The study of aqueous extract of *Ficus religiosa* Linn. on cytokine TNF-α in type 2 diabetic rats

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ABSTRACT

Background: Chronic systemic inflammation is an early process in pathogenesis of type 2 diabetes. Hence the present study was aimed to investigate the effect of traditionally known plant *Ficus religiosa* on elevated glucose and inflammatory marker namely tumor necrosis factor (TNF)-α in type 2 diabetic rats. **Methods:** Type 2 diabetes was induced by administering streptozotocin (90 mg/kg, i.p.) in neonatal rat model. Aqueous extract of *F. religiosa* at a dose of 100 and 200 mg/kg was given orally to desired group of animals for a period of 4 weeks. After 4 weeks of drug treatment, parameters such as fasting blood glucose, postprandial blood glucose and TNF-α in serum were analyzed. **Results:** Aqueous extract of *F. religiosa* at both dose levels i.e., 100 and 200 mg/kg decreased the elevated glucose and TNF-α in type 2 diabetic rats. The extract at 200 mg/kg had more pronounced effect. **Conclusion:** Modulation of cytokine TNF-α by the aqueous extract of *F. religiosa* indicates that the anti-inflammatory and immunomodulatory property of the plant is related with its potential anti-diabetic activity.

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INTRODUCTION

Elevated circulating inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6 are observed in patients with postprandial hyperglycemia.[1-2] Activated innate immune system and chronic systemic inflammation is an early process in the pathogenesis of type 2 diabetes.[3] Cytokine TNF-α has been implicated in insulin resistance.[4] TNF-α stimulate the endothelial production of adhesion molecules such as E-selectin and vascular cell adhesion molecule-1 (VCAM-1).[5] E-selectin and VCAM-1 accelerate the atherosclerosis and vascular complications in diabetes. In this view, development of a drug which modulates the cytokine TNF-α in type 2 diabetes will be a novel approach in early intervention of the disease.

Ficus religiosa Linn. commonly known as pipal is a large perennial tree that grows throughout India.[6] It is widely cultivated in South-east Asia especially in vicinity of temples. In Ayurveda, *F. religiosa* belongs to a class of drugs called rasayana.[7] Rasayana drugs are rejuvenators, immunomodulators and relieve stress in the body.[7,8] Decoction prepared from the bark of *F. religiosa* is traditionally used in treatment of ulcers, inflammation and diabetes.[9,10] Alcoholic extract of the drug has been reported for immunomodulatory activity.[11] Hence the present study has been carried to analyze the effect of *F. religiosa* on elevated glucose and inflammatory marker TNF-α in type 2 diabetic rats.

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MATERIALS AND METHODS

Collection and authentication of plant material

Bark of *F. religiosa* was collected from Udupi which is in Karnataka, India during the month of November-December and dried under shade at temperature not exceeding 40°C. Drug sample was authenticated and deposited (Voucher number: Bark/2005/540/15) at Department of Raw Materials Herbarium and Museum, National Institute of Science Communication and

Information Resources (NISCAIR), New Delhi.

Aqueous extract and phytoconstituents

Dried bark was grounded into a moderately coarse powder (# 22) in domestic electric grinder. One part of the powdered drug was boiled with 16 parts of water for a period of 15 min and filtered hot through muslin cloth. Filtrate was then evaporated under reduced pressure in Rota-rod evaporator (Buchi RE 121, Japan). The dried aqueous extract (9.6%) was packed in air-tight container and stored in desiccators at room temperature for further studies.[7] Preliminary phytochemical analysis[12] of the aqueous extract showed the presence of carbohydrates, tannins, flavonoids, coumarin glycosides and phenolic compounds.

Dose and Drug solution

In Ayurveda, 10-20 g of the powdered bark in the form of decoction is used in treatment of diabetes.[7,9] In the present study, considering the extractive value of the bark and rat metabolic rate (seven times higher than humans), aqueous extract of *F. religiosa* at 100 and 200 mg/kg dose was selected for analyzing the biological activity. To prepare the test drug solution, required quantity of the aqueous extract was dissolved in distilled water to have a desired dose in 1 ml.

Animals

Wistar albino rats weighing 140-160 g of either sex were housed under standard laboratory conditions of temperature 25 ± 2ºC and 55 ± 5% relative humidity with a regular 12 h light:12 h dark cycle. Animals were given standard rat pellet and tap water *ad libitum*. The study protocol (Protocol number: 06/DIPSAR/ IAEC/ 2004) was approved by Institutional Animal Ethical Committee (IAEC), Delhi Institute of Pharmaceutical Sciences and Research (DIPSAR), New Delhi.

