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Anti-diabetic Activity of Ethanolic Extract of *Elaeocarpus serratus* L. in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

To investigate the anti-diabetic activity of *Elaeocarpus serratus* fruit in streptozotocin-induced (STZ) diabetic rats. The dose-dependent effects of 30days oral treatment with ethanol extracts of fruit (200 and 400 mg/kg) of *E. serratus* on body weight, blood glucose level, total protein, albumin, liver marker enzymes and carbohydrate metabolizing enzymes were evaluated in STZ-induced diabetic rats. Oral administration ethanolic extract of fruit of *E. serratus* showed significant restoration of the body weight and decrease in the blood glucose level, liver marker enzymes (ALT, AST ALP) and carbohydrate metabolizing enzymes were observed in diabetic rats. These results suggest that fruit extract of *E. serratus* has valuable anti-diabetic activity in STZ-induced diabetic rats which is comparable to the standard drug glibenclamide and hence might be of use in the management of diabetes.

Keywords: *Elaeocarpus serratus*, Anti-diabetic activity, Ethanolic extract, Streptozotocin, Glibenclamide.

INTRODUCTION

Diabetes mellitus is an endocrine disorder that is characterized by hyperglycemia. [1] This disease is major degenerative ailment in the world today and has affected at least 15 million people. [2] The management of diabetes mellitus is considered a global problem and successful treatment is yet to be discovered. [3] Throughout the world a great number of plants have been suggested as a rich as yet unexplored scientific source of potentially useful anti-diabetic drugs. However, the ultimate objective of their use is that they should interact directly with our body chemistry without side effects. [4-5] Synthetic hypoglycemic agents those are capable of reducing blood sugar level possessed most worrying side effects. Therefore, finding other anti-diabetes agents, especially those made from natural sources is desired. [6]

Elaeocarpus is a genus of tropical and subtropical evergreen trees belonging to family *Elaeocarpaceae*. Studies indicate that various species contain chemical constituent such as triterpenes, tannins, indolizidine alkaloids, flavonoids, and ellagic acid derivatives. [7-8] Several species have been known to possess anti-inflammatory [9], antimicrobial, analgesic [10], antioxidant activity [11] and antihypertensive [12] activities. *Elaeocarpus serratus* leaves are used in the treatment of rheumatism [13], diuretic, cardiovascular stimulant and as antidote to poison, antimicrobial activity [14-15] while the fruits are locally prescribed for the treatment of diarrhoea and dysentery. The fruit juice is given for stimulating secretions from taste buds thus increasing appetite in patients. [16] Leaves contain Myricitrin, Myricetin, Mearnsitrin and Ellagic acid. Fruits contain tannin and large amount of plant acids.

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[17-18] To our knowledge, diverse bioactive compounds attributed to this plant, no biochemical studies have been carried out to shed light on the role of this plant in diabetes. The main objective of this study was to assess the anti-diabetic effect of ethanolic extract of fruit of *E. serratus* in streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Plant materials

The fruit of *Elaeocarpus serratus* L. was collected from Upper Palani Hills of Western Ghats (Kodaikanal Forest Division), Tamil Nadu, India and were authenticated at Botanical Survey of India (BSI), Southern Circle, Coimbatore, India and the herbarium of Voucher specimen number BSI/SRC/5/23/2011-12/Tech. 454 has been deposited at the PG and Research Department of Botany, Vellalar College for Women, Erode (Tamil Nadu), India. Coarse powder from the shade dried fruit (500 g) were exhaustively extracted using Soxhlet apparatus with absolute ethanol (78.5°C). The extract were dried (free of solvent) in a vacuum evaporator and stored in refrigerator at 4°C for further use.

Chemicals

Streptozotocin (glucose oxidase peroxidase reactive strips) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and blood glucose levels were determined using a portable glucometer (Accu- sure, Roche Diagnostics, USA). The levels of protein, albumin and activities of AST, ALT and ALP were analyzed using commercially available test diagnostic kits and their manual (Beacon Diagnostic P. Ltd., India) obtained from E. Merck and HIMEDIA, Pvt. Ltd, India.

