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Effects of Edaravone on Scopolamine Induced-dementia in Experimental Rats

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Abstract: It has been shown that edaravone and scopolamine have contrasting effects on memory; therefore, this research paper was undertaken to evaluate the impact of edaravone treatment on learning and long-term memory shortage coupled with scopolamine induced dementia. The results showed that chronic edaravone treatment averted the deficit of long-term memory as measured by transfer latency using spatial cues in the elevated plus maze task. Moreover, edaravone protected against the weakening of antioxidant defense activity in the areas of hippocampi and cerebral cortices of scopolamine treated rats. Furthermore, the thiobarbituric acid reactive substances test revealed that edaravone prevented the detrimental effects of scopolamine on lipid peroxidation ($p<0.01$). The results suggest that edaravone treatment protected against the scopolamine generated memory deficit probably by preserving the levels of reduced glutathione and TBARS. Hence, it is found to possess neuroprotective effects in scopolamine induced memory impairment model.

Key words: Edaravone, scopolamine, memory impairment, neuroprotective effects

INTRODUCTION

The oxidative stress is one of most important factors for the neurodegenaration of brain leading to various disorders (Facchinetti *et al*., 1998; Southorn and Powis, 1998; Halliwell, 2001). Additionally, oxidative damage is considered as prevailing factor for aging and cells tend to be exposed to free radicals, which in turn lead to permanent damage of brain cells and neuronal death. Currently, Alzheimer's disease accounts for large number of prevalence, morbidity and mortality in United States and it is estimated that one in every three aged adults are dying with Alzheimer's disease (Kehrer, 1993; Southorn and Powis, 1998; McCord, 2000; Devasagayam *et al*., 2004). Free radicals generated within the brain are the major factor in Alzheimer's disease (Ikeda and Long, 1990). Reactive oxygen species causes permanent irreversible oxidative damage to brain cells, which further aggregates to loss of memory or dementia (Stohs, 1995; Valko *et al*., 2007).

The reduced glutathione and lipid peroxidation are well known elements to play an essential role in oxidative stress balance. The reduced glutathione represents the endogenous defense against oxidative stress, while lipid peroxidation illustrates the damage into the cells. Therefore, the decrease in the activity of reduced glutathione and enhancement in the thiobarbituric acid reactive substances may lead to oxidative stress (Freeman and Crapo, 1982; Halliwell, 1992; Reiter, 1995).

Scopolamine, a muscuramic receptor blocker, has been used to asses amnesia in different animals (El-Sherbiny *et al*., 2003). It causes memory impairment by anti-cholinergic actions which is an useful model to check the anti-amnesic effects of drugs (El-Sherbiny *et al*., 2003; Mishima *et al*., 2003). There is considerable evidence that scopolamine causes oxidative stress in rats leading to cognitive impairment (El-Sherbiny *et al*., 2003; Mishima *et al*., 2003). Taken together, scopolamine leads to oxidative stress through the interference with acetylcholine in central nervous system (Shi *et al*., 2010). Hence, we have selected this model as one of most suitable method to study dementia (Kang *et al*., 2003). It is understood from the fact the free radicals increases oxidative stress in brain leading to memory impairment. However, its precise mechanism is still not clear.

Edaravone is a free radical scavenger and several reports have shown that there is inverse correlation between edaravone administration and oxidative stress (Otomo *et al*., 2003; Zhou *et al*., 2013). In fact, investigators have reported that edaravone produces beneficial effects on oxidative stress markers, including glutathione, superoxide dismutase, malonaialdehyde and glutathione peroxidase. Therefore, the use of edaravone as a neuroprotective agent was suggested as a therapeutic drug for recovery of neurological disorders and treatment of neurodegenerative disorders (Otomo *et al*., 2003; Yoshida *et al*., 2011; Kikuchi *et al*., 2012; Zhou *et al*., 2013). However, the mechanism by

which edaravone antagonizes the sequence of free radical cycle and ameliorates the uncontrollable and irreversible oxidative state is under development. On the basis of literature, our study was to investigate the action of edaravone on spatial learning and memory deficit associated with scopolamine.

