ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 15, Issue 11, 2022

Online - 2455-3891
Print - 0974-2441

Research Article

EVALUATION OF ROLE OF FICUS BENGALENSIS IN MODULATION OF COGNITIVE IMPAIRMENT AND OXIDATIVE STRESS IN DIABETIC RATS

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Received: 17 July 2022, Revised and Accepted: 26 August 2022

ABSTRACT

Objectives: Our objective was to study the effects of *Ficus benghalensis* on cognitive behavior and oxidative stress in diabetic rats and to compare with vitamin C and glimepiride.

Methods: Wistar rats of either sex randomized into five groups of diabetic rats by streptozocin (STZ), five groups of non-diabetic rats (distilled water) (n=10). Subgroup division (*F. benghalensis* dose I and II, i.e., 50 mg/kg and 100 mg/kg; Vitamin C 100 mg/kg and glimepiride 0.5 mg/kg) were done. Each drug was given to one diabetic and one non-diabetic group. Other set served as control. Assessment of blood glucose, cognitive function (using continuous avoidance apparatus and Morris water maze test), and oxidative stress (measuring Malondialdehyde (MDA), reduced glutathione (GSH) levels) were done on Day 0 and 30. The acquisition phase of cognitive behavior tests was assessed on 0, 14, and 29 days and retention phase was assessed on 1, 15, and 30 day.

Results: As compared to control group, *F. benghalensis* dose I, dose II, and glimepiride showed significant decrease ($p<0.001$) in blood glucose. *F. benghalensis* dose I, dose II, vitamin C, and glimepiride group showed significant decrease in acquisition and retention of transfer latency on 29 and 30 days. Significant increase in retention of step-down latency on 30 day was shown by both the doses of *F. benghalensis*. Both doses of *F. benghalensis*, vitamin C and glimepiride group showed significant increase in retention of Quadrant-time in comparison to control on 30 days. Significant decrease in brain MDA levels while a significant increase in brain GSH levels was observed in all groups except control.

Conclusion: *F. benghalensis* reverses behavioral and biochemical changes induced by STZ and effects are comparable with that of vitamin C and glimepiride.

Keywords: *Ficus benghalensis*, Diabetic rats, Oxidative stress, Cognitive impairment, Vitamin C, Streptozocin.

 DOI: http://dx.doi.org/10.22159/ajpcr.2022v15i11.45859. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Derangement of carbohydrate metabolism is main mechanism leading to morbidity, disability and hospitalization in diabetes mellitus. It is associated with cognitive dysfunctions due to neurophysiological and structural changes in the central nervous system [1].

Changes in neurochemical, neurophysiological, and structural abnormalities due to diabetes have been described by various studies [2,3]. Cognitive function and memory impairment with reduced mental flexibility and psychomotor slowing are part of neurobiological changes occurring in diabetes [4-7].

Multifactorial process is involved in causing brain damage in diabetes mellitus, these includes the chronic metabolic and vascular disturbances resulting from fluctuations in blood glucose levels [8].

Studies have shown that oxidative stress due to hyperglycemia in various brain regions perhaps results in cognitive impairment [9].

The central nervous system (CNS) is unique in its high metabolic rate which is maintained by continuous supply of glucose and oxygen. The brain is particularly susceptible to free radical attack, because it generates more of these toxicants per gram of tissue than any other organ and has low anti-oxidative capacity. Hence, oxidative stress in CNS has got predominant role in pathophysiology of various neurodegenerative disorders.

Historically, ethnobotanicals and herbal medicines were being used in treatment of blood sugar abnormalities. *Ficus benghalensis* (Banyan tree) belonging to Moraceae family, is a large tree with aerial roots, found all over India particulary in lower Himalayas, and has gotastringent action on bowels; useful in treatment of ulcers, erysipelas (As per Indian system of medicine (Ayurveda)). According to Unani system, its latex is aphrodisiac, tonic, useful in piles, and gonorrhea and possesses anti-inflammatory property. Fruits, aerial roots and bark of *F. benghalensis* have been shown to exhibit anti diabetic property by different group of researchers [10]. Experimental models have demonstrated that the water and alcoholic extract (leucodelphinidin-active compound) of the bark produces hypoglycemic effect. It has also been shown to possess lipids and cholesterol lowering effects. The antioxidant effect of the aqueous extract of the bark of *F. benghalensis* has been reported in hypercholesterolemic rabbits [11].

Considering the hypoglycemic and antioxidant action of *F. benghalensis* and the possible neurobiological changes induced by diabetes, the aim of this study was to study the effect of *F. benghalensis* on the parameters of cognitive behavior in diabetic rats and to compare the effect of *F. benghalensis* with Vitamin C – an antioxidant, and Glimepiride – an oral hypoglycemic agent, on cognitive function and on parameters of oxidative stress, namely, malondialdehyde (MDA) and reduced glutathione (GSH) levels in brain of diabetic rats.

METHODS

Animals

It was an open-label, prospective, randomized, and controlled animal study, in which Wistar rats of 6–8 weeks, either sex weighing 150–200 g were randomly allocated into ten groups of ten each in separate cages.

Mixing of sexes was avoided. Animals were procured from Central Animal House, University College of Medical Sciences, Delhi. The animals were kept under standard laboratory conditions (Temperature: 22±2°C, relative humidity of 50–55% and with natural light/day cycle). Polypropylene cages of standard size 32.5×21 cm, with paper bedding were used with adequate hygiene. Standard pelleted laboratory diet and water ad libitum were fed. Acclimatization of animals to laboratory conditions was done 2 h before the commencement of experiments. The study was approved by the Institutional Animal Ethics Committee (No.-21/IAEC/UCMS/2009) and appropriate care to animals was provided as per "Committee for the Purpose of control and supervision of experiments on animals Guidelines."

Drugs and treatment schedule

Induction of diabetes

After overnight fasting, rats were administered a single intraperitoneal injection of streptozotocin (STZ) (0.2 ml, 50 mg/kg) dissolved in citrate buffer (50 mM) of pH 4.5. Starting from the day of injecting STZ, the fasting blood glucose levels were estimated every morning and stabilization (i.e., similar fasting blood glucose levels in three consecutive sampling) was achieved in 5 days. This period of stabilization was similar for all the rats. The volume of blood withdrawn was limited to 0.5 ml for each sampling. From Day 1 (the next day of blood glucose stabilization), drug treatment was started and continued for 30 days. The extract of *F. benghalensis* was procured from Arjuna Extracts, Kerala in the crude form and was, further, processed by extraction techniques in the local laboratory. Other drugs were provided by local pharmacy.

