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EFFECT OF MITOGEN ACTIVATED PROTEIN KINASE INHIBITOR IN ANIMAL MODEL OF ALZHEIMER'S DISEASES

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Abstract

Stress activated protein kinases such as p38 MAPK have been implicated in the pathogenesis of Alzheimer's disease (AD). The present study was designed to investigate the effect of SB203580 – a p38 MAPK inhibitor-in intracerebroventricular-streptozotocin (i.c.v - STZ) induced cognitive deficits and oxidative-nitritive stress. i.c.v.-STZ, injected twice (3mg/kg) 48h apart, resulted in significant impairment of learning and memory, cholinergic hypofunction and increased oxidative-nitritive stress. Sub-chronic treatment of SB203580 at different doses (0.25, 5 & 1 µg/5µl/ventricle) from day 4 to day 21 following first i.c.v - STZ injection, significantly improved learning and memory in Morris water maze task. Furthermore, SB203580 significantly reduced the oxidative-nitritive stress as evidenced by decrease in malondialdehyde (MDA) and nitrite levels and increased glutathione (GSH) levels. In addition elevation in acetylcholinesterase (AChE) activity following i.c.v.-STZ on day 22 was significantly reduced by SB203580. Results of the present study have thus demonstrated the facilitatory role of p38 MAPK in cognitive deficit, cholinergic hypofunction and increase in oxidative-nitritive stress following i.c.v.-STZ and favour the potential of p38 MAPK inhibition in neurodegenerative disorders associated with oxidative and cognitive dysfunction.

Keywords: -SB203580; p38 MAPK; Alzheimer's disease; i.c.v. STZ; Memory; Oxidative stress.

Introduction

Alzheimer's disease is characterized by progressive memory loss and deterioration of cognitive function. Pathologically, the hallmarks of Alzheimer's disease include a prevalence of amyloid-β deposits, neurofibrillary tangles (NFTs) and neuronal synapse and cell loss, predominantly in the cortex and hippocampus.[31] Although the neuropathological features of Alzheimer's disease have been well defined, the underlying mechanisms responsible for the pathogenic process have not been clearly delineated. Among the hypotheses that have been proposed to explain the pathogenesis of Alzheimer's disease [48,71] is the concept of inflammatory and oxidative stress, now accepted as components of the pathology of Alzheimer's disease.[41] Oxidative stress has been shown to contribute to the neuropathology of a number of neurodegenerative disorders [28] including Alzheimer's disease .[7] This is further being implicated in neuronal loss, associated with age-related cognitive decline [20,62] and neuro inflammation.[43,44] The observation that multiple pathways are sensitive to alteration in intracellular ROS/RNS concentrations indicates that this might represent a common cellular pathway to signal a large diversity of different stressfull stimuli. Accordingly, a large number of redox-responsive transcription factors and genes have been identified .[3]

Many stress stimuli have been demonstrated to be converted into specific cellular responses through the activation of mitogen activated protein kinase (MAPK) signaling pathways.[35] Oxidative stress [12] and inflammation [15,50] seems to be the major stimuli for MAPK signaling cascades with cell survival or death as a possible consequence. [47] After being phosphorylated by upstream kinases, MAPKs become active serine/ threonine protein kinase that can phosphorylate both cytoplasmic and nuclear targets. [79] Among diverse MAPK family members, p38 MAPK subfamilies are preferentially activated by cellular stresses, such as those induced by proinflammatory cytokines and oxidative stress. [53,85] Aberrant activation of p38 MAPK has also been

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implicated in the pathogenesis of Alzheimer's disease. [78,86] Besides, activation of p38MAPK signaling pathway contributes to the onset and progression of various CNS diseases through production of inflammatory mediators, including NO and O_2^- .[85]

Evidence from experimental models of Alzheimer's disease has implicated p38 MAPK in the process of neuroinflammation [15] , oxidative stress [53,12], amyloid beta production [16,67] tau tangle formation [25,63] and neuronal degeneration. [30,5] Activation of p38MAPK was detected in mouse brain after kainic acid-induced seizures [13], after ischemic insult in rat or gerbil brain [57] and after lipopolysaccharide-induced cell death in rat brain.[53] Inhibition of p38 MAPK has been reported to be reduce oxidative damage and neuroinflammatory events in various experimental models. [78,34] Intracerebroventricular streptozotocin (i.c.v.-STZ) injection in rats has been reported to cause increase in oxidative stress, neuroinflammation and produce neuropathological and biochemical alterations similar to those observed in sporadic Alzheimer's disease. It is now considered to be a valid model of the early pathophysiological changes in Alzheimer's disease [27,65,66] However, a role for the p38 MAPK signal in mediating i.c.v. streptozotocin-induced neurocognitive deficit is not known.

The authors have attempted to evaluate the role of p38 MAPK in i.c.v. streptozotocin-induced oxidative damage and spatial cognitive deficit in rats in the present study, using pharmacological inhibitor. The results are suggestive of the role of p38 MAPK signaling pathway in i.c.v. streptozotocin-induced oxidative damage and spatial cognitive deficit in rats.

2. Material And Methods

2.1 Animals

The experiments were carried out in adult (5-7 months old) male wistar rats (220-250 g) obtained from institutional animal house. They were kept in polyacrylic cages and maintained under standard husbandry conditions (room temperature 22 ± 2^0C and relative humidity of 60-65%) with 12h light/dark cycle. The food in the form of dry pallets and water were made available *ad libitum.* All behavioural 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 Materials

Streptozotocin and acetylthiocholine iodide (AChI), 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) were purchased from Sigma–Aldrich, USA. Vinpocetine was provided as *ex-gratia* sample by M/S Covex Pharma Ltd., Germany. All other chemicals used in the study were of analytical grade. Solutions of the drug and chemicals were freshly prepared before use.

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

Male wistar rats weighing 220-250 g were anaesthetized with ketamine (100mg/kg, ip) and xylazine (5mg/kg, ip). The head 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 .[56] Streptozotocin was dissolved in citrate buffer (pH 4.4)] and slowly injected (1 µl/min) using Hamilton microsyringe in a volume of 10 µl into each cerebral ventricle (bilateral i.c.v.) on day 1 and 3.[72]

2.4 Experimental protocol

Animals were divided into seven groups and each group comprised of six animals.

Group 1: Sham-operated rats, where in the midline saggital incision was made but the hole were not drilled.

Group 2: Vehicle treated rats received citrate buffer containing 1% DMSO (pH 4.4) which was injected i.c.v. in a volume of 10 µl on day 1 and 3.

Group 3: Rats were injected STZ 3mg/kg solution i.c.v. in a volume of 10µl on day 1 and 3.

Group 4: Rats were injected with STZ solution i.c.v. on day 1 and 3 and treated with 0.25 µg/5µl/ventricle of SB203580 from day 4 to day 21.

Similarly group 5 and 6 were treated with SB203580 at doses of 0.5 and 1 µg/5µl/ventricle respectively after from day 4 to 21 STZ injection.

Group 7: SB203580 was administered i.c.v. (1 µg/5µl/ventricle) in normal animals to study *per se* effect of the inhibition.

The SB203580 was administered i.c.v. from day 4 to day 21 following first STZ injection and the doses for SB203580 were selected based on earlier reports.[34]

2.5 Behavioral assessment

2.5.1 Passive avoidance task

On day 14 and 15 after i.c.v. 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 40W 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 60s, 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 0.5mA for 2s through the grid floor. The rat was allowed to remain in the dark compartment for 5s 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 600s (Ishrat et al., 2006). Shorter latencies indicated poorer retention.

