An evaluation of the protective role of *Ficus racemosa* Linn. in streptozotocin-induced diabetic neuropathy with neurodegeneration

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**Introduction**

Diabetes mellitus is a metabolic disease which damage different bodily organs, causing kidney failure, vision loss, autonomic and peripheral neuropathy, peripheral vascular disease, myocardial infarction, and cerebrovascular disease with stroke. Diabetes affects the central nervous system and produce disturbances such as behavioral changes, autonomic dysfunctions, altered neuroendocrine functions, and neurotransmitter alterations and thus leading to end organ damage. Various pathways involved in the pathogenesis of diabetic neuropathy and degeneration are polyol, hexosamine, protein kinase C, advanced glycation, poly (ADP-ribose) polymerase, oxidative stress, and inflammation. Oxidative stress

**ABSTRACT**

**Objective:** *Ficus racemosa* (FR) is one of the herbs mentioned in the scriptures of the Ayurveda as Udumbara with high medicinal value. The objective of this study was to estimate the protective effect of FR against streptozotocin (STZ) induced diabetic neuropathy with neurodegeneration (DNN).

**Materials and Methods:** Diabetes was induced in Wistar rats with STZ and were divided into six groups namely diabetic vehicle control, FR (four) and glibenclamide (one) treated rats; while one group was of normal control rats. After the 4th week of diabetes, induction treatment was started for further 28 days (5th to 8th week) with FR aqueous extract (250 mg/kg and 500 mg/kg) and ethanolic extract (200 mg/kg and 400 mg/kg). Investigation of DNN was carried out through biochemical and behavioral parameter assessment in rats.

**Results:** Study showed a significant fall in glycosylated hemoglobin (HbA1c) and blood glucose level by the treatment of FR in diabetic rats. Antioxidant potential of FR showed a great rise in superoxide dismutase, catalase content and reduction observed in serum nitrite level; while significant fall in lipid peroxidation level and of C-reactive protein was observed in FR treated diabetic rats. Further FR treated diabetic rats also showed marked improvement in tail flick latency, pain threshold, the rise in locomotion and fall latency period.

**Conclusion:** Treatment with FR shows protection in the multiple pathways of DNN by improving blood glucose, HbA1c, biochemical, and behavioral parameters, which suggest the protective role of FR in the reversal of DNN.

**KEY WORDS:** Behavioral parameters, biochemical parameters, diabetes mellitus, diabetic neuropathy with neurodegeneration, *Ficus racemosa*
and inflammation play a crucial role in the development and progression of late-stage complications of diabetes,18 an acute phase reactant C-reactive protein (CRP) level rises dramatically during inflammatory processes.19 The antioxidant defenses in humans are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase. Lipid peroxidation (LPO) was one of the characteristic features of the chronic diabetic condition. Plants have been the major source of the treatment for diabetes in the Indian system of medicine and other ancient systems in the world. Ficus racemosa Linn. (FR) (Family Moraceae) is one of the plants mentioned in the ancient scriptures of Ayurveda. The aim of the present study was to investigate the protective role of FR Linn. in streptozotocin (STZ) induced diabetic neuropathy and neurodegeneration (DNN), which has not been evaluated so far.

Materials and Methods

Drugs and Chemicals

All reagents were analytical grades. Biochemical kits were purchased from Span diagnostics, India. STZ was obtained from Sigma chemicals, India. Glibenclamide (GL) was obtained as gratis sample.

Extraction Methods of Ficus racemosa

FR stem bark was collected from the Directorate of medicinal and Aromatic Plants Research, Gujarat. The stem bark was shade dried and converted to powder form by the milling process. An amount of 30 g of powder was dissolved in 3/4th of water; and the mixture was boiled with the temperature not exceeding 50–60°C till the volume of water came down to one-fourth. The mixture was cooled, and concentrated portion was considered as aqueous extract. A total 200 g of powder was dissolved in 99.5% strength with 2/3rd of ethyl alcohol mixed well and refluxed for 3–4 days (3–4 h/day). The alcoholic portion was transferred in rota-vapor (temp of bath 60°C, RPM – 110, Vacuum – 100) until complete drying of alcoholic extract. The aqueous extract was prepared in 10% tween 20 solution prepared in double distilled water while ethanolic extracts were prepared in 5% gum acacia solution for oral administration. Both the vehicles utilized for aqueous and ethanolic extract were inert.

