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Ameliorative Impact of Piracetam on Cognitive Dysfunction Associated with Animal Model of Alzheimer's Disease

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ABSTRACT

Most of the current evidence for the impact of piracetam on memory consolidation originates from animal and human studies. However, the exact mechanism that underlies memory improvement is yet to be determined. The effects of piracetam pre-treatment on scopolamine induced cognitive dysfunction was examined. In the elevated plus maze, piracetam administration protected against the deterioration of short-term and long-term memory in scopolamine treated rats. Molecular experiments from tissue homogenate of cerebral cortex and hippocampus indicated that the marked decrease of the reduced glutathione activity in scopolamine treated rats was prevented by the use of piracetam. Taken together, the current study suggests that piracetam protects learning and memory impairment induced by scopolamine through restoring the anti-oxidant activity into the normal level.

INTRODUCTION

Muscarinic receptors are involved in a variety of physiological processes including, modulation of cognitive function (Caulfield *et al.,* 1993; Felder *et al.,* 2000; Langmead *et al.,* 2008). In fact, scopolamine, a muscarinic receptor blocker, has been reported to moderate the acquisition of new information and retention of memory, negatively (El-Sherbiny *et al.,* 2003; Mishima *et al.,* 2003). Moreover, scopolamine impairs cerebral cortex and hippocampus dependent memory in the Morris water maze, elevated plus maze, radial water maze, passive avoidance, radial arm maze, and inhibitory avoidance tests. Therefore, scopolamine is commonly used as an animal model for induction of amnesia to assess the efficacy of new drugs as memory protector or enhancer agents (Klinkenberg and Blokland, 2010).

Reduced glutathione, which is an antioxidant, plays a central role in the cellular defense against reactive oxygen species in the nervous system. Indeed, increase in the generation of reactive oxygen species and/or decrease in the antioxidants can result in oxidative stress (Freeman and Crapo, 1982; Halliwell, 1992; Reiter, 1995). Several lines of evidence have suggested that reactive oxygen species causes neuronal death in Alzheimer's disease (Bains and Shaw, 1997, Zhou *et al.,* 2013). Moreover, deficiency in the levels of reduced glutathione in newborn animals leads to oxidative damage in the brain and enhances the harmful impact of insults in ischemia (Kumral*et al.,* 2005).

Piracetam (2-oxo pyrrolidone), which is a cyclic derivative of the neurotransmitter gamma-aminobutyric acid, has been found to enhance cognition and avert mild anxiety in clinical trials (Croisile*et al.,* 1993; Waegemans*et al.,* 2003). Although the beneficial effects of piracetam on learning and memory have been extensively investigated, the mechanism of action is still not completely elucidated. Recently, it has been found that adenosinergic system is probably the target of piracetam. In fact, piracetam reverses the decrease in adenosine triphosphate diphosphohyrolase and 5'-neucleotidase and adenosine deaminase activity induced by scopolamine in the areas of hippocampus and cerebral cortex (Mariscos*et al.,* 2013). These enzymes play important roles in the modulation of learning and memory.

Numerous epidemiological and animal reports have revealed that piracetam is well documented to have beneficial effects on cognitive dysfunction in different neurodegenerative disorders such as Alzheimer's disease and epilepsy as well as in traumatic brain injury (Croisile*et al.,* 1993; Waegemans*et al.,* 2003; Rüthrich*et al.,* 1999; Chaudhry *et al.,* 1992; Gualtieri, 1988). The long-term memory potentiation is widely accepted as the

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hypothetical model of learning and memory. Accordingly, previous findings have revealed that piracetam enhances long-term potentiation in hippocampus and cerebral cortex (Satoh *et al.*, 1988). The beneficial effects of piracetam on synaptic plasticity can be explained by its ability to increase the levels of brain-derived neurtrophic factor, which is well known to maintain neuronal growth, neurogenesis and long-term potentiation (Iuluet *al.*, 2009; Kovalchuk*et al.*, 2002; Patterson *et al.*, 1996). The present study investigates the actions of piracetam on the destructive effects of scopolamine on learning and memory.

