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# Effects of *Nepeta menthoides* Aqueous Extract on Retention and Retrieval of Memory in Mice

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**Abstract:** There are several evidences that plants and vegetables with antioxidant activity can reduce oxidative damages in brain and improve cognitive functions. The aim of this study was evaluation of *Nepeta menthoides* aqueous extract on memory retention and retrieval of mice by using passive avoidance apparatus. For this purpose, mice were classified, coded, weighted and grouped (n = 8) as follow as: control group (Only electric shock), blank group (electric shock plus normal saline) and test groups (electric shock plus *Nepeta menthoides* extract by doses: 100, 200, 400 and 800 mg $kg^{-1}$, i.p.). Delay time of leaving the platform was measured for retention and retrieval test of memory in all mentioned groups. In retention test, plant extract was administered immediately after receiving electric shock while it was administered 24 h after receiving electric shock in retrieval. The results revealed that *Nepeta menthoides* aqueous extract significantly ($p<0.05$) increased memory retention and retrieval. The best response for memory retention and retrieval was achieved with 800 mg $kg^{-1}$ of *Nepeta* extract. In conclusion, enhancement of memory retention and retrieval by *Nepeta menthoides* could be cause of antioxidant activity of its components such as rosmarinic acid, caffeic acid and phenolic acids.

**Key words:** *Nepeta menthoides*, memory retention, memory retrieval, step down passive avoidance

## INTRODUCTION

There is a growing demand for medicinal and aromatic plants in national and international markets. Task Force has reported that international market of medicinal plants is over 60 billion US$ per year and it will grow continues at the rate of 7-30% annually (Sujatha *et al*., 2011). Among different kinds of self-medications in different parts of the world, that is a global problem for governments and health systems (Sarahroodi and Arzi 2009; Sarahroodi *et al*., 2010; Sarahroodi 2012; Sarahroodi *et al*., 2012a), self-medication with medicinal plants and plant base medicines are ancient, cheaper and safer (Sarahroodi, 2006).

*Nepeta* (Lamiaceae) is a genus of annual or perennial herbs that are wildly distributed in Asia, North America, Europe and mountains of tropical Africa (Evans, 1996).They have sturdy stems, opposite heart-shaped green to grayish-green leaves and white, blue or pink flowers (Zargari, 1990). About 300 species of this plant are reported and 67 species of them are found in Iran (Jamila *et al*., 2011).

Different spices of *Nepeta* use as traditional medicine in different countries (Ullah, 2011). They are employed as antispasmodic, expectorant, diuretic, antiseptic, antitussive and antiasthmatic (Rabbani *et al*., 2008). In some areas, people use them for treatment of teeth pain, dysentery and kidney or liver ailments (Baser *et al*., 2000). Furthermore, some studies reported that *Nepeta* reduces serum lipids and has anti-inflammatory effect (Hussain *et al*., 2010).

On the other hand, in some countries such as Iran, fresh or dried *Nepeta cataria* is used in cooking of some foods such as soups, cheese or sauces (Duke, 2002).

Literature surveys have showed that *Nepeta* species extracts contain a high level of antioxidant activity, which is attributed to the rosmarinic acid an in lower amounts to caffeic acid and luteolin (Kraujalis *et al*., 2011).

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Moreover, Polyphenolic compounds in *Nepeta* could give the antioxidant effect and most common plant phenolic antioxidants are flavonoids, cinnamic acid derivatives, coumarins, tocopherols and phenolic acids, which could directly lead the antioxidant effect of plants (Cigremis *et al*., 2010).

One of important functions in the brain is memory that is recording, retaining and recalling of information and using them for adapting the response to the environment (Alikatte *et al*., 2012).

Oxidative damages reduce learning and memory, while increase anxiety (Chepulis *et al*., 2009) and there are several evidences that show significant correlation between vegetables intake and improvement of cognitive function improvement in elderly people (Rubio *et al*., 2011).

The objective of present study was evaluation the effect of aqueous extract of *Nepeta menthoides* on memory retention and retrieval in mice and determination of its possible mechanism of action.

