

Determining the Antioxidant Properties of Chamomile and Investigating the Effects of Chamomile Ethanol Extract on Motor Coordination Disorders in Rats

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ABSTRACT

BACKGROUND AND OBJECTIVE: Alzheimer's, a progressive disorder causing the memory and other crucial functions of the brain to deteriorate, is mainly responsible for Dementia (brain deterioration). Most of the available drugs exert little influence on this brain disorder. The purpose of this study was to investigate the effects of Chamomile (*Matricaria chamomilla*) and its ethanol extract on the balance and motor learning of healthy rats and rats receiving Scopolamine.

METHODS: In this experimental study, 42 male Wister rats were divided into 6 groups of 7. They received one milligram of Scopolamine per kilogram of their body weight. The control group received distilled water. Simultaneously, the Amnesiac groups received 200 to 500 milligrams of Chamomile extract in addition to Scopolamine for 20 days. The healthy group only received the extract. The rats' motor balance was measured using the Rotarod device.

FINDINGS: In comparison with the control group, Scopolamine significantly reduced the time of resistance and staying on the Rotarod rolling stock ($p=0/0005$). The resistance time and staying time on the Rotarod rolling stock in the control group and the Scopolamine group were 169/51 and 46/33 seconds, respectively. The time of resistance and staying in the Scopolamine group receiving 200 milligrams of the Chamomile extract was 119/5 seconds which shows a significant increase compared to the Scopolamine-only group ($p=0/044$). Compared to the Scopolamine group receiving the same dose, 200 to 500 milligrams of the extract in healthy rats noticeably increased the time of resistance and staying on the rolling stock (168/3 and 263 seconds, respectively) ($p=0/000$, $p=0/000$).

CONCLUSION: The results of this study indicate that with its antioxidant properties, Chamomile extract could probably enhance motor balance in Scopolamine receiving rats.

KEY WORDS: Motor Coordination Disorders, Chamomile, Scopolamine, Rats.

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Introduction

Alzheimer's disease is the major cause of Dementia among the elderly which will gradually lead to other afflictions such as memory impairment, Aphasia, Apraxia and motion disorders (1). The patient is likely to experience certain problems of muscle coordination and balance. These could be a result of the disease or the medication the patient uses (1).

Clinical studies demonstrate that oxidative stress has a key role in the pathophysiology of brain deterioration as well as other neurological disorders which are mainly associated with the patient's age (2). Oxidative stress is defined as an imbalance between free radicals and the antioxidant system and it is recognized as one of the factors influencing the pathogenesis of the disease (3). Oxygen free radicals are capable of striking at the proteins, nucleic acids and lipid membranes eventually disrupting the cell functions (4, 5).

Brain tissues contain large proportions of polyunsaturated fatty acids which are vulnerable to free radicals. Lipid peroxidation is presumed to be a specific form of neuronal, oxidative damage. It is capable of impairing the membrane creating a number of secondary products. Both the split form and the circled form of the oxygenated fatty acids are known to have neurotoxic properties (6). The oxidized forms of oxygen are produced in the brain during cellular respiration. When the brain is damaged, these forms of oxygen tend to increase. It has been reported that this increase in the production of free radicals is likely to damage the cell membrane, enzymes, DNA, lipids and proteins and interfere with their function (7). Oxidative stress is a disparity between the amount of produced free radicals and elimination through endogenous antioxidant mechanisms such as Superoxide dismutase (SOD), Glutathione peroxidase (Gsgpx), Catalase and even Glutathione (GSH) and Ascorbate (2).

Furthermore, as a reactive oxidative species, the increase in the rate of Malondialdehyde (MDA) has been recognized as a reliable factor in lipid peroxidation in vivo (2, 3). Impairment in the memory and learning can be chemically infused into clinical animals using Scopolamine. Scopolamine blocks Muscarinic acetylcholine receptors which acting as a helpful medicinal tool in creating a model of Amnesia (8).

Scopolamine notably increases MDA in the cortex and hippocampus. The increase in the oxidative stress in brain upon using Scopolamine confirms the value of the Scopolamine-infused amnesia as an animal type (7). The connecting spots were observed to decrease in N-

methyl Scopolamine in the presence of high density H₂O₂ as the lipid peroxidation inducer in the brain membrane leading to the reduction of Thiobarbituric acid reactive substances (TBARS) level (9).

For another thing, the stimulation of cholinergic neurons is proven to exert toxic effects on the front base of the brain and hippocampus and possibly ignite the release of Glutamate by producing free radicals. Therefore, cholinergic neurotransmission is indispensable to oxidative stress (10). Recent studies have confirmed that antioxidant herbal drugs can efficiently cure or prevent such disorders as memory problems (11-13), strokes (14-17), digestive disorders (18,19), diabetes (20,21), cardiovascular diseases (22, 23), cancer (24, 25) and many other medical conditions. Although such effects might be coming from other special compounds found in these herbs, they are mostly associated with their antioxidant properties.

