RESEARCH ARTICLE

AN EXPERIMENTAL STUDY TO EVALUATE THE HYPOGLYCEMIC AND ANTI-INFLAMMATORY EFFECT OF *Ficus religiosa* AND ITS COMPARISON WITH GLIBENCLAMIDE

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ABSTRACT

Ethnopharmacological relevance: *Ficus religiosa* (FR) commonly known as Bo tree or Peepal, has been extensively used in traditional medicine for a wide range of ailments including diabetes mellitus. However, the plant has not been widely studied for its hypoglycemic/ anti-hyperglycemic effects, except for a few preliminary studies. In this study we provide the experimental evidence for the clinical use of FR in the treatment of type 2 diabetes mellitus.
Aims and 0bjectives: The present study was aimed to evaluate the hypoglycemic and anti-inflammatory effects of *Ficus religiosa* and their comparison with the standard oral hypoglycemic (2nd generation sulfonylurea - Glibenclamide) drug in diabetic rats.
Materials and Methods: 30 Wistar Albino rats were included in the study. A base line body weight was recorded and baseline biochemical analysis of fasting plasma glucose (FPG) and plasma TNF-α levels of rats in all the groups was done. Type 2 diabetes mellitus was induced by feeding rats with a high fat diet (HFD) for 4 weeks followed by a single intraperitoneal injection of low dose of streptozotocin (STZ, 30 mg/kg b.w.). Rats with FPG $\geq$ 160 mg/dl were included in the experiment and were randomly divided into 5 groups, each comprising of 6 rats. *Ficus religiosa* powder at a dose of 100, 200, and 400 mg/kg b.w., and standard drug (Glibenclamide) at a dose of 0.3 mg/kg was given orally, once daily, to the desired group of animals for a period of 4 weeks. After 4 weeks of drug treatment, the above mentioned parameters were analyzed again.
Statistical Analysis: The data was expressed as mean $\pm$ SD and was analyzed by one way analysis of variance (ANOVA), multivariate analysis of variance, and one-sample Kolmogorov-Smirnov test. Statistical significance was based on p value < 0.05.
Results *Ficus religiosa* powder at all the three doses i.e. 100, 200, and 400 mg/kg produced a significant decrease in the elevated FPG levels in diabetic rats. A dose dependent response was also noticed, with a more pronounced effect at a dose of 400 mg/kg than at 200 mg/kg which in turn was more effective than 100 mg/kg dose. The hypoglycemic potential of *Ficus religiosa* at a dose of 400 mg/kg was nearly as effective as the standard drug. Plasma TNF-α levels also showed a dose dependent response with a maximum mean percent reduction at a dose of 400 mg/kg and minimum at a dose of 100 mg/kg.
Conclusion: An effective and dose dependent curative effect of *Ficus religiosa* against hyperglycemic and elevated TNF-α in all the three doses i.e. 100, 200, and 400 mg/kg was evident with the most pronounced effect at 400 mg/kg. This favorable modulation of cytokine TNF-α by *Ficus religiosa* may be responsible for its potent anti-diabetic activity.

INTRODUCTION

Diabetes mellitus is defined as a group of disorders characterized by chronic hyperglycemia due to disturbance in carbohydrate, fat and protein metabolism. This disturbance may be associated with either absolute or relative deficiency in insulin secretion or insulin action, or both (Deb and Dutta, 2006). Diabetes mellitus, along with three other diseases namely cardiovascular diseases (CVD), cancer, and chronic respiratory disease has been identified as one of the four priority non-communicable diseases (NCDs) by WHO (Nigel *et al*., 2011). Diabetes mellitus is the most common disease associated with carbohydrate metabolism, and is the most important non-infective epidemic to hit the world in the present millennium. According to the International Diabetes Federation (IDF) report (Nigel *et al*., 2011), in 2011 nearly 366 million people worldwide suffered from diabetes mellitus. This number is expected to increase to 552 million or more by the year 2030. Asian population has a particularly strong ethnic and genetic

