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Research Article

Anti-Diabetic Effects of Traditional Medicinal Plant *Ficus Recemosa* Extracts in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Plants have formed the basis of traditional medicine systems around the world for thousands of years. Fresh leaves of the *F. recemosa* were collected from the forest region of Eturunagaram of Warangal district, Telangana state and extracted with various solvents by sequential extraction method. The methanol crude extracts were tested for evaluation of antidiabetic activity against streptozotocin-induced diabetic rats. The results of the study have shown a significant ($p < 0.01$) difference between the initial and final fasting plasma glucose levels of methanol leaves extract of *F. recemosa* and glibenclamide treated groups. This study concludes that, the methanol crude extract of *F. recemosa* has beneficial effects in reducing the elevated blood glucose level and lipid profile of STZ-induced diabetic rats, but has no effect on normal rats.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic degenerative disease characterized by hyperglycemia, mainly due to defects of insulin secretion and/or action. The disorder is associated with an imbalance of the glycemic index and glucose intolerance, which increases the risk of individuals progressing to T2DM. Complications from diabetes, such as coronary artery and peripheral vascular disease, stroke, diabetic neuropathy, risk of ulcers and amputations, renal failure, sexual dysfunction, and blindness are resulting in increasing disability, reduced life expectancy, and enormous health costs for virtually every society [1,2].

Plants have formed the basis of traditional medicine systems around the world for thousands of years. Even in modern systems, ethnobotanical treatments continue to play an important role in health care. About 80% of the population in developing countries rely on traditional medicine for their health care. In Mexico, there is a great diversity of medicinal plants. Andrade et al. reported on 306 species of Mexican plants used as hypoglycemic agents [3]. Despite the large number of plants that have been reported for their empirical use as antidiabetics, only a small percentage of these plants have been studied in a systematic way, and the molecules responsible for the biological activity of most of them are unknown.

Due to availability in the local area of Warangal, Telangana State, the medicinal plant *Ficus racemosa* was chosen for study. Its medicinal values have been well documented in traditional and folklore medicine. Many *Ficus species* have long been used in folk medicine as astringents, carminatives, stomachics, vermicides, hypotensives, anthelmintics and anti-dysentery drugs [4]. *Ficus racemosa* Linn. (Family; Moraceae) is used in traditional system of medicine for the treatment of several disorders. It is one of the herbs mentioned in all ancient scriptures of Ayurveda, Siddha, Unani and Homeopathy. Various plant parts such as bark, root, leaf, fruits and latex are used as astringent, carminative, vermifuge and anti-dysentery. It is believed to be a good remedy for visceral obstructions and extract of the fruit is used in leprosy, diarrhea, circulatory and respiratory disorders and menorrhagia [5,6,7].

2. Material and Methods

2.1 Collection of Plant Material:

Fresh leaves of the *F. recemosa* were collected from the forest region of Eturunagaram of Warangal district, Telangana state. The collected plant material species was confirmed by Prof. V.S. Raju, Taxonomist, Department of Botany, Kakatiya University, Warangal. After confirming the species, the Voucher specimens have been deposited in the Herbarium of the Department of Zoology, Kakatiya Government College, Warangal. The collected leaves were thoroughly washed under running tap water, dried in shade and then triturated into fine powders by using an electric grinder. These powder was stored in air sealed brown bottles at ambient temperature.

2.2 Preparation of Extract:

The sequential extraction was carried out with the *F. recemosa* leaves powder using solvents of increasing polarity. 500 g of dried fruits of *F. recemosa* powder was sequentially extracted using n-hexane, chloroform, ethyl acetate, acetone and methanol at room temperature and at atmospheric pressure by shaking at 100 rev/min. Solvent extraction will be carried out for 48 h in total.

After each solvent extraction step, the extracts will be filtered by whatman filter paper and cold centrifuge and concentrated by using rotary evaporator. The concentrated extracts will be freeze-dried to remove the solvent and stored in refrigerator. The weight of the residual extract was measured and percent yield was calculated by the following equation:

Extract yield % = W1/W2 X 100 ;
Where, W1= Net wt of powder in grams after extraction
W2= total wt of powder in grams taken for extraction.