In the experiments, the rats were divided into ten groups with ten animals in each group (n=10, each), after simple randomization was done using research randomizer. Group 1 comprised non-diabetic control which was given distilled water p.o., whereas Group 2 served as a diabetic control induced by single injection of Streptozotocin (50 mg/kg, i.p.). Group 3 comprised non-diabetic rats receiving dose I of *F. benghalensis* (50 mg/kg, p.o.), whereas Group 4 comprised diabetic rats receiving dose II of *F. benghalensis* (50 mg/kg, p.o.). Group 5 comprised non-diabetic rats receiving dose II of *F. benghalensis* (100 mg/kg, p.o.), whereas Group 6 comprised diabetic rats receiving dose II of *F. benghalensis* (100 mg/kg, p.o.). Group 7 comprised non-diabetic rats receiving vitamin C (100 mg/kg, p. o.), whereas Group 8 comprised diabetic rats receiving Vitamin C (100 mg/kg, p.o.). Group 9 comprised non-diabetic rats receiving glimepiride (0.5 mg/kg, p.o.), whereas Group 10 comprised diabetic rats receiving glimepiride (0.5 mg/kg, p. o).

The diabetic and normal animals were kept in the separate cages and their body weight, the levels of serum glucose, assessment of cognition, and acetylcholine esterase levels in all animals were done and these quantities were compared.

Assessment of blood glucose levels

Fasting blood sample was collected from the retro-orbital plexus of the rat under mild halothane inhalation 1 day before the induction of diabetes and on 15th day and 30th day after the stabilization of blood glucose levels. The blood glucose levels of 200 mg/dl or more were considered as diabetic rats.

Assessment of cognition

Transfer latency (TL) on elevated plus maze

The animals were placed individually at the end of either of the open arms of elevated plus maze and the **TL** was noted on Day 0 (1 day before induction of diabetes), on 14th day, and 29th day.

Time taken by the animal to move from the open arm to the closed arm is designated as TL. The animals not entering a closed arm within 90 s were excluded from the study. Retention was examined 24 h after each trial, that is, on day 1, 15, and 30.

Step down latency (SDL) in continuous avoidance apparatus

A continuous avoidance apparatus was used having an insulated platform which was placed in the center of a metallic grid floor. The platform served as a shock free zone (SFZ). Electric shock (20 V) through the grid floor was delivered on stepping-down by the rat after being placed on the SFZ. The time taken by the rat to step down was recorded. This is known as SDL. Increase of SDL was used as parameter of learning. Retention was examined 24 h after each SDL trial (i.e., on 1st, 15th, and 30th day).

Morris water maze (Spatial navigation task)

Animals were trained to swim to a visible platform in a circular pool (Diameter – 180 cm and Height – 60 cm), which was filled with water (a non-toxic dye was added to make it opaque).

a) Maze acquisition phase is begun by releasing the animal into the maze facing toward the wall of the pool. For a maximum duration of 3 min, the latency to find the escape platform was recorded. Initial acquisition latency (IAL) was the time taken by rat to reach the platform. It was done on day before induction of diabetes, on 14th day and day 29.
b) Maze retention phase – After maze acquisition phase, testing for retention of the learned task was done. Rats were released randomly and the time taken to find the hidden platform was recorded (on 15th and 30th day of treatment with study drugs). It was termed as first retention latency (RL1) and second RL2, respectively. After removal of platform, the first Quadrant time (QT)-1 and QT-2 were recorded (these were the time spent by the rat in the target quadrant on 15th and 30th days of treatment, respectively).

Assessment of biochemical parameters

The animals were euthanized as per standard guidelines – under mild CO_2 inhalation and brain were taken out, and assessment of biochemical parameters was done on day 31. The parameters of oxidative stress used were MDA and reduced glutathione (GSH).

Estimation of MDA

MDA (indicator of lipid peroxidation) was estimated as described by Okhawa *et al*. [12]. Acetic acid detaches the lipid and protein of the tissue. The protein in the reaction mixture is dissolved by the addition of lauryl sulfate. MDA reacts with the lipid peroxides, hydroperoxides, and oxygen double bonds to form the color adducts with absorption maxima at 532 nm. The readings of absorbance were plotted against the concentration of MDA to produce a standard curve. The concentration of MDA was determined by the linear standard curve and expressed as nmol/g wet brain tissue.

Estimation of reduced glutathione (GSH)

Reduced glutathione was estimated by the method described by Ellman [13]. Glutathione is tripeptide, important for cellular defense against ROS toxicity and is key cellular reductant to maintain the redox state. The readings of absorbance were plotted against the concentration of GSH to produce standard curve. The concentration of GSH was determined by linear standard graph and expressed as μg/g wet brain tissue.

Statistical analysis

The continuous variables are presented in the form of mean±SEM. Comparison among different groups was done using one-way ANOVA followed by *post hoc* Tukey's HSD test using SPSS version 23.

RESULTS

Effect of various drugs on blood glucose levels

F. benghalensis dose I (50 mg/kg), dose II (100 mg/kg), and glimepiride (0.5 mg/kg) in diabetic rats showed significant decrease ($p<0.001$) in blood glucose levels when compared to STZ (diabetic control) group at day "30." Unlike *F. benghalensis* and glimepiride; Vitamin C

(100 mg/kg) did not cause any significant change in blood glucose levels when compared to STZ (diabetic control) group (Table 1 and Fig. 1).

Effect of various drugs on behavioral parameter of learning and cognition

Acquisition of TL in elevated plus-maze

Significant increase in acquisition of TL ($p<0.001$) was observed in diabetic control (STZ) group as compared to non-diabetic control (Distilled water) group at day "29". In comparison with STZ (diabetic control) group, the groups showing significant decrease in acquisition of TL were *F. benghalensis* dose I group ($p<0.05$) and *F. benghalensis* dose II group ($p<0.01$). No significant change was observed in acquisition of TL with glimepiride and vitamin C, when compared to diabetic control (STZ) group (Table 2 and Fig. 2).