predisposition for diabetes mellitus due to which they have a lower threshold for environmental risk factors and adverse lifestyle and behavioural changes like physical inactivity or a "Western-style" high-energy-low-fibre diet (Ambady *et al*., 2012). Despite the great strides made in the understanding of pathophysiology and management of diabetes mellitus the disease and disease- related complications are increasing unabated, due to multiple defects in their pathophysiology. Studies in recent years have shown that chronic low-grade inflammation and inflammatory cytokines released during inflammation (mainly IL 1, TNF-α, and IL 6) (Alexandraki *et al*., 2006) contribute to the development of type 2 diabetes mellitus and its microvascular complications (Jeffcoate *et al*., 2005; Mocan *et al*., 2006; Mora and Navarro., 2006). These findings point towards research into development of new therapeutic approaches for managing type 2 diabetes mellitus and its complications. Conventionally, type 2 diabetes mellitus is managed with oral hypoglycemic/ anti-hyperglycemic agents like sulphonylureas, biguanides, thiazolidinediones, α- glucosidase inhibitors etc. However, modern allopathic drugs, in general have not been a concrete solution till

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Graphical Abstract
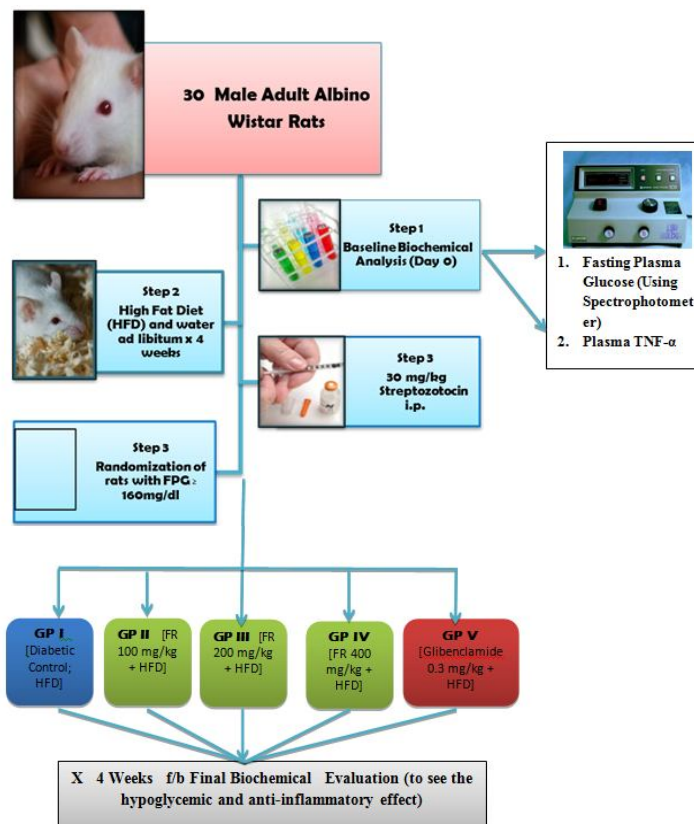
date, because commonly used hypoglycemic/ anti-hyperglycemic drugs are costly and have inadvertently produced serious adverse effects (Chitturi S and George J, 2002). This leads to patient incompliance. Diabetes mellitus has recently been identified by Indian Council of Medical Research (ICMR) as one of the refractory diseases for which satisfactory treatment is not available in modern allopathic system of medicine. It has therefore become evident to look for better options and switch on to safer indigenous system of medicine. According to the World Health Organization (WHO, 1993) estimate, nearly 4 billion people (approximately 80 percent of the world population) presently use herbal medicine for some aspect of their health care. Few of the many advantages of using medicinal plants is that they are cost-effective, provide patients with a complex of many natural compounds, have smoother action and are better tolerated than synthetic drugs and produce only few allergic reactions. Moreover, they don't accumulate in the body and therefore can be administered for a long time. This indigenous pattern of treatment represents the people and has traditionally occupied an important position in the socio-cultural, spiritual and medicinal arena of rural and tribal lives of India. The complex pathophysiology of diabetes mellitus therefore necessitates the researchers to harness and harvest medicinal plants that have multiple beneficial effects which can take care of most of the metabolic abnormalities associated with diabetes mellitus. *Ficus religiosa* is a herb with 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. *Ficus religiosa*, commonly known as Bo or peepal tree, is a large perennial tree that grows throughout India and south-east Asia, especially in the vicinity of temples. In Ayurveda, it belongs to a class of drugs called rasayana. Rasayanas are rejuvinators, antioxidants, and stress