Chamomile is a perennial, flowering plant that grows in Europe, Asia, Africa and many other regions around the world. Chamomile is known to have numerous antioxidant properties. It has a desirable scent and grows in meadows and sandy lands. This plant has long been used in the Iranian traditional medicine as painkiller, antipyretic and antispasmodic (26).

Chamomile has its main source in different Mediterranean regions but nowadays it has been observed to flourish in a wide range of areas worldwide. The capitols of chamomile are the only parts used as medicine. They are gathered when the plant is ripened or when it blossoms. Analgesic and anticonvulsant effects of this herb have mainly been associated with its antioxidant properties as well as Flavonoids (26).

This study aimed to investigate the medicinal effects of the ethanol extract of chamomile on motor coordination disorders of rats by injecting Scopolamine to the groups of Scopolamine receiving and healthy rats using the Rotarod technique.

Methods

In this empirical study, we used freshly dried chamomile flowers. The flowers had been collected during the spring in the Bakhtiari region, Iran. Later on, they were identified by a botanist in the Medicinal Plants Research Centre of Shahr-e-Kurd University of Medical Sciences and were registered under the herbarium number of 423. Firstly, the dried flowers were gently grinded into a powder using electric mills. Afterwards, 50 grams of the powder were mixed with

500 milligrams of Ethanol 70%. After 48 hours, the compound was filtered through a Buchner funnel. Following that, the extract was concentrated by a distilling machine and then, it was dried in a 40-centigrade oven. For achieving different densities, the dried powder of the extract was measured and diluted into suspension using Saline solution. Finally, it was intraperitoneally injected into the rats (27).

This was an empirical study. The animals were classified into different experimental groups using a simple, random method. In this study, 42 mature, male, Wister rats were divided into 6 groups of 7.

The rats weighed between 200 to 250 grams and they had been purchased from Pasteur Institute of Iran. Before and during the study, the animals were kept within a cycle of 12 hours of darkness and 12 hours of light in 22 Celsius degrees. They were given standard rat food manufactured by Pasteur Institute of Iran.

The animals were divided into the following groups:

The Control group received only distilled water and they were put through balance behavior tests using the Rotarod device.

The Scopolamine group (SCOP) received one milligram of Scopolamine intraperitoneally injected per each kilogram of the body weight and they were put through balance behavior tests using the Rotarod device.

The group receiving 200 mg/kg of Chamomile ethanol extract and 1mg/kg of Scopolamine (SCOP+200MC): this medical group received 1 milligram of Scopolamine and immediately after that, they received 200 milligrams of the extract which was intraperitoneally injected. They were then put through balance behavior tests using the Rotarod device.

The group receiving 500 mg/kg of the extract and 1mg/kg of Scopolamine (SCOP+500MC): this medical group received 1 milligram of Scopolamine and immediately after that received 500 milligrams of the extract which was intraperitoneally injected. They were then put through balance behavior tests using the Rotarod device.

The healthy group receiving 200 mg/kg of the extract (Intact+200MC): this group only received the extract intraperitoneally injected for 20 days. They were then put through balance behavior tests using the Rotarod device.

The healthy group receiving 500 mg/kg of the extract (Intact+500MC): this group only received the extract intraperitoneally injected for 20 days. They were then put through balance behavior tests using the Rotarod device.

Balance and motor coordination test by the Rotarod device: The rats' power of balance preservation and motor coordination were surveyed using the Rotarod device which was a model 6700 mt specifically manufactured for rats by Tower Technology Company.

Rotarod is a device used for measuring the ability of balance preservation and motor coordination in animals. It has a rolling rack with a rotating speed range of 0-40 rpm. The rolling rack speed could be adjusted by relocating the device belts.

For the animals to become familiar with the device, they were initially placed on the Rotarod rolling rack so that they could learn how to move on it according to the major protocol (10 rpm rotating speed with 7rpm² velocity). After 30 minutes, we were ready to start the balance test. In this study, the speed was considered to be 10 rpm and the velocity was 7rpm² which was equal to 10-11 rounds per minute. In each of the experimental groups, the rats were placed on the rolling rack one hour after the injection of the extract.

The amount of time that the animal was able to keep its balance and resist the rolling rack's movement was recorded as its resistance time. The maximum time of investigation for each animal in the test was 300 seconds (28). The test was repeated 3 times with an interval of 30 minutes in between the repetitions. Eventually, the average time was calculated.

