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Original Research

Synaptosomal and mitochondrial oxidative damage followed by behavioral impairments in streptozotocin induced diabetes mellitus: restoration by *Malvastrum tricuspidatum*

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Abstract: Synaptosomal and mitochondrial impairments in the brain of diabetic individual manifest metabolic risk factors that most likely affect the brain functions. Diabetes is associated with the structural and functional alterations of the brain and neuronal loss leading to cognitive and other behavioral impairments, thus reducing the quality of life. The biochemical mechanisms underlying the diabetes-associated behavioral decline are poorly understood. In the present study, we examined the effects of hyperglycemia on the oxidative stress and behavioral functions in streptozotocin-induced diabetic mice. Our results showed the increase in oxidative stress in synaptosomes and brain mitochondria marked by an increase in lipid peroxidation and protein carbonyl content and decline in reduced glutathione in mice treated with streptozotocin. The oxidative damage in synaptosomes and brain mitochondria further caused impairment in locomotor and memory behavioral functions. Furthermore, we examined the protective effects of plant extract derived from *Malvastrum tricuspidatum* against STZ induced oxidative stress and behavioural impairments. For the first time we showed that this plant extract attenuate synaptosomal and mitochondrial oxidative stress and behavioral impairments in mice treated with streptozotocin. We suggest that streptozotocin caused impairments in synaptosomes and brain mitochondria and altered behavioral functions via increase in the oxidative stress and decrease in the antioxidant defense system. These impairments in synaptosomes and brain mitochondria and alterations in behavioral functions are significantly prevented by supplementation of *Malvastrum tricuspidatum* extract.

Key words: Oxidative stress; Behavioral impairments; Synaptosomes; Mitochondria; Diabetes mellitus; *Malvastrum tricuspidatum*.

Introduction

Synaptosomes are membrane vesicles consist of the presynaptic nerve terminals including mitochondria and synaptic vesicles, along with the postsynaptic membrane and the postsynaptic density proteins in the brain (1, 2). The synaptosomes are functionally distinct structures that not only permit a neuron to communicate electrical or chemical signals but are also involved in learning and memory (3, 4). Therefore progressive loss of synaptosomes can be directly correlated with severity of cognitive impairment in various neurodegenerative diseases (5, 6, 7). Not only the synaptosomal damage but mitochondrial dysfunctions are also associated with a number of neurodegenerative diseases. Mitochondria are not just ATP generators but are involved in a range of other functions including regulation of redox homeostasis. Mitochondrial dysfunctions may lead to imbalance in redox state of the cells and the imbalance in the redox state of the cells could result in oxidative stress. Biological tissue such as brain is particularly vulnerable to oxidative stress because of its high energy demands and dependence on efficient mitochondrial functions (8). Oxidative stress is one of the causal factors for a variety of neurodegenerative diseases (9). These neurodegenerative diseases are characterized by the progressive neuronal death that results in memory loss, movement problems, cognitive impairment, emotional alterations and other behavioral problems (10).

Streptozotocin (STZ) is a glucosamine-nitrosourea containing antibiotic used to induce insulin-dependent type 1 diabetes mellitus in experimental animals. STZ causes dose dependent damage of pancreatic β cells that results in hypoinsulinemia and hyperglycemia (11). Patients with diabetes mellitus, and in particular hyperglycemic patients, often experience brain complications (12). The complication of diabetic brain includes memory and motor dysfunctions (12, 13, 14) and various clinical studies have established associations between neurodegenerative diseases, insulin resistance and diabetes mellitus (15, 16). Diabetic brain also causes elevated production of reactive oxygen species (ROS) (17) and this hyperglycemia-induced oxidative stress may not only causes mitochondrial dysfunctions but also initiate structural and functional changes in synaptosomes (18). However oxidative stress, diabetic complications and behavioral and cognitive impairment in brain are intimately linked together therefore compounds that can prevent oxidative damage in synaptosomes and brain mitochondria may increase its resistance during diabetes. Various studies have shown that plant derived phytochemicals play important role in the prevention and treatment of such neuronal complications during diabetes (19, 20).

