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Antidiabetic Effect of *Flacourtia indica* Merr in Streptozotocin Induced Diabetic Rats

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Abstract: The present study aimed to evaluate for the antidiabetic effects of ethanolic extracts of leaves of *Flacourtia indica* Merr. (Flacourtiaceae) in streptozotocin (STZ) induced diabetic rats. The diabetes was induced by single dose of STZ (50 mg/kg b.wt.) in citrate buffer, while the normal control group was given the vehicle (citrate buffer) only. After 3 days of induction of diabetes, the diabetic animals were treated further four weeks with ethanolic extract of *Flacourtia indica* Merr. (150 mg/kg and 300 mg/ kg b.wt.) and Glibenclamide (5 mg/kg). STZ-induced diabetic rats showed marked hyperglycemia, hypertriglyceridemia and hypercholesterolemia. Body weight and liver glycogen levels were reduced and glycosylated haemoglobin levels were significantly increased in diabetic rats. The treatment with ethanolic extract of *Flacourtia indica* leaves at the dose of 150 mg/kg and 300 mg/ kg significantly improve the alterations in fasting blood glucose, serum triglyceride, serum cholesterol, liver glycogen, glycosylated haemoglobin and body weight in STZ-induced diabetic rats. Thus present study suggested that ethanolic extract of *Flacourtia indica* have vast therapeutic application against diabetes due to its antidiabetic properties.

Key words: Antidiabetic Activity · *Flacourtia Indica* · Streptozotocin · Diabetes Mellitus

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to secreted insulin [1]. DM is currently one of the most costly and burdensome chronic diseases and is a condition that is increasing in epidemic proportions throughout the world [2]. Diabetes affects about 5% of the global population [3] and the management of diabetes without any side effects is still a challenge to the medical system [4, 5]. Renewed attention in recent decades to alternative medicines and natural therapies has stimulated a new wave of research interest in traditional practices. The plant kingdom has become a target for the search for new drugs and biologically active "lead" compounds [6]. Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes [7, 8], but only a few have received scientific scrutiny. One of such plants is *Flacourtia indica* Merr. has a widespread occurrence in India and believed to have good antidiabetic activity.

Flacourtia indica Merr. (Family: Flacourtiaceae), commonly known as 'Baichi' or 'Katai', is an indigenous medicinal plant widely distributed in Bangladesh and India [9]. This plant has been reported as an effective remedy for the treatment of a variety of diseases. Fruits are used as appetizing and digestive, diuretic, in jaundice and enlarged spleen. Barks are used for the treatment of intermittent fever. Roots are used in nephritic colic and gum is used in cholera [9, 10]. Previous phytochemical investigation on this plant resulted in the isolation of β-sitosterol (a well-known phytosterol), β-sitosterol-β-D-glucopyranoside, ramontoside, butyrolactone lignan

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disaccharide [11] and flacourtin [12]. Recent report shows the presence of coumarin such as scoparone and aesculetin [13].

The objective of this investigation was to ascertain the scientific basis for the use of this plant in the management of diabetes, using streptozotocin (STZ)-induced diabetic rats.

MATERIALS AND METHODS

Collection of Plant Materials: *Flacourtia indica* leaves were collected from the roadside location of the Sagar District, Sagar, Madhya Pradesh, India. The leaves of the plant was collected in third week of December 2009 and preserved in herbarium of institution. The leaves were air dried under shade, powdered mechanically and stored in airtight containers.

Preparation of Extracts: *Flacourtia indica* leaves powder about 500 g was extracted with ethanol by hot extraction process (soxhlet) for 72 hrs. After completion of the extraction the solvent was recovered by distillation and concentrated under vacuum and resulting semisolid mass was vacuum dried using vacuum evaporator to yield a solid residue (ethanolic extract).

Chemicals: Streptozotocin and Glibenclamide were purchased from Sigma Chemical Co. (Saint Louis, MO, USA). All other chemicals used in the study were of analytical grade.

Animals: Wistar albino rats (150-200 g) of both sex were maintained under standard environmental laboratory conditions and fed with laboratory diet and water *ad libitum*. All the protocols were performed in accordance with the Institutional Animal Ethical committee (IAEC) as per the directions of the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Experimental Induction of Diabetes: Diabetes was induced by using streptozotocin as diabetogenic agent. Streptozotocin (50 mg/kg b. wt.) was dissolved in ice cold citrate buffer (pH 4.3) immediately before use. The solution was injected intraperitoneally in the dose of 50 mg/kg b. wt. in rats. 5 % glucose solution was administered orally for 24 hrs to prevent mortality due to initial hypoglycemia induced by streptozotocin. After 72 hrs of STZ injection, fasting blood glucose levels were tested using glucose oxidase- peroxidase reactive strips (Accu-chek, Roche Diagnostics, USA). Rats showing fasting blood glucose more than 200 mg/kg were considered diabetic and used for further study.

