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The effect of hesperetin on depression and anxiety induced by reserpine injection in male rats

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Abstract

Background and Objective: Hesperetin is a citrus skin active ingredient with neuroprotective and antioxidant properties. In this study,we evaluted the therapeutic effect of hesperetin on depression and anxiety induced by reserpine injection according to behavioral tests in rats.

Materials and Methods: For this evaluation, 60 male rats weighing 230-250 mg / kg were divided into six groups, control group receiving saline for 14 days , groups receiving hesperetin (20 and 10 mg / kg for 7 days) , depressed group with reserpine (0.2 mg / kg for 14 days) and depressed groups receiving hesperetin (20 and 10 mg / kg for 7 days). After taking the drugs, the effect of hesperetin was evaluated in behavioral tests.on depression and anxiety.

Results: The results showed that hesperetin significantly reduced the anxiety of depressed animals in Open Field and Elevated Plus Maze Tests ($P < 0.05$). In Forced Swimming Test, hesperetin 20 mg / kg caused a significant decrease in immobility time ($P < 0.001$), and also, hesperetin could decrease the immobility time in depressed groups($P < 0.05$).According to Sucrose Preference Test's data, rats that received 20 mg / kg of hesperetin were more prone to sweet water in compared with reserpine received group.

Conclusion: According to the results of this study, hesperetin is effective in reserpine-induced depressive symptoms in rats.

Keywords: Forced Swimming Test, Sucrose Preference Test, Open Field Test, Elevated Plus Maze Test, Vegetables

1. Introduction

Depression and anxiety are the most common brain disorders worldwide. According to statistics, the risk of depression in women is more than of men, so depression is responsible for 50 to 70 percent of suicides (1). Symptoms of depression may include feeling inadequate, feeling unwell, fatigue, memory impairment, sleep disturbance, suicidal thoughts, and memory impairment, unexplained muscle aches that may lead to decreased quality of life or even mortality (2). There are many theories to explain the pathophysiology of depression and anxiety including the theory of monoamines, the theory of amino acid neurotransmitters(3), (4) neuronal atrophy and decreased synaptic communication in key cortical and limbic regions(5) and exposure to stress and activation of the stress axis (6).

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According to the theory of monoamines, the level of brain monoamines, including noradrenaline, serotonin and dopamine in depression decreases (3, 4). Monoamines in central nervous system structures such as the peripheral, temporal cortex, limbic structures (hippocampus and amygdala), and the basal nuclei are responsible for the formation of the frontal-subcortical connection and for control of tasks such as movement, motivation and motility (6). Two fundamental reasons for the theory of monoamines are the effect of reserpine on serotonin and catecholamines and the mechanism of action of antidepressants. Reserpine is an alkaloid used to treat hypertension, and inhibits storage of serotonin and catecholamines in the synaptic terminals by effect on their transporter, also causes the serotonin and catecholamines decompose under the influence of the enzyme monoamine oxidase (7,8).

Oxygen free radicals are the product of the metabolism of monoamines inside the cell and their auto-oxidation in the body. Research has shown that oxygen free radicals, in the other words oxidative stress, can disrupt the balance of monoamines in the central nervous system, and thus provide a basis for depression(7). Malondialdehyde (MDA) biomarker significantly increases as one of the indicators of oxidative stress in depression (8). It has also been shown that body antioxidant systems such as superoxide dismutase (SOD) and glutathione (GSH) are reduced in depression (10, 11). Studies have also shown that chronic activation of the stress axis and release of cortisol causes neuronal damage in the hippocampus and peripheral cortex, BDNF depletion and neurogenesis restriction (9). Both pharmacological and non-pharmacological treatments are used to treat depression (10). A broad spectrum of drugs increase the function of monoamines by inhibiting the function of monoamine oxidase and thereby enhancing the uptake of monoamines and their storage at synaptic terminals. But despite of many classes of drugs available for depression, due to their side effects, people's interest in using herbal remedies has increased (11).

