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ORIGINAL ARTICLE

Taurine ameliorates neurobehavioral, neurochemical and immunohistochemical changes in sporadic dementia of Alzheimer's type (SDAT) caused by intracerebroventricular streptozotocin in rats

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Abstract Oxidative loads in the brain are involved in age related impairments like learning and memory as well as neurodegeneration. Taurine, the most abundant free amino acid in humans has many potential health benefits through its anti-oxidant and anti-inflammatory properties. Therefore, we investigated the neuroprotective potential of taurine on oxidative stress, neuronal loss and memory impairments in streptozotocin model of cognitive impairments in rats. The cognitive impairment was developed by giving single intracerebroventricular (ICV) injection of streptozotocin (STZ) 3 mg/kg body weight bilaterally. An increased latency and path length was observed in ICV-STZ group animals as compared to sham group animals and these were inhibited significantly in STZ group pre-treated with taurine (50 mg/kg body weight orally once daily for 15 days). Moreover, the significantly depleted content of GSH and elevated level of thiobarbituric acid reactive substances (TBARS) in ICV-STZ group animals were protected significantly with pre-treatment of taurine. The activity of antioxidant enzymes, glutathione peroxidase, glutathione reductase, glutathione-*S*-transferase, catalase, and superoxide dismutase was decreased in STZ group as compared to sham group and pre-treatment of STZ group with taurine has protected their activities significantly. Furthermore, the increased activity of acetylcholine esterase and decreased expression of choline acetyl transferase were attenuated by the pre-treatment of taurine. Taurine also protected the morphology of the hippocampal pyramidal neurons. This study concludes that the prophylactic intervention of taurine may be used to prevent the deterioration of cognitive functions and neurobehavioral activities, often associated with the generation of free radicals.

Keywords Antioxidants · Cognitive impairments · Taurine · Oxidative stress · Streptozotocin

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Introduction

Alzheimer's disease (AD) was first described by Alois Alzheimer in 1907. AD is the main cause of dementia in the elderly people and mainly characterized by the progressive loss of memory and impaired cognitive functions. This disease starts with mild cognitive impairment (MCI), and later affects long-term memory, consequently leads to severe dementia. The major risk factor for the developing AD is the advanced age, it is reported that the incidence is around 1–2 % in people aged around 60–65 years, rising to about 30 % in those aged 85 years or more [1]. The main neuropathological hallmarks of AD are extracellular deposits of amyloid beta (Aβ) peptide, intracellular deposits of neurofibrillary tangles that consist of hyperphosphorylated tau protein and progressive loss of synapses, dendrites and neurons [2]. One of the most important pathological hallmarks of AD is β-amyloid peptide that contributes to progressive loss of neurons and neuronal cell death in various brain regions, particularly in hippocampus, that is responsible for memory and cognition [3]. Oxidative stress is associated with excessive production of free radicals and loss of antioxidant enzymes that play crucial role in the pathogenesis of AD. Antioxidants supplementations are used for the treatment of various neurodegenerative diseases including AD [4].

It is well documented that intracerebroventricular injection of streptozotocin (ICV-STZ) in rats causes desensitization of neuronal insulin receptor that may further leads to prolonged impairment of brain glucose and energy metabolism [5]. Impaired glucose and energy metabolisms accompanied with loss of learning and memory [6–8], additionally, decreased choline acetyl transferase activity and increased oxidative stress in hippocampus [8, 9] have contributed in the dementia in rats. It is reported that ICV-STZ has no effect on blood glucose level [10, 11], indicating that its role is independent of producing hyperglycemia. Experimental ICV-STZ in rats has been shown to induce biochemical changes and neuropathological changes similar to those found in sporadic Alzheimer's disease and therefore considered to be a valid model to study the experimental dementia of Alzheimer's type [5, 12, 13].

Memory impairment can be prevented by the treatment with antioxidants, melatonin and resveratrol [14] indicating the key role played by the reactive oxygen species in the etiology of AD. Taurine (2-aminoethanesulfonic acid) is present in high concentrations in the mammalian brain [15] and plays an important role in intracellular calcium ion homeostasis, transmembrane Cl^- flux and membrane integrity in the brain [16–19]. It is also reported that taurine has antioxidant and osmoregulatory property [20, 21]. In addition it has been shown that taurine is related to neuroprotection against several neurological diseases [22, 23]. Taurine has a modulated action against neurotoxicity [24] and protects the neurons against glutamate neurotoxicity [25, 26]. Taurine may play a role in attenuation of cognitive deficits induced by neurodegenerative diseases, such as AD via its antioxidant and neuroprotective properties.

The ICV-STZ model generates free radicals and causes memory impairments and neuronal damage, thus the oxidative stress emerged as a key factor in consecutive effect of memory impairment. In spite of the good antioxidant potential of taurine, its potential as neuroprotectant against ICV-STZ model of cognitive impairments remained unexplored. Therefore, the present study was designed to evaluate the pre-treatment effect of taurine on memory impairments and oxidative stress followed by ICV-STZ in rats.

Materials and methods

Chemicals and reagents

Oxidized (GSSG) and reduced (GSH) forms of glutathione, glutathione reductase (GR), nicotinamide adenine dinucleotide phosphate reduced form (NADPH), 1-chloro-2,4-dinitrobenzene (CDNB), 5-5′ dithiobis-2-nitrobenzoicacid (DTNB), thiobarbituric acid (TBA), (−) epinephrine, paraformaldehyde, glycine, trichloroacetic acid (TCA), ethylene diamine tetraacetic acid (EDTA), streptozotocin (STZ) and taurine were purchased from Sigma-Aldrich, Chemicals Pvt. Ltd., India. Other chemicals were of analytical reagent grade.

