

EVALUATION OF HYPOGLYCEMIC AND HYPOLIPIDEMIC STUDIES IN ETHANOL LEAF EXTRACT OF *FICUS PUMILA* LINN. ON STREPTOZOTOCIN INDUCED DIABETIC RATS

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Received: 26 Jun 2013, Revised and Accepted: 06 Aug 2013

ABSTRACT

Objective: To find out the hypoglycaemic and hypolipidemic activities of ethanol leaf extract of *Ficus pumila* is the main objective of the present study.

Materials: The acclimatized animals were injected with streptozotocin to induce diabetic condition. Animals were treated with ethanol extract of *Ficus pumila* (EEFP) and standard drug glibenclamide for 21 days. The biochemical parameters such as High density lipoprotein (HDL), Low density lipoprotein (LDL), very low density lipoprotein (VLDL), Total Cholesterol, Triglyceride along with blood glucose level are evaluated.

Results: Decreased blood glucose level of the test animals shows that the extract exhibit significant antidiabetic activity and the levels of LDL, VLDL, TG and TC were reduced after the administration. The HDL level was significantly increased in EEFP administered rats when compared to the diabetic control group.

Conclusion: This finding tends to reveal that the hypoglycemic and hypolipidemic effects of *F. pumila* are similar to the effect of standard drug Glibenclamide. This plant can get in consideration for the searching new drug to treat hyperglycemia from plant source.

Keywords: *Ficus pumila*, Hypolipidemic, Hypoglycaemic, Antidiabetic property.

INTRODUCTION

The usage of herbs to treat a variety of different ailments is universal and exists in every human culture on earth. Every plant on the planet creates specific chemical compounds which is a basic part of their metabolic function. The ethno botanical information reports about 800 plants worldwide possess anti-diabetic potential [1]. But still most of the medicinal plants are not scientifically validated. Scientific studies on those plants are likely to provide valuable medicines [2].

Free radicals play a role in the etiology of several major diseases including Diabetes, Cancer, Atherosclerosis, Parkinsonism and Aging. Free radicals are highly reactive and unstable chemical species of atoms or molecules. Accumulating evidences suggest that oxidative cellular injury caused by free radicals may contribute to the development of diabetes mellitus [3]. The most common lipid abnormality in the diabetes is hypertriglyceridemia, which is associated with metabolic consequences of coagulability, hyperinsulinemia, insulin resistance and insulin intolerance [4].

The number of patients seeking alternate and herbal therapy is growing exponentially. Herbal medicines are now in great demand in the developing world for primary health care not because they are inexpensive but also for better cultural acceptability, better compatibility with the human body and minimal side effects. The growing recognition and world wide acceptance of natural products are due to its lesser side effects, non-toxicity, easily availability and affordable price [5]. Plants have always been great source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The plant *Ficus pumila* belongs to the family Moraceae. The Okinawan folks in Japan use *F. pumila* L. as a beverage or as herbal medicine to treat diabetes, high blood pressure, Dizziness and neuralgia [6].

Besides phenolic acid compounds, flavanoid glycosides with antioxidant properties have been reported from the 50% aqueous and ethanol leaf extract of *F. pumila*. Leaves serve as a good natural source of antioxidants [7]. There is evidence that some of the compounds in the plants regularly consumed by the Okinawans have powerful antioxidant and positive hormonal effects [8]. There is a report showing that oxidative stress is implicated in the development of diabetic complications [9].

These strong reports make emphasize to consider this plant *F. pumila* is taken for evaluation of antidiabetic property for finding alternative for existence. To the best of our knowledge, no information about anti hyperlipidemic and anti hyperglycaemic activities of *F. pumila* ethanol leaf extract in rats. Therefore the present study was aimed to investigate the hypolipidemic and hypoglycaemic activity of *F. pumila* ethanol leaf extract.

MATERIALS AND METHODS

Collection of the plant and authentication

The plant *F. pumila* L. was collected from Nandha College Campus, Erode District. The plant was authenticated by Botanical Survey of India (BSI), Coimbatore. (No -BSI/SRC/5/23/2012-13/Tech-448). The plant material was identified as *F. pumila* L. belonging to the family Moraceae. The study was conducted after obtaining the approval from Committee for the Purpose of Control and supervision on experimental Animals (CPCSEA) and Institutional Animal Ethics Committee (IAEC), Proposal number NCP/IAEC/PG/2010-04.

