Journal of Pharmacological Reports

Dubey et al., J Pharmacol Rep 2017, 2:2

Research Article Open Access

Effects of Nitric Oxide (NO) Modulators on Cognitive Function and Brain Oxidative Stress in Experimental Model of Alzheimer's Disease in Rats

Harikesh Dubey, Kavita Gulati and Arunabha Ray*

Department of Pharmacology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi 110007, India

***Corresponding author:** Arunabha Ray, Department of Pharmacology, Vallabhbhai Patel Chest Institute, University of Delhi, India, Tel: (91) 011 2766 7441; E-mail: arunabha14@yahoo.co.in

Received date: Jul 31, 2017; **Accepted date:** Aug 18, 2017; **Published date:** Aug 25, 2017

Abstract

In present study, we investigated the effects of NO modulators (L-arginine and L-NAME) in intracerebroventricular-streptozotocin (ICV-STZ, 3 mg/kg) induced rat model of sporadic AD (sAD). Pre-treatment with L-arginine (100 mg/kg/ip/day) improved ICV-STZ induced cognitive deficit in the Morris water maze (MWM) test when compared to the control (saline) group. Both memory acquisition (escape latency) and retention (probe trial) were affected by icv-STZ which were reversed after L-arginine treatment. The NO synthase inhibitor, L-NAME (10 mg/kg), on the other hand, did not have much influence on these parameters. In the fear based memory model (Passive Avoidance test, PA), ICV-STZ treated rats showed decreased latency period for entry into the dark chamber, which was reversed after L-arginine pre-treatment. In brain homogenates, there were decreased levels of reduced glutathione (GSH) and increased levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in hippocampus, cortex and amygdala after ICV-STZ injection. Pre-treatment with L-arginine, restored GSH levels in hippocampus and 8-OHdG level in cortex, towards baseline levels. These results provide experimental evidence for the attenuating effects of L-arginine on ICV-STZ induced cognitive dysfunctions which were associated with reduced oxidative stress in this model of sporadic Alzheimer`s Disease (sAD). It is inferred that NO and its interactions with reactive oxygen species could reduce age related cognitive deficits and that L-arginine could be a potential therapeutic supplement in the treatment of sAD.

Keywords: Nitric oxide modulators; Cognitive function; Oxidative stress; Alzheimer's disease

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder which results in dementia and progressive cognitive deficit among the elderly and data indicates that there has been a sharp rise in the number of AD patients worldwide in recent years [1]. Intracellular neurofibrillary tangles and extracellular amyloid β (Aβ) plaques are two neuropathological hallmarks with elevated level of Aβ playing a major role in AD pathophysiology [1,2].

The existing evidence suggests that aging is a major risk factor of AD which is associated with abnormal generation of Reactive Oxygen Species (ROS) and which leads to neuronal damage and such oxidative stress is considered as an important factor in AD pathogenesis [3-5]. It has been observed that in AD brain ROS induces calcium influx, via glutamate receptors and triggers an excitotoxic response leading to cell death [6]. Memory impairment in old age is attributed to a decrease in brain antioxidant levels. It is reported that glutathione, which is important for brain antioxidant defence system, is also responsible for the endogenous redox potential in the cell. Reduced Glutathione (GSH) is involved in the donation of electrons to ROS resulting in free radical scavenging activity, and low GSH levels, particularly in the hippocampus, results in enhanced oxidative stress due to lack of optimal antioxidant defence mechanisms [6]. This consequently leads to various structural and functional changes at the cellular level eg. protein and DNA injury, energy deficiency, inflammation, mitochondrial dysfunction, tau hyperphosphorylation and Aβ overexpression. All of these play important roles in the acceleration of aging and age-related neurodegenerative pathophysiology [7]. Further, to maintain integrity of the genome, cells are well equipped with antioxidant defence mechanism and DNA repair system. It is well established that various neurodegenerative diseases may be related to DNA damage as a result of abnormal DNA repair system. DNA repair system is found to be diminished in AD and higher number of DNA single stand breaks was observed in cortex of such patients [8].

8-hydroxy-2-deoxyguanosine (8-OHdG) is a prominent marker of oxidative DNA damage and higher levels of 8-OHdG has been reported in various AD studies [6,9]. Furthermore, it has also been found that high levels of 8-OHdG are localised around Aβ plaques and NFTs. Though initially considered as part of the apoptotic process, it is now widely accepted that oxidative stress is responsible for DNA damage as higher levels of free carbonyls are present in nuclei of neurons and glial cells in AD [6]. A recent study suggested that in aged persons, there was decreased Nitric Oxide (NO) generation [10]. NO is a unique gasotransmitter with neuromodulatory actions and a prominent role for NO has been proposed in various neurodegenerative diseases [11]. NO has a crucial role in learning and memory, synaptic plasticity, Long-term Potentiation (LTP) and the consolidation of long-term memory [10,12]. NO is generated in the neuronal cells from the amino acid L-arginine by the enzyme, Nitric Oxide Synthase (NOS) [12]. The antioxidant property of L-arginine has been well documented in several reports [10,13,14]. L-arginine has also been shown to have anti-aging effects and cytoprotective activity [10,15]. Hence, L-arginine may have prominent role to attenuate oxidative stress in AD. In view of the importance of oxidative stress and potential involvement of NO in AD, we evaluated the effects of NO

modulators on cognitive parameters and oxidative stress markers in experimental models of sAD in rats. Thus, the effects of L-arginine (NO precursor) and L-NAME (NOS inhibitor) were evaluated in icv-streptozotocin (icv-STZ) induced cognitive deficits and brain oxidative stress markers in hippocampus, cortex and amygdala in rats.

