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SP600125, a competitive inhibitor of JNK attenuates streptozotocin induced neurocognitive deficit and oxidative stress in rats

Nidhi Sharma, Rahul Deshmukh *, K.L. Bedi

Neuropharmacology Div., Department of Pharmacology, I.S. F. College of Pharmacy, Moga-142001, (Punjab), India

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ABSTRACT

Activated JNK has been reported to be located in nucleus in mild cases of Alzheimer's disease (AD), but is exclusively in cytoplasm in more advanced stages of AD and implicated in its pathogenesis, suggesting that activation and re-distribution of JNK correlate with the progress of AD. The present study was designed to investigate the role of JNK in intracerebroventricular streptozotocin (i.c.v. STZ) induced cognitive impairment and oxidative stress. Streptozotocin has been observed to impair learning and memory, increase oxidative–nitritive stress, induce cholinergic hypofunction and neuronal damage in rat brain. Chronic treatment with SP600125 from day 10 to 28 following i.c.v. STZ injections significantly improved spatial memory, attenuate oxidative–nitritive stress. In addition, significant increase in acetylcholinesterase activity and lactate dehydrogenase (LDH) levels was observed in the present model indicating cholinergic hypofunction and increase in neuronal cell damage. Whereas, SP600125 treatment significantly restored acetylcholinesterase activity and reduced LDH levels indicating restorative capacity of SP600125 with respect to cholinergic functions and preventing the neuronal damage. In line with previous report, the current study also supports the potential of JNK inhibition as a possible therapeutic strategy to ameliorate neurodegenerative disorders associated with oxidative stress and cognitive impairment.

1. Introduction

Alzheimer's disease is a progressive neurodegenerative disorder characterized by gradual memory loss and deterioration of cognitive functions (Chong et al., 2005). Although the neuropathological features of Alzheimer's disease including amyloid-β deposits, neurofibrillary tangles (NFTs) and neuronal synapse and cell loss – predominantly in the cortex and hippocampus – have been well defined (Chong et al., 2005; Maiese and Chong, 2004), the underlying mechanisms responsible for these pathogenic processes have not been clearly delineated. Oxidative stress and neuroinflammation found at early stages of Alzheimer's disease have been considered as major factors in neurodegeneration and implicated in its pathogenesis (McGeer and McGeer, 2001; Moreira et al., 2005; Tuppo and Arias, 2005).

The c-Jun N-terminal kinase (JNK) is a member of mitogen-activated protein kinase (MAPK) superfamily and a major cellular stress response protein induced by oxidative stress and plays an important role in Alzheimer's disease (Zhu et al., 2001a,b). Activated JNK has been found in the hippocampal and cortical regions of individuals with severe AD and localized with neurofibrillar alterations (Zhu et al., 2001a,b). Moreover, JNK activation has been considered an early event in Alzheimer's disease (Zhu et al., 2001a). Further, activated JNK has been reported to be located in nucleus in mild AD cases, but is exclusively in cytoplasm in more advanced stages of AD, suggesting that activation and re-distribution of JNK correlate with the progress of Alzheimer's disease (Zhu et al., 2001a,b).

Several agents preventing the activation of JNK or c-Jun phosphorylation have been shown to protect the brain after cerebral ischemia (Bogoyevitch et al., 2004; Kuan and Burke, 2005; Repici and Borsello, 2006). SP600125 is an anthrapyrazolone, a novel inhibitor of JNK catalytic activity. This compound has been reported to inhibits JNK1, JNK2, and JNK3 with a high specificity (IC50: 0.04–0.09 lM) and decreases the phosphorylation of c-jun (Bennett et al., 2001). SP600125 is a competitive inhibitor of the highly conserved ATP-binding pocket of JNK and is widely employed *in vitro* and *in vivo* to implicate JNK. Moreover, inhibition of JNKs by SP600125 has been reported attenuate glutamate-induced excitotoxicity (Johnson and Nakamura, 2007), cerebral ischemic damage (Carboni et al., 2008; Guan et al., 2006) and prevent colchicines and Aβ induced cell death (Morishima et al., 2001; Yang et al., 2007).

JNK plays an integral role in neuronal death and might be operative in various central nervous system (CNS) disease states. However the role of JNK in neurological disorders is least validated and needs to be further explored to identify its role in neurological disorders. Experimental intracerebroventricular (i.c.v.) streptozotocin injection in rats has been reported to cause increase in oxidative stress, glial activation and produce neuropathological and biochemical alterations similar to

* Corresponding author.
E-mail address: login2rd@gmail.com (R. Deshmukh).

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those observed in sporadic Alzheimer's disease and is considered to be a valid model of the early pathophysiological changes in Alzheimer's disease (Grunblatt et al., 2007; Salkovic-Petrisic and Hoyer, 2007). The present study was designed to investigate the role of JNK by its pharmacological inhibition in intracerebroventricular (i.c.v.) streptozotocin induced cognitive impairment and oxidative stress.

2. Materials and method

2.1. Animals

The experiments were carried out in adult (6–7 months old) male wistar rats (220–250 g) obtained from Central Animal House of I.S.F. College of Pharmacy, Moga, Punjab (India). They were kept in polyacrylic cages and maintained under standard husbandary conditions (room temperature 22 ± 1 °C and relative humidity of 60%) with 12 h light/dark reverse cycle. The food in the form of dry pallets and water were made available *ad libitum*. All behavioral experiments were carried out between 10 AM and 4 PM. The protocol was reviewed and approved by the Institutional Animal Ethics Committee and the animal experiments were carried out in accordance with the Indian National Science Academy guidelines for use and care of animals.

2.2. Drugs and chemicals

Streptozotocin, acetylthiocholine iodide (AChI), and 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from Sigma-Aldrich, USA. JNK inhibitor, SP600125 was purchased from LC labs, US. All other chemicals used in the study were of analytical grade. SP600125 was dissolved in citrate buffer (pH 4.4) containing 1% DMSO and it was always prepared afresh before use.

