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Research Article

EVALUATION OF ANTI-DIABETIC POTENTIALS OF METHANOLIC EXTRACT OF FICUS MICROCARPA LEAVES IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Objective: The present study was designed to evaluate the acute and chronic effects of the extract against alloxan induced diabetic rats.

Methods: In acute study, hypoglycemic potency of methanolic extract of *Ficus microcarpa* was assessed by oral glucose tolerance test (OGTT) and in chronic study of 21 days, extract at different doses (ie 100, 200 and 400mg/kg) was screened for its anti-diabetic activity. Blood glucose level had been estimated at 1st, 7th, 14th and 21st and addition to this serum concentrations of insulin, triglycerides, cholesterol, SGOT, SGPT and urea determined at 21st day of the study.

Results: In OGTT, standard glibenclamide and extract (200 and 400mg/kg) have shown significant reduction in blood glucose level compared to control group. In chronic model the methanolic extract was more effective in reducing the blood glucose levels (P<0.001) and effect was comparable to that of standard. The extract could also significantly (P<0.001) reduce concentrations of SGOT, triglycerides, cholesterol and urea in serum and significantly (P<0.001) increased the insulin level in blood which proves beneficial effects of the extract in diabetes. The change in concentrations of SGPT and urea were less significant (P>0.01).

Conclusion: The methanolic extract of *Ficus microcarpa* posses significant antidiabetic activity in alloxan induced diabetic animal model.

Keywords: Anti diabetic, *Ficus microcarpa*, Alloxan, Blood glucose level, Insulin

INTRODUCTION

The diabetis melitus is a non infective pathological condition which could hit the world this millennium. It is estimated that, by 2025 the half the diabetic patients worldwide will be from India and it would become "Diabetic capital of the world" [1,2].

Diabetes is a chronic endocrine disease characterized by persistent hyperglycaemia associated with abnormalities in carbohydrate, protein, and lipid metabolism caused by a failure of insulin

secretion and/or increased cellular resistance to that hormone. This results in severe microvascular and macrovascular problems, including neuropathy, nephropathy, retinopathy, cardiovascular and peripheral vascular disease [3,4].

At present, insulin is the choice of drug for the treatment of insulin dependent diabetis mellitus (Type I IDDM) where as other synthetic drug like sulfonyl ureas and insulin sensitizers are the effective drugs for curing non insulin dependent diabetis mellitus (Type II-NIDDM). But these drugs possess very serious and potential adverse effects like cardiotoxicity, nephrotoxicity and etc. Hence there is no truly satisfactory medicine available for the treatment of diabetis mellitus [2,5,6] .

Herbal remedies are apparently efficient, produce least or no side effects in clinical experience and are comparatively of low costs as compared to oral synthetic antidiabetic agents. Over the years, variety of medicinal plants and their extracts have been reported to be effective in the cure and management of diabetes. Additionally, after the approbations made by WHO on diabetes mellitusexploration on hypoglycemic agents from medicinal plants have become more significant [7].

The *Ficus* is a genus of plant which is of Indian origin. The various species of *Ficus* are medicinally important and have been proved for their several pharmacological activities [8]. The *Ficus hipida* belongs to the same genus used in traditional system, considered to be medicinaly important and proved for many health benefits. A mixture of honey and the juice of these fruit is a good antihemorrhagic [9] but the barks and leaves are of particular interest from a medicinal point of view as antidiarrhoeal [10], cardioprotective[11] and hypoglycemic[12] activity among others. In traditional medicine it is also used for the treatment of leukoderma, psoriasis, hemorrhoids, ulcers, jaundice, inflammations, fever and alopecia. The usefulness of this plant in diabetes is also described in ayurveda and other folk cure books which have no scientific evidence s[9].

The other plant of this genus Ficus microcarpa (*Moraceae)* commonly known as Chinese banyan is grows in tropical and subtropical regions of India. It is an Indigenous plant used and its bark is used to cure sores caused by black magic, ulcers etc [13].

It was also a important component of traditional system of medicine ayurveda for the treatment of diabetis but has lack of scientific evidence for its antidiabetic potential [14]. Hence it was necessary to provide a clear background proof for the beneficial property of the plant in diabetes. In this attempt, study had been conducted to determine anti-diabetic potentials of methanolic extract of *Ficus microcarpa* leaves.

MATERIALS AND METHODS

Plant material

The leaves of *Ficus microcarpa* have been collected from Sri Venkateshwara university, Tirupati, India and dried under shade. The leaves were identified and authenticated by Dr. Madhava chetty Asst. Prof. Dept. of Botany and speciman herbarium were preserved at institute herbarium library. The leaves part were separeted form other parts, washed, cleaned and dried for further use.

