http://jbcp.shahed.ac.ir 10.22070/jbcp.2020.4949.1123

Open Access Research Article

Journal of Basic and Clinical Pathophysiology
Print ISSN: 2322-1895, **Online ISSN:** 2345-4334
Vol.8, No.1, June 2020

A Semiannual Publication of
Shahed University Press

The effects of swimming exercise and *Nepeta menthoides* on depression and anxiety induced by reserpine

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Abstract

Background and Objective: Physical activity has long been associated with mental health. *Nepeta menthoides* is a native Iranian herb recently acknowledged for its anti-depressant properties. This study attempted to determine and compare the effects of swimming exercise and Nepeta menthoides on reserpine-induced depression in rats.

Materials and Methods: Male rats (n=80) were assigned to 8 groups: 1-Saline, 2-Reserpine (0.2 mg/kg, i.p. for 14 days) 3-Swimmig Exercise(30 min swimming sessions daily for 2 weeks) 4-Nepeta (200 mg/kg), 5-Reserpine+Nepeta, 6-Reserpine+Swimming exercise, 7-Reserpine+Nepeta+Swimming exercise, 8-Reserpine+Fluoxetine, Finally, the behavioral tests including sucrose preference, elevated plus maze and open field were performed.

Results: Obtained data showed that depressed rats which were treated with Nepeta, exercise or both of them expressed higher preference for sucrose relative to reserpine group and their locomotor activity was also significantly improved. However, combined Nepeta+Exercise could not significantly antagonize the effect of reserpine on time spent in the open arms of elevated plus maze, but Nepeta in particular displayed marked anti-anxiety effect.

Conclusion: Combined treatment with Nepeta+exercise as the same as only Nepeta or exercise was able to alleviate anhedonia and low activity in depressed rats. Nevertheless, Nepeta alone could significantly improve reserpine-induced anxiety.

Keywords: Depression, Reserpine, Swimming Exercise, *Nepeta menthoides*

1. Introduction

Depression and anxiety are the most common mental disorders affecting millions of people around the world (1). Depressed individuals deal with numerous complications such as anhedonia, low mood and suicidal thoughts which disrupt their daily activities and day to day life (2), however, all of these complications can not be treated by the available anti-depressant treatments (3). It is important to look back on what has been done before and find the missing pieces while searching for effective yet safer alternatives. Physical activity and herbal medicine offer reasonable opportunities in that regard (4). Exercise causes general well-being, positivity and happiness through increasing 5-HT, dopamine and glutamate (5-7). There is clinical evidence of exercise-induced antidepressant effects and animal studies have shown that regular exercise in the form of swimming sessions is able to ameliorate depression-like behaviors caused by chronic stress (8). It is also suggested that vasoactive endothelial growth factor (VEGF) signaling pathway could be another way in which exercise affects depression, not to mention the effects it has on brain derived neurotrophic factor and other neurotrophins (9).

Received: 22 Jan 2020, **Accepted:** 20 May 2020, **Available online:** 23 June 2020
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Nepeta menthoides Boiss & Buhse is a native Iranian herb acknowledged for its therapeutic effects (10). Nepeta menthoides is from the Lamiaceae family of plants and widely distributed throughout the northwest parts of Iran (11). Many reports have been published on its antidepressant and anxiolytic properties (12). Therefore, combining and comparing the effects of exhilarating mild exercise with presumably safe herbal medication for medical purpose against depression comes as a rational plan. In this study, we attempted to determine and compare the effects of swimming exercise and Nepeta menthoides on reserpine-induced depression in rats.

2. Materials and Methods

2.1. Materials

Reserpine was purchased from Sigma Chemicals Co (St. Louis, MO , USA). Fluoxetine was provided from TEMAD Co (Karaj, Iran).

2.1.1. Preparation of Nepeta menthoides

Dried aerial parts of *Nepeta menthoides* were obtained from a local herbal medicine store and was confirmed by the Tehran Medical University Herbarium, where it was recognized under the voucher number PMP-302. Then, 500 g of the milled plant went through the maceration process by adding approximately 2 l of ethanol (70%) and was kept in the dark room for 72 hours. The solution was then filtered and evaporated at 50˚C from which a wax-like extract was obtained to be used at the desired dose of 200 mg/kg.

2.2. Methods

2.2.1. Animals

Male adult Wistar rats (250-300 g) (Razi Vaccine and Serum Research Institute, Alborz province) were housed 10 per cage in a temperature-controlled room under 12 h light/dark cycle. Animals were given free access to water and food all the while kept at their stable body weight throughout the experiment. This study was carried out in accordance with the policies described in the guide for the Care and Use of Laboratory Animals (NIH).