2.3. Methodologies

2.3.1. Intracerebroventricular (i.c.v) infusion of streptozotocin

The rats were anaesthetized with ketamine (100 mg/kg, ip) and xylazine (5 mg/kg, ip). The head of the anaesthetized rat was placed in position in the stereotaxic apparatus and a midline saggital incision was made in the scalp. Two holes were drilled through the skull for placement of injection cannulae into the lateral cerebral ventricles using following coordinates: 0.8 mm posterior to bregma; 1.5 mm lateral to saggital suture; 3.6 mm ventral from the surface of the brain (Paxinos and Watson, 1986). STZ was dissolved in citrate buffer (pH 4.4) just prior to administration and slowly injected (1 µl/min) through the cannula using Hamilton microsyringe in a volume of 5 µl into each lateral cerebral ventricle (bilateral i.c.v) on day 8 and 9 (Sharma and Gupta, 2003; Deshmukh et al., 2009).

2.3.2. Experimental protocol

Animals were divided into six groups and each group comprised of 8 animals. The treatment schedule and the interval for estimation of various parameters are presented in Fig. 1.

Group 1: vehicle treated wherein citrate buffer (pH 4.4) containing 1% DMSO was injected i.c.v (2 µl/ventricle) from day 10 to 28 following i.c.v STZ injection in rats.

Group 2: *per se* study group wherein the normal rats were administered i.c.v SP600125 (10 µg/2 µl/ventricle) for 18 days, in the same way as in group 4 except STZ administration.

Group 3: STZ control wherein the rats were injected with 5 µl (1 µl/min) i.c.v of STZ (3 mg/kg) solution on days 8 and 9.

Group 4: STZ rats were treated with SP600125 [2.5 µg/2 µl/ventricle (i.c.v−1 µl/min)] from days 10 to 28 following STZ administration.

Groups 5 and 6: STZ injected rats treated with SP600125 at doses of 5 and 10 µg/2 µl/ventricle, respectively following STZ administration from days 10 to 28 in the same way as in group 4 above.

2.4. Behavioral assessment

2.4.1. Passive avoidance task

On day 21 and 22 after i.c.v infusion of streptozotocin infusion, the rats were tested for memory retention deficit using a passive avoidance apparatus. The apparatus (Ugo Basile, Type-7552, Italy) consisted of a chamber illuminated with a 40 W bulb and a dark chamber, separated by a guillotine door. The chamber floor consisted of a metal grid with a shock scrambler. During acquisition trial, the rat was placed in the illuminated chamber. After initial habituation period of 60 s, the guillotine door was opened and the time taken by the rat to enter the dark chamber was noted. The latency to step into the dark compartment was recorded as initial trial or pre-shock latency (ITL). As soon as the rat entered the dark chamber, it was given a mild foot shock of 0.5 mA for 2 s through the grid floor. The rat was allowed to remain in the dark compartment for 5 s and then was taken out. After 24 h interval, retention trial was performed and retention trial or post-shock latency (RTL) to step into the dark compartment was noted. The latency time was recorded to a maximum of 600 s (Deshmukh et al., 2009). Short latencies indicated poorer retention.

2.4.2. Spatial navigation task in Morris water maze

Spatial learning and memory of animals were tested in a Morris water maze (Morris, 1984). It consisted of a circular water tank (180 cm diameter, 60 cm height) filled with water (25 ± 1 °C) to a depth of 40 cm. A non-toxic water dispersible emulsion was used to render the water opaque. Four equally spaced locations around the edge of the pool (North, South, East, and West) were used as start points, which divided the pool into 4 quadrants. An escape platform (10 cm in diameter) was placed in the pool 2 cm below the surface of water. The escape platform was placed in the middle of one of the randomly selected quadrants of the pool and kept in the same position throughout the entire experiment (north-east for this study). Before the training started, the rats were allowed to swim freely into the pool for 120 s without platform.

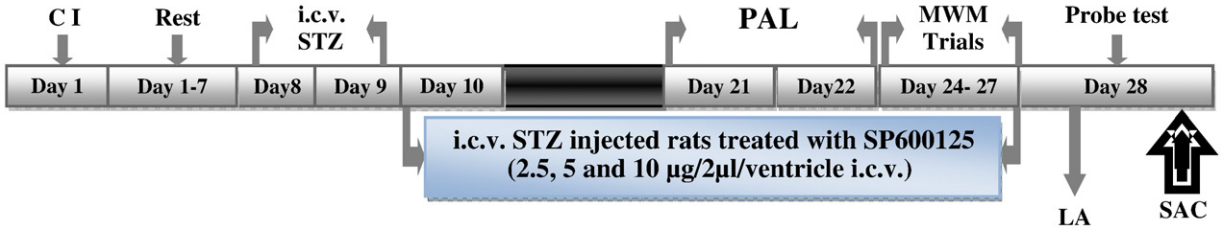

Fig. 1. The design of the treatment schedule and the interval for estimation of various parameters. CI = Cannulae Implantation; days 1–7 = all the animals were given rest; days 10–28 = i.c.v. STZ injected rats treated with SP600125 (SP) at doses of 2.5, 5 and 10 µg/2 µl/ventricle intracerebroventrically; i.c.v. STZ = intracerebroventricular streptozotocin injection; PAL = Passive avoidance learning; MWM = Morris water maze; LA = Locomotor activity; SAC = Sacrificed.

Animals received a training session consisting of 6 trials per session (once from each starting point) for 4 days (days 24, 25, 26 and 27), each trial having a ceiling time of 120 s and a trial interval of approximately 30 s. After climbing onto the hidden platform, the animals remained there for 30 s before commencement of the next trial. If the rat failed to locate the hidden platform within the maximum time of 120 s, it was gently placed on the platform and allowed to remain there for the same interval of time. The time taken to locate the hidden platform (latency in seconds) was measured.

Twenty four hours after the acquisition phase, a probe test (day 28) was conducted by removing the platform. Rats were allowed to swim freely in the pool for 120 s and the time spent in target quadrant, which had previously contained the hidden platform was recorded. The time spent in the target quadrant indicated the degree of memory consolidation which had taken place after the acquisition trial (Deshmukh et al., 2009).

2.4.3. Spontaneous locomotor activity

Each animal was tested for spontaneous locomotor activity on day 28 following 1st i.c.v streptozotocin infusion. Each animal was observed over a period of 10 min in a square closed arena equipped with infrared light sensitive photocells using a digital photoactometer (INCO, India) (Deshmukh et al., 2009).

2.5. Estimation of biochemical parameters

All the biochemical parameters were estimated in the brain homogenate on day 28.

