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Research Article

Antidiabetic Potential of Alcoholic and Aqueous Extracts of *Ficus racemosa* Linn. Bark In Normal and Alloxan Induced Diabetic Rats

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ABSTRACT
The present investigation aims to examine the diabetic potential of the plant *Ficus racemosa* in normal and alloxan induced diabetic rats. The bark extract with water, petroleum ether and with alcohol were screened for blood glucose lowering activity and the alcoholic extract having better therapeutic potential was prepared through Soxhlet extraction for further study. Alcoholic and aqueous extract of bark of *Ficus racemosa* at a dose of 400 mg/Kg was given to normal and alloxan induced diabetic rats and the blood samples taken from the retero-orbital plexus vein were analyzed for blood glucose level as per standard protocol with available kits through Auto-analyzer. The comparison of blood sugar level as per model schedule showed that in normal group the ethanolic extract, at a dose of 400 mg/Kg intra-peritoneal, the blood glucose lowering 28.66 % while in aqueous extract given group it was 25.90 %. In alloxan induced diabetic rats decrease in blood glucose level in aqueous and ethanolic extract group was found to be 27.01 % and 45.03 % respectively. In conclusion, the ethanolic extract of *Ficus racemosa* reflected anti-diabetic potential through its glucose lowering activity in experimental animals. It supported the folklore claim of anti-diabetic activity of the plant.

Keywords: *Ficus racemosa,* antidiabetic, herbal remedy, Goolar.

INTRODUCTION

Diabetes is any disorder characterized by excessive urine excretion. The most common form of diabetes is Diabetes mellitus, a chronic, progressive, systemic condition of impaired carbohydrate metabolism. Diabetes mellitus is characterized by the elevated glucose in plasma and ketoacidosis. Additional symptoms of diabetes mellitus include excessive thirst, glucosuria, polyuria, lipemia and hunger. [1] If left untreated the disease can lead to fatal ketoacidosis. Other forms of diabetes include diabetes insipidus and brittle diabetes. [2]

Criteria, which clinically establish an individual as suffering from diabetes mellitus, include [3]:

- Having fasting glucose level in excess of 140 mg/dL.
- Having plasma glucose level in excess of 200 mg/ dL at two times points during a glucose tolerance test (GTT), one of which must be within two hours of ingestion of glucose.

Its major manifestations include disordered metabolism and inappropriate hyperglycemia. In diabetes, oxidative stress has been found to be mainly due to an increased production of oxygen free radicals and a sharp reduction of antioxidant defenses. [4] The first mention of diabetes (though it was evidently not known as "diabetes" then), found in Indian literature in the works of the physician Susruta (6th century BC), it also finds a mention in Charaka Samhita. [5]

Diabetes seems to be receiving a lot of attention recently in India. Earlier classified as the “rich mans disease” I has now spread amongst masses. So much so, India is slated to be the diabetes capital of the world by 2025. There were 24 million diabetics in the year 2000 and this figure is expected to reach 57.02 million by 2025. [6] The number of people with diabetes will be more than double over the next 25 years, to reach a total of 366 million by 2030. Due to the eminent danger of spreading diabetes mellitus is a research in the development of new drugs than the existing drugs has become a research area of national and international importance. About 15-20 % of the patients with newly diagnosed NIDDM present little or no response to sulphonylureas. Every year, 3-5 % of the patients with NIDDM who have attained acceptable or better glycaemic control are said to lose their responsiveness to sulphonylureas. Adverse effects of oral hypoglycemic agents occur in roughly 3 % of the patients. [7] However, on the other hand a large number of herbal have been given reported to possess hypoglycemic/anti-hyperglycemic properties. These drugs are easily available at low cost, are comparatively safe and people have faith in such remedies. [8]

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The *Ficus racemosa* is a moderate to large sized spreading laticiferous, deciduous tree without much prominent aerial roots, leaves dark green, ovate of elliptic, fruits receptacles 2 – 5 cm in diameter subglobose or pyriform in large clusters on short leafless branches arising from main trunk or large branches. Figs are smooth or rarely covered with minute soft hairs, when ripe they are orange, dull reddish or dark crimson. They have a pleasant smell resembling that of cidar apples. The bark is rusty brown with a thickness from 0.5 – 2 cm according to the age of trunk or bark. The surface is with minute separating flakes of whitish tissues, texture homogeneous leathery. [9] It has been used for the curare of diabetes in some ethenic groups of India. The ayurvedic literature in *'Vanausadhi Chandroday'* also mention its usefulness in *madhumeha i.e.* diabetes. The diabetic potential of the plant have also been investigated in methanolic extracts by Prof. Mandal and supported the ancient claim. The plant is known as *'Goolar'* in the state of Uttar Pradesh, India and is claimed for its utility in treatments of several ailments having multiple medicinal value as antidiabetic, antipyretic, anti-inflammatory, muscle relaxant activity and analgesic potential. In this chain the hypoglycemic activity of aqueous and ethanolic extract of the plat is carried out. The objective of this investigation is to evaluate the effect of aqueous and alcoholic extracts of stem bark of *Ficus racemosa* in normal and alloxan induced diabetic rats to confirm the folklore claim of antidiabetic activity of the plant.