The residue of the different solvent extracts were dissolved in particular solvent and stored in air tight glass vials at 40^0C until further use.

2.3 Animals

Healthy adult male albino wistar rats (150-200 *g*), in house breed at the animal house of Department of Zoology, Kakatiya University, India were used for the study. Rats were housed in polypropylene cages lined with husk in standard environmental conditions. (temperature 25±2° *C*; relative humidity 55±10%; and 12:12 light:dark cycle,) The rats were fed on a standard pellet diet *ad libitum* and had free access to water.

2.4 Evaluation of Antidiabetic Activity

2.4.1 Induction of diabetes:

Diabetes was induced in rats by single intra peritoneal (*i.p.*) injection of steptozotocin (STZ, Sigma chemical Co. USA) at a dose 60 *mg/kg* b.w. freshly dissolved in 0.1 *M* cold citrate buffer of pH 4.5; 48 *hr* later blood samples were collected and blood glucose levels were determined to confirm the development of diabetes. Those animals which showed hyperglycemia (blood glucose levels >240 *mg/dl*) were used in experiment.

2.4.2 Chronic treatment model

The rats were divided into five groups of 6 animals (n = 6) each as below:

- Group I- Normal control (received distilled water 10 *ml/kg* b.w., *p.o.*)
- Group II- Diabetic control untreated (received distilled water 10 *ml/kg* b.w., *p.o.*)
- Group III- Diabetic treated with standard drug glibenclamide (0.25 *mg/kg/day*, *p.o.*)
- Group IV- Diabetic treated with SSAE (250 *mg/kg/day*, *p.o.*)
- Group V-Diabetic treated with SSAE (500 *mg/kg/day*, *p.o.*)

For 30 days blood glucose levels and body weights were measured on 1st, 10th, 20th and 30th day of the study. Finally on day 30, blood was collected to estimate various parameters.

2.4.3 Estimation of Plasma Glucose, Body Weight and Lipid Profile

Every week, following overnight fasting (16 *hr* fasting with free access to water), the blood samples were withdrawn from the animals by retro-orbital puncture under light ether anesthesia.

The plasma glucose estimation was done by the glucose oxidase/peroxidase (GOD/ POD) method using a standard kit obtained from Span Diagnostics, India. Body weight of all experimental animals was recorded using a digital weighing scale. The TG, TC and HDL levels were estimated using standard kits obtained from Span Diagnostics, India.

VLDL = TG/5
LDL = TC - (HDL + VLDL)

2.5 Statistical Analysis

The results were expressed as mean± S.E.M. Statistical difference was tested by using one-way analysis of variance (ANOVA) followed by Dunnette's multiple comparison test. A difference in the mean p value <0.05 was considered as statistically significant.

3. Results and Discussion

3.1 Effect of Methanol Extract in Normoglycemic Rats

The results from the study clearly indicated that there was no significant effect observed on normoglycemic rats when treated with the single dose of *F. recemosa* methanol extract (Table-1).

Table-1 Effect of methanol extract of *F. recemosa* leaves in normoglycemic rats

Group treatment (n = 6)	Fasting plasma glucose level (*mg/dl*) at (*hrs*)				
	0	1	2	3	4
I Normal	95.00±0.73	94.16±0.65	92.50±1.05	91.83±1.07	91.33±0.49
II Glibenclamide	95.16±0.70	92.50±0.67	89.16 ±0.47*	88.50±0.67*	85.33±0.95**
III Methanol extract	95.66±1.05	94.16±0.60	90.00±0.57	89.33±0.33	88.50±0.42*
IV Methanol extract	94.83±1.01	93.83±0.79	90.66±0.91	89.83±0.70	88.62±0.42*

*p < 0.05' **p < 0.01; Values are mean±SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnette's multiple comparison test

Table-2. Effect of methanol extract of *F. recemosa* leaves on OGTT in STZ-induced diabetic rats