MATERIALS AND METHODS

Animals: Adult male albino rats were distributed randomly and a set of six animals were housed together. The cages were in animal facility with a temperature of about 24°C. All rats were allowed to acclimate for one week before the beginning of treatment. Three experimental classes were assigned; control, scopolamine, scopolamine and edaravone before the acclimation of seven days.

Drugs and chemicals: Edaravone and scopolamine were purchased from M/s. Sigma Chemicals, India.

Preparation of dosage form: Edaravone (15 mg kg^{-1} orally) was dissolved in DMSO and administered for seven days. The DMSO was used as solvent for scopolamine (1mg kg^{-1}), which was administered intraperitoneally on 7th day of the experiments.

Experimental protocol

- **Group 1:** Received vehicle (Saline with CMC) for seven consecutive days
- **Group 2:** Scopolamine hydrobromide 1 mg kg^{-1} intraperitoneal at 7th day)
- **Group 3:** Alzheimer induced+(Edaravone 15 mg kg^{-1} for seven days)

Elevated plus maze model: The cognitive function of all three groups were assessed through the elevated plus maze task. Several lines of evidence have shown that elevated plus-maze model can be used to test the hippocampus-dependent memory using spatial cues. The elevated plus-maze technique was composed of a central platform linked into four metal pieces, which form plus sign shape. The diameter was 50×10 cm for the opened metal and 50×40×10 cm for the closed one. The height of central platform and four limbs was 50 cm from the ground (Itoh *et al*., 1990). During the learning phase (i.e., seven days after the beginning of edaravone administration), each rat was located in one of the sides which was opened. Transfer latency was considered as the required time (in seconds) by which the rat moves from the open limb until it reaches any one of the closed limbs. Transfer latency was documented during the learning phase for each rat on day 7th. Twenty four hours later, the long-term memory test was performed (on the 8th day). The decrease and increase in the transfer latency indicate memory improvement and impairment, respectively.

Lipid peroxidation assay: In the current study, the thiobarbituric acid reactive substance was used to as an indicator find out lipid peroxidation (Ohkawa *et al*., 1979; Mattson, 2009). The supernatant of homogenate was added to 0.2 mL of 8.1% sodium dodecyl sulphate, 1.5 mL of 30% acetic acid (pH 3.5), 1.5 mL of 0.8% of thiobarbituric acid in a test tube. Then after, The test tubes were kept for one hour at 95°C, then 1 mL of distilled water was added, subsequently 5 mL of n-butanol-pyridine mixture (15:1 v/v) was added. The mixture was then centrifuged at 4000 g for 10 min. After that, the absorbance was determined at 532 nm using spectrophotometer.

Reduced glutathione level: The level of reduced glutathione within the hippocampus and cerebral cortex was calculated based on technique described by Beutler *et al.* (1963). In brief, the combination of trichloroacetic acid (10% w/v) and supernatant of homogenate in a ratio of 1:1 was centrifuged at 1000 g at 40°C. Then, 0.5 ml of supernatant has been added into 2 mL of 0.3 M disodium hydrogen phosphate and 0.25 mL of 0.001 M freshly prepared [5, 5`-dithiobis (2-nitrobenzoic acid) dissolved in 1% w/v citric acid]. The absorbance was measured at 412 nm through spectrophotometer.

RESULTS

Elevated plus maze studies: Edaravone averts learning and long-term memory deficit associated with scopolamine induced dementia.

In this experiment, we examined the impact of edaravone on scopolamine-induced spatial learning and long-term memory impairment using the elevated plus maze method. Transfer latency on seventh day of edaravone represented the learning acquisition, while transfer latency of next day (eighth day) indicates the long-term memory performance.

In spatial learning test, administered 45 min at day 7th, the scopolamine treated group committed significantly ($p<0.01$) more time (seconds) in finding the closed arm in the elevated plus maze than the control group (control: 22.87±4.41; scopolamine: 61.00±9.16) (Fig. 1a) indicating marked impairment of learning. Chronic edaravone treatment prevented the increase in the number of seconds in the scopolamine group (18.21±6.12) as