Malvastrum tricuspidatum (MT) of family Malvaceae is a weed of waste places, distributed worldwide and also in the Indian subcontinent. It is commonly known as False mallow or Kharenti in hindi (21). The whole plant contains β-phenylethylamine, dotriacontane, dotriacontanol, β-sitosterol, stigmasterol, cam-

pesterol, lutein, N-methyl-β-phenylethylamine, indole alkaloid and fatty acids like palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, malvalic acid and sterculic acid (21, 22). Its leaves contain malvastrone and are applied to inflamed sores and wound (23) while flowers are given as pectoral and diaphoretic (24) and used in cough, chest and lung diseases (25). Various pharmacological studies have confirmed that MT posses anti-inflammatory activity, analgesic activity (26), antibacterial activity (27), hypoglycemic (28) and antipyretic activity (29). However, little is known about the role of MT in the amelioration of oxidative stress. In addition, there are no reports appeared in the literature on the neuroprotective effects of MT. Hence, the present study has been conducted to evaluate the protective role of MT on the oxidative stress induced by STZ in synaptosomes and brain mitochondria. In addition we also evaluated the protective effects of MT on the behavioral impairments caused by STZ. Data obtained from the present study showed that STZ caused impairments of synaptosomes and brain mitochondria by increase in the oxidative damage and that the MT supplementation significantly ameliorated the effects of STZ in the brain.

Materials and Methods

Reagents

STZ was purchased from Sigma Chemical Company Ltd., USA. Sodium dodecyl sulphate (SDS), acetic acid, n-butanol and pyridine were obtained from Glaxo Chemicals, India. Thiobarbituric acid (TBA) was purchased from Loba Chemicals, India. Streptomycin sulphate, trichloroacetic acid (TCA), 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) and di-nitrophenyl-hydrazine (DNPH) were purchased from Sigma Chemical Company Inc. (St. Louis, MO, USA). Acetone, methanol, hexane, sucrose, EGTA, Hepes buffer, HCl, ethyl acetate, ethanol, sodium hydroxide (NaOH) were purchased from HiMedia Laboratories (Mumbai, India). Potassium dihydrogen orthophosphate (KH_2PO_4), dipotassium hydrogen orthophosphate (K_2HPO_4), metaphosphoric acid, and sodium chloride were obtained from Merck, India. All other used chemicals were of analytical grade.

Experimental Animal

Adult male Swiss albino mice (*Mus Musculus albinus*) weighing 25 gm were obtained from College of Veterinary Science and Animal Husbandry, Mhow, India and all experimental animals housed and monitored in environmentally regulated room (under pathogen free conditions) on a 12 h light: 12 h dark cycle with a temperature of 25±1°C and had free access to food and water. The use of these mice in experimental protocol was approved by the research committee of the Vikram University, Ujjain, India in accordance with the international guidelines for the care and use of laboratory animals.

Experimental design

A total of 24 experimental mice were randomly divided into three groups with 8 mice in each group. The first group of animal belongs to control. The second group was treated with different STZ concentrations (40, 60, 80 and 120 mg/kg b.w.). The third group was treated with different STZ concentrations (40, 60, 80 and 120 mg/kg b.w.) + MT (28 mg/kg b.w.) extract.

Plant materials and fractionation of extracts

Powdered plant material (500 gm) of MT was extracted in acetone at room temperature to obtain (after solvent evaporation) a brown solid extract. Fractionation of the extract was done by using methanol (90%); hexane (1:1) liquid–liquid extraction as described by Danizu et al. (30) and Mitscher et al. (31). The alcoholic fraction was dried and dissolved in distilled water before mixing into diet.

Treatments

Diabetes was induced in adult male Swiss albino mice by concentration dependant intraperitoneal injection of STZ (40, 60, 80 and 120 mg/kg b.w.) in citrate buffer solution (pH 4.0) and monitored for a month. Control mice were injected only with normal saline/ citrate buffer solution instead of STZ under the same experimental conditions. The amount of 28 mg/kg b.w. of MT extract was given to experimental mice mixed with the diet daily for one month. The diet of mice was prepared by mixing 10g of standard food with freeze dried MT extract. All experimental mice were housed individually in cages and checked daily to assure the consumption of full daily diet.

Synaptosomes preparation

Crude synaptosomes were prepared from each group according to the method of Hajos (32) and Duarte et al. (33). Experimental mice were decapitated and the whole brains were removed, immersed in cold saline. Tissues were homogenized at 0°C in 0.32 M sucrose buffered with tris (pH 7.4). The homogenate was centrifuged at 1000g for 10 min. and the synaptosomal fraction isolated from the supernatant by centrifugation at 12000g for 20 min. The white and fluffy synaptosomal layer was then re-suspended, re-spun and re-suspended in the sucrose medium at a protein concentration of 15-20 mg /ml.