Experimental Design: The Streptozocin-induced diabetic Wistar rats were randomly assigned into Seven groups, each group contain five rats (n = 5).

Group I: As normal control where rats received citrate buffer daily.

Group II: as diabetic control where diabetic rats received citrate buffer daily.

Group III: Diabetic rats received 150 mg/kg EEFI.

Group IV: Diabetic rats received 300 mg/kg EEFI.

Group V: Diabetic rats received 5 mg/kg of glibenclamide, an oral hypoglycemic agent.

Group VI: Normal rats received 150 mg/kg EEFI.

Group VII: Normal rats received 300 mg/kg EEFI.

Experimental Procedure: Blood glucose estimation Fasting blood glucose levels were determined in all experimental rats initially to determine the diabetic status and thereafter every week during the 28 days study period. Blood was obtained by snipping tail of rat with the help of sharp razor and blood glucose levels were determined using glucometer (Ultra Touch Two, Johnson and Johnson). Each time the tail of the rat was sterilized with spirit. Serum lipid profile estimation At the end of 28 days, blood was collected from inferior vena cava, serum separated for determination of parameters like total cholesterol, HDL- cholesterol and triglycerides using commercially available kits (Span diagnostics). VLDL cholesterol and LDL-cholesterol were calculated using the Friedewald's formula [16].

VLDL = Triglycerides / 5
LDL = Total cholesterol - (HDL-CH + VLDL-CH)

Liver Glycogen Estimation: Liver of individual animal was homogenized in 5% w/v trichloroacetic acid and its glycogen content was determined by the method of Carrol [17].

Glycosylated Haemoglobin Determination: At the end of 28 days, blood was collected from retro-orbital plexus and subjected for the determination of glycosylated haemoglobin.

Statistical Analysis: All results are expressed as the mean ± SEM. The results were analysed for statistical significance by one way ANOVA followed by Dunnet's Multiple Test for comparison.

RESULTS

Phytochemical Analysis: Many secondary metabolites participate in a variety of anti-diabetic functions *in vivo* [18]. Freshly prepared extracts were subjected to preliminary phytochemical screening test for various constituents. This revealed the presence of alkaloids, tannins, saponins, flavonoids, glycosides, phenolic compounds, terpenoids and steroids. Results indicate that ethanolic extract of *Flacourtia indica* (EEFI) possess significant antidiabetic activity.

Anti-Diabetic Activity: The effect of STZ and plant extracts on blood glucose level is shown in Table 1. On repeated administration of EEFI for 28 days, a sustained and significant ($p<0.01$) decrease in blood glucose level of diabetic rats was observed in dose dependent manner as compared to diabetic control group. In diabetic rats blood glucose level was reduced by 30.3% and 45.79% at 150 and 300 mg/kg doses of the extract respectively. The standard oral hypoglycemic drug glibenclamide (5 mg/kg) showed more potent antidiabetic activity by reducing blood glucose level by 59.25% as compared to diabetic control group. However there was no significant effect of the extract on the blood glucose level of normoglycemic rats. As shown in Table 2, STZ diabetic rats treated with extract showed significant ($p<0.01$) reduction in the elevated levels of total cholesterol and triglycerides in diabetic rats. Chronic treatment of extract (300 mg/kg) and glibenclamide (5 mg/kg) reduced the LDL-cholesterol by 53.79% and 64.13% respectively as compared to diabetic control group. Also the extract significantly ($p<0.01$) improved the HDL-cholesterol

Table 1: Effect of chronic administration (28 days) of ethanolic extract of *Flacourtia indica* leaves on fasting blood glucose in STZ diabetic rats