Streptozotocin-induced neonatal rat model for type 2 diabetes

Type 2 diabetes was induced by administering streptozotocin at a dose of 90 mg/kg i.p., in two-day-old neonatal rats. After 6 weeks of streptozotocin injection, rats showing the fasting blood glucose more than 160 mg/dl were considered as type 2 diabetes positive.[13]

Experimental groups

Wistar albino rats of either sex were randomly allotted into five groups of six animals (n=6) each. Group I served as normal and received distilled water. Group II served as type 2 diabetic control and received distilled water. Group III was type 2 diabetic treated with 100 mg/kg of aqueous extract of *F. religiosa*. Group IV was type 2 diabetic treated with 200 mg/kg of aqueous extract of *F. religiosa*. Group V was type 2 diabetic treated with 10 mg/kg of gliclazide. Drug treatment was given on each morning with the help of oral catheter for a period of 4 weeks. Body weight was determined at the end of every week. After 4 weeks of drug treatment, parameters such as fasting blood glucose, postprandial blood glucose and TNF-α in serum were analyzed.

Estimation of fasting blood glucose

Blood samples were withdrawn from overnight-fasted animals and glucose in serum was estimated by Glucose Oxidase and Peroxidase (GOD-POD kit) method[14] using autoanalyzer (Logotech, Tecno 168, Italy).

Estimation of postprandial blood glucose

Blood samples were withdrawn from overnight-fasted animals and glucose in serum was estimated for basal reading (0 min). Then the animals were treated with respective drug solutions. Thirty minutes after the drug treatment, glucose solution at a dose of 2.5 g/kg body weight was administered orally with the help of oral catheter.[15] Blood samples were withdrawn after 120 min of oral glucose load (postprandial) and glucose in serum was estimated by GOD-POD kit method.

Estimation of TNF-α

TNF-α in serum was estimated by ELISA (Rat TNF- α ELISA KIT, DIACLONE, France). Sufficient microwell strips were taken out of the pouch. One hundred microliters of standard diluent and 100 µl of serum were added into the blank and sample well, respectively. Fifty microliters of diluted biotinylated anti-rat TNF-α was added to all the wells. Wells were incubated for 3 h at 37ºC. Plate was then removed and liquid from the wells were aspirated. 0.3 ml of washing solution was added into each well and aspirated. Washing was repeated two more times. One hundred microliters of streptavidin-HRP solution was added to all the wells including blank. The wells were incubated at 37ºC for 30 min. 0.3 ml of washing solution was added into each well and aspirated. Washing was repeated two more times. One hundred microliters of chromogen-TMB (substrate) solution was added to all the wells including blank. The wells were again incubated at 37ºC for 15 min. The enzyme-substrate reaction was stopped by quickly adding 100 µl of sulfuric acid. Absorbance of the color developed in the wells was measured at 420 nm in ELISA reader (Awareness Technology, Mumbai, India).[16] TNF-α in the sample was analyzed from the standard curve plotted with a limit of detection equivalent to 20 pg/ml [Figure 1].

Statistical analysis

Data are expressed as mean ± SEM. Statistical comparison between different groups was done using One-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. $P<0.05$ was considered as statistically significant.

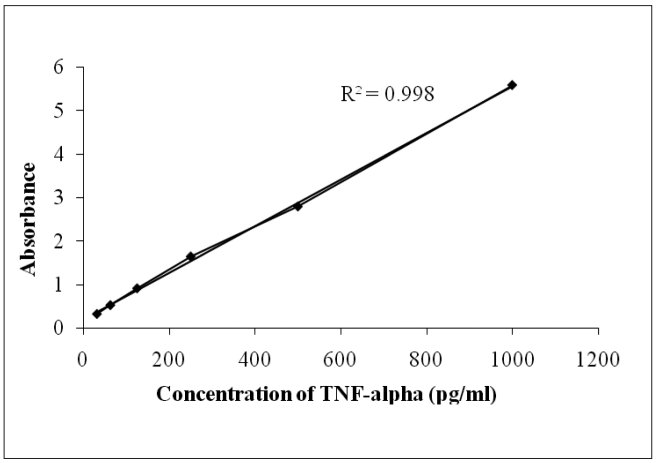

Figure 1: Standard curve of tumor necrosis factor (TNF)-α

RESULTS

Effect on fasting blood glucose

Aqueous extract of *F. religiosa* at both doses, i.e. 100 and 200 mg/kg significantly ($P<0.001$) decreased the elevated fasting blood glucose in type 2 diabetic rats. However, the difference between 100 and 200 mg/kg was found to be significant ($P<0.05$) when analyzed for inter-group comparison. Gliclazide used as the standard drug for comparison also significantly ($P<0.001$) decreased the fasting blood glucose of type 2 diabetic rats. Statistical data of fasting blood glucose in various experimental groups is shown in Table 1.