Mitochondrial preparation

The mice were euthanized by decapitation. Brains were rapidly excised and placed into ice cold isolation medium containing 0.25 M sucrose, 1.0 mM EGTA and 5.0 mM Hepes buffer (pH 7.2). Tissues were weighed, minced and homogenized in 10% w/v isolation medium. Mitochondria were isolated from mice brain of all groups by conventional differential centrifugation method as described (34, 35). The homogenate was centrifuged at 3000 rpm for 10 min to discard nuclei and cell debris and the pellet was washed and centrifuged at 12000 rpm to obtain mitochondria. The pellet was then re-suspended in 1 ml isolation buffer to obtain approximately 80-100 mg mitochondrial protein/ml. The experiments were performed immediately after purification of the mitochondria.

Lipid peroxidation

LPO was determined by measuring thiobarbituric acid reactive substance (TBARS) in terms of malondialdehyde equivalent (MDA) using the molar extinction coefficient of 1.56×10^5 $min^{-1}.cm^{-1}$ as described by

Ohkawa et al. (36). Previously prepared homogenate was centrifuged at 3,000 x g for 15 min, and the supernatant was used for the assay. Samples of 0.1 ml supernatant were mixed with 0.2 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml 20% glacial acetic acid, and 1.5 ml of 0.8% thiobarbituric acid (TBA). Now tubes containing reaction mixture were shaken and heated at 95 °C for 60 min in a water bath, and then cooled under tap water before mixing with 1 ml distilled water and 5 ml mixture of n-butanol and pyridine (15: 1). The reaction mixture was centrifuged at 2,200 x g for 10 min. The upper organic layer was isolated for the measurement of TBARS value by taking absorbance at 532 nm in a Perkin-Elmer UV-Spectrophotometer. The results were expressed as nM TBARS/mg protein.

Protein carbonyl content

Protein carbonyl content was evaluated according to Levine et al. (37) with some modifications. Briefly, 0.5 ml sample is mixed with streptomycin sulphate solution (10% w/v) up to a final concentration of 1% to precipitate DNA. The solution was mixed and left to stand for 15 min at room temperature, and then it was centrifuged at 2,800 x g for 10 min at room temperature. The supernatant is removed and 0.2 ml is divided equally between two test tubes. Both the tubes were incubated with DNPH (1.6 ml, 10 mM in 2 M HCl) and 1.6 ml of 2M HCl respectively for 60 minutes and subsequently, the proteins were precipitated using 20% trichloroacetic acid (TCA) and centrifugation at 3,400 x g for 10 min at room temperature. The pellet was suspended in phosphate buffer and washed thrice with ethyl acetate: ethanol (1:1 v/v) to remove excess DNPH. The final protein pellet was dissolved in 1.5 ml of 6 M guanidine hydrochloride. The carbonyl content was evaluated by detecting the absorbance at 370 nm in a Perkin-Elmer UV-Spectrophotometer. A standard curve was obtained using bovine serum albumin (BSA) and included in each assay to determine linearity and the extent of derivatization. Results were expressed in nM carbonyl group/mg protein.

Reduced glutathione

The GSH content was quantitated according to Jollow et al. (38), that involves the spectrophotometric assessment of the 5-thio-2-nitrobenzoate in the presence of NADPH and glutathione reductase. Briefly, homogenates were mixed with 0.5 M ice cold metaphosphoric acid and centrifuged for 15 min at 16,000 x g at 4 ºC. The 0.5 ml supernatant was mixed with 4 ml of ice cold 0.1 mM solution of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) in 0.1 M phosphate buffer pH 8.0 and the optical density was obtained at 412 nm in a Perkin-Elmer UV-Spectrophotometer. A standard calibration curve was prepared using GSH.

Total protein assay

Total protein of brain homogenates was measured by Folin-phenol reaction as described by Lowry et al. (39). Briefly protein sample was prepared by precipitation with TCA. The precipitate was dissolved in 0.4 N NaOH and 5 ml protein reagent with 0.5 ml Folin phenol reagent was mixed and the absorbance was measured at 625 nm. A standard curve was prepared using bovine serum albumin (BSA) as a standard to determine linearity and to measure the extent of derivatization.

Behavioral test

For behavioral tests we used 4 different concentrations of STZ (40, 60, 80, 120 mg/kg b.w.). Individual memory and locomotors functions were observed after starving the control and all experimental mice. T-Maze and an active avoidance apparatus were used for locomotor and memory tests respectively as described (40).

Measurement of locomotor performance

Locomotor performances of all groups of mice were evaluated by T-Maze. Before test session, the mice were habituated to the experimental conditions by allowing them to spend 1–2 hrs daily in the T-Maze. After 24 hrs of the last training session mice were placed in a T-Maze box for test session and the time taken to cross the serpentine path (s) was evaluated from the front-view images.