Animal Model

Adult male Wistar rats 150-200 g were procured from the Small Animals Breeding Station, Mannuthy, Kerala, India. Animal experiments were done in compliance with the Institutional Ethical Committee-CPCSEA (Reg.No.722/02/a/CPCSEA). The animals were housed in polypropylene cages (38 × 23 × 10 cm) with not more than six animals per cage and maintained under standard environmental conditions (14 h dark/ 10 h light cycles; temp.25+ 2^{o}C; 35-60% humidity, air ventilation) and were fed with standard pellet diet (M/s. Hindustan Lever Ltd., Mumbai, India) and fresh water *ad libitum*. The rats were acclimatized to the environment for two weeks prior to experimental use. Animals were fasted overnight before the experimental schedule, but had free access for water *ad libitum.*

Acute toxicity study

Acute oral toxicity study was carried out according to Organization for Economic Co-operation and Development (OECD) guidelines 423. The animals were divided into 5 groups (n= 6) and were orally fed with first group served as control and was treated with normal water. Groups 2, 3, 4, 5 and 6 were treated with the single graded dose ethanol extract of *E. serratus* fruit in increasing dose levels of (1000, 2000, 3000, 4000 and 5000 mg/kg b. wt.) respectively. Monitoring of the parameters commenced immediately after the administration of the drug. The animals were observed continuously for 2 hours under behavioral, neurological, autonomic profiles. After a period of 24 and 72 hours, they were observed for any lethality or death.

Experimental induction of diabetes

Insulin dependent Diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection of streptozotozin (STZ) (50 mg/kg b. wt.) dissolved in citrate buffer (0.01M, pH 4.5). The rats were given 5% glucose orally on the first day to stave off hypoglycaemic shock after STZ administration. Three days after STZ-induction, fasting blood glucose levels were determined using glucose oxidase peroxidase reactive strips and a portable glucometer (Accu-sure, Roche Diagnostics, USA). Animals with blood glucose levels above 250mg/dl were considered diabetic and selected for the study.

Experimental design

A total of 30 rats (24 diabetic surviving rats and 6 normal rats) were used. The animals were divided into five groups containing six animals each as follows: group I: normal control rats; group II: STZ-induced diabetic rats; group III: diabetic animals treated with standard drug glibenclamide (600µg/kg b. wt.); group IV: diabetic animals treated with ethanolic extract of fruit sample (200 mg/kg b. wt.); group V: diabetic animals treated with ethanolic extract of fruit sample (400 mg/kg b. wt.). [19]

The standard drug and ethanolic extract of fruit sample were administrated orally to group III, groups IV and group V animals respectively with oral gavage once in a day for 30 days. Third day after the induction of STZ was considered as the first day for extract administration. Soon after the last dosage, all the animals were fasted but had free access of water for 24 hours. The fasting blood glucose levels were detected on the initial day, third day of STZ-induction and on 10th, 20th and 30th day of extract administration using glucose oxidase peroxidase reactive strips and a portable glucometer. At the end of the experiment, the fasting blood glucose levels of the animals were measured before they were sacrificed.

Biochemical estimations

Twenty four hours after the treatment with plant sample, blood samples were collected from all groups by puncturing retro-orbital plexus. The blood samples were allowed to clot for 45 minutes and the serum was separated by centrifugation at 2500 rpm for 15 minutes and analyzed for the levels of protein, albumin and activities of AST, ALT and ALP. They were analyzed using commercially available test diagnostic kits and their manual (Beacon Diagnostic P. Ltd., India). Later, the animals were euthanized and liver was excised, perfused with ice cold normal saline, dried with blotting paper, weighed, cut into pieces and 1 g of it was homogenized in 0.25M Tris Hcl buffer of pH 7.5 to

give 10% homogenate. The homogenate was centrifuged at 10,000 rpm for 20 minutes at 4°C to obtain a clear supernatant which was used for the assay of hexokinase, phosphoglucoisomerase, glucose-6-phosphatase and fructose-1,6-diphosphatase were estimated the following the protocols of Branstrup *et al.*, 1957; Horrocks *et al.*, 1963; King 1965; and Giancarlo *et al.*, 2006. [20-23]

Statistical analysis

Result were presented as mean (n=6) ± Standard Deviation (SD). Statistical significance of difference between groups was determined by one way analysis of variance (ANOVA). *P* values of <0.05 are considered significantly different.