relievers (Arora *et al*., 1999; Govindrajan *et al*., 2005). However, the plant has not been widely studied for its hypoglycemic/ anti-hyperglycemic effects, except for a few preliminary studies (Kirana *et al*., 2009; Pandit *et al*., 2010; Kirana *et al*., 2011). The current study therefore, was envisaged to investigate the hypoglycemic effect of *Ficus religiosa* and its possible mechanism of action in high fat diet and low dose streptozotocin induced diabetic rat models in order to rationalize its medicinal use in type 2 diabetes mellitus. Since both, type 2 diabetes mellitus and a pro-inflammatory state are components of the 'Platinum standard definition' (Zimmet *et al*., 2006) of Metabolic Syndrome or the Insulin Resistance Syndrome (Ford *et al*., 2008), therefore the parameters, namely plasma blood glucose and cytokine TNF-α became the focus of our study.

MATERIALS AND METHODS

Drugs: Sources, Doses and Dosage Forms

Dried powder form of *Ficus religiosa* (commonly known as Peepal) was procured from Vyas Pharmaceuticals, Indore (Batch No. 0084). It was administered orally in three doses i.e. 100 mg/ kg b.w (Pandit *et al*., 2010; Kirana *et al*., 2011), 200 mg/ kg b.w (Kirana *et al*., 2011), and 400 mg/ kg b.w. The purified dried powder form was used in our study as powder form usually contains most of the active compounds and is a safer form to consume as a drug as compared to other forms, like extract. The oral route was chosen for giving the test drug, as it is the usual and convenient method of taking a drug and ensures better patient compliance. This route does not need any kind of assistance and is quite easy in terms of intake. The standard drug (Tab. Glibenclamide) was administered orally in a dose of 0.3 mg/ kg b.w. (Gupta and Gupta, 2011). Streptozotocin was administered in a low dose of 30 mg/kg for inducing type 2 diabetes mellitus. It was procured from Merck, KGaA, Germany.

(Cat# 572201, Lot# D0011626; stored at -20° C). Glucose detection kit (Eco-Pak Glucose Kit) was procured from Accurex Biomedical Pvt. Ltd. The Avibion TNF-α ELISA Kit for TNF-α estimation was procured from Orgenium Laboratories Buisness Unit, Finland.

Experimental Animals

Thirty (30) healthy adult Albino Wistar rats of male sex, weighing between 140-160 gms and bred in the 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, Lucknow) under standard laboratory condition of temperature and humidity ($25 \pm 2°C$, 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 experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC), King George's Medical University, Lucknow. Care of animals was taken as per internationally accepted guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Diet

High Fat Diet (HFD) was manufactured and supplied by Dayal Industries Pvt. Ltd. Barabanki Road, Lucknow, Uttar Pradesh. The contents were as follows: crude protein (16%), crude fat (prepared from rice bran, 15%), acid insoluble ash (2.30%), moisture (8%), vitamins and minerals (in appropriate quantity).

Acute toxicity study of *Ficus religiosa*

Toxicity study to determine the safe oral dose of *Ficus religiosa* was not done in our study because acute toxicity tests, as per OECD 423 guidelines (2010) have already been conducted in many previous studies. Khan *et al*. (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. Based on the findings of these studies, three doses of *Ficus religiosa* (100, 200, and 400 mg/kg b.w.) were chosen.

Induction of Type 2 Diabetes mellitus

Diabetes mellitus was induced by feeding rats with a high-fat diet for 4 weeks followed by an intraperitoneal injection of a low dose of streptozotocin (STZ). STZ was administered intraperitoneally in overnight fasted rats in a dose of 30 mg/ kg b.w. (Hui *et al*., 2007; Wang *et al*., 2011), dissolved in 0.1 M sodium citrate buffer (pH 4.5). Rats were then given standard diet and water ad libitum for 1 week. One week after STZ injection, fasted plasma glucose (FPG) levels were measured using reagent kits and rats with FPG ≥ 160 mg/dl were included in the experiment.