Table 1: Effect of various drugs (×30 days) on blood glucose levels of streptozotocin (STZ) induced diabetic rats

Group	Treatment and Route (mg/kg/day) p.o.	Blood glucose (mg/dl) Mean±SEM	
		Day 0	Day 30
Distilled water (non-Diabetic healthy Control)	0.2 ml	81±2.69	79.7±2.34
Streptozotocin (Diabetic Control)	50 mg/kg, single i.p. injection	275.3±3.61[a]	268.9±4.87[a]
Ficus bengalensis Dose I	50	276±4.49[a]	188.5±3.38[a,b]
Ficus bengalensi Dose II	100	274.5±5.42[a]	164.2±4.60[a,b]
Vitamin C	100	272.1±5.34[a]	275.7±6.16[a]
Glimepiride	0.5	273.5±4.27[a]	171.6±3.43[a,b]
		F=285.71	F=298.14
		Df=6, 63	Df=6,63

n=10, [a]$p<0.001$ as Compared to the non-diabetic control (Distilled water) group, [b]$p<0.001$ as Compared to diabetic control (STZ) group, one-way ANOVA followed by *post hoc* Tukey's test

Table 2: Effect of various drugs (×30 days) on the acquisition of transfer latency in streptozotocin (STZ) induced diabetic rats

Groups	Treatment and route (mg/kg/day) p.o	Transfer latency (Seconds) Mean±SEM		
		Day 0	Day 14	Day 29
Distilled water (non-Diabetic healthy Control)	0.2 ml	72.6±6.55	55.4±6.83	28.1±3.20
Streptozotocin (Diabetic Control)	50 mg/kg, single i.p.inj	80.1±4.43	70.3±6.91	63.7±5.02[a]
Ficus bengalensis Dose I	50	73.4±5.75	51.1±6.25	40.6±5.16[c]
Ficus bengalensis Dose II	100	77.3±4.46	42±3.88	36.4±3.25[b]
Vitamin C	100	72.6±6.35	61.6±5.88	47.6±4.69[a]
Glimepiride	0.5	68.5±5.76	58.9±6.07	46.4±5.01
			F=41.477	F=6.042
			Df=6,63	Df=6,63

n=10, [a]$p<0.001$ as Compared to the non-diabetic control (Distilled water) group, [b]$p<0.01$ as compared to diabetic control (STZ) group, [c]$p<0.05$ as compared to the diabetic control (STZ) group, one-way ANOVA followed by *post hoc* Tukey'stest

Retention of TL in elevated plus-maze (after 24 h of acquisition)

Significant increase in retention of TL was seen in STZ group ($p<0.001$) as compared to non-diabetic control group at day "30." Significant decrease in retention of TL was seen in *F. benghalensis* dose I group ($p<0.01$), *F. benghalensis* dose II group ($p<0.01$), and glimepiride group ($p<0.05$). However, vitamin C did not cause any significant change in retention of TL when compared to STZ (diabetic control) group (Table 3 and Fig. 3).

Acquisition of SDL in continuous avoidance apparatus

Significant increase in acquisition of SDL was seen in *F. benghalensis* dose II group ($p<0.05$) as compared to diabetic control (STZ) group at day "14." Significant decrease in acquisition of SDL was seen in STZ group ($p<0.001$) as compared to non-diabetic control (Distilled water) group at day "30." In comparison with STZ (diabetic control) group, the groups which showed significant increase in acquisition of SDL are *F. benghalensis* dose II group ($p<0.01$) and glimepiride group ($p<0.01$). Whereas, *F. benghalensis* dose I and Vitamin C did not cause any significant change in acquisition of TL when compared to STZ (diabetic control) group (Table 4 and Fig. 4).

Retention of SDL in continuous avoidance apparatus

Significant increase in retention of SDL was seen in *F. benghalensis* dose I group ($p<0.001$), *F. benghalensis* dose II group ($p<0.001$) and glimepiride group ($p<0.05$) as compared to STZ (diabetic control) group at day "15." Significant increase in retention of SDL was observed in *F. benghalensis* dose I group ($p<0.001$), *F. benghalensis* dose II group ($p<0.001$), vitamin C group, and glimepiride group ($p<0.001$) at day "30" (Table 5 and Fig. 5).

Retention of spatial navigation task in Morris water maze

Significant decrease in RL-1 was shown by *F. benghalensis* dose I group ($p<0.001$), *F. benghalensis* dose II group ($p<0.01$), vitamin C group ($p<0.001$), and glimepiride group ($p<0.001$) as compared to STZ (diabetic control) group at day '15'.

Significant decrease in RL-2 was shown by *F. benghalensis* dose I group ($p<0.001$), *F. benghalensis* dose II group ($p<0.001$), vitamin C group ($p<0.001$), and glimepiride group ($p<0.001$) at day '30'(Table 6 and Fig. 6).

Table 3: Effect of various drugs (×30 days) on retention of transfer latency in streptozotocin (STZ) induced-diabetic rats

Group	Treatment and route	Transfer latency (Seconds) Mean±SEM		
		Day 1	Day 15	Day 30
Distilled water (non-diabetic healthy Control)	0.2 ml	51±8.97	33.8±7.39	19.6±2.51
Streptozotocin (Diabetic Control)	50 mg/kg, single i.p. injection	70.8±4.65	54.3±7.45	60.4±5.58[a]
Ficus bengalensis Dose I	50	58.4±6.81	41.7±3.82	33.9±4.56[c]
Ficus bengalensis Dose II	100	54.5±5.00	40.9±3.79	30.4±4.08[c]
Vitamin C	100	65.3±5.46	50.3±5.15	42.5±5.24[b]
Glimepiride	0.5	63.2±7.31	48.6±5.12	36.7±4.23[d]
				F=7.152
				Df=6,63

n=10, [a]$p<0.001$ as compared to the non-diabetic control (Distilled water) group, [b]$p<0.001$ as compared to diabetic control (STZ) group, [c]$p<0.01$ as compared to the diabetic control (STZ) group, [d]$p<0.05$ as compared to the diabetic control (STZ) group, one-way ANOVA followed by *post hoc* Tukey'stest