Measurement of memory response

Memory responses were observed by active avoidance method by using locally designed active avoidance apparatus. This apparatus was made up of two compartments i.e. light and dark compartment separated by doors in each compartment. Avoidance schedule consist of an inter trial interval of 30 s and a warning period of 10 s. In the training session visual (2 s) and auditory (2 s) stimuli were given with electric shock (1.5 mA for 2 s). As soon as the warning signal ceases, a 1.5 mA foot shock is delivered until the animal crosses the mid line; if the animal crosses to the other side of the box during the warning signal, the shock is interrupted and the trial program was returned to starting point of the 30 inter trial intervals. One avoidance session consisting of 25 training session per day and the avoidance session was conducted every week during the experimental period. The active avoidance rate was expressed as the mean time taken as correct avoidance responses.

Statistical analysis

Results are expressed as means ± SE. Statistical analysis was performed by ANOVA and student's t-test. The 0.05 level was selected as a point of minimal statistical significance.

Results

Lipid peroxidation

STZ is a broad spectrum antibiotic having diabetogenic properties via inducing oxidative stress in experimental animals. The peroxidation products formed as a result of oxidation of membrane lipids can serve as potential oxidative stress biomarkers. Fig. 1 (A and B), showed the concentration dependant increase in the LPO in synaptosomes and mitochondria of the STZ treated mice as compared to the control. In synaptosomes, the level of LPO was significantly increased from 1.34 ± 0.3 nM TBARS/mg protein in control to 3.33 ± 0.12 nM TBARS/mg protein at 40 mg/kg STZ, 4.87 ± 0.3 nM TBARS/mg protein at 60 mg/kg STZ, 8.75 ± 0.57 nM TBARS/mg protein at 80 mg/kg STZ and 9.23 ± 0.7 nM TBARS/mg protein at 120 mg/kg STZ treatments

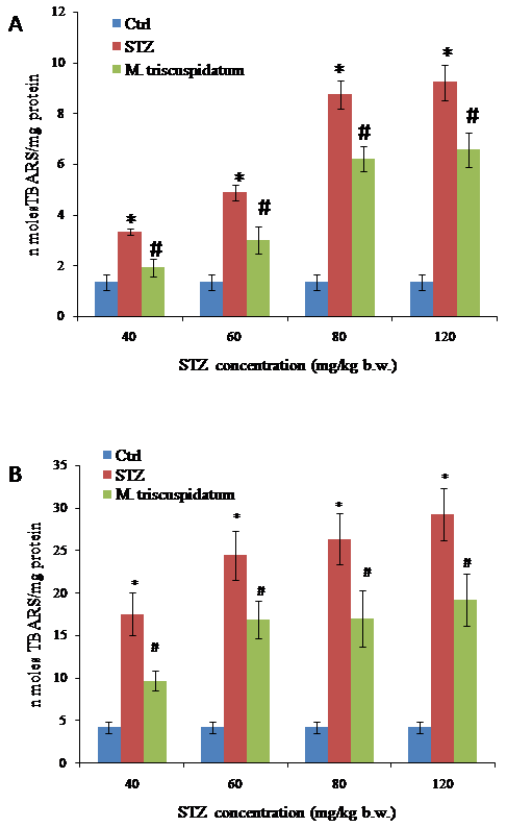

Figure 1. Effect of extract of Malvastrum tricuspidatum (MT) on lipid peroxidation (nM TBARS/mg protein) in synaptosomes (Fig. 1A) and brain mitochondria (Fig. 1B) of streptozotocin (STZ) induced diabetic mice. Mice were treated with different doses of STZ (40, 60, 80, 120 mg/kg b.w.) and provided with daily the dietary supplementation of extract of MT (28 mg/kg b.w.) for one month. Values represent the mean ± SE. *p < 0:05 significantly different from control. #p < 0:05 significantly different from STZ treatment as determined by ANOVA (n=5).

