Aqueous extract of *Ficus religiosa* Linn. reduces oxidative stress in experimentally induced type 2 diabetic rats

H Kirana, S S Agrawal & B P Srinivasan*

Delhi Institute of Pharmaceutical Sciences and Research (DIPSAR), Sector-III, Pushp Vihar, New Delhi 110 017, India

Received 24 March 2009; revised 8 June 2009

One of the major etiologies in pathogenesis of type 2 diabetes especially complications is oxidative stress. Aqueous extract of *Ficus religiosa* at a dose of 100 and 200 mg/kg orally decreased the fasting blood glucose in streptozotocin induced type 2 diabetic rats. The drug had enzyme induction effect with respect to catalase (CAT) and glutathione peroxidase (GSH-Px) activity, however decreased the exaggerated activity of superoxide dismutase (SOD) in type 2 diabetic rats. *F. religiosa* modulated the enzymes of antioxidant defence system to combat oxidative stress. As a result, glutathione (GSH-reduced form) was restored and inhibited the formation of malondialdehyde. Drug at higher dose (200 mg/kg) had more pronounced effect.

*F. religiosa*, a rasayana group of plant drug having anti-diabetic activity along with antioxidant potential was beneficial in treatment of type 2 diabetes.

**Keywords:** *Ficus religiosa*, Oxidative stress, Rasayana, Type 2 diabetes

Oxidative stress is the major cause and consequence of type 2 diabetes. In hyperglycemia, autooxidation of glucose increases the formation of free radicals beyond the capacity of defence system to neutralize it and cause oxidative stress\(^1\). Oxidative stress declines the biosynthesis of insulin in pancreas\(^2\). Free radicals generated during oxidative stress damage the insulin receptors and thereby decrease the number of sites available for insulin action\(^3,4\). Free radical induced endothelial dysfunction is a pathology associated with vascular complications. Diabetic vascular complications are leading cause of end-stage renal failure, acquired blindness, cardiovascular diseases and a variety of neuropathies\(^5\). It follows that the therapies aimed at reducing oxidative stress would benefit patients in type 2 diabetes.

*Ficus religiosa* Linn. commonly known as pipal is a large perennial tree grows throughout India. 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. Rasayana are rejuvenators, antioxidants and relieve stress in the body\(^6,7\). Decoction prepared from the bark is used in treatment of diabetes\(^8\). Hence, we studied the effect of aqueous extract of *F. religiosa* (bark) on experimentally induced type 2 diabetic rats and related oxidative stress.

**Materials and Methods**

Collection and authentication of plant material—Bark of *F. religiosa* was collected from local market of Udupi, India during the month of November-December. It was dried under shade at 40ºC. 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.

Preparation of aqueous extract—Dried bark was grounded into a moderately coarse powder (# 22) in domestic electric grinder. One part of the powdered drug was boiled with sixteen parts of water for 15 min and filtered hot through muslin cloth. Filtrate was then lyophilized by continuous freeze drying process for 36 h. Temperature at -50ºC and pressure of 0.030 to 0.038 TORR was maintained during the operation of freeze drier (Allied Frost SZ 7510, New Delhi, India). The dried aqueous extract (9.6%) was packed in air tight container and stored in desiccators at room temperature for further studies\(^9\). Preliminary phytochemical analysis of the aqueous extract showed the presence of carbohydrates, tannins, flavonoids and polyphenolic compounds.

---

*Correspondent author
Telephone: + 9810972672; Fax: 91-11-29554503
E-mail: bpsrinivasan@yahoo.com
Dose and drug solution—Traditionally 1 to 2 g of the powdered bark is used in diabetes. Alcoholic extract of the drug at 100 mg/kg dose has been reported for biological activity. Since decoction of the drug is suggested, aqueous extract at 100 and 200 mg/kg dose was studied. To prepare the test drug, required quantity of the aqueous extract was dissolved in distilled water to have a desired dose in 1 ml solution.

Animals—Wistar albino rats weighing 140-160 g of either sex were housed under standard laboratory conditions at 25 ± 2°C and 55 ± 5% relative humidity with a regular light/dark cycle of 12 h. 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 (ip), in 2-day old neonatal rats. After six weeks of streptozotocin injection, rats showing the fasting blood glucose more than 160 mg/dl were considered as type 2 diabetes positive.

Experimental groups—Wistar albino rats of either sex were randomly allotted into four 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 everyday morning with the help of oral catheter for a period of four weeks. Body weight was determined at the end of every week. After four weeks of drug treatment, parameters such as fasting blood glucose, activity of enzymes namely superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) in erythrocyte lysate, erythrocyte glutathione (GSH: reduced form) and plasma malondialdehyde (MDA) were analyzed.

Estimation of fasting blood glucose—Glucose in serum was estimated by Glucose Oxidase and Peroxidase (GOD-POD kit) method. Intensity of the red quinoneimine was measured at 540 nm in autoanalyzer (Logotech, Tecno 168, Italy).