Experimental Protocol

Prior to the experiment, animals were acclimatized to the surroundings for a period of 7 days during which they were given a standard pellet diet and water ad libitum. A baseline body weight of all the rats was recorded on day 0 of the study and simultaneously blood sample was withdrawn for baseline biochemical evaluation of fasting plasma glucose (FPG) and
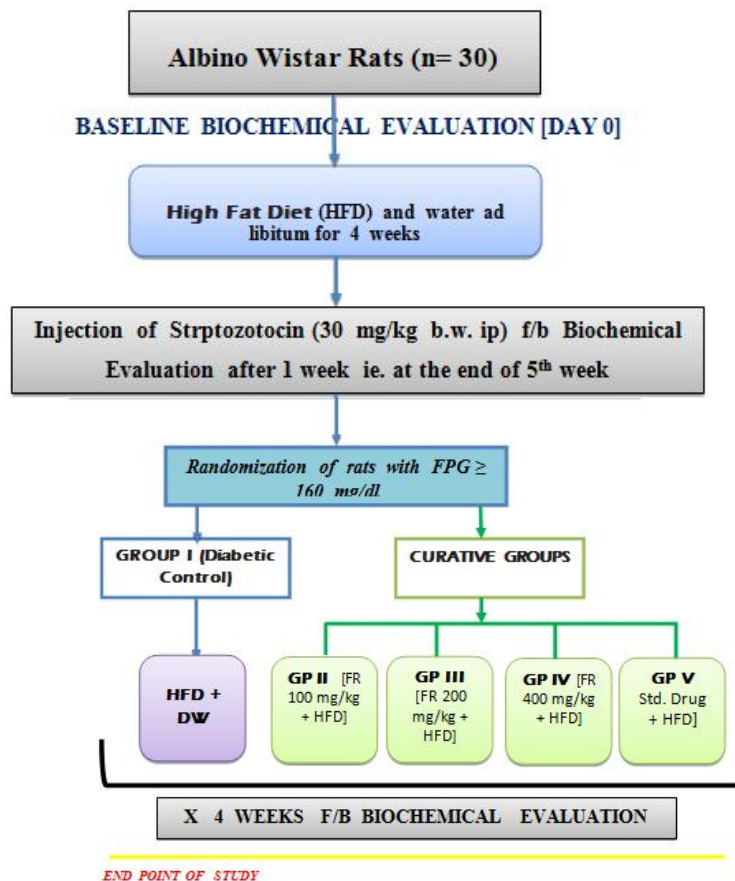

Figure 1. Flow diagram depicting the experimental protocol

plasma TNF-α levels. Rats were then given a high fat diet (HFD) and water ad libitum for a period of 4 weeks, starting from day 1 of the study. After 4 weeks they were given an intraperitoneal injection of a low dose of Streptozotocin (STZ) for the induction of type 2 diabetes mellitus. One week later, i.e. at the end of the 5th week of the study, body weight of all the rats was recorded and simultaneously blood sample was withdrawn for biochemical analysis. Rats with FPG ≥ 160 mg/dl were included in the study and were divided randomly into 5 groups, each comprising 6 rats. The five groups were then given the following treatment (Figure 1):

Group I (diabetic control): HFD + distilled water
Group II: 100 mg/ kg b.w. Ficus religiosa + HFD
Group III: 200 mg/ kg b.w. Ficus religiosa + HFD
Group IV: 400 mg/ kg b.w. Ficus religiosa + HFD
Group V: Glibenclamide (0.3 mg/ kg b.w) + HFD

Both the above mentioned drugs were formulated as a suspension using distilled water just prior to administration. The strength of the suspension was adjusted in a way that 1 ml of the suspension contained the desired dose that was to be administered in an individual rat. The drugs were given once daily orally with the help of a feeding cannula. All the drugs were administered for a period of 4 weeks. At the end of the treatment (9th week of the study), body weight was recorded and blood sample was withdrawn for biochemical analysis.

Sample Collection

Blood sample was withdrawn at three point of time during the study i.e. day 0 (for baseline biochemical evaluation), at the end of 5th week (after STZ injection), and at the end of 9th week (at the end of the treatment). Blood sample of 1 ml volume was withdrawn from overnight fasted rats through retro- orbital route. An intraperitoneal injection of pentobarbitone in a dose of 35 mg/kg was used for producing light anaesthesia during the procedure. The sample (0.5 ml) for FPG estimation was collected in tubes containing sodium fluoride (NaF) while that for TNF-α estimation was collected in centrifugation tubes. The samples were left at room temperature for 30 min for coagulation and plasma was then separated by centrifuging the sample at a rate of 2000 r.p.m. for 15 minutes. FPG was estimated immediately following serum separation while the serum for TNF-α estimation was stored at -20 °C until use.