MATERIALS AND METHODS

Drug and Chemicals: The drug tolbutamide was kindly gifted by the Apco Pharmaceuticals, Ltd. Haridwar, alloxan monohydrate (Loba Chemi, Mumbai) purchased from commercial sources. All other chemicals were analytical grade laboratory reagent and were used as such without further testing.

Animals: Sprague-Dawley rats, weighing 200-220 g of either sex were used in this investigation. They were kept in the departmental animal house at 26 ± 2 °C and relative humidity 44 – 56 %, light and dark cycles of 10 and 14 h respectively for one week before and during the experiments. Animals were provided with standard rodent pellet diet (Amrut, India) and the food was withdrawn 18-24 h before the experiment though water was allowed *ad libitum*. All experiments were performed in the morning according to current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals. [10] Approval for the study protocol was granted by the Institutional Animal Ethics Committee of Raja Muthiah Medical College, Annamalai University, India (Reg.160/1999/CPCSEA).

Collection and authentication of plant material: The plant material was collected from wild sources around tehsil Sikandara Kanpur-Dehat, authenticated by the taxonomist at department of botany Annamalai University, and the voucher specimens were deposited in the departmental herbarium for future reference. The bark was cut into the pieces and shade dried at room temperature; the dried bark were subject to size reduction to a coarse powder by using a dry grinder (Philips, India) and passed through the sieve before being stored in a closed vessel for further use.

Preparation of extract: The powdered bark was extracted by Soxhlet extraction apparatus using the 90 % ethanol. The extracts were dried in a rotary vacuum evaporator and successively in a hot air oven till solid to semisolid mass. Aqueous extracts were prepared by using distilled water as solvent for the experiment. Extracts were stored in an airtight container in refrigerator below 10°C.

Preliminary phyto-chemical screening: The bark extracts of *Ficus racemosa* were subjected to qualitative tests for the identification of various active constituents viz. carbohydrate, glycoside, alkaloid, amino acids, flavanoids, fixed oil, tannins, gum and mucilage and phytosterols using standard test procedures. [11-12]

Acute toxicity study: The acute toxicity studies were conducted using adult Swiss albino mice of both sexes taking the bark extract at various dose levels (5, 50, 300, 2000 mg/Kg), by adopting fixed dose method as per the OECD guidelines. [13] The animals were observed continuously for 2 hours and then occasionally for further 4 hours and finally overnight mortality/survival was recorded and LD_{50} was extrapolated graphically.

Experimental induction of diabetes:

Diabetes was induced in rats by tail vein injection of alloxan monohydrate (150 mg/kg, i.v.) dissolved in normal saline to the overnight fasted rats (One group of 6 identical rats was kept without streptozotocin administration as normal control, group 1). Forty eight hours after alloxan administration blood samples were drawn and glucose levels determined to confirm diabetes induction. The diabetic rats exhibiting blood glucose levels in the range of 250 and 280 mg/100 ml were selected for the studies. [14]

Experimental Design: Seven groups of rats were used to study the effect of aqueous and alcoholic extracts of *Ficus racemosa* on diabetes so induced. The rats were grouped and labeled as below.

Group 1 – Normal untreated rats

Group 2 – Normal rats treated with 400 mg/kg of alcoholic extract

Group 3 – Normal rats treated with 400 mg/kg of aqueous extract

Group 4 – Diabetic control

Group 5 – Diabetic rats treated with 100 mg/kg of tolbutamide.

Group 6 – Diabetic rats treated with 400 mg/kg of alcoholic extract

Group 7 - Diabetic rats treated with 400 mg/kg of aqueous extract

After a week of treatment the rats were fasted overnight, the aqueous extract and alcoholic extract (solution in distilled water) was fed by gastric intubations. [15] Group 1 and group 4 were given with distilled water alone. The blood samples were taken for the glucose level monitoring from the tail vein at 0, 1, 2, and 3 hours interval. The blood glucose level was determined through an electric semi-auto analyzer and data so obtained was used for analysis.

Statistical Analysis:

The data were analysed statistically using analysis of variance (ANOVA) follwed by Dunnett's `*t*' test to confirm about the statistical significance of the results. The values are expressed as mean ± standard errors of the mean (S.E.M.). At 99 % confidence interval p-values less than 0.01 were considered to be significance.

RESULTS

The hypoglycemic effects produced by the aqueous and ethanolic extracts of Ficus *racemosa* in normal and diabetic induced rats are summarized in the table 1 and 2.

