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Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtbOriginal article <http://dx.doi.org/10.1016/j.apjtb.2015.06.006>Effects of *Matricaria chamomilla* extract on motor coordination impairment induced by scopolamine in ratsSamira Asgharzade^{1,2}, Zahra Rabiei¹, Mahmoud Rafeian-Kopaei^{1*}¹Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran²Department of Molecular Medicine, Faculty of Advanced Medical Technologies, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:

Received 19 May 2015

Received in revised form 28 May 2015

Accepted 3 Jun 2015

Available online 7 Aug 2015

Keywords:

Motor coordination impairment

Chamomile plant

Scopolamine

Rat

ABSTRACT

Objective: To evaluate the effect of ethanolic extract of chamomile on balance and motor learning in rats receiving scopolamine and intact rats.**Methods:** Forty-two rats were divided into 6 groups ($n = 7$). Control group received distilled water. Rats in Group 2 were given 1 mg/kg scopolamine. Groups 3 and 4 received chamomile extract 200 mg/kg and 500 mg/kg, respectively, and scopolamine simultaneously for 20 days. Intact groups (Groups 5 and 6) only received chamomile extract 200 mg/kg and 500 mg/kg, respectively. Motor coordination of rats was assessed with rotarod apparatus.**Results:** According to the obtained results, compared with the control group, scopolamine significantly decreased time spent on rotarod performance ($P < 0.001$). Compared with scopolamine group, the strength and staying on rotarod apparatus in Group 3 significantly increased ($P < 0.05$). The results of this research showed that intact groups that received only chamomile extract at doses of 200 mg/kg and 500 mg/kg significantly increased time spent on rotarod, compared with scopolamine group ($P < 0.001$).**Conclusions:** The results of this study indicated the high antioxidant property and protective effect of chamomile extract on motor coordination in the groups that received scopolamine.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by cognitive deterioration together with behavioral disturbances and declining activities of daily living. Other features associated with the later stages of AD include language deficits, depression, behavioral problems including agitation, mood disturbances and psychosis [1].

AD is the most common form of dementia in middle age and aging, and currently more than 1.5% of people in developed countries suffers from AD. Patients get involved in chronic and progressive loss of memory and cognitive intelligence analysis and other higher functions such as reasoning that is associated

with neuronal death and formation of extracellular protein aggregates called amyloid plaques or senile plaques throughout the cerebral cortex.

Researchers at the annual meeting of the Alzheimer's population in Vancouver, British Columbia present research that demonstrates the power walk of the people or the walking gait is perhaps indicative of Alzheimer's disease. The investigation showed that the slow movement and the change of gait are associated with exacerbation of mental deterioration. Alzheimer's patients walk more slowly than patients who suffer from moderate mental problems, the patients, in turn, are moving slower than healthy people [2].

Reactive oxygen radicals are involved in brain injuries such as AD [3,4]. The increase in production of free radicals has been reported to cause damage to cell membranes, enzymes, DNA, lipids and proteins, impairing their functions [5].

Oxidative stress is a disparity between the rates of free radical production and elimination through endogenous antioxidant mechanism [6]. These constantly produced reactive oxygen species (ROS) are scavenged by the enzyme superoxide dismutase, glutathione peroxidase and catalase [7,8].

*Corresponding author: Mahmoud Rafeian-Kopaei, Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran.

Tel: +98 381 334 6692

Fax: +98 381 3330709

E-mail: rafeian@yahoo.com

Peer review under responsibility of Hainan Medical University.

Foundation Project: Supported by the Shahrekord University of Medical Sciences, Shahrekord, with Iran foundation number of 1756.

Lipid peroxidation is thought to be a prominent and especially deleterious form of neuronal oxidative injury damaging membranes and generating several secondary products. Increased level of malondialdehyde (MDA) as one of the ROS has been shown to be a reliable index of *in vivo* lipid peroxidation [9,10].

Impairment of learning and memory can be induced chemically by scopolamine in laboratory animals [11]. Scopolamine is the blocker of muscarinic acetylcholine receptor that acts as useful pharmacological tools to produce a model of amnesia [12]. Scopolamine significantly increases acetylcholinesterase and MDA levels in the cortex and hippocampus [8]. The elevation of brain oxidative stress after administration of scopolamine further substantiates the value of scopolamine-induced amnesia as an animal model [12].

ROS plays a role not only in dementia, but also in many incurable diseases like diabetes [13,14], atherosclerosis [15], cardiovascular diseases [15,16], neurological diseases [17,18], cancer [19,20], and also creates complications and toxins [21,22].

Today, herbs are an important part of traditional medicine in many countries and various studies have shown that plants with high antioxidant effects could be effective in treatment or prevention of these diseases [23–27].

Chamomile is a kind of perennial flowering herb that grows in widespread regions, including Europe, Africa and Asia. Chamomile is widely distributed in Iran, and it is used in Iranian traditional medicine as reliever.

