

## Effects of Erythropoietin on Memory Deficits and Brain Oxidative Stress in the Mouse Models of Dementia

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The present study was undertaken to explore the potential of erythropoietin in memory deficits of mice. Memory impairment was produced by scopolamine (0.5 mg/kg, *i.p.*) and intracerebroventricular streptozotocin (*i.c.v.* STZ, 3 mg/kg, 10  $\mu$ l, 1<sup>st</sup> and 3<sup>rd</sup> day) in separate groups of animals. Morris water-maze test was employed to assess learning and memory. The levels of brain thio-barbituric acid reactive species (TBARS) and reduced glutathione (GSH) were estimated to assess degree of oxidative stress. Brain acetylcholinesterase enzyme (AChE) activity was also measured. Scopolamine/streptozotocin administration induced significant impairment of learning and memory in mice as indicated by marked decrease in Morris water-maze performance. Scopolamine/streptozotocin administration also produced a significant enhancement of brain AChE activity and brain oxidative stress (an increase in TBARS and a decrease in GSH) levels. Treatment of erythropoietin (500 and 1,000 IU/Kg *i.p.*) significantly reversed scopolamine- as well as streptozotocin-induced learning and memory deficits along with attenuation of those-induced rise in brain AChE activity and brain oxidative stress levels. It may be concluded that erythropoietin exerts a beneficial effect in memory deficits of mice possibly through its multiple actions including potential anti-oxidative effect.

**Key Words:** Erythropoietin, Memory, Scopolamine, Streptozotocin, Morris water-maze

### INTRODUCTION

The human brain is a very complex and intricate machine and many factors can interfere with its functioning. With age, time and medical conditions such as atherosclerosis, stroke, high levels of cholesterol, plasma homocysteine, diabetes and genetic factors a person may lose his ability to solve problems and maintain emotional control, therefore experience personality changes and behavioral problems such as agitation, delusions, hallucinations and cognitive deficits. Such a mental condition characterized by impairment of memory and loss of intellectual ability, sufficiently severe to interfere with one's occupational or social activities is termed as dementia (Sharma et al, 2008a; 2008b). Alzheimer's disease (AD) is the most common serious type of dementia effecting elderly population. AD is a progressive neurodegenerative disorder associated with loss of neurons in distinct brain areas. Currently, over 50% cases of memory impairment account for dementia of AD (Torre et al, 2004). Clinical management of memory dysfunction associated with dementia is still a nightmare for the neurobiologist because of only limited therapeutic modalities. Currently only two categories of the drugs are available in the market *i.e.* cholinesterase inhibitors (such as donepezil,

rivastigmine and galantamine) and N-methyl-D-aspartate (NMDA) receptor antagonist (memantine). Scientists are looking for an agent who will not only reverse memory dysfunction but also progression of dementia.

Erythropoietin (EPO) is basically a glycoprotein that increases red cell mass to improve tissue oxygenation. It is produced by the kidney in response to hypoxia. Recombinant human EPO (r-Hu-EPO) is effective and widely used in treatment of anemia associated with renal failure, HIV infection, cancer and surgery. Like other members of the cytokine family to which it and its receptor belong, both are expressed within the CNS in response to hypoxia (Masuda et al, 1994; Marti et al, 1996; Juul et al, 1999). Hypoxia may not be the only relevant stimulus for brain EPO production, however, as metabolic disturbances, including hypoglycemia and strong neuronal depolarization, generate mitochondrial reactive oxygen species that may increase brain EPO expression through hypoxia inducible factor-1 (Chandel et al, 1998). EPO may thus protect nervous tissue under any condition characterized by a relative deficiency of ATP in the face of increased metabolic demands. EPO has been shown to exhibit classic neurotrophic effects *in vivo* and *in vitro* (Konishi et al, 1993;

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**ABBREVIATIONS:** STZ, streptozotocin; *i.c.v.*, intracerebroventricularly or intracerebroventricular; EPO, erythropoietin; ACSF, artificial cerebrospinal fluid; AChE, acetylcholinesterase; TBARS, thiobarbituric acid reactive species; GSH, glutathione; AD, Alzheimer's disease; HIV, human immunodeficiency virus; NMDA, N-methyl-D-aspartate; ATP, adenosine triphosphate; MWM, Morris water-maze; ELT, escape latency time; TSTQ, time spent in target quadrant; AChE, acetyl cholinesterase; LTP, long term-potential.