Animals and Experimental Protocol

The animal protocol was approved by the Institutional Animal Ethics Committee. Male Wistar rats (300 ± 50 g) were provided the standard pelleted diet and ad libitum water, were kept at environmental temperature (23°C ± 3°C) and under 12 h light-dark cycle. The animals were acclimatized to the experimental conditions a week before the study. Acute toxicity study of FR was performed with a dose level of 2000 mg/kg,20 which formed the basis for dose selection in the efficacy study.

Induction of Diabetic Complication with Treatment Schedule

Diabetes was induced in overnight fasted rats by a single intraperitoneal injection of fresh STZ7,26 (60 mg/kg body weight) in citrate buffer (0.1M, pH 7.4). After 48 h of STZ injection, fasting blood samples were withdrawn from tail vein and blood glucose level was measured by use of a glucometer (Accu-Chek, Johnson and Johnson, India). The animals having fasting blood glucose level ≥230 mg/dl were randomized in groups. The development of DNN was confirmed by basal nociceptive reaction at the 4th week of STZ injection, and all treatments were started thereafter from 5th to 8th week. GL is widely used as second-line therapy in diabetes and can lower glycosylated hemoglobin (HbA1c) by 1–2%,28 several research reports had mentioned the use of GL as a positive control.18,19,20 At the end of 8th week, behavioral analysis were done and the blood sample was collected, while brain and sciatic nerve were isolated and washed with ice-cold saline, then homogenate was prepared and stored at −80°C till further analysis. No mortality was observed during the study period.

The animals were divided into seven groups (n = 5):

- Normal control (NC) animals received saline
- Diabetic control (DC) animals received saline
- FR aqueous extract treated diabetic animals (250 mg/kg) p.o (DAFR-250)
- FR aqueous extract treated diabetic animals (500 mg/kg) p.o (DAFR-500)
- FR ethanolic extract treated diabetic animals (200 mg/kg) p.o (DEFR-200)
- FR ethanolic extract treated diabetic animals (400 mg/kg) p.o (DEFR-400)
- GL-treated diabetic rats (2.5 mg/kg) (DGLY).

Biochemical Markers

Analysis of blood glucose and glycosylated hemoglobin level

The blood glucose level was analyzed with glucometer using glucose reagent strip while HbA1c level was measured as reference cited.121

Serum protein level and C-reactive protein

Serum protein (SP) level (g/dl) was estimated by a method as per assessment kit.13 CRP level (mg/l) was determined by particle enhanced immunoturbidometry method.14

Serum antioxidant catalase and superoxide dismutase

CAT activity of serum samples was expressed in µmol H₂O₂ utilized per min/mg of protein.15 SOD level in serum was expressed as mU of SOD/mg protein.16

Serum nitrite level

The serum nitrite level was estimated using greiss reagent which served as an indicator of nitric oxide production.17

Tissue homogenate level of malonaldehyde

LPO product as malonaldehyde (MDA) level estimated in tissue homogenate by method cited in reference.18

Behavioral Markers

Tail immersion (hot water) test

The tail of the rat was immersed in a hot water bath (55°C ± 0.5°C) until withdrawal or signs of struggle were observed (cutoff 10 s). Shortening of the tail-withdrawal time indicated hyperalgesia.19

Hot plate test

In this test, animals were individually placed on a hot plate with the temperature adjusted to 43°C ± 1°C. The latency to the first sign of paw licking or jump response to avoid the heat was taken as an index of the pain threshold.19

Motor coordination activity

The motor coordination and performance of each rat was evaluated using rota-rod apparatus. Latency to fall from the rotating bar was registered in seconds.22
Locomotion activity

Photoactometer test was performed to study the effect of drug treatment on spontaneous motor activity and cutoff of the photocell beam was recorded.\cite{27}

Statistical Analysis

Values in the result were expressed as a mean ± standard error of the mean. Differences between groups mean were estimated using one-way analysis of variance. Paired t-test was applied for the comparison of groups at different time interval. The result was considered statistically significant at $P < 0.05$, 0.01, and 0.001.

Results

STZ injected Wistar rats had produced cardinal signs of diabetes, i.e., weekly change in body weight [Figure 1], polyphagia, and polydipsia [Table 1], which persisted throughout the period of study.

