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Research Article

EVALUATION OF ANTIDIABETIC ACTIVITY OF METHANOLIC LEAF EXTRACT OF *FICUS CARICA* IN ALLOXAN - INDUCED DIABETIC RATS

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ABSTRACT

Ficus carica Linn. (Moraceae) is commonly known as Edible fig. The leaves, roots, fruits and latex of the plant are medicinally used in different diseases. In traditional medicine the roots are used in treatment of leucoderma and ringworms and its fruits which are sweet, have antipyretic, purgative, aphrodisiac properties and have shown to be useful in inflammations and paralysis. The Present investigation was carried out to study the antidiabetic effect of the methanolic extract of Ficus *carica* (MEFC) in alloxan induced diabetic rats. The LD50 determination was done in mice as per OECD guidelines 423. The rats were divided into five groups. Diabetes was induced using alloxan and the treatment was continued for 21 days using Metformin (500 mg/kg p.o) as a standard drug. Blood glucose level, bodyweight, biochemical parameters and histopathological observation were done. The methanolic extract (200 mg/kg p.o) had shown significant ($p < 0.01$) antidiabetic activity than (100 mg/kg p.o) by showing a reduction in blood glucose levels and triglycerides compared to pretreatment levels. The results indicate that, methanolic extract of *Ficus carica* have prominent antidiabetic effect and can therefore be used as an alternative remedy for the treatment of diabetes mellitus and its complications.

Keywords: *Ficus carica* , alloxan -induced diabetic rats, LDL,TG, Total cholesterol.

INTRODUCTION

Diabetes mellitus (DM) is common endocrine disorder affecting more than 200 million people worldwide[1]. According to the International Diabetes Federation, India has been declared as the diabetes capital of the world[2]. Plant materials which are being used as traditional medicine for the treatment of diabetes are considered one of the good sources for a new drug or a lead to make a new drug .Despite the introduction of many anti hyperglycemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major medical problem[3]. Many indigenous Indian medicinal plants have been found to be useful in managing diabetes. One of the great advantages of medicinal plants is that these are readily available and have no side effects.

Diabetes mellitus (DM) comprises a group of common metabolic disorders characterized by hyperglycemia. Many distinct types of DM exist and the etiology being a complex interaction of genetics, environmental factors, and life-style choices. Depending on the cause for the DM, factors contributing to hyperglycemia may include reduced insulin secretion, decreased glucose utilization, and increased glucose production[4,5]. The metabolic dysregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system.

Figs (*Ficus carica* L.) are a widespread species commonly grown, especially in warm, dry climates. Figs have been grown for centuries and are the most frequently mentioned fruit in the Bible6. *Ficus carica* Linn. (Syn: *Ficus sycomorous*; Family: Moraceae) is commonly referred as "Fig". Its fruit, root and leaves are used in the native system of medicine in different disorders such as gastrointestinal (colic, indigestion, loss of appetite and diarrhea), respiratory (sore throats, coughs and bronchial problems), inflammatory and cardiovascular disorders[6-8]. Fig has been traditionally used for its medicinal benefits as metabolic, cardiovascular, respiratory, antispasmodic and anti-inflammatory[9-11] remedy.

The root is tonic, useful in leucoderma and ringworm. The fruit is sweet, antipyretic, tonic, purgative useful in inflammation, weakness, paralysis, thirst "*Vatta* diseases" of head, diseases of liver and spleen, pain in chest, cures piles, stimulate growth of hair. The milky juice is expectorant, diuretic, and dangerous for eye. Fig latex is used as an anthelmintic[12].The *Ficus carica* leaf has been reported hypoglycemic, hepatoprotective[13] and latex reported the anthelmintic activity[14].

MATERIALS AND METHODS

Plant collection

Fresh leaves of the plant were collected from Chirala, Andrapradesh, India during the month of August 2011. The plant was identified and authenticated by Dr.M.Raghuram, Assistant professor, Department of Botany & Microbiology, Acharya Nagarjuna University, Guntur, Andrapradesh, India.

Preparation of extracts

The leaves of plant were dried in shade, separated and made to dry powder. It was then passed through the 40 # sieve. A weighed quantity (200g) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. Percentage yield of methanolic extract *Ficus carica* of was found to be 16.5% w/w.

Animals Used

Albino-Wistar rats (200-250g) and Swiss albino mice (20-25g) of either sex were used. The selected animals were housed in Polypropylene cages in standard environmental conditions (20–25ºC), fed with standard rodent diet and water ad labium. The animals were exposed to alternate 12 hrs of darkness and light each. All experiments were performed in the morning according to current guidelines for investigation of experimental pain in conscious animals. The experiment protocols were approved by Institutional Animal Ethical Committee.

Chemicals

Alloxan Monohydrate was purchased from Sigma Aldrich Chemicals Pvt, Ltd, Bangalore. All other chemicals and reagents used were of analytical grade.