Preparation of plant extract

About 500 gm of dried powdered material was taken in 1000ml soxhlet apparatus and extracted with petroleum ether for 18 hours till the solvent became colourless. At the end of the extraction process the marc was taken out and it was dried. After drying, the powdered marc was weighed & again packed and extracted with Ethanol for another 18 hours till it became colourless. Then the extract was concentrated by distillation and final solution was evaporated to obtain dry mass. The ethanol leaf extract of *F. pumila* (EEFP) was suspended in 0.5% CMC solution and used for the experiment.

Acute oral toxicity studies

The animals were divided into six groups separately and were treated orally with ethanolic extracts of *Ficus pumila* at 50, 100 and 200 mg/kg, body weight doses. The animals were continuously observed for 1 hour then frequently for 14 days. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsion [10].

Anti-diabetic activity

Selection of animals

Wistar Albino Rats (Male) weighing around 150-200 gm was selected for the experiment. The animals were checked for the free of any disease, only healthy rodent is accepted for the experiments. The male rodents are preferred so that there has not been interference in between the experiment because of the pregnancy. The rodents are collected from the animal house of Nandha College of pharmacy and Research Institute, Erode.

Maintenance of animals

The selected rodents are brought to the laboratory two days before the commencement of the experiment and provided with standard laboratory rodent chow diet obtained from (Pranav Agro Industries Ltd, Bangalore) and free access of water, 12hrs day/ dark cycle and room temperature is maintained 27°C. The night before the commencement of the experiment food is withdrawn but free access of water is provided [11].

Induction of Diabetes

The acclimatized animals were kept fasting for 24 hrs with water *ad libitum* and the initial blood glucose levels were checked. Intravenous injection of 60mg/kg dose of Streptozotocin in buffer pH 4.5, makes pancreas swell and at last causes degeneration in Langerhan islet beta cells and induces experimental diabetes mellitus in the 2-4 days [12]. On set of diabetes was confirmed in the experimental rats by measuring the blood glucose levels after 72 hrs of STZ injection. The animals were considered diabetic when the blood glucose level was raised beyond the 200mg/dl.

Experimental procedure

After confirmation of increased hyperglycemias the diabetic rats were divided into different groups as mentioned below.

Groupings of animals

- Group I = Control (Normal saline- 1ml/kg)
- Group II = Diabetic Control (STZ- 60mg/kg)
- Group III = STZ + EEFP leaf extract (200mg/kg)
- Group IV = STZ + EEFP leaf extract (400mg/kg)
- Group V = STZ + Glibenclamide (10mg/kg)

The drugs were dissolved in 0.5% CMC and it was administered orally via a standard orogastric cannula, Anti-hyperglycaemic activity in diabetic rats was assessed by fall in Fasting Blood Glucose level [13]. Blood samples were collected from the tip of the tail on 0th, 7th, 14th and 21st days [1].

Estimation of Biochemical parameters in Blood serum

After the completion of experiment, the blood samples were collected through the retro orbital puncture of eye of animals under

mild ether anesthesia in Eppendorff's tube (1ml) containing 50µl of anticoagulant (10% trisodium citrate) and the serum was separated by centrifuging at 3000rpm for 15min. The biochemical parameters HDL, LDL, VLDL, Total Cholesterol, Triglyceride [14] were determined by using the commercial kit available (Ecoline, Manufactured by Merck Specialties, Private limited, Ambernath).

Statistical analysis

Statistical significance was determined by one way analysis of variance (ANOVA) followed by Dunnet's t - test.