Tabira et al, 1995; Campana et al, 1998; Juul et al, 1999; Brines et al, 2000). In the light of above, the present study has been undertaken to investigate the effects of EPO on memory deficits and brain oxidative stress in scopolamine- and streptozotocin (STZ)-induced dementia mouse models (Lannert and Hoyer, 1998; Sharma and Gupta 2001; Parle and Singh, 2004).

## METHODS

### Animals

Swiss albino mice, weighing 20~30 g were employed in the present study (procured from Central Research Institute, Kasauli). They were maintained on standard laboratory pellet chow diet (Kisan Feeds Ltd, Chandigarh, India) and water ad libitum. The animals were exposed to natural cycles of light and dark. The mice were acclimatized to the laboratory conditions five days prior to behavioral study. The experimental protocol was duly approved by Institutional animal ethics committee and care of the animals was carried out as per the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of Environment and Forest Government of India (Reg. No. CPCSEA/107/1999).

### Drugs and reagents

Erythropoietin was purchased from Claris (India) Pvt. Ltd. Folin-Ciocalteu's Phenol reagent was purchased from Merck Limited, Mumbai, India. 5,5, dithiobis (2-nitro benzoic acid) (DTNB), reduced glutathione (GSH), Bovine serum albumin (BSA) and Thiobarbituric acid were obtained from Loba Chem, Mumbai, India. Streptozotocin (STZ) and scopolamine were purchased from Sigma-Aldrich, St. Louis, U.S.A. All the reagents used in this study were of analytical grade. STZ was dissolved in artificial cerebro spinal fluid (ACSF) prepared according to the method as described by Sakurada et al, (1999). EPO and scopolamine were dissolved in distilled water and given intraperitoneally (i.p.). STZ and ACSF were delivered intracerebroventricularly (i.c.v.).

### Intracerebroventricular administration of STZ

Mice were anesthetised with anaesthetic ether for intracerebroventricular (i.c.v.) administrations. Ether has been preferred here due to its ultrashort action and fast reversibility. Moreover brief extent of ether exposure for i.c.v. injection has been reported to exert no significant effect on learning and memory behavior of animals (Haley and McCormick, 1957). Intracerebroventricular injections were made with hypodermic needle of 0.4 mm external diameter attached to a 10  $\mu$ l Hamilton microlitre syringe (Top Syringe, Mumbai, India). The needle was covered with a polypropylene tube except 3 mm of the tip region so as to insert this much portion of the needle perpendicularly through the skull into the brain of mouse. STZ was dissolved in artificial CSF (25 mg/ml) solution, made freshly. The injection site was 1 mm to right or left midpoint on the line drawn through to the anterior base of the ears. Injections were performed into right or left ventricle randomly. Two doses of STZ (3 mg/kg) were administered by i.c.v. injection bilaterally. The second dose was administered after 48 h of first dose. The concentration was ad-

justed so as to deliver 10  $\mu$ l in an injection. Control group mice were given i.c.v. injection of artificial cerebrospinal fluid (CSF) in a similar fashion.

### Intra peritoneal (i.p.) administration of scopolamine

Mice were administered scopolamine (0.5 mg/kg, i.p.) dissolved in distilled water (10 ml/kg, i.p.) daily for 4 days, 30 min prior to acquisition trials on Morris water-maze.

### Morris water maze (MWM)

Morris water-maze test was employed to assess learning and memory of the animals (Parle and Singh, 2004; Morris, 1984). MWM is a swimming based model where the animal learns to escape on to a hidden platform. It consisted of large circular pool (150 cm in diameter, 45 cm in height, filled to a depth of 30 cm with water maintained at 28 $\pm$ 1 $^{\circ}$ C). The water was made opaque with white colored non-toxic dye. The tank was divided into four equal quadrants with the help of two threads, fixed at right angle to each other on the rim of the pool. A submerged platform (10 $\times$ 10 cm), painted in white was placed inside the target quadrants of this pool, 1 cm below surface of water. The position of platform was kept unaltered throughout the training session. Each animal was subjected to four consecutive training trials on each day with inter-trial gap of 5 min. The mouse was gently placed in the water between quadrants, facing the wall of pool with drop location changing for each trial, and allowed 120 sec to locate submerged platform. Then, it was allowed to stay on the platform for 20 sec. If it failed to find the platform within 120 sec, it was guided gently onto platform and allowed to remain there for 20 sec. Day 4 escape latency time (ELT) to locate the hidden platform in water maze was noted as an index of acquisition or learning. Animal was subjected to training trials for four consecutive days, the starting poison was changed with each exposure as mentioned below and target quadrant (Q4 in the present study) remained constant throughout the training period.