Group treatment (n = 6)	Fasting plasma glucose level (*mg/dl*) at (*hrs*)				
	0	1	2	3	4
I Normal	95.00±0.73	123.17±2.72	134.83±1.35	144.83±1.35	154.83±1.35
II Diabetic control	259.17±1.16	269.50±0.95	279.50 ±0.95	289.50±0.95	297.83±0.83
III Positive control	255.17±1.01	265.17±1.01*	275.17±1.01*	285.17±1.01*	265.00±1.15**
IV Methanol extract	259.67±1.02	266.33±1.30	276.33±1.30	286.33±1.30	274.67±1.17*
V Methanol extract	258.50±2.04	265.17±1.13*	275.37±1.13*	285.47±1.13*	265.17±1.13**

*p < 0.05; **p < 0.01; Values are mean±SEM, n = 6, when compared with diabetic control by using one way ANOVA followed by Dunnette's; multiple comparison test

Table-3. Effect of methanol extract of *F. recemosa* leaves on serum glucose level

Group treatment (n = 6)		Fasting plasma glucose level (*mg/dl*)			
		1st day	10th day	20th day	30th day
I	Normal control	94.50±2.07	94.33±2.10	96.33±1.89	95.16±2.02
II	Diabetic control	255.00±1.18	286.67±1.22	312.67±4.58	387.67±2.83
III	Diabetic + glibenclamide (0.25 *mg/kg*)	255.67±1.33	265.67±1.33**	210.67±2.84**	115.83±1.53**
IV	Diabetic + methanol extract (250 *mg/kg*)	255.83±0.79	275.83±0.79**	235.83±0.79**	135.83±0.79**
V	Diabetic + methanol extract (500 *mg/kg*)	256.33±2.65	268.67±1.02**	223.67±2.01**	123.67±2.01**

*p < 0.05; **p < 0.01; Values are mean±SEM, n = 6, when compared with diabetic control by using one way ANOVA followed by Dunnette's multiple comparison test

3.2 Effect of Methanol Extract on Oral Glucose Tolerance Test in STZ-Induced Diabetic Rats (OGTT)

The results from the study clearly indicated that the methanol extract of *F. recemosa* leaves at 250 and 500 mg/kg reduced the blood glucose level (hyperglycemia due to glucose load 2 g/kg p.o.) significantly and glibenclamide (0.25 mg/kg) after 60 min of oral administration, when compared to diabetic control (Table-2).

3.3 Hypoglycemic Effect of the Methanol Extract

The results from the study clearly indicated that the methanol extract exhibited significant hypoglycemic activity in STZ-induced diabetic rats, whilst there was no significant effect observed on normoglycemic rats. However, at the end of 30 days of treatment, there was a 70.12%, 64.96% and 68.09% ($p < 0.01$) decrease of serum glucose levels with the glibenclamide and methanol extract (250 and 500 mg/kg) respectively when compared with diabetic control after 30 days (Table-3).

The present study was undertaken to evaluate the antidiabetic activity of methanol leaves extract of *F. recemosa* in normal, glucose-loaded hyperglycemic and STZ induced diabetic rats. There was no lethality or no toxic reactions were found with the selected doses until the end of study period. The results of the study have shown that the methanol extract of leaves at dose 500 mg/kg has a marked hypoglycemic activity by improvement of the glucose tolerance test in normoglycemic rats and by lowering the blood glucose levels in STZ-induced diabetic rats. The results of the study have shown a significant ($p < 0.01$) difference between the initial and final fasting plasma glucose levels of methanol leaves extract of *F. recemosa* and glibenclamide treated groups (Table-3). Induction of diabetes by STZ leads to loss of body weight due to increased muscle wasting and loss of tissue proteins [7]. The results obtained with the methanol extract treatment in chronic diabetic model further clarified the antidiabetic effect of the extract. After 30 days of methanol extract treatment, gain in body weight was observed in diabetic rats and the results were comparable with that of the standard drug glibenclamide.

4. Conclusions

In conclusion, it can be stated that the methanol leaves extract of *F. recemosa* has beneficial effects in reducing the elevated blood glucose level and lipid profile of STZ-induced diabetic rats, but has no effect on normal rats. Thus justifying the claim made by ayurvedic classics.

Competing Interests

The authors have declared that no competing interests exist.

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