## MATERIALS AND METHODS

**Animals:** Male albino NMRI mice weighing 20-25 g were used throughout the study. The animals were purchased from Pasture Institute (Tehran, Iran) and were housed in an animal house with controlled temperature (22±1°C), relative humidity of 45-55% and a 12-h light/12-h dark cycle. Mice were housed in groups of 5 in Plexiglas animal cages and were given free access to food (Pars Khorakdam, Shushtar, Iran) and tap water. All experiments were performed between 8:00 a.m. and 2:00 p.m. in December 2012, in Qom University of Medical Sciences, School of Medicine. Each animal was used once. The experimental protocol was carried out in accordance with the Research and Ethics Committee of Qom University of Medical Sciences.

**Plant material and preparation of extracts:** *Nepeta menthoides* leaves were obtained from grocery in Tehran, Iran and identified at Traditional Medicine and Materia Medica Research Center herbarium, Shahid Beheshti University of Medical Sciences, Tehran, Iran. The dried leaves were crushed into powder and stored at room temperature in appropriate container. 100 g of the powder was infused for 30 min in 100 mL of boiled distilled water by filtration. The prepared extract concentrated by vacuum evaporation and dried in low temperature.

**Dose preparations:** The doses of 100, 200, 400 and 800 mg $kg^{-1}$ of *Nepeta menthoides* aqueous extract were used. Sodium chloride 0.9% was used as solvent and mentioned doses were selected on the basis of extract dry weight. All doses administered intraperitoneally as mentioned in passive avoidance test.

**Passive avoidance test:** Step-down latency in passive avoidance is a proper test for memory retention and retrieval in mice (Zarrindast *et al*., 2010).

The step-down apparatus used to test passive avoidance, consisted of a Plexiglas box (25×25×30 $cm^3$) with a floor that consisted of parallel stainless steel rods (0.3 cm in diameter, spaced 1 cm apart). There was a round Plexiglas platform 1 cm high and 9 cm in diameter which could be enclosed by a 20 cm long hollow glass cylinder with an inner diameter of 10 cm. Electric shocks (1 Hz, 0.5 sec and 45 V DC) were delivered to the grid floor by an isolated stimulator (Pashmforush Co, Ahwaz, Iran).

Two groups of animals used for retention and retrieval experiments. Each group subsequently divided into six sub-groups (each of 5 animals). The first to fourth groups received purple basil extract 100, 200, 400 and 800 mg $kg^{-1}$, respectively. The fifth group received normal saline (1 mL/100 g) as control group and the sixth group was untreated (Blank). Each animal was used only once.

On first day, groups of 5 animals were given access to learning apparatus for 3 min to familiarize them with the new environment. On second day, mice were individually placed on the round Plexiglas platform inside the cylinder. After 10 sec the cylinder was removed and the step-down latency was measured. Animals that had latencies longer than 30 sec were discarded (Arzi *et al*., 2010). On third day, the same procedure was followed as on second day, except that a 1 sec foot shock (1 mA) was administered as soon as the animals left the platform with all 4 legs. For study the effects of *Nepeta menthoides* on memory retention, the animals were injected (ip.) with normal saline or *Nepeta* extract immediately after foot shock and on fourth day, the mice step-down latency was measured.

For memory retrieval study, anything was injected to mice on third day of study while on fourth day, normal saline or *Nepeta* extract were intraperitoneally injected 30 min before mice step-down latency test.

**Statistical analysis:** Results are expressed as Means±SEM. Data were analyzed using one-way ANOVA followed by LSD test. Calculations were performed using the SPSS statistical package (SPSS Inc., Chicago, Illinois, USA) for windows version 16 and a difference with $p<0.05$ between the experimental groups was considered statistically significant.

## RESULTS

**Effect of *Nepeta menthoides* aqueous extract on memory retention:** Figure 1 shows the effects of *Nepeta menthoides* aqueous extract (100, 200, 400 and 800 mg $kg^{-1}$ ip.) on memory retention in the passive avoidance step down test in 4th day of study.