RESULTS

The acute toxicity studies revealed the nontoxic nature of the *E. serratus*. There was no lethality or any toxic reactions found at any of the high dose (5000 mg/kg b. wt. *p.o.*) until the end of the study period.

Table 1 and 2 showed changes in the body weight and blood glucose levels of normal and experimental rats. There was a significant decrease in the body weight in diabetic rats as compared with normal rats. Thus, the administration of glibenclamide and *E. serratus* at high dose (400 mg/kg b. wt.) diabetic rats restored the changes in the body weight near normal. The blood glucose level in the normal control group did not show any significant variation in the blood glucose throughout the experimental period. Administration of streptozotocin led to a significant elevated glucose level almost four times on 30th day than control group. In diabetic rats, when treatment with glibenclamide and high dose of the plant extract showed maximum reduction in the blood glucose level.

Table 1: Effect of ethanolic extract of fruit of *E. serratus* on body weight of normal and diabetic rats

Groups	Initial	Body weight (g)		
		10th day	20th day	30th day
Control	153.01 ± 1.01	155.04 ± 0.98	159.40 ± 6.40	162.07 ± 1.18
Induced (50 mg/kg)	152.15 ± 0.79*	151.35 ± 1.35*	148.43 ± 1.15*	145. 34 ± 1.06*
Standard (600µg/kg)	158.20 ± 1.95*	159.28 ± 1.63*	161.27 ± 2.30*	163.49 ± 2.10*
ESF LD (200 mg/kg)	156.05 ± 1.63*	157.39 ± 0.98*	160. 04 ± 1.85*	163.78 ± 1.02*
ESF HD (400 mg/kg)	162.25 ± 2.38*	164.78 ± 1.17*	167.12 ± 1.05*	170.09 ± 0.01*

Values are expressed as mean ± SD for six animals. Statistical significance: * - Significant at 5% level (p<0.05)

Table 2: Effect of ethanolic extract of fruit of *E. serratus* on plasma blood glucose levels in normal and diabetic rats

Groups	Initial	STZ induction	10th day	20th day	30th day
Control (G-I)	107.67± 3.61	108.33 ± 3.83	105.50 ± 4.14	105.50 ± 4.93	105.83 ± 2.86
Induced (G-II) (50 mg/kg)	108.16 ± 2.32ns	477.0 ± 10.16*	464.33 ± 7.50*	468.33 ± 4.97*	470.83 ± 6.31*
Standard (G-III) (600µg/kg)	110.83 ± 2.64*	484.17 ± 7.22ns	380.50 ± 6.92*	255.83 ± 6.65*	142.83 ± 5.67*
ESF LD (G-IV) (200 mg/kg)	105.17 ± 2.48ns	483.17 ± 7.73 ns	457.83 ± 5.53*	359.00 ± 7.38*	253.17 ± 3.71*
ESF HD (G-V) (400 mg/kg)	106.67 ± 1.97ns	482.17 ± 5.27 ns	417.50 ± 3.08*	319.17 ± 4.36*	202.50 ± 5.79*

Values are expressed as mean ± SD for six animals. Statistical significance: * - Significant at 5% level (p<0.05)

Table 3: Effect of ethanolic extract of fruit of *E. serratus* on serum liver marker enzymes in normal and diabetic rats