Effect on postprandial blood glucose

Aqueous extract of *F. religiosa* at 100 and 200 mg/kg dose decreased the elevated postprandial blood glucose significantly ($P<0.001$) as compared to untreated diabetic control group. Difference between 100 and 200 mg/kg dose was significant ($P<0.05$) when analyzed for inter-group comparison. *F. religiosa* at 100 mg/kg did not reduce the postprandial blood glucose below 140 mg/dl (normal).

Table 1: Effect of aqueous extract of *Ficus religiosa* on blood glucose in type 2 diabetic rats

Group (n=6)	Fasting blood glucose (mg/dl)	Postprandial blood glucose (mg/dl)
Normal	89.0 ± 2.7	112.5 ± 2.8
Type 2 diabetic control	186.5 ± 4.1	301.4 ± 5.6
Type 2 diabetic treated with 100 mg/kg of *F. religiosa*	124.7 ± 3.6$^{\#,c}$	155.2 ± 4.1$^{\#,c}$
Type 2 diabetic treated with 200 mg/kg of *F. religiosa*	110.4 ± 3.2$^{\#,b,ns}$	137.0 ± 3.5$^{\#,b,@}$
Type 2 diabetic treated with 10 mg/kg of gliclazide	105.2 ± 2.5$^{\#,a}$	132.9 ± 3.7$^{\#,a}$

Values are expressed as mean ± SEM; $^{\#}P<0.001$ as compared to type 2 diabetic control; $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$ as compared to normal; $^{ns}P>0.05$, $^{@}P<0.05$ as compared between type 2 diabetic treated with 100 and 200 mg/kg of *F. religiosa*

However, 200 mg/kg was able to reduce the postprandial blood glucose below 140 mg/dl indicating that the aqueous extract at higher dose is better effective. Gliclazide reduced the postprandial hyperglycemia below 140 mg/dl. Statistical data of postprandial blood glucose in various experimental groups is shown in Table 1.

Effect on body weight

Two weeks of drug treatment did not improve the body weight of type 2 diabetic rats. By the end of third week, 100 and 200 mg/kg of aqueous extract of *F. religiosa* increased the body weight at $P<0.05$ and <0.01 level of significance, respectively, as compared to untreated diabetic control group. Progress in weight gain of drug-treated animals was continued for further weeks. By the end of 4 weeks, 100 and 200 mg/kg showed a significant ($P<0.001$) increase in body weight of treated animals. Body weight of various experimental groups at basal level, i.e., before drug treatment and at the end of 1st, 2nd, 3rd and 4th week of drug treatment is shown in Figure 2.

Effect on TNF-α

TNF-α was found be elevated in type 2 diabetic rats as compared to normal group. Aqueous extract of *F. religiosa* at 100 and 200 mg/kg dose significantly ($P<0.001$) decreased the elevated TNF-α in type 2 diabetic rats. Difference between 100 and 200 mg/kg dose is statistically significant ($P<0.05$) when analyzed for inter-group comparison. Drug at higher dose, i.e. 200 mg/kg had more pronounced effect on elevated TNF-α. TNF-α level in various experimental groups is shown in Figure 3.

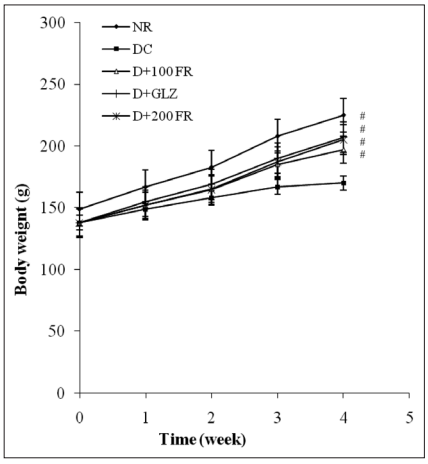

Figure 2: Effect of aqueous extract of *F. religiosa* (FR) on body weight of type 2 diabetic rats; NR: Normal, DC: Type 2 diabetic control, D+100 FR: Type 2 diabetic treated with 100 mg/kg of FR, D+200 FR: Type 2 diabetic treated with 200 mg/kg of FR, D+GLZ: Type 2 diabetic treated with gliclazide; Values are mean ± SEM; n=6; $^{\#}P<0.001$ as compared to type 2 diabetic control

