

NEUROPROTECTIVE EFFECT OF RESVERATROL AGAINST SCOPOLAMINE-INDUCED COGNITIVE IMPAIRMENT AND OXIDATIVE STRESS IN RATS

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Abstract - The objective of this study was to examine the neuroprotective effect of resveratrol on cognitive impairment induced by scopolamine, a muscarinic antagonist, in rats. Memory impairment was induced by administration of scopolamine (1 mg/kg) intraperitoneally. Cognitive functions were assessed using radial arm maze, an active avoidance paradigm. Oxidative stress parameters like malondialdehyde, catalase and superoxide dismutase were assessed and acetylcholinesterase activity was estimated. More working and reference memory errors in the radial arm maze test and fewer avoidances in the active avoidance test were observed with scopolamine in the 1 mg/kg i.p.-treated animals. This phenomenon is a clear indication of memory impairment. Oral administration of resveratrol (20 mg/kg) inhibited the occurrence of higher working, reference memory errors and prevented the incidence of less avoidances. Resveratrol appeared to have exerted memory-enhancing effects by inhibiting acetylcholinesterase activity and prevented the rise in malondialdehyde levels and loss of antioxidant enzymes catalase and superoxide dismutase, showing antioxidant potential. Based on the above results of behavioral and biochemical studies, it can be concluded that resveratrol protected against scopolamine-induced loss of cognition. The results also indicate that resveratrol is an antioxidant and an acetylcholinesterase inhibitor, and it is likely that resveratrol's protective effect is related to its antioxidant and cholinesterase inhibitory effects.

Key words: Resveratrol, cognitive impairment, oxidative stress

INTRODUCTION

The brain is the central part of our body that controls physiological and cognitive functions. Billions of neurons in the brain connect each other to form communication networks. Normal brain functioning, including memory, is impaired when connections in the neurons are lost. Oxidative stress is one of the factors causing the death of neurons. Acetylcholine is the neurotransmitter present in cholinergic neurons responsible for memory. A decreased level of

acetylcholine due to the death of central cholinergic neurons is thought to be one of the factors for loss of memory (Ramakrishna et al., 2010). Loss of memory is the main symptom of brain damage and for a variety of disorders including Alzheimer's disease.

Scopolamine is a muscarinic receptor antagonist that blocks cholinergic neurotransmission, leading to memory impairment in rats. Recent studies have reported that scopolamine induces oxidative stress leading to memory impairment (Fan Y et al., 2005)

Many studies report that polyphenols have antioxidant capacity neutralizing free radicals by crossing the blood-brain barrier to protect the brain and nervous system. The main functions of polyphenols include improvements in memory, the immune system and heart. Resveratrol is one of the naturally occurring polyphenolic antioxidant found in red wine, grape skins, blueberries, cranberries and peanuts.

The present study was undertaken to evaluate the neuroprotective effect of resveratrol against scopolamine-induced cognitive impairment and oxidative stress. The objectives include evaluation of the effect of resveratrol on a scopolamine-induced cognitive impairment model, evaluation of the antioxidant property of resveratrol and an estimation of the acetylcholine esterase activity of resveratrol.

MATERIALS AND METHODS

Animals

All experiments were conducted using male albino Wistar rats (150-200 g), 6-8 weeks of age. All animals were obtained from Sanzyme Limited, Hyderabad. The animals were maintained with free access to food and water and kept at $25 \pm 2^\circ\text{C}$ under a controlled 12 h light/dark cycle.

Treatment schedule

Animals were weighed and divided into five groups, each group containing 6 animals. The animal groups and their treatment are given in Table 1.

The animals were trained by conducting one daily training trial during which they did not receive any drug. Completely trained animals were chosen for the study. These animals were dosed once a day with the respective drugs for 8 days, along with the daily training trial. Scopolamine was given on the eighth day 45 min after treatment. After one hour, all animals were tested on radial arm maze performance and the active avoidance test.

Behavioral models

Radial arm maze

Behavioral training began after the rats were food-deprived to 85% of their original body weight. Each animal was weighed daily and manually fed with 2-3 food pellets. The food-deprived weight of the animals was attained within 6 days (Russell et al., 2005)

For the first phase of behavioral training, rats were habituated to the maze for four consecutive days. During habituation, food pellets dipped in sucrose solution were placed in food cups of all eight arms. Rats were released on the center platform and given 10 min (or until all food pellets were consumed) to explore all eight arms.

Finally, on the third and fourth days of habituation, the rats were placed in the maze for 5 min with one drop of sucrose solution in the food well at the end of each arm. On all days, the arms the rats visited were recorded to ensure that the rats were visiting all arms of the maze.