2.5.2 Morris water maze test

Spatial learning and memory of animals were tested in a Morris water maze.[49] It consisted of a circular water tank (180cm diameter, 60cm height) filled with water (22±1°C) to a depth of 40cm. 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 (10cm in diameter) was placed in the pool 2cm 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 120s without platform. Animals received a training session consisting of 4 trials per session (once from each starting point) for 4 days (day 17, 18, 19 and 20), each trial having a ceiling time of 120s and a trial interval of approximately 30s. After climbing onto the hidden platform, the animals remained there for 30s before commencement of the next trial. If the rat failed to locate the hidden platform within the maximum time of 120s, it was gently placed on the platform and allowed to remain there for the same interval of time i.e. 30s. The time taken to locate the hidden platform (latency in seconds) was measured.

Twenty four hours after the acquisition phase, a probe test (day 21) was conducted by removing the platform. Rats were allowed to swim freely in the pool for 120s 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 learning .[33]

2.5.3 Spontaneous locomotor activity

Each animal was tested for spontaneous locomotor activity on day 21 following 1st i.c.v. streptozotocin infusion. Each animal was observed over a period of 5 min in a square closed arena equipped with infrared light sensitive photocells using a digital photoactometer (INCO, India).[73]

2.6 Estimation of biochemical parameters

All the biochemical parameters were measured in the brain homogenate on day 22 following 1st streptozotocin infusion.

2.6.1 Brain tissue 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 0.1M 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.

2.6.2 Protein estimation

Protein was measured in all brain samples by the method of .[39] using bovine serum albumin (BSA) (1 mg/ml) as a standard.

2.6.3 Acetylcholinesterase assay

The quantitative measurement of acetylcholinesterase activity in brain was performed according to the method of .[18] The assay mixture contained 0.05 ml of supernatant, 3 ml of 0.01M 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 412nm spectrophotometrically (Shimadzu, UV-1700). The acetylcholinestrase activity in the supernatant was expressed as nmol per mg protein.

2.6.4 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 (1996). The amount of MDA was measured after its reaction with thiobarbituric acid at 532nm using spectrophotometer (Shimadzu, UV-1700). The concentration of MDA was determined from a standard curve and expressed as nmol per mg protein.

2.6.5 Estimation of reduced glutathione

Reduced glutathione in brain was estimated according to the method of .[19] One ml supernatant was precipitated with 1 ml of 4% sulfosalicylic acid and cold digested at 4°C for 1h. The samples were centrifuged at 1200×g for 15 min. To 1 ml of the supernatant, 2.7ml of phosphate buffer (0.1M, pH 8) and 0.2 ml of 5,5′dithiobis (2-nitrobenzoic acid) (DTNB) were added. The yellow color of the reaction mixture was measured immediately at 412nm using a spectrophotometer (Shimadzu, UV-1700). The concentration of glutathione in the supernatant was determined from a standard curve and expressed as µmol per mg protein.

2.6.6 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 .[26] 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 540nm spectrophotometrically (Shimadzu, UV-1700). The concentration of nitrite in the supernatant was determined from sodium nitrite standard curve and expressed as µmol per mg protein.

2.6.7 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 2M HCl and kept in a dark place for 1h 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 360nm in a spectrophotometer (Shimadzu, UV-1700). Protein carbonyl level was expressed as nmol carbonyl mg−1 protein, using a molar extinction coefficient of $22\times104M^{-1}cm^{-1}$.

2.6.8 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; Maharaj et al., 2003).

2.7 Statistical analysis

The results are expressed as means±SD. 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 considered statistically significant.

3. Results

3.1 Behavioural Parameters

3.1.1 Effect of SB203580 on memory performance in passive avoidance task in streptozotocin injected rats

On day 14 following 1st i.c.v. streptozotocin infusion, the mean initial latency in the acquisition trial remained unchanged among all the groups ($F(6, 69) = 0.2101$, $p > 0.05$). But the mean retention latency was significantly

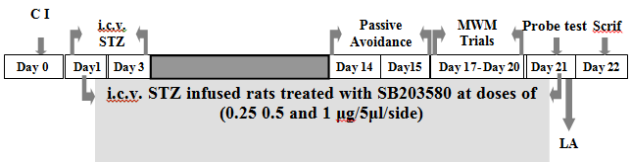


Fig.1 – The design of the treatment schedule and the interval for estimation of various parameters.

Day 0 - Refers to the day of surgery**; CI =** Cannulae Implantation; **i.c.v. STZ** = intracerebroventricular streptozotocin; **MWM** = Morris water maze; **LA =** Locomotor activity; **Scrif =** Sacrificed.

decreased ($P < 0.001$) in i.c.v. streptozotocin group compared with the sham and citrate buffer groups on the next day (Fig. 2). However the decreased mean retention

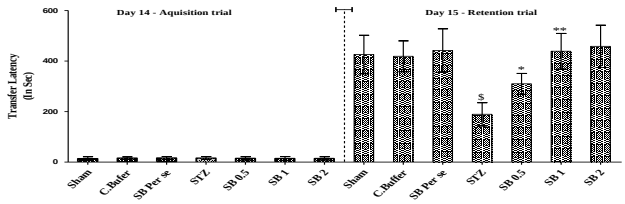


Fig. 2 - Effect of SB203580 on memory performance in passive avoidance task in i.c.v. streptozotocin infused rats.

Values are expressed as mean±SD (n = 10). Retention latency was recorded to a maximum of 600s. Streptozotocin infused rats showed shorter retention latency compared with sham or citrate buffer group ($ $P < 0.05$ vs sham or citrate buffer group). SB203580 treatment in i.c.v. streptozotocin infused rats dose dependently significantly increased retention latencies compared with i.c.v. Streptozotocin group [*$P < 0.05$ vs i.c.v. Streptozotocin group, **$P < 0.05$ vs i.c.v. Streptozotocin and SB 0.5 µg/5µl/ventricle groups].

C.Buffer = Citrate buffer; SB 0.5, SB 1 and SB 2 = SB203580 at 0.25, 0.5 and 1 µg/5µl/ ventricle dose respectively, *Per se* = administered SB203580 at a dose of 1 /5µl/ ventricle in normal animals.

latency in i.c.v. streptozotocin rats was significantly attenuated by SB203580 treatment ($F(6, 69) = 20.82$, $P < 0.05$) indicating improved acquisition or retention of memory. Maximum improvement in retention latency on day 15 was observed with vinpocetine treatment in i.c.v. streptozotocin rats at a dose of 0.5 µg/5µl/ventricle ($P < 0.001$). On the other hand, normal rats treated with SB203580 *per se* did not show any significant change in retention latency, as compared with those of sham or citrate buffer groups ($p > 0.05$).

3.1.2 Effect of SB203580 on memory performance in Morris water maze task in streptozotocin injected rats

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). But the mean latencies were found to be significantly

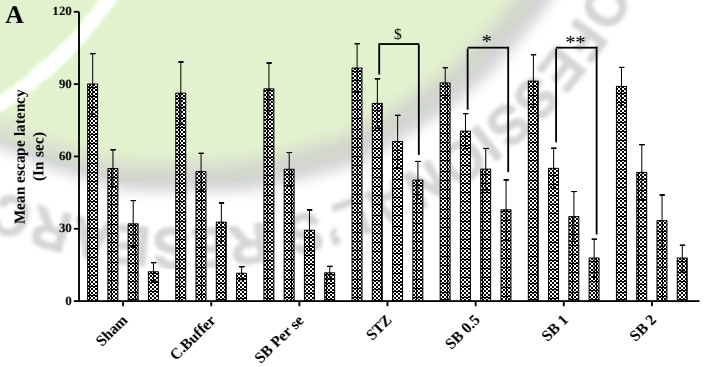


Fig. 3 - (A). Effect of SB203580 on escape latency to find the hidden platform in Morris water maze test in i.c.v. streptozotocin rats. Mean escape latency to locate the hidden platform was recorded on day 17, 18, 19 and day 20 to a maximum of 120s. Values are expressed as mean±SD (n = 10). The mean escape latency (days 18 - 20) to find the hidden platform was significantly prolonged in i.c.v. streptozotocin group compared with sham group ($ $P < 0.05$ vs sham group). SB203580 significantly reversed streptozotocin induced learning deficit compared with streptozotocin group [*$P < 0.05$ vs i.c.v. streptozotocin group, ** $P < 0.05$ vs i.c.v. streptozotocin and SB 5 (SB203580 at 0.25 µg/5µl/ ventricle) groups on day 18, 19 and day 20].