Preparation of erythrocyte lysate (E. lysate)—Blood was collected into a vacutainer pre-coated with anticoagulant. It was centrifuged at 3000 rpm for 15 min, at 4°C in cooling centrifuge (Remi, C-24 BL, Mumbai, India). Plasma was separated and the packed cell volume (PCV) was washed three times with saline. To 0.5 ml of 5% suspension of PCV in saline, 0.5 ml of ice cold distilled water was added and kept aside for 5 min at 4°C. To this, 0.4 ml mixture of chloroform:ethanol (3:5) was added. Contents were mixed well and centrifuged again. The water layer separated was used as E. lysate.

Estimation of haemoglobin—Haemoglobin (Hb) was estimated by Drabkin’s method. Intensity of the colour formed by oxidized haemoglobin with potassium ferricyanide was measured at 530 nm in UV-Visible Spectrophotometer (Shimadzu 1601, Japan).

Determination of CAT activity in E. lysate—Assay was based on the ability of CAT to induce disappearance of H$_2$O$_2$. The reaction mixture contained 30 mM of hydrogen peroxide (H$_2$O$_2$) and 0.05 M phosphate buffer (pH 7.0). Decrease in absorbance of H$_2$O$_2$ was measured at 240 nm. One unit of CAT is equal to the number of µmole of H$_2$O$_2$ decomposed per min at 25°C.

Determination of SOD activity in E. lysate—Assay was based on the ability of SOD to inhibit the spontaneous oxidation of adrenaline to adrenochrome. The reaction mixture contained 3 × 10$^{-4}$ M adrenaline and 0.05 M carbonate buffer (pH 10.2). Decrease in absorbance of adrenochrome was measured at 480 nm. One unit of SOD activity is equal to the amount of enzyme required to inhibit 50% auto-oxidation of adrenaline at 25°C.

Determination of GSH-Px activity in E. lysate—The reaction mixture contained 0.1 M reduced glutathione, 10 U/ml of glutathione reductase, 2 mM nicotinamide adenine dinucleotide phosphate reduced (NADPH), 0.05 M phosphate buffer (pH 7.0) and 7 mM t-butyl hydroperoxide. Decrease in absorbance of NADPH was measured as GSH-Px activity at 340 nm. One unit of GSH-Px is equal to the number of nano moles of NADPH oxidized/utilized per min at 25°C.

Estimation of GSH in erythrocytes—In haemolysates, proteins were precipitated and filtered. GSH (thiol) in the filtrate was treated with 5, 5'-dithiobis-2-nitrobenzoic acid [DTNB-Ellmans reagent]. A stable yellow coloured compound formed was measured at 412 nm.
Estimation of MDA in plasma—MDA with thiobarbituric acid reagent at 95°C for 60 min (pH less than 2) develops a pink coloured compound. It was extracted in butanol for colour stability and measured at 532 nm. Statistical analysis—Data are expressed as mean±SE. Statistical comparison between different groups were done using One-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. *P<0.05 was considered as statistically significant.

Results

Effect on fasting blood glucose—Aqueous extract of F. religiosa at both doses, (100 and 200 mg/kg) significantly decreased the fasting blood glucose in type 2 diabetic rats. However, the difference between 100 and 200 mg/kg dose was significant when analyzed for inter-group comparison. Gliclazide, a standard drug of comparison decreased significantly the fasting blood glucose (Fig. 1).

Effect on body weight—By the end of fourth week, aqueous extract of F. religiosa at 100 and 200 mg/kg dose increased significantly the body weight of diabetic rats as compared to untreated rats. Gliclazide also significantly increased the body weight of diabetic rats. Body weight of various experimental groups at basal level, i.e. before drug treatment (0 time) and at the end of 1st, 2nd, 3rd and 4 weeks of drug treatment is shown in Fig. 2.

Effect on oxidative stress—SOD activity was exaggerated in type 2 diabetic rats as compared to normal group. F. religiosa at 100 mg/kg dose decreased significantly (P<0.05) SOD activity and 200 mg/kg dose decreased the SOD activity significantly at P<0.01. Decrease in CAT and GSH-Px activity was found in type 2 diabetic rats as compared to normal group. Aqueous extract of F. religiosa at 200 mg/kg significantly enhanced the CAT (P<0.05) and GSH-Px (P<0.01) activity in type 2 diabetic rats. F. religiosa at 100 mg/kg dose was unable to modulate the CAT activity but enhanced the GSH-Px (P<0.01) activity. Gliclazide was found to be significant (P<0.01) in modulating the enzymes. Enzyme activity expressed as units/mg of Hb in various experimental groups is shown in Table 1.

