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Research Article

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Investigation of Increasing Learning under Stress Conditions with the Effect of Rosemary Extract and Alprazolam in Mice

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Abstract Current study was carried out to compare the effects of hydroalcoholic extract of rosemary and alprazolam on learning under stress in mice. Sixty female mature mice from 25-30g weight range were divided into six groups: control, placebo, alprazolam and 50, 100, and $200_{mg/kg}$ doses of extract that were injected intraperitoneal. To experience the stress, 30 minutes after injection mice were placed in dark boxes for 50 minutes. After that, each mouse was placed in a T-shaped maze and its behavior was recorded. Obtained data were analyzed using SPSS program. Results showed that the extract in various doses reduced the time of reaching to target box in T-shaped maze, which indicates stress reduction. In addition, the number of errors in reaching target was not significantly different in extract groups and placebo group (stress). Overall, effective compounds of rosemary extract are able to increase learning by reducing stress.

Keywords rosemary, learning, stress, alprazolam, T-shaped maze, laboratory mice

Introduction

Learning and memory ability are very important for survival of human. The importance of learning lies in improving human performance. Learning is training which leads to changes in behavior. The importance of learning in all aspects of life is obvious [1].

Learning is about not only special subjects or lessons, but also learning affects but also learning in emotional development, personality development, and human social interaction. It is closely related to behavior and actions of live creatures and is a neural phenomenon in which live creatures change their behavior via practicing. Learning is the ability of changing behavior because of experience. Learning and memory are from the highest levels of performance of central nervous system. It is a process in which people obtain information about their surrounding environment and has a wide range from the most basic creatures to human [1].

One affecting factor of learning and reminding is stress. If stress is not severe or chronic, individual can grow in stressful situations. Positive stress is a stimulating source for people to achieve maximum efficiency and ability.

Chronic stress causes the arousal and body reflexes and creates functional changes in perceptual system and body's reactions. Stress is a term to describe unpleasant emotional and physical symptoms of people who are under excessive pressure [2]. It is called to situations and events which person face with, but are not in agreement with his current abilities or facilities; therefore, person suffers from psychological conflicts [3].

To reduce the stress, many drugs have been used in psychiatric treatments including alprazolam. This drug is a benzodiazepine drug that has a proven soothing effect on the central nervous system. On the other side, it is proposed as an anti- anxiety drug. Alprazolam appears its palliative and anti-anxiety effects via interacting with GABA receptors in the brain, particularly in the midbrain reticular formation, to reduce the stress [4].

Considering side effects of chemical drugs, addiction probability, and prohibition during pregnancy, late studies have showed that replacing them with drugs with fewer side effects are necessary. Nowadays, pharmaceutical plants are important part of traditional medicine of many countries and have special places in modern approaches.

Romaren or rosemary (*Rosmarinus officinalis*) is native to the Mediterranean region and Uruguay. This fragrant plant is from mint family, which is shrub with wooden stalks (0.5-1 m). Narrow needle shaped leaves with no end, dark green flowers and rarely pink or white flowers.

Leaves and flowering branches of plant are pharmaceutical part of it. Essence, depend on growth region has different compounds but the most important compounds arecamphon, limonene, borneol, cineole, linalool and *Robinul*. Flavonoids of this plant include diosmetin, diosmin, luteolin, etc... Rosemary is used in traditional medicine as anti-asthma, for digesting food, as sedative drug, for treating disorders of blood circulation, for enhancing eyesight, as anti-rheumatism, and Memory Booster.

Many properties have been ascribe to rosemary in various studies. Aquatic extract of rosemary decreased muscle jump in morphine withdrawal syndrome and had effects such as diazepam in reducing withdrawal syndrome symptoms in morphine-dependent rats [5].

In another study (2002-Mashhad), rosemary was compared with phenobarbital for curing convulsion and all aerial products of plant-reduced animals' convulsion [6]. Another study showed that the essence of rosemary could increase healthy or damaged memory, Dose dependently [7].

Rosemary has therapeutic effects in reducing opium signs (insomnia, muscle pains and convulsion[8].In a study in Brasilia (2009), rosemary could treat mood disorders such as depression via affecting monoaminergic receptors [9].

In a study (2015), photochemical compounds in hydroalcoholic extract of leaves had considerable effects on primary stages of virus reproduction and apparentlycouldtreatvirus reproduction [10]. In a study in 2008, aquatic and hydroalcoholic extracts of aerial parts had analgesic effects [11].

Specific doses of rosemary extract increased spatial memory capacity, memory improvement and increased anti-oxidant hippocampal activity in young adults [12]. Extract of this plant has anti convulsion, anti-anxiety effects. Recent effect is because of reducing GABA activity and increasing chloride ions and blocking sodium stream in neurons. In addition, rosemary extract increases acetylcholine activity and improves memory [13].