Note- Four bars per group indicate - Each bar represents mean escape latency of

four trials per day i.e. on day 17, 18, 19 and day 20 respectively.

prolonged even on day {18 [Total: $F(6, 69) = 16.70$], 19 [$F(6, 69) = 21.85$] & 20 [$F(6, 69) = 48.69$], $P < 0.001$} in the i.c.v. streptozotocin group of animals as compared to those of sham group, showing poorer learning performance. But the poorer performance of this group was significantly improved by chronic treatment (21 days) with SB203580 ($p < 0.001$). Amongst the doses of SB203580 used in the present study, the dose of 0.5µg/5µl/ventricle was found to be most effective in ameliorating streptozotocin induced spatial memory deficit. On the other hand, administration of SB203580 *per se* at a dose of 1 µg/5µl/ ventricle in normal animals did not modify basal learning performance of animals during 4 days of training as compared to sham or citrate buffer injected groups ($P > 0.05$).

During the probe trial, with the platform removed, i.c.v. streptozotocin injected rats failed to remember the precise location of the platform, spending significantly less time in the target quadrant than sham or citrate buffer group ($P < 0.001$). The mean percent time spent in the target quadrant in i.c.v. streptozotocin group treated with SB203580 was significantly increased as compared to streptozotocin control group indicating improved consolidation of memory [$F(6, 69) = 22.58$, $P < 0.001$] (Fig. 3b).

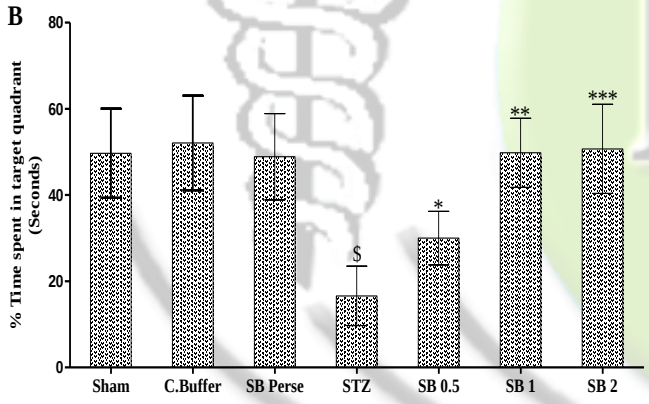
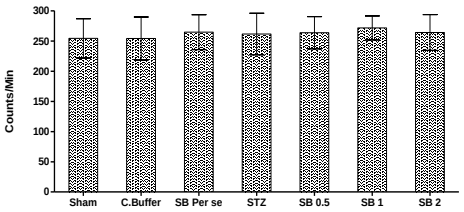


Fig. 3 - (B). Effect of SB203580 on percentage of time spent in target quadrant in i.c.v. streptozotocin infused rats. Values are expressed as mean±SD (n = 10). The percentage of time spent in target quadrant was significantly lesser in i.c.v. streptozotocin infused group compared with sham group ($ $P < 0.05$ vs sham group). SB203580 treatement in i.c.v streptozotocin infused rats significantly attenuated streptozotocin induced memory deficits compared with i.c.v. streptozotocin group [* $P < 0.05$ vs i.c.v. streptozotocin group, ** $P < 0.05$ vs i.c.v. streptozotocin and SB 0.5 (SB203580 at 0.25 µg/5µl/ ventricle) groups on day 21] and *** $P < 0.05$ vs i.c.v. streptozotocin and SB 1 (SB203580 at 0.5 µg/5µl/ ventricle) groups on day 21]

C.Buffer = Citrate buffer; SB 0.5, SB 1 and SB 2 = SB203580 at 0.25, 0.5 and 1 µg/5µl/ ventricle dose respectively, *Per se* = administered SB203580 at a dose of 1 µg/5µl/ ventricle in normal animals.

3.1.3 Effect of SB203580 on spontaneous locomotor activity in i.c.v. streptozotocin injected rats.

The spontaneous locomotor activity on day 21 did not differ significantly among all the groups [Total: $F(6, 69) = 0.4190$, $P > 0.05$] (Fig. 4), suggesting no effect whatsoever of SB203580 on this parameter.



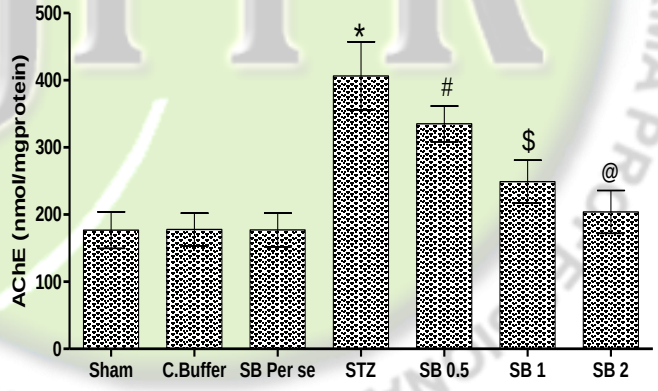
3.1.2. Effect of SB203580 on spontaneous Locomotor activity in i.c.v. streptozotocin -injected rats.

Values are expressed as mean±SD (n = 10). The spontaneous locomotor activity on day 21 did not differ significantly among all the groups [Total: $F(6, 69) = 0.199$, $p > 0.05$].

3.2 Biochemical parameters

3.2.1 Effect of SB203580 on brain acetyl cholinesterase activity in i.c.v. streptozotocin injected rats

The activity of acetylcholinesterase was increased significantly in brain homogenate of i.c.v. streptozotocin injected rats compared with those of sham group ($P < 0.001$). SB203580 treatment in streptozotocin injected rats dose dependently decreased the enhanced acetylcholinesterase activity compared with streptozotocin injected rats [Total: $F(6, 69) = 79.53$, $P < 0.05$]. SB203580 at a dose of 1 µg/5µl/ ventricle was found to be comparatively most effective in ameliorating streptozotocin induced increase in acetylcholinesterase activity ($P < 0.001$). However, the same treatment (1 µg/5µl/ ventricle) in normal animals did not modify the basal acetylcholinesterase activity compared with those of sham and citrate buffer group of animals ($P > 0.05$, Fig. 5).



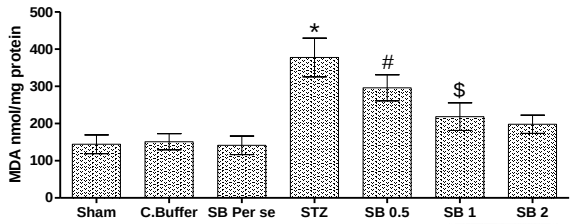
3.2.1 Effect of SB203580 on brain acetylcholinesterase activity in streptozotocin-injected rats.