Recent studies have shown that depression is associated with dietary patterns and food intake. For example, fruits and vegetables such as citrus can be effective in treating brain disorders such as depression and anxiety (1). Chemicals in plants (fruits and vegetables), including alkaloids, polyphenols, triterpenoids, essential oils, fatty acids, flavonoids, have anxiolytic and antidepressant properties(1,8). Flavonoids have useful biological properties such as neutralizing free radicals, antibacterial and antiviral activity. Hesperetin is a flavonoid in citrus peels such as lemon and grapefruit, which is cholesterol-lowering, lipid-lowering, anti-inflammatory, anti-cancer and neuroprotective. Hesperetin increases the activity of the antioxidant enzymes such as superoxide dismutase and catalase and glutathione reductase and glutathione peroxidase in the hippocampus and decreases MDA (12). Studies have also shown that hesperetin improves the decreased levels of BDNF in brain disorders, for example in Alzheimer's disease (13).

In this study, due to the neuroprotective and antioxidant properties of hesperetin, we evaluated the effect of hesperetin on the treatment of depression and anxiety induced by reserpine injection in male rats by behavioral tests.

2. Materials and Methods

Healthy male adult Wistar rats (n=100), weighing 230-250 g, were obtained from Karaj Razi Vaccine and Serum research institute and were maintained in a standard environment for one week acclimatization period before experiments at the Shahed University Behavioral Testing Center. All of the experimental procedures were approved by the Ethical Committee of Shahed Medical University and carried out in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals. The animals were kept in separate groups of five and had access to adequate water and food except for behavioral tests.

In this study, reserpine and hesperetin (Sigma-Aldrich, USA) were used and dissolved in normal saline and ethanol (13). The animals were divided into the following ten groups:

The control group received saline via gavage equivalent to the other volume of injection (0.3 ml) daily for two weeks.

The second group received saline (0.3 ml) for 14 days and then received Hesperetin 10 mg/kg (0.3 ml) for 7 days by gavage (14).

The third group received saline (0.3 ml) for 14 days then received Hesperetin 20 mg/kg (0.3 ml) for 7 days by gavage (14).

The forth group received a low dose of reserpine 0.2 mg/kg intraperitoneally for two weeks (14).

The fifth group received a low dose of reserpine 0.2 mg/kg intraperitoneally for two weeks then received hesperetin 10 mg/kg (0.3 ml) for 7 days by gavage.

The sixth group received a low dose of reserpine 0.2 mg/kg intraperitoneally for two weeks then received hesperetin 20 mg/kg (0.3 ml) for 7 days by gavage.

2.1. Behavioral tests

2.1.1. Open Field Test (OFT)

The open field environment is a means of measuring anxiety, a square environment measuring 72.72 inches wide and 36 cm walls that divided into 16 squares (18.18) and a central square (18.18) in the middle of the open box. At the beginning of the experiment, the rat is gently placed in the center of the environment

and allowed for 5 minutes to explore the area freely. Evaluation indices in this test are: number of central entries, central box spending (min) and number of line crossing entries (13, 15).

2.1.2. Elevated Plus Maze Test (EPMT)

This test was performed to assess the level of anxiety in the animal. The plus maze is a tall black wood and has four arms, two open arms and two closed arms. The dimensions of the open and closed arm are 10 x 50, on both sides and end of the closed arm are walls 40 cm high. Four arms reach a central range of 10 x 10 cm. The maze is positioned at a height of 50 cm above the ground by a stand. Suitable light is provided by a 60-watt bulb that is illuminated by a maze arm (16). For behavioral testing, mice were placed individually in the center of the maze plus an open arm and allowed to search for 5 minutes freely (17). During this time, an observer sitting in a maze of logs recorded the number of arrivals and the time spent in each arm for 5 minutes. Increasing the presence of open arms or entering the open arms is considered as an anxiolytic activity and decreasing these two indices is considered as anxious behavior (16, 17).