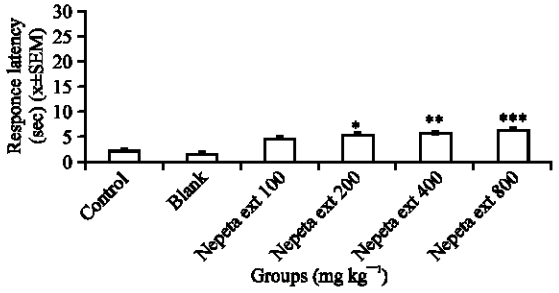


Fig. 1: Comparison of step down latency between 4th days in mice receiving *Nepeta menthoides* extract (100, 200, 400 and 800 mg $kg^{-1}$), normal saline and untreated in memory retention test. Significant difference between day 4 of extract groups with control is shown as ***p<0.001, **p<0.01 and *p<0.05, Control: No injection, Blank: Normal saline (1 mL/100 g), data are Mean±SEM (n = 8)

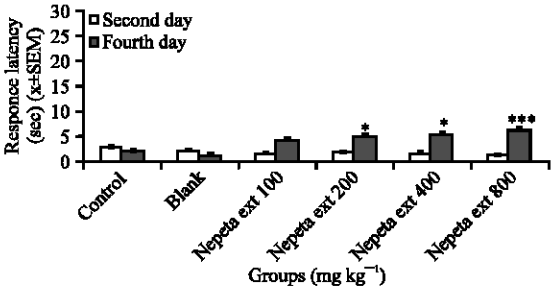


Fig. 2: Comparison of step down latency between day 4 and day 2 in mice received *Nepeta menthoides* extract (100, 200, 400 and 800 mg $kg^{-1}$), Normal saline and untreated in memory retention test. Significant difference between day 2 and 4 is shown as ***p<0.001 and *p<0.05. Control: No injection, Blank: Normal saline (1 mL/100 g), Data are Mean±SEM (n = 8)

A one-way ANOVA showed that *Nepeta* extract significantly (p<0.05) increased step-down latency in passive avoidance task. Post hoc analysis by LSD indicated that 800 mg $kg^{-1}$ extract is the most effective dose as memory retention enhancer (Fig. 1).

Figure 2 reveals comparison of step-down latency between 2nd and 4th day in mice, received *Nepeta* extract (100, 200, 400 and 800 mg $kg^{-1}$ ip.), normal saline and untreated group, in memory retention test.

A one-way ANOVA showed that extract of *Nepeta*, significantly (p<0.001) increased step-down latency on 4th day in comparison with 2nd day, indicating memory retention enhancer. Post hoc analysis by LSD revealed that intraperitoneal administration of all doses of *Nepeta* extract significantly (p<0.05) increased step-down latency on day 4 in comparison with day 2 and 800 mg $kg^{-1}$ of the plant extract was the most effective dose as memory retention enhancer.

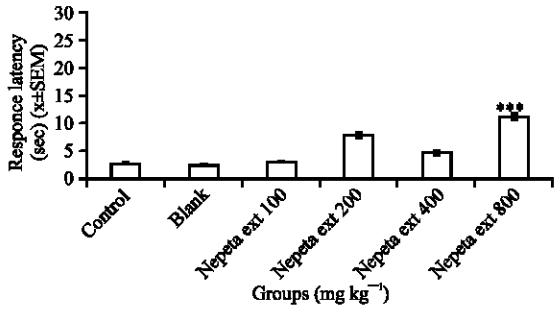


Fig. 3: Comparison of step down latency between 4th days in mice receiving *Nepeta menthoides* extract (100, 200, 400 and 800 mg $kg^{-1}$), normal saline and untreated in memory retrieval test. Significant difference between day 4 of extract groups with control is shown as **p<0.01. Control: No injection, Blank: Normal saline (1 mL/100 g). Data are Mean±SEM (n=8)

**Effect of purple *Nepeta menthoides* aqueous extract on memory retrieval:** Figure 3 shows effects of *Nepeta menthoides* aqueous extract (100, 200, 400 and 800 i.p. mg $kg^{-1}$) on memory retrieval in passive avoidance step down test, in 4th day of study.