Biochemical Analysis

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

Fasting Plasma Glucose Level

The plasma glucose level was estimated using Eco- Pak Glucose kit (Accurex Biomedical Pvt. Ltd.). It was based upon an enzymatic method using Glucose Oxidase and Peroxidase enzyme and a spectrophotometer.

Estimation of TNF- α

The plasma TNF-α level was estimated by an enzyme-linked immune-sorbent assay (ELISA) using a commercial TNF-α ELISA kit (AviBion TNF-α ELISA kit, Orgenium Laboratories Buisness Unit, Finland). Principle of the test: An antibody specific for rat TNF- α remains coated onto the wells of the microtiter strips that binds to the rat TNF-α antigen. A biotinylated antibody is then added which binds to the immobilized rat TNF-α antigen. Horseradish Peroxidase (HRP)-Streptavidin solution is then added which binds to the biotinylated antibody to complete the four-member sandwich. This HRP catalyzes the oxidation of the substrate solution, TMB (3,3′,5,5′-tetramethylbenzidine) by hydrogen peroxide resulting in the formation of a blue colored product, if TNF-α is present. The intensity of this colored product is directly proportional to the amount of TNF-α bound. The formation reaction is terminated by addition of a stop solution that changes the color from blue to yellow. 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 data were expressed as mean ± 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. The percent change from baseline to follow-ups was also calculated for each group. p value < 0.05 was considered as statistically significant. Analyses were performed by using the SPSS software package (WINDOWS version 15.0; SPSS Inc, Chicago).

RESULTS

The baseline (at week 0), post-STZ (at week 5), and post treatment (at week 9) body weight, fasting plasma glucose (FPG), and fasting TNF-α levels in rats of five groups are summarized in Table 1 and shown graphically in Figures 1- 3.

Table 1. Body weight (g), Fasting plasma glucose (FPG, mg/dl), and TNF-α (pg/ml) summary (Mean ± SD) in rats of five groups

Variable	Group	Baseline	Post STZ (At week 5)	At week 9	Baseline to 5 week p value	% mean change	p-value[1]
			Periods			5 week to 9 week	
Body Weight	I	227.3±25.9	257.8±1.9	261.8±5.0	0.03*	1.52	0.87
	II	205.2±18.6	235.0±10.0	244.5±9.6	0.02*	4.11	0.46
	III	214.2±10.2	236.5±20.8	247.7±20.2	0.004*	5.36	0.32
	IV	196.7±16.3	236.0±17.1	250.8±24.0	0.01*	6.65	0.13
	V	220.8±1	252.7±22.4	264.0±18.0	0.01*	5.41	0.31
FPG	I	90.7±17.2	184.7±17.4	202.3±9.4	0.001*	10.47	0.11
	II	99.3±8.1	175.5±11.7	136.8±24.0	<0.0001*	-21.31	0.03*
	III	95.2±19.0	168.5±20.0	114.8±16.0	0.001*	-30.84	0.01*
	IV	100.3±20.9	187.8±34.7	114.0±17.8	0.01*	-38.33	0.01*
	V	91.5±11.3	173.7±18.8	96.2±18.9	<0.0001*	-48.76	0.0001*
TNF-α	I	5.5±1.8	7.6±1.3	9.9±1.0	0.002*	41.48	0.02*
	II	5.5±2.0	5.3±0.4	5.7±1.9	0.88	-7.69	0.69
	III	4.7±1.0	5.7±1.5	4.7±1.8	0.11	-12.22	0.36
	IV	4.7±1.4	5.1±0.7	4.0±1.0	0.01*	-18.67	0.17
	V	6.0±2.2	7.4±2.2	5.7±1.4	0.001*	-13.80	0.26

Table 2. Significance of fasting plasma glucose (FPG, mg/dl) and TNF-α in five groups of rats

Variables	Groups		Baseline		Week 5		Week 9	
			Mean difference	p-value	Mean difference	p-value	Mean difference	p-value[1]
FPG	Gr II vs	I	8.66	0.98	9.16	0.99	65.50	<0.0001*
		II	4.50	1.00	16.16	0.88	87.500	<0.0001*
		III	9.66	0.97	3.13	1.00	88.33	<0.0001*
		IV	0.83	1.00	11.00	0.98	113.50	<0.0001*
TNF-α	Gr II vs	I	0.01	1.00	2.28	0.12	4.91	<0.0001*
		II	0.78	0.99	1.87	0.31	5.20	<0.0001*
		III	0.75	0.99	2.52	0.08	5.82	<0.0001*
		IV	0.49	1.00	0.20	1.00	4.19	<0.0001*

Within Groups

The significance of mean difference of FPG and TNF-α within the five groups (at week 5 and at week 9) are summarized in Table 2.