2.1.3. Sucrose Preference Test (SPT)

This test is used to assess depression. In this test, after a twenty-three-hour period of food and water deprivation, each rat was given free access to two pre-weighed bottles of water and 1% sucrose for one hour separately. Each bottle was weighed before and after one hour access to water and then calculated as a percentage of sucrose preference:

Sucrose Preference Percentage (SPP); Freshwater Consumption / Total Water Consumption (Normal Water + Freshwater) x 100 (18).

2.1.4. Forced Swimming Test (FST)

This test is done in two days to assess depression. The test uses cylinders 50 cm high and 19 cm in diameter. The cylindrical material is plexiglass and filled with 25ºC water up to 35 cm high.

On the first day of acclimatization, place the animal in a water-containing cylinder for 15 minutes and, after the desired time, remove the animal at 30 ° C to dry and place the animal in the water-containing cylinder again 24 hours later. Data and measure the immobilization time of the animal in water for 5 minutes. The animal's immobility index is that the animal is floating in the water so that it only gets out of the water with very minor movements. This immobility is interpreted as a strategy to cope with depression (6, 19).

3. Results

Number of central entries and central box spending in OFT showed no significant differences (P> 0.05) among the experimental groups (Figures 1 and 2).

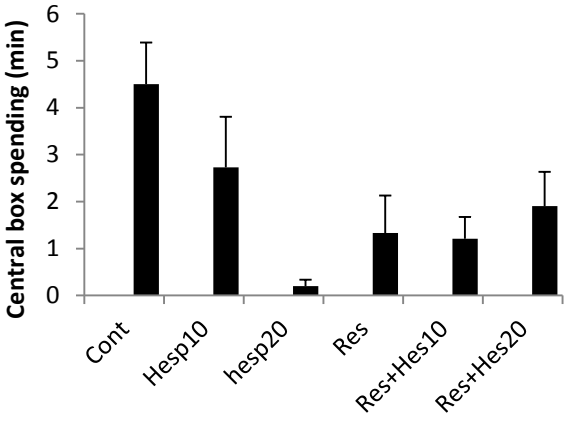

Figure. 1: Central box spending (min) in experimental groups of OFT: The columns represent the center box spending ± standard deviation. There was no significant difference between groups. Con=control hes=hesperetin res=reserpine

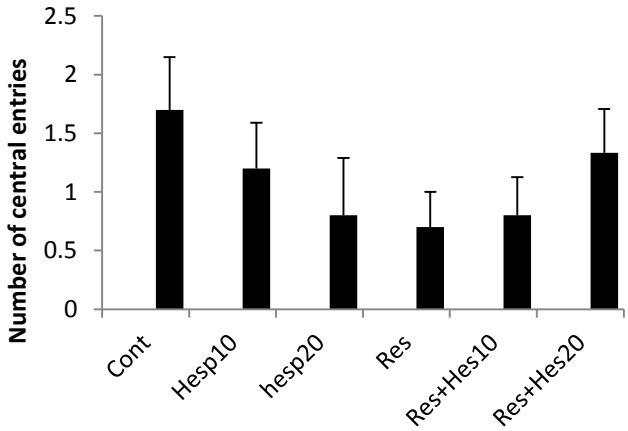

Figure. 2: Number of centeral entires in experimental groups of OFT: The columns represent number of centeral entires ± standard deviation. There was no significant difference among groups. Con=control hes=hesperetin res=reserpine

In the OFT, the number of line crossing in the reserpine receiving group (30.2±5.66) in compare to the other groups significantly decreased (*p<0.05). Cont "46.5 ± 5.516", Hes10 "51.6 ± 5.474", Hes20 "52.9± 7.173", Res+ Hes 10 "51 ± 6.721", Res+Hes20 "44.556±5.603") (Fig. 3).