Animals

One-year-old male Wistar rats weighing 480–520 g were used in this study. They were kept in the Central Animal House of Hamdard University in cages at an ambient temperature of 25 ± 2 °C and relative humidity 45–55 % with 12 h light/dark cycles and had free access to food and water. The food was withdrawn 18–24 h before the surgical procedure. Experiments were conducted in accordance with the Animal Ethics Committee of the University.

Experiment I

Experiment I was carried out to evaluate the pre-treatment effect of taurine (50 mg/kg, body weight, orally in normal saline once daily for 15 days) on thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), antioxidant enzymes, glutathione peroxidase (GP_x), glutathione reductase (GR), glutathione-*S*-transferase (GST), super oxide dismutase (SOD) and acetylcholine esterase (AChE) in ICV-STZ-infused rats. Rats were separated into four groups of ten animals each. Group I was sham operated vehicle-treated control (S) group. Group II was sham-

operated and taurine pre-treated (T + S) group. Group III was ICV-STZ-infused and vehicle-treated lesioned (L) group. Group IV was ICV-STZ-infused and taurine pre-treated (T + L) group. The dose used in the present study was employed from the earlier study performed by Sankar Samipillai et al. [27].

Experiment II

Experiment II was carried out to evaluate the pre-treatment effect of taurine on histopathological and immunohistochemical parameters in ICV-STZ-infused rats and divided into four groups and each group has eight rats.

Post-operative care

After surgery, the animals took approximately 1–2 h to recover from anaesthesia. The rats were kept in a well-ventilated room at 25 ± 2 °C in individual cages and had access to food and water ad libitum, until they recovered full consciousness and then were housed together three animals per cage. Food and water was placed inside the cage for 2–3 days so that the animals could easily access it without any physical trauma due to head surgery.

Surgical procedure

The animals were anesthetized with 400 mg/kg body weight chloral hydrates intraperitoneally (i.p.) and placed on a stereotaxic frame (dual manipulator model 51600 Stoelting Co., IL, USA) and the skin overlying the skull was cut to expose it and the coordinates for the lateral ventricle [28] were measured accurately as anterio-posterior −0.8 mm, lateral 1.5 mm and dorso-ventral −4.0 mm relative to bregma and ventral from dura with the tooth bar set at 0 mm. Burr hole was made in skull by automatic micro drilling machine attached on stereotaxic apparatus. Through the hole, a 28-gauge Hamilton® syringe of 10 µl attached to a micro-injector unit and piston of the syringe was lowered manually into each lateral ventricle. The lesioned groups received a bilateral ICV injection of STZ (3 mg/kg, body weight in saline, 5 µl/injection sites). The sham groups underwent the same surgical procedures, but same volume of saline was injected instead of STZ. After surgery, the rats were housed individually and had access to food and water ad libitum.

Behavioural testing

The behavioural tests were started 2 weeks after lesioning. The experiment was performed between 9.00 am and 4.00 pm at standard laboratory conditions. All the tests were performed and analyzed by the subject blind to the experiment.

Morris water maze test

Spatial learning and memory of animals were tested in Morris water maze [29]. It consisted of a circular water tank (132 cm diameter and 60 cm height) that was filled till 40 cm with water. The water temperature was 25 ± 2 °C. A non-toxic paint was used to render the water opaque. The pool was divided virtually into four equal quadrants, labelled south west, south east, and north east, and north west. The rats were started from four different randomly chosen start positions such as south west (SW), south east (SE), north east (NE), and north west (NW) and trained to find an invisible rectangular platform (10 × 5 cm) at a fixed position in the water tank, 2 cm below the surface of the water. A trial finished until the rats had found the platform or until a time period of 60 s elapsed. Rats did not find the platform within 60 s, were placed on the platform for 3 s and then removed from the water tank. On day first, the rats were given three trials and on five subsequent days, the trials were four per day. The time interval between subsequent trials was 10 min. An overhead video camera was connected to a video monitor and computer software was used to track the animal's path and to calculate the escape latency and travelled distance (path length).

Biochemical analysis

Tissue preparation

After 3 weeks of lesioning, animals were killed and their brains were taken out quickly on ice to dissect hippocampus. The dissected brain parts were homogenized in 10 mM phosphate buffer (PB, pH 7.0) having 10 µl/ml protease inhibitor to get 5 % w/v homogenate. The homogenate was centrifuged at 800×*g* for 5 min at 4 °C to separate the nuclear debris. The supernatant S1 was used for estimation of lipid per oxidation in terms of TBARS content and acetylcholine esterase. The remaining S1 was further centrifuged at 10,500×*g* for 30 min at 4 °C to get the post-mitochondrial supernatant (PMS) which was used for estimation of reduced glutathione and antioxidant enzymes.

TBARS content

TBARS content was estimated by the method of Utley et al. [30]. S1 0.1 ml was pipette into a 15 × 100 mm test tube and incubated at 37 °C in a metabolic shaker for 1 h. An equal volume of homogenate was pipette into a centrifuge tube and placed at 0 °C and marked at 0 h incubation. After 1 h of incubation, 0.45 ml of 5 % (w/v) chilled TCA and 0.45 ml 0.67 % TBA were added and

centrifuged at 4,000×*g* for 10 min. Thereafter, supernatant was transferred to other test tubes and placed in a boiling water bath for 10 min. The absorbance of pink colour produced was measured at 535 nm. The TBARS content was calculated by using a molar extinction coefficient of 1.56×10^5/M/cm and expressed as nmol of TBARS formed per hour per milligram protein.

Reduced glutathione (GSH) content

GSH content was measured by the method of Jollow et al. [31] with slight modification. PMS was mixed with 4.0 % sulfosalicylic acid (w/v) in 1:1 ratio (v/v). The samples were incubated at 4 °C for 1 h, and centrifuged at 4,000×*g* for 10 min at 4 °C. The assay mixture contained of 0.1 ml of supernatant, 1.0 mM DTNB and 0.1 M phosphate buffer pH 7.4 in a total volume of 3.0 ml. The yellow colour developed was read immediately at 412 nm. The GSH content was calculated as nmol GSH mg^{-1} protein, using a molar extinction coefficient of $13.6 \times 10^3 M^{-1} cm^{-1}$.