2.5.1. Brain homogenate preparation

Animals were sacrificed by decapitation and brains were removed and rinsed with ice-cold isotonic saline. Brain tissue samples were then homogenized with ice-cold 0.1 M phosphate buffer (pH 7.4) in a volume 10 times the weight of the tissue. The homogenate was centrifuged at 10,000×*g* for 15 min and aliquots of supernatant separated and used for biochemical estimation.

2.5.2. Protein estimation

Protein was measured in all brain samples by the method of Lowry et al. (1951) using bovine serum albumin (BSA) (1 mg/ml) as a standard.

2.5.3. Measurement of acetylcholinesterase activity

The quantitative measurement of acetylcholinesterase activity in brain was performed according to the method described by Ellman et al. (1961). The assay mixture contained 0.05 ml of supernatant, 3 ml of 0.01 M sodium phosphate buffer (pH 8), 0.10 ml of acetylthiocholine iodide and 0.10 ml of DTNB (Ellman reagent). The change in absorbance was measured immediately at 412 nm spectrophotometrically. The acetylcholinestrase activity in the supernatant was expressed as nmol per mg protein.

2.5.4. Estimation of malondialdehyde (MDA)

The quantitative measurement of malondialdehyde (MDA) – end product of lipid peroxidation – in brain homogenate was performed according to the method of Wills (1966). The amount of MDA was measured after its reaction with thiobarbituric acid at 532 nm using spectrophotometer (Shimadzu, UV-1700). The concentration of MDA was determined from a standard curve and expressed as nmol per mg protein.

2.5.5. Protein carbonyl assay

Protein carbonyl levels were measured by the method of Levine et al. (1990). The PMS (0.5 ml) was treated with an equal volume of 20% TCA for protein precipitation. After centrifugation, the pellet was resuspended in 0.5 ml of 10 mM DNPH in 2 M HCl and kept in a dark place for 1 h by vortexing repeatedly at 10 min intervals. This mixture was treated with 0.5 ml of 20% TCA. After centrifugation at 10, 000×*g* at 4 °C for 3 min, the precipitate was extracted three times with 0.5 ml of 10% TCA and dissolved in 2.0 ml of NaOH at 37 °C. Absorbance was recorded at 360 nm in a spectrophotometer (Shimadzu, UV-1700). Protein carbonyl level was expressed as nmol carbonyl mg^{-1} protein, using a molar extinction coefficient of $22\times104\ M^{-1}\ cm^{-1}$.

2.5.6. Estimation of nitrite

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide (NO), was determined by a colorimetric assay using Greiss reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid) as described by Green et al. (1982). Equal volumes of supernatant and Greiss reagent were mixed, the mixture incubated for 10 min at room temperature in the dark and the absorbance determined at 540 nm spectrophotometrically. The concentration of nitrite in the supernatant was determined from sodium nitrite standard curve and expressed as μmol per mg protein.

2.5.7. Estimation of reduced glutathione

Reduced glutathione in brain was estimated according to the method described by Ellman (1959). One ml supernatant was precipitated with 1 ml of 4% sulfosalicylic acid and cold digested at 4 °C for 1 h. The samples were centrifuged at 1200×*g* for 15 min. To 1 ml of the supernatant, 2.7 ml of phosphate buffer (0.1 M, pH 8) and 0.2 ml of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) were added. The yellow color that developed was measured immediately at 412 nm using a spectrophotometer. The concentration of glutathione in the supernatant was determined from a standard curve and expressed as μmol per mg protein.

2.5.8. Estimation of Lactate dehydrogenase

A diagnostic kit (Raken Pharma, Mumbai, India) was used to measure lactate dehydrogenase activity in rat brain homogenate and expressed as IU/ mg protein (Choi and Lee, 2004; Hoyer and Lannert, 2007; Deshmukh et al., 2009).

2.6. Statistical analysis

The results are expressed as means ± S.D. The behavioral and biochemical values were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. $p<0.05$ was set to be statistically significant.

3. Results

3.1. Behavioral parameters

3.1.1. Effect of SP600125 on memory performance in passive avoidance task in i.c.v. streptozotocin injected rats

On day 21 following 1st i.c.v. streptozotocin injection, the mean initial latency in the acquisition trial remained unchanged among all the groups ($F(5, 47)=2.52$, $p>0.05$). But the mean retention latency was significantly decreased ($p<0.001$) in i.c.v. streptozotocin control group compared with those of the sham and vehicle groups on the next day (Fig. 2). However, the i.c.v. streptozotocin induced which decreased in mean retention latency was significantly attenuated by SP600125 treatment ($F(5, 47)=94.46$, $p<0.05$) indicating an improved acquisition/retention of memory. Maximum improvement in retention latency on day 22 was observed with SP600125 treatment at a dose of 10 μg/2 μl/ventricle ($p<0.001$). Moreover, normal rats treated with SP600125 *per se* 10 μg/2 μl/ventricle did not show any significant difference in retention latency as compared with those of sham operated or vehicle treated groups ($p<0.05$).

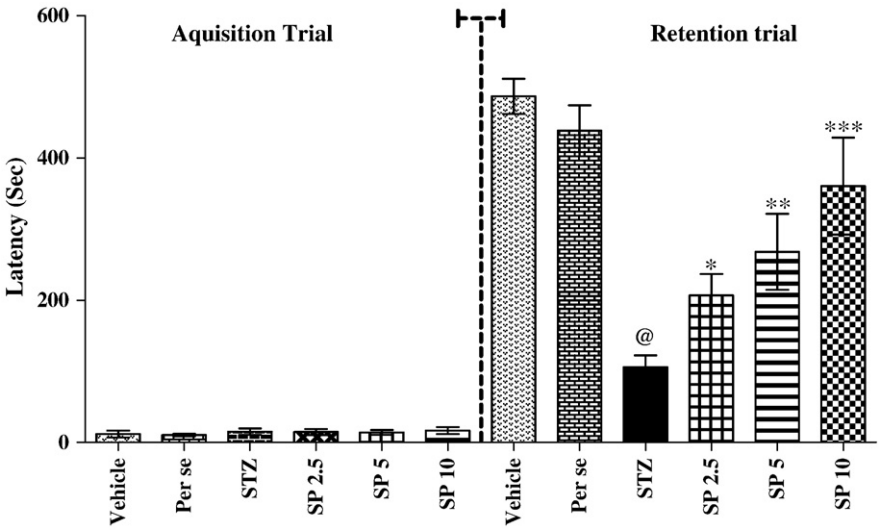

Fig. 2. Effect of SP600125 on memory performance in i.c.v. streptozotocin injected rats in passive avoidance task. Values are expressed as mean ± S.D. (n = 8). Retention latency was recorded to a maximum of 600 s. Streptozotocin injected rats showed shorter retention latency compared with sham, vehicle group or *per se* (@ $p<0.05$). SP600125 treatment in i.c.v. STZ injected rats dose dependently and significantly increased retention latencies compared with STZ control [* $p<00.05$ vs STZ control, ** $p<0.05$ vs SP600125 at 2.5 mg/kg (SP2.5) and STZ control, *** $p<0.05$ vs. SP600125 at 5 mg/kg (SP5) and STZ control]. Note — SP2.5, SP5 and SP10 = SP600125 at a dose of 2.5, 5 and 10 μg/2 μl/ventricle, respectively. SP *per se* = SP600125 administered at a dose of 10 μg/2 μl/ventricle in normal animals.