Material and Methods

Animals

Inbred male Wistar rats (200-300 g) were used for the study (n=8/group). The rats were housed under standard laboratory conditions (temperature 22 ± 2°C, 12 hr light/dark cycle-lights on at 0800 h). Care of animals was as per guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi. The research protocol was approved by the Institutional Animals Ethics Committee (IAEC) of the institute.

Drugs and chemicals

The drugs used were: Streptozotocin (STZ), L-arginine HCl and L-NAME (all procured from Sigma-Aldrich, India). ELISA kit for 8-OHdG were purchased from USCN Life Science (China). All other chemicals required for biochemical assay were of analytical grade and obtained from Sisco Research Laboratories (SRL, New Delhi). STZ was dissolved in artificial CSF (aCSF), whereas both L-arginine and L-NAME were dissolved in distilled water.

Experimental induction of AD

The animals were randomly divided in four groups and anesthetized with a combination of ketamine (65 mg/kg b.w.) and xylazine (5 mg/kg b.w.) i.m. They were placed on a Stereotaxic (Inco, Ambala) frame, and the skull was exposed. The stereotaxic coordinates for the lateral ventricle were measured accurately as antero-posterior 0.8 mm, lateral 1.5 mm and dorso-ventral 3.6 mm, all relative to the bregma [16]. After making proper marking for lateral ventricle in both sides (left and right) a hole was made with the help of a motorized drill and a guide cannula (20-gauge) was fixed to the skull with help of dental cement. Postoperative care was done by daily application of antiseptic powder (Neosporin) to the site of surgery and animals were housed individually. To confirm the accurate implantation of cannula, after a 7-day post-operative recovery of animals, icv angiotensin II (100 ng/rat) induced drinking response was tested, and animals with positive drinking response were selected for experiments. The animals were then injected with STZ (3 mg/kg, 10 µl/injection site at the rate of 1 µl/min) into the lateral cerebral ventricle (left side, on day 1), and then again on right side (on day 3) with the help of injector cannula and Hamilton syringe. STZ was dissolved in freshly prepared aCSF. Various drug treatments were started from the next day following STZ administration [17].

Experimental protocol

Animals were divided into four groups (n=8/group) and assigned as follows. Group I (received only aCSF ICV+normal saline; Group II (ICV-STZ injected+normal saline; Group III (ICV-STZ injected+L-NAME (10 mg/kg/ip/day) [18]; and Group IV (ICV-STZ injected+L-arginine (100 mg/kg/ip/day) [19] Groups I and II received normal saline (0.1 ml/ip/day) during the course of the entire study of 90 days. All groups of animals were subjected to the behavioural studies for cognitive function on last week of 1st, 2nd and 3rd months post icv-STZ.

Behavioural studies

Morris water maze (MWM) test: MWM was used to assess spatial memory in animals. MWM apparatus consisted of a circular tank (160 cm diameter and 50 cm height) filled with warm water (22-24°C) to a height of 30 cm. The water was made opaque with a black non-toxic dye. The tank was divided into four quadrants (NE, SE, SW and NW) by four points (E, S, W and N), which were equally spaced along the circumference of the tank. A circular escape platform (12 cm diameter and 28 cm height) was fixed in the middle of quadrant NE (target quadrant), which was 2 cm below the surface of the water for the non-visible trials. A video camera was mounted above the centre of the water maze and was used to track and capture the swimming path of each rat using video tracking software (ANY MAZE software, version 4.72, Stoelting Co. USA). Animals received four trials during four daily acquisition sessions. Each of the four starting positions (N, S, W, E) was used once in a series of trials. The platform was always in the same quadrant (NW). The trial was terminated automatically as soon as the rat reached the platform and situated for 10 seconds or when 60 s had elapsed. The rat was allowed to stay on the platform for 10 s. Rats that did not find the platform within 60 s were put on the platform for 10 sec. The next trial was started after 20 s. On day 5, a spatial probe trial (60s) was given to detect the spatial memory of the rat. On this day, the same protocol as described earlier was followed. However, the platform was removed from the pool and the time spent in the target quadrant was recorded. Latency to find the platform, (acquisition trial) and time spent in target quadrant were recorded [20,21].

Passive avoidance test

Passive Avoidance (PA) (step-through) rat apparatus (Ugo Basile S.R.L. Gemonio-Italy) was used and standardized by using male rats. The instrument consisted of two chambers–the first one a light chamber and second one a dark compartment containing an electric shock grid floor. Both chambers are connected by automated sliding-door at floor level called guillotine door. During the acquisition trial, each animal was placed in the light chamber. After a 60s habituation period, the guillotine door was opened, and the initial latency of animals to enter the dark chamber was recorded. Rats with an initial latency time of more than 60s were excluded from further experiments. Immediately after the rat entered the dark chamber, the guillotine door was closed and an electric foot shock of 1.5 mA for 3s was delivered to the floor grids with a stimulator. Five seconds later, the rat was removed from the dark chamber and returned to its home cage. After 24 h, the retention latency time was measured in the same way as in the acquisition trial, but foot shock was not delivered and the latency time was recorded to a maximum of 600s. Short latencies indicated poorer retention [22].