Day1	Q1	Q2	Q3	Q4
Day2	Q2	Q3	Q4	Q1
Day3	Q3	Q4	Q1	Q2
Day4	Q4	Q1	Q2	Q3

On fifth day, platform was removed and each mouse was allowed to explore the pool for 120 sec. Mean time spent in all four quadrants was noted. The mean time spent by the animal in target quadrant searching for the hidden platform was noted as index of retrieval or memory.

The experimenter always stood at the same position. Care was taken that relative location of water maze with respect to other objects in the laboratory serving, as prominent visual clues were not disturbed during the total duration of study. All the trials were completed between 09.00 to 17.00 h.

### Collection of samples

Animals were sacrificed by cervical dislocation; brains were removed and homogenized in phosphate buffer (pH=7.4). The homogenates were then centrifuged at 3,000 rpm for 15 min. The supernatant of homogenate was used for biochemical estimation as per the following methods.

**Estimation of brain acetyl cholinesterase (AChE) activity**

The whole brain AChE activity was measured by the method of Ellman, et al, (1961) and Voss and Sachsse, (1970). Change in absorbance per min of the sample was read spectrophotometrically at 420 nm.

**Estimation of brain thiobarbituric acid reactive species (TBARS) level**

The whole brain TBARS level was measured by the method of Okhawa et al, (1979). The absorbance was measured spectrophotometrically (DU 640B spectrophotometer, Beckman Coulter Inc., CA, USA) at 532 nm.

**Estimation of brain reduced glutathione (GSH) level**

The whole brain GSH level was measured by the method of Beutler et al, (1963). The absorbance was measured spectrophotometrically at 532 nm.

**Estimation of brain total protein**

Total brain protein was estimated using method of Lowry et al, (1951). The absorption was read spectrophotometrically at 750 nm.

**Experimental protocol**

In total 70 mice were employed in the study. The animals were divided into 10 groups, each comprising 07 mice.

**Group I (Control)**

Mice were administered distilled water (10 ml/kg, i.p.) 30 min before acquisition trials conducted from day 1 to day 4 and 30 min before retrieval trial conducted on day 5.

**Group II (Artificial cerebrospinal fluid {ACSF} control)**

Mice were injected intracerebroventricularly artificial cerebrospinal fluid (i.c.v., ACSF, 25 mg/ml, 10  $\mu$ l) in two dosage schedules i.e. on first and third day followed by exposure to Morris Water Maze (MWM) test after 15 days.

**Group III (scopolamine control)**

Mice were administered scopolamine (0.5 mg/kg, i.p.) 30 min before acquisition trial conducted from day 1 to day 4 and then vehicle (distilled water) only 30 min prior to retrieval trial conducted on day 5.

**Group IV (streptozotocin control)**

Mice were injected intracerebroventricularly streptozotocin (STZ i.c.v., 3 mg/kg, 10  $\mu$ l) in two dosage schedules i.e. on first and third day followed by exposure to MWM test after 15 days.

**Group V (EPO per se- Low dose)**

Mice were administered EPO (500 IU/Kg, i.p.) 30 min before acquisition trial conducted from day 1 to day 4. The animals were administered vehicle (distilled water) only 30 min before retrieval trial conducted on day 5.

**Group VI (EPO per se- High dose)**

Mice were administered EPO (1,000 IU/Kg, i.p.) 30 min before acquisition trial conducted from day 1 to day 4. The

animals were administered vehicle (distilled water) only 30 min before retrieval trial conducted on day 5.

**Group VII (EPO {Low dose}+scopolamine)**

Mice were treated with EPO (500 IU/kg, i.p.) and then after 30 min with scopolamine (0.5 mg/kg, i.p.) After 30 min of scopolamine treatment these animals were then subjected to acquisition trials on MWM. The treatment of EPO+scopolamine was continued during acquisition trials from day 1 to day 4. The animals were administered vehicle (distilled water) only 30 min before retrieval trial conducted on day 5.