In a study (2012), dose-dependent effects of hydroalcoholic extract of rosemary in improving short time memory was showed. It also improved long-term memory in consolidation phase [14]. Results of that study showed that extract was effective in improving memory and preventing brain neural damages [15].

In a study, oral use of extract containing carnocic acid had conservative effect on hypocampus and prevented neural death caused by neurotoxim-OHDA-6 induction. So, rosemary extract in 100 $_{mg/kg}$ dose can be introduced as a drug with high medical potential to treat memory disorders [16].

A study in 2016 showed that rosemary extract improved spatial memory deficits associated with mild traumatic brain injuries. This is because of antioxidant, anti-inflammation effects of rosemary. Therefore, it can be a potential treatment to improve cognitive impairment in patients with mild brain damage.

Considering that hydroalcoholic extract of rosemary has not compared with alprazolam, this study was carried out to compare the effects of hydroalcoholic extract of rosemary and alprazolam on learning under stress in mice.

Materials and Methods

Treatment groups

Sixty female mature mice from 25-30g weight range were selected. Animals were kept in special cages with controlled temperature and humidity and 12:12 hours photoperiod. Samples had free access to food and water. Food was compressed pellets. Mice were kept for 7 days before experiment to adapt to environment.

Mice were divided into six groups: control (no injections), placebo (physiological serum), alprazolam ($1/2_{mg/kg}$) and 50, 100, and $200_{mg/kg}$ doses of extract, which were injected intraperitoneal.

T-shaped maze was used to evaluate learning under stress that is standard model for evaluating learning level of rodents. This apparatus has two wooden arms with 55x15x10 cm dimensions and entrance arm with 55x15x10. Two arms are tangent to each other and the input arm is placed on two arms.

This evaluating model is experimental and does not need to train animal. To enforce the stress, dark box (15x10x10 cm) was used to increase searching activities.

Evaluating extract effects was done along three day as follows:

1. First day (discovery stage): Mice were placed in entrance arm of T-maze and allowed to search maze environment for five minutes. The arm which mouse entered was recorded.

2. Second day (learning stage):

a. Food was placed in target box in another arm (opposite to arm which mouse had entered first day) and that arm was closed. Mouse was allowed to enter food arm and east for 30 seconds.

b. five minutes later without-food arm was opened and food containing arm was closed. Mouse was placed into maze and allowed to enter maze arm and stay for 30 seconds in target box without food.

c. Five minutes after second stage, first stage was repeated and maze was transferred to cage.

3. Third day:On the morning of the test, animal was injected and was placed in dark box under stress to increase searching activities. Then, animal was placed in T-maze and learning amount was evaluated according to entering target arm.

Animal was placed for five minutes in T-shaped maze and standard learning evaluation indices under stress were recorded via observation. Indices were:

1) Reaching time to target box comparing to placebogroup.

2) Determining the direction of arm entries recorded on the memory in comparison with learning stage.

Reduced reaching time to target box is considered as stress reduction. Reduced both indices (reaching time to target box and reducing errors in selecting box) at the same time or at least significant difference of one of them with placebo (stress) group is considered as significant difference of stress level on learning.

Obtained data were analyzed using SPSS in two descriptive and inferential levels; average and standard deviation were calculated and one-way analysis of variance was used. Groups compared using LSD test and for selecting target box (reaching target) Chi^2 was used.

Results and Discussion

Experimental groups (100 and $200_{mg/kg}$ of extract) spent less time to reach box in proportion to placebo (stress) group (Figure 1).