Depletion of erythrocyte GSH was observed in type 2 diabetic rats as compared to normal group. Aqueous extract of F. religiosa at both doses inhibited significantly the formation of MDA in type 2 diabetic rats. Gliclazide also significantly improved GSH and inhibited MDA (Table 1).

Discussion

Results from this study demonstrate the aqueous extract of F. religiosa had significant anti-diabetic activity. The drug has been reported to contain...
phytosterols, flavonoids, tannins, furanocoumarin derivatives namely bergapten and bergaptol. Since sterols and coumarins are insoluble in water, activity may attribute to tannins, flavonoids and polyphenols present in the aqueous extract. Flavonoids isolated from F. bengalensis have insulin secretagogue action. Antioxidant property of flavonoids coupled with their nutritional value may be responsible for rejuvenation of pancreas. Rejuvenation increases the biosynthesis and secretion of insulin. F. religiosa being a rasayana drug possibly rejuvenates the pancreas.

Type 2 diabetic rats gained relatively less weight during the course of development as compared to normal rats. Decrease in uptake of glucose, free fatty acids from circulation and accelerated β-oxidation in adipose tissue leads to weight loss in diabetes. It was observed that aqueous extract of F. religiosa improved the body weight in diabetic rats.

Superoxide anion radical (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (OH$^-$) are the major reactive oxygen species (ROS) generated during oxidative stress. Free radicals decrease the insulin receptor substrate (IRS) tyrosine phosphorylation and in turn the activity of phosphatidyl inositol (PI) 3-kinase. Altered insulin signaling pathway exerts insulin resistance, a state of type 2 diabetes. Aerobic cells are endowed with extensive antioxidant defence mechanisms including both low molecular weight scavengers such as reduced glutathione (GSH), ascorbic acid (vitamin C), Vitamin E and enzyme system namely SOD, CAT and GSH-Px. The exaggerated activity of SOD in type 2 diabetic rats is probably an adaptive response towards oxidative stress. F. religiosa modulated the SOD activity in dose-dependent manner. Decrease in CAT activity could be possibly due to less availability of NADPH or gradual decrease in erythrocyte CAT concentration by excessive generation of O$_2^-$ that inactivates the enzyme. Since the activity of an enzyme depends upon its substrate, depletion of GSH may be the reason for decreased GSH-Px activity. Aqueous extract of F. religiosa had up-regulated the CAT and GSH-Px activity. Drug at higher dose (200 mg/kg) was better effective in modulating the enzymes.

GSH plays a major role in cellular protection against oxidative damage. Significant depletion of erythrocyte GSH in type 2 diabetic rats is due to oxidative stress. Aqueous extract of F. religiosa restored the erythrocyte GSH. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids. Lipid peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of lipids.

### Table 1—Effect of aqueous extract of Ficus religiosa (FR) on oxidative stress status of type 2 diabetic rats

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>SOD U/mg Hb</th>
<th>CAT U/mg Hb</th>
<th>GSH-Px U/mg Hb</th>
<th>GSH nmole/g Hb</th>
<th>MDA nmole/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1.24 ± 0.12</td>
<td>0.133 ± 0.012</td>
<td>0.053 ± 0.003</td>
<td>6.53 ± 0.12</td>
<td>1.57 ± 0.05</td>
</tr>
<tr>
<td>Type 2 diabetic control</td>
<td>2.11 ± 0.13</td>
<td>0.082 ± 0.003</td>
<td>0.028 ± 0.002</td>
<td>4.17 ± 0.12</td>
<td>6.31 ± 0.21</td>
</tr>
<tr>
<td>Type 2 diabetic treated with FR (100 mg/kg)</td>
<td>1.55 ± 0.12</td>
<td>0.095 ± 0.004</td>
<td>0.040 ± 0.002</td>
<td>4.80 ± 0.13</td>
<td>2.46 ± 0.11</td>
</tr>
<tr>
<td>Type 2 diabetic treated with FR (200 mg/kg)</td>
<td>1.28 ± 0.11</td>
<td>0.110 ± 0.006</td>
<td>0.043 ± 0.002</td>
<td>5.68 ± 0.15</td>
<td>1.88 ± 0.06</td>
</tr>
<tr>
<td>Type 2 diabetic treated with Gliclazide (10 mg/kg)</td>
<td>1.26 ± 0.12</td>
<td>0.123 ± 0.005</td>
<td>0.045 ± 0.003</td>
<td>5.79 ± 0.14</td>
<td>1.85 ± 0.05</td>
</tr>
</tbody>
</table>

Values are mean ± SE from 6 animals in each group. Significant at *P<0.05, **P<0.01 and Non-significant at *NS*P>0.05 as compared to type 2 diabetic control.

SOD-Superoxide dismutase; CAT-Catalase; GSH-Px-Glutathione peroxidase; GSH-Glutathione and MDA-Malondialdehyde.
Acknowledgement

Authors are thankful to AICTE, New Delhi for financial assistance.

References