Values are expressed as mean±SD (n = 10). The acetylcholinesterase activity was significantly increased in i.c.v. streptozotocin group compared with sham group (*$P < 0.05$ vs sham group). SB203580 significantly decreased streptozotocin induced increase in acetylcholinesterase activity compared with streptozotocin group [#$P < 0.05$ vs i.c.v. streptozotocin group, $ $P < 0.05$ vs i.c.v. streptozotocin and SB203580 at SB 0.25 groups, @ $P < 0.05$ vs i.c.v. streptozotocin and SB203580 at 0.25 µg/5µl/ ventricle groups]. C.Buffer = Citrate buffer; SB 0.5, SB 1 and SB 2 = SB203580 at 0.25, 0.5 and 1 µg/5µl/ ventricle dose respectively, *Per se* = administered SB203580 at a dose of 1 µg/5µl/ ventricle in normal animals.

3.2.2 Effect of SB203580 on brain malondialdehyde (MDA) levels in i.c.v. streptozotocin injected rats

The levels of MDA rose significantly in i.c.v. streptozotocin injected rats as compared to those of sham control and citrate buffer injected rats ($P < 0.001$). But the

treatment of these animals with SB203580 significantly decreased MDA levels in a dose dependent manner as compared with those of streptozotocin injected control rats [Total: $F(6, 69) = 72.88$, $P < 0.001$]. SB203580 treatment in normal rats, however, did not modify the basal MDA levels which remained similar to those of sham and citrate buffer groups ($P > 0.05$, Fig. 6).



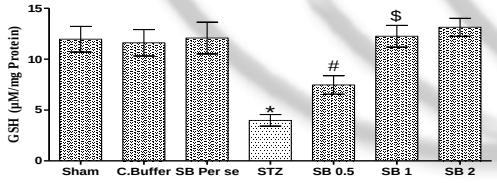
3.2.2 Effect of SB203580 on brain malondialdehyde (MDA) levels in streptozotocin injected rats.

Values are expressed as mean±SD (n = 10). The levels of malondialdehyde was significantly increased in i.c.v. streptozotocin group compared with sham or citrate buffer group (*P < 0.05 vs sham or citrate buffer group). SB203580 significantly decreased streptozotocin induced increase in malondialdehyde levels compared with streptozotocin group [$^{\#}$ P < 0.05 vs i.c.v. streptozotocin group, $^{\$}$ P < 0.05 vs i.c.v. streptozotocin and SB 0.25 µg/5µl/ventricle groups].

C.Buffer = Citrate buffer; SB 0.5, SB 1 and SB 2 = SB203580 at 0.25, 0.5 and 1 µg/5µl/ ventricle dose respectively, *Per se* = administered SB203580 at a dose of 1 µg/5µl/ ventricle in normal animals.

3.2.3 Effect of SB203580 on brain glutathione (GSH) levels in i.c.v. streptozotocin injected rats

The levels of GSH were found to be significantly depleted on day 22 following 1st streptozotocin infusion as compared to sham group animals ($P < 0.001$). Chronic treatment of streptozotocin injected rats with SB203580 was able to raise the levels of GSH significantly compared with those of streptozotocin group animals [Total: $F(6, 69) = 89.56$, $P < 0.001$]. However, the similar chronic treatment with SB203580 *per se* in normal rats did not modify the basal levels of GSH compared with sham group ($P > 0.05$, Fig. 7).



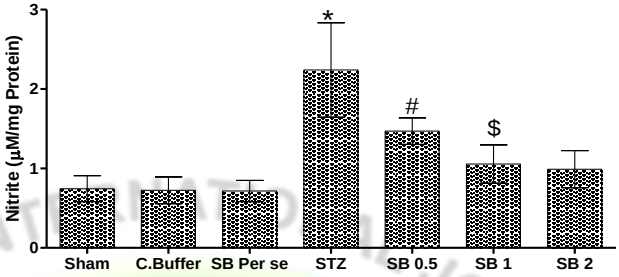
3.2.3 Effect of SB203580 on brain of glutathione (GSH)levels in streptozotocin -injected rats

Values are expressed as mean±SD (n = 10). The level of glutathione was significantly decreased in i.c.v. streptozotocin group compared with sham or citrate buffer group (*P < 0.05 vs sham or citrate buffer group). SB203580 significantly restored streptozotocin induced decrease in glutathione levels compared with streptozotocin group [# P < 0.05 vs i.c.v. streptozotocin group, $ P < 0.05 vs i.c.v. streptozotocin and SB 0.25 µg/5µl/ ventricle groups].

C.Buffer = Citrate buffer; SB 0.5, SB 1 and SB 2 = SB203580 at 0.25, 0.5 and 1 µg/5µl/ ventricle dose respectively, *Per se* = administered SB203580 at a dose of 1 µg/5µl/ ventricle in normal animals.

3.2.4 Effect of SB203580 on brain nitrite levels in i.c.v. streptozotocin injected rats

The level of nitrite rose significantly on day 22 following 1st streptozotocin infusion as compared to those of sham group animals ($P <$ 0.001). However, these animals when treated chronically (21 days) with SB203580 showed dose dependent decrease in the nitrite levels compared with those of streptozotocin group [Total: $F(6, 69) = 38.19$, $P < 0.05$]. But the chronic SB203580 *per se* treatment in normal rats did not modify the basal nitrite levels when compared with those of sham group ($P > 0.05$, Fig. 8).



3.2.4 Effect of SB203580 on brain of nitrite levels in streptozotocin -injected rats

Values are expressed as mean±SD (n = 10). The level of nitrite was significantly increased in i.c.v. streptozotocin group compared with sham or C.Buffer group (*P < 0.05 vs sham or C.Buffer group). SB203580 significantly decreased streptozotocin induced increase in nitrite levels compared with streptozotocin group [$^{\#}P$ < 0.05 vs i.c.v. streptozotocin group, $^{\$}$ P < 0.05 vs i.c.v. streptozotocin and SB 0.25 µg/5µl/ ventricle groups].

C.Buffer = Citrate buffer; SB 0.5, SB 1 and SB 2 = SB203580 at 0.25, 0.5 and 1 µg/5µl/ ventricle dose respectively, *Per se* = administered SB203580 at a dose of 1 µg/5µl/ ventricle in normal animals.

3.2.5 Effect of SB203580 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 22 following 1st streptozotocin infusion as compared to sham and citrate buffer group of animals ($P < 0.001$) indicating extensive neuronal cell damage in these animals. Chronic treatment of these animals with SB203580 significantly decreased the LDH levels compared with those of the streptozotocin group [Total: $F(6, 69) = 400.5$, $P < 0.001$]. On the other hand, chronic vinpocetine treatment *per se* in normal rats did not modify the basal nitrite levels compared with those of the sham group ($p > 0.05$, Fig. 9).

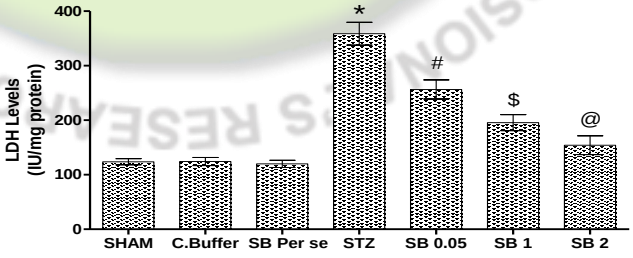


Fig. 3.2.6 - Effect of SB203580 on brain lactate dehydrogenase activity in i.c.v. streptozotocin infused rats.

Values are expressed as mean±S.D. (n = 10). The activity of LDH was significantly increased in i.c.v. streptozotocin group compared with sham or aCSF group (*P < 0.05 vs sham or C.Bufer group). SB203580 significantly decreased streptozotocin induced increase in LDH activity compared with streptozotocin group [$^{\#}$ P < 0.05 vs i.c.v. streptozotocin group, $^{\$}$ P < 0.05 vs i.c.v. streptozotocin and SB 0.25 µg/5µl/ ventricle groups].