Biochemical Assays

On the next day after the last behavioural testing, all animals were decapitated under anaesthesia (urethane) and the brains were quickly removed and rinsed with ice-cold Phosphate Buffered Saline (PBS) solution, pH 7.4 to remove any red blood cells and clots. Then, the hippocampus, amygdala and cortex were dissected out and tissues were homogenized in 10% (w/v) cold PBS (0.1 M, with a pH of 7.4), to which a protease inhibitor cocktail was added. Homogenates were centrifuged for 20 min at 10000 × g at 4°C. The supernatants were

collected and stored at -80°C for estimation of the GSH and 8-OHdG levels [23,24].

Reduced Glutathione (GSH) was measured according to the method of Ellman [25]. In brief an equal quantity of homogenate was mixed with 10% trichloroacetic acid and centrifuged to separate the proteins. To 50 µL of the sample supernatant or standard, 200 µL of phosphate buffer (pH 8.4) and 50 µL of 5,5-dithiobis (2-nitrobenzoic acid) was added. The mixture was slightly shaken and absorbance read at 412 nm within 15 min. The concentration of reduced glutathione was expressed as µmoles/g tissue. 8-OHdG level was estimated from various homogenised supernatants, according to the manufacturer's ELIZA kit (Qayee-Bio, China), All samples were estimated in duplicate and the average of the two findings were used for comparative purposes in data analysis.

Statistical Analysis

The statistical analysis was performed using GraphPad Prism 5 (GraphPad Software Inc., CA, USA). Data were expressed as Mean ± SEM. The behavioral data of learning in Morris water maze were analyzed by repeated measures two-way Analysis of Variance (ANOVA) followed by Bonferroni's post hoc test for multiple comparisons. The data of the passive avoidance and probe trial test in MWM as well as those of the biochemical studies were analyzed by one-way ANOVA followed by Tukey's test for multiple comparisons test. A p value of at least 0.05 ($p \leq 0.05$) was considered as the level of significance in all statistical tests.

Results

Assessment icv-STZ induced cognitive deficit was performed by the Morris Water Maze (MWM) and Passive Avoidance (PA) tests on the last week of 1st, 2nd, and 3rd months (post icv-STZ). Preliminary data showed that the most consistent changes were apparent after the 3rd month, and hence for all further studies this time internal was used for assessing the various drug effects on cognitive functions.

Effect of NO modulators on escape latency time in MWM test (acquisition trials)

In order to investigate the effects of NO modulators on escape latency time for spatial memory in STZ induced AD, animals were administered L-arginine (100 mg/kg/ip/day) or L-NAME (10 mg/kg/ip/day) for 90 days following which they were tested for spatial memory in the MWM. In the Icv-STZ+saline treated animals (experimental controls), there were marked increases in the mean escape latencies at both day 1 and day 4 of testing ($p<0.02$). Pre-treatment with L-arginine (100 mg/kg) attenuated the icv-STZ induced changes in spatial memory, and the data of the L-arginine+icv-STZ group were significantly different from the saline+icv-STZ group of rats on both day 1 and day 4 ($p<0.05$, in each case). However, L-NAME (10 mg/kg) treatment did not appreciably influence the mean escape latencies in the MWM as compared to the saline+icv-STZ group ($p>0.05$). In the probe trial test, which was performed on day 5 to test memory retention, icv-STZ+saline treated rats showed marked reductions in the time spent in the target quadrant of the MWM ($p<0.02$ as compared to controls). Pre-treatment with L-arginine (100 mg/kg) induced significant attenuations in the target quadrant time as compared to the icv-STZ+saline group ($p<0.05$). On the other hand, pre-treatment with L-NAME (10 mg/kg) did not influence the icv-STZ induced reductions in target quadrant time by any appreciable extent ($p>0.05$). These results are summarized in Table 1.

Treatment groups	Acquisition trial-Mean escape latency time (s) in MWM		Probe trial-Time spent in target quadrant (s) in MWM
	Day 1	Day 4	Day 5
Controls	17.69+3.02	8.37+1.79	20.55+1.63
icv-STZ+Saline	54.13+2.72#	48.50+7.91#	11.90+0.68#
icv-STZ+L-NAME 10mg/kg)	45.76+11.81	34.44+8.60	13.54+1.94
icv-STZ+L-Arginine (100 mg/kg)	30.75+7.36*	19.25+8.38**	17.27+2.02*

Table 1: Effect of NO modulators on cognitive performance in Morris Water Maze (MWM) test (Acquisition trial and Probe trial) in experimentally induced AD in rats.

Effect of NO modulators on the PA test (fear based memory)

In the PA test, the latency of entry into the dark chamber of the PA apparatus was recorded. Icv-STZ+saline treated animals showed decreased latency period as compared to controls ($p<0.02$). However, L-NAME (10 mg/kg) did not influence this reduction by any significant extent ($p>0.05$). On the other hand, though L-arginine (100 mg/kg) pre-treatment attenuated the icv-STZ induced decrease in the latency to enter the dark chamber by approx. 40%, these differences were not statistically significant ($p>0.05$). These results are summarized in Figure 1.