Effect of Ficus racemosa on Body Weight, Food, and Water Intake

Chronic treatment with FR significantly in diabetes prevented weight loss as compared to DC rats [Figure 1]. FR treated diabetic rats also showed a reduction in food consumption and increased water intake during the study period.

Effect of Ficus racemosa on Biochemical Markers

Effect of Ficus racemosa on blood glucose and glycosylated hemoglobin level

Sustained hyperglycemia was observed in STZ induced diabetic rats during the study. There was significant reduction seen in blood glucose level of FR treated diabetic animals as compared to DC rats [Figure 2]. HbA1c level was significantly increased in diabetic animals (8.14 ± 0.34), while aqueous (5.66 ± 0.78, 5.44 ± 0.73) and ethanolic extract of FR (6.38 ± 1.02, 5.47 ± 1.84) treated animals showed a significant reduction in HbA1c level [Table 2].

Effect of Ficus racemosa on Antioxidant Level

Antioxidant enzyme (CAT and SOD) levels were significantly reduced in diabetic animals ($P < 0.05$) as compared to normal animals, while FR treated groups showed significant rise in CAT and SOD enzyme levels as of DC rats.

Effect of Ficus racemosa on Serum Protein, C-reactive Protein, and Nitrite Level

Protein present in the blood was significantly higher in diabetic rats as compared to NC animals [Table 2]. Treatment

Figure 1: Effect of Ficus racemosa aqueous and ethanolic extracts administration on weekly changes in body weight (g). Each value was considered as mean ± standard error of mean. ($n = 5$) statistical significant difference was mentioned for Ficus racemosa treated groups ($P < 0.05$) as compared to diabetic control animals

Table 1: Effect of FR Linn. Aqueous and ethanolic extracts administration on food and water intake

| Groups   | Weekly food intake (g) | Weekly water intake (mL) |
|----------|------------------------|--------------------------|
|          | 2nd week               | 4th week                 | 6th week | 8th week | 2nd week               | 4th week                 | 6th week | 8th week |
| DAFR-250 | 245±100                | 237.5±96.93              | 216.6±88.43 | 192.5±78.57 | 300±123                | 312.5±127               | 283.3±115 | 310±126  |
| DAFR-500 | 245±100                | 225±91.83                | 210±85.71  | 197.5±80.61  | 350±143                | 412.5±168               | 375±153  | 335±136  |
| DFR-200  | 230±93.87              | 217.5±88.75              | 220±89.79  | 187.5±76.53  | 325±132                | 312.5±127               | 343±140  | 357.5±145 |
| DFR-400  | 170±69.38              | 207.5±84.69              | 203±83.8   | 197.5±80.61  | 265±108                | 335±136               | 356.6±145 | 332.5±135 |
| DGLY     | 165±67.34              | 245±100                 | 223.3±91.15 | 182.5±74.48  | 305±125                | 305±124               | 316.6±129 | 342.5±139 |
| DC       | 310±126.53             | 245±100                 | 216.6±88.43 | 200±16.63   | 290±118                | 425±173               | 400±163  | 392.5±160 |
| NC       | 170±69.38              | 232.5±94.89             | 206.6±84.35 | 220±87.9    | 250±102                | 275±112               | 326.6±133 | 268.7±109 |

Each value was considered as mean±SEM ($n=5$). SEM=Standard error of mean, FR=Ficus racemosa
with FR showed a significant reduction in SP content as compared to DC rats. CRP levels were significantly increased in diabetic rats as compared to normal rats, while FR treated diabetic rats showed a significant reduction in CRP level as compared to DC rats [Table 2].

Nitrite levels were significantly increased in diabetic rats as compared to NC rats, while FR treated animals showed a significant reduction in nitrite level as compared to DC rats [Table 2].

**Effect of Ficus racemosa on Tissue Malondialdehyde Level**

Tissue MDA level was significantly raised in brain and nerve tissues of diabetic rats. Two-fold decrease in the level of brain MDA was observed in FR (aqueous extract) treated diabetic rats, while four-fold decrease in brain MDA level was observed in FR (ethanolic extract) treated diabetic rats as compared to DC rats [Table 2]. Nerve MDA level were reduced four-fold with FR treated (ethanolic extract) diabetic rats as compared to DC rats.

