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Research Article

An Evaluation of the Preventive Role of *Ficus religiosa* on High Fat Diet and Low Dose Streptozotocin Induced Diabetes Mellitus in Rats

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Abstract

Objective

To evaluate the protective role of *Ficus religiosa* in the development of diabetes and inflammation in high fat diet and low dose Streptozotocin (STZ) induced diabetic rat models.

Materials and methods

30 male adult Wistar rats were divided into 5 groups (n = 6). Rats in group I were fed normal pellet diet for 4 weeks and were taken as normal control. Rats in groups II-V were first fed with high fat diet for 4 weeks and were then given an intraperitoneal injection of low dose of Streptozotocin (30 mg/kg body weight). Group II was taken as diabetic control while rats in groups III-V were treated with *Ficus religiosa* in a dose of 100, 200, and 400 mg/kg body weight respectively. Body weight, fasting plasma glucose, and plasma Tumor Necrosis Factor (TNF)-α levels were measured for evaluation.

Results

Ficus religiosa in all the three doses was found to be effective in preventing a significant increase in fasting plasma glucose while it was effective in preventing a significant increase in body weight and plasma tumor necrosis factor-α level in a dose of 400 mg/kg body weight.

Conclusion

Treatment with *Ficus religiosa* shows protection against elevation of fasting plasma glucose and plasma Tumor necrosis factor -α level in rats exposed to risk factors like high fat diet and streptozotocin and suggest a protective role in the development of diabetes mellitus.

Keywords: Diabetes mellitus; *Ficus religiosa*; High Fat Diet (HFD); Streptozotocin (STZ); TNF-α

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Introduction

Diabetes mellitus is an 'iceberg' disease, as a large number of individuals who meet the current criteria for diabetes mellitus remain asymptomatic and unaware of having this disorder. Once regarded as a single disease entity, diabetes is now seen as a heterogenous group of diseases, characterized by a state of chronic hyperglycemia resulting from a diversity of etiologies, both environmental and genetic, acting jointly. Depending on the etiology, factors contributing to this state of chronic hyperglycemia include reduced insulin secretion, decreased glucose utilization, and increased glucose production. These metabolic dysregulations further cause secondary pathophysiologic changes in multiple organ systems which impose a tremendous burden on the individual with diabetes and also on the national health care system [1]. Studies in recent years have shown that chronic low-grade inflammation, more specifically inflammatory cytokines (mainly IL 1, TNF-α, and IL 6) [2] also contribute to the development of type 2 diabetes mellitus and its microvascular complications [3-5].

Diabetes mellitus has recently been identified by Indian Council of Medical Research (ICMR) as one of the refractory diseases for which satisfactory treatment is not available in modern allopathic system of medicine. Furthermore, allopathic system do not have any safer and effective drug which could reduce the risk or delay the development of diabetes mellitus in high risk individuals. WHO (1980) has also recommended the evaluation of the effectiveness of plants in conditions where there are no safe modern drugs [6]. Moreover the diabetes mellitus type-2 is increasing as an epidemic in developed and developing countries across the world and there is no satisfactory preventive treatment except the life style changes.

It is therefore evident to harness and harvest medicinal plants having multiple beneficial effects which can not only take care of most of the metabolic abnormalities associated with diabetes [7,8] but can also reduce the risk of developing or delay the onset of diabetes mellitus amongst susceptible persons. Therefore in search of effective alternative therapy we have taken the plant herb *Ficus religiosa* having multiple beneficial medicinal effects [9-11] but the work for its role in diabetes mellitus type-2 is in infancy.

The present study was therefore envisaged to discern the protective role of *Ficus religiosa* in the development of diabetes and inflammation in high fat diet and low dose streptozotocin induced diabetic rat models, which has not been evaluated so far.

Materials and Methods

Experimental animals

Thirty (30) healthy adult male Wistar rats having similar physical constitution (in terms of age, body weight), weighing between 140-160 gms and bred in the Committee for the Purpose of Control and Supervision of Animals (CPCSEA) certified animal house

(Indian Institute of Toxicology Research (IITR), Lucknow) were used in the study. They were housed in well-ventilated Institutional Animal House Facility (King George's Medical University (KGMU), Lucknow) under standard laboratory condition of temperature and humidity (25 ± 2°C, 70%). They were provided pellet food (either normal or 15% high fat diet) and water *ad libitum* with 12 hours light/ dark cycle. The use of these animals and the study protocol was approved by the Institutional Animal Ethics Committee (IAEC), KGMU, Lucknow.