MATERIALS AND METHODS

Animals

Adult albino Wistar rats (175-200 g body weight) were housed in a controlled room temperature of 24-26 C° on a 12/12-hr light/dark cycle. The rats were adapted for seven days after arrival and had free access to standard rodent food and water.

Drugs and chemicals

Piracetam (Sigma Chemicals, India) and scopolamine (Sigma Chemicals, India) were used in this study.

Experimental protocol

The experimental animals were randomly allocated into three groups (n=6) namely; control, Scopolamine, and Piracetam/Scopolamine. Control rats received 0.1% DMSO orally for 7 days. The second group was received Scopolamine (1mg/kg) in 0.1% DMSO in day seven by i.p. injection. The third group was received Piracetam, 200 mg/kg orally every day for 7 days and i.p. injection of Scopolamine (1mg/kg) in 0.1% DMSO in day seven.

Elevated plus maze model

Elevated plus-maze (EPM) served as hippocampus-dependent memory test using spatial cues. The EPM consisted of a central platform linked into four arms making a plus sign shape. The maze height was 50 cm from the flour. The end of two arms was closed (50 cm x 40 cm x 10 cm) while the other two were opened (50 cm x10 cm) (Itoh *et al.*, 1990). During learning phase and long-term memory test phase (i.e., seven days after the beginning of Piracetam administration), rats were located individually at the end of one of the open arms. Transfer latency is defined as the time taken by the rat, with all of its legs, to reach the covered arm. The significant decrease in the transfer latency suggests improvement of memory.

Lipid peroxidation assay

Thiobarbituric acid-reactive substances (TBARS) were quantitated as an indicator for lipid peroxidation (Ohkawa*et al.*, 1979, Mattson *et al.*, 2009). To the brain tissue supernatant , 0.2 mL of 8.1% sodium dodecyl sulphate , 1.5 mL of 30% acetic acid (pH 3.5), and 1.5 mL of 0.8% of thiobarbituric acid were added. The resultant mixture was then kept for 60 min at 95 °C. Then after, 1 mL of distilled water and 5 mL of n-butanol-pyridine mixture (15:1 v/v) were added respectively. The mixture was centrifuged at 4000 g for 10 min. Finally, TBARS levels were determined spectrophotometrically at 532 nm and expressed as nanomoles per mg of protein.

Reduced glutathione level

The reduced glutathione (GSH) level in the brain tissue samples was measured according to the standard method (Beutler *et al.*, 1963, Zhang *et al.*, 2012). The supernatant of brain tissue homogenate was mixed with trichloroacetic acid (10% w/v) in 1:1 ratio. The resultant mixture was then centrifuged at 1000 g for 10 min at 40°C. Thereafter, 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] were added to 0.5ml of the supernatant. The absorbance was measured spectrophotometrically at 412 nm.

Statistical analysis

Results were descriptively reported as mean ± standard error of the mean (SEM). Differences amongst the three experimental groups were investigated using one-way analysis of variance (ANOVA), and post-hoc Tukey's multiple range test using GraphPad Prism. Statistical significance was predetermined as ($p<0.05$).

RESULTS

Behavioral tests

Pre-treatment of piracetam ameliorates cognitive loss associated with Alzheimer's disease

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In this section, I examined the impact of piracetam administration on scopolamine-induced memory loss using the elevated plus maze task.

Learning trails

During the learning (acquisition) phase, control scopolamine and piracetam\scopolamine groups were trained to locate the covered arm within the same situation. Rats in the control, and