Groups	Protein (g/dl)	Albumin (g/dl)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control (G-I)	9.45 ± 0.41	3.44 ± 0.16	53.35 ± 0.88	45.78 ± 0.70	57.40 ± 0.95
Induced (G-II) (50 mg/kg)	4.51 ± 0.12*	1.44 ± 0.06*	158.60 ± 1.79*	103.79 ± 1.97*	116.92 ± 1.78*
Standard (G-III) (600µg/kg)	9.53 ± 0.15*	3.65 ± 0.17*	55.10 ± 0.95*	54.32 ± 0.88*	63.13 ± 0.89*
ESF LD (G-IV) (200 mg/kg)	6.63 ± 0.23*	2.19 ± 0.06*	122.32 ± 0.57*	96.90 ± 1.83*	90.85 ± 1.25*
ESF HD (G-V) (400 mg/kg)	8.07 ± 0.10*	3.62 ± 0.03*	68.29 ± 1.75*	75.66 ± 1.04*	78.05 ± 1.36*

Values are expressed as mean ± SD for six animals. Statistical significance: * - Significant at 5% level (p<0.05)

Table 4: Effect of ethanolic extract of fruit of *E. serratus* on carbohydrate metabolizing enzymes in the liver of normal and diabetic rats

Groups	HK µmoles/min/mg protein	PGI µmoles/min/mg protein	G-6-P µmoles/min/mg protein	F-1,6- DP µmoles/min/mg protein
Control (G-I)	0.32 ± 0.03	1.82 ± 0.14	1.34 ± 0.09	1.46 ± 0.09
Induced (G-II) (50 mg/ kg)	0.11 ± 0.01*	0.51 ± 0.01*	3.42 ± 0.22*	3.28 ± 0.11*
Standard (G-III) (600 µg/kg)	0.33 ± 0.03*	1.53 ± 0.09*	1.34 ± 0.06*	1.54 ± 0.18*
ESF LD (G-IV) (200 mg/kg)	0.21 ± 0.01*	0.82 ±0.02*	2.81 ± 0.13*	2.30 ± 0.04*
ESF HD (G-V) (400 mg/kg)	0.30 ± 0.01*	1.33 ± 0.06*	2.03 ± 0.06*	1.81 ± 0.01*

Values are expressed as mean ± SD for six animals. Statistical significance: * - Significant at 5% level (p<0.05)

The levels of total protein, total albumin and liver function enzymes aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) of normal and experimental rats were given in Table 3. It could be observed that diabetes caused a significant decrease in total protein and total albumin levels (mg/dI) in serum of diabetic rats, and these values were reversed by treatment with high dose (400 mg/kg *p.o.*) of plant extract and glibenclamide. Diabetic rats showed a significant elevation in the activities of serum liver marker enzymes AST, ALT and ALP as compared to normal rats. However, treatment with high dose of plant extract for 30th days brought back the activities of these markers to near normal levels comparable to the control and standard drug treated group.

Table 4 shows the activities of hexokinase, phosphoglucoisomerse, glucose-6-phosphatase and

fructose-1, 6-diphosphatase in the blood serum of control and experimental rats.

There was a significant decrease in the activities of hexokinase and glucose-6- phosphatase and a significant increase in the activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase in STZ treated diabetic rats. This condition was reverted to near-normal by the administration of high dose of fruit extract which was comparable with the standard drug glibenclamide.

DISCUSSION

Diabetes is one of the most dreaded ailments afflicting the mankind today. It is a slow killer which when come's stay permanently, weakens the immune system and predisposes human beings for greater health hazards and clinical complications. In India, many plants have been used traditionally for this purpose but, there is hardly any attempt to consolidate the widely scattered large mass of data for information on the inexpensive phytotherapeutic agents for alleviating the suffering of mankind due to this dreaded ailment. Streptozotocin is a commonly used chemical to generate diabetic animals in the laboratory for its ability to destroy insulin producing β-cells. [24-25] Glibenclamide is a sulfonylurea that decreases blood glucose levels in diabetic subjects by increasing insulin secretion from pancreatic beta cells decreasing blood glucagon concentrations and improving insulin action on target tissues. [26] In the present study, untreated diabetic rats showed severe body weight loss could be due to degradation and catabolism of fats and proteins. [27-28] However, the administration of ethanolic extract of fruit increased the body weight of the STZ treated animals in a dose-dependent pattern which suggested the protective effect of the extract by preventing it from muscle wastage and other macromolecular degradations. These findings are in agreement with those suggested by Rattima *et al*., 2007; Sridhar *et al., 2011.* [29-30]