Diet

High Fat Diet (HFD) containing 16% crude protein, 15% crude fat (prepared from rice bran), 2.30% acid insoluble ash, and 8% moisture was manufactured and supplied by Dayal Industries Pvt. Ltd. Barabanki Road, Lucknow, Uttar Pradesh.

Drugs and chemicals

Dried powder form of *Ficus religiosa* (Peepal) was procured from Vyas Pharmaceuticals, Indore. It was administered in three doses i.e., 100 mg/ kg body weight [12,13], 200 mg/ kg body weight [13], and 400 mg/ kg body weight. Streptozotocin (STZ) was procured from Merck, KGaA, Germany. It was reconstituted in Na-Citrate buffer of strength 0.1 M and pH 4.5 and given intraperitoneally in a dose of 30 mg/kg body weight. Eco- Pak Glucose kit for plasma glucose estimation was procured from Accurex Biomedical Pvt. Ltd. For the estimation of plasma TNF-α level, AviBion TNF-α ELISA kit was used which was procured from Orgenium Laboratories Business Unit, Finland.

Route of drug administration

The test drug was formulated as a suspension using distilled water in a way that 1 ml of the suspension contained the desired dose that was to be administered in an individual rat. The test drug was given once daily orally with the help of a feeding cannula.

Experimental protocol

After 7 days of acclimatization, the rats were divided randomly into 5 groups and were given the following treatment and water *ad libitum* for 4 weeks:

Group I/ *Ficus Religiosa* Control (FRC, n = 6): *Ficus religiosa* (200 mg/kg) + normal pellet diet

Group II/ Diabetic Control (DC, n = 6): Distilled water + High Fat Diet (HFD)

Group III (n = 6): *Ficus religiosa* (100 mg/kg) + HFD

Group IV (n = 6): *Ficus religiosa* (200 mg/kg) + HFD

Group V (n = 6): *Ficus religiosa* (400 mg/kg) + HFD

A baseline body weight of all rats was recorded on day 0 of the study and blood sample was drawn for baseline biochemical evaluation of fasting plasma glucose and plasma TNF-α levels.

After 4 weeks of treatment, the body weight of rats in group I was recorded and blood sample was withdrawn for fasting plasma glucose levels while rats in groups II- V were given an intraperitoneal injection of a low dose (30 mg/ kg body weight) of Streptozotocin (STZ) for induction of type 2 diabetes mellitus [14-16] which is as follows:

Group II/ Diabetic Control (Distilled water and HFD treated group) + STZ (30 mg/kg)

Group III, IV & V (*Ficus religiosa* and HFD treated group) + STZ (30 mg/kg)

One week after STZ injection i.e., at the end of the 5th week of the study, body weight of rats of groups II- V was recorded and blood sample was drawn for biochemical evaluation of fasting plasma glucose and plasma TNF-α levels.

Biochemical Analysis

The biochemical evaluation of the samples was done in the Department of Pathology, King George's Medical University, Lucknow.

Fasting plasma glucose level

The estimation was done using Eco-Pak Glucose kit based upon an enzymatic method using Glucose Oxidase and Peroxidase enzyme and a spectrophotometer.

Estimation of plasma TNF-α

The plasma TNF-α level was estimated by an Enzyme-Linked Immune-Sorbent Assay (ELISA) using a commercial TNF-α ELISA kit, according to instructions given in it. The absorbance is then measured at 450 nm by a microplate photometer. The standard curve is generated and used to determine the amount of TNF-α present in an unknown sample.

Statistical Analysis

All values were expressed as means ± SD. The data was analyzed by one way Analysis of Variance (ANOVA) and one-sample Kolmogorov-Smirnov test. Independent t test/ Tuke's pairwise comparison was used for comparison between the treatment groups. Differences in treatment effects within groups and between the treatment and control groups were tested by a multivariate analysis of variance repeated-measures design with treatment type as a between-subject factor (2 groups) and treatment effect (baseline compared with follow-ups) as a within-subject factor. Statistical significance was based on a two-tailed p value < 0.05.