	Fasting Blood Glucose (mg %)				
Experimental Groups	Day 0	Day 7	Day 14	Day 21	Day 28
Normal control	78.16±4.96	71.16±3.06	74.16±3.11	70.33±3.07	73.5±3.470
Diabetic control	297.5±5.680	308.66±8.08	300.66±7.70	292.66±5.9	297.83±5.25
Diabetic + 150 mg/kg EEFI	304.5±8.420	267.5±10.11	238.5±6.730	222.16±7.12	207.16±7.24
Diabetic + 300 mg/kg EEFI	292.5±7.360	248.83±5.91	223.5±6.210	186.33±8.86	161.16±8.60
Diabetic + GL (5 mg/kg)	295.16±6.63	219.16±6.11	195.16±6.96	149±7.88	121.5±7.510
Normal + 150 mg/kg EEFI	75.16±3.82	72.5±2.750	76.66±4.20	78.16±4.96	74.16±3.11
Normal + 300 mg/kg EEFI	74.16±3.47	76.16±3.47	75.33±3.66	72.33±3.42	75.66±4.52

Values are mean ± S.E.M., EEFI: Ethanolic Extract of Flacourtia indica; GL: Glibenclamide, Data analysed by one way Analysis of Variance (ANOVA) followed by Dunnet's Multiple Test for comparison

Table 2: Effect of chronic administration (28 days) of ethanolic extract of *Flacourtia indica* leaves on lipid profile in STZ diabetic rats.

Experimental Groups	T-CH	TG	HDL	VLDL	LDL	Atherogenic index
Normal control	80.2±2.77	66.5±1.91	28.8±1.01	13.3±0.38	38±2.6	1.79±0.12
Diabetic control	198.0±2.5	169.2±2.33	18.8±0.7	33.8±0.47	145.3±2.79	9.96±0.45
Diabetic + 150 mg/kg EEFI	143.3±3.42	130.8±2.14	22.2±1.58	26.2±0.43	95±2.71	5.63±.039
Diabetic + 300 mg/kg EEFI	110.3±2.36	90.6±1.2	24.3±0.91	18.1±0.29	67.8±2.84	3.56±0.22
Diabetic + GL (5 mg/kg)	93.3±1.23	74.2±1.14	26±0.85	14.8±0.27	52.4±2.22	2.62±0.18
Normal + 150 mg/kg EEFI	78.2±1.64	70.6±1.45	30.3±1.28	14.1±0.3	33.7±1.56	1.59±0.09
Normal + 300 mg/kg EEFI	82.3±2.06	65.5±2.17	28.2±2.12	13.1±0.43	41±1.11	1.97±0.13

Values are mean ± S.E.M., EEFI: Ethanolic extract of Flacourtia indica leaves, GL: Glibenclamide, T-CH: Total cholesterol, TG:Triglycerides, HDL-CH:High density lipoprotein cholesterol, LDL-CH:Low density lipoprotein cholesterol, VLDL- CH:Very low density lipoprotein cholesterol. Data analysed by one way Analysis of Variance (ANOVA) followed by Dunnet's Multiple Test for comparison

Table 3: Effect of chronic administration (28 days) of ethanolic extract of *Flacourtia indica* leaves on liver glycogen and glycosylated haemoglobin in STZ diabetic rats

Experimental Groups	Liver Glycogen (g/100gm)	Glycosylated Haemoglobin (%)
Normal control	3.50±0.1	5.38±0.21
Diabetic control	0.83±0.01	8.53±0.22
Diabetic + 150 mg/kg EEFI	1.90±0.09	7.86±0.11
Diabetic + 300 mg/kg EEFI	2.46±0.11	6.91±0.21
Diabetic + GL (5 mg/kg)	3.10±0.16	6.13±0.25
Normal + 150 mg/kg EEFI	3.60±0.14	5.73±0.25
Normal + 300 mg/kg EEFI	3.42±0.12	5.28±0.21

Values are mean ± S.E.M., EEFI: Ethanolic extract of Flacourtia indica leaves, GL: Glibenclamide, Data analysed by one way Analysis of Variance (ANOVA) followed by Dunnet's Multiple Test for comparison

Table 4: Effect of chronic administration (28 days) of ethanolic extract of *Flacourtia indica* leaves on body weight in STZ diabetic rats

	Body Weight (g)	
Experimental Groups	Initial	Final
Normal control	197.7±5.12	243.7±4.720
Diabetic control	203.2±5.24	158.7±4.160
Diabetic + 150 mg/kg EEFI	202.7±3.74	170.8±6.580
Diabetic + 300 mg/kg EEFI	205.0±5.16	185.16±2.77
Diabetic + GL (5 mg/kg)	207.7±6.37	210.3±6.880
Normal + 150 mg/kg EEFI	205.5±4.28	250.7±5.810
Normal + 300 mg/kg EEFI	210.3±6.88	240.7±6.580

Values are mean ± S.E.M., EEFI: Ethanolic extract of Flacourtia indica leaves, GL: Glibenclamide, Data analysed by one way Analysis of Variance (ANOVA) followed by Dunnet's Multiple Test for comparison

level at 300 mg/kg. In addition the EEFI in dose of 300 mg/kg showed significant ($p<0.01$) reduction in atherogenic index as comparable to glibenclamide (5 mg/kg b. wt.).