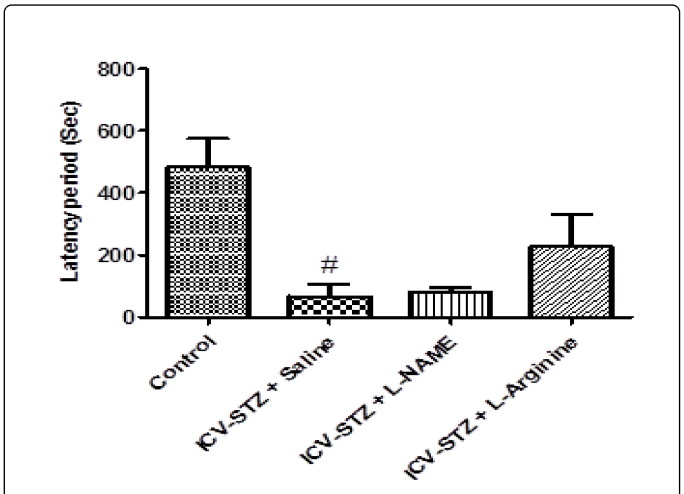

Figure 1: Effect of NO modulators on passive avoidance test (3rd month after treatment) in icv-STZ treated rats. Data expressed as mean ± SEM. # $p<0.02$ (compared to controls).

Effect of NO modulators on oxidative stress markers in brain

Following the behavioural testing for cognitive functions at the end of 3 months post icv-STZ, the rat brains were removed, the

hippocampi, the cortices and the amygdalae were dissected out and separately homogenized for various biochemical estimations.

8-OHdG: In the hippocampus, icv-STZ+saline treated animals showed significant increases in the 8-OHdG levels as compared to controls ($p<0.02$). Pre-treatment with L-NAME (10 mg/kg) did not influence the icv-STZ induced increases in 8-OHdG levels by any appreciable extent. However, L-arginine (100 mg/kg) treatment tended to reverse the increased 8-OHdG levels, and though a near 15% decrease was seen as compare to saline+STZ treated group, these differences were not statistically significant ($p>0.05$). Similar increases in 8-OHdG levels were also seen in cortical homogenates of saline +STZ treated rats as compared to controls ($p<0.02$). L-arginine pre-treatment induced appreciable reductions in the 8-OHdG levels as compared to experimental controls (saline+STZ) ($p<0.05$), whereas, L-NAME treatment did not influence this parameter by any significant extent. As seen in the hippocampal and cortical homogenates, significant elevations in 8-OHdG levels were seen in amygdalar homogenates after saline+icv-STZ when compared to controls ($p<0.02$). Neither, L-arginine nor L-NAME treatment of rats were able to influence the 8-OHdG levels in amygdalar homogenates by any appreciable extent. These results are summarized in Figure 2.

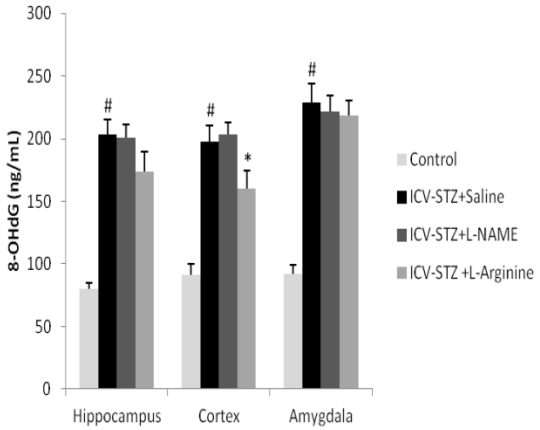

Figure 2: Effects of NO modulators on icv-STZ induced changes in 8-OHdG levels in brain homogenates of rats. Data are expressed as Mean ± SE. #p<0.02 (compare to controls; *p<0.05 (compared to icv-STZ+saline group).

GSH: In the hippocampus, icv-STZ+saline treated animals showed significant decreases in the GSH levels as compared to controls ($p<0.02$). Pre-treatment with L-NAME (10 mg/kg) did not influence the icv-STZ induced increases in 8-OHdG levels by any appreciable extent. However, L-arginine (100 mg/kg) treatment tended to reverse the decreased GSH levels, and these were significantly increased as compared to the values of the saline+STZ group ($p<0.05$). Similar decreases in GSH levels were also seen in cortical homogenates of saline+STZ treated rats as compared to controls ($p<0.02$). Neither L-arginine nor L-NAME pre-treatment induced changes in the GSH levels as compared to experimental controls (saline+STZ) ($p<0.05$). As seen in the hippocampal and cortical homogenates, significant reductions were seen in GSH levels in amygdalar homogenates after saline+icv-STZ when compared to controls ($p<0.02$). Neither, L-arginine nor L-NAME treatment of rats were able to influence the GSH levels in amygdalar homogenates, and though an 18% enhancement was seen after L-arginine, these differences were not statistically significant ($p>0.05$). These results are summarized in Figure 3.