Observations and Results

Baseline study

The baseline (day 0) mean body weight, Fasting Plasma Glucose (FPG), and plasma TNF-α level of 5 groups (groups I-V) of rats are summarized in table 1. The one way Analysis of Variance (ANOVA) indicated that the baseline mean body weight, Fasting Plasma Glucose (FPG), and TNF-α levels did not differ significantly between all the five groups (p > 0.05). Hence, all the groups were comparable (Figure 1).

Variables	Controls		Treatment Groups		
	Group I (FRC)	Group II (DC)	III (FR 100 mg/kg)	IV (FR 200 mg/kg)	V (FR 400 mg/kg)
BW	227.3 ± 26.1	227.3 ± 25.9	203.5 ± 10.1	212.3 ± 29.9	201.5 ± 32.7
FPG	126.8 ± 13.0	90.7 ± 17.2	84.8 ± 14.3	102.7 ± 15.5	108.7 ± 15.6
TNF-α	4.68 ±1.04	5.5 ± 1.8	6.3 ± 0.5	4.7 ± 2.1	7.2 ± 1.8

Table 1: Baseline (day 0) Mean (± SD) Body Weight (BW, grams), Fasting Plasma Glucose (FPG, mg/dl), and Plasma TNF-α (pg/ml) levels in 5 groups of rats (n = 6).

DC: Diabetic and hyperlipidemic Control; FRC: *Ficus Religiosa* Control; III: *Ficus religiosa* 100 mg/kg; IV: *Ficus religiosa* 200 mg/kg; V: *Ficus religiosa* 400 mg/kg

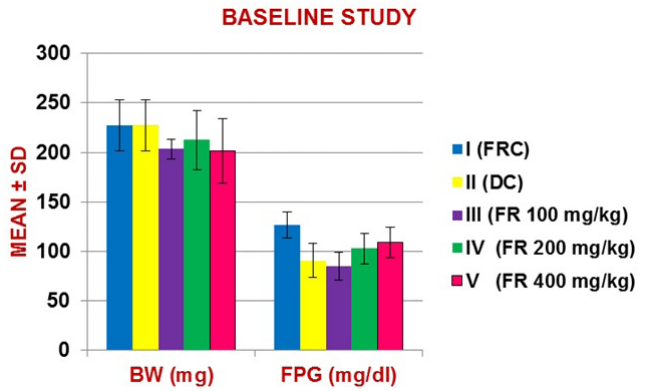

Figure 1: Baseline (day 0) Mean (± SD) body weight and Fasting Plasma Glucose (FPG, mg/dl) levels in 5 groups of rats (n=6).

Effect of *Ficus religiosa* on normal rats

The effect of test drug (*Ficus religiosa*) on Fasting Plasma Glucose (FPG) in normal rats (group I) is depicted graphically in figure 2, which shows that after 4 weeks of drug treatment there was a marginal, non-significant decrease in FPG (- 2.37%).

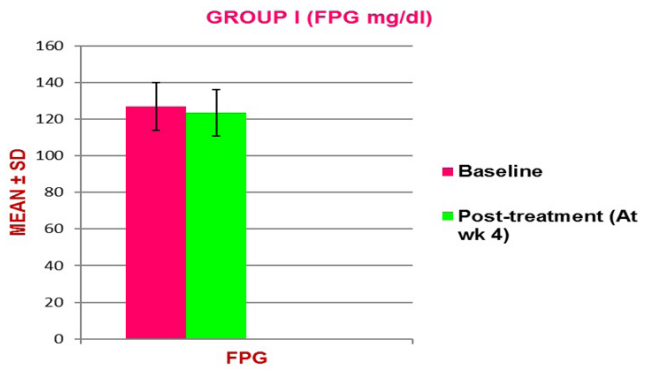

Figure 2: Baseline and post-treatment mean (± SD) Fasting Plasma Glucose (FPG) in normal rats (group I).