**Discussion**

STZ causes direct DNA damage to the pancreatic islets of beta cells, which leads to hyperglycemic state. Increase in blood glucose and HbA1c levels following STZ treatment observed in our study was supported by other work.\[^{10,22}\] Coscinium fenestratum stem and Catharanthus roseus brought back the status of blood glucose and HbA1c to normal range in diabetic rats,\[^{10}\] supporting our study results. Alteration in antioxidant defense in the diabetic rats was evidenced by a significant reduction in serum antioxidant enzyme activity in diabetic rats. The decrease in antioxidant enzymes activity in the hyperglycemic rats could be due to oxidative stress induced inactivation\[^{23}\] which was observed in present study. Antioxidant levels were increased with FR treatment in DNN supported

**Table 2:**

| Groups       | SP analysis (g/L) | Serum catalase analysis (µmol/ mg of protein) | Serum SOD analysis (mU/ mg protein) | Serum CRP analysis (µg/mL) | Serum nitrite level (µg/mL) | Tissue MDA analysis in brain homogenate (mmol/g) | Tissue MDA analysis in nerve homogenate (µmol/g) | HbA1c estimation (%) |
|--------------|------------------|---------------------------------------------|-------------------------------------|---------------------------|-----------------------------|-----------------------------------------------|-----------------------------------------------|---------------------|
| DAFR-250     | 3.22±0.375*      | 571.48±92.13**                             | 398.58±103.24**                     | 4.8±0.84**                | 3.99±1.28**                 | 5.58±0.28                                     | 2.099±0.56                                     | 5.66±0.78*          |
| DAFR-500     | 6.73±0.513       | 487.72±96.58**                             | 440.33±122.06**                     | 4.32±1.33**               | 4.60±1.36**                 | 3.95±0.92                                     | 1.71±0.64                                     | 5.44±0.73*          |
| DEFR-200     | 5.51±1.271**     | 363.77±90.74**                             | 295.28±76.97**                      | 2.61±0.46**               | 7.90±1.26**                 | 0.73±0.10                                     | 0.90±0.44                                     | 6.38±1.02           |
| DEFR-400     | 5.11±0.498**     | 1072.17±387.40**                           | 434.10±190.33**                     | 2.40±0.37**               | 5.57±1.76**                 | 2.03±0.09                                     | 1.59±0.60                                     | 5.47±1.84*          |
| DGLY         | 3.19±0.557**     | 587.17±201.82**                            | 284.80±88.36**                      | 4.54±1.14**               | 6.72±2.43**                 | 2.51±0.47                                     | 1.01±0.38                                     | 7.56±0.93           |
| DC           | 8.33±1.344**     | 182.82±25.56**                             | 182.76±25.56**                      | 8.06±2.80**               | 22.26±2.98**                | 8.03±1.53                                     | 5.19±2.64                                     | 8.14±0.34*          |
| NC           | 3.61±0.653**     | 758.01±202.99**                            | 679.08±242.57**                     | 3.89±0.63**               | 3.53±1.12**                 | 4.27±0.77                                     | 1.33±0.93                                     | 3.86±0.08**         |

Each value was considered as mean±SEM (n=5). Statistical significant difference of treated groups at *P<0.05, **P<0.01 compared to DC rats, statistical significant difference of disease group at *P<0.05, **P<0.01 compared to NC rats. SEM=Standard error of mean, HbA1c=Glycosylated hemoglobin, SOD=Superoxide dismutase, CRP=C-reactive protein, MDA=Malondialdehyde, FR=Ficus racemosa, SP=Serum protein

**Table 3:**

| Groups       | Photoactometer test | Rotarod test | Hot plate assay | Tail immersion test |
|--------------|---------------------|--------------|-----------------|---------------------|
|              | Number of cut-off observed (s) | Fall latency from bar (s) | Jump time (s) | Tail flick latency before treatment (s) | Tail flick latency after treatment (8 weeks) (s) |
| DAFR-250     | 90.2±6.36*          | 5.60±4.196   | 5.46±4.029**    | 6.4±3.068          | 7.38±3.083**               |
| DAFR-500     | 95.4±6.98*          | 16.07±5.09   | 5.03±6.62**     | 5.16±0.7          | 7.27±0.43**                |
| DEFR-200     | 77.5±3.57*          | 9.90±1.95**  | 4.51±0.816**    | 4.98±0.28         | 6.03±0.11**                |
| DEFR-400     | 78.75±7.43          | 15.27±2.53** | 5.48±0.818**    | 3.86±0.39         | 7.37±0.25**                |
| DGLY         | 106±13.5*           | 8.20±2.05   | 3.94±0.127      | 5.2±0.22          | 5.09±0.48*                 |
| DC           | 59.8±2.2*           | 2.20±0.24**  | 2.09±2.31**     | 3.95±0.29         | 3.01±0.1**                 |
| NC           | 100.2±2.03*         | 38.27±4.62** | 4.61±4.836      | 6.81±1.09         | 6.56±0.86**                |