Determination of the Phenolic compounds: For measuring the Phenolic compounds, 0.1 millilitre of diluted extract (0/01 gr in 10 millilitre of 60 degree methanol) was added to 0/5 millilitre of Folin Ciocalteu and after 3-5 minutes, 0/4 milligrams of Sodium carbonate %7/5 was added to the compound.

After 30 minutes of incubation in the room temperature, the absorption was read in comparison to blank, distilled water. Gallic acid was also produced while the test was being conducted. Gallic acid was tested using the method above and the standard curve was prepared. The samples' absorption was compared to the standard curve and the amount of the total phenols of each extract was measured at 1 milligram for each gram of the dried extract (29).

Determination of Flavonoid compounds: For measuring the Flavonoid compounds briefly, 0/5 millilitres of the solution of each extract (0/01 gr in 10 millilitre of 60 degree methanol) was combined with 0/5 millilitres of Aluminium chloride %2 and 3 millilitre of Potassium acetate 5% was also added to the mix. After 40 minutes, the absorption of the samples against distilled water was regarded at a wavelength of 415

nanometres. At the same time, different dilutions were prepared and tested using the method above and the standard curve was also confirmed. The samples' absorption was compared to the standard curve and the amount of Flavonoid was measured at 1 milligram per gram of the dried extract (30).

Determination of Flavonol compounds: For measuring these compounds, 0/5 millilitres of the solution of each extract (0/01 gr in 10 millilitre of 60 degree methanol) was combined with 0/5 millilitres of Aluminium chloride %2 and 3 millilitres of Sodium acetate 5% was added to the mix as well.

After 40 minutes, the absorption of the samples against distilled water was regarded at a wavelength of 440 nanometres. At the same time, different dilutions were prepared and tested using the method above and the standard curve was also confirmed. The samples' absorption was compared to the standard curve and the amount of Flavonol was measured at 1 milligram per gram of the dried extract (31).

Investigating the antioxidant properties by testing 2, 2-Diphenyl-1-picrylhydrazyl (DPPH): Through this method, DPPH was introduced as an identifier for combining sustainable radicals. The method consisted of adding 50 micro litres of different densities like 10, 15, 20, 25, 30, 40, 50, 60, 70 and 80 micrograms per CC of the extracts in methanol into 5 millilitres of 0/0004% DPPH in methanol. After 30 minutes in room temperature, the optical density of the samples was regarded in the wavelength of 517 nanometres against blanc. The percentage of the inhibiting DPPH free radicals was calculated using the following formula:

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

In this formula, A_{control} is the optical density of the negative control which does not contain the extract while A_{sample} is the optical density of different concentrations of the extract. Following that, the density of the extract which contained 50% of the free radicals was measured using the diagram. Evidently, with a smaller number there was a higher antioxidant power or free radicals. In this study, synthetic antioxidant-butyl hydroxytoluene was used as the positive control and all the tests were repeated twice (32).

Statistical Analysis: ANOVA was used for the statistical analysis of the obtained data. In case there was a level of significance, Fisher's Least Significant Difference (LSD) test was used to determine the different significance levels between the groups and $p < 0/05$ was regarded as the level of significance.

Results

Standardization of Matricaria chamomilla: The rate of Phenolic, Flavonoid and Flavonol compounds were 78/4, 26/5, and 47/6 milligrams per 1 gram of the dried extract, respectively.

Antioxidant activities of Chamomile: IC_{50} was reported as the amount of free radical activities in the Chamomile extract (table 1).

Table 1. Comparing Antioxidant Activities in Chamomile with Hydroxyltoluene as the Positive Control using DPPH (32)

Sample	Density (µg/ml)	Percentage of DPPH radical scavenging (%) IC_{50} (µg/ml)
Chamomile extract	80	74.8
	70	65.3
	60	56.9
	50	49.2 (IC_{50})
	40	41.29
	30	32.4
	25	27.5
	20	22.15
	15	18
	10	5.8
Hydroxyl toluene	20	8.8
	50	14.4
	100	30.7(IC_{50})
	250	68.2
	500	79.3
	700	95.3

Statistical tests revealed no significant differences in the time delay of falling off the rolling rack between the Scopolamine receiving group and the control group ($p < 0/001$).

It was also confirmed that Scopolamine could significantly decrease this time. Compared to the Scopolamine receiving group ($p < 0/001$), the results indicate that injecting 200 and 500 milligrams of the Chamomile ethanol extract to the healthy group which received only the extract (Intact 200MC, intact 500MC) could significantly increase the resistance time as well as the ability to remain on the Rotarod's rolling rack. Furthermore, the Scopolamine group which received 200 milligrams of the extract could stay on the rolling stack for a longer time in comparison with the Scopolamine-only group ($p < 0/05$) (fig 1).