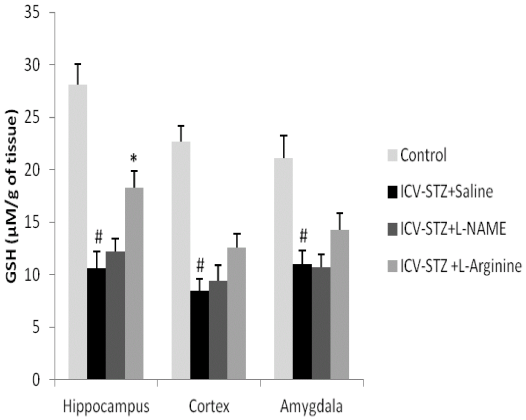

Figure 3: Effects of NO modulators on icv-STZ induced changes in GSH levels in brain homogenates of rats. Data are expressed as Mean ± SE. #p<0.02 (compare to controls; *p<0.05 (compared to icv-STZ+saline group).

Discussion and Conclusion

The present study evaluated the effects of NO modulators in ICV-STZ induced changes is cognitive parameters and brain oxidative stress markers in rats. The data clearly demonstrates the neuroprotective effect of L-arginine in the experimental model of AD and brain oxidative stress markers. Studies have already demonstrated that ICV infusion of STZ results in cognitive deficit and other neuropathological changes similar to sAD, hence it is considered as a relevant animal model for sAD [2,26]. In accordance with previous studies [17,27], ICV-STZ infusion induced significant impairment in learning and memory, and our data showed increased mean escape latency period during acquisition trial and decreased in time spent in target quadrant during probe trial of MWM test. Further, there was a decrease in retention latency period for entering the dark chamber in the PA test. This cognitive deficit behaviour for spatial memory in MWM test was significantly attenuated by L-arginine. However, L-arginine did not have any significant influence on retention latency period during contextual fear conditioning memory performance in PA test. It is well known that spatial memory as tested by MWM which is regulated primarily by the hippocampus [28], while contextual fear conditioning memory by PA test is governed by amygdala [29,30]. Further, the cortex has a prominent role in memory storage and helps in recalling or remembering [31]. Various reports have also suggested that hippocampus, amygdala and cortex play complimentary roles in learning and memory [32,33].

Interestingly, in our study, we have observed that ICV-STZ infusion increased DNA damage and increased 8-OHdG level in homogenates of hippocampus, amygdala and cortex. Similarly, decreased cellular GSH levels were seen in all the above three parts of the brain. Further, administration of the NO precursor, L-arginine, significantly attenuated the icv-STZ induced changes in brain oxidative stress markers in hippocampus and cortex–two brain areas crucial for spatial memory. This suggested that L-arginine via NO plays an important role in preventing the loss of spatial memory. However, L-arginine did

not show any significant improvement in 8-OHdG and GSH levels in amygdala, an area which deals with contextual fear memory. This could explain why L-arginine was not effective in the PA test. Another probable reason could be that L-arginine administration inhibits hippocampal NA-ATPase activity which impairs retention of an inhibitor avoidance task in rats [34]. It is also reported that hippocampal NO acts as inhibitory for avoidance learning task in rats which may be triggered via the calcium/calmodulin-dependent protein kinase II activation [35]. Irrespective of the reason, it was clear that L-arginine treatment sustainably improved spatial memory, as tested by MWM, during long duration of treatment [36].

It is also illustrated that appropriate concentrations of NO in brain is neuroprotective. This is supported by the observation of Kwak et al. [37] that NO possesses potent anti-amyloidogenic activity by inhibiting BACE1 enzyme, the rate-limiting enzyme responsible for the β-cleavage of APP to Aβ peptides. It is also reported that Long Term Potentiation (LTP) and synaptic plasticity in relevant neurons of brain are most important steps for learning and memory. Disruption of these steps leads to memory deficits [38]. It has been shown that ICV-STZ, infusion causes disruption in LTP and synaptic plasticity [39]. It is also demonstrated that NO as well as L-arginine promotes synaptic plasticity and LTP [40]. Hence, it could be the reason why L-arginine treated animals attenuated cognitive deficits in behavioural studies while L-NAME treatment was ineffective. Interestingly, the experimental data did not show any significant worsening of behavioural parameters after L-NAME, though some changes were seen (approx. 25%, $p>0.05$). This could have been due to the fact that already maximal cognitive deficits would have been induced after icv-STZ, and hence L-NAME could not induce any further change in this parameter. The same explanation could be forwarded to explain the lack of change in the 8OHdG and GSH levels. It may be pertinent to point out here that, L-NAME treatment did not show ameliorative effects in ICV-STZ induced cognitive deficits and brain oxidative stress.