DISCUSSION

The study was conducted to discern the curative effect of *Ficus religiosa* on type 2 diabetes mellitus and elevated TNF-α levels, both being components of Metabolic Syndrome (Zimmet *et al*., 2005). The curative effect was compared with standard drug (Glibenclamide). Insulin resistance is one of the most important risk factors associated with type 2 diabetes mellitus and is polygenic and multifactorial in origin. Lifestyle factors such as obesity and physical inactivity are known to play a vital role in the development of insulin resistance. Many recent studies have also provided evidence that adipose tissue plays a crucial role in the development of insulin resistance and type 2 diabetes mellitus through an imbalanced secretion of a variety of adipocytokines. This imbalance may result either due to increased TNF-α, IL-6 (Ouchi *et al*., 2011), and NFκB (Yin *et al*., 1998) levels or decreased adiponectin, leptin, and omentin (Ouchi *et al*., 2011; Shibata *et al*., 2009) levels. Apart from obesity, the concept of chronic low grade inflammation and activation of innate immunity as underlying factors in the pathogenesis of insulin 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) (Alexandraki *et al*., 2006) are independent predictors of type 2 diabetes mellitus (Miles *et al.,* 1997; Frank *et al*., 2004). Elevation in TNF-α levels therefore reflects both an upregulated adipocyte signaling and systemic inflammation. TNF-α may cause insulin resistance through the following possible mechanisms:

a) **D*irect 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 CRP), or impairment of endothelial function.

Rat Model of Type 2 Diabetes Mellitus

The serum biochemical analysis was done 1 week after STZ treatment i.e at week 5. Results, as compared to baseline, showed a significant increase in FPG and TNF-α level in all the groups (Tables 1 and 2 and Figures 1- 3). These findings validated the induction of a state of type 2 diabetes mellitus and inflammation in our diabetic control group (group I) and treatment groups (groups II-V) and are in agreement with

previous studies (Maiti *et al*., 2005; Hui *et al*., 2007). The high fat diet feeding induced a mild state of insulin resistance at first (Hui *et al*., 2007). The excessive circulating lipids from high fat diet (HFD) stimulates lipoprotein lipase (LPL) overexpression in skeletal muscle and liver leading to increased cellular stores of triglycerides that are known to cause insulin resistance. The hypertrophic adipose tissue also promotes a state of chronic mild inflammation which further worsens insulin resistance (Weisberg *et al*., 2006). A low dose injection of STZ then leads to partial destruction of pancreatic β cells, suppressing insulin secretion.
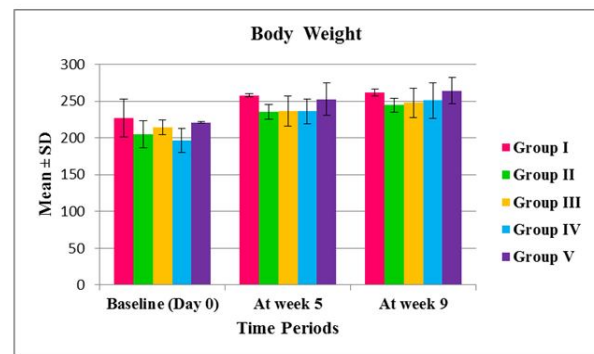

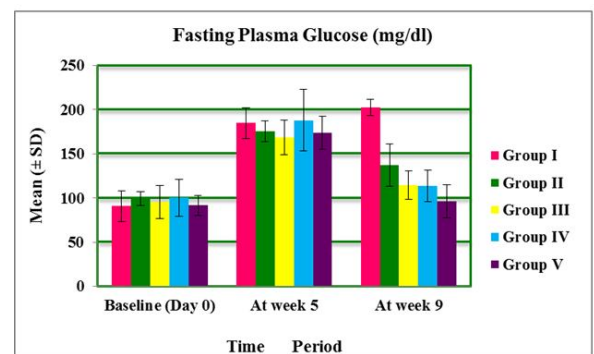**Figure 1. Pre and post treatment mean (± SD) Body Weight in five groups of rats**