Chamomile has several flavonoids one of which is apigenin that is the most important one. Chamomile has been known traditionally for its mild relaxing effect and candidate constituent for this effect is apigenin [28].

This study aimed to investigate the therapeutic effect of alcoholic extract of chamomile on motor coordination impairment caused by the injection of scopolamine.

2. Materials and methods

2.1. Preparation of *Matricaria chamomilla* (*M. chamomilla*) extract

In this study, fresh and dried chamomile flowers were collected in spring and were identified by botanist of Medicinal Plants Research Center, Shahrekord University of Medical Sciences and herbarium number was 423. Dried chamomile flowers were crushed by electric mill. The powder of chamomile flowers was extracted by ethanol 70% as solvent. This extract was filtered and concentrated under reduced pressure on a rotary evaporator. In order to prepare different concentrations, dried extract was weighed and diluted with saline to form a suspension and then the rats were injected intraperitoneally [29].

2.2. Animals and treatment

In this study, animals were divided into different experimental groups by the simple random method. In this study, 42 adult Wistar rats at the weight range of 200–250 g purchased from Pasture Institute, Tehran, Iran were used. The rats were maintained in an air-conditioned animal room with a 12 h light–dark cycle under controlled humidity (10%) and temperature [(25 ± 3) °C]; the rats had free access to standard laboratory diet. Animals were divided into the following groups:

- (1) Group 1 (control group) that received only the distilled water and the behavioral balance test was performed on them by using rotarod apparatus ($n = 7$).
- (2) Group 2 (scopolamine group) that received scopolamine 1 mg/kg via intraperitoneal injection and the behavioral balance test was performed on them by using rotarod apparatus ($n = 7$).
- (3) Group 3 that received scopolamine (1 mg/kg) and chamomile extract 200 mg/kg simultaneously and the behavioral balance test was performed on them by using rotarod apparatus ($n = 7$).
- (4) Group 4 that received scopolamine (1 mg/kg) and chamomile extract (500 mg/kg) simultaneously and the behavioral balance test was performed on them by using rotarod apparatus ($n = 7$).
- (5) Group 5 that received only chamomile extract 200 mg/kg for 20 days and the behavioral balance test was performed on them by using rotarod apparatus ($n = 7$).
- (6) Group 6 that received only chamomile extract 500 mg/kg for 20 days and the behavioral balance test was performed on them by using rotarod apparatus ($n = 7$).

2.3. Motor coordination test by the rotarod apparatus

The power of the rats to maintain balance and motor resistance was studied. Rotarod is a device that measures the motor resistance and balance maintenance of animals. This device has a carousel with rotation speed at the range of 0–40 r/min. This device has a belt and the rotation speed can be regulated by replacing it on its place. To make the animal familiar with the device, they were placed on the trundling shaft and they were trained to move on it. The rotation speed was considered 10 r/min with the 7 r/min² acceleration in this study, which was almost 10–11 revolutions per minute. In each of the experimental groups, the rats were placed on carousel of the rotarod device 1 h after injection of the extract. The carousel turned for 300 s and the duration that animal could maintain his balance and resist against the movement of carousel was considered as the rat's resistance time. The maximum time considered for each animal in this test was 300 s [30].

2.4. Total phenolic compounds

The concentration of total phenolic compounds in *M. chamomilla* extract was determined by using Folin–Ciocalteu reagent. Briefly, 0.5 mL of a 5.5 g/L diluted extract, and 2.5 mL of Folin–Ciocalteu reagent (diluted 10 times with water) were added. The standard curve was plotted by using 12.5, 25.0, 50.0, 62.5, 100.0, and 125.0 mg/L solutions of gallic acid in methanol and water (60:40, v/v). The absorbance was measured at 760 nm. The total phenolic contents of the extract were expressed as gallic acid equivalent (mg/g extract) [31].

2.5. Total flavonoids and flavonols

The amount of total flavonoids and flavonols in the *M. chamomilla* extract was determined calorimetrically as described by Sharafati-Chaleshtori *et al.* [32]. Total flavonoids and flavonols were expressed in terms of rutin equivalent (mg/g), which is a common reference compound [32].

2.6. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

Antioxidant capacity of the *M. chamomilla* extract was measured by using the DPPH assay based on the scavenging ability to DPPH stable radical [33]. Butylated hydroxytoluene (BHT) was used as a positive control. The samples at different concentrations were mixed with DPPH solution and ethanol. After vortexing, the tubes were left in the dark at room temperature after which the absorbance was measured at 517 nm by using a UV–vis spectrophotometer (Biochrom Ltd., England). Each measurement was performed in triplicate under identical conditions. Antioxidant activities were expressed as the IC₅₀ values. Inhibition of free radical by DPPH was calculated as follows:

$$\text{Inhibition (\%)} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

The concentration of antioxidant was required to cause 50% reduction in the original concentration of DPPH.