C.Buffer = Citrate buffer; SB 0.5, SB 1 and SB 2 = SB203580 at 0.25, 0.5 and 1 µg/5µl/ ventricle dose respectively, *Per se* = administered SB203580 at a dose of 1 µg/5µl/ ventricle in normal animals.

3.2.6 Effect of SB203580 on brain protein carbonyl levels in i.c.v. streptozotocin injected rats

The protein carbonyl level significantly increased on day 22 following 1st streptozotocin infusion as compared to those of sham group animals ($P < 0.001$). However, these animals when treated chronically (21 days) with SB203580 showed dose dependent significant decrease in the protein carbonyl levels compared with those of streptozotocin group [Total: $F(6, 69) = 195.8$, $P < 0.05$]. But the chronic SB203580 *per se* (1 µg/5µl/ ventricle) treatment in normal rats did not modify the basal protein carbonyl levels when compared with those of sham group ($p > 0.05$, Fig. 8).

3.2.6 Effect of SB203580 on brain protein carbonyl levels in i.c.v. streptozotocin injected rats

The protein carbonyl level significantly increased on day 22 following 1st streptozotocin infusion as compared to those of sham group animals ($P < 0.001$). However, these animals when treated chronically (21 days) with SB203580 showed dose dependent significant decrease in the protein carbonyl levels compared with those of streptozotocin group [Total: $F(6, 69) = 195.8$, $P < 0.05$]. But the chronic SB203580 *per se* (1 µg/5µl/ ventricle) treatment in normal rats did not modify the basal protein carbonyl levels when compared with those of sham group ($p > 0.05$, Fig. 8).

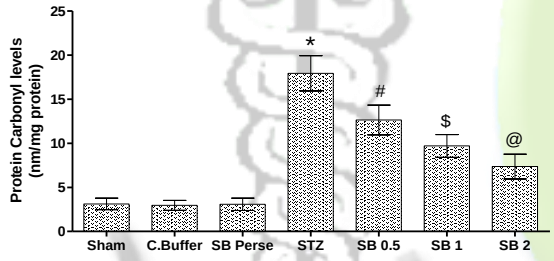


Fig. 3.2.6 - Effect of SB203580 on brain protein carbonyl levels in i.c.v. streptozotocin infused rats.

Values are expressed as mean±SD (n = 10). The level of nitrite was significantly increased in i.c.v. streptozotocin group compared with sham or aCSF group (*$P <$ 0.05 vs sham or C.Buffer group). SB203580 significantly decreased streptozotocin induced increase in nitrite levels compared with streptozotocin group [$^{\#}P < 0.05$ vs i.c.v. streptozotocin group, $^{\$}P < 0.05$ vs i.c.v. streptozotocin and SB 0.25 µg/5µl/ ventricle groups]. C.Buffer = Citrate buffer; SB 0.5, SB 1 and SB 2 = SB203580 at 0.25, 0.5 and 1 µg/5µl/ ventricle dose respectively, *Per se* = administered SB203580 at a dose of 1 µg/5µl/ ventricle in normal animals.

Note: Out of the three doses (0.25, 0.5 and 1 µg/5µl/ ventricle) of SB203580 studied in the present investigation, the middle dose of 0.5 µg/5µl/ ventricle was found to be comparatively most effective with respect to all the biochemical parameters, except acetyl cholinesterase, LDH activity and in protein carbonyl levels mentioned above.

4. Discussion

The current study, presented in this thesis, has revealed that i.c.v.-STZ significantly increased the levels of malondialdehyde (MDA), nitrite, protein carbonylation and decreased the levels of antioxidant defence viz., glutathione, SOD and catalase. The increase in oxidative-nitritive stress was found to be associated with increase in acetylcholinesterase activity and spatial cognitive deficit. Present findings are in tune with previous reports. [72,76,33,80,69] But the pharmacological inhibition of p38 mitogen activated protein kinase (p38 MAPK) using SB203580, a synthetic compound, attenuated the oxidative damage and spatial cognitive deficit induced by intra cerebroventricular streptozotocin (i.c.v.-STZ) injection in rats.

The exact mechanism of streptozotocin-induced oxidative damage is not known. The direct cytotoxic action [74] or streptozotocin induced glial activation [75] may possibly be responsible for increase in oxidative damage. Indeed, astrocytic and microglial activation has been reported to release veriety of neurotoxic chemicals including ROS/RNS and other pro-inflammatory mediators.[77] Oxyradical-induced damage to macromolecules (lipid, protein and nucleic acids, etc.) is considered an important factor in the acceleration of aging and age-related neurodegenerative disorders such as Alzheimer's disease.[38,83]

Second interrelated mechanism that might account for oxidative damage is through the generation of nitric oxide. NO is an important biological mediator in the living organism that is synthesized from L-arginine using NADPH and molecular oxygen. However, overproduction of NO, catalyzed by iNOS, is cytotoxic, especially when it reacts with superoxide anion to produce peroxynitrite. Moreover, it is well known that both NO and $ONOO^-$ act as main toxicants in the brain under neuropathological conditions.[10] One of the well known toxicities caused by NO and $ONOO^-$ is the disruption of cellular energy metabolism: inhibition of mitochondrial respiratory chain, TCA cycle components and glycolysis.[54] Besides these toxicants, superoxide can also exert the same toxic effects as mentioned above through the formation of peroxinitrite or hydrogen peroxide.[81] Moreover, superoxide generating system (i.e. NADPH oxidase activation) is as much important as NO system in generating $ONOO^-$ in the brain damage.[9] Infact, NADPH oxidase-derived superoxide in microglia, astrocytes or neurons themselves can cause neuronal death under neuropathological conditions.[61]

In the present study, i.c.v. STZ-injection in rats significantly induced peroxidation of lipids and proteins and increased nitrite levels and reduced antioxidant defense indicating increased oxidative-nitritive stress. The 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[14,40] and streptozotocin injected rats showed extensive cell damage as evidenced by increase in LDH levels.[32] Recently, p38 MAPK pathway has been proposed to be involved in neuronal death by oxidative stress that can be induced by amyloid beta [34], LPS [53] or ischemic challenge.[57] In line with these findings, it was observed here in the present study that the pharmacological inhibition of p38MAPK by SB203580 significantly reduced streptozotocin induced oxidative-nitritive stress and attenuated oxidative damage. The observed results on i.c.v. STZ-injection rat model are in tune with previous reports concerning other models [78,57,34,68] and indicate neuroprotective properties of SB203580 in the presently used i.c.v.-STZ model. The results of the present study further, suggest that p38 MAPK may play a role in STZ-induced oxidative brain damage model too.

Indeed, activation of the p38 MAPK pathway in neurons and glial cells has been reported to stimulate the production of pro-inflammatory mediators, reactive oxygen (ROS) and nitrogen species (RNS)[60], or further activation of these cells.[8] Further, ROS have been implicated as second messengers that trigger mitogen activated protein kinase signaling cascade[82,64] as the incubation with H_2O_2 increases phosphorylation of p38 in rat cortical and hippocampal tissue causing neuronal cell death. Whereas, SB203580, a p38 MAPK inhibitor reported to abolish this H_2O_2 induced neuronal cell death.[53] On the other hand, p38 MAPK activation has been well known to induce iNOS derived NO production[6,52] while inhibition of p38 MAPK has been reported to negatively regulate iNOS at mRNA as well as protein levels. In addition, NADPH oxidase mediated O_2^- production has been shown to be attenuated by inhibiting this signaling pathway.[85]

A growing body of evidence now further supports the concept of reactive oxygen species and its involvement in oxidative pathway of memory impairment.[11] Indeed, oxidative stress has been implicated in acceleration of aging and age-related neurodegenerative disorders such as Alzheimer's disease.[38,83] 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 avoidance tasks which is in accord with earlier studies.[59,76,33,69] The changes in locomotor activity have also been suggested to modulate the learning and memory in passive avoidance and Morris water maze paradigms.[74] However no significant difference in spontaneous locomotor activity was observed in any of the animal groups in the present study. Further, pharmacological blockade of p38 MAPK significantly ameliorated acquisition and retention deficits without modifying the cognitive functions in normal animals (per se study group).