Figure 2. Pre and post treatment mean (± SD) Fasting Plasma Glucose levels in five groups of rats

Effect on Body Weight

The body weight showed a significant increase in all the groups at week 5 as compared to the baseline. This increase in body weight is in accordance with a previous study (Yang *et al*., 2012) and may be attributed to the high fat feeding of these rats which is known to cause a rapid onset visceral obesity in as early as 2 weeks' time. The HFD was continued in all the

groups for further 4 weeks post STZ treatment (from week 5 to week 9). As compared to the values at week 5, diabetic control (group I) showed a further increase in FPG at week 9, but it was not found to be statistically significant. However, a statistically significant increase in plasma TNF-α (p= 0.02), levels was observed at week 9 as compared to the values at week 5 (Table 1 and 2 and Fig. 1- 3). These findings suggest that continuation of HFD caused a further worsening of serum biochemical parameters which is in accordance with a previous study (Hui *et al*., 2007).
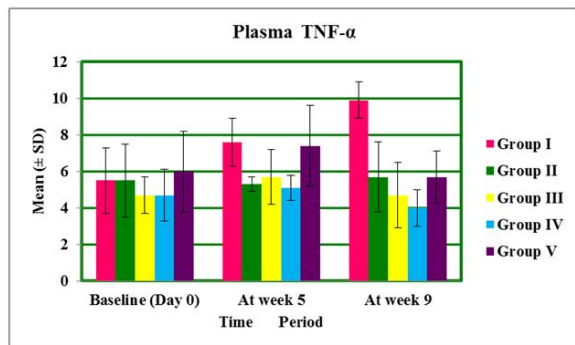

Figure 3. Pre and post treatment mean (± SD) TNF-α levels in five groups of rats

This can be explained by the fact that the partial destruction of pancreatic β cells, caused by low dose STZ, led to suppression of insulin secretion, and the subsequent high energy feeding further worsened the state of insulin resistance. This resulted in further dysfunction of pancreatic β cells (Hui *et al*., 2007) and persistent hyperglycemia. Results showed that the percent mean increase in body weight at 9 weeks was maximum in group IV (FR 400 mg/kg; +6.65%), followed in descending order by group V (standard treatment; +5.41%), III (FR 200 mg/kg; +5.36%), II (FR 100 mg/kg; +4.11 %) and minimum in group I (diabetic control; +1.52%) (Table 1and Fig. 1). The improved body weight of Ficus religiosa treated animals may be due to its direct lipid and glucose lowering activity or indirectly due to its effect on reducing the elevated TNF- α levels. Elevated TNF- α has been reported to inhibit the uptake of free fatty acids from circulation and accelerate the lipolysis in adipose tissue, leading to weight loss. This, in addition to decreased glucose uptake and accelerated β-oxidation in adipose tissue (You and Nicklas, 2006) may explain the poor weight gain in group I in spite of being fed on HFD.

Effect on Fasting Plasma Glucose (FPG)

FPG at week 9 was found to decrease significantly in all the curative groups i.e. II to V as compared to their respective values at week 5 (Post STZ) (Table 1 and Fig. 2b). This suggests that the treatments given to all the four groups i.e. II to V have effective hypoglycemic action. When compared with group I (diabetic control), the individual mean difference between group I and groups II to V was found to be significant (p<0.001) in all the curative groups, further suggesting a significant curative hypoglycemic effect in all the curative groups. When analyzed for inter group comparison between the three Ficus religiosa treated groups i.e groups II-IV, a maximum reduction in FPG from week 5 to week 9 was observed in group IV (FR 400 mg/kg; -38.33 %) followed by Group III (FR 200 mg/kg; -30.84 %) with least reduction in group II (FR 100 mg/kg; -21.31 %) (Table 1 and Fig. 2). This finding suggests a dose dependent hypoglycemic effect of *Ficus religiosa* with a more pronounced effect at a dose of 400 mg/kg than at 200 mg/kg which in turn is more effective than 100 mg/kg dose. When compared with the standard drug group (group V), the percent mean reduction in FPG at week 9 as compared to week