(Fig. 1A). Whereas in brain mitochondria, the LPO was significantly increased from 4.23 ± 0.72 nM TBARS/mg protein in control to 17.54 ± 2.5 nM TBARS/mg protein at 40 mg/kg STZ, 24.45 ± 2.9 nM TBARS/mg protein at 60mg/kg STZ, 26.35 ± 3.0 nM TBARS/mg protein at 80 mg/kg STZ and 29.26 ± 3.07 nM TBARS/mg protein at 120 mg/kg STZ treatments (Fig. 1B). The supplementation of MT to STZ treated mice was found to prevent the oxidation of membrane lipids. In synaptosomes, the LPO was decreased from 3.33 ± 0.12 to 1.92 ± 0.34 nM TBARS/mg protein at 40 mg/kg STZ+MT, 4.87 ± 0.3 to 3.02 ± 0.54 nM TBARS/mg protein at 60 mg/kg STZ+MT, 8.75 ± 0.57 to 6.2 ± 0.49 nM TBARS/mg protein at 80 mg/kg STZ+MT and 9.23 ± 0.7 to 6.56 ± 0.69 nM TBARS/mg protein at 120 mg/kg STZ+MT supplemented mice (Fig. 1A). In mitochondria, the LPO was significantly decreased from 17.54 ± 2.5 to 9.71 ± 1.2 nM TBARS/mg protein at 40 mg/kg STZ+MT, 24.45 ± 2.9 to 16.87 ± 2.24 nM TBARS/mg protein at 60 mg/kg STZ+MT, 26.35 ± 3.0 to 17.0 ± 3.34 nM TBARS/mg protein at 80 mg/kg STZ+MT and 29.26 ± 3.07 to 19.23 ± 3.08 nM TBARS/mg protein at 120 mg/kg STZ+MT supplemented mice (Fig. 1B).

Protein carbonylation

Carbonylated proteins are relatively stable group of oxidized proteins targeted by ROS and therefore serve as valuable biomarkers of oxidative stress. We measured STZ induced protein carbonyl content in the synaptosomes and brain mitochondria. As shown in Fig. 2 (A and B), the levels of protein carbonyl were significantly increased by STZ in the concentration dependant manner as compared to the control. In synaptosomes, the level of protein carbonyl was significantly increased from 39.76 ± 2.12 nM carbonyl group/mg protein (control) to 67.43 ± 2.48 nM carbonyl group/mg protein at 40 mg/kg STZ, 88.87 ± 4.7 nM carbonyl group/mg protein at 60 mg/kg STZ, 97.32 ± 6.9 nM carbonyl group/mg protein at 80 mg/kg STZ and 111.87 ± 5.9 nM carbonyl group/mg protein at 120 mg/kg STZ treatments (Fig. 2A). In mitochondria, the protein carbonyl level was increased from 22.9 ± 2.1 nM carbonyl group/mg protein (control) to 52.31 ± 2.0 nM carbonyl group/mg protein at 40 mg/kg STZ, 96.39 ± 3.9 nM carbonyl group/mg protein at 60 mg/kg STZ, 111.01 ± 4.7 nM carbonyl group/mg protein at 80 mg/kg STZ and 119.85 ± 4.2 nM carbonyl group/mg protein at 120 mg/kg STZ treatments (Fig. 2B). MT supplementation caused significant restorative effect on the level of protein carbonyl content in both synaptosomes and brain mitochondria. In synaptosomes, the level of protein carbonyl was significantly decreased from 67.43 ± 2.48 to 42.79 ± 1.98 nM carbonyl group/mg protein at 40 mg/kg STZ+MT, 88.87 ± 4.7 to 52.98 ± 4.2 nM carbonyl group/mg protein at 60 mg/kg STZ+MT, 97.32 ± 6.9 to 63.21 ± 4.93 nM carbonyl