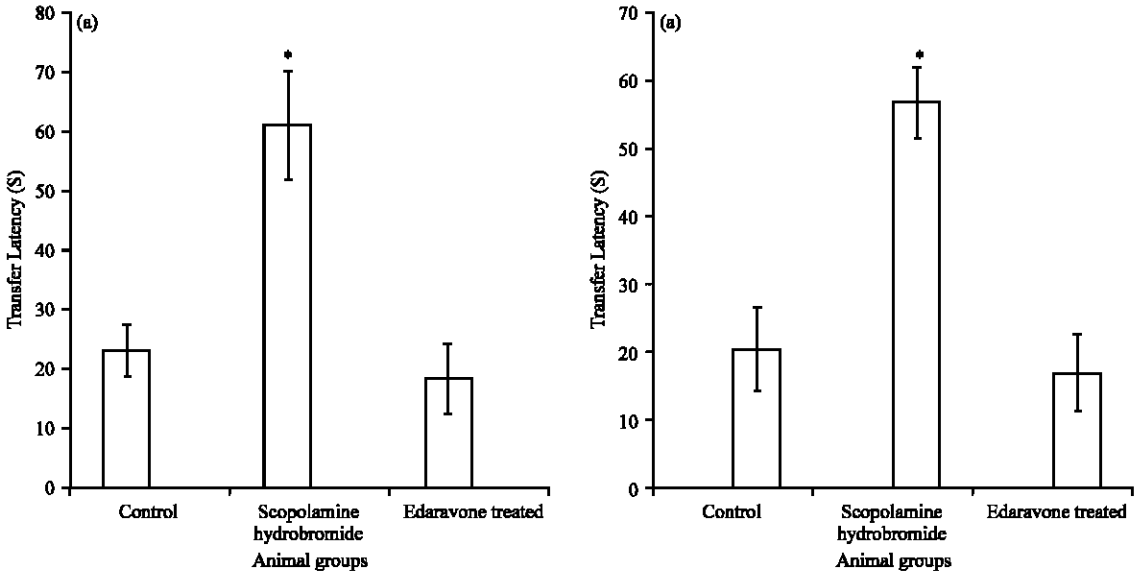

Fig. 1(a-b): The impact of edaravone on transfer latency in scopolamine-induced amnesia (a) Transfer latency on Day 7th and (b) Transfer latency on Day 8th (TL after 24 h (s)) # Diseased vs all groups, *p<0.001

revealed by the lack of significant difference from the control group (Fig. 1b).

Twenty four hours after the acquisition phase, the edaravone group made significantly (p<0.001) less time (16.91±5.62) to locate the covered arm in the long-term memory trial than the scopolamine group (Fig. 1b). Also, scopolamine administration (on the 7th day) significantly increased (p<0.01) the time required for transfer latency compared to the untreated rats (Fig. 1b).The findings suggest that chronic treatment of edaravone protects against the deleterious effects of scopolamine on cognitive function.

Antioxidant activity: To reveal the alterations in the levels of oxidative stress-related molecules that may account for the ability of edaravone to avert memory impairment in scopolamine induced cognitive loss, we determined the levels of reduced glutathione and thiobarbituric acid reactive substances from the tissue homogenate of hippocampus and cerebral cortex.

Effect of edaravone on oxidative damage marker: The Thiobarbituric Acid Reactive Substances (TBARS) is widely considered as the most common assay to measure the lipid peroxidation. In the current study, the scopolamine group revealed an increase in the TBARS levels compared to control group. In edaravone-treated rats, chronic treatment of edaravone for seven days prevented the increased in the levels of TBARS (p<0.001) as a result of single i.p. injection of scopolamine, but did not restore it as in control rats (p<0.01) (Table 1).

Table 1: The effect of edaravone on lipid peroxidation levels

Groups	TBARS (nM MDA/g of protein)	(%) Inhibition
Control group	34.41± 0.339	
Scopolamine	100.12±0.713	
Diseased+edaravone	87.56±0.339#a	12.54

Statistical analysis of data was carried by one-way ANOVA followed by Tukey's multiple range test, The values are Mean±SEM for each group (n = 6), p value less than 0.05 was considered as significant, # Diseased vs all groups, [a]p<0.001

Table 2: The effect of edaravone on reduced glutathione levels

Groups	Reduced glutathione (μmol mg^{-1} of protein)	(%) activity
Control group	10.11±0.03	
Scopolamine	6.93±0.03	
Diseased+edaravone	10.76±0.018#a	35.59