Preparation of extract

The shade dried leaves were pulverised into powder and sieved through No. 22 mesh. About 350 g (appx.) of coarse powder was subjected to successive solvent extraction using 2.4 L of petroleum ether, 2.4 L of benzene, 2.2 L of chloroform and 2.2 L methanol in soxhlet's apparatus (8-10 hours for each extraction) [15]. The percentage yield of methanolic extract of *Ficus microcarpa* was found to be 6.77 % W/W.

Preliminary phytochemical investigation

The preliminary phytochemical investigation for the methanolic extract of *Ficus microcarpa* had been conducted as per procedure prescribed by Khandelwal [16].

Drugs and chemicals

Glibenclamide was procured from Aventis Pharma Ltd., India. alloxan was obtained from Sigma Laboratory, India**.** All other reagents and chemicals were obtained commercially and were of analytical grade.

Animals

Wistar male rats (180-220g) were procured from Yash farm, Pune. The animals were housed under standard conditions of temperature (22 ± 1.0C), relative humidity (55 ± 10%), 12 hr light/dark cycles and fed with standard pellet diet (Amrut, Pranav Agro Industries Ltd., Sangli, India) and water ad libitum. After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under above said environmental conditions. The experimental protocol has been approved by the Institutional Animals Ethics Committee (Ref.no.IJAHSM/IAEC/2011/012) with the permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Acute Oral Toxicity Studies

The acute oral toxicity study was done according to the OECD guidelines 423 (Acute Toxic Method). A starting dose used was 2000 mg/kg body weight p.o. of extract (FMcME) was administered to 3 male rats, observed for 14 days. The experiments were repeated again with the same dose level, 2000 mg/kg body weight p.o. of extracts for 3 days more, and observed for 14 days [17]. The doses of extracts for the anti-diabetic study were selected as 100mg/kg, 200mg/kg and 400mg/kg based on the ratio 1/20 , 1/10 and 1/20 of safest dose [17].

Evaluation of antidiabetic activity

Induction of Diabetes in Experimental Animals

In both acute and chronic models, rats were made diabetic by a single intra peritoneal injection of alloxan monohydrate (150 mg/kg). Alloxan was first weighed individually for each animal according to the body weight and then solubilized with 0.2ml saline (154mM NaCl) just prior to injection. Two days after alloxan injection, rats with plasma glucose levels of >140 mg/dl were included in the study. Treatment with plant extracts was started 48 h after alloxan injection [18].

Oral glucose tolerance test [OGTT]

The animals were divided into six groups consisting of six animals in each and all the animals except normal (Group I) were induced diabetes by administering single dose of alloxan as explained above. At third day, the suspensions of standard drug glibenclamide and extract were prepared using Tween20 as suspending agent and administered to respective animals with help of oral feeding tubes according to below protocol [19].

Group I served as Normal control treated with normal saline (10ml p.o) alone, Group II served as Diabetic control treated with alloxan (150mg/kg) and vehicle, Group III was Standard treated alloxan and Glibenclamide, Group IV, V and VI were test groups treated with alloxan and 100mg/kg, 200mg/kg and 400mg/kg of methanolic extract of *Ficus microcarpa* (FMcME) respectively.

One hour after administration of extract all rats were fed with oral glucose solution (2g/kg) and blood samples from each rat were collected at different intervals of 0mins, 30 mins, 60 mins, 90mins and 120 mins and estimated for blood glucose.

Evaluation of antidiabetic activity by chronic study model

The animals were divided into six groups consisting of six animals and all the animals except normal (Group I) were induced diabetes by administering single dose alloxan before two days of the study as explained above. From the third day of the study, the suspensions of standard drug glibenclamide and extract were prepared using Tween20 as suspending agent and administered for 21 days to respective animals with help of oral feeding tubes according to below protocol.

Group I served as Normal control treated with normal saline (10ml p.o) alone, Group II served as Diabetic control treated with alloxan and vehicle, Group III was Standard treated alloxan and Glibenclamide (5mg/kg) , Group IV, V and VI were test groups treated with alloxan and 100mg/kg, 200mg/kg and 400mg/kg of methanolic extract of *Ficus microcarpa* (FMcME) respectively [20].

Blood samples from each rat were collected on day 1st, 7th, 14th and 21st and estimated for blood glucose. On last day of study blood samples had been also estimated for serum alanine transferase (SGPT or ALT), serum aspartate transferase (SGOT or AST), cholesterol, Triglycerides, urea and insulin.