Effect of *Ficus religiosa* on biochemical markers

The baseline (day 0) and post-treatment (week 5) mean Body Weight (BW), Fasting Plasma Glucose (FPG), and plasma TNF-α levels of four groups of rats (groups II-V) are summarized in table 2, and shown graphically in figures 3,4.

TNF-α level was significantly increased from baseline to week 5 (5.46 ± 1.79 to 7.61 ± 1.27, p = 0.002) in group II. Table 2, depicts the average percent change in the level of TNF-α from baseline to week 5 in four groups, II-V.

Body weight was significantly increased from baseline (day 0) to week 5 in Groups II (p = 0.03), III (p = 0.001), and IV (p = 0.04).

There was an increase in the level of FPG from baseline to week 5 in groups II-IV. Fasting Plasma Glucose (FPG) level was significantly increased from baseline to week 5 (90.67 ± 17.15 to 184.67 ± 17.41, p = 0.001) in Group II. Table 2, depicts the average percent change in the level of FPG from baseline to week 5 in groups II-V.

Variables	Groups	Periods		Baseline to Week 5	
		Baseline (day 0)	Post-treatment (week 5)	% mean change	p-value[1]
Body Weight	II	227.3 ± 25.9	257.8 ± 1.9	6.11	0.03*
	III	203.5 ± 10.1	243.3 ± 7.1	19.87	0.001*
	IV	212.3 ± 29.9	247.3 ± 26.1	17.98	0.04*
	V	201.5 ± 32.7	213.8 ± 17.0	11.32	0.46
FPG	II	90.7 ± 17.1	184.7 ± 17.4	107.03	0.001*
	III	84.8 ± 14.3	101.2 ± 15.5	22.63	0.15
	IV	102.7 ± 15.5	111.8 ± 30.7	13.06	0.58
	V	108.7 ± 15.6	105.5 ± 4.4	-2.83	0.73
TNF-α	II	5.5 ± 1.8	7.6 ± 1.3	47.49	0.002*
	III	6.3 ± 0.5	7.4 ± 0.7	35.96	0.03*
	IV	4.7 ± 2.1	5.0 ± 2.1	28.93	0.04*
	V	7.2 ± 1.8	6.0 ± 0.9	26.46	0.83

Table 2: Baseline (week 0) and Post-treatment (week 5) Mean (± SD) body weight (grams), Fasting Plasma Glucose (FPG, mg/dl), and Plasma TNF-α (pg/ ml) level summary in rats of groups II-V.

[1]Paired t-test, *Significant

II: Diabetic and hyperlipidemic control; III: Ficus religiosa 100 mg/kg; IV: Ficus religiosa 200 mg/kg; V: Ficus religiosa 400 mg/kg

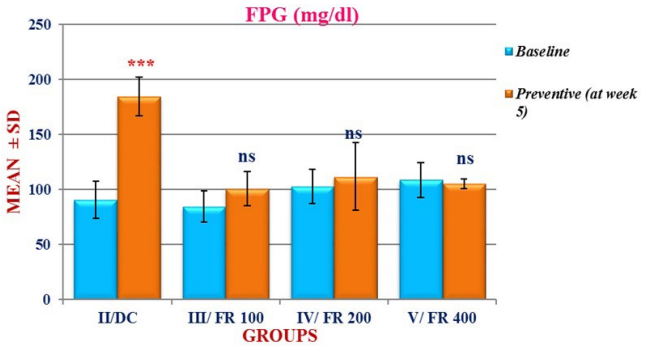

Figure 3: Baseline and Post-treatment mean (± SD) Fasting Plasma Glucose (FPG) in four groups of rats (groups II-V).

***p< 0.001 (highly significant); ns: p > 0.05 (not significant)

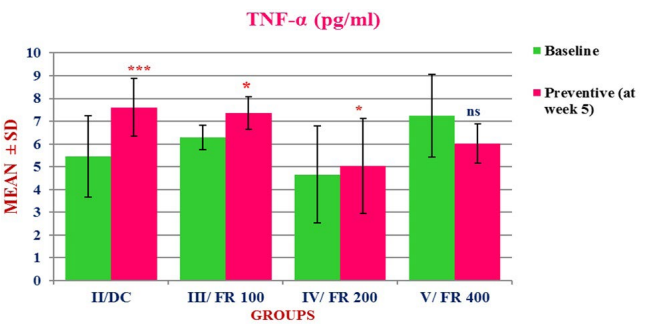

Figure 4: Baseline and Post-treatment mean (± SD) plasma TNF-α in four groups of rats (groups II-V).