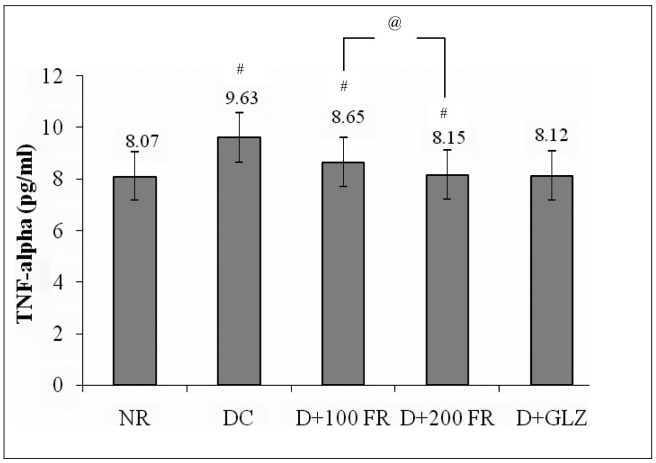

Figure 3: Effect of aqueous extract of *Ficus religiosa* (FR) on TNF-α in type 2 diabetic rats; NR: Normal, DC: Type 2 diabetic control, D+100 FR: Type 2 diabetic treated with 100 mg/kg of FR, D+200 FR: Type 2 diabetic treated with 200 mg/kg of FR, D+GLZ: Type 2 diabetic treated with gliclazide; Values are mean ± SEM; n=6; #*P*<0.001 as compared to type 2 diabetic control; @*P*<0.05 when analyzed for inter-group comparison between D+100 FR and D+200 FR

DISCUSSION

Postprandial hyperglycemia is an earliest metabolic abnormality to occur in type 2 diabetes.[17] This state initiates the development of microvascular and macrovascular complications. Most of the currently available anti-diabetic therapies reduce the fasting blood glucose but have a little impact on postprandial hyperglycemia. In this view, aqueous extract of *F. religiosa* at a dose of 200 mg/kg was found to be better in controlling postprandial hyperglycemia of type 2 diabetes. Reduction in blood glucose may be mediated through the enhanced insulin secretion. A leucopelargonin compound isolated from *Ficus bengalensis* has insulin secretagogue action in alloxan-induced diabetic dogs.[18] Coumarins in the form of glycosides are soluble in water and are identified in the aqueous extract of *F. religiosa*.[19] Coumarins along with flavonoids and polyphenols may be responsible for the above biological activity of aqueous extract. Since *F. religiosa* belongs to a rasayana group of plant drugs there may be rejuvenation of pancreas.[7,8] Indeed rejuvenation of pancreas enhance the secretion of insulin.

Body weight of type 2 diabetic rats was found to be less during the course of development as compared to normal animals. Elevated TNF-α inhibits the uptake of free fatty acids from circulation and accelerate the lipolysis in adipose tissue, which leads to weight loss in type 2 diabetes. Paracrine effect of TNF-α is high in obesity and type 2 diabetes.[20] Weight loss in diabetes is also generally due to continuous excretion of glucose from the body. Improved body weight of drug-treated animals is due to the effect of drug on reducing elevated glucose and TNF-α in type 2 diabetic rats.

A variety of stressors such as infection, tissue injury and food cause macrophages, adipocytes, endothelial cells etc., to secrete inflammatory cytokines.[21] Cytokines are the small soluble peptides released by the cells of immune system to communicate and influence their function. Cytokine TNF-α has been implicated in insulin resistance.[4] Insulin resistance is a primary metabolic defect in type 2 diabetes. Binding of insulin to its receptor induces autophosphorylation at multiple tyrosine sites is a key element in insulin signaling pathway.[22] TNF-α has direct inhibitory effect on tyrosine kinase and phosphorylation cascade.[3,4] TNF-α mediate insulin resistance also through indirect effects including increasing the free fatty acids in circulation, stimulation of insulin counter-regulatory hormones, impairment of endothelial function or inhibiting the glucose-stimulated insulin release by pancreatic β-cells.[23] The above interference of TNF-α in various pathways is justified by its elevated levels in type 2 diabetic rats. Decrease in elevated TNF-α by the aqueous extract of *F. religiosa* indicate the anti-inflammatory and immunomodulatory (rasayana) property of the plant drug is related with its potential glucose lowering effect in type 2 diabetic rats. Modulation of TNF-α is possibly due to flavonoids present in the aqueous extract. As natural modulators of pro-inflammatory gene expression, flavonoids are considered as potential drugs for immunomodulatory agents.[16,24] Multi-dimensional activity of the plant drugs is beneficial in treatment of type 2 diabetes where the cause and consequence are multi-factored.[25]

CONCLUSION

F. religiosa modulates the inflammatory cytokine TNF-α in type 2 diabetic rats. Drug at 200 mg/kg dose has more pronounced effect. Rasayana property of *F. religiosa* is related with its potential anti-diabetic activity.