Oral acute toxicity studies

Acute toxicity was generally carried out for the determination of LD_{50} value in experimental animals. The LD_{50}determination was done in mice by OECD guidelines 423. The aim of performing acute toxicity study is for establishing therapeutic index of particular drug and to ensure safety *invivo.*

Antidiabetic Activity

Experimental induction of diabetes

All animals were allowed to adapt to cages for 3 days, after which they were fasted overnight and 150 mg/kg of alloxan monohydrate freshly dissolved in normal saline was injected intra-peritoneally. Alloxan induces the diabetes by damaging the insulin secreting cells of the pancreas leading to hyperglycemia. After alloxan treatment, all animals were given free access to food and water. Blood glucose levels were measured 2 days after alloxan injection and used as parameters to obtain matching pairs of rats with diabetes of similar level of severity. Only rats with fasting blood glucose levels greater than 200 mg/dl were considered to be diabetic and were used in the experiment.

Experimental Design

Experimental rats were divided into five groups of six animals each and treated for 21 days as follows.

Group I: Normal control received 1% CMC (10 ml/kg)

Group II: Diabetic controls received 1% CMC (10 ml/kg)

Group III: Animals Received Methanolic extract of *Ficus Carica* (100 mg/kg, p.o)

Group IV: Animals Received Methanolic extract of *Ficus Carica* (200 mg/kg, p.o)

Group V: Animals Received Metformin (500 mg/kg p.o).

The values of sample treated were compared with that of the standard group which was treated with Metformin.

Testing of fasting blood glucose level

Fasting blood glucose levels were measured on 0, 3, 14, and 21 days of treatment of methanol extract of *Ficus carica* Linn from the animals of all these groups. Blood was collected from tip of the tail vein and fasting blood glucose level was measured using single touch glucometer .The results were expressed in terms of milligram per deciliter (dL) of blood. The body weight of each animal was noted. At the end of the experimental period, all the animals were sacrificed by decapitation and blood was collected with anti-coagulant and the serum was used for the estimation of total cholesterol and triglycerides.

Histopathological study of pancreas

Pancreatic tissues from all groups were subjected to histopathological studies. The whole pancreas from each animal was removed after sacrificing the animal and was collected in 10% formalin solution and immediately processed by paraffin technique. Sections of 5µm thickness was cut and stained by hematoxyllin and eosin.

Statistical analysis

All the values were expressed as mean ± SEM (standard error mean) for six rats. Statistical analysis was carried out by using PRISM software package (version 5.0). Statistical significance of differences between the control and experimental groups was assessed by One-way ANOVA followed by Dunnets Multiple Comparison Test. The value of probability less than 5% ($P < 0.05$) was considered statistically significant.

RESULT AND DISCUSSION

Phytochemical analysis

Preliminary Phytochemical screening of methanol extract tested positive for alkaloids, flavonoids, coumarins, saponins, sterols and terpenes.

Oral Acute Toxicity Studies

Oral administration of methanolic extract of *Ficus carica* in mice caused death and behavioural changes at 2000mg/kg b.wt. (table.1). LD_{50} value was found to be 1000 mg/kg.

Antidiabetic Activity

The effect of repeated oral administration of methanolic extract of Ficus carica(MEFC) on blood glucose levels in alloxan-diabetic rats is presented in table- 2 , and the effect on body weight is presented in table- 3. MEFC, administered at doses of 100 & 200 mg/kg to alloxan-treated diabetic rats caused significant ($p < 0.01$) reduction of blood glucose levels which was related to dose and duration of treatment (fig.No.1). Maximum reduction was observed on day 21.gradual increase in body weight was also observed. MEFC 200 mg/kg exhibited maximum glucose lowering effect in diabetic rats. Metformin exhibited significant reduction in blood glucose levels at the end of the study when compared to diabetic control.

Estimation Of Biochemical Parameters

MEFC showed a dose related significant ($p < 0.01$) reduction in triglycerides compared to pretreatment levels (Table 4). MESR at the doses of 100 and 200 mg/kg was dose dependently reduced the Total cholesterol, LDL, VLDL, TG levels than diabetic control rats.

Table 1: **LD_{50} of methanolic extract of *Ficus carica***

Sl. No	NO OF ANIMALS	DOSE	No. of death of animals
1	3	5 mg/kg	0
2	3	50 mg/kg	0
3	3	300 mg/kg	0
4	3	2000mg/kg	1

LD 50 =1000mg/kg ; ED 50 =100mg/kg

Table 2 : Effect Of MEFC On Blood Glucose Level In Alloxan Induced Diabetic Rats (24 Hrs Study).

GROUPS	TREATMENT	DOSE	0HR	2HR	4 HR	6 HR	24 HR
I	Normal Control (1 % CMC)	10 ml/kg	87.16±1.5	87.16±2.8	88±2.1	88±2.9	88.83±2.6
II	DiabeticControl (1% CMC)	10 ml/kg	271.9.5±5.3***	282.3±1.6***	332±1.8**	379±2.7**	407±2.5***
III	Methanolic extract of *Ficus carica*	100 mg/kg	262.2±3.5##	257.4±1.6	248.3±1.4###	224±2.1#	215±2.4##
IV	Methanolic extract of *Ficus carica*	200 mg/kg	264.7±2.7##	253±2.1##	241±1.5#	219±1.4##	201±2.1###
V	Metformin	500 mg/kg	269.5±1.1#	249±1.3##	221±1.4##	211±1.6##	181±2.5###

All Values are expressed as Mean ±SEM (n=6) , *p≤0.05, **p≤0.01, ***p≤0.001
***Group I compared with Group II, # Group II compared with Group III, IV, and V.**

Table 3: Effect of MEFC on body weight in alloxan induced diabetic rats (21day study).

