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Antihyperglycemic and protective effects of *Trigonella foenum graecum* seed powder on biochemical alterations in alloxan diabetic rats

P. KUMAR, R.K. KALE, N.Z. BAQUER

School of Life Sciences, Jawaharlal Nehru University, New Delhi (India)

Abstract. – BACKGROUND, *Trigonella foenum-graecum*, an annual herb belonging to the family Leguminosae, commonly known as fenugreek, has been reported to have hypoglycemic, hypocholesterolemic, hyperinsulinemic and antidiabetic properties. In the present study, the effect of oral feeding of *Trigonella foenum-graecum* seed powder (TSP) has been studied on blood glucose, monoamine oxidase (MAO), membrane fluidity, neurolipofuscin content, DNA degradation and glucose transporter-4 (GLUT4) accumulation in the alloxan-induced diabetic rat brain.

METHODS, Diabetes was induced by administration of alloxan monohydrate (15 mg/100 g body weight) and diabetic rats were treated with 2 IU insulin, per day and 5% TSP in the diet for 21 days.

RESULTS, Diabetic rats showed hyperglycemia with almost four fold high blood glucose levels. Increased MAO activity with correlated increase in genomic DNA degradation in the diabetic brain supports the hypothesis that catecholamine oxidation is an important source of oxidative stress, causing loss of membrane fluidity, increased neurolipofuscin and decreased of GLUT4 expression with diabetes in the brain. The present study showed that TSP treatment reversal the changes to near normal levels in diabetic rat brain.

CONCLUSIONS, The present findings indicate that the TSP exerts its anti-diabetic and neuroprotective effects, probably mediated through a decrease in hyperglycemia and oxidative stress thereby ameliorating the control and management of diabetic complications.

Key Words:

Alloxan diabetes, glucose transporter, monoamine oxidase, DNA degradation, *Trigonella foenum-graecum* seed powder.

Abbreviations

GLUT4 = Glucose transporter-4
C = Control
D = Diabetic untreated
D+I = Diabetic treated with insulin
D+T = Diabetic treated with *Trigonella foenum-graecum* seed powder
H_2O_2 = Hydrogen peroxide
MAO = Monoamine oxidase
TSP = *Trigonella foenum-graecum* seed powder

Introduction

It is projected that the worldwide prevalence of diabetes is likely to increase to more than 439 million by the year 2030[1]. Although sulphonylureas, biguanides, insulin sensitizers (thiazolidinediones) and other current drugs are valuable in the treatment of type 2 diabetes mellitus, their use is restricted by cost, limited pharmacokinetic properties, secondary failure rates and accompanying side-effects[2].

Trigonella foenum-graecum Linn. is an annual herb belonging to the family Leguminosae, widely grown in India, Egypt, and Middle Eastern countries[3]. Traditional medicines in India and other countries since ancient days, have employed hypoglycemic plants such as *Trigonella foenum-graecum* to protect against diabetic pathogenesis[4,5]. Various reports have demonstrated that the *Trigonella foenum-graecum* seed powder (TSP) have hypoglycemic, hypocholesterolemic and hyperinsulinemic effects on type-1 and type-2 diabetes mellitus (DM) patients and experimental diabetic animals[5-7]. The seeds are rich in protein and contain an unique major free amino acid 4-hydroxyisoleucine (4-OH-Ile), which has been characterized as one of the active ingredients in TSP[8,9].

The neurological consequences of diabetes mellitus in the central nervous system are now receiving considerable attention[10,11]. Glucose utilization is decreased in the diabetic brain, providing a po-

Corresponding Author: Najma Zaheer Baquer, Ph.D; e-mail: nzbaquer@mail.jnu.ac.in

tential mechanism for increased vulnerability to acute pathological events[12]. Disturbances in catecholamines metabolism and monoamine oxidase (MAO) activity play a role in the pathogenesis of the acute and chronic complications of diabetes[11,13,14]. Oxidative stress causing loss of neural membrane fluidity and inhibition of glucose transporter (GLUT4) and increased genomic DNA degradation and neurolipofuscin content[12,15,16].

Glucose is the major source of energy in the brain. The transport of glucose into most mammalian cells occur by facilitated diffusion, mediated by a family of glucose transporter proteins. Insulin stimulates increased glucose uptake by promoting a translocation of GLUT4 from their basal, intracellular location to the plasma membrane[17]. In the last few years, however, there have been several reports of GLUT4 mRNA and GLUT4-immunoreactive cells in regions of rat brain, including cerebellum and several cortical regions[18-20].

Previous studies from our laboratory have demonstrated that oral administration of *Trigonella foenum graecum*, seed powder (TSP) to diabetic animals has been shown to lower blood glucose levels and partially restore the activities of key enzymes of carbohydrates and lipid metabolism to near normal levels in various animal models[6,12,21-25].

The aim of the present study was to investigate the anti-diabetic and neuroprotective potential of TSP on physiological, biochemical and molecular parameters like glucose levels, MAO activity, membrane fluidity, neurolipofuscin, DNA degradation and GLUT4 expression in rat brain.

Materials and Methods

Animals

Adult female albino rats of the Wistar strain, weighing 180-220 g were used for all the experiments. Animals were kept in the animal house maintained at temperatures of 22-26°C and relative humidity of 55% with a 12 h each of dark and light cycle. The animals were fed standard chow (Hindustan Lever Ltd., India) and given tap water ad libitum for the time of treatment before sacrifice. All the animal procedures were approved by the Institutional Animal Ethical Committee (IAEC) of Jawaharlal Nehru University, New Delhi, India

Induction of Diabetes

A group of 60-70 overnight-starved rats were made diabetic by a single subcutaneous injection of alloxan monohydrate (15 mg/100 g body weight) freshly prepared in 0.154M sodium acetate buffer (pH 4.5) according to the method of Raju et al[21]. The alloxan induced diabetic rats were injected i. p. with 2 IU of protamine-zinc insulin for the next 7 days, this procedure decreased the mortality of the diabetic animals. The severity of diabetes was checked in alloxan diabetic rats by using glucose strips (Diastix, Bayer Diagnostic, India). Control animals were given only the vehicle.