After habituation was completed, the second phase of behavioral training began. Four arms were randomly selected and baited. Rats were released from the center platform and arm visits were recorded. The training trial was considered complete when all four pellets were consumed or 5 min had passed. Once all the rats had reached a set criterion of a minimal (average) 75% correct attempts, training with drugs began. These animals were dosed once a day with the respective drugs for 8 days, along with daily training trial. Scopolamine was given on eighth day 45 min after treatment. After one hour, all animals were tested on the radial arm maze.

Active avoidance

Prior to avoidance training, each rat was habituated to the apparatus for 2 min. At the beginning of each session a rat was placed in the left compartment close to and facing the end wall. In each trial the animal is subjected to a light for 30 s, followed by a sound

stimulus for 10 s. Immediately after the sound stimulus, the rat received a single low intensity foot shock (0.5 mA; 3 s) through the grid floor. Each animal received a daily session of 15 trials with an inter-trial duration of 15 s for 5 days, i.e. a maximum of 75 trials. Transfer time from one compartment to another, number of avoidances (after the stimulus of either light alone or both light and sound) and escape (after the foot shock) response were recorded. The criterion for improved cognitive activity was taken as a significant increase in the avoidance response on 5th session (retention) as compared to 1st session (training). All the behavioral models were carried out in a semi-dark soundproof room in order to overcome external interferences in the experiment (Alikatte et al., 2012).

Biochemical estimation of markers of oxidative stress

Biochemical tests were conducted 24 h after the last behavioral test. The animals were sacrificed by decapitation. Brains were removed and rinsed with ice-cold isotonic saline. Brains were then homogenized with ice-cold phosphate buffer (pH 8). The homogenates (10% w/v) were then centrifuged at 10,000rpm for 15 min and the supernatant formed was used for the biochemical estimations.

Estimation of acetylcholinesterase activity

The acetylcholinesterase activity was estimated using Ellman's method (Ellman et al., 1959).

Estimation of superoxide dismutase

The enzyme superoxide dismutase (SOD) was determined in brain homogenate using a photo-oxidation method, which is briefly described (Arutla et al., 1998): 0.88ml of riboflavin solution (1.3×10^{-5} M in 0.01M potassium phosphate buffer, pH 7.5) was added to 60 ml of o-dianisidine solution (10^{-2} M in ethanol), and to this 100 ml of clear separated SOD (brain homogenate) was added; optical density was measured at 460 nm. The cuvette containing reaction mixture was transferred to the illuminating box, illuminated for 4 min, and the optical density was remeasured against

a blank containing ethanol in place of enzyme. The change in optical density was determined.

Estimation of lipid peroxidation (LPO)

The amount of lipid peroxidation products present in the homogenate samples of brain was estimated by the thiobarbituric acid reactive substances (TBARS) assay (Okhawa et al., 1979) which measures the malondialdehyde (MDA) reactive products by using UV-visible spectroscopy.

Estimation of catalase activity

Catalase activity was assessed by the method of Luck (Luck et al., 1971) wherein the breakdown of hydrogen peroxide is measured.

Statistical analysis

The results were presented as the mean \pm SEM. Statistical analysis was done by ANOVA followed by Bonferroni's test. $P < 0.05$ was considered as statistically significant.

RESULTS

Effect of resveratrol on reference memory

The occurrence of reference memory errors was more in the scopolamine (1 mg/kg) treated group compared to the control group, indicating an induction of memory impairment. Oral administration of resveratrol (20 mg/kg) showed a protective effect against scopolamine-induced memory impairment by preventing more reference memory errors (Table 2).

Effect of resveratrol on working memory

More working memory errors were observed in the scopolamine-treated group when compared to the control group, indicating memory impairment. Oral administration of resveratrol (20 mg/kg) exhibited a protective effect against scopolamine-induced memory impairment (Table 2).

Table 1. Animal groups and treatments.

Groups		Treatment
Group-I	Normal control	Vehicle
Group-II	Disease control	Scopolamine 1 mg/kg i.p.
Group-III	Standard	Piracetam (200mg/kg) i.p. + Scopolamine (1mg/kg) i.p.
Group-IV	Test 1	Resveratrol (10 mg/kg) oral + Scopolamine (1mg/kg) i.p.
Group-V	Test 2	Resveratrol (20 mg/kg) oral + Scopolamine (1mg/kg) i.p.

Table 2. The effect of resveratrol on various behavioral parameters and AchE levels.

Groups	No. of reference memory errors	No. of working memory errors	No. of avoidances	AchE(μ moles/min/mg protein)
Normal control	0.8 \pm 0.16	0.50 \pm 0.22	10.83 \pm 0.30	118 \pm 1.76
Scopolamine	2.5 \pm 0.22 *	2.00 \pm 0.26 *	6.167 \pm 0.30 *	206 \pm 3.11*
Piracetam	1.0 \pm 0.25 #	0.83 \pm 0.16 #	9.500 \pm 0.22 #	128 \pm 1.20 #
Test drug (lower dose)	1.5 \pm 0.22 #	0.33 \pm 0.22 #	8.167 \pm 0.16 #	165 \pm 2.17 #
Test drug (higher dose)	1.2 \pm 0.16 #	1.00 \pm 0.00 #	9.000 \pm 0.25 #	138 \pm 1.66 #

*P< 0.05 compared with normal control group; #p<0.05 compared with scopolamine-treated group.