Table 4: Effect of various drugs (×30 days) on acquisition of step-down latency in streptozotocin (STZ) induced-diabetic rats

Group	Treatment and route (mg/kg/day) p.o	Step-down latency (Seconds) Mean±SEM		
		Day 0	Day 14	Day 29
Distilled water (Non-diabetic healthy Control)	0.2 ml	173.4±13.45	214.8±12.86	267.3±9.87
Streptozotocin (Diabetic Control)	50 mg/kg, single i.p. injection	187.4±14.19	179.5±14.87	185.5±20.04[a]
Ficus bengalensis Dose I	50	192.5±16.76	229.6±10.20	266.2±9.16
Ficus bengalensis Dose II	100	190.6±7.85	232.9±10.15[c]	257.7±10.71[b]
Vitamin C	100	191.8±11.29	216.2±9.14	239.4±13.20
Glimepiride	0.5	192.2±14.65	218.2±10.93	252.7±10.61[b]
			F=2.394	F=4.575
			Df=6,63	Df=6,63

n=10, [a]p<0.001 as compared to the non-diabetic control (Distilled water) group, [b]p<0.01 as compared to diabetic control (STZ) group, [c]p<0.05 as compared to the diabetic control (STZ) group, one-way ANOVA followed by *post hoc* Tukey's test

Table 5: Effect of various drugs (×30 days) on retention of step-down latency in streptozotocin (STZ) induced-diabetic rats

Group	Treatment and route (mg/kg/day) p.o	Step-down latency (Seconds) Mean±SEM		
		Day 1	Day 15	Day 30
Distilled water (non-Diabetic healthy Control)	0.2 ml	237.3±14.59	256.7±14.37	293.2±2.74
Streptozotocin (Diabetic Control)	50 mg/kg, single i.p. injection	185.7±11.55	183.7±15.03[a]	182.4±22.51[a]
Ficus bengalensis dose I	50	221.7±12.47	258.1±8.07[b]	266.2±9.16[b]
Ficus bengalensis dose II	100	212.5±10.67	258.5±9.34[b]	271.8±10.20[b]
Vitamin C	100	222±12.82	229±11.16	243±8.77[b]
Glimepiride	0.5	218.5±12.97	241.5±9.18[c]	275.9±6.54[b]
			F=5.479	F=9.681
			Df=6,63	Df=6,63

n=10, [a]p<0.001 as compared to the non-diabetic control (Distilled water) group, [b]p<0.001 as compared to diabetic control (STZ) group, [c]p<0.05 as compared to the diabetic control (STZ) group, one-way ANOVA followed by *post hoc* Tukey'stest

Table 6: Effect of various drugs (×30 days) on retention of spatial navigation task in streptozotocin (STZ)-induced diabetic rats

Group	Treatment and route (mg/kg/day) p.o	Latency (Seconds) Mean±SEM		
		Day 1: IAL	Day 15: RL-1	Day 30: RL-2
Distilled water (Non-diabetic healthy control)	0.2 ml	37±6.93	38.7±4.14	49.2±4.62
Streptozotocin (Diabetic control)	50 mg/kg, singlei.p. injection	50.2±2.83	67.9±2.65[a]	93.6±2.88[a]
Ficus bengalensis; Dose I	50	55.8±3.63	46.9±3.60[b]	44.3±3.70[b]
Ficus bengalensis; Dose II	100	55.3±3.23	50.4±3.03[c]	37.9±3.19[b]
Vitamin C	100	54.9±3.01	45.9±2.54[b]	40.5±2.52[b]
Glimepiride	0.5	55.6±3.58	46.8±3.55[b]	40.3±3.55[b]
			F=7.166	F=30.643
			Df=6	Df=6, 63

n=10, IAL: Initial acquisition latency, RL: Retention latency, [a]p<0.001 as compared to the non-diabetic control (Distilled water) group, [b]p<0.001 as compared to diabetic control (STZ) group, [c]p<0.01 as compared to the diabetic control (STZ) group, one-way ANOVA followed by *post hoc* Tukey's test

QT in spatial navigation task in Morris water maze

Significant increase in retention of QT-1 was shown by *F. benghalensis* dose I group (p<0.05) and glimepiride group (p<0.05) as compared to STZ (diabetic control) group, but *F. benghalensis* dose II and Vitamin C did not cause any significant change in retention of QT-1 when compared to STZ (diabetic control) group at day "15." Significant increase in QT-2 was shown by *F. benghalensis* dose I group (p<0.001), *F. benghalensis* dose II group (p<0.001), vitamin C group (p<0.001), and glimepiride group (p<0.001) at day "30" (Table 7 and Fig. 7).

Effect of various drugs on biochemical parameters

MDA and GSH levels in brain

Among STZ-induced diabetic groups, significant increase in brain MDA levels was seen in STZ group (p<0.001) as compared to non-diabetic control (Distilled water) group. In comparison to STZ (diabetic control) group; the groups which showed significant decrease in brain MDA levels were – *F. benghalensis* dose I group (p<0.001), *F. benghalensis* dose II group (p<0.001), vitamin C group (p<0.001), and glimepiride group (p<0.05).

Significant decrease in brain GSH levels among diabetic rats was seen in STZ group (p<0.001) as compared to non-diabetic control (Distilled water) group. In comparison to STZ (diabetic control) group; the groups which showed significant increase in brain GSH levels were – *F. benghalensis* dose I group (p<0.001), *F. benghalensis* dose II group (p<0.001), vitamin C group (p<0.001), and glimepiride group (p<0.01) (Table 8 and Fig. 8).

DISCUSSION

Diabetes is a complex metabolic disorder which is associated with cognitive dysfunctions due to neurophysiological and structural changes in the CNS [1]. Multifactorial process is involved in cognitive and neurophysiological changes in diabetes mellitus, these includes the chronic metabolic and vascular disturbances resulting from fluctuations in blood glucose levels [14]. In the background of diabetes induced neurobehavioral changes, the present study analyzes the role of *F. benghalensis* in modulation of cognitive impairment and oxidative stress in brain of diabetic rats. Standard drugs such as vitamin C – an antioxidant and glimepiride – an oral hypoglycemic drug were included for the comparison with *F. benghalensis*.