As per free radical hypothesis, ageing is a progressive and unavoidable process which is related to increased oxidative stress and incidence of neurodegenerative disorders. Increasing evidence suggests that oxidative stress is a major cause of AD pathogenesis. This happens due to an imbalance between pro-oxidants and anti-oxidant forces, which is associated with increased production of ROS including superoxide radical anion (O_{2^-}), hydrogen peroxide (H_2O_2), hydroxyl radical (HO-), nitric oxide (NO), and peroxynitrite (ONOO-). Various free radicals/ROS are involved in AD, and from the above mentioned free radical moieties, HO- plays a prominent role in AD pathogenesis, due to its higher toxicity and role in various chemical reactions e.g. Fenton's. Further, H_2O_2 is related to Aβ toxicity and ONOO- increases neurological damage by further increasing NFTs level in brain [41,42]. ICV-STZ infusion causes neuronal damage by overproduction of free radicals, which might have caused oxidative damage to membrane lipid and protein and decreases in cellular antioxidants like GSH [43,44]. Importance of GSH in cellular antioxidant system has been already established in various studies. Reduced GSH is the most abundant cellular non-protein thiol, which neutralizes free radicals in brain tissue. It eliminates H_2O_2 and organic peroxide from brain tissue, while decreased levels of GSH results in increased OH- formation, thus leading to more oxidant load and oxidative damage to brain tissues which could initiate neuropathological changes seen in early stage of AD [45]. In the present study with sAD rat model, ICV-STZ administration deceased GSH levels in all areas (hippocampus, cortex, amygdala) of the rat brain. Further, L-arginine treatment significantly restored the GSH level in cortex ($p<0.05$), while in hippocampus and amygdala minor ameliorative effects were seen compared to that of the (ICV-STZ+Saline) group. The possible mechanism for the observed ameliorative effects of GSH level by the NO precursor could be that L-Arginine induced glutathione synthesis and improved antioxidant defence system [46,47]. Additionally, NO also increases GSH levels by activating the mRNA expression of glutamate-cysteine ligase, as rate limiting enzyme in GSH synthesis.

L-arginine treatment significantly attenuated the 8-OHdG up regulation in the cortex seen in the experimental control group (ICV-STZ+Saline). However, in hippocampus and amygdala smaller reductions in 8-OHdG levels were seen after L-arginine when compared with experimental control (ICV-STZ+Saline) group. As mentioned initially, oxidative stress induced DNA damage plays an important role in AD pathogenesis and similar results was observed in our study. However, L-arginine treatment showed ameliorative effects on such DNA damage. The possible explanation could be that L-arginine has potent antioxidant activity which leads to neuroprotective effects [10,48]. It is also documented that NO levels are decreased in aged patient`s brain tissue, and that NO has potent antioxidant, anti-apoptosis and neuroprotective activity. A prominent role for NO has been reported in regulation of learning and memory. L-arginine has ant-aging and potent antioxidant property and experimental studies have shown that the NO precursor attenuates ageing induced memory impairment in rats by increasing NO levels in rat hippocampus [10,12,49,50]. It is also suggested that NOS is involved in buffering of oxidative stress, as L-NAME reduced NO levels, and may lead to oxidative stress and increased 8-OHdG levels [48]. Furthermore, L-Arginine also participates in the synthesis of creatine, which is a guanidinic compound that is considered a nutritional supplement with potent antioxidant and neuroprotective effects [51,52]. This may play a critical role in improvement of cognitive deficits due to oxidative stress. Several studies have proposed that there is a strong correlation between oxidative stress and AD pathogenesis and ROS increases Aβ level in brain, which is responsible for AD [53,54]. Further, it is also reported that ICV-STZ increases Aβ level in brain [31]; Aβ blocks NO production leading to cognitive impairment [55], and L-arginine increases NO concentration and consequently improves cognitive function. Taken together, our results demonstrate that L-arginine ameliorated the behavioral cognitive deficit, attenuated cellular oxidative stress and DNA damage in rat brain produced due to ICV-STZ administration–an experimental model for sAD. This study also indicates a role for L-arginine as a potential therapeutic agent in the treatment of age-related cognitive deficit related to sAD.

References

1. Wirz KTS, Keitel S, Swaab DF, Verhaagen J, Bossers K (2014) Early molecular changes in Alzheimer disease: can we catch the disease in its presymptomatic phase? J Alzheimers Dis 38: 719-740.
2. Kosaraju J, Gali CC, Khatwal RB, Dubala A, Chinni S, et al. (2013) Saxagliptin: a dipeptidyl peptidase-4 inhibitor ameliorates streptozotocin induced Alzheimer's disease, Neuropharmacol 72: 291-300.
3. Markesbery WR (1999) The role of oxidative stress in Alzheimer disease. Arch Neurol 56: 1449-1452.
4. Su B, Wang X, Nunomura A, Moreira PI, Lee HG, et al. (2008) Oxidative stress signaling in Alzheimer's disease. Curr Alzheimer Res 5: 525-532.
5. Moslemnezhad A, Mahjoub S, Moghadasi M (2016) Altered plasma marker of oxidative DNA damage and total antioxidant capacity in patients with Alzheimer's disease. Caspian J Intern Med 7: 88-92.