Table 1: Effect of alcoholic and aqueous extracts of *Ficus racemosa* on fasting blood glucose levels of normal rats

Treatment groups	Dose (mg/kg)	Blood glucose (mg/dl) at different hours after the treatment			
		0 h	1 h	2 h	3 h
Control (Distilled water)	400	86.96 ± 1.14	86.85 ± 0.96	88.58± 0.64	86.76 ± 1.49
Alcoholic Extract	400	85.38 ± 1.6	76.56 ± 2.23	72.41 ± 1.98	67.43± 1.61
Aqueous Extract	400	84.35 ± 1.08	76.38 ± 1.13	73.3 ± 1.03	68.91 ± 0.45

Table 2: Effect of alcoholic and aqueous extracts of *Ficus racemosa* on fasting blood glucose levels of alloxan induced diabetic rats

Treatment groups	Dose (mg/kg)	Blood glucose (mg/dl) at different hours after the treatment			
		0 h	1 h	2 h	3 h
Diabetic Control	-	317.4 ± 3.17	321.6 ± 1.3	320.4 ± 1.3	320.3 ± 1.2
Alcoholic Extract	400	304.8 ± 1.3	204.6 ± 1.5	160.8 ± 2.5*	120.3 ± 2.7*
Aqueous Extract	400	324.2 ± 3.1	258.2 ± 1.5	199 ± 1.3*	160.4 ± 1.5*

ANOVA *p< 0.01 compared with the initial level of blood glucose (0 h) in the respective group. Values are expressed as mean ± SEM

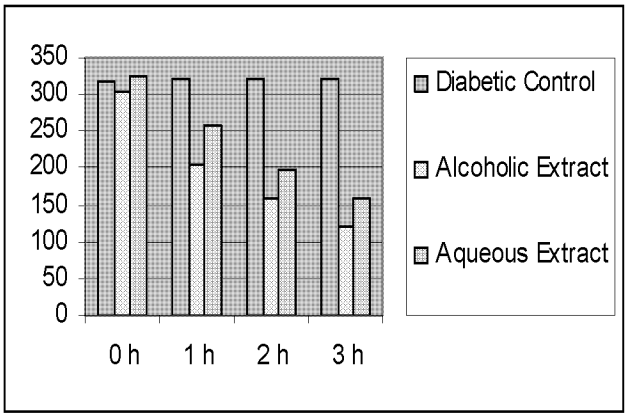

Fig. 1: Effect of alcoholic and aqueous extracts of *Ficus racemosa* on fasti blood glucose levels

DISCUSSION

Diabetes mellitus is the most important and common heterogenous metabolic syndrome disease involving the endocrine pancreas. Diabetes mellitus, prevalent throughout the world and has been projected to become one of the world's major disablers and killers within next 25 years. [16] Its major manifestations include disordered metabolism and inappropriate hyperglycemia. It is through that the many stresses inherent in the modern lifestyle may cause an increased incident of diseases such as cancer, heart disease and hypertension. The rising incidence of such diseases is alarming and becoming a serious public health problem. Diabetes one such disease and it is estimated that the number of diabetes patients will continue to increase in future. [17]

Therefore, an attempt has been made in scientifically validated experimental animal models to investigate the *F. racemosa* against diabetes condition through its blood glucose lovering activity. In present investigation, the *per se* effect and toxicological studies of *F. racemosa* were investigated for their safety and toxicity (acute and subacute) in mice at different dose levels. The results, showed no abnormal symptoms when administered orally, reflected therapeutically acceptable safety profile. Several researchers have reported that plant drugs are safe and effective in treatment of incurable diseases.

The oral administration of *F. racemosa* extract 400 mg/kg, showed the significant decrease in the glucose level at the end of study after 21 days. The mechanism of *F. racemosa* extract to bring about its hypoglycemic action is still to be explored, may be due to the increase in peripheral utilization of glucose or by stimulating the secretion of insulin by the remaining intact b cells that may likely be present and potentiation of its effect. In this context a number of other plants have also been observed to have hypoglycemic and insulin release stimulatory effects. [18] On the other hand flavonoids were isolated from the leaves of *F. racemosa* that may trigger some Ca^{+2} mediated mechanism for insulin release, or may modulate the voltage dependant channel inactivation mechanism by altering voltage sensitivity.

Glucose metabolism and Ca^{+2} concentration ($[Ca^{+2}]$ C) oscillate in cells. Because $[Ca^{+2}]$ C controls insulin secretion, cells secrete insulin in an oscillatory manner. [19] Depolarized cell membranes activate their voltage-dependent Ca^{+2} channels leading to an influx of Ca^{+2}. Increases in the cytosolic $[Ca^{+2}]$C direct the fusion of insulin-containing vesicles with plasma membrane and the expulsion of insulin. [20] Further investigation using biochemical tracing technique are sought to identify the exact mechanism of action of the natural drug.

In conclusion, this can be stated that the ethanolic extract of *Ficus racemosa* is having potential of being used for anti-diabetic activity in herbal treatment. It supported the folklore claim of antidiabetic activity of the plant; further research is sought to explore the exact mechanism of action and phyto-constituent responsible for the pharmacological response. Further it should be investigated for the antioxidant and free radical scavenger activities as the results have given a clue to such action.

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