Experimental Design

Animals were divided into four groups of six rats each. Group I– control (C), Group II– diabetic untreated (D), Group III– diabetic treated with insulin (D+I) and Group IV-diabetic treated with TSP (D+T). Protamine zinc insulin (2 IU) suspension was administered intraperitoneally to diabetic animals (D+I), each day for 21 days. The diabetic treated with TSP (D+T) were given 5% finely powdered *Trigonella* seeds in powered rat feed (5 g of dry TSP was mixed with 95 g of powdered rat feed), each day for 21 days. Seeds of *Trigonella foenum-graecum* (Agmark Brand) purchased from local market, New Delhi, India. The plant material was identified by local experts of herbal gardens and further taxonomically validated by Dr. Gita Mathur, Department of Botany, Gargi College, University of Delhi, New Delhi. A voucher specimen (No. 106) has been kept at the Herbarium of the University. The most effective dose of 5% TSP in the diet identified in previous studies[6,12,21].

Preparation of Homogenate and Subcellular Fractions

Animals were sacrificed by cervical dislocation. Whole brains and brain regions of control and diabetic and treated diabetic rats were rapidly excised, and washed with chilled normal saline. The tissues homogenates and subcellular fractions were prepared as described earlier, the pellet obtained at 12,000 g containing synaptosomes and mitochondria were used for enzyme assays. The supernatant fraction was separated from the pellet and was used as the soluble fraction[18].

Biochemical Assays

Assay of Monoamine Oxidase

MAO activity was measured in the synaptosomal and supernatant fractions isolated from brains according to the method of Mayanil et al[14]; the

activity was measured as amount of the reaction product, 4-hydroxyquinoline and was determined spectrophotometrically (Shimadzu UV-160, Nakagyo-ku, Kyoto, Japan) at 330 nm. One unit of enzyme is defined as 1 μmol of 4-hydroxyquinoline produced/mg protein/min at 37°C.

Membrane Fluidity

The synaptosomal fractions were labelled with 1,6-diphenyl-1, 3, 5-hexatriene (DPH), a fluorescent probe by incubating equal volume of a membrane suspension containing 100 μg.ml^{-1} of protein in phosphate buffer and 2 μM DPH suspension in the same buffer. Excitation and emission wavelengths were, respectively, 365 and 428 nm. Anisotropy (r) measurements were carried out on a model SLM 4800 polarization Cary Eclipse spectrofluorimeter (Varian Inc., Palo Alto, CA, USA) as described by Kumar et al[18].

Neurolipofuscin Content

The extraction of neurolipofuscin in whole homogenate and measurement of its fluorescence were essentially similar to the methods described by Tappel et al[26]. Quinine sulphate (0.1 μg/ml) in 0.05 M H2SO4 was used as a standard. The results were expressed as relative percentage fluorescence of control from 1 ml of 10% homogenate (w/v).

Protein Estimation

Protein was estimated in brain subcellular fractions by the method of Bradford[27] using bovine serum albumin (BSA) as standard.

Blood Glucose

Blood glucose was estimated by Glucose Enzokit from Ranbaxy Laboratories, Gurgaon, India, using glucose oxidase method.

Genomic DNA Degradation

Laddering of genomic DNA was carried out as described by Sandberg et al[28] with minor modifications. The phenol method was used to isolate the DNA samples from cerebral cortex of control and different experimental animals and analyzed by horizontal gel electrophoresis as described by Nagata[29].

Semiquantitative Detection of GLUT4 mRNA

Total RNA was isolated and purified from the cerebral cortex of rats using TRI-Reagent (Sigma-Aldrich Company, St Louis, MO, USA) according to manufacturer's instructions. RT-PCR detection was carried out using one step RT-PCR kit (Applied Biosystems Company, St Louis, MO, USA). Gene specific primers (synthesized by Sigma Aldrich Co., St Louis, MO, USA) were designed as follows: 5′-CAGATCGGCTCT-GAAGATGG-3′ and 5′-CTGAGTAGGCGC-CAATGAG-3′ sense and antisense primers for GLUT4 5′-AACGACCCCTTCATTGAC-3′ and 5′-TCCACGACATACTCAGCAC-3′ sense and antisense primers for glyceraldehyde-3-phosphate dehydrogenase. PCR products were electrophoresed on 1.5% agarose gel and gel images were captured and quantified with Gel Doc Imaging System (Alpha Innotech Corporation, Santa Clara, CA, USA).

Western Blot Analysis

Total membrane fractions prepared from the cerebral cortex was subjected to sodium dodecyl sulphate-polyacrylamide gel (SDS-PAGE) on a 10% polyacrylamide gel and Western blotting (BIORAD, Trans Blot, Semidry transfer cell, Hercules, CA, USA) was performed as described by Towbin and Gordon[30] and modified by Alonso et al[31]. Protein was estimated in brain cortex fraction by the method of Bradford[27]. After blocking with tris buffer saline (TBS) containing 0.1% Tween-20 and 5% BSA at 4°C overnight, the filter was incubated with 1:500 dilution of polyclonal anti-GLUT4 antibody for 2 h at room temperature. Polyclonal antibodies against rat GLUT4 were a kind gift from Dr. Sameul Cushman (National Institute of Health, Bethesda, MD, USA). After washing with TBST (Tris Buffer Saline-Tween 20). This was followed by the incubation with secondary antibody [anti-rabbit IgG conjugated with horse radish peroxidase (HRP)] for 2 h at room temperature. The signals were detected using 3,38-diaminobenzidine (DAB) tetrahydrochloride as the coloring reaction. The Western blot revealed a single band of 45 kDa, compatible with the molecular weight of the protein. Membrane is further incubated with a positive control gene i.e. glyceraldheyde-3-phosphate dehydrogenase (GAPDH) after stripping the GLUT4 antibody. Quantification was performed by densitometry after scanning the blots with gel documentation system (alpha imager).