Streptozotocin significantly increased the blood glucose levels and the levels remained same throughout the study period. *F. benghalensis*

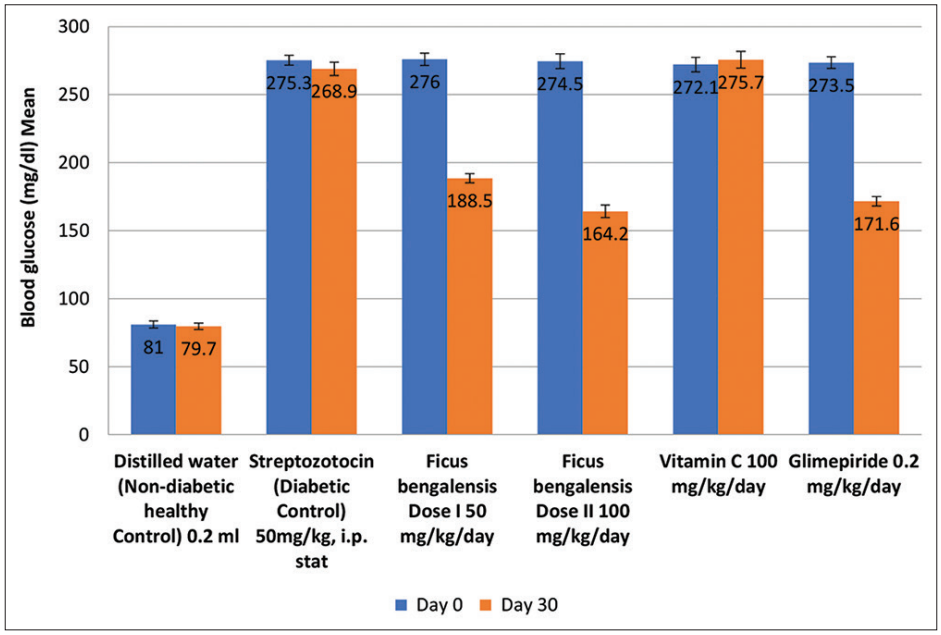

Fig. 1: Effect of various drugs (×30 days) on blood glucose levels of streptozotocin-induced diabetic rats

Table 7: Effect of various drugs (×30 days) on "Quadrant-time" of spatial navigation task in streptozotocin (STZ)-induced diabetic rats

Group	Treatment and route (mg/kg/ day) p.o	Time (Seconds) Mean±SEM		
		Day 1: IAL	Day15:QT-1	Day 30:QT-2
Distilled water (Non-diabetic healthy Control)	0.2 ml	37±6.93	63.3±3.89	60.5±3.51
Streptozotocin (Diabetic Control)	50 mg/kg, single i.p. injection	50.2±2.83	26.2±2.27a	17.5±2.16[a]
Ficus bengalensis Dose I	50	55.8±3.63	39.9±2.86[a,d]	46.2±3.48[b,c]
Ficus bengalensis Dose II	100	55.3±3.23	36.8±2.72[a]	49±3.26[c]
Vitamin C	100	54.9±3.01	35.4±2.75[a]	41.3±2.80[a,c]
Glimepiride	0.5	55.6±3.58	41.1±4.08[a,d]	47.1±3.51[a,c]
			F=13.981	F=18.760
			Df=6, 63	Df=6, 63

n=10, initial acquisition latency (IAL), Quadrant-time (QT), [a]p<0.001 as compared to the non-diabetic control (Distilled water) group, [b]p<0.05 as compared to non-diabetic control (Distilled water) group, [c]p<0.001 as compared to the diabetic control (STZ) group, [d]p<0.05 as compared to the diabetic control (STZ) group, one-way ANOVA followed by *post hoc* Tukey's test

Table 8: Effect of various drugs (×30 days) on brain levels of MDA and GSH in streptozotocin (STZ)-induced diabetic rats

Group	Treatment and route (mg/kg/ day) p.o	MDA (nmol/g wet brain tissue) Mean±SEM	GSH (μg/g wet brain tissue) Mean±SEM
Distilled water (Non-diabetic healthy Control)	0.2 ml	176±8.61	381.4±10.63
Streptozotocin (Diabetic Control)	50 mg/kg, single i.p. injection	321.6±18.11[a]	207.8±16.59[a]
Ficus bengalensis Dose I	50	264.9±6.50[a,c]	306.6±13.08[a,c]
Ficus bengalensis Dose II	100	245.3±11.85[b,c]	313±9.30[b,c]
Vitamin C	100	214.7±12.63[c]	351.9±9.87[c]
Glimepiride	0.5	257.2±13.04[a,d]	263.7±7.98[a,d]
		F=14.114	F=25.945
		Df=6, 63	Df=6, 63

n=10, [a]p<0.001 as compared to non-diabetic control (Distilled water) group, [b]p<0.01 as compared to non-diabetic control (Distilled water) group, [c]p<0.001 as compared to diabetic control (STZ) group, [d]p<0.05 as compared to diabetic control (STZ) group, one-way ANOVA followed by *post hoc* Tukey's test

(in both 50 mg/kg and 100 mg/kg doses) significantly decreased the blood glucose levels by day 30, indicating the antihyperglycemic activity of *F. benghalensis*. Similar effect was seen with the standard oral hypoglycemic drug glimepiride also.

Learning and memory impairment in the animals was indicated by the significant increase in both acquisition and retention components of TL in STZ-induced diabetic rats. *F. benghalensis* dose I (50 mg/kg) and dose II (100 mg/kg) (×30 days) significantly decreased the acquisition of TL, along with these groups glimepiride (×30 days) significantly decreased the retention component of TL. However, no change in acquisition or retension of TL was observed in vitamin C group. These findings are indicative of significant reversal and/or decrease in learning and memory impairment (caused by STZ) by *F. benghalensis* (in both 50 mg/kg and 100 mg/kg doses).

Another test indicating learning and memory impairment in animals was SDL in continuous avoidance apparatus, the impairment was depicted by significant decrease in both acquisition and retention components of SDL in STZ-induced diabetic rats, significant increase in acquisition of SDL was shown by *F. benghalensis* dose II and glimepiride group, in addition to these groups, *F. benghalensis* dose I and Vitamin C group showed significant increase in retention of SDL. The results of SDL test, like the TL test, also suggested that *F. benghalensis* (in both doses) can significantly reverse and/or decrease the learning and memory impairment caused by STZ.