RESULTS

Blood glucose level

The initial blood glucose level before STZ administration was found in the range of 106-112 mg/dL in all the five groups. STZ has administered to all the groups except normal control, there is a rapid increase in blood glucose level while the animals in the normal control maintained the same level. The glucose level was significantly high in STZ treated group when compared to that of control and drug treated group. After the confirmation of diabetes, *F. pumila* plant extracts of 200 mg/kg and 400 mg/kg as well as standard drug (Glibenclamide) are given to experimental group of animals. The results indicated that there was slow decrease in animals treated with *F. pumila* extract-I (200 mg/kg) while there was rapid depletion of blood glucose level in animals treated with *F. pumila* extract-II (400 mg/kg) and standard drug (Glibenclamide 10mg/kg), when compared with diabetic control group. This reveals that the given EEFP extract possess hypoglycemic activity. On repeated administration of the extract and standard drug for 21 days, a significant decrease in glucose level was observed in diabetic rats. The readings are ascertained in table 1.

Serum lipid profile

The extract and standard treated group's shows significant balance in the biochemical parameters when compared to that of diabetic rats. Animals in Diabetic control group decrease in HDL levels compared to normal control group. Animal groups treated with Standard drug (Glibenclamide 10mg/kg), EEFP- 200mg/kg, EEFP-400mg/kg, show significant increase in HDL levels compared to diabetic control group.

Animals in Diabetic control group increase in LDL and VLDL levels compared to normal control group. Animal groups treated with Standard drug (Glibenclamide 10mg/kg), EEFP- 200mg/kg, EEFP-400mg/kg, show considerable reduction in LDL levels compared to diabetic control group. Animals in Diabetic control group exhibited very significant increase in TC and TG levels compared to normal control group. Animals groups treated with Standard drug (Glibenclamide 10mg/kg), EEFP- 200mg/kg, EEFP-400mg/kg, show significant reduction in TC levels compared to diabetic control group. Table 2 shows biochemical parameters of the blood serum in different groups.

Table 1: Blood glucose (mg/dl) levels of tested animal groups on successive days

S. No.	Groups	0th Day	7th Day	14th Day	21st Day
1	Control (Normal saline 1ml/kg)	111.01 ± 3.05	110.53±2.96	109.12 ± 2.79	110.01 ± 1.80
2	Diabetic Control (STZ 60mg/kg)	250.83±3.468	297.33± 3.86	321.83 ± 2.94	386.16 ± 3.27
3	STZ + EEFP extract 200 mg/kg	283.33 ± 3.97	268.33± 2.61	210.60±2.70*	164.52±2.46**
4	STZ + EEFP extract 400mg/kg	279.66 ± 2.67	245.16± 3.27	183.83±2.574**	131.83± 3.77**
5	STZ +Glibenclamide 10mg/Kg	277.83±3.28	228.66± 1.87	158.01 ± 3.31**	126.33± 3.21**

Data represents mean ± SEM. (n=5); *p<0.05; **p<0.01

Table 2: Serum Lipid Profile (mg/dl) of *Ficus pumila* leaf extract in blood serum of experimental rats

Grouping	HDL	LDL	VLDL	TC	TG
Control (Normal Saline 1ml/kg)	35.73 ± 1.47	60.69 ± 2.73	15.81 ± 0.18	75.55 ± 1.52	74.32 ± 5.57
Diabetic Control (STZ 60 mg/kg i.p.)	28.66 ± 1.29	290.13 ± 1.40	35.93 ± 2.83	147.20 ± 1.58	167.11 ± 5.57
STZ + EEFP 200 mg/Kg p.o	29.11±4.56*	143.56±3.56*	25.21±1.43*	117.57 ± 6.38*	87.24±5.38*
STZ + EEFP 400 mg/Kg p.o	31.11±3.11*	101.31±3.51**	19.23±1.50**	98.72±5.53**	83.50±5.96**
STZ+ Glibenclamide (10mg/kg p.o)	32.53±1.47**	90.73±3.34**	18.03±0.17**	89.43 ± 2.51**	78.01±8.01**