The current research showed that the high doses of the plant extract (400 mg/kg b.wt.) significantly decreased the blood glucose level when compared to diabetic-control rats. This is due to the pancreatic secretion insulin from beta cells of the Islets of Langerhans. This is in agreement with Kaleem *et al*., 2008. [31] In diabetic condition, occurrence of reduction in protein and albumin may be due to proteinuria, albuminuria or increased protein catabolism, which is a clinical marker in diabetic nephropathy. [32] Administration of ethanolic extract of fruits caused significant increase in total protein and total albumin levels compared with control diabetic rats. This attributed effect of insulin-like factors in the plant extract, since insulin is reported to increase protein synthesis. Similar results found by Reda *et al*., 2010; Mohan *et al*., 2014. [33-34]

The liver is regarded as the central metabolic organ in the body, with an important role in glucose and lipid homeostasis. [35] AST, ALT and ALP are considered as liver toxicity markers. [36] The increase in the activities of plasma AST, ALT and ALP indicated that diabetes may be induced hepatic dysfunction. Therefore the increment of the activities of AST, ALT and ALP in plasma may be mainly due to the leakage of these enzymes from the liver cytosol to blood stream, which indicate the hepato toxic effect of STZ. [37] In the current investigation, diabetic rats showed a significant elevation in the activities of serum AST, ALT and ALP as compared to normal rats. However, treatment with the high dose of plant extract significantly reduced the enzyme activities to near normal levels. The curative effect of ethanol extracts of *E. serratus* fruit could easily be noticed through the normalization of all enzymes tested returned more or less to the level of normal control and standard drug treated group. The obtained result is similar to Pari and Rajarajeswari, 2009. [38]

In experimental diabetes, enzymes of glucose metabolism are markedly altered. Persistent hyperglycemia is a major contribution to such metabolic alterations that lead to pathogenesis of diabetic complications, especially, neuropathy and micro vascular diseases. One of the key enzymes in the catabolism of glucose is hexokinase, which phosphorylates glucose and converts it into glucose-6-phosphate. [39] The current study shows, the decreased activity of carbohydrate metabolizing enzymes like hexokinase and phosphoglucoisomerse and increased activity of glucose 6 phosphate and fructose 1, 6 diphosphatase was observed in the diabetic rats as compared with normal control rats. These values were reversed by treatment with ethanolic extract of fruit (high dose) and the standard glibenclamide restored the level of these carbohydrate metabolizing enzymes to normalcy. Hexokinase, which brings about the first phosphorylation step of glucose metabolism, is reduced significantly in the diabetic group of rats. [40] In the present study the observed decrease in the activity of hexokinase in the diabetic rat liver might be due to the diminished consumption of glucose in the system and increased blood sugar level. This is in par with the findings of Singh and Kakkar, 2009. [41]

The liver is an important organ that plays a vital role in glycolysis and gluconeogenesis pathways and also maintenance of blood glucose level by regulating its metabolism. Glucose-6-phosphatase and fructose-1, 6-bisphosphatase are the key enzymes in homeostatic regulation of blood glucose level in liver and kidney through a mechanism involving gene expression or biochemical inhibition of its enzymatic activity. [42-43] The level of these hepatic gluconeogenic enzymes were increased significantly in diabetic rats. More over increased level of these enzymes in diabetic rats provides hydrogen, which binds with $NADP^+$ in the form of NADPH and enhances the synthesis of fats from carbohydrates i.e. lipogenesis [44] and finally contributes to increased levels of glucose in blood.

Activation of gluconeogenic enzymes is due to the state of insulin deficiency, because under normal conditions, insulin functions as a suppression of gluconeogenic enzymes. The present study was in agreement with Balamurugan *et al*., 2012. [45]

In conclusion, our results clearly showed that the ethanolic extract of fruit of *E. serratus* possesses potent anti-diabetic activity in STZ-induced diabetic rats and further study is needed to identify the compounds responsible for its promising *in vivo* anti-diabetic activity.

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