Collection of blood sample and estimation of parameters

Blood samples were collected from retro-orbital plexus under mild ether anesthesia from rats. The blood glucose estimation done by GOD-POD kit using UV spectrophotometer (Shimatzu 1700). On the 21st day, serum was separated from blood samples and analyzed for serum cholesterol, serum triglycerides by enzymatic DHBS colorimetric method serum SGOT, serum SGPT, serum urea and serum insulin was estimated using standard kits.

Statistical Analysis

All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean±standard error of mean (S.E.M.) and analyzed for ANOVA and post hoc Dunnet's t-test using Graphpad prism5 software.

RESULTS

Preliminary phytochemical study

The percentage yield of the FMcME was found to be 6.67% w/w. The preliminary phyto-chemical investigation for the methanolic extract of *Ficus microcarpa* reveals the presence of flavonoids, tannins, poly phenols, steroids and carbohydrates in the leaves.

Acute toxicity studies

The single dose of 2000 mg kg^{-1} b.w. of FMcME was safe and caused neither mortality nor any signs of clinical abnormality in the tested animals during the observation period of 14 days after administration of highest dose. There was no considerable change in body weight before and after treatment of the experiment and no signs of toxicity were observed. When the experiments were repeated again with the same dose level, 2000 mg/kg body weight p.o. of extracts for 3 days more, no changes were observed for 14 days. As per the results obtained in acute oral toxicity study doses were selected as 100, 200 and 400mg/kg.

Evaluation of antidiabetic activity

Oral glucose tolerance test

In oral glucose tolerance test, animals of diabetic control group have shown significant elevation in blood glucose level through entire study when compare to normal animals. But treatment with standard drug glibenclamide and methanolic extract (200mg/kg and 400mg/kg) of *Ficus microcarpa* could able to reduce significantly (P<0.001) blood glucose level in therapeutic groups after 60 mins and 120 mins. The results of OGTT have shown in [Table No 1].

Determination of chronic antidiabetic activity

In chronic study, there was significant increase in blood glucose level (P <0.001) in diabetic control animals compare to normal animals while extract treated animals at 200mg/kg and 400mg/kg have shown significant (P <0.001) reduction in blood glucose concentrations at 14th and 21st day when compare to diabetic control animals. [Table No .2]

The concentration of insulin was found to be declined significantly (P <0.001) in diabetic control animals compare to normal animals due to the administration of alloxan. In animals treated with glibenclamide and FMcME (100mg/kg and 200mg/kg) there was significant (P<0.001) increasing in blood insulin level compare to diabetic control animals and the results were comparable to normal animals. There was increasing in the concentration of liver enzymes

in the blood was found in diabetic animals compare to normal animals. The increase in SGOT concentration was more significant (P<0.001) whereas increase SGPT concentration was less significant (P<0.01). In therapeutic animals treated with glibenclamide and FMcME (200mg/kg), there was significant.

The concentration of serum cholesterol and triglycerides in the blood was significantly (P<0.01) increased in diabetic animals compare to normal animals and there was reduction in the serum cholesterol and triglycerides concentration found in glibenclamide and FMcME (400mg/kg) treated animals when compare to diabetic control animals but the effect was less significant (P<0.01).

It is found that there is no significant change in blood urea (P>0.01) concentration in animals of diabetic control compare to normal animals and in therapeutic animals treated with glibenclamide and FMcME when compare to diabetic animals. [Table No: 3]

Table 1: Effect of methanolic extract of *Ficus microcarpa* on oral glucose administration in rats in OGTT

Treatment	Concentration of Blood glucose (mg/dl)				
	0 mins	30 mins	60 mins	90 mins	120 mins
Normal Control	91.02± 0.853	160.9± 0.886	149.9± 1.003	121.1± 1.315	141.8± 1.344
Diabetic control	316.8^{+++}± 3.696	378.6^{+++}± 1.517	387.3^{+++}± 2.267	275.6^{+++}± 17.52	333.9^{+++}± 1.614
Glibenclamide (5mg/kg)	121.8***± 1.237	180.4***± 1.077	167.2***± 1.818	142.3***± 0.759	129.0***± 1.253
FMcME 100mg/kg	162.9***± 4.9	235.9***± 1.12	225.7***± 6.85	219.0***± 2.5	209.2***± 3.78
FMcME 200mg/kg	148.8***± 1.23	203.1***± 5.32	183.3***± 1.82	171.4***± 3.32	161.2***± 1.65
FMcME 400mg/kg	121.1***± 1.85	183.0***± 2.0	160.4***± 1.98	153.6***± 1.98	149.1***± 4.62

Values are mean ± S.E.M, n=6 symbols represent statistical significance.