Estimation of glutathione peroxidase (GP_x)

GP_x activity was determined by the method of Mohandas et al. [32]. The reaction assay consisted of phosphate buffer (0.05 M, pH 7.0), EDTA (1 mM), sodium azide (1 mM), glutathione reductase (1 EU/ml), glutathione (1 mM), NADPH (0.2 mM), hydrogen peroxide (0.25 mM) and 0.1 ml of PMS in the final volume of 2 ml. The disappearance of NADPH at 340 nm was recorded at room temperature. The enzyme activity was calculated as nmol NADPH oxidized/min/mg/protein using molar extinction coefficient of 6.22×10^3 M^{-1} cm^{-1}.

Glutathione reductase (GR)

Glutathione reductase activity was measured by the method of Carlberg and Mannervik [33] as modified by Mohandas et al. [32]. The reaction mixture consisted of phosphate buffer (0.1 M, pH 7.6), NADPH (0.1 mM), EDTA (0.5 mM) and oxidized glutathione (1 mM) and 0.05 ml of PMS in total volume of 1 ml. The enzyme activity was quantified at room temperature by measuring the disappearance of NADPH at 340 nm and calculated as nmol NADPH oxidized/min/mg protein using molar extinction coefficient of 6.22×10^3 M^{-1} cm^{-1}.

Glutathione-S-transferase (GST)

GST activity was measured by the method of Habig et al. [34] in a reaction mixture consisting of 0.1 M phosphate buffer (pH 6.5), 1.0 mM reduced glutathione, 1.0 mM CDNB and 0.1 ml of PMS in a total volume of 3 ml. The change in absorbance was recorded at 340 nm, and the enzymatic activity was calculated as nmole CDNB conjugate formed per minute per milligram protein, using a molar extinction coefficient of 9.6 x 10^3 M^{-1} cm^{-1}.

Superoxide dismutase (SOD)

Superoxide dismutase activity was measured spectrophotometrically as described previously by Stevens et al. [35] by monitoring the auto oxidation of (−)-epinephrine at pH 10.4 for 3 min at 480 nm. The reaction mixture contained glycine buffer (50 mM, pH, 10.4) and 0.2 ml of PMS. The reaction was initiated by the addition of (−)-epinephrine. The enzyme activity was calculated in terms of nmol (−)-epinephrine protected from oxidation per min per mg protein using molar extinction coefficient of 4.02×10^3 M^{-1} cm^{-1}.

Catalase (CAT)

Catalase activity was assayed by the method of Claiborne [36]. In Brief, the assay mixture consisted of 0.05 M phosphate buffer (pH 7.0), 0.019 M H_2O_2, and 0.05 ml PMS in a total volume of 3.0 ml. Changes in absorbance were recorded at 240 nm. Catalase activity was calculated in terms of nmol H_2O_2 consumed per min per mg protein using molar extinction coefficient of 43.6×10^3 M^{-1} cm^{-1}.

Acetylcholinesterase (AChE) activity

AChE activity was determined by a modified method of Ellman et al. [37]. Briefly 2.84 ml of PB (0.1 M, pH 8.0), 40 µl of 0.075 M acetylthiocholine iodide and 0.1 ml of buffered Ellman's reagent (DTNB 10 mM, $NaHCO_3$ 15 mM) were mixed and allowed to incubate for 5 min at room temperature. Enzyme sample (20 µl) was added and optical density was measured at 412 nm within 5 min. AChE activity was expressed as nmole thiocholine formed per minute per milligram protein, using a molar extinction coefficient of 1.36 x 10^4 M^{-1} cm^{-1}.

Hemataoxylin and eosin stain

The animals were anaesthetized with chloral hydrate (400 mg/kg body weight i.p.) on 22nd day of lesioning and perfused transcardially through ascending aorta with 100 ml ice cold phosphate buffered saline (PBS 0.1 M, pH 7.4) followed by 4 % paraformaldehyde in cold PBS (0.1 M, pH 7.4). Brains were removed quickly, post fixed in the paraformaldehyde solution for 48 h, and embedded with wax. Cronal sections having hippocampus 10 µm thickness were dewaxed and stained with hematoxylin and eosin.

Immunoflourescence staining of choline acetyl transferase (ChAT)

Immunoflourescence staining was performed to detect the expression of choline acetyl transferase (ChAT). Cronal sections of hippocampus (10 µm thick) were dewaxed by the gradient ethyl alchohol and processed for immunostaining. The sections were blocked in 10 % normal goat serum for 45 min at room temperature, thereafter washed the slides with PBST 0.05 % and then incubated with primary antibody, anti ChAT polyclonal rabbit (Abcam, USA. dilution 1:200) at 4 °C overnight. Thereafter, sections were incubated with Alexa flour 488 (Invitrogen, USA) goat anti-rabbit IgG at dilution 1:200 for 1 h. Thereafter, slides were washed three times with PBS 0.01 M, pH 7.4 and coversliped with 10 % glycerol in PBS and sealed by nail polish and store in dark at 4 °C.

Protein content

Protein content was determined by the method of Lowry et al. [38], using bovine serum albumin as a standard.

Statistical analysis

Results are expressed as mean ± SE. Statistical analysis of the data was done by applying the analysis of variance (ANOVA), followed by Tukey's test for biochemical parameters and for behavioral observations. The p value <0.05 was considered statistically significant.