Statistical analysis of data was carried by one-way ANOVA followed by Tukey's multiple range test, The values are Mean±SEM for each group (n = 6), p value less than 0.05 was considered as significant, #Diseased vs all groups, [a] p<0.001

Effect of Edaravone on Reduced glutathione levels: Reduced glutathione is an indicator for the ability of the tissues to naturalize the free radicals (Table 2), the scopolamine treated rats showed that the levels of reduced glutathione were markedly (p<0.01) decreased (6.93±0.03) compared to the control rats (10.11± 0.03). However, chronic i.p administration of edaravone prevented the effects of scopolamine on the reduced glutathione, which was significantly (p<0.001) increased the edaravone treated rats (10.76±0.018).

DISCUSSION

Various reports have shown that edaravone and scopolamine produce beneficial and detrimental actions

on cognitive function, respectively. However, the collective impacts of edaravone and scopolamine on cognition have not been studied. I assessed the impact of edaravone administration on memory deficit produced by scopolamine through two procedures, behavioral and molecular. The findings suggest that administration of edaravone for seven days prevents scopolamine-induced long-term memory through normalizing the balance between reduced glutathione and lipid peroxidation.

There is growing body of evidence in the literature that indicates oxidative stress as crucial factor for several disorders including, dementia. Furthermore, data indicates that oxidative stress is one of the earliest events in pathogenesis of memory impairment (Facchinetti *et al*., 1998; Southorn and Powis, 1998; Halliwell, 2001; Butterfield *et al*., 2002; Devasagayam *et al*., 2004; Silva *et al*., 2004; Suzuki *et al*., 2006). The cognitive deficit in several diseases demonstrates oxidative damage via inequity on the oxidative stress balance Castellani *et al*., 2001; Halliwell, 2001; Bradley *et al*., 2010).

Scopolamine has been commonly used to induce memory deficits in experimental rats through antagonizing muscarinic cholinergic receptors. Several reports have shown that single intraperitoneal (i.p.,) injection of scopolamine blocks cholinergic transmission and impairs cognition in rats (Fan *et al*., 2005; Alikatte *et al*., 2012). In consistent with that our findings show that an i.p. dose of scopolamine damage learning and long-term memory as indicated by the increase in the transfer latency compared to control rats. Lately, it has been found that the decline in cognition as a result of single injection of scopolamine has been accompanied with changes in the brain oxidation condition (Wang *et al*., 2014). In our study, the markers of oxidative stress were measured to investigate the mechanism underlies memory impairment in scopolamine group. The results suggest that the memory deficit was as a result of an enhancement of lipid peroxidation and reduction in reduced glutathione within the areas of hippocampus and cerebral cortex.

Edaravone, a chemical with antioxidant effect, has been studied to explore its sequences at the behavioral level. The edaravone prevents long-term memory impairment induced by Alzheimer's disease using Morris water maze procedure (Yoshida *et al*., 2006; Kamida *et al*., 2009; Zhou *et al*., 2013). In the present experiments, the elevated plus maze was used to evaluate the action of edaravone on memory deficit associated with scopolamine. Edaravone protected against the decrease in the transfer latency in scopolamine group. Similar result was reported by Ueno in Japan which showed that protective action after 3 day treatment in rat chronic hypoperfusion model (Ueno *et al*., 2009). A study on Indian patients showed significant improvement after two weeks of treatment with edaravone. Furthermore and found to be harmless and efficient in treatment of neurodegenrative disorders (Sinha *et al*., 2009).

Various reports have suggested that edaravone inhibits vascular endothelial damage and hinders neurodegeneration. Indeed, pretreatment of edaravone has been found to reduce the apoptosis in hypoxic-ischemic rats (Yasuoka *et al*., 2004). Furthermore, several lines of evidence have demonstrated the positive effects of edaravone on lipid peroxidation and antioxidant enzymes. The use of edaravone has been found to decrease hydroxyl radicals and superoxide anions levels (Pan *et al*., 2010). The current findings in hippocampus and cerebral cortex support the role of edaravone on oxidative stress. The current study shows that edaravone enhanced the activity of reduced glutathione and reduced the activity of TBARS. Therefore, the protective effects of edaravone on spatial memory impairment as demonstrated by elevated plus maze could be as a result of the antioxidant activity.

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