2.2.2. Experimental procedure

Rats (n=80) were randomly divided into 8 groups each one 10 numbers:1. Control 2. Exercise 3. Nepeta 4. Reserpine 5. Reserpine + Exercise 6. Reserpine + Nepeta 7. Reserpine + Exercise + Nepeta and 8. Reserpine + Fluoxetine. In order to induce depression like behavior in rats, reserpine was administered chronically at a dose of 0.2 mg/kg, i.p. for 14 days (13) while the exercise groups went through swimming sessions 30 minutes each daily for 2 weeks (14). At the same time, Nepeta groups received Nepeta extract at a dose of 200 mg/kg and the fluoxetine group were treated with fluoxetine at a dose of 10 mg/kg for two consecutive weeks.

2.3. Behavioral tests

2.3.1. Sucrose preference test

Three days prior to the test, animals were adapted to the existence of two bottles in their cages, starting from the fourth day animals were given the choice between water and sucrose 2% in 8-hour trials for four consecutive days. The volume of the bottles was measured before and after each trial. Sucrose solution intake and total consumption was recorded at the end of each trial and reported in percentage. Low sucrose solution intake is interpreted as anhedonia, which is a main sign of depression (15).

2.3.2. Open Field Test

Total locomotor activity was assessed through the open field test. The open field was an enclosed box of 60*60 square consisting of one big central square and 16 peripheral squares. The activity of animals was recorded for a total of 10 minutes each. The space was cleaned with ethanol 70% in between each test to avoid any traces of smell and presence. Total number of line crossings were reported as an index of locomotor activity. Decreased locomotor activity is attributed to anxiety and depression (16).

2.3.3. Elevated plus maze

The elevated plus maze (EPM) is a widely used model for the study of anxiety-like behavior in rodents. This plus shaped apparatus is elevated from the floor and consists of two arms with walls of 40 cm height and two arms which are open platforms. Each arm is 40 cm in length and 10 cm in width. There is a central 10*10 square at the intersection area of the four arms in which the rats are left to explore the maze for 5 minutes. The time spent in the open arms was recorded and then reported in percentage. Increased activity and time spent in the open arms is a sign of anti-anxiety behavior in rodents (17).

2.4. Statistical analysis

All the results were expressed as mean ± S.E.M and analyzed for statistical significance by one-way ANOVA and Tukey post hoc test. Kruskal-Wallis test was used to analyze non-parametric data and $P<0.05$ was assumed as significant.

3. Results

3.1. The effect of swimming exercise and Nepeta on depression in sucrose preference test

Figure 1 shows that reserpine reduced the sucrose preference ratio significantly compared to the control, exercise and Nepeta groups and all of the treatment groups were able to antagonize the effect of reserpine. However, there was not a significant difference among the experimental treatment groups.

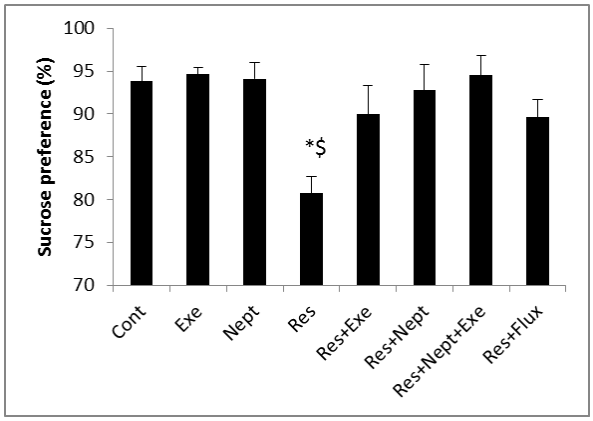

Figure 1. The effect of swimming exercise and Nepeta on sucrose preference ratio after i.p. injection of reserpine in rats. Sucrose preference enhancement shows antidepressant effect. Values are expressed as means ± S.E.M. * P<0.05 (reserpine in comparison with control, exercise and Nepeta groups). $ P<0.05 (reserpine in comparison with reserpine+exercise, reserpine+Nepeta, reserpine+Nepeta+exercise and reserpine+fluoxetine groups).

3.2. The effect of swimming exercise and nepeta on depression in the open field test

Figure 2 shows mean± SEM of total locomotor activity in all of the experimental groups, according to which reserpine has reduced the number of line crossings markedly compared to the control, exercise and Nepeta groups. However, the experimental treatment groups as well as fluoxetine significantly ameliorated the decreased activity due to reserpine, however there was not a significant difference between the treatment groups themselves. Also, exercise seems to have markedly increased locomotor activity of the animals compared to the control group.

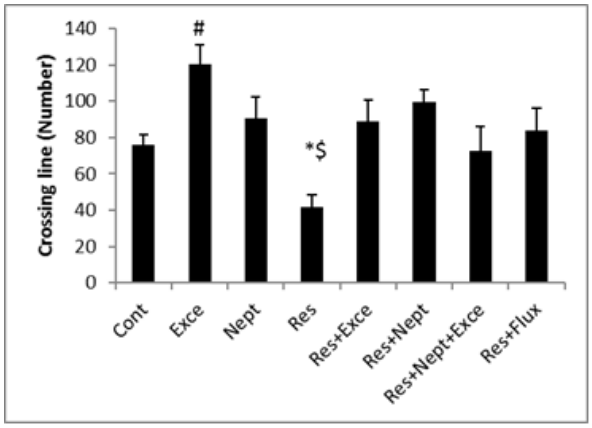

Figure 2. The effect of swimming exercise and Nepeta on locomotor activity in the OFT after injection of reserpine in rats indicated by the number of line-crossings. Values are expressed as means ± S.E.M. * P<0.05 (reserpine compared with control, exercise and Nepeta groups). $ P<0.05 (reserpine compared with Reserpine+exercise, reserpine+Nepeta, res+exercise+Nepeta and reserpine+fluoxetine). # P<0.05 (exercise vs. control).