To investigate the effect of p38 MAPK inhibition on cholinergic function, that governs vital aspects of memory and other cognitive functions, brain acetylcholinesterase activity was also measured in the present study. The hippocampus, amygdala and cortical regions of the brain are mainly involved in cholinergic transmission to monitor learning and memory processing and seem to be more prone to oxidative damage. [51,37,45,22,4] Moreover, oxidative damage to the rat synapse in these regions of brain has been reported to contribute to cognitive deficits. [29,58] In addition, overproduction of NO is reported to be neurotoxic to the cholinergic neurons .[17,23]

It has been well documented that activation of p38 MAPK in response to microglial activation has been reported to increase production of pro-inflammatory mediators viz., cytokines and further activation of these cells. [1,2,15] A strong and long lasting activation of p38 MAPK has been demonstrated to cause cholinergic dysfunction[23,70], while inhibition of p38 MAPK has shown to inhibit cytokine release and reverse cholinergic dysfunction.[24]

In the present study, STZ was found to significantly elevate acetylcholinesterase activity, an enzyme responsible for degradation of acetylcholine, which is in tune with earlier report.[76] This increase in acetylcholinesterase activity was, however, significantly restored dose dependently by SB203580. These observations suggest the modulation of cholinergic neurotransmission and/or prevention of cholinergic neuronal loss.

In conclusion, pharmacological inhibition of p38 MAPK by SB203580 following i.c.v. streptozotocin injection significantly attenuated oxidative damage, restored acetylcholinesterase activity and improved spatial cognitive functions in rats. The results of the present study suggest that p38 MAPK signaling pathway may mediate streptozotocin-induced neurotoxic effects and support the reported potential of p38 MAPK inhibition in amelioration of brain disorders associated with oxidative insult and cognitive impairment . [78,57,34,68]

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6. References

1. Ajome-Cat, M.A., Nicolini, A., Mingheti, L., 2003. Prolonged exposure of microglia to lipopolysaccharidemodifies the intracellular signaling pathways and selectivity promotes prodtaglandins E2synthesis. J. Neurochem. 87, 1193-1103.
2. Akundi, R.S., Candelario-Jalil, E., Hess, S., Hull, M., Lieb, K., Gebicke-Haerter, PJ., Fiebich, B.L., 2005. Signal transduction pathways regulating cyclooxygenase-2 in lipopolysaccharide-activated primary rat microglia. Glia. 51:199-208.
3. Allen, R.G., Tresini, M., 2000. Oxidative stress and gene regulation. Free. Radic. Biol. Med. 60, 409-470.
4. Arendt, T., 2001. Alzheimer's disease as a disorder of mechanisms underlying structural brain self-organization. Neuroscience. 102, 723-765.
5. Atzori, C., Ghetti, B., Piva, R., Srinivasan, A.N., Zolo, P., Delisle, M.B., Mirra, S.S., Migheli, A., 2001. Activation of the JNK/p38 pathway occurs in diseases characterized by tau protein pathology and is related to tau phosphorylation but not to apoptosis. J Neuropathol. Exp. Neurol. 60, 1190–1197.
6. Barone, F.C., Irving, E.A., Ray, A.M., Lee, J.C., Kasis, S., Kumar, S., Badger, M.A., White, R.F., Mcvey, M.J., Legos, J.J., Erhardt, J.A., Nelson, H.A., Ohlstein, E.H., Hunter, A.J., Ward, K., Smith, B.R., Adams, J.L., Parsons, A.A., 2001. SB239063, a second-generation p38 mitogen-activated protein kinase inhibitor, reduces brain injury and neurological deficits in cerebral focal ischemia. J. Pharmacol. Exp. Therap. 296, 312-321.
7. Behl, C., 1999. Alzheimer's diseaseand oxidative stress: implications for novel therapeutic approaches. Prog. Neurbiol. 57, 301-323.
8. Bhat, N.R., Zhang, P., Lee, J.C., Hogan, E.L. 1998. Extracellular signalregulated kinase and p38 subgroups of mitogen-activated protein kinases regulate inducible nitric oxide synthase and tumor necrosis factor-alpha gene expression in endotoxin-stimulated primary glial cultures. J. Neurosci. 18, 1633–1641.
9. Bianca, V.D., Dusi S, Bianchini, E., Dal, Pra. I., Rossi, F., 1999. Beta-amyloid activates the O-2 forming NADPH oxidase in microglia, monocytes, and neutrophils. A possible inflammatory mechanism of neuronal damage in Alzheimer's disease. J. Biol. Chem. 274, 15493-15499.
10. Block, M.L., Hong, J.S., 2007. Microglia-mediated neurotoxicity:uncovering the molecular mechanisms Nat. Rev. Neurosci. 8, 57-69.
11. Bruce-Keller, AJ., Li, YJ., Lovell, A., Kramer, P.J., Gray, D.S., Brown, R.R., Marksberry, W.R., Mattson, MP., 1998. 4-hydroxinonenal, a product of lipid peroxidation, damages cholinergic neurons and impairs visuospatial memory in rat. J. Neuropathol. Exp. Neurol. 57, 257-267.
12. Carracedo, A., Egia, A., Guzman, M., Velasco, G., 2006. p8 upregulation sensitizes astrocytes to oxidative stress. FEBS. Lett. 580, 1571-1575.
13. Che, Y., Yu, Y.M., Han, P.L., Lee, J.K., 2001. Delayed induction of p38 MAPKs in reactive astrocytes in the brain of mice after KA-induced seizure. Mol. Brai. Res. 94, 157-165.
14. Choi,, E.J., Lee, B.H., 2004. Evidence for genistein mediated cytotoxicity and apoptosis in rat brain. Life. Sci. 75, 499-502.
15. Culbert, A.A., Skaper, S.D., Howlett, D.R., Evans, N.A., Facci, L., Soden, P.E., Seymour, Z.M., Florence, G., Gaestal, M., Richardson, J.C., 2006. MAPK-activated protein kinase 2 deficiency in microglia inhibits proinflammatory mediator release and resultant neurotoxicity. J. Biol. Chem. 281, 23658-23667.
16. Daniels, W.M.U., Hendricks, J., Salie, R., Taljard, J.J.F., 2001. Role of the MAP-kinase superfamily in β-amyloid toxicity. Mol. Brain. Dis. 16, 175-185.
17. Dawson, T.M., Dawson, V.L., 1997. Protection of the brain from ischemia in cerebrovascular disease. ed. Batjer HH Philadelphia: Lippincott-Raven 1997; 319-25.
18. Ellman, G.L, Courtney, K.D., Andres, V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88–95.
19. Ellman, G.L., 1959. Tissue sulfidryl groups. Arch. Biochem. Biophys. 82, 70-77.
20. Finkel, T., Holbrook, N.J., 2000. Oxidants, oxidative stress and the biology of ageing. Nature. 408, 239-247.
21. Fass, U., Panickar, K., Personett, D., Bryan, D., Williams, K., Gonzales, J., Sugaya, K., McKinney, M., 2003. Differential vulnerability of primary cultured cholinergic neurons to nitric oxide excess. Neuroreport. 11, 931–936.
22. Francis, P.T., Palmer, A.M., Snape, M., Wilcock, G.K., 1999. The cholinergic hypothesis of Alzheimer's disease: a review of progress (Review). J. Neurol. Neurosurg. Psych. 66, 137-147.
23. Giovanelli, L., Scali, C., Faussone-Pellergrini, M.S., Pepeu, G., Casamenti, F., 1997. Long term changes in the aggregation state and toxic effects of β-amyloid