**Group VIII (EPO {High dose}+scopolamine)**

Mice were treated with EPO (1,000 IU/kg, i.p.) and then after 30 min with scopolamine (0.5 mg/kg, i.p.) After 30 min of scopolamine treatment these animals were then subjected to acquisition trials on Morris water maze. The treatment of EPO+scopolamine was continued during acquisition trials from day 1 to day 4. The animals were administered vehicle (distilled water) only 30 min before retrieval trial conducted on day 5.

**Group IX (STZ+EPO {Low dose})**

STZ i.c.v treated mice, were administered EPO (500 IU/kg, i.p.) starting after 2<sup>nd</sup> dose of STZ, daily for 15 days and then subjected to Morris water maze test. The administration of EPO (administered 30 min before) was also continued during acquisition trials conducted from day 1 to day 4. On day 5 animals were administered vehicle (distilled water) only, and then subjected to retrieval test after 30 min.

**Group X (STZ+EPO {High dose})**

STZ i.c.v treated mice, were administered EPO (1,000 IU/kg, i.p.) starting after 2<sup>nd</sup> dose of STZ, daily for 15 days and then subjected to Morris water maze test. The administration of EPO (administered 30 min before) was also continued during acquisition trials conducted from day 1 to day 4. On day 5 animals were administered vehicle (distilled water) only, and then subjected to retrieval test after 30 min.

**Statistical analysis**

All the results were expressed as mean $\pm$ standard error of mean (S.E.M). The data of ELT and time spent in quadrants in various groups were statistically analyzed using two-way ANOVA followed by Bonferonni test and rests of the data was analyzed using one-way ANOVA followed by Tukey's multiple range test.  $p < 0.05$  was considered to be statistically significant. Sigma Stat Statistical software version 3.5 was used for data processing.

**RESULTS**

Various pharmacological interventions employed in the present study did not show any significant mortality. Further, no significant difference was observed between the results obtained from mice of either sex.

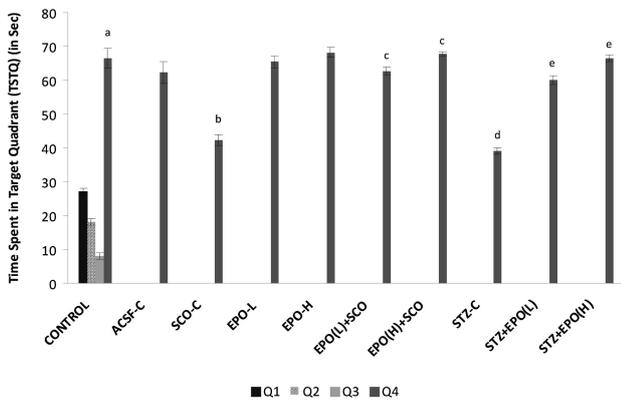
**Effect on escape latency time (ELT) and time spent in target quadrant (TSTQ) using Morris water maze**

Control animals, showed a significant decrease in their

**Table 1.** Effect of erythropoietin on scopolamine/streptozotocin-induced changes in escape latency time (ELT) using Morris water-maze