6. Gella A, Durany N (2009) Oxidative stress in Alzheimer disease. Cell Adh Migr 3: 88-93.
7. Zhou B, Liu Y, Zhang Z, An N, Yao H (2013) Impaired functional connectivity of the thalamus in Alzheimer's disease and mild cognitive impairment: a resting-state fMRI study. Curr Alzheimer Res 10: 754-766.
8. Koppele JMT, Lucassen PJ, Sakkee AN, Van Asten JG, Ravid R, et al. (1996) 8OHdG levels in brain do not indicate oxidative DNA damage in Alzheimer's disease. Neurobiol Aging 17: 819-826.
9. Lan J, Henshall DC, Simon RP, Chen J (2000) Formation of the base modification 8-hydroxyl-2′-deoxyguanosine and DNA fragmentation following seizures induced by systemic kainic acid in the rat. J Neurochem 74: 302-309.
10. Hosseini M, Pourganji M, Khodabandehloo F, Soukhtanloo M, Zabihi H (2012) Protective Effect of L-Arginine against Oxidative Damage as a Possible Mechanism of its Beneficial Properties on Spatial Learning in Ovariectomized Rats. Basic Clinical Neurosci 3: 36-44.
11. Ray A, Gulati K (2007) Nitric oxide and central nervous system: perspectives and trends, Current Trends in Pharmacology, I. K. International Pvt Ltd, New Delhi, Banglore, Mumbai pp: 367-381.
12. Paul V, Ekambaram P (2011) Involvement of nitric oxide in learning & memory processes. Indian J Med Res 133: 471-478.
13. Boger RH, Bode Boger SM, Mugge A, Kienke S, Brandes R, et al. (1995) Supplementation of hypercholesterolaemic rabbits with L-arginine reduces the vascular release of superoxide anions and restores NO production. Atherosclerosis 117: 273-284.
14. Lubec B, Hayn M, Kitzmuller E, Vierhapper H, Lubec G (1997) L-arginine reduces lipid peroxidation in patients with diabetes mellitus. Free Radic Biol Med 22: 355-357.
15. Gad MZ (2010) Anti-aging effects of l-arginine. J Adv Res 1: 169-177.
16. Paxinos G, Watson C (1997) The rat brain in stereotaxic coordinates (2nd edn.). Academic Press, San Diego.
17. Ozkay UD, Can OD, Ozkay Y, Ozturk Y (2012) Effect of benzothiazole/piperazine derivatives on intracerebroventricular streptozotocin-induced cognitive deficits. Pharmacol Rep 64: 834-847.
18. Misra S, Tiwari V, Kuhad A, Chopra K (2011) Modulation of nitrergic pathway by sesamol prevents cognitive deficits and associated biochemical alterations in intracerebroventricular streptozotocin administered rats. Eur J Pharmacol 659: 177-186.
19. Chakraborti A, Gulati K, Ray A (2011) Involvement of nitric oxide in the protective effects of dehydroepiandrosterone sulphate on stress induced neurobehavioral suppression and brain oxidative injury in rats. Eur J Pharmacol 652: 55-59.
20. Sharma M, Briyal S, Gupta YK (2005) Effect of alpha lipoic acid, melatonin and trans resveratrol on intracerebroventricular streptozotocin induced spatial memory deficit in rats. Indian J Physiol Pharmacol 49: 395-402.
21. Zhang T, Pan BS, Zhao B, Zhang LM, Huang YL, et al. (2009) Exacerbation of poststroke dementia by type 2 diabetes is associated with synergistic increases of β-secretase activation and β-amyloid generation in rat brains. Neuroscience 161: 1045-1056.
22. Flint RW, Noble LJ, Ulmen AR (2013) NMDA receptor antagonism with MK-801 impairs consolidation and reconsolidation of passive avoidance conditioning in adolescent rats: evidence for a state dependent reconsolidation effect. Neurobiol Learn Mem 101: 114-119.
23. Mohamed AR, Soliman GY, Ismail CA, Mannaa HF (2015) Neuroprotective role of vitamin D3 in colchicine-induced Alzheimer's disease in rats. Alexandria J Med 51: 127-136.
24. Chakraborti A, Gulati K, Banerjee BD, Ray A (2007) Possible involvement of free radicals in the differential neurobehavioral responses to stress in male and female rats. Behav Brain Res 179: 321-325.
25. Ellman GL (1959) Tissue sulfydryl group. Arch Biochem Biophys 82: 70-77.
26. Nazem A, Sankowski R, Bacher M, Al-Abed Y (2015) Rodent models of neuroinflammation for Alzheimer's disease. J Neuroinflammation 12: 74.
27. Ishrat T, Khan MB, Hoda MN, Yousuf S, Ahmad M, et al. (2006) Coenzyme Q10 modulates cognitive impairment against intracerebroventricular injection of streptozotocin in rats. Behav Brain Res 171: 9-16.
28. Broadbent NJ, Squire LR, Clark RE (2004) Spatial memory, recognition memory, and the hippocampus. PNAS 101: 14515-14520.
29. Slotnick BM (1973) Fear behavior and passive avoidance deficits in mice with amygdala lesions. Physiol Behav 11: 717-720.
30. Russo II NJ, Kapp BS, Holmquist BK, Musty RE (1976) Passive avoidance and amygdala lesions: Relationship with pituitary-adrenal system. Physiol Behav 16: 191-199.
31. Bear MF (1996) A synaptic basis for memory storage in the cerebral cortex. Proc Natl Acad Sci 93: 13453-13459.
32. D'Hooge R, De Deyn PP (2001) Applications of the Morris water maze in the study of learning and memory. Brain Res Brain Res Rev 36: 60-90.
33. Lombroso PJ, Ogren MP (2008) Learning and memory, part I: brain regions involved in two types of learning and memory. J Am Acad Child Adolesc Psychiatry 47: 1228-1232.
34. Dos Reis EA, de Oliveira LS, Lamers ML, Netto CB, Wyse AY (2003) L-arginine administration inhibits hippocampal NA (+)-ATPase activity and impairs retention of an inhibitor avoidance task in rats. Brain Res 951: 151-157.
35. Tan SE (2007) Roles of hippocampal nitric oxide and calcium/calmodulin-dependent protein kinase II in inhibitory avoidance learning in rats. Behav Pharmacol 18: 29-38.
36. Calabrese V, Mancuso C, Calvani M, Rizzarelli E, Butterfield DA, et al. (2007) Nitric oxide in the central nervous system: neuroprotection versus neurotoxicity. Nat Rev Neurosci 8: 766-775.
37. Kwak YD, Wang R, Li JJ, Zhang YW, Xu H, et al. (2011) Differential regulation of BACE1 expression by oxidative and nitrosative signals. Mol Neurodegener 6: 17.
38. Huang YY, Pittenger C, Kandel ER (2004) A form of long-lasting, learning-related synaptic plasticity in the hippocampus induced by heterosynaptic low-frequency pairing. PNAS 101: 859-864.
39. Xu ZP, Li L, Bao J, Wang ZH, Zeng J (2014) Magnesium Protects Cognitive Functions and Synaptic Plasticity in Streptozotocin-Induced Sporadic Alzheimer's Model. PLoS ONE 9: e108645.
40. Austin SA, Santhanam AV, Hinton DJ, Choi DS, Katusic ZS (2013) Endothelial nitric oxide deficiency promotes Alzheimer's disease pathology. J Neurochem 127: 691-700.
41. Christen Y (2000) Oxidative stress and Alzheimer disease. Am J Clin Nutr 71: 621S-629S.
42. Barnham KJ, Masters CL, Bush AI (2004) Neurodegenerative diseases and oxidative stress. Nat Rev Drug Discov 3: 205-214.
43. Giasson B, Ischiropoulos H, Lee V, J Trojanowski (2002) The relationship between oxidative/nitrative stress and pathological inclusions in Alzheimer's and Parkinson's diseases. Free Radic Biol Med 32: 1264-1275.
44. Rajasekar N, Dwivedi S, Nath C, Hanif K, Shukla R (2014) Protection of streptozotocin induced insulin receptor dysfunction, neuroinflammation and amyloidogenesis in astrocytes by insulin. Neuropharmacol 86: 337-352.
45. Dringen R (2000) Metabolism and functions of glutathione in brain. Prog Neurobiol 62: 649-671.
46. Krause MS, McClenaghan NH, Flatt PR, Homem de Bittencourt PI, Murphy C (2011) L-Arginine is essential for pancreatic β-cell functional integrity, metabolism and defense from inflammatory challenge. J Endocrinol 211: 87-97.
47. Petrovic V, Buzadzic B, Korac A, Vasilijevic A, Jankovic A et al. (2009) L-Arginine supplementation induces glutathione synthesis in interscapular brown adipose tissue through activation of glutamate-cysteine ligase expression: The role of nitric oxide. Chem Biol Interact 182: 204-212.
48. Tsukahara H, Hiraoka M, Kobata R, Hata I, Ohshima Y, et al. (2000) Increased oxidative stress in rats with chronic nitric oxide depletion: measurement of urinary 8-hydroxy-2'-deoxyguanosine excretion. Redox Rep 5: 23-28.