Immunohistochemistry of GLUT4

The immunohistochemical detection of GLUT4 in cerebral cortex was performed by using the technique as described by El Messari et al[20]. Paraffin-embedded brain 5 micron sections

were deparaffinized in xylene, rehydrated through a series of graded ethanol solutions, and washed with cold 0.01 MPBS (skimmed milk phosphate buffered saline) containing 0.5% Tween 20 (PBST: phosphate buffered saline with Tween 20). Polyclonal antibodies against rat GLUT4 were a kind gift from Dr. Sameul Cushman (National Institute of Health, Bethesda, MD, USA). Secondary antibodies (goat anti-rabbit IgG conjugated with HRP) were from Sigma-Aldrich Company respectively. Slides were incubated for 2 h at 37°C with polyclonal rabbit GLUT4 antisera, diluted 1:100 and followed by a 1-hwash in TBS. After washing, sections were incubated with biotinylated secondary antibodies at a dilution of 1:100 for 30 min. The signal was detected and amplified using the avidin-biotin-peroxidase method (Vector, Burlingame, CA, USA) and visualized with 3,38-diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, MO, USA) as chromogens. Tissue sections were finally dehydrated and mounted in permount to be observed in light microscopy. Immunostaining was evaluated by examination of slides under a bright field microscope (Carl Zeiss Axioskop 2 Mot, Thornwood, NY, USA) at a magnification of 400× and images were captured through a digital camera for measurement of intensity. All images were reduced to half of the original size using Adobe Photoshop Elements 3 software (Adobe, San Jose, CA, USA) to facilitate measurements on the image. Intensities of immunostained cells were estimated by densitometry using morphometric Scion Image software v. Alpha 4.0.3.2 (Scion Corp., Frederick, ML, USA).

Statistical Analysis

Results were analyzed by means of Prism 5.0 (GraphPad, San Diego, CA, USA). All data were calculated as means ± SEM of 4-6 separate values. The ANOVA test followed by Dunnet Multiple Comparison test was employed for statistical comparison between control and various experimental groups. Values with $p < 0.05$ were considered as statistically significant.

Chemicals

All purified enzymes, coenzymes, substrates, standards and buffers were from Sigma chemicals Company (St Louis, MO, USA). All other chemicals were of analytical grade and were from Super Religare Laboratories (SRL), Mumbai, India and (Qualigens, Mumbai, India).

Results

Effect of TSP on General Parameters

Body weights were significantly decreased in the diabetic groups ($p < 0.01$) as compared to controls. After 21 days of insulin and TSP administration, the body weights of diabetic rats increased. There was a four-fold increase ($p < 0.001$) in blood glucose concentration in alloxan diabetic rats when compared to control rats. Three weeks of treatment with insulin and TSP separately (5% in diet) resulted in a significant ($p < 0.05$) reduction in hyperglycemia in the diabetic rats. Results are shown in Figure 1.

Effect of TSP on Monoamine Oxidase Activity

The changes in MAO activity from brain synaptosomal and supernatant fractions of diabetic and diabetic rats treated with insulin, and TSP are shown in table I. The induction of diabetes resulted in a significant ($p < 0.001$) increase in the activity of MAO from synaptosomal and supernatant fractions. The treatment of diabetic animals with insulin and TSP separately

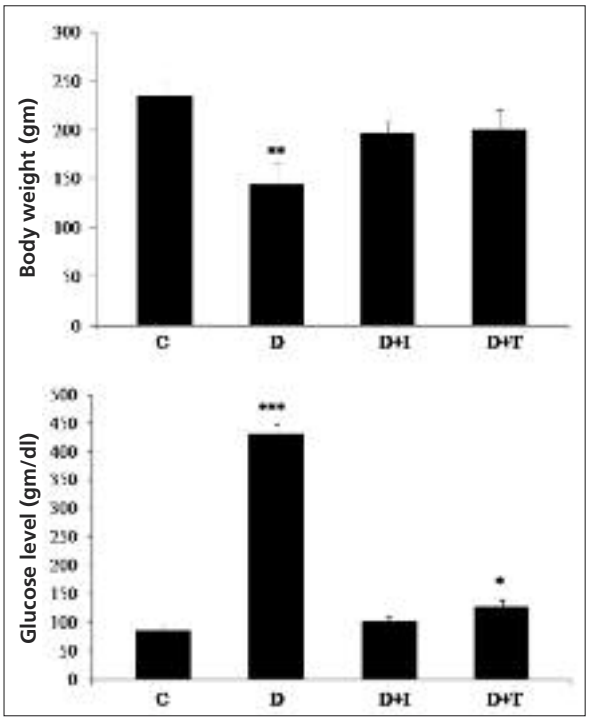

Figure 1. Changes in body weight and glucose levels of control (C), diabetic (D), and diabetic rats treated with insulin (D+I) and Trigonella (D+T). Each value is a mean of ± SEM of five or more separate values from two to three experiments. The comparisons of experimental values are with the control values. Statistical significance: ***$p < 0.001$,**$p < 0.01$, *$p < 0.05$.

brought the increased activity of MAO to nearly control levels. There was a significant ($p < 0.05$) decrease in MAO in diabetic with TSP treatment when compared with control groups. The percentage of MAO activity in the supernatant fractions as compared to the synaptosomal MAO was 29% in control, 40% in diabetic, 34% in D+I and 35% in D+T, showing that diabetes induced a change in MAO distribution between synaptosomes and soluble fraction.

Effect of TSP on Neurolipofuscin Content

The neurolipofuscin content increased with diabetes. Consequent to 21 days of insulin withdrawal, the diabetic rats showed a significant increase of 76% ($p < 0.01$) in the neurolipofuscin content in the brain. Treatment of diabetic rats with antidiabetic compounds, insulin and TSP separately, showed a significant ($p < 0.01$) reversal of the increased neurolipofuscin content when compared with control groups. The results are presented in Table I.