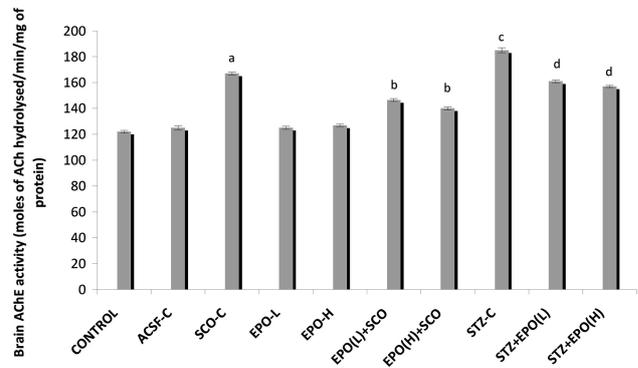
Sr. No.	Group	Dose	Day 1 ELT (sec)	Day 4 ELT (sec)
I	Control	10 ml/kg, i.p.	95.5±4.11	38.5±1.04 <sup>a</sup>
II	ACSF control	25 mg/ml, 10 $\mu$ l, i.c.v	97.2±3.64	40.2±1.40 <sup>a</sup>
III	scopolamine	0.5 mg/kg, i.p.	92.8±1.01	66±0.69 <sup>b</sup>
IV	EPO-L	500 IU/kg, i.p.	94.1±1.5	39.4±0.97 <sup>a</sup>
V	EPO-H	1000 IU/kg, i.p.	95.1±1.47	41.1±1.4 <sup>a</sup>
VI	EPO-L+scopolamine	500 IU/kg, i.p.+0.5 mg/kg, i.p.	95.4±1.96	45.1±0.76 <sup>c</sup>
VII	EPO-H+scopolamine	1000 IU/kg i.p.+0.5 mg/kg, i.p.	97.7±1.04	40±1 <sup>c</sup>
VIII	STZ	3 mg/kg, 10 $\mu$ l, i.c.v	99.1±1.10	75.5±1.41 <sup>d</sup>
IX	STZ+EPO-L	3 mg/kg, 10 $\mu$ l, i.c.v+500 IU/kg, i.p.	96.5±1.67	49.1±1.03 <sup>e</sup>
X	STZ+EPO-H	3 mg/kg, 10 $\mu$ l, i.c.v+1,000 IU/kg, i.p.	97.5±0.78	42.5±0.89 <sup>e</sup>

ACSF-C: artificial cerebrospinal fluid-control; EPO-L and H: erythropoietin (low and high); STZ: streptozotocin; ELT: escape latency time. Each group (n=7) represents mean±standard errors of means. Two-way ANOVA followed by Bonferonni's post hoc test. F (3, 24)=11.100,  $p < 0.001$  for evaluating the effect of days and F (9, 60)=50.740,  $p < 0.001$  for evaluating the effect of treatment on ELT, <sup>a</sup> $p < 0.05$  Vs Day 1 ELT in control group, <sup>b</sup> $p < 0.05$  Vs Day 4 ELT in control, <sup>c</sup> $p < 0.05$  Vs Day 4 ELT in scopolamine group, <sup>d</sup> $p < 0.05$  Vs Day 4 ELT in ACSF group, <sup>e</sup> $p < 0.05$  Vs Day 4 ELT in STZ group.



**Fig. 1.** Effect of erythropoietin on scopolamine and streptozotocin induced memory impairments using Morris water-maze. ACSF-C: artificial cerebrospinal fluid-control; SCO-C: scopolamine-control; EPO-L and H: erythropoietin (low and high); STZ: streptozotocin. Each group (n=7) represents mean±standard errors of means. Two-way ANOVA followed by Bonferonni's post hoc test. F (3, 24)=6.455,  $p < 0.0001$  for evaluating the difference in time spent in various quadrants; F (9, 60)=26.231,  $p < 0.001$  for evaluating the effect of treatment on difference in time spent in target quadrant. <sup>a</sup> $p < 0.05$  Vs time spent in other quadrants in control group, <sup>b</sup> $p < 0.05$  Vs time spent in Target Quadrant (TSTQ) of control, <sup>c</sup> $p < 0.05$  Vs TSTQ of scopolamine group, <sup>d</sup> $p < 0.05$  Vs TSTQ of ACSF group, <sup>e</sup> $p < 0.05$  Vs TSTQ of STZ group.

day 4 ELT as compared to its value noted on day 1, reflecting acquisition (learning). Further these mice significantly spent more time in the target quadrant (Q4) in search of missing platform as compared to the time spent in other quadrants (Q1, Q2, Q3) during retrieval trial conducted on day 5, indicating memory or retrieval. Treatment of vehicle artificial cerebrospinal fluid (ACSF) did not show any significant effect on day 4 ELT (Table 1) and day 5 mean time spent in target quadrant (TSTQ) of control animals (Fig. 1). Administration of scopolamine (0.5 mg/kg, i.p.)/streptozotocin (3 mg/kg, 10  $\mu$ l, i.c.v) significantly prevented decrease in day 4 ELT of respective control group (Table 1) and markedly reduced time spent time in target quadrant (Q4) in search of missing platform during retrieval trial reflect-