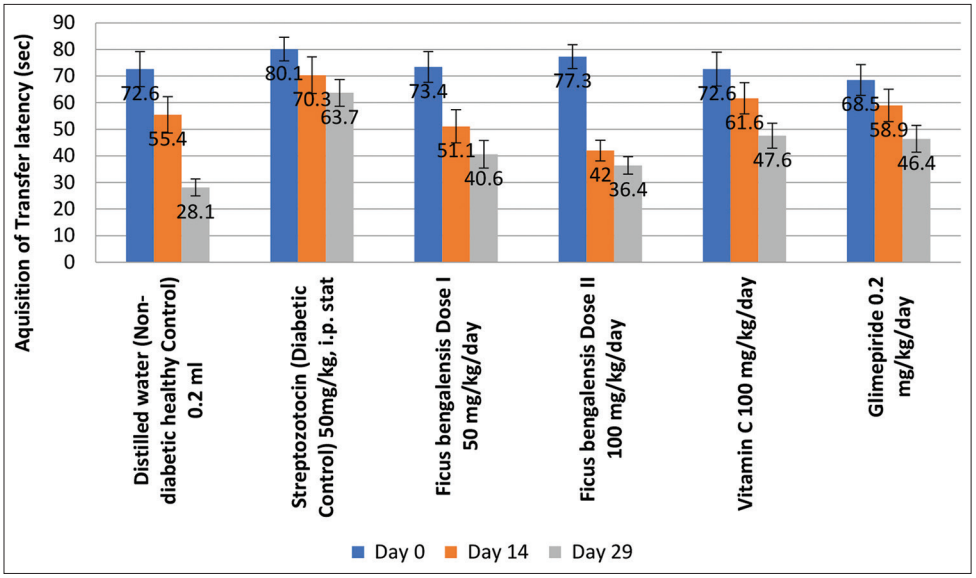

Fig. 2: Effect of various drugs (×30 days) on acquisition of transfer latency in streptozotocin-induced diabetic rats

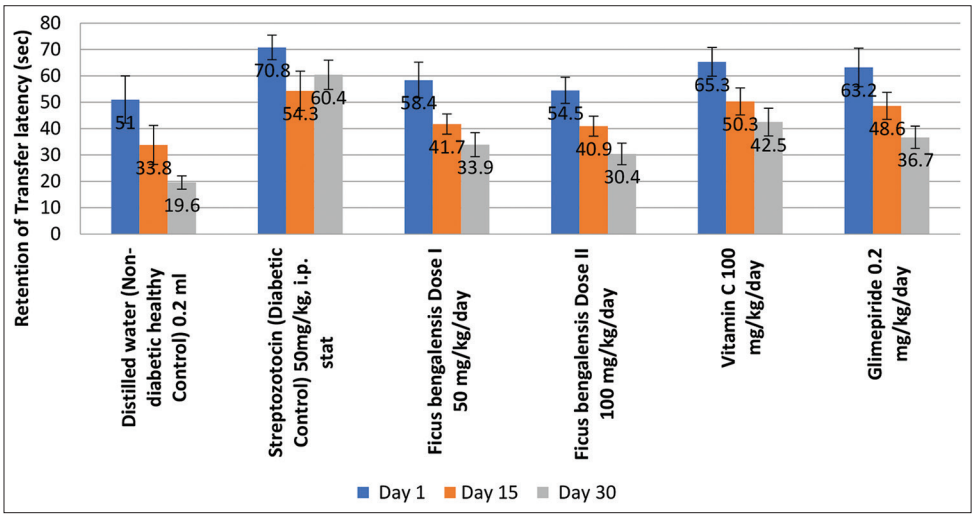

Fig. 3: Effect of various drugs (×30 days) on retention of transfer latency in streptozotocin-induced diabetic rats

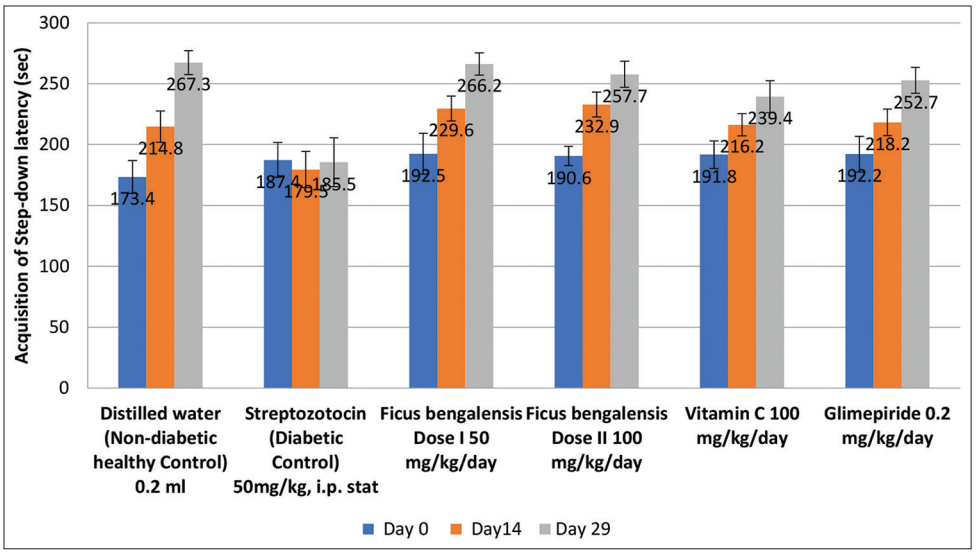

Fig. 4: Effect of various drugs (×30 days) on acquisition of step-down latency in streptozotocin-induced diabetic rats