injected into the rat brain. Neurosci. 87, 349-357.
24. Giovannini, M.G., Scali, C., Prosperi, C., Bellucci, A., Vannucchi, M.G., Susanna, R., Giancarlo, P., Casamrnti, F., 2002. β-Amyloid-induced inflammation and cholinergic hypofunction in rat brain in- vivo: involvement of the p38 MAPK pathway. Neurobiol. Dis. 11, 257-274.
25. Goedert, M., Hasegawa, M., Jakes, R., Lawler, S., Cuenda, A., Cohen, P., 1997. Phosphorylation of microtubule-associated protein tau by stress-activated protein kinases. FEBS. Lett. 409, 57-62.
26. Green, L.C., Wagner, D.A., Glgowski, J., Skipper, P.L., Wishnok, J.S., Tannebaum, S.R., 1982. Analysis of nitrate, nitrite and [^{15}N] nitrate in biological fluids. Ann. Biochem. Exp. Med. 126, 131-135.
27. Grunblatt. E., Salkovic-Petrisic, M., Osmanovic, J., Riederer, P., Hoyer, S., 2007. Brain insulin system dysfunction in streptozotocin intracerebroventricularly treated rats generates hyperphosphorylated tau protein. J. Neurochem. 101, 757-770.
28. Halliwell, B., 1992. Reactive oxygen species and the central nervous system. J. Neurochem. 59, 1609-1623.
29. Hartman. S., 1995. Free radicals theory of aging: Alzheimer's diseases pathogenesis. Age. 18, 97-119.
30. Hensley, K., Floyd, R.A., Zheng, N.Y., Nael, R., Robinson, K.A., Nguyen, X., Pye, Q.N., Stewart, C.A., Geddes, J., Markesbery, W.R., Patel, E., Johnson, G.V., Bing, G., 1999. p38 kinase is activated in the Alzheimer's disease brain. J. Neurochem. 72, 2053–2058.
31. Higgins, L.S., Rodems, J.M., Catalano, R., Quon, D., Cordell, B., 1995. Early Alzheimer disease-like histopathology increases in frequency with age in mice transgenic for beta-APP751. Proc. Natl. Acad. Sci. USA. 92, 4402–4406.
32. Hoyer, S., Lanner, H., 2007. Long-term abnormalities in brain glucose/energy metabolism after inhibition of the neuronal insulin receptor: implication of tau-protein. J. Neural. Trans. [Suppl 72], 195-202.
33. Ishrat, T., Khan, M.B., Hoda, M.N., Yousuf, S., Ahmad, M., Ansari, M.A., Ahmad, A.S., Islam, F., 2006. Coenzyme Q10 modulates cognitive impairment against intracerebroventricular injection of streptozotocin in rats. Behav. Brain. Res. 171, 9-16.
34. Jin, Y., Yan, E.Z., Fan, Y., Zong, Z.H., Qi, Z.M., Li, Z., 2005. Sodium ferulate prevents amyloid-beta-induced neurotoxicity through suppression of p38 MAPK and upregulation of ERK-1/2 and AKT/protein kinase B in rat hippocampus. Acta. Pharmacol. Sinica. 26, 943-951.
35. Kyriakis, M.J, Joseph, A., 2001. Mammalian Mitogen Activated Protein Kinase Signal Transduction Pathways Activated By Stress And Inflammation, Physiological Reviews, 81, 807-869.
36. Levine, R.L., Garland, D., Oliver, C.N., Amici, A., Climent, I., Lenz, A.G., Ahn, B.W., Shaltiel, S., Stadtman, E.R., 1990. Determination of carbonyl content in oxidatively modified proteins. Meth. Enzymol. 186, 464-487.
37. Liang, K.C., McGaugh, J.L., Martinez, J.R., Jensen, R.A., Vasquez, B.J., Messing, R.B., 1982. Post-training amygdaloid lesions impair retention of an inhibitory avoidance response. Behav. Brain. Res. 4, 237–249
38. Liu, R., Liu, I.Y., Bi, X., Thompson, R.F., Doctrow, S.R., Malfroy, B., Baudry, M., 2001. Reversal of age-related learning deficits and brain oxidative stress in mice with superoxide dismutase/catalase mimetics. Proc. Natl. Acad. Sci. USA. 100, 8526-8531.
39. Lowry, O.H., Roseburgh, N.J., Farr, A.L., Randal, R.L., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 205-215.
40. Maharaj, S.D.B., Walker, R.D., Glass, B., Daya, S., 2003. 6-Hydroxymelatonin protects against cyanide induced oxidative stress in rat brain homogenates. J. Chem Neuro. 26, 103-107.
41. Markesbery, W.R., 1997. Oxidative stress hypothesis in Alzheimer's disease. Free. Rad. Biol. Med. 23, 134-147.
42. McGeer, P.L., McGeer, E.G., 2001. Inflammation, autotoxicity and Alzheimer disease. Neurol. Biol. Ageing. 22, 799-809.
43. McGeer. E.G., McGeer. P.L., 2003. Inflammatory processes in Alzheimer's diseases. Prog. Neuropsychopharmacol. Biol. Psych. 27, 741-749.
44. McIntosh, L.J., Trush, M.A., Troncoso, J.C., 1997. Increased susceptibility of Alzheimer's disease temporal cortex to oxygen free radical-mediated processes. Free. Radic. Biol. Med 23, 183-190.
45. Meister, A., 1998. Glutathione metabolism and its selective modification. J. Biol. Chem. 263, 17205-17208.
46. Miekle, K., Herdegen, T., 2000. JNK and p38 stresskinases- degenerative effectors of signal-transduction-cascades in the nervous system. Prog. Neurobiol. 61, 45-60.
47. Moore, AH., O'Banion, M.K., 2002. Neuroinflammation and anti-inflammatory therapy for Alzheimer's diseases. Adv. Drug. Del. Rev. 54, 1627-1656.
48. Morris, R.G.M., 1984. Development of a water –maze procedure for studying spatial learning in the rats. J. Neurosci. Meth. 11, 47-60.
49. Munoz, L., Ranaivo, H.R., Roy, S.M., Hu, W., Craft, J.M., McNamara, L.K., Chico, L.W., Van, Eldik. L.J., Watterson, D.M., 2007. A novel p38 alpha MAPK inhibitor suppresses brain