Results

Effect of taurine on performance in Morris water maze task

Latency

The animals of all groups have improved Morris water maze acquisition performance. S and T + S group animal's shows decreased latency to find the platform from the second to last day of experiment (Fig. 1). However, L group animals presented significantly higher latency ($p < 0.01$) to find the platform than S, but T + L group animals has shown a significant ($p < 0.01$) improvement as compared to L group animals (Fig. 2a).

Path length

The path length was improved in the animals of all groups. S and T + S group animals show decreased path length to find the platform from the second to last day of experiment. However, L group animals presented significantly ($p < 0.01$) higher path length to find the platform than S but T + L group animals has shown a significant ($p < 0.01$) improvement as compared to L group animals (Fig. 2b).

Biochemical observations

Effect of taurine on TBARS content

The effect of taurine on TBARS content was measured to demonstrate the oxidative damage on lipids in hippocampus of ICV-STZ rats. There was no significant alteration in TBARS content in T + S group animals as compared to S group animals, while it was elevated significantly ($p < 0.001$) in L group animals as compared to S group animals and significantly ($p < 0.05$) reversed by T + L group animals (Fig. 3).

Effect of taurine on GSH

Protective effect of taurine on GSH level was observed. The level of GSH was not elevated significantly in T + S group as compared to S group animals but it was depleted significantly ($p < 0.05$) in L group animals as compared to S group animals, and significantly ($p < 0.05$) protected by the pre-treatment of taurine in T + L group animals (Fig. 4).

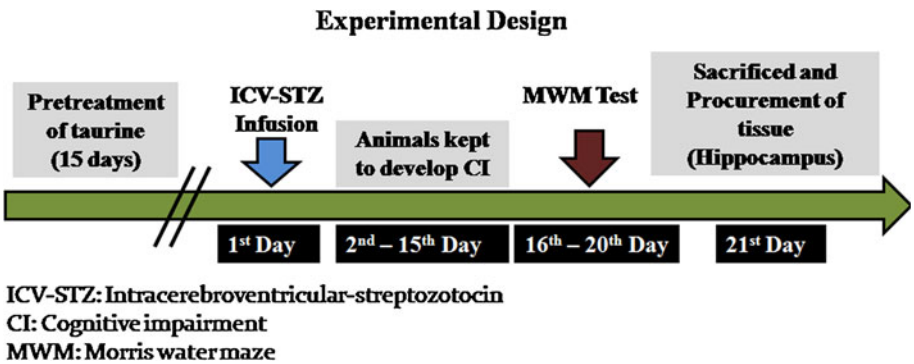

Fig. 1 Schematic representation of experimental design

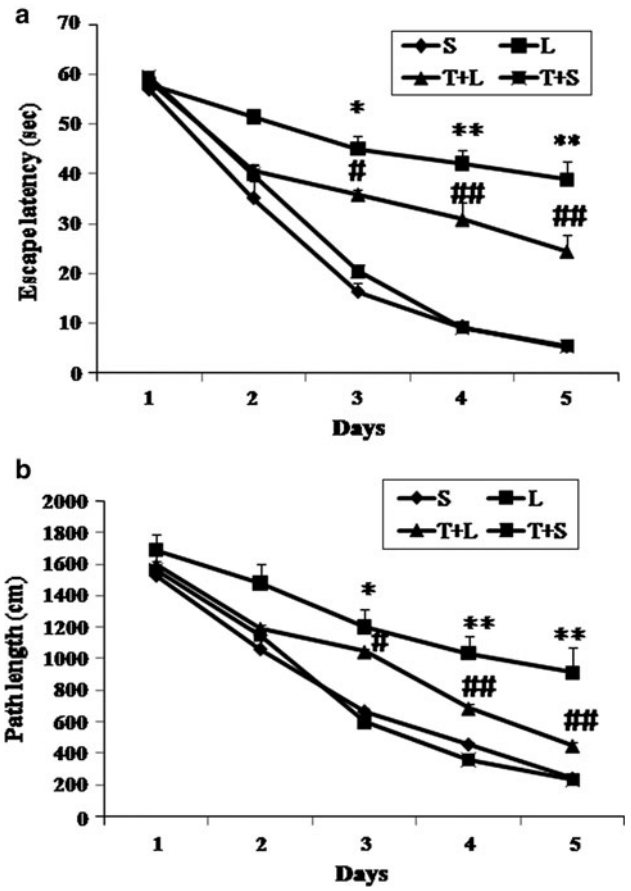

Fig. 2 **a** Effect of taurine administration on escape latency to find the plateform in Morris water maze test in ICV-STZ-infused rats. Values are expressed as mean ± SEM ($n = 10$). Swimming times of four trials per day for 5 days to each group animals are shown. Average escape latency time from day second to fifth to find the submerged platform was significantly ($^{**}p < 0.01$) prolonged in the L group animals when compared to the S group animals. Pre-treatment with taurine has reversed it significantly ($^{\#\#}p < 0.01$) in T + L group animals as compared with the L group animals. **b** Effect of taurine administration on path length to find the plateform in Morris water maze test in ICV-STZ-infused rats. Values are expressed as mean ± SEM ($n = 10$). Swimming times of four trials per day for 5 days to each group animals are shown. Average distance travelled from day second to fifth to find the submerged platform was significantly ($^{**}p < 0.01$) prolonged in the L group animals when compared to the S group animals. Pre-treatment with taurine has reversed it significantly ($^{\#\#}p < 0.01$) the learning deficits in T + L group animals as compared with L group animals