Effect of TSP on Membrane Fluidity

The DPH fluorescence anisotropy (r) values were found to be increased in 21 days diabetic groups when compared with the control groups, indicating significant ($p < 0.01$) decrease in the membrane fluidity in the diabetic brain. Treatment of the diabetic animals with insulin and TSP ($p < 0.01$) restored the change in the membrane fluidity when compared with diabetic groups bringing it closer to the control groups. Results are presented in Table I.

Effect of TSP on genomic DNA Degradation

The DNA degradation was assessed by DNA laddering method of the genomic DNA isolated from cerebral cortex from control and different experimental groups. Genomic DNA isolated from control animals showed no evidence of DNA fragmentation. In contrast, genomic DNA isolated from alloxan diabetic animals showed the banding pattern, which is a characteristic of DNA fragmentation and apoptosis. Furthermore, the treatment of the diabetic rats with insulin and *Trigonella* prevented genomic DNA fragmentation, to some extent bringing it nearer to control levels. Result are shown in Figure 2.

Effect of TSP on GLUT4 mRNA

Semi Quantitative mRNA Analysis

To ascertain the presence of GLUT4 mRNA in the rat diabetic rat brain, polymerase chain reaction (RT-PCR) analysis was performed on total RNA extracted from cerebral cortex using specific primers. After 35 cycles of amplification, a single band (411 bp) was observed in the brain examined. There was a significant decrease 45% ($p < 0.01$) in mRNA levels in diabetic rat brain when compared with control groups. Consequent to treatment with the antidiabetic compounds insulin and TSP to diabetic rats, a reversal of the GLUT4 mRNA levels to control levels was observed. There was a significant ($p < 0.05$) reversal in GLUT4 mRNA in diabetic animals with TSP treatment when compared with control groups. Results are shown in Figure 3.

Effect of TSP on GLUT4 Protein Expression

Change in GLUT4 protein expression in cerebral cortex membrane fraction by Western blotting from diabetic and diabetic rats treated with

Table I. Changes in monoamine oxidase activity , membrane fluidity and neurolipofuscin content in brains of control (C), diabetic (D), and diabetic rats treated with insulin (D+I) and Trigonella (D+T).

Conditions	Monoamine oxidase		Membrane fluidity (anisotropy)	Neurolipofuscin
	Synaptosomal	Supernatant		
C	2.20 ± 0.039 (100)	0.648 ± 0.023 (100)	0.178 ± 0.0034 (100)	47.68 ± 5.33 (100)
D	3.22 ± 0.051 (147)[a]	1.294 ± 0.047 (199)[a]	0.224 ± 0.0051 (126)[b]	84.22 ± 5.10 (176)[b]
D+I	2.34 ± 0.069 (107)	0.802 ± 0.069 (124)	0.187 ± 0.0026 (105)[b]	61.33 ± 2.67 (128)[b]
D+T	2.67 ± 0.022 (121)[c]	0.941 ± 0.015 (145)[c]	0.191 ± 0.0061 (108)[b]	64.63 ± 2.52 (135)[b]

Each value is a mean of ± SEM of five or more separate values from two to three experiments. The comparisons of experiment values are with the control values. *Statistical significance*: [a]$p < 0.001$; [b]$p < 0.01$; [c]$p < 0.05$. MAO activity as one μmole 4-hydroxyquinoline produced/mg protein/minute at 37°C. The neurolipofuscin content expressed as relative percentage fluorescence of control from 1 ml of 10% homogenate (w/v). Values in parentheses are percentage change taking control as 100%.

insulin and TSP are shown in Figure 4. There was a significant decrease by 54% ($p < 0.01$) in GLUT4 protein in the diabetic rats. Treatment with TSP and insulin, showed a reversal in the GLUT4 protein levels. There was a significant ($p < 0.05$) increase in GLUT4 expression in the diabetic animals with TSP treatment when compared with control groups.

Effect of TSP on Distribution of GLUT4

Immunohistochemistry was used to determine the possible presence and distribution of the GLUT4 transporter protein in cerebral cortex of control and diabetic and diabetic treated with insulin and with TSP rat brains. GLUT4-like immunoreactivity was present in numerous punctate and linear process-like structures. GLUT4 distribution was observed on the neuronal membrane in control cerebral cortex; while it localized to the cytoplasm in cortex of diabetic rat. Immunohistochemical quantification of GLUT4 showed that it appears to be relatively more abundant in the control rat than the diabetic rats. With diabetes there was a significant decrease of ($p < 0.05$) in the GLUT4 immunoreactivity of the cortex of diabetic rats as compared to controls. Treatment with insulin and TSP to the diabetic animals restored the distribution of GLUT4

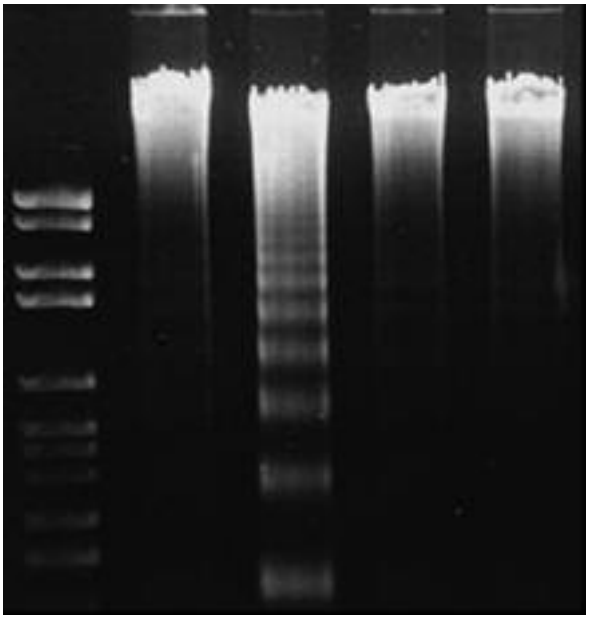

Figure 2. DNA degradation study in brain of control (C) diabetic (D) and diabetic treated with insulin (D+I) and Trigonella (D+T). Genomic DNA was extracted from brains of control (C) and diabetic treated rats by phenol-chloroform as described in methods.