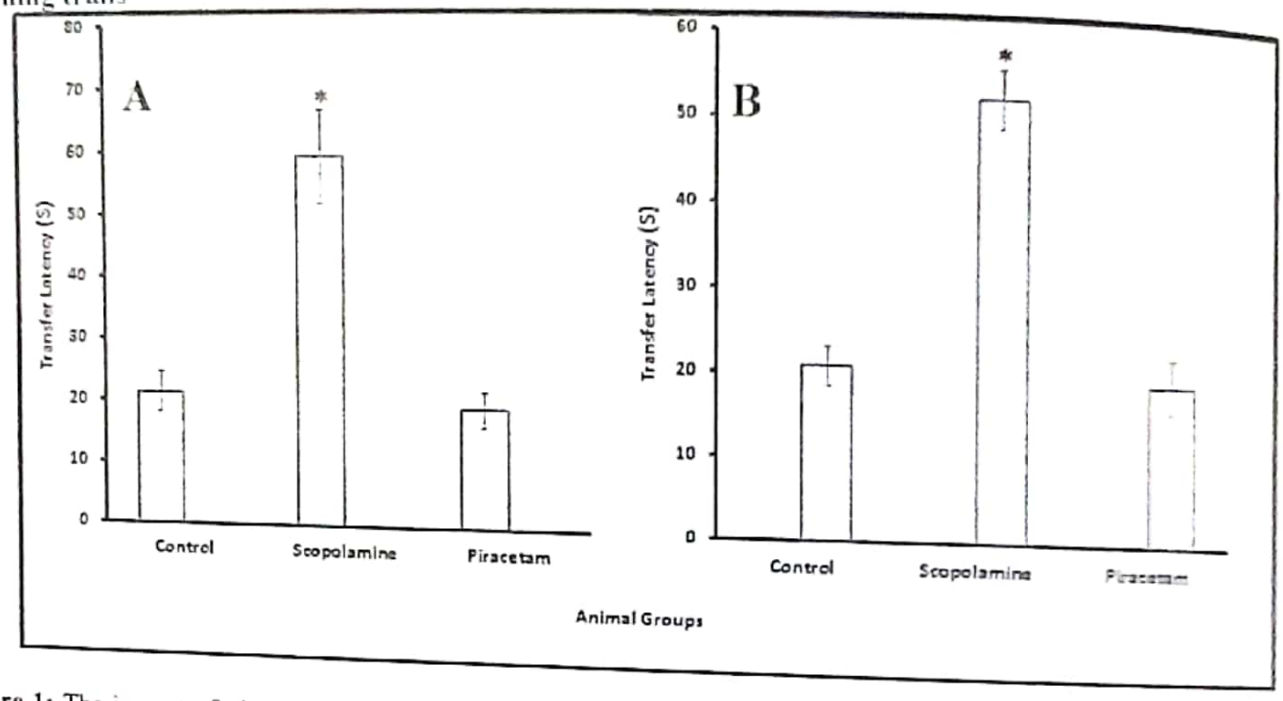

Figure 1: The impact of piracetam on transfer latency in scopolamine-induced memory loss. (A)Transfer latency on Day 7th. (B) Transfer latency on Day 8th (TL after 24 h (s)). Diseased vs all groups, * $p < 0.001$.

piracetam/scopolamine groups found the location of the covered arm at equivalent rates of 45 min after the learning phase (21.45 ± 3.22; 19.25 ± 2.90). In contrast, the scopolamine group ability to find the closed arm was significantly impaired (59.40 ± 7.40) as indicated by more time needed as compared to the other two experimental groups (fig. 1A).

Spatial long-term memory

In the long-memory test administered 24 hour after the end of the acquisition phase, the scopolamine group used more time (52.15 ± 3.50) in finding the closed arm in the elevated plus maze than the control group (20.75 ± 2.35) (fig. 1B) suggesting significant deficit of long-term memory. Sub-chronic piracetam treatment ameliorated long-term memory impairment in scopolamine-treated rats as found by the insignificant difference (19.10 ± 3.20) from control group (fig. 1B). These results suggests that pre-treatment with piracetam averts the deleterious effect of scopolamine on learning and memory.

Molecular experiments

Levels of oxidative stress markers

This section was carried out to validate the behavioral results and to elucidate the changes in the levels of reduced glutathione and thiobarbituric acid reactive substances that are indices for antioxidant and lipid peroxidation.

Effect of piracetam on reduced glutathione and thiobarbituric acid reactive substances

In the current study, the effect of scopolamine on the basal levels of reduced glutathione in mixed tissue homogenate of hippocampus and cerebral cortex regions was evaluated. It was found that there was significant reduction in the levels of reduced glutathione of scopolamine rats (6.43 ± 0.05) compared to control rats (12.11 ± 0.07) (Fig. 2). However, piracetam pre-treated rats showed no difference in the levels of reduced glutathione (12.74 ± 0.01) compared to control rats. Therefore, piracetam pre-treatment prevented the decrease in the levels of reduced glutathione associated with scopolamine administration.