3.1.2. Effect of SP600125 on memory performance of streptozotocin injected rats in Morris water maze task

The latencies to reach the submerged platform decreased gradually in experimental animals of all the groups during 4 days of training in Morris water maze (MWM) task (Fig. 3A) except those of the i.c.v. STZ injected group of animals wherein the mean latencies were found to be still significantly prolonged even on day 25 [Total: [$F(5, 47) = 25.99$]; 26 [$F(5, 47) = 25.45$] ; 27 [$F(5, 47) = 49.70$]; $p<0.001$] as compared to those of sham group, indicating poorer learning performance of this group of animals. But their performance was significantly improved by chronic treatment (18 days i.e. from days 10 to 27 after the 1st i.c.v. STZ injection) with SP600125 ($p<0.001$). Amongst the doses of SP600125 investigated in the present study, the 10 μg/2 μl/ventricle dose was found to be the most effective in ameliorating STZ induced spatial memory deficit.

During the probe trial, with the platform removed, i.c.v. STZ injected rats failed to remember the precise location of the platform, spending significantly less time in the target quadrant than sham or vehicle group [$p<0.001$, Fig. 3B]. But the mean percent time spent in the target quadrant by i.c.v. STZ injection group of animals treated with SP600125 was significantly increased as compared to STZ control group indicating improved consolidation of memory [$F(5, 47) = 46.67$, $p<0.001$].

3.1.3. Effect of SP600125 treatment of i.c.v. streptozotocin injected rat on their spontaneous locomotor activity

The spontaneous locomotor activity on day 21 did not differ significantly among all the groups [Total: $F(5, 47) = 0.56$, $p>0.05$] (Fig. 4), suggesting no effect whatsoever of SP600125 on this parameter.

3.2. Biochemical parameters

3.2.1. Effect of SP600125 on acetylcholinesterase activity (AChE) in i.c.v. streptozotocin injected rats

The acetylcholinesterase activity was found to be increased significantly in i.c.v. STZ injected rats as compared to those of sham and vehicle injected rats ($p<0.001$). But the treatment of i.c.v. STZ injected animals with SP600125 significantly restored acetylcholinesterase activity in a dose dependent manner as compared with those of STZ injected control rats [Total: $F(5, 47) = 109.4$, $p<0.001$]. SP600125 treatment in normal rats, however, did not modify the basal acetylcholinesterase activity which remained similar to those of sham and vehicle groups ($p>0.05$; Fig. 5).

3.2.2. Effect of SP600125 treatment on brain malondialdehyde (MDA) levels in i.c.v. streptozotocin injected rats

The level of MDA rose significantly in i.c.v. STZ injected rats as compared to those of sham and vehicle injected rats ($p<0.001$). But the treatment of i.c.v. STZ injected animals with SP600125 significantly decreased MDA levels in a dose dependent manner as compared with those of STZ injected control rats [Total: $F(5, 47) = 106$, $p<0.001$]. Further, SP600125 treatment in normal rats did not show any significant difference in MDA levels as compared to sham operated animals ($p>0.05$; Fig. 6).

3.2.3. Effect of SP600125 on brain protein carbonyl levels in i.c.v. streptozotocin injected rats

The protein carbonyl formation significantly increased on day 28 following 1st STZ injection as compared to those of sham group of animals ($P<0.001$). However, these animals when treated chronically with SP600125 showed dose dependent significant decrease in the protein carbonyl levels compared with those of STZ control group [Total: $F(5, 47) = 117.1$, $P<0.05$]. But the chronic SP600125 *per se* (10 μg/2 μl/ventricle) treatment in normal rats did not modify the basal protein carbonyl levels when compared with those of sham group ($p>0.05$, Fig. 7).

3.2.4. Effect of SP600125 treatment on brain nitrite levels in i.c.v. streptozotocin injected rats

The levels of nitrite rose significantly on day 28 following 1st STZ injection as compared to those of sham group of animals ($p<0.001$). However, these animals when treated chronically with SP600125 at different doses showed dose dependent significant decrease in the nitrite levels compared with those of STZ injected control group [Total: $F(5, 47) = 78.90$, $p<0.05$]. But the chronic SP600125 treatment in normal rats, *per se,* did not modify the basal nitrite levels when compared with those of sham group ($p>0.05$, Fig. 8).

3.2.5. Effect of SP600125 treatment on brain glutathione (GSH) levels in i.c.v. streptozotocin injected rats

The levels of GSH were found to be significantly depleted on day 28 following 1st STZ injection as compared to sham or vehicle treated animals ($p<0.001$). Whereas, chronic treatment of STZ injected rats with SP600125 was able to restore the levels of GSH significantly compared with those of STZ injected group animals [Total: $F(5, 47) = 48.97$,