49. Paul V, Reddy L, Ekambaram P (2005) A reversal by L-arginine and sodium nitroprusside of ageing-induced memory impairment in rats by increasing nitric oxide concentration in the hippocampus. Indian J Physiol Pharmacol 49: 179-186.

50. Wei XM, Yang W, Liu LX, Qi WX (2013) Effects of L-arginine and Nω -nitro-L-arginine methylester on learning and memory and α7 nAChR expression in the prefrontal cortex and hippocampus of rats. Neurosci Bull 29: 303-310.

51. Dos Santos FS, Da Silva LA, Pochapski JA, Raczenski A, Da Silva WC, et al. (2014) Effects of L-arginine and creatine administration on spatial memory in rats subjected to a chronic variable stress model. Pharm Biol 52: 1033-1038.

52. Silva EP Jr, Borges LS, Mendes-da-Silva C, Hirabara SM, Lambertucci RH (2017) L-Arginine supplementation improves rats' antioxidant system and exercise performance. Free Radical Res 51: 281-293.

53. Zuo L, Hemmelgarn BT, Chuang CC, Best TM (2015) The role of oxidative stress-induced epigenetic alterations in amyloid- production in Alzheimer's disease. Oxid Med Cell Longev 2015: 13.

54. Cai Z, Zhao B, Ratka A (2011) Oxidative stress and β-amyloid protein in Alzheimer's disease. Neuromol Med 13: 223-250.

55. Lamoke F, Mazzone V, Persichini T, Maraschi A, Harris MB, et al. (2015) Amyloid β peptide-induced inhibition of endothelial nitric oxide production involves oxidative stress-mediated constitutive eNOS/HSP90 interaction and disruption of agonist-mediated Akt activation. J Neuroinflamm 12: 84.