Data represents mean ± SEM. (n=5); *p<0.05; **p<0.01

DISCUSSION

Commonly, *Ficus* species are rich source of naturally occurring antioxidants of which phenolic compounds and flavanoids play a vital role in preventing innumerable health disorders [13]. The plant *F. pumila*, worldwide growing plant exhibits potent anti-inflammatory, antihypertensive activity, antioxidant [6] and antiulcer [15]. In the present study, we have tested the hypoglycemic potency of *F. pumila* ethanol leaf extract in STZ induced diabetic rats as well as hypolipidemic activity. Streptozotocin is selectively accumulated in pancreatic beta cells via the low-affinity GLUT 2 glucose transporter in the plasma membrane [16, 17]. The transfer of the methyl group from streptozotocin to the DNA molecule causes damage, which along a defined chain of events [18], results in the fragmentation of the DNA [19]. The administrations of EEFP recuperate all the symptoms of STZ treated animals. By the way it may be, In *F. pumila*, the presence of flavanoids such as rutin, apigenin 6-neohesperidose, kaempferol 3-robinobioside and kaempferol 3-rutinoside which could be the reason for strongest anti oxidant property is confirmed in both DPPH and superoxide radical inhibition assessments [6]. Recent study revealed that Flavanoids, Tannins, Triterpens also exhibits hypolipidemic activity. The responsible compounds may inhibit the beta cells of islets destruction.

In the present experiment, administration of *F. pumila* ethanol extract has prevented the elevation of glucose levels when compared to diabetic control group animals. The ethanol extract of *F. pumila* produced a significant reduction in blood glucose level in hyperglycemic animals $p < 0.01$. The pronounced hypoglycemic effect may be either due to the increase in glycogenesis, decrease in glycogenolysis or increase in entry of glucose molecules to various skeletal muscles [20]. The result is suggested that the hypoglycemic and hypolipidemic activities of the plant are due to the active principles of the plant.

In this family, the evaluation indicates the hypoglycemic, antiperoxidative and ameliorative potential of *F. bengalensis* bark aqueous extract on STZ -induced diabetic rats [21]. Similarly antihyperglycemic activity of *F. glomerata* stem bark in streptozotocin induced diabetic rats exposed the percentage reduction in blood glucose of *F. glomerata* was 50% and 52% for the first and second week respectively [22] has beneficial effects on blood glucose levels as well as improving hyperlipidemia [23]. Related species like *F. glomerata* and *F. religiosa* Linn. ethanol extracts of the leaves and fruits [24] were comparatively evaluated for their blood glucose lowering activity in normal and STZ induced diabetic rats. Ethanol extract of *F. glomerata* (at 250 and 500 mg/kg) exhibited a dose dependent significant anti- hyperglycemic activity [23]. The reduced glucose levels suggested that *F. nervosa* might exert insulin-like effect on peripheral tissues by either promoting glucose uptake metabolism by inhibiting hepatic gluconeogenesis [25, 26] or by absorption of glucose into the muscle and adipose tissues [27].

When we focus the hypolipidemic potency of the plant, the atherogenic index i.e. TC/HDL ratio, which is a useful determinant of cardiovascular risk [28] can be effectually achieved by the lipid oscillations of each group during the experiment. Insulin deficiency is also responsible for dyslipidemia, because insulin has an inhibitory action on HMG-CoA reductase, a key enzyme that is rate limiting in the metabolism of cholesterol rich LDL particles [29]. The levels of LDL, VLDL, TG, and TC were reduced after the administration EEFP and the HDL level was significantly increased when compared to the diabetic control group. This finding tends to suggest that the hypoglycemic and hypolipidemic effects of EEFP are similar to the effect of standard drug Glibenclamide and which could amend the atherogenic ratio. As a need for developing antidiabetic drugs which may also protect against diabetic complications, it is necessary to understand pathophysiological mechanisms involved in them [30].

CONCLUSION

Generally, all *Ficus* species having rich source of antioxidants of which their phenolic compounds are taking main role in treatment of many health problems including diabetes. Hence, the findings of

the present study is also a march to discloses that administration of EEFP in two doses as 200 mg/kg and 400 mg/kg b.w p.o significantly decreased the blood glucose level in STZ induced diabetic rats. Accumulation of bad cholesterol is being one of the main hypoglycemic drug contradictions. Administration of EEFP has shown significant increase in HDL levels and decreased levels of LDL, VLDL, TC and TG when compared to the diabetic control group. The *F. pumila* plant extract is efficient and may fulfill all the health expectations along with hypoglycemic nature hence isolation of active phytochemical and clinical pharmacology studies has to be progressed to elucidate in detail the active principle and real mechanism of action. This could reveal the plant potency and will make this plant well worthy in medicinal field.

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