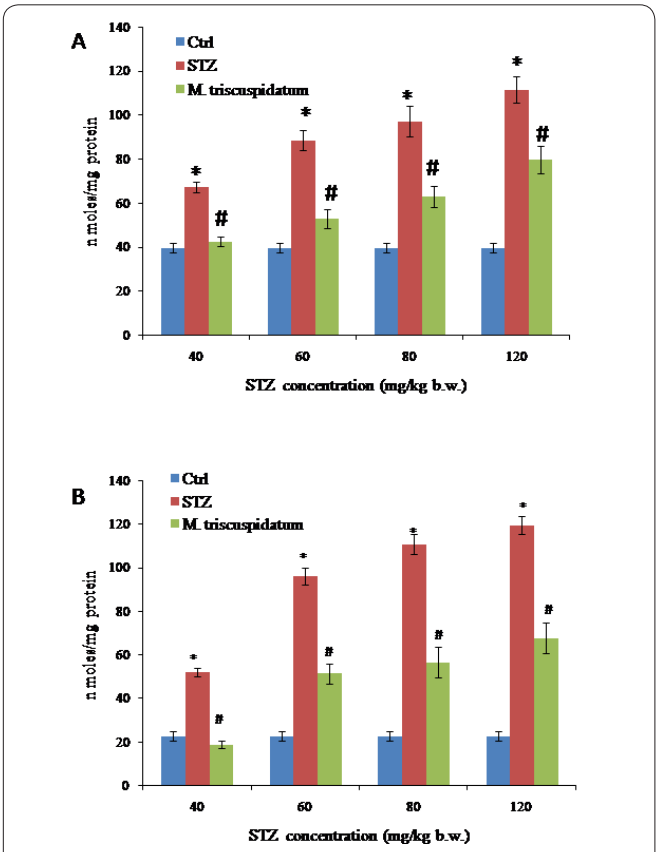

Figure 2. Effect of extract of Malvastrum tricuspidatum (MT) on protein carbonyl (nm/mg protein) in synaptosomes (Fig. 2A) and brain mitochondria (Fig. 2B) of streptozotocin (STZ) induced diabetic mice. Mice were treated with different doses of STZ (40, 60, 80, 120 mg/kg b.w.) and provided with daily the dietary supplementation of extract of MT (28 mg/kg b.w.) for one month. Values represent the mean ± SE. *p<0:05 significantly different from control. #p<0:05 significantly different from STZ treatment as determined by ANOVA (n=5).

group/mg protein at 80 mg/kg STZ+MT and 111.87 ± 5.9 to 80.12 ± 6.2 nM carbonyl group/mg protein at 120 mg/kg STZ+MT supplemented mice (Fig. 2A). In mitochondria, the level of protein carbonyl was significantly decreased from 52.31 ± 2.0 to 19.11 ± 1.64 nM carbonyl group/mg protein at 40mg/kg STZ+MT, 96.39 ± 3.9 to 51.64 ± 4.59 nM carbonyl group/mg protein at 60 mg/kg STZ+MT, 111.01 ± 4.7 to 56.85 ± 6.98 nM carbonyl group/mg protein at 80 mg/kg STZ+MT and 119.85 ± 4.2 to 67.91 ± 7.2 nM carbonyl group/mg protein at 120 mg/kg STZ+MT supplemented mice (Fig. 2B). These results suggest that MT extract is effective in reducing STZ-induced protein carbonyl in both synaptosomes and brain mitochondria.

Reduced glutathione

GSH is the most abundant non protein thiol that acts as a free radical scavenger. Therefore endogenous level of GSH is analyzed as oxidative stress markers. We measured the levels of GSH in synaptosomes and mitochondria of mice after treatment of STZ. The level of GSH was significantly reduced in synaptosomes and mitochondria in the concentration dependant manner in comparison to the control. As shown in Fig. 3A, in synaptosomes, STZ treatment caused a significant decrease in the GSH level from 7.5 ± 0.1nmol/mg protein (control) to 2.9 ± 0.08 nmol/mg protein at 40 mg/kg STZ, 3.02 ± 0.21 nmol/mg protein at 60 mg/kg STZ, 6.2 ± 0.17 nmol/mg protein at 80 mg/kg STZ and 6.56 ± 0.23 nmol/mg protein at 120 mg/kg STZ treatments. In mitochondria, the level of GSH was significantly decrease from 9.98 ± 1.0 nmol/mg protein (control) to 4.5 ± 0.32 nmol/mg protein at 40 mg/kg STZ, 4.0 ± 0.43 nmol/mg protein at 60 mg/kg STZ, 3.21 ± 0.56 nmol/mg protein at 80mg/kg STZ and 4.86 ± 0.72 nmol/mg protein at 120 mg/kg STZ treatments (Fig. 3B). However, the supplementation of MT significantly increased the level of GSH. In synaptosomes, the GSH level was significantly increased from 2.9 ± 0.08 to 6.5 ± 0.08 nmol/mg protein at 40 mg/kg STZ+MT, 3.02 ± 0.21 to 4.87 ± 0.15 nmol/mg protein at 60mg/kg STZ+MT, 6.2 ± 0.17 to 8.75 ± 0.25 nmol/mg protein at 80 mg/kg STZ+MT and 6.56 ± 0.23 to 9.23 ± 0.29 nmol/mg protein at 120 mg/kg STZ+MT supplemented mice (Fig. 3A). In mitochondria, the level of GSH was significantly increased from 4.5 ± 0.32 to 7.9 ± 0.8 nmol/mg protein at 40 mg/kg STZ+MT, 4.0 ± 0.43 to 6.1 ± 0.4 nmol/mg protein at 60 mg/kg STZ+MT, 3.21 ± 0.56 to 5.0 ± 0.47 nmol/mg protein at 80 mg/kg STZ+MT and 4.86 ± 0.72 to 5.53 ± 0.41 nmol/mg protein at 120 mg/kg STZ+MT supplemented mice (Fig. 3B). These results suggest that MT increases the endogenous level of GSH and prevents the oxidative stress in both synaptosomes and brain mitochondria of STZ treated mice.