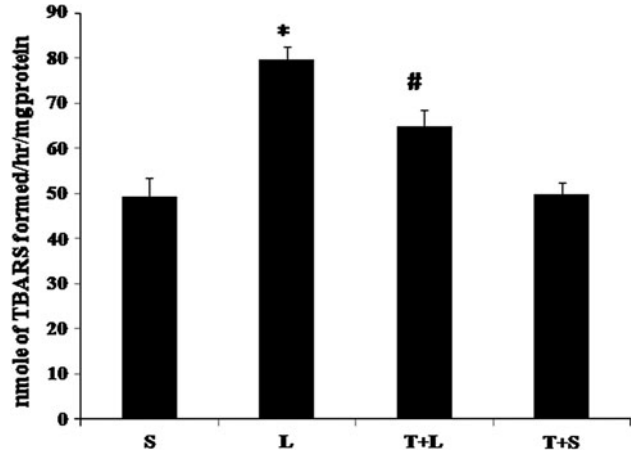

Fig. 3 Effect of taurine pre-treatment on TBARS content in the hippocampus of ICV-STZ-infused rats. Values are expressed as mean ± SEM. TBARS content was significantly increased in the L group as compared to S group ($^{*}p < 0.001$ L vs. S group). Taurine pre-treatment significantly decreased TBARS content in the T + L group animals compared with L group animals ($^{\#}p < 0.05$ L vs. T + L group)

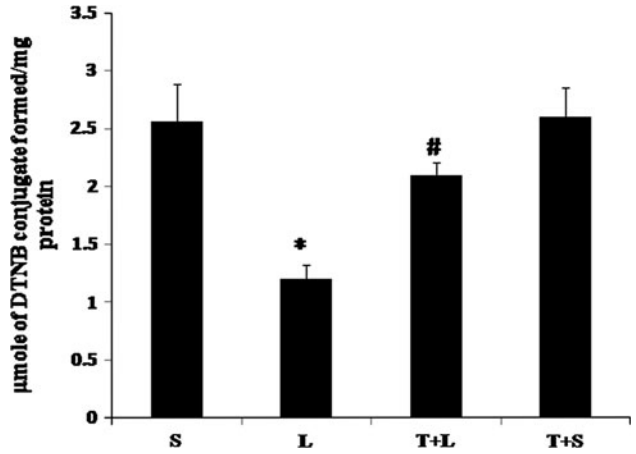

Fig. 4 Effect of taurine pre-treatment on GSH level in the hippocampus of ICV-STZ-infused rats. Values are expressed as mean ± SEM. GSH content was significantly decreased in the L group as compared to S group ($^{*}p < 0.01$ L vs. S group). Taurine pre-treatment significantly increased GSH content in the T + L group animals compared with L group animals ($^{\#}p < 0.05$ L vs. T + L group)

Effect of taurine on activity of antioxidant enzymes

The activity of antioxidant enzymes (GP_x GR, GST, SOD and CAT) was decreased significantly in L group animals as compared to S group animals (Table 1) and was protected significantly by the pre-treatment of taurine in T + L group animals as compared to L group animals. No significantly change was observed in T + S group as compared to S group (Table 1).

Effect of taurine on AChE activity in hippocampus

The activity of AChE was increased significantly ($p < 0.001$) in L group as compared to S group. The pre-treatment with taurine has protected its activity significantly ($p < 0.05$) in T + L group as compared to L group (Fig. 5).

Effect of taurine on the histopathological evaluation:

High power photomicrograph of the brain showing a portion of CA1 region. In S group, all the neurons have large

Table 1 Protective role of taurine on the activities of antioxidant enzymes (GP_x, GR, GST, SOD and Catalase) in hippocampus of ICV-STZ-infused animals

Parameters	S	L	T + L	T + S
GP_x (nmol NADPH oxidized/min/mg/protein)	447.88 ± 18.23	225.89 ± 13.22** (−49.56 %)	397.99 ± 25.49$^{\#\#}$ (43.24 %)	466.20 ± 23.20 (4.09 %)
GR (nmol NADPH oxidized/min/mg/protein)	754.62 ± 33.89	365.01 ± 24.05** (−51.62 %)	611.33 ± 24.65$^{\#\#}$ (40.29 %)	790.18 ± 33.54 (4.71 %)
Catalase (nmol of H_2O_2 consumed/mg/protein)	25.03 ± 2.11	11.88 ± 1.86** (−52.53 %)	22.15 ± 2.50$^{\#}$ (46.36 %)	25.36 ± 2.20 (1.31 %)
SOD (nmol of epinephrine protected from oxidation/min/mg/protein)	735.89 ± 64.13	499.33 ± 28.06* (−32.14 %)	645.51 ± 36.50$^{\#}$ (22.64 %)	777.83 ± 58.66 (5.69 %)
GST (nmol of CDNB conjugate formed/min/mg/protein)	1,205.34 ± 20.99	649.33 ± 56.60** (−46.12 %)	923 ± 40.01$^{\#\#}$ (29.65 %)	1,233.80 ± 25.80 (2.36 %)

STZ infusion significant depleted the activities of antioxidant enzymes (GP_x, GR, GST, SOD and Catalase) in hippocampus of L group animals as compared to S group animals. Administration of taurine has significantly attenuated these activities in T + L group animals as compared to L group animals. Values in parentheses show the percentage increase or decrease with respect to their control. Values are expressed as mean ± SEM