REFERENCES

1. Manning PJ, De Jong SA, Sultherland WHF, McGrath M, Williams SM. Changes in circulating postprandial proinflammatory cytokine concentration in diet-controlled type 2 diabetes and the effect of ingested fat. Diabetes Care 2004;27:2509-11.
2. Spranger J, Kroke A, Mohlig M, Hoffmann K, Manuela M, Bergmann M, *et al*. Inflammatory cytokines and the risk to develop Type 2 Diabetes. Diabetes 2003;52:812-7.
3. Pickup JC. Inflammation and activated immunity in the pathogenesis of Type 2 Diabetes. Diabetes care 2004;27:813-23.
4. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and Insulin resistance. J Clin Invest 1995;95:2409-15.

5. Koga M, Otsuki M, Kubo M, Hashimoto J, Kasayama S. Relationship between circulating vascular cell adhesion molecule-1 and microvascular complications in type 2 diabetes mellitus. Diabet Med 1998;15:661-7.
6. The Wealth of India. A Dictionary of Indian Raw Materials. New Delhi; 2002. p. 131-2.
7. Agnivesha. Prameha Chikitsa, In: Charaka Samhita. 5th ed. Varanasi: Choukamba Sanskrita Samsthana; 2001. p. 446-7.
8. Govindarajan R, Pushpangadan P, Vijayakumar M. Anti-oxidant approach to disease management and role of rasayana herbs of Ayurveda. Ethnopharmacol 2005;99:165-78.
9. The Ayurvedic Pharmacopoeia of India. Ministry of Health and Family Welfare, Government of India: New Delhi; 1999. p. 17-8.
10. Lansky EP, Paavilainen HM, Pawlus AD, Newman RA. Ficus spp. (fig): Ethnobotany and potential as anticancer and anti-inflammatory agents. J Ethnopharmacol 2008;119:195-213.
11. Mallurwar VR, Pathak AK. Studies on Immunomodulatory activity of *Ficus religiosa*. Indian J Pharm Educ Res 2008;42:341-3.
12. Evans WC. Trease and Evans Pharmacognosy. 15th ed. Philadelphia: Elsevier Science Ltd; 2002. p. 224-8.
13. Bonner-Weir S, Trent DF, Honey RN, Weir GC. Responses of neonatal rat islets to streptozotocin. Diabetes 1981;30:64-9.
14. Trinder P. Determination of blood glucose using an oxidase-peroxidase system with a non carcinogenic chromogen. J Clin Pathol 1969; 22:158-61.
15. Baron AD. Postprandial hyperglycemia and α-glucosidase inhibitors. Diabetes Res Clin Pract 1998; 40: S51-5.
16. Rao YK, Fang SH, Tzeng YM. Anti-inflammatory activities of flavonoids isolated from Caesalpinia pulcherrima. J Ethnopharmacol 2005;100:249-53.
17. Ratner RE. Controlling postprandial hyperglycemia. Am J Cardiol 2001;88:26-31.
18. Augusti KT, Daniel RS, Cherian S, Sheela CG, Nair CRS. Effect of leucopelargonin derivative from Ficus bengalensis Linn. on diabetic dogs. Indian J Med Res 1994;99:82-6.
19. Swami KD, Bisht NPS. Constituents of *Ficus religiosa* and Ficus infectoria and their biological activity. J Indian Chem Soc 1996;73:631-2.
20. You T, Nicklas BJ. Chronic inflammation: Role of adipose tissue and modulation by weight loss. Curr Diab Rev 2006;2:29-34.
21. Van de Kar LD, Carrasco GA. Neuroendocrine pharmacology of stress. Eur J Pharmacol 2003;463:235-72.
22. Virkamaki A, Ueki K, Kahn CR. Protein-Protein interaction in insulin signaling and the molecular mechanism of insulin resistance. J Clin Invest 1999;103:931-43.
23. Tataranni PA, Ortega E. Does an adipokine induced activation of the immune system mediate the effect of over nutrition on Type 2 Diabetes? Diabetes 2005;54:917-27.
24. Devasagayam TPA, Sainis KB. Immune system and antioxidants, especially those derived from Indian medicinal plants. Indian J Exp Biol 2002;40:639-55.
25. Arora D, Dubey SD, Ojha JK. Role of rasayana in preventing the complications of diabetes mellitus, J Diab Assoc India 1999;39:47-50.

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