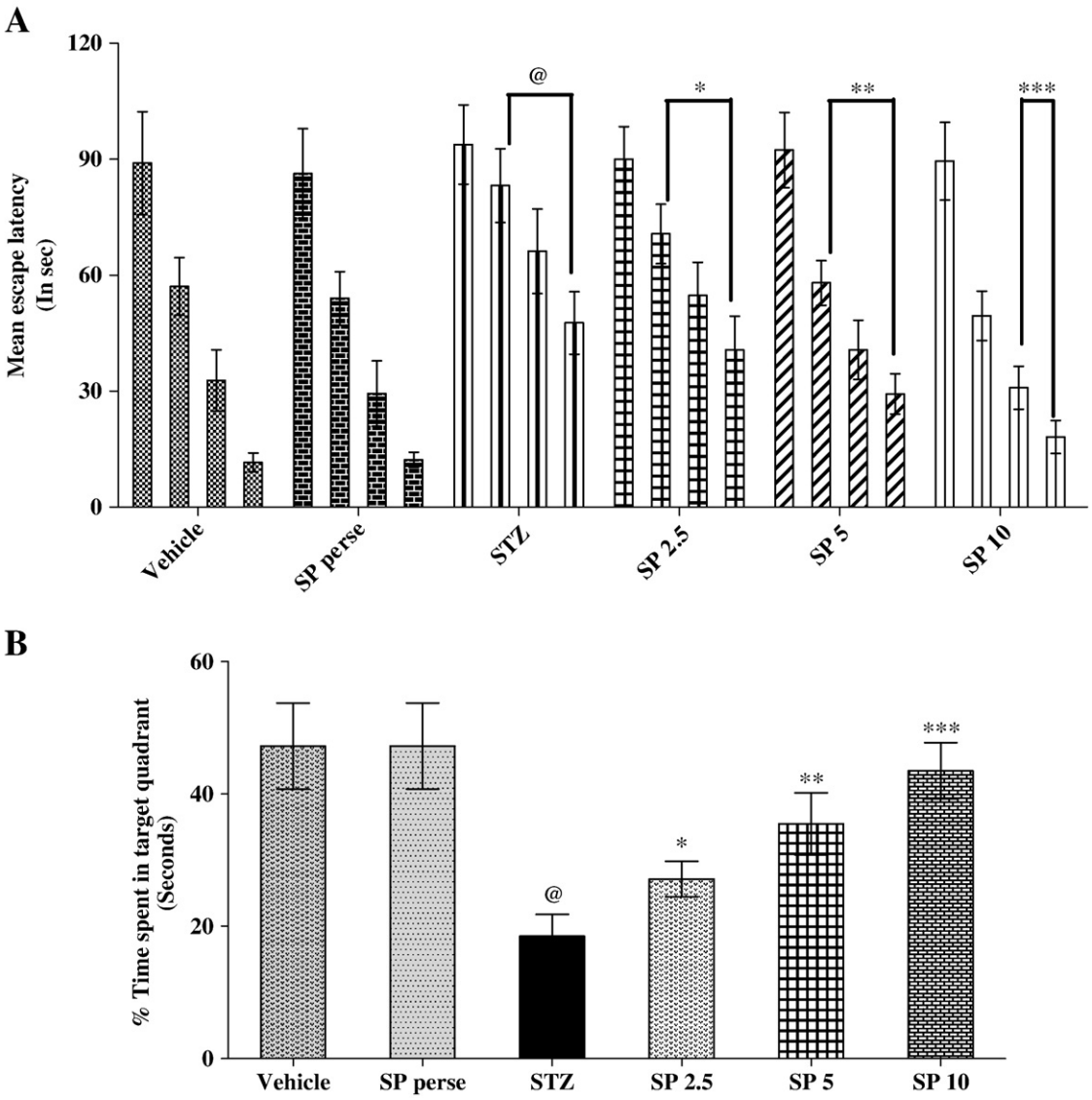

Fig. 3. (A)—effect of SP600125 on memory performance in i.c.v. streptozotocin injected rats in Morris water maze task. Mean escape latency to locate the hidden platform was recorded on days 24, 25, 26 and day 27 to a maximum of 120 s. Values are expressed as mean ± S.D. (n = 8). The mean escape latency (days 24–27) to find the hidden platform was significantly prolonged in i.c.v. STZ group compared with sham operated animals or vehicle injected group (@ $p<0.05$). SP600125 treatment significantly and dose dependently attenuated STZ induced learning/acquisition deficit compared with STZ control [* $p<0.05$ vs STZ control, ** $p<0.05$ vs STZ control and SP 2. 5, *** $p<0.05$ vs STZ control and SP 5]. Note — four bars per group represent mean escape latency of six trials per day i.e. on day 24, 25, 26 and day 27 respectively. (B) — values are expressed as mean ± S.D. (n = 8). The percentage of time spent in target quadrant was significantly lesser in i.c.v. STZ control rats compared with sham operated or vehicle treated group (@ $p<0.05$). SP600125 treatment in i.c.v. STZ injected rats significantly and dose dependently attenuated STZ induced retention deficit compared with i.c.v. STZ control group [* $p<0.05$ vs STZ control, ** $p<0.05$ vs STZ control and SP 2. 5, *** $p<0.05$ vs STZ control and SP 5].

$p<0.001$]. However, the similar chronic treatment with SP600125 *per se* in normal rats did not show any significant effect on basal GSH levels when compared with those of the sham group ($p>0.05$, Fig. 9).

3.2.6. Effect of SP600125 on brain lactate dehydrogenase (LDH) levels in i.c.v.streptozotocin injected rats

The levels of lactate dehydrogenase (LDH) in brain homogenate of experimental animals were found to be raised significantly on day 28 following 1st STZ injection as compared to sham or vehicle treated animals ($P<0.001$) indicating extensive neuronal cell damage in STZ control. Whereas, chronic treatment of STZ injected rats with SP600125 significantly decreased the LDH levels compared with those of the STZ control [Total: $F(5, 47) = 290.9$, $P<0.001$]. On the other hand, *per se* group of animals did not show significant difference in LDH levels when compared with those of sham or vehicle treated group ($p>0.05$, Fig. 10).

4. Discussion

Present study has been designed to investigate the role of JNK in intracerebroventricular streptozotocin (i.c.v. STZ) induced cognitive dysfunction and oxidative stress. The i.c.v. STZ model has been described as an appropriate animal model for sporadic Alzheimer type dementia (Lannert et al., 1998). Since both are associated with progressive deterioration in memory, cerebral glucose and energy metabolism and presence of oxidative stress (Lannert et al., 1998; Grunblatt et al., 2007; Salkovic-Petrisic and Hoyer, 2007). Oxidative damage to macromolecules (lipid, protein and nucleic acids, etc.) has been considered as an important factor in the acceleration of aging and age-related neurodegenerative disorders (Liu et al., 2001; Wickens, 2001).