2.7. Statistical analysis

All the results were expressed as mean \pm SE of the mean and statistical analyses were performed by using SPSS 11.0 statistical software. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Standardization of *M. chamomilla* extract

Total phenolics, measured by Folin–Ciocalteu, were reported 79 mg/g, containing 48.2 mg/g flavonoid and 26.2 mg/g flavonol compounds.

3.2. Antioxidant activity of chamomile

The antioxidant activity of chamomile extract was assessed on the basis of scavenging of the stable DPPH free radicals. Chamomile extract showed strong free radical scavenging activity against DPPH radicals, with an IC₅₀ value of 50 $\mu\text{g}/\text{mL}$ (Table 1).

Table 1

Antioxidant activity of *M. chamomile* extract, with BHT used as positive control.

Sample	Concentration ($\mu\text{g}/\text{mL}$)	Radical scavenging activity DPPH (IC ₅₀) ($\mu\text{g}/\text{mL}$)
Chamomile extract	80	74.80
	70	65.30
	60	56.90
	50	49.20
	40	41.29
	30	32.40
	25	27.50
	20	22.15
	15	18.00
	10	5.80
	BHT	20
50		14.40
100		30.70
250		68.20
500		79.30
700		95.30

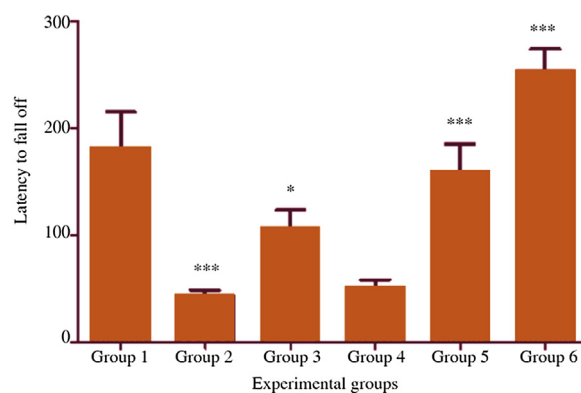


Figure 1. Motor resistance and time of maintaining on the carousel of rotarod apparatus in experimental groups.

*** $P < 0.001$; * $P < 0.05$.

There was a significant difference in the resistance time and the maintaining on the carousel of the rotarod apparatus between the scopolamine group and control group ($P < 0.001$), with significantly less time in scopolamine group.

The results of this study showed that chamomile extract at doses of 200 mg/kg and 500 mg/kg in intact groups (Groups 5 and 6) significantly increased the motor resistance and the time of maintaining on the carousel, compared to scopolamine group ($P < 0.001$). Motor resistance and time of maintaining on the carousel increased in Group 3 compared with scopolamine group ($P < 0.05$) (Figure 1).

4. Discussion

One of the problems in AD is apraxia which means that the patient's ability to perform purposeful movements and coordinate proficiently loses without paralysis or sensory and motor impairment [34].

Scopolamine, a muscarinic receptor antagonist, is reported to impair long-term potentiating, and it is frequently used as amnesic agent for evaluation of anti-amnesic effect of new drugs [35]. Scopolamine significantly increases acetylcholinesterase and MDA levels in the cortex and hippocampus. The elevation of brain oxidative stress after administration of scopolamine further substantiates the value of scopolamine-induced amnesia as an animal model [12].

Brain oxidative stress was reported following intraventricular administration of ethylcholine aziridinium, a toxic analogue of choline that disrupts high-affinity choline transport producing a persistent presynaptic cholinergic hypofunction with the induction of amnesia [36].

Decreased numbers of [3H]-N-methylscopolamine binding sites, observed in the presence of high concentrations of H₂O₂ as an inducer of lipid peroxidation in rats' cerebral cortex membranes, was accompanied by decrease of thiobarbituric acid-reactive substances levels [37].

Cholinergic nerve stimulation in basal forebrain and hippocampus was reported to be excitotoxic, causing tonic-clonic convulsions due to the release of glutamate mediated through the production of ROS [38].

Cerebral cortex and hippocampus are linked together to control cognitive and motor functions, and appear to be sensitive to oxidative stress. Oxidative stress is thought to be a factor in the decline in cognitive and motor performance seen in aging [39].

The main constituent of the essential oil of *M. chamomilla* is flavonoids of which the most important ones are apigenin and chamazulene [40].

In this study, the amount of phenolic and flavonoid compounds which have antioxidant activity were measured. These results suggest that chamomile extract demonstrates antioxidant properties by protecting rat brain from elevated oxidative status due to administration of scopolamine. Most of the studies also needed to investigate the role of cholinergic neurotransmission in mediation of brain oxidative stress [41–45]. The result of this study demonstrated that *M. chamomilla* extract significantly increased the motor resistance and increased time of maintaining on the carousel of rotarod apparatus.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This research was financially supported by the Shahrekord University of Medical Sciences, Shahrekord, with Iran foundation number of 1756.

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