50. proinflammatory cytokine up-regulation and attenuates synaptic dysfunction and behavioral deficits in an Alzheimer's disease mouse model. J. Neuroinflamm. **4**, 1-14
51. Nagel, J.A., Kemble, E.D., 1976. Effects of amygdaloid lesions on the performance of rats in four passive avoidance tasks. Physiol. Behav. 17, 245-50.
52. Niikura, T., Yamada, M., Chiba, T., Aiso, S., Matsuoka, M., Nishimoto, I., 2004 Characterization of V6421-AβPP-induced cytotoxicity in primary neurons. J. Neurosci. Res. 77, 54-62.
53. Nolan, Y., Vereker, E., Lynch, A.M., Lynch, M.A., 2003. Evidence that lipopolysaccharide-induced cell death is mediated by accumulation of reactive oxygen species and activation of p38 in rat cortex and hippocampus. Exp. Neurol. 184, 794-804.
54. Parihar, M.S., Brewer, G.J., 2007. Mitogenergetic failure in Alzheimer's disease. Am. J. Physiol. Cell. Physiol. 292, C8-C23.
55. Pathan, A.R., Viswanad, B., Sonkusare, S.K., Ramarao, P., 2006. Chronic administration of pioglitazone attenuates intracerebroventricular streptozotocin induced-memory impairment in rats. Life. Sci. 79, 2209-22016.
56. Paxinos, G., Watson, C., 1986. The rat brain in stereotaxic coordinates, (2nd Eds), Academic Press, Sydney. Piao, C.S., Kim, J.B., Han, P.L., Lee, J.K., 2003. Administration of the p38 MAPK inhibitor SB203580 affords brain protection with a wide therapeutic window against focal ischemic insult. J. Neurosci. Res. 73, 537-544.
57. Pratico, D., Delanty, N., 2000. Oxidative injury indiseases of the central nervous system: focus on Qin. B., Cartier, L., Dubois-Dauphin, M., Li. B., Serrander, L., Krause, K.H., 2006. A key role for the microglial NADPH oxidase in APP-dependent killing neurons. Neurobiol. Aging. 27, 1577-1587.
58. Quinn, J., Kaye, J., 2001. The neurology of aging. Neurologist. 7, 98-112.
59. Reynolds, C.H., Betts, J.C., Blackstock, W.P., Nebreda, A.R., Anderton, B.H., 2000. Phosphorylation sites on tau identified by nanoelectrospray mass spectrometry: differences in vitro between the mitogen-activated protein kinases ERK2, c-Jun N-terminal kinase and P38, and glycogen synthase kinase-3beta. J. Neurochem. 74, 1587–1595.
60. Rosenberger. J., Petrovics, G., Buzas, B., 2001. Oxidative stress induces proorphanin FQ and proenkephalin gene expression in astrocytes through p38- and ERK-MAP kinases and NF-[kappa]B. J. Neurochem. 79, 35-44.
61. Salkovic-Petrisic, M., 2008. Amyloid cascade hypothesis: is it true for sporadic Alzheimer's diseases. Periodicum. Biolgorum. 110, 17-25.
62. Salkovic-Petrisic, M., Hoyer, S., 2007. Central insulin resistance as a trigger for sporadic Alzheimer-like pathology: an experimental approach. J. Neural. Transm. (Suppl.) 72, 217–233.
63. Savage, M.J., Lin, Y.G., Ciallella, J.R., 2002. Flood CG, Scott RW. Activation of c-jun N-terminal kinase and p38 in an Alzheimer's disease model is associated with amyloid deposition. J. Neurosci. 22, 3376–3385.
64. Savage, M., Gingrich, DE., 2009. Advances in the development of kinase inhibitor therapeutics for Alzheimer's disease. Drug. Dev. Fres. 70, 124-44.
65. Alzheimer's diseases. Am. J. Med. 109, 577-585.
66. Prickaerts, J., Fahrig, T., Blokland, A., 1999. Cognitive performance and biochemical markers in septum, hippocampus and striatum of rats after an i.c.v. injection of streptozotocin: a correlation analysis. Behav. Brain. Res. 102, 73–88.
67. Pyo. H., Jou, I., Jung, S., Hong, S., Joe, E.H., 1998. Mitogen-activated protein kinases activated by lipopolysaccharide and beta-amyloid in cultured rat microglia. Neuro. Report. 9:871-874.
68. Saxena, G., Pratap, S.S., Agrawal, R., Nath,.C., 2007. Effect of denepezil and tacrine on oxidative stress in intracerebral streptozotocin-induced model of dementia in mice. Eur. J. Pharmacol. 581, 283-289.
69. Scali, A., Giovannini, M.G., Prosperi, C., Bellucci, A., Pepeu, G., Casamenti, F., 2003. The selective cyclooxygenase-2 inhibitor refecoxib suppresses brain inflammation and protects cholinergic neurons from excitotoxic degeneration in vivo. Neurosci. 117, 909-919.
70. Selkoe, D.J., 2001. Alzheimer's disese: Genes, Proteins and Therapy. Physiol Rev., 81, 741-765.
71. Sharma, M., Gupta, Y.K., 2001. Effect of chronic treatment of melatonin on learning, memory and oxidative deficiencies induced by intracerebroventricular streptozotocin in rats. Pharmacol. Biochem. Behav. 70, 325–331.
72. Sharma, M., Gupta, Y.K., 2003. Effect of alpha lipoic acid on intracerebroventricular streptozotocin model of cognitive impairment in rats. European Neuropsychopharmacol. 13, 241–247.
73. Shoham, S., Bejar, C., Kovalev, E., Weinstock, M., 2003. Intracerebroventricular injection of streptozotocin causes neurotoxicity to myelin that contributes to spatial memory deficits in rats. Exp. Neurol. 184, 1043–1052.
74. Shoham, S., Bejar, C., Kovalev, E., Schorer-Apelbaum, D., Weinstockm., 2007. Ladostigil prevents gliosis, oxidative-nitrative stress and memory deficits induced by intracerebroventricular injection of streptozotocin in

rats. Neuropharmacol. 52, 836–43.
75. Sonkusare, S.K., Kaul, C.L., Ramarao, P., 2005. Dementia of Alzheimer's disease andother neurodegenerative disorders memantine, a new hope. Pharmacol. Res. 51, 1–17.
76. Stuchbury, G., Munch, G., 2005. Alzheimer's associated inflammation, potential drug targets and future therapies. J. Neural. Transm. 112, 429-453.
77. Sugino, T., Nozaki, K., Takagi, Y., Hattori, I., Hashimoto, N., Moriguchi, T., Nishida, E., 2000. Activation of mitogen-activated protein kinases after transient forebrain ischemia in gerbil hippocampus. J. Neurosci. 20, 4506-4514.
78. Sun, A., Liu, M., Nguyen, X.V., Bing, G., 2003. p38 MAP kinase is activated at early stages in Alzheimer's disease brain. Exp. Neurol. 2003, 183:394-405.
79. Tahirovic, I., Sofic, E., Sapcanin, A., Gavrankapetanovic, E., Bach-Rojecky, L., Salkovic-Petrisic, M., Lackovic, Z., 2007. Reduced Brain Antioxidant Capacity in Rat Models of Betacytotoxic-Induced Experimental Sporadic Alzheimer's Disease and Diabetes Mellitus. Neurochem. Rev. 32, 1709-1717.
80. Torreilles, F., Salman-Tabeheh, S., Guerin, M.C., Torreilles, J., 1999. Neurodegenerative disorders: the role of peroxynitrite. Brain. Res. Rev. 30, 153-163.
81. Vereker, E., O'Donnell., Lynch, M.A., 2000. The inhibitory effect of interleulin-1β on long-term potentiation is coupled with increased activity of stress-activated protein kinases. J. Neurosci. 20, 6811-6819.
82. Wickens, A.P., 2001. Ageing and the free radical theory (Review). Respir. Physiol 128, 379-391.
83. Wills, E.D., 1996. Mechanism of lipid peroxide formation in animal. Biochem.J. 99, 667–676.
84. Yoo, B.K., Choi, J.W., Shin, S.L., Jeon, S.J., Park, S.J., Cheong, J.H., Han, S.Y., Ryu, J.R., Song, M.R., Ko, K.H., 2008. Activation of p38 MAPK induced peroxynitritre generation in LPS plus IFN-γ-stimulated rat primary astrocytes via activation of iNOS and NADPH oxidase. Neurochem. Int. 52, 118-1197.
85. Zhu, X., Mei, M., Lee, H.G., Wang, Y., Han, J., Perry, G., Smith, M.A., 2005. p38 activates mediated amylid-β cytotoxicity. Neurochem. Res. 30, 791-796.