3.3. The effect of swimming exercise and Nepeta on anxiety in the elevated plus maze

Figure 3 shows mean ± SEM of time spent in the open arms (%OAT) of elevated plus maze. The more the animal stays in the open arms, the less are the anxiety levels. Exercise and Nepeta significantly increased the time spent in the open arms compared to control group. Reserpine reduced %OAT markedly compared to Exercise and Nepeta group. Nepeta was able to antagonize anxiety behavior in contrast with fluoxetine.

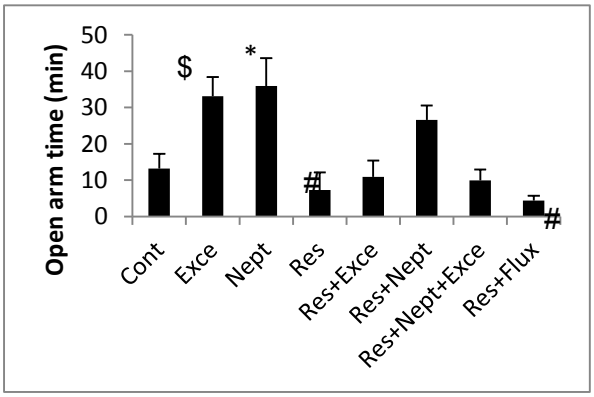

Figure 3. The effect of swimming exercise and Nepeta on percent of the time spent in open arms of the EPM after injection of reserpine in rats. Values are expressed as means ± S.E.M. * P<0.05 (Nepeta in comparison with all the groups except for exercise and reserpine+Nepeta). $ P< 0.05 (exercise in comparison with all groups except for Nepeta and reserpine+Nepeta). # P<0.05 (reserpine and reserpine+fluoxetine in comparison with exercise, Nepeta and reserpine+Nepeta groups).

4. Discussion

The results of the present study demonstrated that repeated administration of a low dose of reserpine caused a significant decrease in locomotor activity of the rats in open field test and reduced sucrose preference which is considered a case of anhedonia; one of the main symptoms of depression. On the other hand, the experimental treatments suggested by the present study including swimming exercise and nepeta were able to attenuate depression like behavior induced by reserpine in the rats.It is agreed upon for years that reserpine which was primarily an antihypertensive drug induces depression like behavior (18). Repeated treatment with reserpine can be used as a progressive model of depression (13, 19). Many studies have assessed the effects of a single dose and chronic administration of reserpine through different behavioral tests. They have described these effects as increased immobility time and reduced active swimming time in the forced swim test, low ratio of preference for sucrose solution and overall inadequate locomotor activity in the open field test (20-22).

The results of present study show that chronic administration of low dose of reserpine caused significant decrease in locomotor activity of the rats in the open field test. Also, reserpine decreased sucrose preference which is an index of depressive behavior. Reserpine decreased the time spent in the open arms of the EPM that is considered an indication of anxiety in the animals.The behavioral effects of reserpine are attributed to its biochemical properties. It is well-known that reserpine depletes monoamine stores in the brain (23). Monoamine depletion in brain is a well-recognized hypothesis for the pathophysiology of depression and anxiety that has been studied upon over the years (24). Depleted levels of monoamines (including serotonin and dopamine) lead to depression like behaviors which makes reserpine a common animal model of depression (20). The results of present study is in the same direction with previous studies regarding reserpine-induced depression. On the other hand, Exercise and Nepeta were shown to have clear anti-depressant effects either alone or combined, while Nepeta in particular was effective against anxiety induced by reserpine. It seems that anti-depressant effects of exercise are mediated by increased serotonin levels (25) and regulation of many different biochemical mechanisms including antioxidant defense enzymes (26), while anti-depressant and anti-anxiety effects of Nepeta are attributed to its anti-inflammatory and antioxidant properties (27, 28). Increasing brain monoamine levels by Nepeta has not been investigated before which must be studied in the future.

In conclusion, this study clearly demonstrated that treatment with either exercise or nepeta or both of them could significantly antagonize depression symptoms caused by reserpine which is comparable to Fluoxetine, while only nepeta was able to exert significant anxiolytic effects against reserpine. Nevertheless, in order to determine other behavioral and biochemical effects and therapeutic potential of exercise and nepeta as a combined treatment program on different layers of depression, performing further studies are necessary. These studies should include different behavioral tests such as the tail suspension tests and biochemical tests to measure brain monoamine levels and oxidative stress markers.

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