Behavioral test

We assessed the locomotor and memory behavioral tests in STZ induced mice. To test the locomotor function in the T-maze, the mice were randomly divided in three groups: control, STZ treated and STZ + MT treated. Further STZ treated mice were again divided in four subgroups according to the concentration of STZ. At the learning trial, mice of all groups entered the compartment randomly, and there were no significant differences in mean time taken by three groups. In the testing trial, mice in the STZ treated group showed significant increases in the mean time as compared to those of control group (Fig 4). MT supplemented group showed significant decreases in the mean time taken as compared to those of STZ treated groups.

The effect of STZ and STZ + MT on memory func-

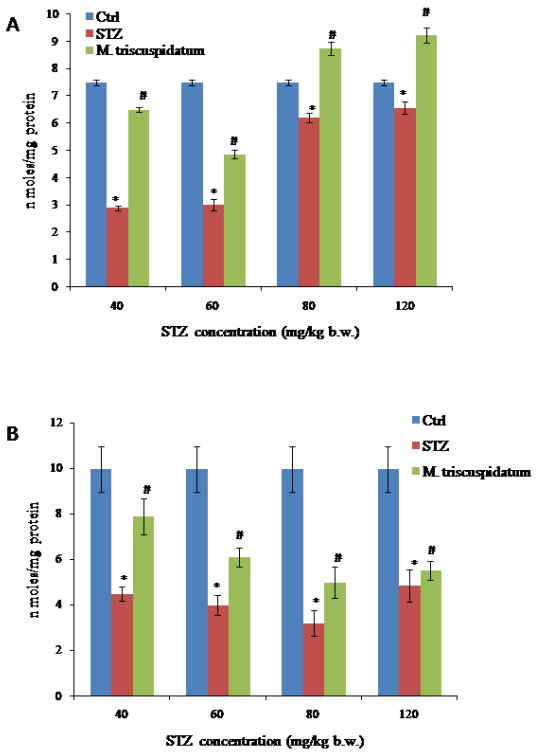

Figure 3. Effect of extract of Malvastrum tricuspidatum (MT) on reduced glutathione (GSH; nmol/mg) in synaptosomes (Fig. 3A) and the brain mitochondria (Fig. 3B) of streptozotocin (STZ) induced diabetic mice. Mice were treated with different doses of STZ (40, 60, 80, 120 mg/kg b.w.) and provided with daily the dietary supplementation of extract of MT (28 mg/kg b. w.) for one month. Values represent the mean ± SE. *p<0:05 significantly different from control. #p<0:05 significantly different from STZ treatment as determined by ANOVA (n=5).

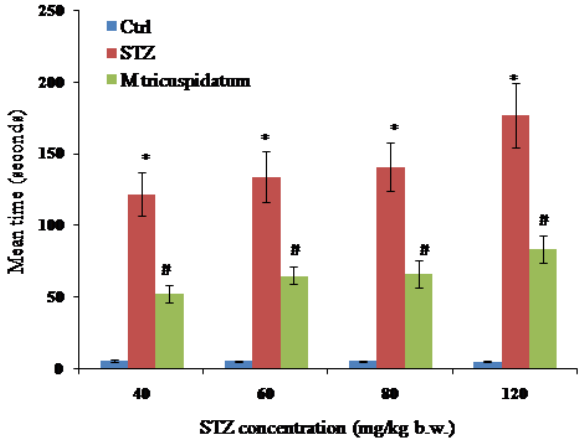

Figure 4. Showing the locomotor behavioral response of control, streptozotocin (STZ) diabetic mice and STZ + Malvastrum tricuspidatum (MT) supplemented mice as measured by T-Maze. Data are expressed in terms of SEM. The values represented the mean time (in s) taken to cross the serpentine path in each trial. *p<0:05 significantly different from control. #p<0:05 significantly different from STZ treated diabetic mice (n=5).