5 was found to be more in group V (standard drug; -48.76%) as compared to group IV (FR 400 mg/kg; -38.33%), III (FR 200 mg/kg; -30.84%), and II (FR 100 mg/kg; -21.31%). The above observation suggests that *Ficus religiosa* in a dose of 100 and 200 mg/kg is less effective than the standard drug but nearly as effective as the standard at a dose of 400 mg/kg. These findings are in accordance with the previous studies (Kirana *et al*., 2009; Pandit *et al*., 2010; Choudhary *et al*., 2011; Kirana *et al*., 2011; Nishant *et al*., 2012) which have shown that *Ficus religiosa* possess significant antidiabetic activity. Kirana *et al*. (2009) reported that *Ficus religiosa,* in a dose of 200 mg/kg had a more pronounced antidiabetic effect than at 100 mg/kg dose. Our results also suggest the same, however we had also taken a higher dose in our study which has shown a more pronounced effect than 200 mg/kg dose. The proposed mechanism for this effect as reported in various studies done so far are as follows:

Enhanced insulin secretion - *Ficus religiosa* has been reported to contain tannins, saponins, flavonoids, phytosterols, terpenoids, cardiac glycosides, and furancoumarin derivatives namely bergapten and bergaptol, β-sitosteryl-D-glucoside, vitamin K, n-octacosanol, methyl oleanolate, lanosterol, stigmasterol, lupen-3-one (Jiwala *et al*., 2008; Sheetal *et al*., 2008; Uma *et al*., 2009; Babu *et al*., 2010). Coumarins along with flavonoids, tannin, β-sitisteryl-D-glucoside (Kameswararao *et al*., 1997) and polyphenols have insulin secretagogue property (Wadood *et al*., 2003). The hypoglycemic effect of FR may therefore be due to the presence of more than one hypoglycemic/ anti-hyperglycemic principles and their synergistic properties.

Rejuvenation of pancreas- Flavanoids may be responsible for rejuvenation of pancreas and increased biosynthesis and secretion of insulin (Arora *et al*., 1999; Govindranjan *et al*., 2005).

Anti-oxidant property- *Ficus religiosa* modulates the enzymes of antioxidant defense system to combat oxidative stress leading to its anti-diabetic activity (Catopano, 2009).

Modulation of TNF-α- TNF-α has a direct inhibitory effect on insulin signaling pathway leading to insulin resistance. Flavonoids possibly modulate TNF- α (Kirana *et al*., 2011).

Effect on Plasma TNF-α level

Plasma TNF-α level at week 9 was found to be lower than the values at week 5 in all the 4 curative groups i.e. groups II-V. Inter group comparison analysis showed a dose dependent response with maximum mean percent reduction in group IV (-18.67%) and minimum in group II (-7.69%) (Table 1 and Fig. 3). When compared with the standard drug group (group V) group IV showed a higher percent reduction while that of groups II and III was less than group V. We may thus conclude that *Ficus religiosa* in a dose of 400 mg/kg had the most pronounced effect in lowering plasma TNF α level (Table 1 and Fig. 3). The above findings are in agreement with a recent study (Kirana *et al*., 2011) which reported that Ficus caused a significant reduction in TNF level which could be possibly related with its potential anti-diabetic property. The effect was found more pronounced at 200 mg/kg than at 100 mg/kg which is also seen in our study,

Conclusion

Keeping in view the results obtained in the present study, the following conclusions may be drawn regarding the potential effectiveness of *Ficus religiosa* against diabetes mellitus and pro-inflammatory markers: An effective and dose dependent curative hypoglycemic/ anti-hyperglycemic effect of *Ficus religiosa* in all the three doses i.e. 100, 200, and 400 mg/kg was evident with the most pronounced effect at 400 mg/kg. Ficus religiosa in a dose of 400 mg/kg had nearly similar curative hypoglycemic/ anti-hyperglycemic effect as that of the

standard drug (Glibenclamide). With the above documented curative effects against diabetes mellitus, *Ficus religiosa* might have a potential usefulness as an adjunct to conventional therapy in the future management of these metabolic derangements.

The results of the present study are encouraging and may reveal the importance of Ficus religiosa as an economical antidiabetic 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.

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