Each value was considered as mean±SEM (n=5). Statistical significant difference of treated groups at *P<0.05, **P<0.01 compared to DC rats, statistical significant difference of disease group at *P<0.05, **P<0.01 compared to NC rats. SEM=Standard error of mean, FR=Ficus racemosa
by result of carnitine and lipoic acid had reduced free radical production and increased in antioxidant status thereby lowering oxidative stress,\textsuperscript{159} while ginger had significantly improved the level of SOD, CAT in diabetic rats.\textsuperscript{111} The excessive production of superoxide and peroxynitrite in sciatic nerve have been linked with altered vasorelaxation responsible for nerve perfusion irregularities.\textsuperscript{225} In hyperglycemia induced oxidative injury, key mediator is peroxynitrite formed by the combination of superoxide with nitric oxide that exerts detrimental effects on the nerve tissue leading to neuropathic pain.\textsuperscript{290} Thus formed peroxynitrite further initiates the pathways implicated in the development of diabetes neuropathy and degeneration.\textsuperscript{227} Present study showed reduced nitrite level by FR in diabetic rats, supported by a work that showed reduced neural nitrite level in naringin-treated diabetic rats.\textsuperscript{291} In another study, cannabis exhibited significantly lower levels of nitrite in diabetic condition.\textsuperscript{292} CRP, an acute phase reactant, was a highly sensitive marker of inflammation.\textsuperscript{293} Treatment with FR extracts showed anti-inflammatory potential, which is supported by results of berberine, which had reduced plasma CRP levels.\textsuperscript{294} LPO was one of the characteristic features of chronic diabetes. The overall effect of LPO was to decrease the membrane fluidity, deformability, visco elasticity of tissues, which was improved by treatment with FR extracts in diabetic rats, result of other studies showed decreased LPO level in diabetic rats when treated by ginger\textsuperscript{295} and Erythrina variegate also showed reduction in basal LPO as compared to DC.\textsuperscript{296} The nociceptive threshold was significantly lower in diabetic animals than nondiabetic animals indicating that diabetics exhibited thermal hyperalgesia. Diabetic rats showed a significant reduction in paw withdrawal threshold, which indicates the development of hyperalgesia. Present study revealed that treatment with FR prevents allodynia which further reduces neuropathic pain in diabetic rats, in scientific evidence naringin-treated diabetic rats attenuated reduced mean tail withdrawal latency as compared to diabetic rats.\textsuperscript{297} Present study also evaluated the behavioral response in motor and lo performance of diabetic and NC rats through rota‑rod and photoactometer test. Diabetic rats showed lower fall off time from the rotating rod when compared to NC and suggesting impairment in their ability to integrate sensory input with appropriate motor commands to balance their posture as showed in present study supported by literature.\textsuperscript{298} Present study showed reduced motor performance (mp) and lo in STZ‑induced diabetic rats, and FR treated rats showed improvement for mp and lo in diabetic rats. In one study, fall latency was improved, motor incoordination was prevented, and lo counts were improved when diabetic rats treated by the extract of Parkinsonia aculeata\textsuperscript{299} supports our study results.

**Conclusion**

The data of present study suggest that FR exhibit protective effect by reducing complications of DNN through preventing a rise in glycated hemoglobin content, reduced oxidative-nitrosative stress level, and decreased early inflammation level. FR treatment also showed excellent antioxidant potential with a low level of LPO thus provided protection in diabetic tissue. Behavioral aspects were improved during treatment by FR in diabetic animals. FR may be considered as a future option due to varieties of pharmacological actions with proven results in DNN for its reversal. However, further studies are required for the better understanding of the mechanism of action of FR.

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**Conflicts of Interest**

There are no conflicts of interest.

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