Thiobarbituric acid reactive substances play an important role in hippocampal and cortical dependent-memory. The present results show that scopolamine increased the activity of thiobarbituric

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acid reactive substances (101.22 ± 0.766; Fig. 3) compared to the control (33.11 ± 0.419). Even though sub-chronic piracetam treatment did not restore the activity of thiobarbituric acid reactive substances (84.48 ± 0.462) to that in control rats, it prevented the scopolamine-induced increase in the levels of thiobarbituric acid reactive substances in the scopolamine group.

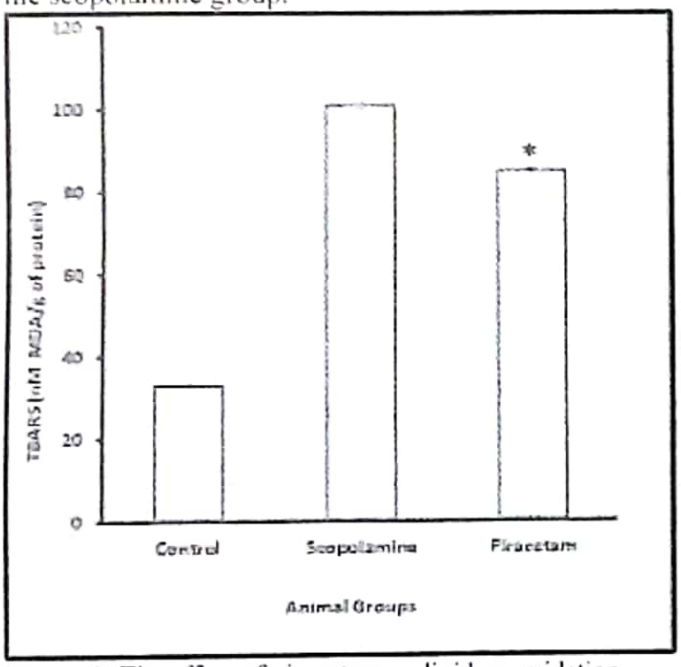

Figure 2: The effect of piracetam on lipid peroxidation levels. # Diseased vs all groups, * $p < 0.001$.

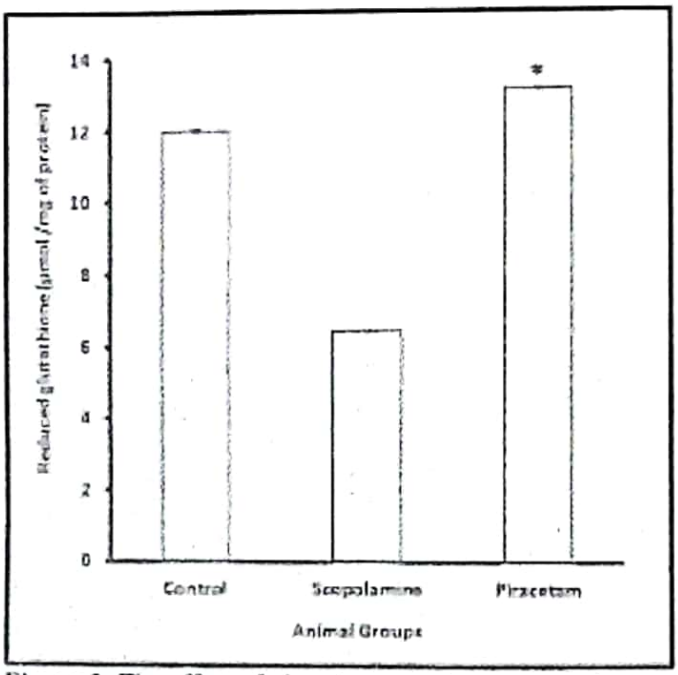

Figure 3: The effect of piracetam on reduced glutathione levels. Diseased vs all groups, * $p < 0.001$.