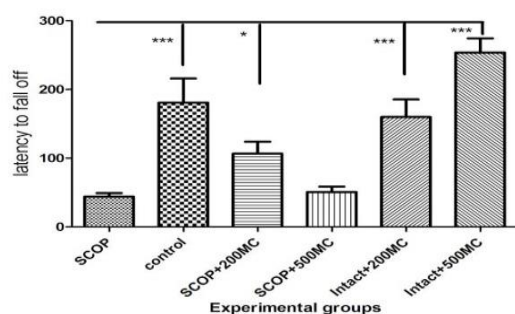


Figure 1. Comparison of Average Motion Test between SCOP group and other experimental groups using the Rotarod device $p<0/001$ ^A, $p<0/05$ ^B

Discussion

The results of the current study indicated that Scopolamine could significantly decrease the resistance time in the Scopolamine receiving animals. Compared to the control group, they could stay on the rolling rack longer ($p<0/05$). Moreover, curing by Chamomile ethanol extract increased the resistance time in the Scopolamine receiving rats enabling them to stay on the rolling rack for a longer time. One of the most evident features of brain deterioration is impaired memory and disordered learning which could be chemically infused in experimental animals using Scopolamine.

Scopolamine is recognized as a cholinergic antagonist which interferes with the transmission of Acetylcholine in the central nervous system (8). As an important marker in the lipid peroxidation, receiving Scopolamine is proven to increase the level of Malondialdehyde in rats' brain as well as decrease the level of cerebral Glutathione. Moreover, when compared to healthy people, lipid peroxidation in the brains of the Alzheimer's patients is observed to increase significantly (7). The findings of the present study confirmed that Chamomile ethanol extract exerts antioxidant effects protecting the rat's brain from high levels of oxidative conditions by using Scopolamine. Brain cortex and hippocampus are in correlation with the control cognitive and motor functions. They seem to be quite sensitive to oxidative stress which increases their need for antioxidants. Furthermore, it is assumed that oxidative stress acts as a decreasing factor of cognitive and motor functions among the elderly (2). One of the major problems in the Alzheimer's disease is Apraxia. It is a condition in which the patient loses the ability to perform purposeful and coordinated movements without being paralyzed, suffering sensory impairments or having difficulty moving the limbs (28). Among the most essential compounds of Chamomile are Flavonoids and other necessary essences like Musin, Coumarin, Carboxyl acid phenolic, Amino acids, Choline and Phytosterols (29). For another thing, Chamomile hydro alcoholic extract (500,600,800 and

1000 mg/kg) is proven to play a key role in preventing seizures caused by the existence of nicotine in white small mice which reveals the fact that hydro alcoholic extract has anticonvulsant properties as well (33). There is ample evidence indicating that Scopolamine is capable of generating oxidative stress in brain. This phenomenon happens through the interference with Acetylcholine in the brain which could give rise to certain cognitive impairments (7). Chamomile contains polyphenolic compounds as well as substantial amounts of antioxidants which are a testament to this plant's many beneficial effects. Normally, Alzheimer's patients receive large proportions of chemical drugs to cure the symptoms of their illness. Nevertheless, these drugs might produce many reverse and adverse effects. Such examples are dizziness, anxiety, sleepiness, amnesia, mood swings, memory impairment or digestive disorders (34). These negative side effects convinced us to investigate the effects of herbal medication as an alternative treatment for this disease. Laboratory analysis and pharmacological evaluations have realized that Chamomile contains oily Terpenoides (Azulene, Chamazulene, Bisabolol oxide, Sesquiterpene A and B), Flavonoids (Chrysene, Luteolin, Quercetin), Coumarins, and other beneficial compounds such as Mucilage, minerals, Polysaccharides, Tannins and fatty acids (35). For another thing, previous studies have claimed that the compounds found in the Chamomile extract are likely to have anxiolytic and soothing effects. Additionally, they are known to increase the level of physical activity in the model of anxiety evaluation (36). During our experiment, we also concluded that injecting Scopolamine could cause motor coordination impairments in rats through creating oxidative stress in the brain.

Moreover, we discovered that the ethanol extract of chamomile has strong antioxidant properties and can noticeably decrease the level of oxidative stress. Eventually, it will be able to enhance the motor coordination effectually. So far, there are a limited number of studies conducted on this subject. According to our findings, there were improvements in the resistance time and the fact that the rats were able to stay longer on the Rotarod's rolling rack in the Scopolamine group receiving the extract could be a result of the favourable Acetylcholinesterase features of chamomile in addition to its antioxidant properties.

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