In line with previous reports, i.c.v. STZ injection has shown to cause peroxidation of membrane lipids and proteins as evidenced by significant increase in malondialdehyde levels and protein carbonyl formation in the present study (Sharma and Gupta, 2003; Deshmukh

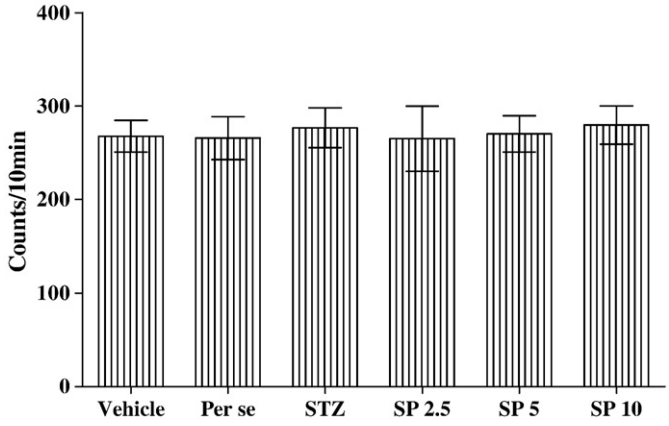

Fig. 4. Effect of SP600125 on spontaneous locomotor activity in i.c.v.Streptozotocin injected rats Values are expressed as mean ± S.D. (n = 8). The spontaneous locomotor activity on day 28 did not differ significantly among all the groups [Total: $F(7,63) = 0.4777$, $p > 0.05$].

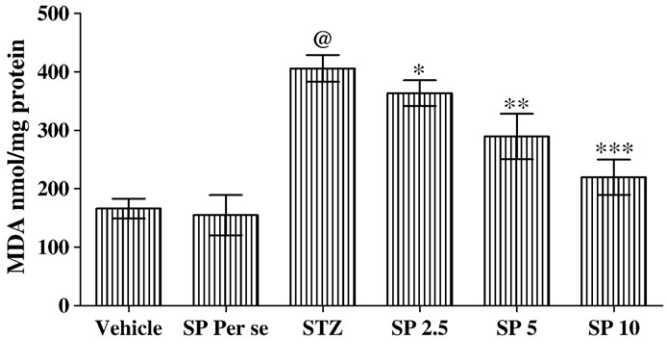

Fig. 6. Effect of SP600125 on brain malondialdehyde (MDA) levels in i.c.v. streptozotocin injected rats. Values are expressed as mean ± S.D. (n = 8). The level of malondialdehyde was significantly increased in i.c.v. STZ control compared with sham operated or vehicle treated group (@ $p < 0.05$). SP600125 treatment in i.c.v. STZ injected rats significantly and dose dependently attenuated STZ induced increase in malondialdehyde levels compared with i.c.v. STZ control group [* $p < 0.05$ vs STZ control, ** $p < 0.05$ vs STZ control and SP 2. 5, *** $p < 0.05$ vs STZ control and SP 5].

et al., 2009). Further it has also caused a significant increase in nitrite and decrease in GSH levels indicating increased oxidative stress. Furthermore, oxidative damage to the cell integrity was assessed by quantitative estimation of lactate dehydrogenase (LDH) activity in rat brain. LDH is a non-specific marker of cell membrane integrity and streptozotocin injected rats showed extensive cell damage as evidenced by significant increase in LDH activity (Figs. 6–10) (Choi and Lee, 2004; Hoyer and Lannert, 2007; Deshmukh et al., 2009).

In the present study, pharmacological inhibition of JNK by administration of SP600125 in i.c.v. STZ injected rat showed significant dose dependent reduction in the levels of malondialdehyde, protein carbonyl formation and nitrite levels and restored glutathione levels. Further, SP600125 treatment in i.c.v. STZ rats has shown to significantly attenuate neuronal damage as evidenced by significant reduction in LDH activity.

Streptozotocin induced impairment of energy metabolism has been considered as an initiating factor in free radical production and subsequent oxidative stress (Sharma and Gupta, 2001). On the other hand, i.c.v. STZ has been shown to cause direct neurotoxic effects and glial activation (Shoham et al., 2003, 2007). However, microglial activation has been reported to release variety of neurotoxic chemicals including reactive oxygen species (ROS), reactive nitrogen species (RNS) and other pro-inflammatory mediators (Stuchbury and Munch, 2005). Even if free radical generation is secondary to other initiating causes they are deleterious and a part of cascade of events that can lead to neuronal death (Markesbery, 1999). Moreover, free radicals have been reported to cause cell death through activation of JNK (Davil and Torres-Aleman, 2008; Lehtinen, 2006) and aberrant activation of JNK signaling pathway in neurons and glial cells has been reported to be neurotoxic and stimulate the production of pro-inflammatory cytokines, induction of iNOS, and COX-2 in microglial cells (Hunot et al., 2004; Waetzig et al., 2005), and even further activation of these cells (Waetzig et al., 2005). Whereas, inhibition of JNK, on the other hand, has been reported to be neuroprotective (Guan et al., 2006) and attenuate microglial activation (Waetzig et al., 2005). SP600125, competitive inhibitor of ATP-binding pocket of JNK, has been used as a pharmacological tool to implicate JNK in physiologic and pathologic conditions (Bennett et al., 2001; Guan et al., 2006). Further, SP600125 has been reported not to have an antioxidant activity (Yeste-Velasco et al., 2009). Thus the attenuation of oxidative stress by SP600125 in the present study may be solely due to inhibition of deleterious JNK signaling pathway.

Accumulating evidence now further supports the concept of reactive oxygen species and its involvement in oxidative pathway of memory impairment (Bruce-Keller et al., 1998). Indeed, STZ induced oxidative stress has been reported to be associated with cognitive impairment (Sharma and Gupta, 2001). In the present study, i.c.v. STZ injected animals had deficits in spatial learning and memory as indicated by impaired acquisition and retention in Morris water maze and passive