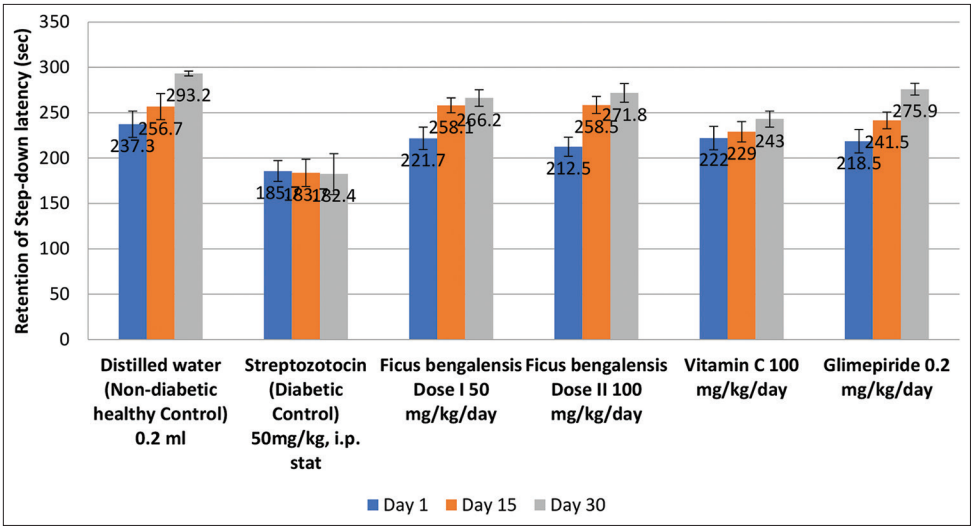

Fig. 5: Effect of various drugs (×30 days) on retention of step-down latency in streptozotocin-induced diabetic rats

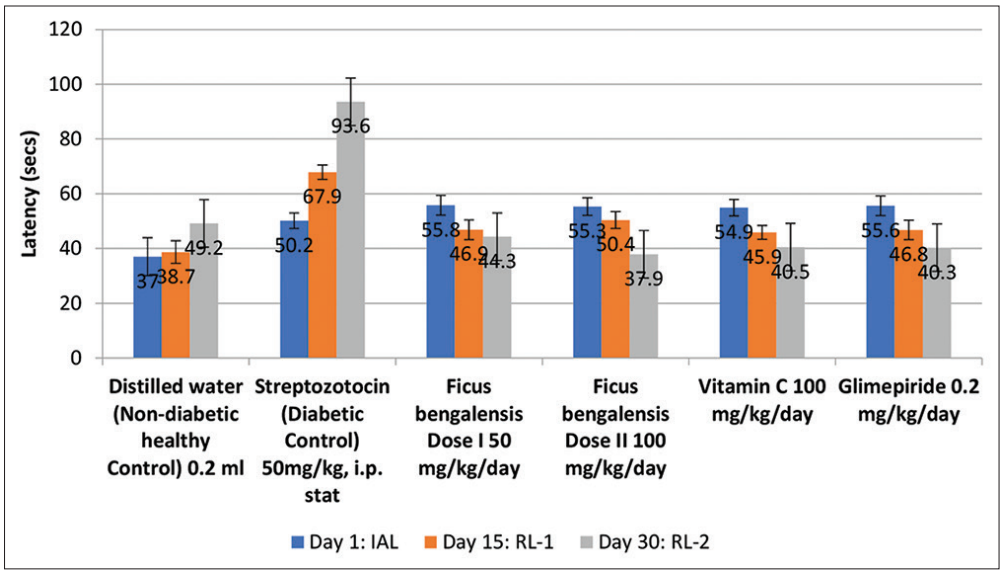

Fig. 6: Effect of various drugs (×30 days) on retention of spatial navigation task in streptozotocin-induced diabetic rats

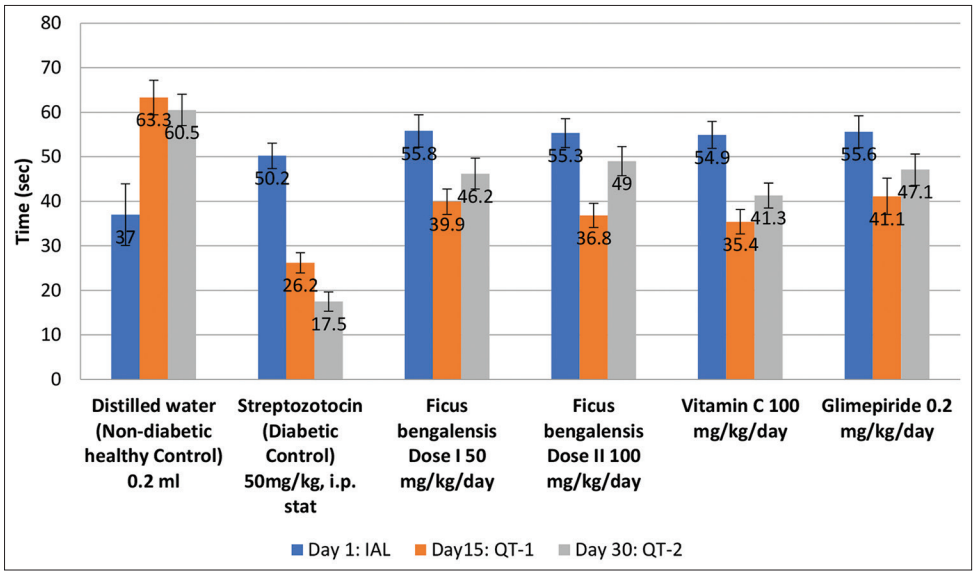

Fig. 7: Effect of various drugs (×30 days) on "Quadrant-time" of spatial navigation task in streptozotocin-induced diabetic rats

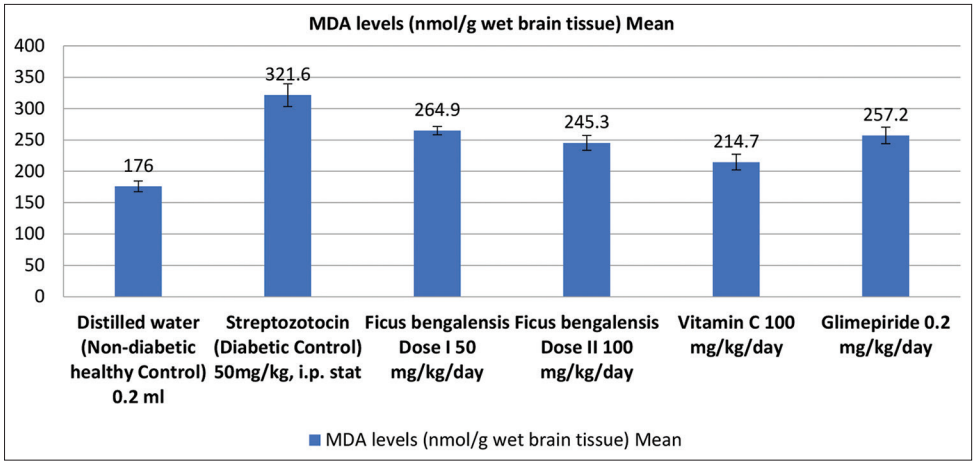

Fig. 8: Effect of various drugs (×30 days) on brain levels of MDA levels in streptozotocin-induced diabetic rats