GROUPS	TREATMENT	DOSE	0 Day	7Day	14Day	21Day
I	Normal Control (1 % CMC)	10 ml/kg	230±2.1	233.6±2.4	240.7±2.2	244±1.4
II	Diabetic Control (1% CMC)	10 ml/kg	230.5±3.5***	228±4.3***	225.9±4.1***	212±3.1***
III	Methanolic extract of *Ficus carica*	100 mg/kg	229.5±3.2	230.6±2.7	234.5±2.1##	237.5±2.3##
IV	Methanolic extract of *Ficus carica*	200 mg/kg	230.4±4.2	232±3.6#	238.2±2.7#	240.6±2.2###
V	Metformin	500 mg/kg	230±2.4	232.2±2.4	239.4±2.1	242.5±1.7###

All Values are expressed as Mean ±SEM (n=6) , *p≤0.05, **p≤0.01, ***p≤0.001
***Group I compared with Group II, # Group II compared with Group III, IV, V.**

Table 4: Effect of MEFC on biochemical parameters measured in alloxan induced diabetic rats (21day study).

GROUPS	TREATMENT	DOSE	CHOLESTEROL	TRIGLYCERIDES	HDL	LDL
I	Normal Control (1 % CMC)	10 ml/kg	107.66±6.62	80.66±5.60	66.5±5.85	22.23±3.82
II	Diabetic Control (1% CMC)	10 ml/kg	144±4.56***	179.33±8.26***	48.33±6.2***	52.66±7.52***
III	Methanolic extract of *Ficus carica*	100 mg/kg	119.6±4.88###	113.66±5.64###	50.5±7.91#	41±4.42 ###
IV	Methanolic extract of *Ficus carica*	200 mg/kg	114.66±4.92###	103.83±6.70###	53.66±6.43###	32.33±4.31###
V	Metformin	500 mg/kg	110.16±5.41###	91.33±7.31#	57.66±7.78###	25±6.24###

All Values are expressed as Mean ±SEM (n=6) , *p≤0.05, **p≤0.01, ***p≤0.001
***Group I compared with Group II, # Group II compared with Group III, IV, V**

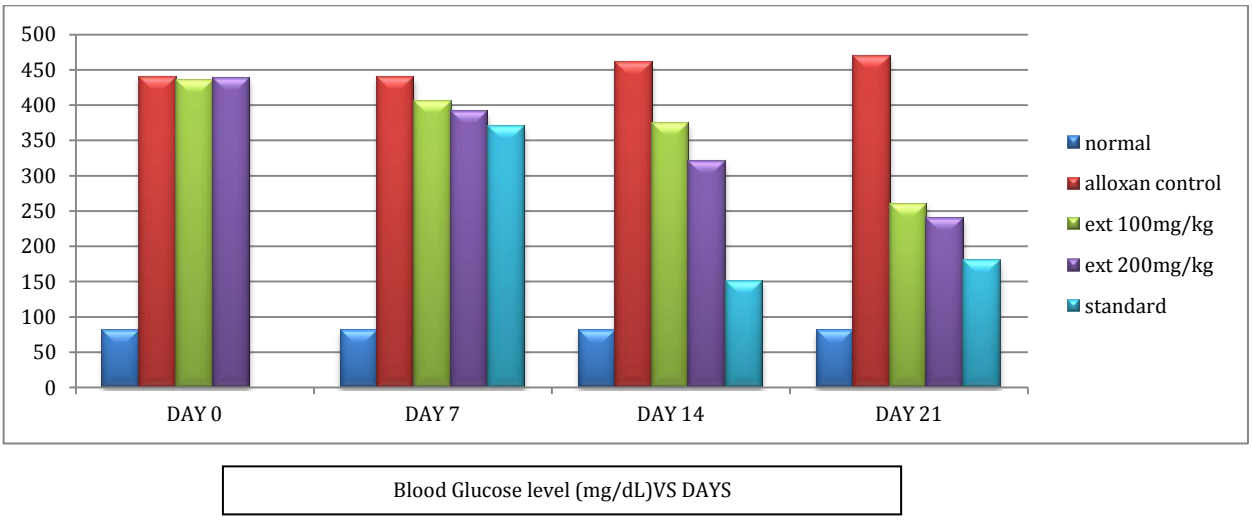

Fig 1: Effect of MEFC on blood glucose level in alloxan induced diabetic rats (21day study).

CONCLUSION

The hypothesis of obtaining plant based medicine is beneficial to human health. Based on the *in vivo* experimental study and the active profile exposed through various biochemical parameters it can be concluded that the methanolic extract of leaves of Ficus carica showed significant antidiabetic activity. Further investigations on the isolation and identification of Bio active components on the plant would help to ascertain its potency.

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