**Fig. 2.** Effect of erythropoietin on scopolamine/streptozotocin-induced changes in brain AChE activity. ACSF-C: artificial cerebrospinal fluid-control; SCO-C: scopolamine-control; EPO-L and H: erythropoietin (low and high); STZ: streptozotocin. Each group (n=7) represents mean±standard errors of means. One-way ANOVA followed by Tukey's multiple range test; F (9, 60)=15.851,  $p < 0.001$ . <sup>a</sup> $p < 0.05$  Vs control group, <sup>b</sup> $p < 0.05$  Vs scopolamine, <sup>c</sup> $p < 0.05$  Vs ACSF control, <sup>d</sup> $p < 0.05$  Vs STZ.

ing impairment of both learning as well as memory (Fig. 1).

#### **Effect of erythropoietin on scopolamine/streptozotocin-induced impairment of learning and memory using Morris water maze**

Erythropoietin (500 IU/kg i.p.; 1,000 IU/kg, i.p.) *per se* did not produce any significant effect on day 4 decrease in ELT (Table 1) and day 5 increase in TSTQ of control mice (Fig. 1). However administration of EPO to scopolamine/streptozotocin treated animals, significantly attenuated day 4 rise in the ELT (Table 1) as well as day 5, decrease in TSTQ, indicating reversal of scopolamine/streptozotocin-induced memory deficits (Fig. 1).

#### **Effect of erythropoietin on scopolamine/streptozotocin-induced changes in acetylcholinesterase (AChE) activity of brain**

Scopolamine (0.5 mg/kg, i.p.)/streptozotocin (3 mg/kg,

10  $\mu$ l, i.c.v) significantly, increased the brain AChE activity when compared to control mice. EPO (500 and 1,000 IU/kg, i.p.) did not produce any significant *per se* effect on the brain AChE level as compared to control mice. Treatment of EPO significantly attenuated scopolamine as well as streptozotocin-induced rise in brain AChE activity (Fig. 2)

#### Effect of erythropoietin on scopolamine/streptozotocin-induced changes in oxidative stress levels of brain

Scopolamine (0.5 mg/kg, i.p.)/streptozotocin (3 mg/kg, 10  $\mu$ l, i.c.v) significantly, increased the brain thio barbituric acid reactive species (TBARS) level and reduced the brain GSH levels, compared to control group of animals, reflecting enhanced oxidative stress. EPO (500 and 1,000 IU/kg, i.p.) did not produce any significant *per se* effect on brain TBARS and GSH levels of control group animals. Treatment of EPO (500 and 1,000 IU/kg, i.p.) significantly reversed scopolamine as well as streptozotocin-induced rise in brain oxidative stress as reflected by a rise in brain TBARS levels and a fall in GSH levels (Fig. 3 and 4).

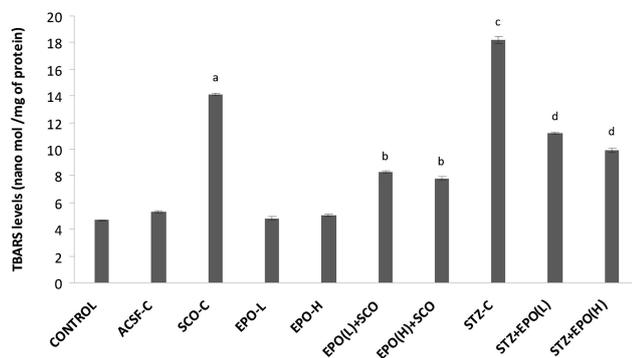
## DISCUSSION

Morris Water Maze test employed in present study is one of the most widely accepted models to evaluate learning and memory of the animals (Morris, 1984; Parle and Singh, 2004). A significant decrease in day 4 escape latency time (ELT) of control animals during ongoing acquisition trials denoted normal acquisition of memory and an increase in time spent in target quadrant (TSTQ), in search of missing platform during retrieval trial indicated, retrieval of memory. These results are consistent to our earlier findings (Parle and Singh, 2007; Sharma et al, 2008a; 2008b) and reports from other laboratory (Packard et al, 1996).