^{ns} P>0.05, $^{+,*}$ P <0.05, $^{++,**}$ P <0.01, $^{+++,***}$ P<0.001 vs positive control

Table 2: Effect of prolonged treatment of *Ficus microcarpa* extract on blood glucose in alloxan induced diabetic animals.

Treatment	Concentration of Blood glucose (mg/dl)			
	Day 1	Day 7	Day 14	Day 21
Normal Control	119.1±1.533	120.7±0.941	121.0±1.585	122.3±1.394
Diabetic control	334.6^{+++}±3.773	345.8^{+++}±4.094	362.4^{+++}±3.198	416.8^{+++}±4.561
Glibenclamide (5mg/kg)	370.5±1.218	282.7**±4.229	248.0***±2.166	170.2***±3.932
FMcME 100mg/kg	392.9±5.52	361.1*±5.66	349.7*±3.42	279.1***±2.25
FMcME 200mg/kg	403.2±6.65	325.0*±7.32	297.7***±2.81	197.7***±1.91
FMcME 400mg/kg	390.2±8.12	320.3***±4.85	281.20***±3.29	191.1***± 0.78

Values are mean ± S.E.M, n=6 symbols represent statistical significance.

^{ns} P>0.05, * P <0.05, ** P <0.01, *** P<0.001 vs positive control

Table 3: Effect of prolonged treatment of *Ficus microcarpa* extract Insulin, lipid profile, SGPT, SGOT and urea in alloxan induced diabetic animals.

Treatment	Insulin (µU/ml)	Cholesterol mg/dl	Triglycerides mg/dl	SGPT U/L	SGOT U/L	Urea Mg/dl
Normal Control	134.3±0.945	81.32± 1.447	103.6±1.031	72.35±0.722	136.4±0.568	78.18±1.276
Diabetic control	59.67^{+++}±0.966	104.8^{++}± 1.62	125.4^{++}±1.324	96.98^{++}±0.686	192.7^{+++}±3.170	93.27^{++}±1.060
Glibenclamide (5mg/kg)	120.4***±0.858	84.65**±0.888	104.1**±0.990	81.27**±0.681	148.9***±0.876	79.82*±0.881
FMcME 100mg/kg	82.13***±6.9	94.87^{ns}±2.25	120.08^{ns}±7.29	92.75^{ns}±5.71	186.5*±2.25	84.67^{ns}±5.71
FMcME 200mg/kg	103.8***±7.29	87.9*± 1.9	113.9*±7.41	89.03*±7.241	165.3***±1.91	81.22^{ns}±1.93
FMcME 400mg/kg	108.5***± 0.41	85.59*± 0.349	108.8*± 0.53	83.8**± 0.44	160.7***± 0.99	80.17*± 0.31

Values are mean ± S.E.M, n=6 symbols represent statistical significance.

^{ns} P>0.05, * P <0.05, ** P <0.01, *** P<0.001 vs positive control

DISCUSSION

Diabetes mellitus is a debilitating and often life threatening disorder with increasing incidence throughout the world [21]. Alloxan, a cyclic urea derivative, which selectively destroys insulin-producing pancreatic cells by free radical mediated damage and when administered to rodents cause an insulin-dependent diabetes mellitus. Hence it was reported as a potent diabetogenic agent [22] and has been widely used for the induction of experimental diabetes in animals.

In the current study, set of experiments were designed to explore the in vivo antidiabetic potential of methanolic extract of *Ficus microcarpa* leaves against alloxan induced diabetic rat model. The study was designed to evaluate the acute and chronic effects of extract in animals induced with diabetis and to compare the results with anti-diabetic potentials of reference drug glibencalmide. In the present study, the acute effect of extract was studied by Oral Glucose Tolerance Test (OGTT). For the chronic study, we have made the animals diabetic by administering Alloxan and treated the animals with varying doses of extract for 21 days. The blood glucose level in animals were estimated at 1st, 7th, 14th and 21st day of the study to evaluate the potency of the extract in clearing blood glucose in animals induced with diabetis. In additionally, we evaluated methanolic extract of *Ficus microcarpa* leaves for the enhancement of insulin secretion and hence ability to lower the blood glucose level in diabetic rats by estimating concentration of insulin in blood. We have also studied the potentials of the extract to lower the diabetic complications by assessing the lipid profile, liver and kidney function tests. For the evaluation of lipid profile, concentrations of total serum cholesterol and triglycerides were estimated. The liver enzymes alanine transferase and aspartate transferase were estimated to study the hepatic function and blood urea was estimated to study the renal function.