Table 3. The effect of resveratrol on oxidative stress and antioxidant enzymes.

Groups	SOD(% superoxide ion scavenging activity)	Catalase (% H ₂ O ₂ scavenging activity)	Lipid Peroxidation (nmoles/g brain tissue)
Normal control	60.99 \pm 0.49	71.92 \pm 0.42	26.25 \pm 0.51
Scopolamine	43.78 \pm 0.84 *	54.26 \pm 0.76 *	46.32 \pm 0.81 *
Piracetam	57.85 \pm 0.65 #	68.62 \pm 0.67 #	30.29 \pm 0.66 #
Test (lower dose)	50.77 \pm 0.33 #	60.60 \pm 0.62 #	38.99 \pm 0.60 #
Test (higher dose)	54.58 \pm 0.55 #	64.28 \pm 0.83 #	33.62 \pm 0.79 #

*P< 0.05 compared with normal control group. #p<0.05 compared with scopolamine-treated group

Effect of resveratrol on the avoidance paradigm against scopolamine-induced cognitive impairment.

In active avoidance test, the number of avoidances was less in the scopolamine-treated group when compared with the control group, indicating memory impairment. Resveratrol (20 mg/kg) apparently possessed a protective effect against scopolamine-induced memory impairment by inhibiting the incidence of a lower number of avoidances (Table 2).

Effect of resveratrol on acetylcholinesterase activity in scopolamine-treated rats

In the present study, the results show that scopolamine significantly elevated brain acetylcholinesterase activity. However, resveratrol at a dose of 20mg/kg seemed to have memory-enhancing effects by inhibiting the elevation of acetylcholinesterase activity when compared with the scopolamine-treated group (Table 2).

Effect of resveratrol on oxidative parameters in scopolamine-treated rat brain

The antioxidant activity of enzymes such as superoxide dismutase (SOD) and catalase were significantly inhibited in the scopolamine-treated group when compared with the control group. Resveratrol significantly prevented loss of activity of these antioxidant enzymes when compared to scopolamine-treated group.

Scopolamine treatment significantly increased the brain malondialdehyde (MDA) levels compared to control group. Resveratrol significantly prevented the rise in brain MDA levels compared to scopolamine-treated group (Table 3).

DISCUSSION

Formation of memory is a very complex process involving multiple neuronal pathways and neurotransmitters. The cholinergic neuronal system is known to play an important role in memory in humans and animals (Blokland et al., 1996). Based on the cholinergic hypothesis, many attempts have been made to reverse cognitive deficits by increasing brain cholinergic activity via acetylcholinesterase (AChE) inhibitors.

In this neurobehavioral study, the effects of resveratrol on cognitive parameters, the radial arm maze and active avoidance task, after scopolamine-induced memory loss in rats. Radial arm maze performance is an appetitive motivation task that is useful to assess the spatial reference as well as spatial working memory performance (Kulkarni et al., 2005). Results of this study showed that oral administration of resveratrol significantly prevented the higher reference memory and working memory errors, suggesting that resveratrol ameliorated the memory impairment.

From the active avoidance test, it was clearly seen that resveratrol had a protective effect, indicating a therapeutic efficacy of resveratrol against memory loss. Oxidative stress is a critical detriment factor

stimulating neuronal cell death. Through generation of reactive oxygen species (ROS) resulting in oxidative damage within the cell, it leads to memory deficits. In the present study, resveratrol was found to prevent the rise in malondialdehyde levels and loss of antioxidant enzymes catalase and superoxide dismutase, showing an antioxidant potential.

In the present study, resveratrol seemed to prevent the memory-disturbing effect of scopolamine, probably by acting on acetylcholinesterase (AChE). The results of this study suggest that a decrease of acetylcholinesterase activity by resveratrol can contribute to increased levels of acetylcholine and consequently improve cognitive functions.

CONCLUSION

The present study demonstrates the beneficial effect of resveratrol on scopolamine-induced cognitive impairment. Resveratrol significantly ameliorated the cognitive deficit. Resveratrol protected memory as shown by behavioral tests using the radial arm maze and active avoidance test. Based on the results of behavioral and biochemical studies, it can be concluded that resveratrol protected against scopolamine-induced loss of cognition. The results also indicated that resveratrol is an antioxidant and an acetylcholinesterase inhibitor; suggesting that resveratrol's protective effect were related to its antioxidant and cholinesterase inhibitory effects.

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