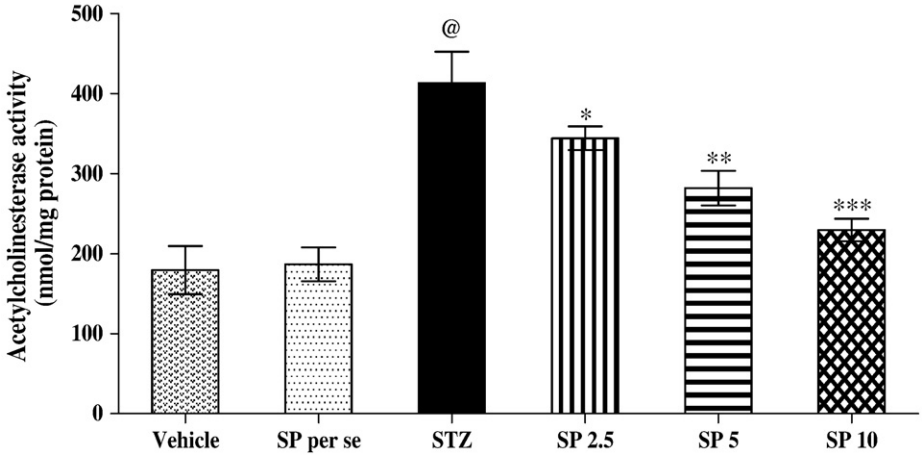

Fig. 5. Effect of SP600125 on acetylcholinesterase activity (AChE) in i.c.v. streptozotocin injected rats. Values are expressed as mean ± S.D. (n = 8). The acetylcholinesterase activity was significantly increased in i.c.v. STZ control compared with sham operated or vehicle treated group (@ $p < 0.05$). SP600125 treatment in i.c.v. STZ injected rats significantly and dose dependently attenuated STZ induced increase in acetylcholinesterase activity compared with i.c.v. STZ control group [* $p < 0.05$ vs STZ control, ** $p < 0.05$ vs STZ control and SP 2. 5, *** $p < 0.05$ vs STZ control and SP 5].

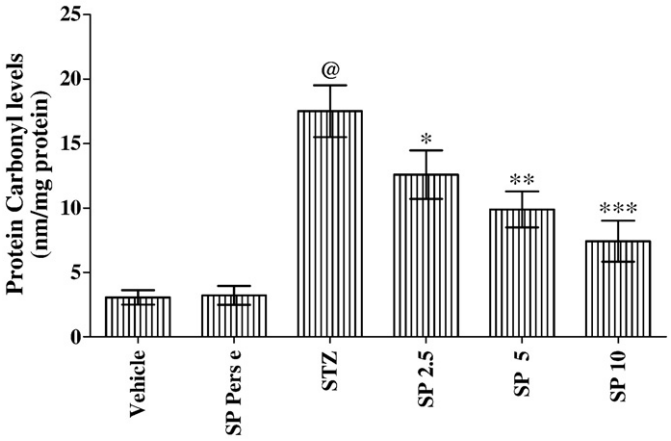

Fig. 7. Effect of SP600125 on brain protein carbonyl levels in i.c.v.streptozotocin injected rats. Values are expressed as mean ± S.D. (n = 8). The level of protein carbonyl was significantly increased in i.c.v. STZ control compared with sham operated or vehicle treated group (@ $p<0.05$). SP600125 treatment in i.c.v. STZ injected rats significantly decreased STZ induced increase in protein carbonyl formation compared with i.c.v. STZ control group [* $p<0.05$ vs STZ control, ** $p<0.05$ vs STZ control and SP 2. 5, *** $p<0.05$ vs STZ control and SP 5].

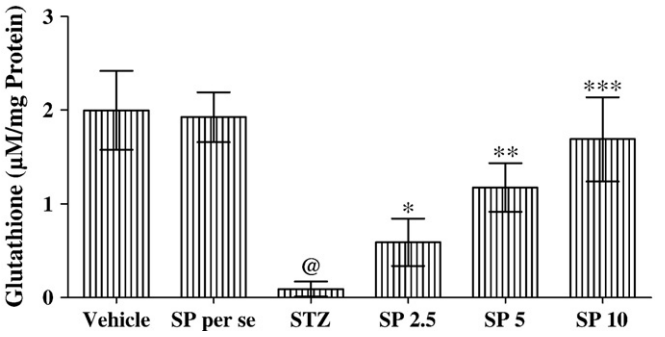

Fig. 9. Effect of SP600125 treatment on brain glutathione (GSH) levels in i.c.v. streptozotocin injected rats. Values are expressed as mean ± S.D. (n = 8). The level of glutathione was significantly decreased in i.c.v. STZ control compared with sham operated or vehicle treated group (@ $p<0.05$). SP600125 treatment in i.c.v. STZ injected rats significantly restored STZ induced depletion of glutathione levels compared with with i.c.v. STZ control group [* $p<0.05$ vs STZ control, ** $p<0.05$ vs STZ control and SP 2. 5, *** $p<0.05$ vs STZ control and SP 5].

avoidance tasks, which is in accord with earlier studies (Deshmukh et al., 2009). The changes in locomotor activity have also been suggested to modulate the learning and memory in passive avoidance and Morris water maze paradigms (Sharma and Gupta, 2003; Deshmukh et al., 2009). However no significant difference in spontaneous locomotor activity was observed in any of the animal groups in the present study. This excludes the possibility that the locomotor activity *per se* may have contributed to the changes in passive avoidance and Morris water maze in vehicle treated and SP600125 treated i.c.v. STZ injected rats. However, inhibition of JNK has been observed to significantly attenuate acquisition and retention deficits in STZ injected rats (Figs. 2–4) without modifying the cognitive functions in normal animals *per se*.

Aberrant activation of JNK has been reported to be associated with tau-induced neurodegeneration (Dias-Santagata et al., 2007) and with Abeta pathology (Colombo et al., 2007). Despite the fact that JNK responsible for phosphorylating c-jun (one of the best-characterized transcription factor), JNK has been only tangentially studied in relation to its participation in cognition. The definite role of JNK has shown in hippocampal long-term potentiation (LTP—a physiology correlate of learning and memory) in adult mice (Bevilaqua et al., 2003). In contrast, JNK inhibition does not affect young mice (Costello and Herron, 2004; Li et al., 2007). On the other hand, JNKs have been reported to be involved in the inhibition of LTP by beta-amyloid (Abeta) (Costello and Herron, 2004) or by lipopolysaccharide-induced cytokines (Barry et al., 2005). Furthermore, the pharmacological inhibition of hippocampal JNK with SP600125 has been reported to enhance short-term memory but appears to block the long-term memory formation and retrieval of an inhibitory avoidance task (Bevilaqua et al., 2003). However, complete deletion of the c-jun gene using its neural excision with nestin did not interfere with spatial learning in Morris water maze or with fear conditioning paradigms (Raivich et al., 2004). Similar disparity has also been reported in stroke and ischemia models, where deletion of JNK2 or JNK3 confers protection, whereas, deletion of JNK1 actually enhances the forebrain tissue loss (Kuan et al., 2003; Brecht et al., 2005). As discussed earlier, for short-term synaptic plasticity, the data has been controversial. SP600125 has been reported to enhance the formation of short-term memory (Bevilaqua et al., 2003), whereas JNK1-deficiency blocks it (Li et al., 2007). More recently D-JNKi, a peptide inhibitor of JNK has further been reported to improve cognitive performance in object recognition task following focal cerebral ischemia in rats (Esneault et al., 2008). In the present study, SP600125 has shown to attenuate streptozotocin induced cognitive impairment. Discussing these discrepancies, we should be aware that inhibition of all JNK isoforms by SP600125 may easily masks isoform-specific actions. Further, these complex findings emphasize again the need to analyze the isoform-specific contributions to cognitive processes.