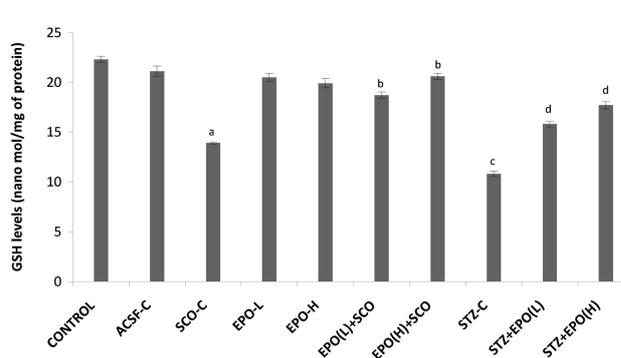
In the present study scopolamine produced impairment of acquisition and retrieval of memory as reflected by significant increase in day 4 ELT and decrease in day 5 TSTQ respectively. Acetylcholine (ACh) is a classic mediator of learning and memory (Blokland, 1995). Drugs that reduce

cholinergic function such as muscarinic receptor antagonist scopolamine, cause profound memory impairments in animals and humans (Deutsch and Rocklin, 1967; Deutsch, 1971). The degeneration and dysfunction of cortical cholinergic neurons is closely associated with cognitive deficits of AD (Bartus et al, 1982; Coyle et al, 1983). These findings provided a cholinomimetic rationale for treatment of dementia closely related to AD (Becker, 1991) and support the use of animal models using muscarinic receptor antagonists (Yamazaki et al, 1995). This contention is further supported by our study, whereby a significant impairment of learning and memory in mice treated with scopolamine has been observed. Moreover, scopolamine treatment also produced a significant enhancement of brain acetylcholinesterase (AChE) activity and increase in oxidative stress as indicated by rise in brain thioarbituric acid reactive species (TBARS) and reduction in reduced glutathione (GSH) levels which is in line with previous findings (El-Sherbiny et al, 2003; Khalifa, 2004; Agrawal et al, 2008).

In the our investigation, pretreatment of erythropoietin (EPO) significantly reversed the memory deficits induced by scopolamine along with significant attenuation of scopolamine mediated rise in brain AChE activity and brain oxidative stress levels. EPO is a hematopoietic cytokine which has recently been shown to be expressed in the nervous system (Dame et al, 2000; Siren et al, 2001; Juul, 2002). The expression of EPO and its receptor in the nervous system are modulated by hypoxia and metabolic insult (Siren et al, 2001a; 2001b; 2001e). Recent studies suggest that EPO has neuroprotective effects against various insults such as glutamate-induced excitotoxicity, serum deprivation, hypoxia, and growth factor deprivation (Morishita et al, 1997; Lewczuk et al, 2000; Siren et al, 2001a). EPO has been shown to protect primary hippocampal neurons by increasing the expression of brain-derived neurotrophic factor (Viviani et al., 2005). EPO in-vitro has also been observed to exert neurotrophic effect on cholinergic and other neurons (Tabira et al, 1995). In addition erythropoietin has also been found to exert stimulatory effect on the release of dopamine and acetylcholine in rat brain slices (Yamamoto et al, 2000). Many studies have also implicated



**Fig. 3.** Effect of erythropoietin on scopolamine/streptozotocin-induced changes in brain thio-barbituric acid reactive species (TBARS) levels. ACSF-C: artificial cerebrospinal fluid-control; SCO-C: scopolamine-control; EPO-L and H: erythropoietin (low and high); STZ: streptozotocin. Each group (n=7) represents mean $\pm$ standard errors of means. One-way ANOVA followed by Tukey's multiple range test; F (9, 60)=10.721, p<0.001. <sup>a</sup>p<0.05 Vs control group, <sup>b</sup>p<0.05 Vs scopolamine, <sup>c</sup>p<0.05 Vs ACSF control, <sup>d</sup>p<0.05 Vs STZ.