* $p < 0.01$

** $p < 0.001$ L vs. S

$^{\#}$ $p < 0.05$

$^{\#\#}$ $p < 0.001$ L vs. T + L

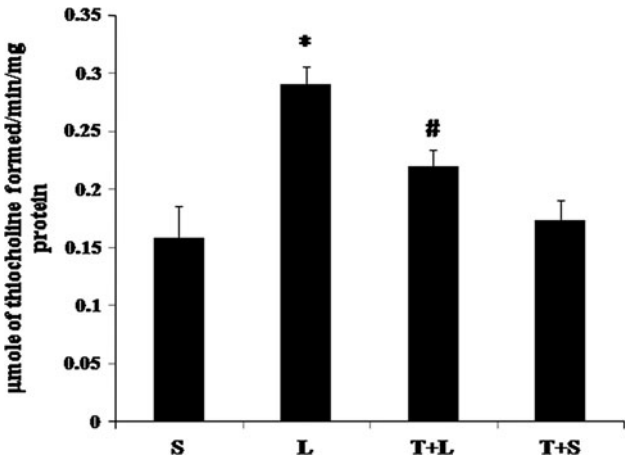

Fig. 5 Effect of taurine pre-treatment on AChE activity in the hippocampus of ICV-STZ-infused rats. Values are expressed as mean ± SEM. AChE activity was significantly increased in the L group as compared to S group (*$p < 0.001$ L vs. S group). Taurine pre-treatment significantly decreased AChE activity in the T + L group animals compared with L group animals ($^{\#}p < 0.05$ L vs. T + L group)

round vesicular nuclei with prominent nucleoli and amphophilic cytoplasm. The neurons of lesion group have shown dense and hyper chromatic nuclei and condensed cytoplasm (arrow). T + L group neurons have normal size and shape with partial neuronal loss (Fig. 6).

Effect of taurine on the expression of choline acetyl transferase (ChAT)

Cholinergic deficiency is supported by the reduced expression of ChAT in CA1 hippocampal region of the brain. Reduced expression of ChAT in lesion group animals was observed as compared to S group animals. Taurine pre-treated group animals has restored the expression of ChAT in T + L group animals as compared to L group animals (Fig. 7).

Discussion

The present study was undertaken to investigate the neuroprotective effect of taurine on cognitive dysfunctions, histopathological and biochemical alterations in hippocampus. In neurodegenerative disorders, the targets of oxidative alterations induced by reactive oxygen species (ROS) are lipids and proteins which are the main structural and functional components of cell membrane [39]. It is well known that lipid peroxidation and protein oxidation play an important role in the aging and other neurodegenerative diseases. The ICV-STZ rat model is an appropriate animal model used for the study of sporadic dementia of Alzheimer's type. ICV administration of STZ in subdiabetogenic dose interferes with brain insulin receptor system and a critical reduction in brain glucose utilization, resulting in oxidative stress consequently leads to learning and memory impairment. Cholinergic function is important for the learning and memory and it is well understood that its alteration is one of the main causes of cognitive impairment in AD. In the present study Morris water maze test was employed to confer the memory impairment followed by ICV-STZ infusion. Our Morris water maze data showed that lesioned group rats took long time (escape latency) and travelled long distance (path length) to find the submerged platform, located in

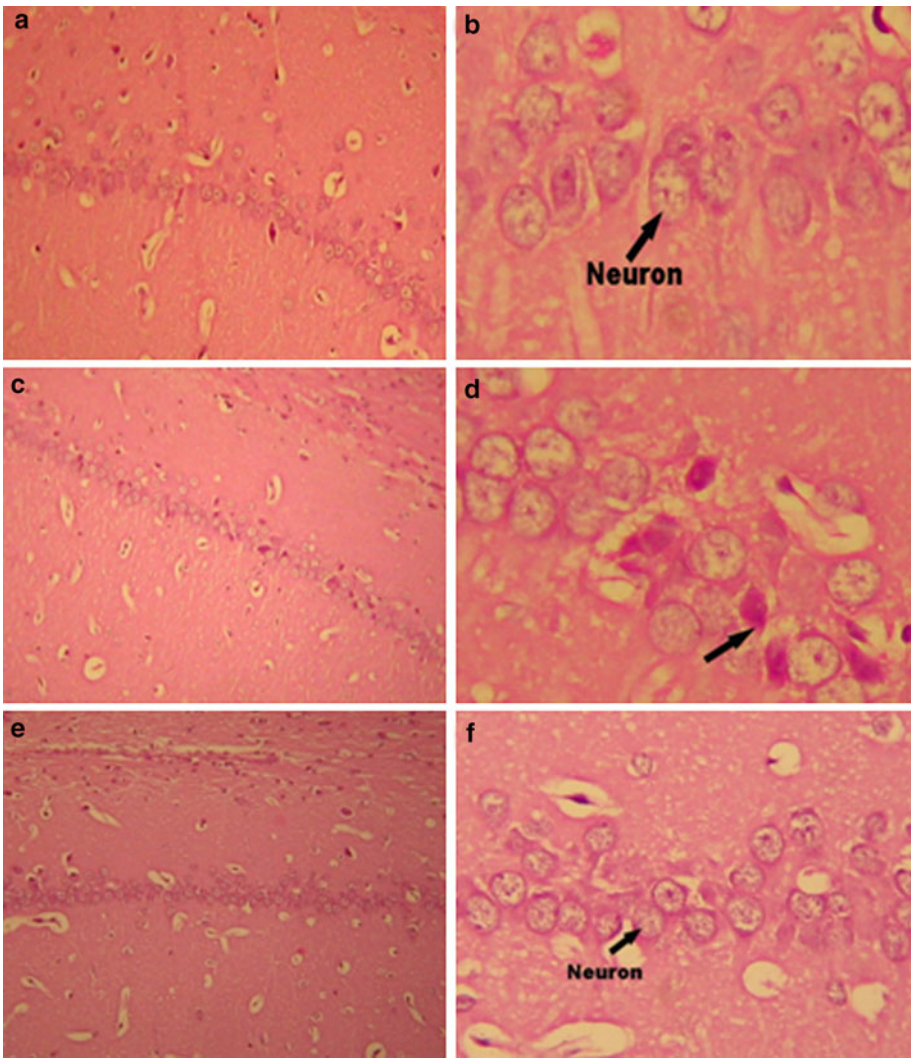

Fig. 6 Histopathological changes in the CA1 region of hippocampus. Sections were stained with hematoxylin and eosin. *Black arrows* with neuron indicate the normal pyramidal neuron in sham group (**b**) and *black arrow* indicates the degenerated pyramidal neuron in L group (**d**) while T + L group shows normal pyramidal neuron staining (**f**). Magnification ×10 (**a**, **c**, **e**) and ×40 (**b**, **d**, **f**)

particular quadrant in water pool, indicating the impairment in learning and memory process, which is consistent with the earlier findings [8, 9]. Meanwhile increased latencies and path length were significantly protected in taurine pre-treated group animals. This improvement in the learning and memory with the supplementation of taurine is possibly due to its antioxidant activity and important action in improving memory and long term potentiation [40]. It could be suggestive that taurine has the beneficial effect on memory improvement.