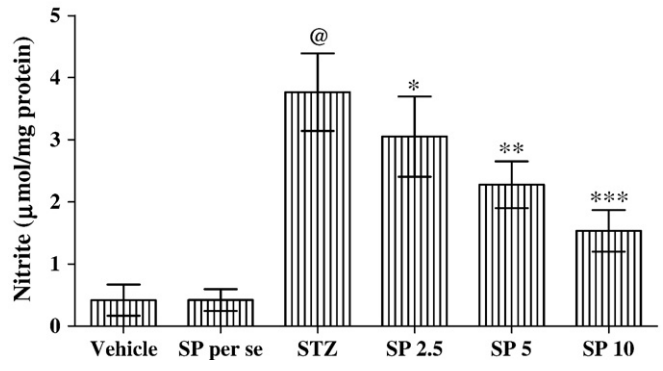

Fig. 8. Effect of SP600125 treatment on brain nitrite levels of i.c.v. streptozotocin injected rats. Values are expressed as mean ± S.D. (n = 8). The levels of nitrite were significantly increased in i.c.v. STZ control compared with sham operated or vehicle treated group (@ $p<0.05$). SP600125 treatment in i.c.v. STZ injected rats significantly decreased STZ induced increase in nitrite levels compared with i.c.v. STZ control group [* $p<0.05$ vs STZ control, ** $p<0.05$ vs STZ control and SP 2. 5, *** $p<0.05$ vs STZ control and SP 5].

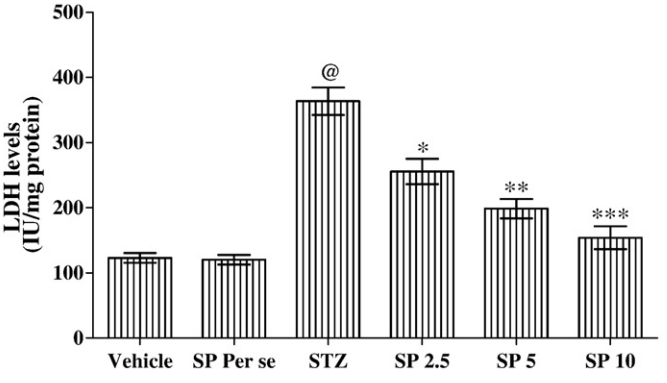

Fig. 10. Effect of SP600125 on brain lactate dehydrogenase levels in i.c.v. streptozotocin injected rats. Values are expressed as mean ± S.D. (n = 8). The level of LDH in brain homogenate was found to be significantly increased i.c.v. STZ control compared with sham operated or vehicle treated group (@ $p<0.05$). Whereas, SP600125 treatment in i.c.v. STZ injected rats significantly decreased STZ induced increase in LDH levels compared with i.c.v. STZ control [* $p<0.05$ vs STZ control, ** $p<0.05$ vs STZ control and SP 2. 5, *** $p<0.05$ vs STZ control and SP 5].

Object recognition, which is a non-spatial task, appears to be more or less independent of the hippocampus (Mumby, 2001; Hartman et al., 2005). A recent study has shown the importance of the perirhinal cortex, but not of the prefrontal cortex for the discrimination of novel and familiar objects (Barker et al., 2007). On the other hand, Morris water maze (MWM) is employed in the present study as an exteroceptive model to evaluate spatial learning and memory. One of the brain structures, especially CA1 region of hippocampus is believed to have critical role in the processing of spatial information (Morris, 1984). Most importantly, spatial learning in general and MWM performance in particular appear to depend upon the coordinated action of different brain regions constituting a functionally integrated neural network (Hooge and Deyn, 2001). Whereas, passive avoidance learning (PAL) refers to the learned inhibition of behavior in order to avoid punishment. Both hippocampus and amygdala are thought to be involved in fear conditioning (passive avoidance) (Lenard and Kertes, 2002). Moreover, application of SP600125 has been reported to prevent hippocampal neuronal death following cerebral ischemia (Guan et al., 2006).

The hippocampus, amygdala and cortical regions of the brain are mainly involved in cholinergic transmission and play vital role in learning and memory processing, and seem to be more prone to oxidative damage and pathogenesis of Alzheimer's disease (Nagel and Kemble, 1976; Francis et al., 1999; Arendt, 2001). Cholinergic system plays an important role in memory formation and retrieval (Butcher et al., 1986; Popic et al., 1994). Oxidative damage to the rat synapse in these regions of brain has been previously reported to contribute to cognitive deficits (Hartman, 1995; Pratico and Delanty, 2000). Elevation in the acetyl cholinesterase activity has observed following STZ injection in rat brain which is accord with earlier report (Deshmukh et al., 2009). Whereas, treatment with SP600125 in STZ injected rats has significantly restored the acetyl cholinesterase activity (Fig. 5). These results suggest that SP600125 can reverse or prevent cognitive deficit induced by STZ in rat.

In conclusion, aberrant activation of JNK has been reported following cerebral ischemia (Zhao and Herdegen, 2009). Here, in the present study, pharmacological inhibition of JNK signaling pathway by SP600125 following i.c.v. STZ injection significantly improved spatial cognitive functions and attenuate oxidative damage in rat. JNK has also been reported to aberrantly get activated in AD pathology (Zhu et al., 2001a,b). In line with these evidences, the results of the present study also suggest that the JNK signaling pathway may get dysregulated following i.c.v. STZ injection in rat and contributing to cognitive impairment and oxidative damage. The study also supports the potential of JNK inhibition as a possible therapeutic strategy to ameliorate neurodegenerative disorders associated with oxidative stress and cognitive impairment.

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