***p< 0.001 (highly significant); *p< 0.05 (significant); ns: p> 0.05 (not significant)

Discussion and Conclusion

The present study was conducted to discern the protective role of *Ficus religiosa* in development of diabetes and inflammation in high fat diet and low dose streptozotocin induced diabetic rat models.

Insulin resistance is one of the most important risk factors associated with type 2 diabetes and is polygenic and multifactorial in origin. Chronic low grade inflammation and activation of innate immunity as underlying factors in the pathogenesis of insulin

Variables	Groups		Baseline		At week 5	
			Mean difference	p-value	Mean difference	p-value
FPG	Gr II vs	III	5.83	0.99	83.5	<0.001*
		IV	12	0.91	72.83	<0.001*
		V	18	0.53	79.16	<0.001*
TNF- α	Gr II vs	III	0.83	0.99	0.24	1
		IV	0.8	0.99	2.57	0.05
		V	1.78	0.61	1.59	0.64

Table 3: Significance of fasting plasma glucose (FPG, mg/dl) and plasma TNF-α (pg/ml) in four groups of rats (II to V).

resistance and type 2 diabetes mellitus is also now well established. Various studies have suggested that elevated levels of inflammatory markers (mainly IL 1, TNF-α, and IL 6) are independent predictors of type 2 diabetes mellitus [17,18]. TNF-α may cause insulin resistance through the following possible mechanisms: a) Direct inhibitory effect on glucose transporter protein GLUT4, insulin receptor substrates, or glucose-stimulated insulin release by pancreatic β-cells, or b) Indirect effect by increasing free fatty acid oxidation, stimulation of insulin counter-regulatory hormones or cytokines [e.g., IL-6, TNF-α and C-Reactive Protein (CRP), or impairment of endothelial function.

Modern allopathic drugs till date have not been a concrete solution in reducing the risk of developing diabetes mellitus or delaying the onset of diabetes mellitus in susceptible individuals. It is therefore evident to look for options that belong to safer indigenous system of medicines. *Ficus religiosa* is known to have multiple beneficial effects and has been extensively used in traditional medicine for a wide range of ailments of the central nervous system, endocrine system, gastrointestinal tract, reproductive system, respiratory system, and infectious disorders. However, this plant has not been studied for its preventive effect in diabetes mellitus. With the ayurvedic concept of management in mind we have chosen to evaluate the effect of *Ficus religiosa* in the prevention of development of diabetes mellitus in the present study.

Effect of *Ficus religiosa* on normal rats (Group I, *Ficus religiosa* 200 mg/kg body weight)

Results showed that there was no significant change in FPG level in rats of group I after 4 weeks of administration of 200 mg/ kg body weight of *Ficus religiosa* (Figure 2). This suggests that *Ficus religiosa* do not have hypoglycemic effect on normal rats. Based upon this observation, we may therefore conclude that *Ficus religiosa* did not lower the plasma glucose level in normal rats.

Preventive Study

Groups III, IV, and V were evaluated for the preventive effects of *Ficus religiosa* in the development of diabetes mellitus in susceptible subjects exposed to risk factors like high fat diet and low dose STZ. They were given *Ficus religiosa* in a dose of 100, 200, and 400 mg/kg body weight respectively along with High Fat Diet (HFD) for 4 weeks. We used the purified dried powder form in our study as powder form usually contains most of the active compounds. Moreover it is a safer form to consume as a drug as compared to other forms like extract.

Toxicity study to determine the safe oral dose of *Ficus religiosa* was not done in our study because acute toxicity tests, as per Organisation for Economic Co-operation and Development (OECD) 423 guidelines (2010) have already been conducted in many previous studies. Khan et al., [19] (2010) reported that methanolic extract of *Ficus religiosa* did not produce any acute toxicity, or significant behavioral change, or mortality up to an oral dose of 2000 mg/ kg in male Wistar rats.