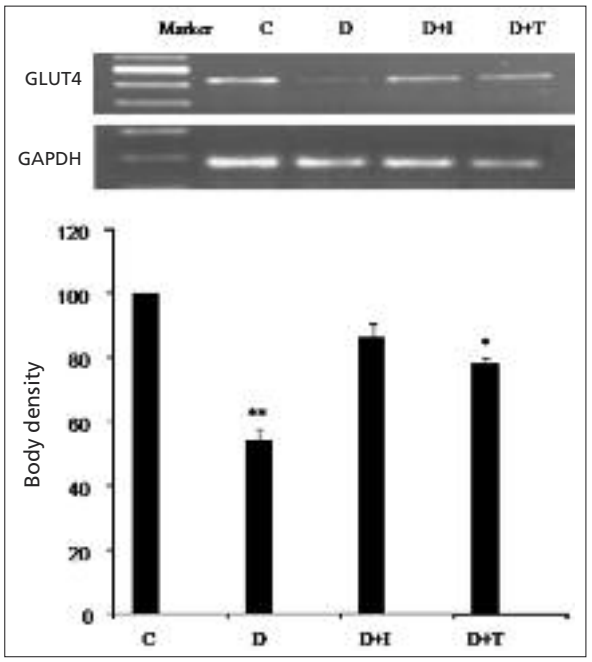

Figure 3. Glucose transporter (GLUT4) mRNA levels in the cerebral cortex of control (C) diabetic (D) and diabetic treated rats with insulin (D+I) and *Trigonella* (D+T). Each value is the mean ± SEM of more than six values from 5 separate experiments. Total RNA was extracted from rat cerebral cortex and amplified by RT-PCR as described in methods. The bands at the expected sizes, 411 and 198 bp, correspond to GLUT4 and GAPDH mRNAs, respectively. Expression of GLUT4 and GAPDH mRNA in brain of rats analyzed by RT-PCR after 35 cycles of amplification. Statistical significance: $**p < 0.01$, $*p < 0.05$. Values in bar diagram are percentage changes, taking control as 100%.

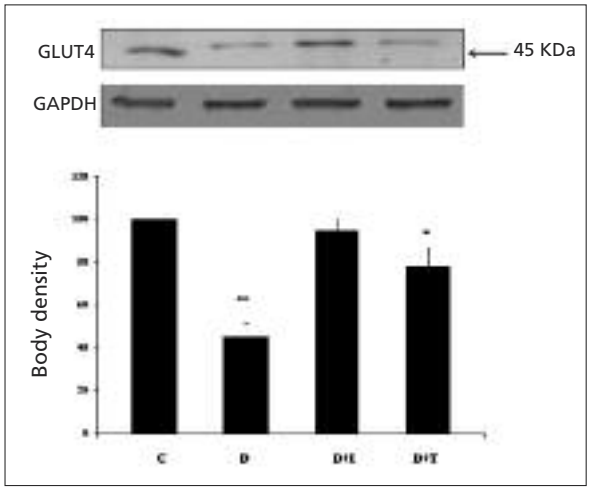

Figure 4. Glucose transporter (GLUT4) protein levels in the cerebral cortex of control (C) diabetic (D) and diabetic treated rats with insulin (D+I) and Trigonella (D+T). Values are given as mean ± SEM and statistical significance: $**p < 0.01$, $*p < 0.05$. Values in bar diagram are percentage changes, taking control as 100%.

to the membranes. Results are presented in Figure 5A and 5B.

Discussion

This study was undertaken to investigate the antidiabetic and neuroprotective properties of TSP on MAO activity, membrane fluidity, neurolipofuscin, DNA degradation and GLUT4 expression in rat brain. Hyperglycemia during diabetes has been shown to generate free radicals from auto-oxidation of glucose and increased polyol pathway, with concomitant increase in cellular lipid peroxidation and damage to membranes[21,22,32]. Our results showed that three weeks treatment of the diabetic group with insulin, and TSP separately resulted in a marked reduction in hyperglycemia in the diabetic animals. TSP improved glucose homeostasis in diabetes by decreasing oxidative stress, probably by enhancing or mimicking insulin action[4,12,33,34].

Neurodegenerative processes may preferentially affect the brain as a result of the production of free radicals associated with catecholamine metabolism[35]. The hydrogen peroxide (H_2O_2) derived from MAO represents a special source of oxidative stress in brain. Hence, an increased MAO activity during diabetes in the brain could be an important mechanism for increasing H_2O_2 production in the diabetic rats, thereby, increasing oxidative stress[11,14]. The treatment of diabetic animals with insulin and TSP normalized the increased activity of MAO to nearly control levels with insulin and TSP administration. TSP might also protect the enzyme activities by preventing hyperglycemia-induced.

Present results showed that diabetes induces a significant decrease in the membrane fluidity and the bilayer lipid membrane becomes more rigid than normal. The decrease in membrane fluidity in synaptosomes from the diabetic brain could be due to the peroxidation of membrane phospholipids through free radicals, generated by persistent hyperglycemia[8,12,15,34,36]. Insulin and TSP treatment brought the membrane fluidity values close to controls. Diabetes could, therefore, affect the overall physiological responses in the central nervous system.