Oxidative stress is defined as imbalance between the reactive oxygen species (ROS) and antioxidant system which may originate from an overproduction of ROS or from a reduction in antioxidant defences [41]. Brain has multiple sources of ROS and a large oxidative ability, but its capacity to fight against oxidative stress is limited [42]. It is reported that oxidative stress normally associated with aging is a prominent and early pathological feature of Alzheimer's disease [43]. Oxidative stress caused the loss of antioxidant enzymes that alter the cellular redox status of the neuron, and treatment with antioxidants boost up the immune system and combat with the variety of neurodegenerative diseases. The hippocampal cells are primarily responsible for spatial learning and memory [44] and more vulnerable to oxidative damage.

ICV-STZ infusion in subdiabetogenic dose leads to failure of cellular energetic due to reactive ROS production and increasing lipid peroxidation, which leads to cellular disintegrity and progressive dementia. Lipid peroxides and hydroperoxides cause secondary injury by further generating relatively more stable and diffusible cytotoxic agents like malondialdehyde (MDA) and 4-hydroxy-*trans*-2-nonenal (4-HNE), respectively, and amplify oxidative cascade. Increased content of TBARS has been reported in AD brains [45]. The present study showed that lipid peroxidation level (in terms of TBARS content) was significantly increased with remarkable impairments in learning and memory in lesion group, which is consistent with the earlier reports [8, 9].

Glutathione (GSH) is a well-known antioxidant that is synthesized in the cytoplasm and is present in higher

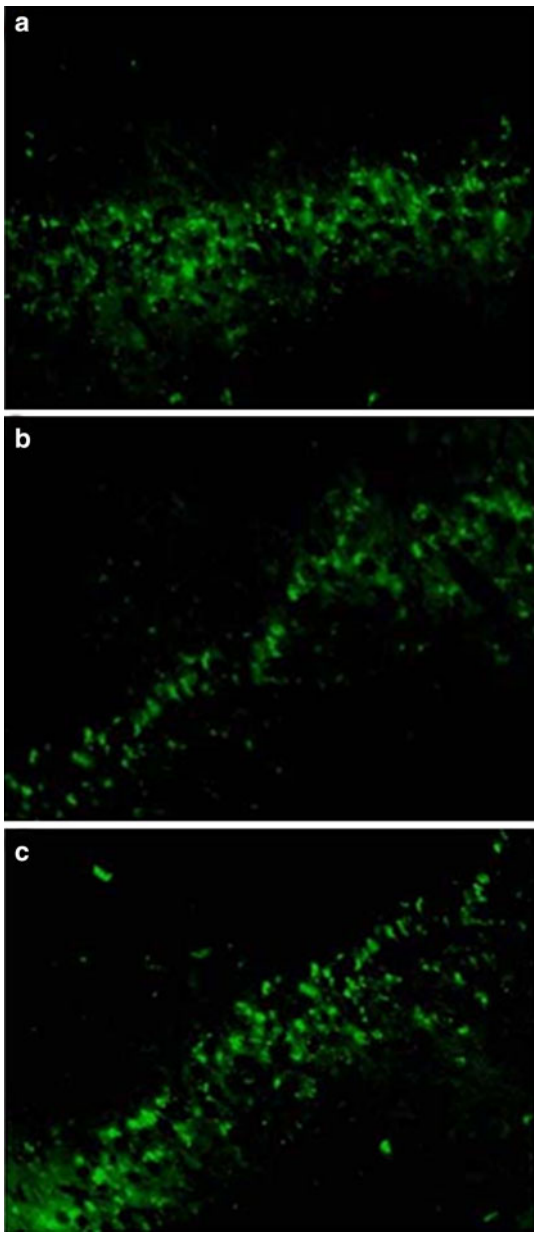

Fig. 7 Representative photomicrograph illustrating the expression of ChAT in hippocampus area (CA1 region). Prominent ChAT expression was observed in S group animals (**a**) although L group animals showed lower expression of ChAT (**b**). Considerably more ChAT expression was found in taurine pre-treated group animals (**c**) as compared to L group animals (magnification 40×)

concentration in the mitochondrial matrix. The low levels of GSH may be directly related to increased ROS, lipid peroxides, and highly reactive hydroxyl radicals [47, 48]. The lipid peroxidation reacts avidly with cellular nucleophiles such as glutathione (GSH), and causes continuous decrease in its level through increased oxidant content or protein modification. To eliminate the peroxides, GSH works in conjunction with GP_x to form glutathione disulfide (GSSG), which is reduced to GSH by GR at the consumption of one molecule of NADPH. A reduction in GSH may hamper H_2O_2 clearance and promote $^{\bullet}OH$ formation, thus increasing the free radical formation, which triggers oxidative stress [47, 49] associated pathological pathways in AD [50]. GST catalyse the conjugation of GSH via sulfhydryl group to the electrophilic centre of peroxides which can alleviate damage from lipid peroxidation. Thus, GP_x, GR and GST are secondary antioxidant enzymes that play an important role in detoxifying ROS by maintaining a ready supply of GSH [49, 51]. Decreased activity of GP_x and GR would directly affect GSH level and reduction in the activity of GST, resulting in overall low-levels of antioxidant system, thus predicting the uncontrolled influences of ROS in AD. The enzyme superoxide dismutase (SOD) catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide (H_2O_2), which is one of the most toxic molecules in the brain. Moreover, CAT participates in the detoxification of H_2O_2 into O_2 and H_2O. The depletion of CAT activity represents the loss of one of the major defence against ROS. In the present study ICV-STZ infusion causes oxidative damage of membranous lipid, that further leads to decrease in glutathione and its dependent enzymes (GR, GP_x and GST) as well as superoxide dismutase and catalase. There are several reports about the protective effect of taurine on lipid peroxidation, glutathione and antioxidant enzymes following brain injury [23, 46]. In agreement with this finding, we also found that taurine significantly reduced the TBARS level along with increase in level of glutathione and antioxidant enzymes activity. Therefore, it seems that protective effect of taurine against ICV-STZ induced free radical generation, which may be mediated through suppression of oxidative stress.