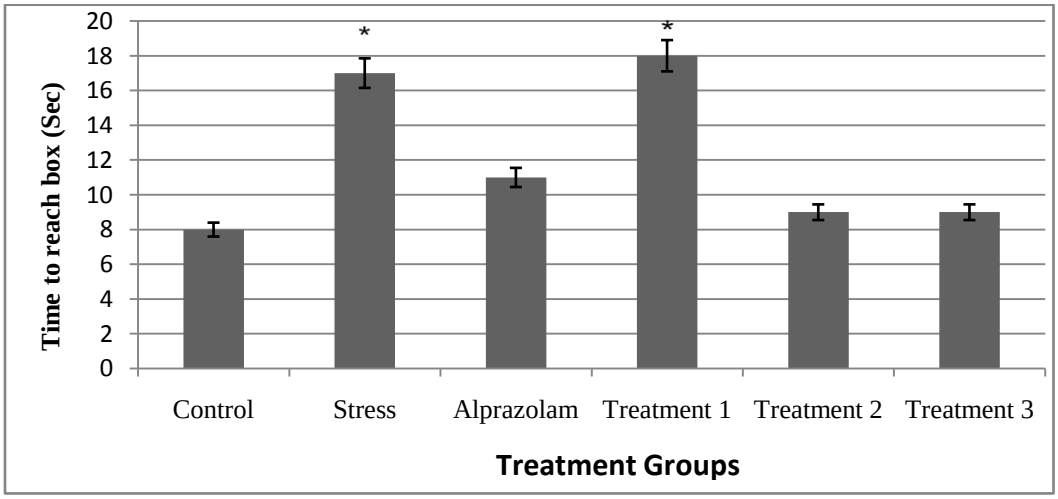

Figure 1: Average reaching time of treatment groups

Mice of treatment 3 and alprazolam groups did not have fewer errors to select target box in comparison with placebo (stress) group (Figure 2).

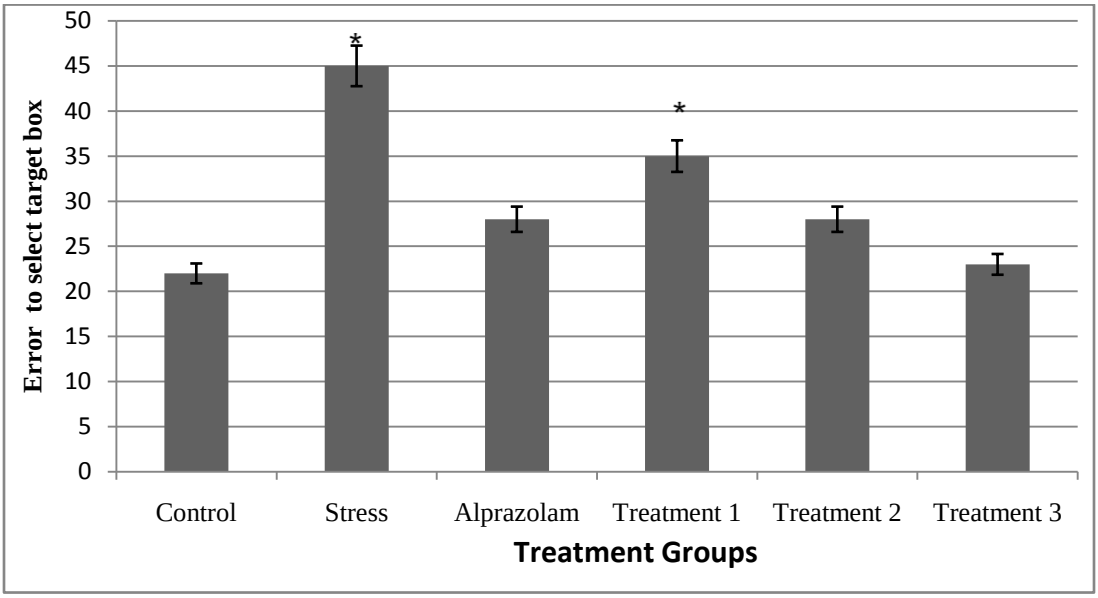

Figure 2. Mice errors to reach the target in all groups

According to results, intraperitoneal injection of rosemary's hydroalcoholic extract in all doses reduced reaching time to target box that was similar to alprazolam performance and can be considered as stress reduction and learning increment under stress.

However, mice of extract groups did not have fewer errors to select target box in comparison with placebo group. Therefore, extract did not cause considerable effect on learning amount (selecting target box in T-maze).

Hosseinzadeh et al. showed that rosemary essence increased the ability of healthy and destroyed memory, dose despondently [4]. Abedelkader et al. (2010) studied anti-epileptic and anti-anxiety effect of rosemary essence and showedanti-anxiety effect due to reducing GABA activity and increasing chloride ions and blocking sodium stream in neurons [12].

Tamaddaoniet al (2013) studied the effects of oral administration extract containing carnocic acid had conservative effect on hypocampus and announced that extract in $100_{mg/kg}$ dose can be introduced as a drug with high medical potential to treat memory disorders [16].

Zanella et al. (2012) reported that hydro-alcoholic extract of rosemary in is effective improved short-term memory dose dependently and improved learning in long-term memory in consolidation phase [13].

Researches of Mahdizadeh et al. (2015), Far et al. (2016), and Arashpour et al. (2016) showed that rosemary extract is effective in improving memory and preventing neuronal damage in the brain. Sung (2016) investigate the effect of rosemary extract to improve spatial memory deficits associated with mild traumatic brain injuries and showed that rosemary extract has antioxidant properties and anti-inflammatory. Therefore, rosemary extract may be a potential treatment for improving cognitive impairment in patients with mild brain damage [12].

Conclusion

Results of this study showed that hydroalcoholic Extract of rosemaryin 100 and 200 $_{mg/kg}$ does reduce the stress and therefore increase learning ability.

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