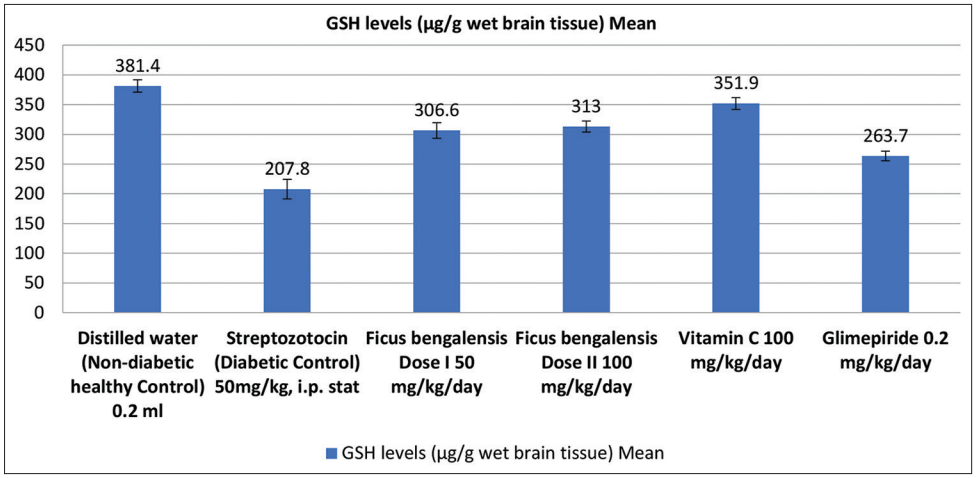

Fig. 9: Effect of various drugs (×30 days) on brain levels of GSH levels in streptozotocin-induced diabetic rats

Learning and memory behavior were assessed using the spatial navigation task in Morris water maze, the parameters assessed two components of spatial navigation, which were retention latency at day 15 and day 30 (RL-1 and RL-2, respectively) and time spent in target quadrant (QT-1 and QT-2, respectively). Findings indicated that both RL-1 and RL-2 were significantly increased and both QT-1 and QT-2 were significantly decreased by STZ. Both RL-1 and RL-2 were significantly decreased by *F. benghalensis* (in both 50 mg/kg and 100 mg/kg doses), vitamin C, and glimepiride. QT-1 was significantly increased by *F. benghalensis* (50 mg/kg) and glimepiride, whereas QT-2 was significantly increased by *F. benghalensis* (100 mg/kg) and vitamin C, in addition to the above-mentioned groups. The test results indicate that *F. benghalensis* (in both 50 mg/kg and 100 mg/kg doses) can significantly reverse and/or decrease the learning and memory impairment caused by STZ. This behavioral paradigm represents the positive modulation of cognitive impairment.

The results of all the behavioral test in our study match with various studies that have shown the STZ-induced cognitive impairment in diabetic rats [3,8,15].

The brain MDA and GSH levels were assessed as markers of oxidative stress. In non-diabetic rats, no study agent significantly altered brain MDA levels, but *F. benghalensis* (in both 50 mg/kg and 100 mg/kg doses) and vitamin C increased brain GSH significantly above the normal range, the possible mechanism might be that the above-mentioned drugs must have caused the addition of glutathione groups to the normal reserve of the body GSH in the absence of any oxidative stress condition. In diabetic rats, STZ significantly increased brain MDA and significantly decreased brain GSH. *F. benghalensis* (in both 50 mg/kg and 100 mg/kg doses), vitamin C, and glimepiride caused significant decrease in brain MDA and significant increase in brain GSH levels. These findings indicate the protective effect of *F. benghalensis* in the condition of increased oxidative stress. The antioxidant effect of *F. benghalensis* may be responsible for the reversal of levels of MDA and GSH in STZ induced diabetic rats. Various researchers have shown similar changes in MDA and GSH levels proving the antioxidant potential of *F. benghalensis* [11,16].

The phytochemical screening of aqueous extract of *F. benghalensis* showed the presence of flavonoids, tannins, reducing sugars, and sterols. The flavonoids and tannins isolated from various plant extracts have demonstrated antioxidant activity [17-19]. It is likely that tannins and flavanoids present in bark extract of *F. benghalensis* are responsible for observed antioxidant effect. Reducing sugars must be responsible for the antihyperglycemic action of *F. benghalensis*. However, detailed studies are warranted in this direction to decode the exact nature of the phytochemical compounds responsible for above-mentioned effects.

The results of biochemical tests are in agreement with the other studies which have verified the STZ-induced cognitive impairment in diabetic rats [5,20].

CONCLUSION

The present study demonstrates the cognitive impairment, increased oxidative stress caused by STZ-induced diabetes. The study strengthens

the correlation between cognitive dysfunction and oxidative stress in the background of diabetes. The present study shows that *F. benghalensis* promptly reverses and/or decreases the behavioral and biochemical changes induced by STZ. The effects of *F. benghalensis* on modulation of cognitive impairment and oxidative stress were comparable with that of vitamin C – an antioxidant and glimepiride – an oral hypoglycemic drug.

DECLARATIONS

Authors' contributions

Dr Umesh D Suranagi, Dr Ankit Arora and Dr Bhupinder Solanki contributed the concept, design, and experimental procedure of the study.

Dr Sanjib Gogoidid assessment of biochemical parameters

Dr.RavikantNirala contributed toward statistical analysis of data.

Conflicts of interests

The authors declare that they have no competing interests.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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