The oral glucose tolerance test is performed to study the acute effects of extract in diabetic animals and it based on ability of the

body to utilize or tolerate the glucose load administered orally [23,24].

In OGTT, induction of diabetes in toxic control rats resulted in increased concentration of blood glucose due to inability of the system to utilize glucose in the absence of insulin. But in extract and glibenclamide treated therapeutics animals blood glucose level was significantly reduced than control group which clearly shows the ability of the extract to increase the utilization of the glucose by cells and tissues.

In chronic study, diabetes was induced in all animals except normal group by administering alloxan before three days of study. Hence there was significant decrease in insulin secretion in diabetic animals due to the destruction pancreatic cells which resulted in decreased utilization of glucose and hence the blood glucose level was elevated. But in therapeutic groups treated with standard drug glibenclamide, FHME (200mg/kg and 400mg/kg), significant increase in insulin release and subsequent decrease in blood glucose concentration was found.

The diabetes mellitus is a chronic metabolic disorder and it is also associated with several secondary complications such as hyperlipidemia, atherosclerosis, hypertension, diabetic nephropathy, diabetic neuropathy and diabetic keto acidosis. Hyperlipidemia is one of such common complication of diabetes which is characterized by increase in serum total cholesterol (TC), triglycerides (TG), LDL and VLDL. The azotemia is condition which is due to the accumulation of nitrogenous waste products like urea and creatinine in blood and usually found during diabetic nephropathy [25,26].

Accelerated Coronary and peripheral vascular atherosclerosis is one of the most common and serious chronic complications of long term diabetes mellitus. Along with other risk factors such as hypertension, smoking, obesity etc., increasing importance has been given to secondary hyperlipidaemias in the causation of accelerated atherosclerosis. Hyperlipidaemia as a metabolic abnormality is frequently associated with diabetes mellitus. The most characteristic lipid abnormality in diabetics is hypertriglyceridaemia, with or without associated increase in plasma cholesterol [27, 28, 29].

In our present study administration of alloxan in control animals caused elevation of serum cholesterol, triglycerides, and urea as a consequence of secondary complications of diabetes. In animals of therapeutic groups treated with FMcME (200mg/kg and 400 mg/kg) have shown significant reduction in above serum parameters.

The association between liver disease and diabetes mellitus is well known, the overall prevalence being significantly higher than that expected by a chance association of two very common diseases. More recently, new insights into this association came from the recognition that diabetis mellitus itself may be a cause of liver disease, via non-alcoholic fatty liver disease, nonalcoholic steatohepatitis, cirrhosis, and ultimately hepatocellular carcinoma. The liver damage leads to its malfunctioning and it is characterized by the increased concentrations of liver enzymes ALT and AST in the blood [30,31]. In the present study, elevation of the liver enzymes AST and ALT was found in diabetic control animals but in animals treated with extract at higher dose have shown the normal blood concentrations of those enzymes.

The kidney acts as a filter of the blood, removing the wastes from the blood. Over years of high blood sugar levels, damage occurs to the fine blood vessel walls in the kidney filters, and these filters become leaky. The earliest sign of diabetic damage to kidneys is protein in the urine. A urine and blood test is performed to check for this. The presence of microalbuminuria, or an albumin : creatinine ratio greater than 2.5 in a diabetic is suggestive of diabetic kidney damage (diabetic nephropathy). The reduced renal function is always associated with azotaemia which is characterized by accumulation of nitrogenous waste creatinine, urea and uric acid in the blood [32].

In the above study, concentration of blood urea was estimated to evaluate renal function in diabetic animals. In control animals treated with alloxan alone there was significant increase in blood urea level whereas chronic treatment with our methanolic extract for 21 days could able to normalize blood urea concentration in test animals and the results were comparable to standard group treated with glibenclamide.

Although the exact mechanism of action of alloxan is not fully understood, evidences indicate that the alloxan causes pancreatic β cell damage followed by insulin deficiency and diabetes mellitus [33, 34, 35]. In the present study the extract had been successful to maintain the normal glucose level in the therapeutic animals and increased insulin secretion.

CONCLUSION

The methanolic extract of *Ficus microcarpa* posses significant antidiabetic activity in alloxan induced diabetic animal model. But further study is required to evaluate the antidiabetic activity from the isolated compounds from the plant extract.

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