DISCUSSION

According to the recognized influence of scopolamine and piracetam on the brain, I proposed that pretreatment with piracetam reverses cortex and hippocampus dependent cognitive loss produced by an intra-peritoneal injection of scopolamine. The findings of the present investigations include in the elevatedplus maze approach, sub-chronic piracetam treatment protected against the decline in the acquisition as well as in long-term memory trials induced by scopolamine. In addition, piracetam treatment prevented the reduction in the activity of reduced glutathione generated by scopolamine.

Scopolamine, a commonly used drug for nausea and vomiting, is implicated in the alteration of the acquisition and retention of information (Fan *et al.*, 2005; Oh *et al.*, 2009). Growing body of evidence has demonstrated the effectiveness of scopolamine use as a model for amnesia (Chen *et al.*, 2008). The deficit in cognition has been reported at the behavioral, synaptic plasticity, and molecular levels. The detrimental impact of scopolamine on cognitive function is credited to its influence on muscarinic cholinergic system as receptor antagonist (Mariscoet *al.*, 2013). Moreover, scopolamine has been demonstrated to affect synaptic plasticity. In hippocampus, scopolamine harms long-term potentiation in granule cells of dentate gyrus and pyramidal cells of Ammon's horn (CA1) (Ito *et al.*, 1988). At the behavioral level, loss of learning and memory as a result of scopolamine administration have been shown in various animal investigation (Oh *et al.*, 2009; Golechhaet *al.*, 2012; Mariscoet *al.*, 2013). In agreement, the current research paper shows that scopolamine increased the transfer latency in the elevated plus maze task suggestion memory impairment.

A growing body of evidence has shown that there is converse relationship between piracetam treatment and cognitive dysfunction at the behavioral level (Winblad, 2005; Malykhet *al.*, 2010). Indeed, piracetam has been demonstrated to attenuate the loss of cognitive function in various central nervous system disorders such as Alzheimer's disease, stroke, and inflammation (Malykhet *al.*, 2010). The findings of the present experiments therefore validate the efficacy of piracetam as memory protector. Actually, the amnesic consequences of scopolamine as revealed by the increase in the transfer latency in the elevated

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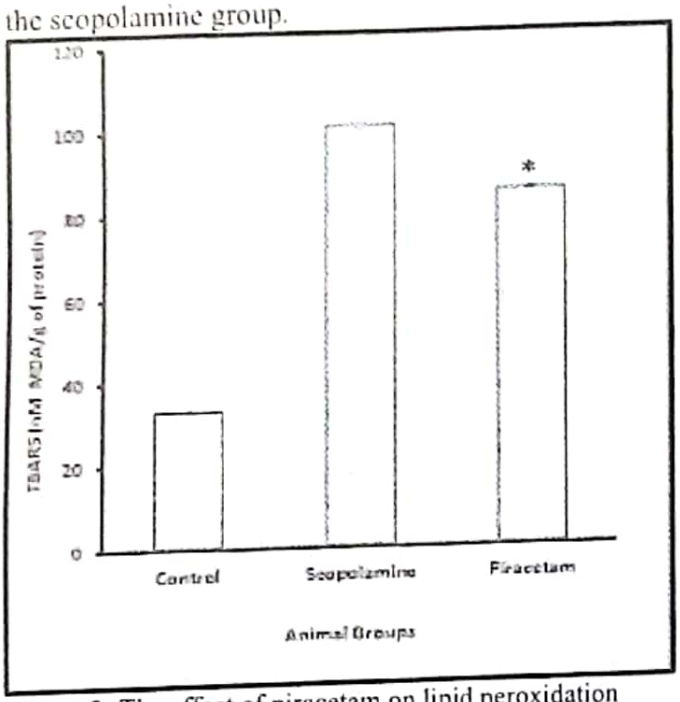