Cholinergic neuronal systems play an important role in the cognitive deficits associated with aging and neurodegenerative diseases [52]. Beside oxidative damage, decreased activity of glycolytic enzymes are reported in ICV-STZ model of memory impairments, resulting in reduction of acetylcholine level a neurotransmitter plays an important role in memory and cognition. Pivotal role of cholinergic system in memory is further underlined by use of acetylcholine esterase inhibitors in AD to prevent memory decline. Acetylcholine esterase is present within the synaptic cleft that hydrolyses acetylcholine to choline and acetic acid, thus preparing the synapse for the passage of new impulse. In AD patients the activity of acetylcholine esterase is increased which is closely associated with reduced amount of acetylcholine available for the brain. It is reported that acetylcholine esterase activity increased following ICV-STZ administration in rats [8, 53, 54]. Increased acetyl cholinesterase activity may lead to diminished cholinergic transmission due to decrease in acetylcholine level. In the present study, we observed

significant increase in acetyl cholinesterase activity in lesion group, which is restored by the taurine pre-treatment. The restoration of AChE activity by taurine may be due to amelioration of insulin signalling and disturbed glucose metabolism induced by STZ. It is reported that taurine increases the brain levels of acetylcholine in experimental animals, and decreased levels of taurine have been found in Alzheimer's patients [55].

Synthesis of acetylcholine is carried out by the presence of acetyl Co-A provided by the breakdown of glucose and insulin, which control the activity of choline acetyl transferase is one of the specific cholinergic marker proteins for the functional state of cholinergic neurons, and synthesizing enzyme for acetylcholine [8]. It has been suggested that ICV-STZ injection disturbs glucose energy metabolism by inhibiting the insulin receptor system and reduces the expression of ChAT in the hippocampus [8, 9]. Hence hippocampus is responsible for the formation of various types learning and memory process in mammal including rats; therefore in the present study, we examined the expression of ChAT in hippocampus. Our results showed that ICV-STZ infused animals had deficits in spatial learning and memory as analysed by Morris water maze test, also decreased expression of ChAT in hippocampus (Fig. 7). Moreover, taurine supplementation significantly restored ChAT expression and improved the deficits in learning and memory in ICV-STZ-infused rats. Our present finding suggests that taurine may serve as an antioxidant to reduce oxidative stress by up-regulation of antioxidant defense system to the brain and thereby improves learning and memory.

Histopathological study was performed to see the hippocampal neuronal damage and efficacy of taurine against neuronal loss. It is evidenced that hippocampus is responsible for the memory and cognition and ICV-STZ causes neurodegeneration in hippocampus. In the present study histopathological analysis shows that neuronal layers of CA1 region of the hippocampus did not reveal any evidence of neuronal loss and neuronal densities in significant manner between the groups. However, distinct differences in neuronal structure were seen. The neurons of the sham group were large, conical shaped cells with well delineated amphophillic cytoplasm and round vesicular nuclei with prominent nucleoli (Fig. 6). In the lesion group, the CA1 layer neurons showed pronounced shrinkage of the neuronal bodies with the nuclei losing their regular outlines and becoming hyper chromatic. This feature was seen in all the neurons in the CA1 region in a scattered fashion in L group animals. Taurine pre-treatment has protected the neuronal morphology which parallel to behavioural improvements and restoration of biochemical changes.

The antioxidant effect of the oral administration of taurine to ICV-STZ infused animals is clearly shown in the present study. It significantly attenuated the loss of neuronal cell death, biomarkers of oxidative stress, and improvements in learning and memory. The effective treatments of AD patients are still limited; but the taurine, a natural antioxidant and anti-inflammatory that is able to attenuate neuronal death and has shown the mechanisms of protection in ICV-STZ infused animals, may be used for effective treatment in sporadic dementia of Alzheimer's type.

The ICV-STZ model has limitations. It initiates amyloid plaque deposition in meningial capillaries at 3 months, but is more prominent at 6–9 months after ICV-STZ injection [56]. The period (21 days) in the present study is less than the period (9 months) to develop a complete pathology of Alzheimer's disease (amyloid beta production and hyperphosphorylation of Tau). But our study makes emphasis on the early intervention of sporadic dementia of Alzheimer's type induced by ICV-STZ. However, this must be explored in further studies in animal models based on amyloid beta treatment.

Conclusion

The present study demonstrated that ICV-STZ causes learning and memory deficits and neuronal injury in rat hippocampus and prophylactic treatment with taurine significantly improved learning and memory deficits by inhibiting oxidative stress and ameliorating neuronal injury in hippocampus. Our results suggested that taurine is effective in ameliorating ICV-STZ induced behavioural alterations, cholinergic dysfunction and oxidative stress. Further understanding the mechanism underlying the neuroprotection of taurine may have important clinical ramifications and will provide an avenue to disclose both the pathogenesis and therapeutic mechanisms underlying current AD paradigm.

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Conflict of interest None.

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