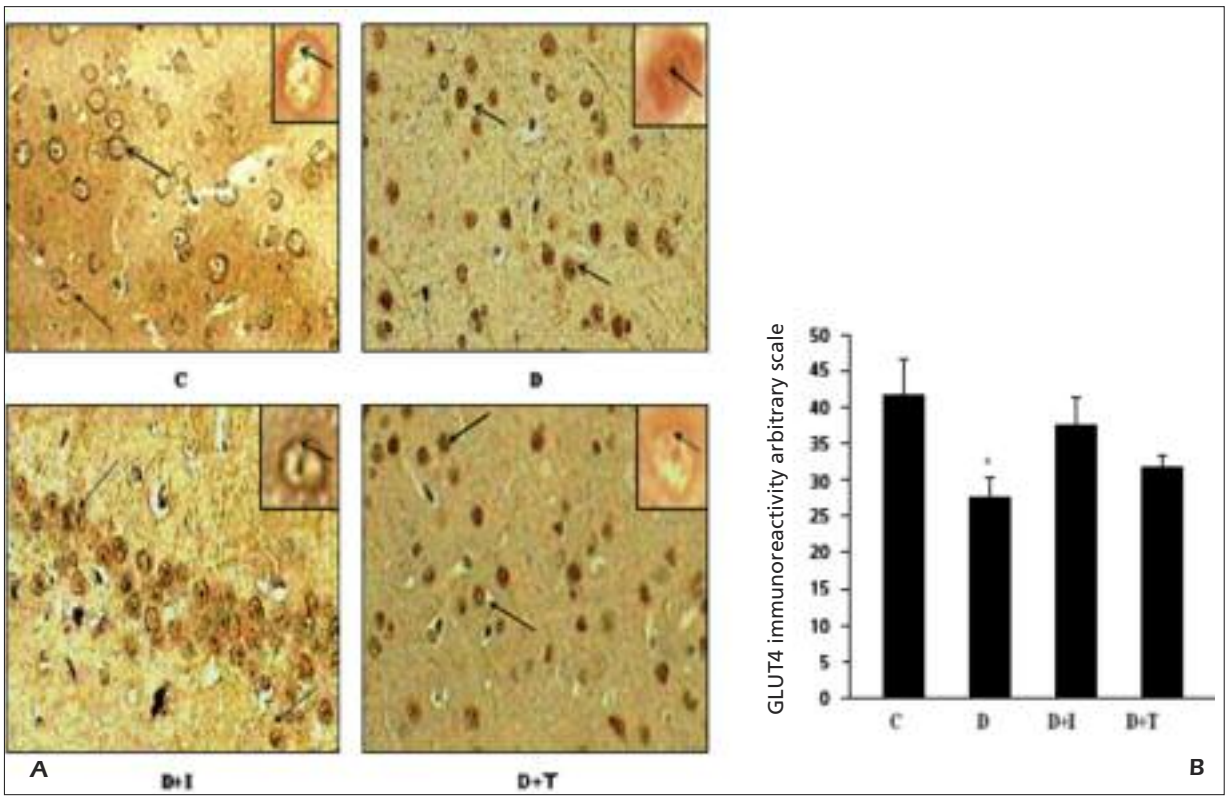

Figure 5. Immunohistochemical analysis ***(A)*** of glucose transporter (GLUT4) (shown by black arrowheads) in the cerebral cortex of control (C) diabetic (D) and diabetic treated rats with insulin (D+I) and *Trigonella* (D+T). Immunohistochemical quantification ***(B)*** of GLUT4 in cerebral cortex of control (C) diabetic (D) and diabetic treated rats with insulin (D+I) and *Trigonella* (D+T). Values are given as mean ± SEM and statistical significance: $*p < 0.05$.

The formation of neurolipofuscin content was increased in experimental diabetes as reported earlier[16,37], in the brain, suggesting that diabetes could have functional change in the neural tissue. Insulin and TSP treatment to diabetic animals resulted in a decrease in neurolipofuscin content. Inhibition of neurolipofuscin content and restoration of membrane fluidity by insulin administration and TSP strongly suggests anti-lipidperoxidative abilities of TSP[21,38].

Oxidative stress is known to play an important role in the pathogenesis and complications of diabetes and as a result increased risks of oxidative DNA degradation have been observed in diabetic patients[39]. In the present results, oxidative DNA degradation was increased in the DNA sample isolated from cortex of diabetic animals as compared to the normal controls, as has been shown earlier[40,41]. Administration of TSP and insulin restored the DNA degradation in diabetic groups close to controls.

Glucose is the major source of energy in the mammalian brain. In the present study expression of GLUT4 was decreased in diabetes, which showed that there was an alteration in the glucose transporter in brain, which is supported earlier study of Vannucci et al[17]. The reduction in the GLUT4 level results in decreased uptake of glucose and, therefore, contributes in the increased blood glucose levels in diabetic conditions.

GLUT4 distribution in brain tissue of control, diabetic, and diabetic treated rats was also monitored by immunohistochemistry and analyzed by light microscopy. The results are in agreement with those obtained with RT PCR and Western blotting. GLUT4 was localized predominantly in the membrane in control rat brain. With diabetes there was a marked decrease in the GLUT4 distribution in the membranes. GLUT4 appears to be relatively abundant in the control rat than in diabetic rats. Treatment with insulin and TSP separately corrected the alterations in the distribution of GLUT4 and expression of GLUT4 mRNA and protein in the rat brain[18,31].

In agreement with earlier findings, our findings showed that the GLUT4 diminished in diabetic rats when compared with respective control (Figures 3, 4, 5). A possible role for TSP on GLUT4 during diabetes, may result in an impairment of GLUT4 function emphasizing effects of TSP in GLUT4 action. Administration of TSP and insulin restored the GLUT4 protein expression in diabetic groups. Earlier from our laboratory Mohammad et al[6] showed that TSP treatment restored the GLUT4 in skeletal muscle of alloxan diabetic rats[19,20].

The components responsible and the mechanism by which TSP exerts these effects in not clearly understood. However, as mentioned earlier, several studies have shown the presence of steroid saponins in TSP seeds. 4-hydroxyisoleucine, a modified amino acid extracted and purified from *Trigonella* seeds, stimulated insulin secretion. The active component of *Trigonella foenum-graecum* seeds has been found to be associated with the defatted part of the seeds, rich in fibre containing steroidal saponins and proteins comparable to those of soybean[12,42,43]. A possible mode of action of TSP suggested was an effect on intestinal carbohydrate digestion; it was found to decrease digestion of starch and also glucose absorption both *in vivo* and *in vitro*, may be as a result of a direct inhibitory effect on the digestive enzymes[7,44,45]. *Trigonella* is also known to rejuvenate the beta cells of pancreas[9,12].