As compared to baseline, the body weight in diabetic control group i.e. group II (p=0.03) and in groups III (p = 0.001) and IV (p = 0.04) showed a significant increase while the increase in body weight of group V was not found to be statistically significant (Table 2).

On comparing the results between the three treatment groups, the percent increase in mean body weight was found to be least in group V (+ 11.32%) followed by group IV (+ 17.98%), with a maximum increase in group III (+ 19.87%) (Table 2). These results suggest that *Ficus religiosa* at a higher dose (400 mg/kg) may have a protective effect in preventing high fat diet induced weight gain (Table 2).

As compared to baseline, although the FPG at week 5 showed an increase in groups II, III, and IV, but the increase was found to be statistically significant only in group II (Table 2). In group V, a decrease in FPG was observed which was statistically not significant. When compared with group II, the FPG values of groups III, IV, and V were found to be significantly (p<0.001) less (Table 2 and Figure 3). Thus, as hypothesized in our study, *Ficus religiosa* in all the three doses was found to be effective in preventing a significant increase in FPG after 4 weeks of HFD f/b STZ injection.

If we see the comparison between the three treatment groups (i.e., III-V) then it was observed that though both groups III and IV did not show a significant increase in FPG from baseline, but the percent mean increase from baseline in group III (+ 22.63 %) was higher than group IV (+ 13.06 %) (Table 2). The best response was however seen in group V, which did not cause any increase in FPG levels from baseline.

We may thus infer from these findings that although all the three doses of *Ficus religiosa* were effective in preventing the development of a state of hyperglycemia, but *Ficus* at a dose of 400 mg/kg was found to be more effective than 200 mg/kg which in turn had a better effect than at a dose of 100 mg/kg (Table 2).

Plasma TNF-α level at week 5 increased significantly in group II (p = 0.002), III (p =0.03), and IV (p = 0.04) as compared to baseline (Table 2 and Figure 4). However, an insignificant increase in TNF-α levels was observed in group V at week 5 as compared to the baseline (Table 2). This suggests that *Ficus* in a dose of 400 mg/kg was effective in preventing a significant increase in TNF-α levels.

As compared to the control group, which showed a + 47.49% mean increase from baseline, the percent mean increase in group III, IV, and V was found to be + 35.96%, + 28.93%, and + 26.46% respectively (Table 2). We may therefore conclude from these results that although only group V treatment could succeed in preventing a significant increase in TNF-α levels, but the remaining two preventive groups (III and IV) also managed to cause a less increase as compared to the control (group II). Thus, as hypothesized in our study, *Ficus religiosa* was found to be effective in preventing a significant increase in TNF-α level.

Since no comprehensive planned study has been done so far to see the preventive effect of *Ficus religiosa* in the development of diabetes in rats exposed to risk factors like HFD and STZ, our findings could find no resemblance to the recent literature.

Keeping in view the results obtained in the present study, the following conclusions may be drawn regarding the potential

protective role of *Ficus religiosa* against development of diabetes mellitus and pro-inflammatory markers:

- *Ficus religiosa* did not produce any significant hypoglycemic effect in normal rats, therefore it may be prescribed to normal individuals prophylactically.
- *Ficus religiosa* in all the three doses i.e., 100, 200, and 400 mg/kg b.w., is effective in preventing the onset of hyperglycemia when exposed to risk factors like high fat diet. A dose dependent response was observed with different doses used. The dose of 400 mg/kg lead to the most pronounced effect.
- *Ficus religiosa* in a dose of 400 mg/kg had a significant preventive effect against the elevation of inflammatory marker TNF-α as well.

In light of the above evidences, *Ficus religiosa* may be recommended prophylactically along with proper diet and exercise for preventing the onset of diabetes mellitus. The results of the present study are encouraging and may reveal the importance of *Ficus religiosa* as an economical prophylactic agent but details of the complete mechanism have yet not been explored. Therefore, further experiments are required to elucidate the exact mechanism of action. Also, more specific and longer duration animal and human studies are required to further substantiate the findings of the present study.

Ethical Clearance

This work has been approved by Institutional Animal Ethical Committee (IAEC) and registered under Registration No. 646/02/a//CPCSEA for conducting animal tissue experiment.

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