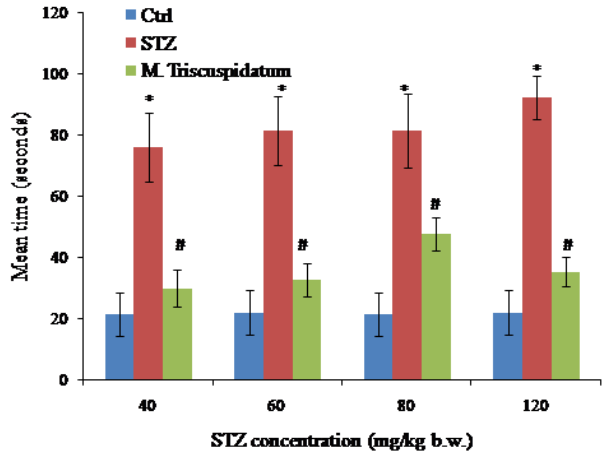

Figure 5. Showing the measurement of active avoidance response of control, streptozotocin (STZ) treated and STZ + Malvastrum tricuspidatum (MT) supplemented mice. Data are expressed in terms of SEM. One avoidance session consisting of 25 times training per week. The active avoidance rate was expressed as the mean time taken in avoidance response.

tions were assessed by active avoidance performance. Fig 5 showed the increase in time taken during active avoidance performance with respect to increase in the concentration of STZ. STZ treated mice were taken maximum time in active avoidance performance at the dose of 120 mg/kg than the controls ($P<0.05$). However, the administration of MT significantly enhanced the memory functions as compared to STZ treated diabetic mice as measured by the decrease in the time taken during the active avoidance performance.

Discussion

STZ induces diabetes in experimental animals via increase in the endogenous oxidative stress in the pancreas. Besides pancreatic tissue, other body organs including the brain are also the target of STZ induced oxidative stress. Oxidative stress is attributed to the formation of ROS that causes the oxidative damage of lipids, proteins and nucleic acids (41). LPO and protein carbonylation are considered as the early markers of the oxidative stress (42, 43). In the present study, we have provided evidence of increased LPO and protein carbonylation in the synaptosomes and brain mitochondria of mice treated with STZ. An increase in lipid peroxidation and protein carbonylation suggest increased oxidative damage of the synaptosomes and brain mitochondria. The GSH is a non protein thiol associated with providing the endogenous antioxidant defense mechanism against the ROS. The GSH content provides the valuable indicator of oxidative stress in the brain thus it can also be an important biomarker of the oxidative stress. The level of GSH has been significantly reduced in the synaptosomes and brain mitochondria further confirming increased oxidative stress and associated complications in mice treated with STZ. Previous work from our laboratory suggested that LPO and protein carbonylation are the major mechanisms by which various brain regions are damaged during acute GSH depletion (40). Since the synaptosomes are involved in signals communication between two neurons and the mitochondria provides energy for this communications therefore the increase in oxidative damage in the synaptosomes and mitochondria may limit the intra neuronal communications thus affecting the brain functions. The antioxidant properties of many plant extracts have been reported in several studies and have been attributed to the inhibition of ROS-generating enzymes as antioxidants through cell signaling mechanisms, scavenging of ROS and decreased mitochondrial superoxide production. Various studies have deduced the effects of different polyphenols and plant extracts in the reduction of STZ induced oxidative stress in the synaptosomes and brain mitochondria (19, 20). However, this is the first study where we report the effects of MT extracts in the reduction of LPO and protein carbonylation in the synaptosomes and brain mitochondria of STZ induced diabetic mice. Our findings suggest that regular supplementation of plant extract may be crucial in managing oxidative damage in the synaptosomes and brain mitochondria.

Synaptosomes are the presynaptic terminal that includes mitochondria and synaptic vesicles, postsynaptic membrane and the postsynaptic density proteins (2). Functionally, synaptosomes transmits electrical or chemical signal to another neuronal cell. Therefore synaptosomal mediated information flow through neural circuits is necessary for behavioral and memory functions (44). The brain is highly susceptible to oxidative damage and mitochondria are the major producer of ROS. We conclude that STZ caused significant increase in the oxidative stress and decrease in the antioxidant defense system in the synaptosomes and brain mitochondria. STZ not only reduced antioxidative responses in the brain but also impaired the memory and locomotor functions. However supplementation of MT not only ameliorated the impaired memory and locomotor functions but also increased the endogenous antioxidant level with the decrease in oxidative stress. Our study suggests that MT extracts may be beneficial in reducing the synaptosomal and the brain mitochondrial impairments against the STZ induced oxidative stress. Our results may help in better understanding of the mechanism of synaptosomes and mitochondrial damage and protection by MT extract in diabetes.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

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