As shown in Table 3, there was a significant elevation in the blood glycosylated haemoglobin and a decrease in liver glycogen levels in STZ diabetic rats as compared to normal rats. Oral administration of the extract (300 mg/kg) significantly ($p<0.01$) restored the increased glycosylated haemoglobin and decreased liver glycogen level in streptozotocin-diabetic rats as comparable to glibenclamide. As shown in Table 4, STZ diabetic rats showed significant ($p<0.01$) reduction in body weight from 203.2 g to 158.7 g as compared to normal group. Oral administration of EEFI (300 mg/kg) significantly ($p<0.01$) and periodically improved the body weight after 28 days as compared to diabetic control.

DISCUSSION

The study indicates that the ethanolic extract of *Flacourtia indica* leaves possess anti-diabetic properties which suggest the presence of biologically active components. The extract might be promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis. Result from the phytochemical analysis of *Flacourtia indica* revealed the presence of flavonoids, which has also been isolated from the other plant and found to stimulate secretion or possess an insulin-like effect [19]. In type-II diabetes, more often the cause is the lack of insulin sensitivity or resistance to insulin action at the receptor or post-receptor level, rather than lack of insulin. New drugs are required for treatment of type-II diabetes, which increase insulin sensitivity or decrease insulin resistance. Direct effect (20% increase in 30 min) in the absence of insulin indicates that the extract has either insulin-like effect on psoas muscle (skeletal muscle) or direct stimulatory effect on the enzymes involved in the metabolism of glucose. Increase of glucose uptake in the presence of insulin suggests the possibility of increased binding of insulin to receptor in the muscle or increase in the number of insulin receptors. The enhanced uptake of glucose would lead to increased utilization of glucose from the blood. Hyperglycemia results in free radical formation through various biochemical reactions. Free radicals may also be formed via the auto-oxidation of

unsaturated lipids in plasma and membrane lipids. The free radical produced may react with polyunsaturated fatty acids in cell membranes leading to lipid peroxidation. Lipid peroxidation will in turn results in elevated production of free radicals [20]. Diabetes is now considered to be a vascular disease. The cost of treating the microvascular component (retinopathy, nephropathy and neuropathy) and controlling the macrovascular component is a serious drain on health resources, particularly in developing countries. It is expected that by the year 2025 India will have 57.2 million diabetics (one sixth of the world total) [21]. Besides the prevention strategies proposed [21], the use of cost-effective therapies goes a long way towards the aforementioned goal. The authors contend that patient preferences for therapies are guided by cultural heritage and by the natural environment of the region they live in.

It could be concluded that, *Flacourtia indica* plant is safe and rich in many constituents that are pharmacologically active and can be used in various therapeutic purposes as treatment of diabetes mellitus.

CONCLUSION

The present study showed that ethanolic extracts of leaves of *Flacourtia indica* Merr significantly reduced elevated blood glucose level in STZ diabetic rats without showing any hypoglycemic effect in normal rats. Since STZ effectively destroys pancreatic beta cells and causes persistent hyperglycemia, the mechanism of action of *Flacourtia indica* Merr might involve actions other than pancreatic β cells insulin release or secretion. The antidiabetic effect of the extract could be due to increased utilization of glucose by peripheral tissues, improved sensitivity of target tissues for insulin or it may be due to improved metabolic regulation of glucose. Our findings reported that leaves of *Flacourtia indica* significantly reduced serum triglyceride levels in STZ diabetic rats support its long term use not only for better control of blood glucose but also for normalization of disturbances in lipid metabolism which may prevent further predisposition of the patients to cardiovascular complications. Thus the present study showed that leaves of *Flacourtia indica* possesses antidiabetic and antihyperlipidemic effects in STZ diabetic rats. The antiatherogenic potential of the EEFI indicates its usefulness not only in diabetes mellitus but also in long term complications associated with diabetes mellitus. However comprehensive research is required to identify the active constituents responsible for this effect.

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