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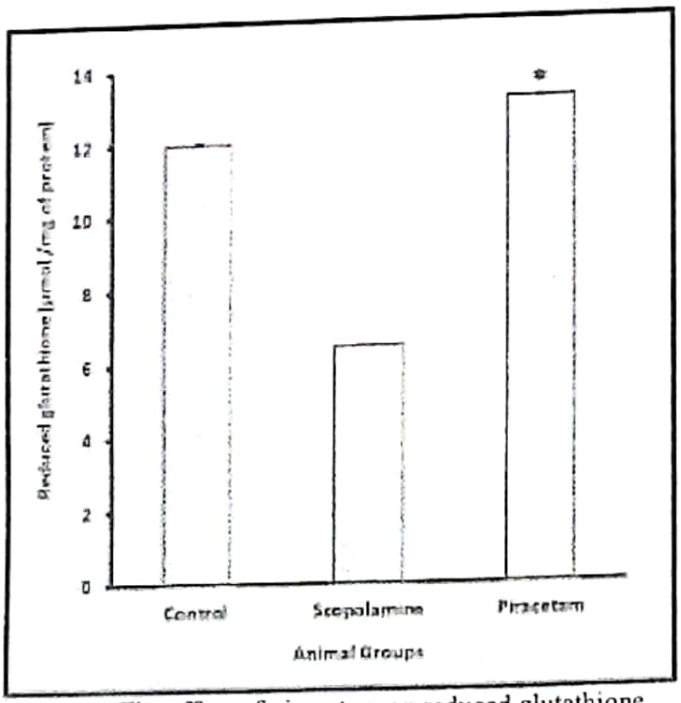

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plus maze procedure was averted by sub-chronic treatment of piracetam. Furthermore, researchers have shown that piracetam affects the long-term potentiation, which is the cellular model of learning and memory, positively (Loscertales*et al.*, 1998; Fesenko, 2009). All together, the behavioral and cellular results validate the neuroprotective role of piracetam on the function of brain. However, the molecular mechanism underlies the action of piracetam is still a matter of debate.

Several lines of evidence have shown that the increase in the reactive oxygen species, which results in lipid peroxidation and/or decrease in reduced glutathione, which reflects the antioxidant defense can cause oxidative damage (Schulz *et al.*, 2000; Abdel-Salam *et al.*, 2011; Goverdhan*et al.*, 2012). The oxidative imbalance can underlie the process of neurodegenerative disorders. In this paper, scopolamine led to oxidative stress as indicated by the levels of reduced glutathione and TBRAS.

The influence of reduced glutathione as an antioxidant on central nervous system is well documented (Schulz *et al.*, 2000: Abdel-Salam *et al.*, 2011). Several lines of evidence have shown the decreased reduced glutathione levels in oxidative stress induced free radicals in the brain. Evidence suggests that deficiency in the levels of reduced glutathione causes a gradual deterioration of cortical neurons. In view of that, both working and reference classes of memory, and synaptic transmission have been found to be impaired. Similarly, the results of my study suggest that reduced glutathione is an essential contributor for the reversal of memory damage. Consistent with in this findings, is the fact that piracetam is implicated in memory modulation particularly in the elderly.

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التأثير التحسيني للبيراسيتام على الاختلال الوظيفي المعرفي المرتبط بنموذج حيواني لمرض الزهايمر

ابراهيم عبدالرحمن الحيدر

كلية الصيدلة الاكلينيكية- جامعة الملك فيصل-الاحساء-المملكة العربية السعودية

ان معظم الدلائل الحالية على تأثير البيراسيتام على تقوية الذاكرة ينشأ من دراسات على الحيوان والانسان. وبرغم ذلك فان الآلية المؤكدة التي توضح تحسن الذاكرة ما زالت بحاجة للتحديد. ولذا فقد تم فحص تأثيرات المعالجة القبلية بالبيراسيتام على الاختلال الوظيفي المعرفي المحدث بواسطة السكوبولامين. ووجد أنه في حالة استخدام المتاهة فان استخدام البيراسيتام قد وقى من تدهور الذاكرة القصيرة المدى والطويلة المدى في الجرذان المعالجة بالسكوبولامين. ووجد بالتجارب الجزينية على نسيج القشرة المخية وقرن آمون أن النقص الحاد في نشاط الجلوتاثيون المنخفض في الجرذان المعالجة بالسكوبولامين قد تم منعه بستخدام البيراسيتام. وتقترح الدراسة الحالية أن البيراسيتام يقي من تدهور القدرة على التعلم والذاكرة المحدثة بالسكوبولامين من خلال استعادة النشاط المضاد للاكسدة للمستوى الطبيعي.

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