A one-way ANOVA revealed that *Nepeta* extract significantly (p<0.05) increased step-down latency in passive avoidance task. Post hoc analysis by LSD indicated that 800 mg $kg^{-1}$ of plant extract was the most effective dose as memory retrieval enhancer.

Figure 4 reveals comparison of step-down latency between day 2 and day 4 in mice, received plant extract (100, 200, 400 and 800 mg $kg^{-1}$ i.p.), normal saline or untreated group.

A one-way ANOVA showed that *Nepeta* extract significantly (p<0.05) increased step-down latency on 4th day in comparison with 2nd day, indicating memory retrieval enhancer. Post hoc analysis with LSD revealed that intraperitoneal administration of Ocimum extract (200 and 800 mg $kg^{-1}$) increased step-down latency on 4th day in comparison with 2nd day.

## DISCUSSION

There are several evidences that foods, which are rich in antioxidants, can protect us from various diseases such as cancer, cardiovascular diseases as well as neurodegenerative diseases like Parkinson's and Alzheimer (Cigremis *et al*., 2010).

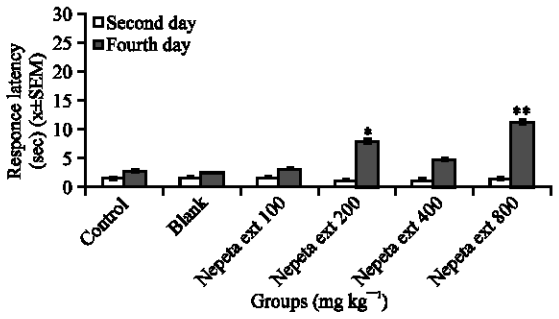


Fig. 4: Comparison of step down latency between day 4 and day 2 in mice received *Nepeta menthoides* extract (100, 200, 400 and 800 mg kg$^{-1}$), Normal saline and untreated in memory retrieval test. Significant difference between day 2 and 4 is shown as **p<0.01 and *p<0.05. Control: No injection, Blank: Normal saline (1 mL/100 g), Data are Mean±SEM (n = 8)

The present study investigated the effect of *Nepeta menthoides* on memory retention and retrieval using the passive avoidance step down model.

The results achieved in this study reveals that different doses of *Nepeta menthoides* increased memory retention and retrieval significantly (p<0.05). It seems that these effects increased dose dependently.

These findings are in agreement with the other study on hydroalcoholic extract of *Nepeta menthoides* on memory which enhanced memory retention and retrieval in Y-maze task (Kiyani *et al*., 2012).

As we mentioned before *Nepeta extract* contain a high level of antioxidant activity, which is attributed to the rosmarinic acid an in lower amounts to caffeic acid and luteolin (Kraujalis *et al*., 2011).

Previous studies on memory retention and retrieval indicated that plants contain large amounts of antioxidants improve memory. For example, various pieces of Ocimum (Joshi and Parle 2006; Kumar *et al*., 2007; Dokania *et al*., 2011; Sarahroodi *et al*., 2012b), Saffron (Abe and Saito, 2000), *Ginkgo biloba* (Rai *et al*., 1991; Rigney *et al*., 1999), green tea (Wu *et al*., 2012), Magnolia officinalis (Lee *et al*., 2012), Curcumin (Bishnoi *et al*., 2008), *Anacyclus pyrethrum* (Sujith *et al*., 2012) and other plants with antioxidant activity improve memory.

We believe that memory retention and retrieval enhancement by *Nepeta* hydroalcohlic extract could be due to the presence of antioxidants such as rosmarinic acid, caffeic acid, luteolin, flavonoids, cinnamic acid derivatives, coumarins, tocopherols and phenolic acids and their power in scavenge reactive oxygen species.

In conclusion, this study provides experimental evidence for *Nepeta menthoides* as a memory enhancer but further studies are required to fractionate *Nepeta menthoides* extract and study each fraction separately for their effect on memory and even other neurodegenerative disorders.

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