**Fig. 4.** Effect of erythropoietin on scopolamine/streptozotocin-induced changes in brain reduced glutathione (GSH) levels. ACSF-C: artificial cerebrospinal fluid-control; SCO-C: scopolamine-control; EPO-L and H: erythropoietin (low and high); STZ: streptozotocin. Each group (n=7) represents mean $\pm$ standard errors of means. One-way ANOVA followed by Tukey's multiple range test; F (9, 60)=16.522, p<0.001. <sup>a</sup>p<0.05 Vs control group, <sup>b</sup>p<0.05 Vs scopolamine, <sup>c</sup>p<0.05 Vs ACSF control, <sup>d</sup>p<0.05 Vs STZ.

potential anti-oxidative (Genc et al, 2004; Signore et al, 2006; Xue et al, 2007; Wang et al, 2009) actions of EPO. Few recent reports have documented a prominent role of erythropoietin in process of long term-potential (LTP) hence memory formation (Adamcio et al, 2008; El-Kordi et al, 2009). Erythropoietin probably exerts its actions via acting as a ligand for erythropoietin receptors and stimulating erythropoietin receptor mediated signaling cascade, which certainly plays a prominent role in process of LTP and memory (Adamcio et al, 2008; El-Kordi et al, 2009). Therefore it may be put forth that EPO in our investigation attenuated scopolamine -induced memory deficits and associated biochemical changes by virtue of its multiple actions including anti-oxidative, neuroprotective, and anti-cholinesterase actions. Anti-cholinesterase activity of EPO in brain tissue perhaps, being first report in this study. However it is difficult say at this point whether it is a direct or indirect effect of EPO.

Intracerebroventricular streptozotocin (STZ, i.c.v) in our study has also impaired learning and memory along with significant rise in brain oxidative stress levels and brain AChE activity of mice. The STZ i.c.v model has been described an appropriate animal model of dementia closely related to Alzheimer's disease, typically characterized by progressive impairment of learning abilities and memory capacities (Lannert and Hoyer, 1998). Cerebral glucose and energy metabolism is associated with oxidative stress. After i.c.v administration, the highest concentration of STZ (3 mg/kg) reaches the fornix and periventricular white matter at the level of 3<sup>rd</sup> ventricle, which shows the greatest damage (Shoham et al, 2003) and STZ i.c.v induced memory impairment is independent of its hyperglycemic effect (Mayer et al, 1990). Although the mechanism of action of STZ i.c.v on memory impairment is not yet known, it probably involves the induction of oxidative stress (Feillet-Coudray et al, 1999; Reagan et al, 2000) to which myelin is particularly vulnerable (Smith et al, 1999). Damage to myelin by oxidative stress is seen in disorders such as dementia of AD type with cognitive impairment (Braak et al, 2000). It causes desensitization of insulin receptors and biochemical changes similar to AD or ageing brain (Hoyer, 2000a; 2000b). In addition, reduced energy metabolism and synthesis of acetyl-CoA ultimately results in cholinergic deficiency and thereby memory deficit in STZ treated animals. Recent animal studies have demonstrated that STZ i.c.v produces brain changes that are hallmark of human AD (Salkovic-Petrisic et al, 2006; Grünblatt et al, 2007). This observation is in line with our previous reports whereby intracerebroventricular administration of streptozotocin at sub-diabetogenic dose has been shown to induce memory deficits along with increase in brain oxidative stress levels and brain AChE activity (Sharma et al, 2008a; 2008b; Kaur et al, 2009).

Administration of EPO to STZ i.c.v treated mice significantly prevented memory deficits as well as STZ induced elevation in brain oxidative stress levels and brain AChE activity. As mentioned above EPO in various studies has been reported to exert number of important actions like anti-oxidative (Genc et al, 2004; Signore et al, 2006; Xue et al, 2007; Wang et al, 2009), neuroprotective (Morishita et al, 1997; Lewczuk et al, 2000; Siren et al, 2001b), neurotrophic (Viviani et al, 2005) effects and a vital role in hippocampal LTP (Adamcio et al, 2008; El-Kordi et al, 2009). EPO has also shown to exert potential anti-inflammatory actions (Rui et al, 2005; Liu et al, 2006) and role of in-

flammation in the pathobiology of AD is well documented (Granic et al, 2009; Salminen et al, 2009). Therefore, with support from literature and data in hand it may be proposed that EPO mediated beneficial effect in STZ (i.c.v) dementia may be attributed to its multiple effects including anti-oxidative, anti-cholinesterase, anti-inflammatory and neuroprotective actions. Hence, it may be concluded that EPO has shown ameliorative effect in memory deficits and brain oxidative stress associated with experimental dementia. Nevertheless further studies including brain histopathological analysis are needed and underway to explore full potential of erythropoietin in dementia and to find out exact mechanism of erythropoietin transduction system in its memory preserving/improving effect.

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