The experimental animal model of diabetes mellitus of the present study is similar to type-1 diabetes, in which insulin secretion is defective due to alloxan induction. Earlier studied showed that hyperglycemia during diabetes has been shown to generate free radicals, increase damage to membranes and decreased antioxidant status[10,12,15]. Administration of TSP for three weeks to alloxan diabetic rats stabilize glucose homeostasis in brain by normalizing the glucose and membrane linked and antioxidant enzymes[6,33,34]. *Trigonella* seed supplementation in the diet normalizes the free radical metabolism in alloxan diabetic rats[4, 35,42].

Our findings showed that three weeks treatment of the diabetic group with insulin, and TSP separately resulted in a marked reduction in hyperglycemia in the alloxan diabetic animals. TSP improved glucose homeostasis in diabetes by decreasing oxidative stress, probably by enhancing or mimicking insulin action[6,12,22,43]. Present findings indicate that the TSP exerts its antidiabetic effects, probably mediated through a decrease in hyperglycemia and oxidative stress, thereby, ameliorating the control and management of diabetic complications.

Conclusions

Our investigation leads us to conclude that TSP administration to diabetic rats significantly and effectively reversed the diabetic aberrations ob-

served, in the brain. *Trigonella*, therefore, represents a potentially useful dietary adjunct in the treatment of diabetes and a potential source of a new orally active antidiabetic dietary supplement.

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References

1) Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010; 87: 4-14.
2) Krentz AJ, Bailey CJ. Oral antidiabetic agents: current role in type 2 diabetes mellitus. Drugs 2005; 65: 385-411.
3) Alarcon-Aguilara FJ, Roman-Ramos R, Perez-Gutierrez S, Aguilar Contreras A, Contreras-Weber CC, Flores-Saenz JL. Study of the anti-hyperglycemic effect of plants used as antidiabetics. J Ethnopharmacol 1998; 61: 101-110.
4) Puri D, Prabhu KM, Murthy PS. Hypocholesterolemic effect of the hypoglycemic principle of fenugreek (TSP foenum graecum) seeds. Ind J Clin Biochem 1995; 9: 13-16.
5) Gupta S, Prakash J. Studies on indian green leafy vegetables for their antioxidant activity. Plant Foods Hum Nutr 2009; 64: 39-45.
6) Mohammad S, Taha A, Akhtar K, Bamezai RN, Baquer NZ. *In vivo* effect of Trigonella foenum graecum on the expression of pyruvate kinase, phosphoenolpyruvate carboxykinase and distribution of glucose transporter (GLUT4) in alloxan diabetic rats. Can J Physiol Pharmacol 2006; 84: 647-654.
7) Hannan JMA, Ali L, Rokeya B, Khaleque J, Akhter M, Flatt P, Abdel-Wahab Y HA. Soluble dietary fibre fraction of *Trigonella foenum-graecum* (fenugreek) seed improves glucose homeostasis in animal models of type 1 and type 2 diabetes by delaying carbohydrate digestion and absorption, and enhancing insulin action. Br J Nutr 2007; 97: 514-521.
8) Kaviarasan S, Vijayalakshmi K, Anuradha CV. Polyphenol-rich extract of fenugreek seeds protect erythrocytes from oxidative damage. Plant Foods Hum Nutr 2004; 59: 143-147.
9) Broca C, Gross R, Petit P, Sauvaire Y, Manteghetti M, Tournier M, Masiello P, Gomis R, Ribes G. 4-Hydroxyisoleucine: experimental evidence of its insulinotropic and antidiabetic properties. Am J Physiol 1999; 277: 617-623.
10) Kamal A, Biessels GJ, Gispen WH, Ramakers GM. Synaptic transmission changes in the pyramidal cells of the hippocampus in streptozotocin-induced diabetes mellitus in rats. Brain Res 2006; 1073-1074: 276-280.
11) Baquer NZ, Taha A, Kumar P, McLean P, Cowsik SM, Kale RK, Singh R, Sharma D. A Metabolic and functional overview of brain aging linked to neurological disorders. Biogerontology 2009; 10: 377-413.
12) Baquer NZ, Kumar P, Taha A, Kale RK, Cowsik SM, McLean P. Metabolic and molecular action of *Trigonella foenum-graecum* (fenugreek) and trace metals in experimental diabetic tissues. J Biosci 2011; 36: 386-396.
13) Lakhman SS, Kaur G. Effect of experimental diabetes on monoamine oxidase activity from discrete areas of rat brain: relationship with diabetes associated reproductive failure. Mol Cell Biochem 1997; 177: 15-20.
14) Mayanil CS, Kazmai SMI, Baquer NZ. Na+,K+-ATPase and Mg2+-ATPase activities in different regions of rat brain during alloxan diabetes. J Neurochem 1982; 39: 903-908.
15) Kamboj SS, Chopra K, Sandhir R. Hyperglycemia-induced alterations in synaptosomal membrane fluidity and activity of membrane bound enzymes:beneficial effect of N-acetylcysteine supplementation. Neuroscience 2009; 162: 349-358.
16) Alvarez EO, Beauquis J, Revsin Y, Banzan AM, Roig P, De Nicola AF, Saravia F. Cognitive dysfunction and hippocampal changes in experimental type 1 diabetes. Behav Brain Res 2009; 198: 224-230.
17) Vannucci SJ, Koehler-Stec EM, Li K, Reynolds TH, Clark R, Simpson IA. GLUT4 glucose transporter expression in rodent brain: effect of diabetes. Brain Res 1998; 22: 1-11.
18) Kumar P, Taha A, Kale RK, Cowsik SM, Baquer NZ. Physiological and biochemical effects of 17β estradiol in aging female rat brain. Exp Gerontol 2011; 46: 597-605.
19) Bakirtzi K, Belfort G, Lopez-Coviella I, Kuruppu D, Cao L, Abel ED, Brownell AL, Kandror KV. Cerebellar neurons possess a vesicular compartment structurally and functionally similar to Glut4-storage vesicles from peripheral insulin-sensitive tissues. J Neurosci 2009; 29: 5193-5201.
20) El Messari S, Leloup C, Quignon M, Brisorgueil MJ, Pe´nicaud L, Arluison M. Immunocytochemical localization of the insulin-responsive glucose transporter 4 (Glut 4) in the rat central nervous system. J Comp Neurol 1998; 399: 492-512.

