg shows a consistent decrease in blood glucose level.

The important pathways which regulate blood glucose and insulin levels are glucose regulated insulin secretion, hepatic glucose synthesis and insulin mediated glucose uptake in the tissues. Gluconeogenesis and glycolysis are two main metabolic pathways regulating the glucose level in liver and blood. Gluconeogenic pathway is mainly regulated by the enzymes namely G6Pase and F-1,6-DPase. Glucose-6-phosphate converted to glucose mediated by the enzyme G6Pase and also lipogenesis process that is conversion of carbohydrate or protein to fat (Krebs et al., 1965). F-1,6-DPase mediate the formation of fructose-6-phosphate from fructose-1,6-diphosphate, which determines whether or not a tissue is capable of resynthesizing glycogen from pyruvate triosephosphates (Cox et al., 2008). G6Pase, F-1,6-DPase enzymes level considerably augmented in diabetic animals. Further, the treatment with EEPL decreased the activity of these enzymes in the liver of diabetic animals.

Glycolytic pathway is mainly regulated by the enzyme PGI which is important for glucose utilization. Glucose-6-phosphate gets converted to fructose 6-phosphate with the mediation of PGI during the second step glycolysis. Aldolase is an enzyme (protein) that produces energy by breaking some sugars, by forming triose phosphates dihydroxyacetone phosphate and glyceraldehydes 3-phosphate from fructose 1,6-bisphosphate. The hepatic PGI and aldolase level decreases and increases respectively in diabetic animals. The EEPL treatment enhanced the PGI activity and reduced aldolase activity. G6PDH acts by maintaining the levels of nicotinamide adenine dinucleotide phosphate (NADPH), a co-enzyme that guards the cells against oxidative damage (Vinay et al., 1999). The G6PDH enzyme level was found to be significantly reduced in diabetic animals. The EEPL protects the cells against oxidative damage by increasing the activity of enzyme G6PDH in diabetic animals.

Amylase hydrolyses the starch, a polysaccharide into simpler forms namely disaccharides and trisaccharides which further get converted into glucose by activity of several other enzymes. Treatment with EEPL decreased the amylase level which was elevated in diabetic animals. This indicates normalization of cells those which were secreting amylase in the pancreas. Lipase is another enzyme involved in hydrolysis of lipids. Reports indicate that lipase level in the blood was measured to investigate acute pancreatitis and other disorders of the pancreas (Casas et al., 2012). Our study shows that, treatment with EEPL normalized the lipase level, which may indicate the normalization of lipase secreting cells in pancreas.

Augmented chances of atherosclerosis and coronary heart diseases are complications associated with diabetes. LDL is bad cholesterol as it gets deposited on walls of blood vessels increasing the risks of heart attack. Increased level of cholesterol causes clots and/or plaques in the blood vessels leading to hypertension, angina, heart attacks, strokes and peripheral vascular diseases. The diabetes induced by STZ significantly varies the lipid profile which is attributed to increased serum lipase enzyme activity. The EEPL had a beneficial effect on the STZ-induced hyperlipidemia. This may be due to its effect on lipid metabolizing enzymes and normalization of liver and pancreas function.

CONCLUSION

The possible mechanism by which EEPL shows antihyperglycemic activity may be due to potentiation of pancreatic β-cells of islets leading to secretion of insulin and increase in insulin mediated uptake of glucose by cells. This possible mechanism was supported by elevated levels of insulin in diabetic animals treated with EEPL. The steroids, terpenoids, tannins and polysaccharides present in EEPL may be responsible for the antidiabetic activity.

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