21) Raju J, Gupta D, Rao AR, Yadava PK, Baquer NZ. TSP foenum graecum (fenugreek) seed powder improves glucose homeostasis in alloxan diabetic rat tissues by reversing the altered glycolytic, gluconeogenic and lipogenic enzymes. Mol Cell Biochem 2001; 224: 45-51.

22) Preet A, Gupta BL, Siddiqui MR, Yadava PK, Baquer NZ. Restoration of ultrastructural and biochemical changes in alloxan-induced diabetic rat sciatic nerve on treatment with Na_3VO_4 and Trigonella–a promising antidiabetic agent. Mol Cell Biochem 2005; 278: 21-31.

23) Kumar P, Kale RK, Mukherjee S, Prakash K, McLean P, and Baquer NZ. Antidiabetic effects of Trigonella foenum-graecum seed powder in a rat model. Toxicol Environ Chem 2011; 93: 2085-2097.

24) Kumar P, Kale RK, McLean P, Baquer NZ. Antidiabetic and neuroprotective effects of Trigonella foenum-graecum seed powder in diabetic rat brain. Prague Med Rep 2012; 113: 33-43.

25) Kumar P, Kale RK, Baquer NZ. Membrane linked functions in diabetic and aging female rat brain. LAP Lambert Academic Publishing GmbH & Co. KG, Saarbrücken Germany, 2011.

26) Tappel A, Fletcher B, Deamer D. Effects of antioxidants and nutrients on lipid peroxidation fluorescent product and aging parameters in the mouse. J Gerontol 1973; 28: 415-424.

27) Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72: 248-254

28) Sandberg EM, Sayeski PP. Jak2 tyrosine kinase mediates oxidative stress-induced apoptosis in vascular smooth muscle cells. J Biol Chem 2004; 279: 34547-34552.

29) Nagata S. Apoptotic DNA fragmentation. Exp Cell Res 2000;10; 256: 12-18.

30) Towbin H, Gordon J. Immunoblotting and dot immunobinding–current status and outlook. J Immunol Methods 1984; 72: 313-340.

31) Alonso A, Moreno M, Ordóñez P, Fernández R, Pérez C, Díaz F, Navarro A, Tolivia J, González C. Chronic estradiol treatment improves brain homeostasis during aging in female rats. Endocrinology 2008; 149: 57-72.

32) Udayakumar R, Kasthurirengan S, Vasudevan A, Mariashibu TS, Rayan JJ, Choi CW, Ganapathi A, Kim SC. Antioxidant effect of dietary supplement Withania somnifera L. reduces blood glucose levels in alloxan-induced diabetic rats. Plant Foods Hum Nutr 2010; 65: 91-98.

33) Losso JN, Holliday DL, Finley JW, Martin RJ, Rood JC, Yu Y, Greenway FL. Fenugreek bread: a treatment for diabetes mellitus. J Med Food 2009; 12: 1046-1049.

34) Tripathi UN, Chandra D. The plant extracts of Momordica charantia and *Trigonella foenum-graecum* have anti-oxidant and anti-hyperglycemic properties for cardiac tissue during diabetes mellitus. Oxid Med Cell Longev 2009; 2: 290-296.

35) Kumar P, Kale RK, Baquer NZ. Effect of Estradiol administration in normalizing age related neuronal parameters in female rats. New Frontiers in Molecular Mechanisms of Neurological and Psychiatric Disorders. - 1. Martin: Jesseniova Lekárska Fakulta, Comenius University, Jessenius Faculty of Medicine in Martin, SLOVAKIA, 2011, pp. 182-218.

36) Anuradha, CV, Ravikumar P. Restoration on tissue antioxidants by fenugreek seeds (Trigonella Foenum Graecum) in alloxan-diabetic rats. Indian J Physiol Pharmacol 2001; 45: 408-420.

37) Sugaya A, Sugimioto H, Mogi N, Tsujigami H, Deguchi S. Experimental diabetes accelerates accumulation of fluorescent pigments in rat trigeminal neurons. Brain Res 2004; 27: 132-134.

38) Vats V, Yadav SP, Grover JK. Effect of *T. foenum graecum* on glycogen content of tissues and the key enzymes of carbohydrate metabolism. J Ethnopharmacol 2003; 85: 237-242.

39) Li ZG, Sima AA. C-peptide and central nervous system complications in diabetes. Exp Diabesity Res 2004; 5: 79-90.

40) Farhangkhoee H, Khan ZA, Mukherjee S, Cukiernik M, Barbin YP, Karmazyn M, Chakrabarti S. Heme oxygenase in diabetes-induced oxidative stress in the heart. J Mol Cell Cardiol 2003; 35: 1439-1448.

41) Murata M, Ohnishi S, Seike K, Fukuhara K, Miyata N, Kawanishi S. Oxidative DNA damage induced by carcinogenic dinitropyrenes in the presence of P450 reductase. Chem Res Toxicol 2004; 17: 1750-1756.

42) Kumar P. Membrane-linked functions and glucose transporter (GLUT4) in diabetic and aging female rats: Effect of *Trigonella foenum-graecum* and estradiol, Ph.D thesis, School of Life Sciences, Jawaharlal Nehru University, New Delhi, India; 2011.

43) Srinivasan K. Fenugreek (*Trigonella foenum-graecum*): A Review of health beneficial physiological effects. Food Rev Intern 2006; 22: 203-224.

44) Kumar GS, Shetty AK, Salimath PV. Modulatory effect of fenugreek seed mucilage and spent turmeric on intestinal and renal disaccharidases in streptozotocin induced diabetic rats. Plant Foods Hum Nutr 2005; 60: 87-91.

45) Al-Habori M, Raman A, Lawrence MJ, Skett P. In vitro effect of fenugreek extracts on intestinal sodium-dependent glucose uptake and hepatic glycogen phosphorylase A. Int J Exp Diabetes Res 2001; 2: 91-99.