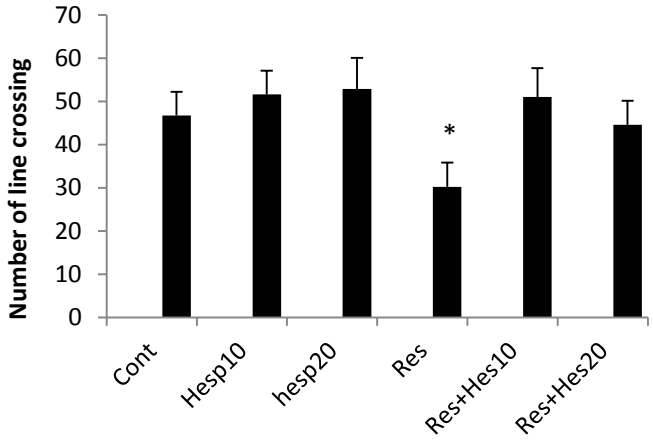

Figure. 3: Number of line crossing entires in experimental groups of OFT: The columns represent number of line crossing entires ± standard deviation. * Significant with other groups (p<0.05).

Open arm time in EPMTshowed no significant differences (P> 0.05) among the experimental groups (Figure 4).

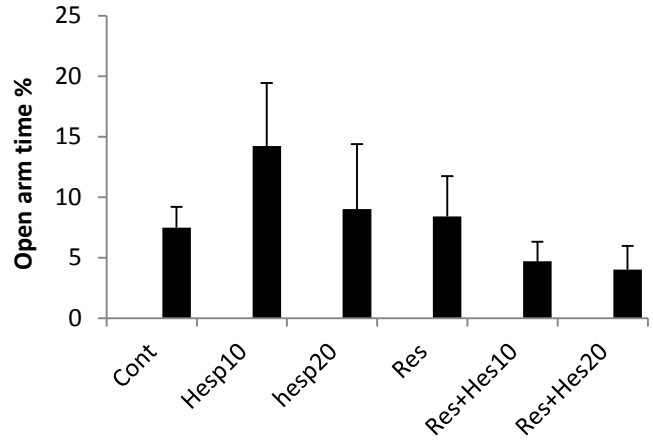

Figure. 4: Open arm time percent in experimental groups of EPMT: The columns represent open arm time percent ± standard deviation. There was no significant difference among groups. Con=control hes=hesperetin res=reserpine

Open arm entry in EPMT, reserpine-treated group (6.64± 2.13) showed significant changes (* P< 0.05) as compared to Control group (21.49±4.223) and Hes 10 and Hes 20 (29.29±7.297, 25.82±8.71) and Res+Hes 10 and Res+Hes 20 groups (27.44±5.408 and 29.98±8.18) (Figure 5).

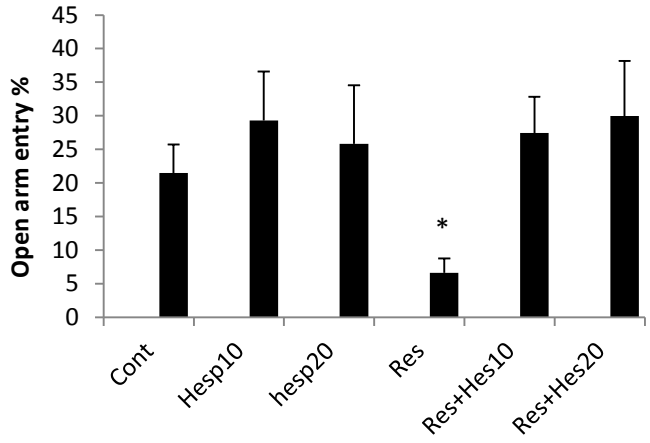

Figure. 5: Open arm entry percent in experimental groups of EPMT: The columns represent open arm entry percent ± standard deviation. * Significant with other groups (p<0.05). Con=control hes=hesperetin res=reserpine

One-way analysis of variance in SPT showed significant differences (* P< 0.05) between reserpine receiving group (41.68±5.757) as compared to Control group (67.69±3.33) and depressed group treated with Hes 20 (60.956±7.769) (Figure 6).

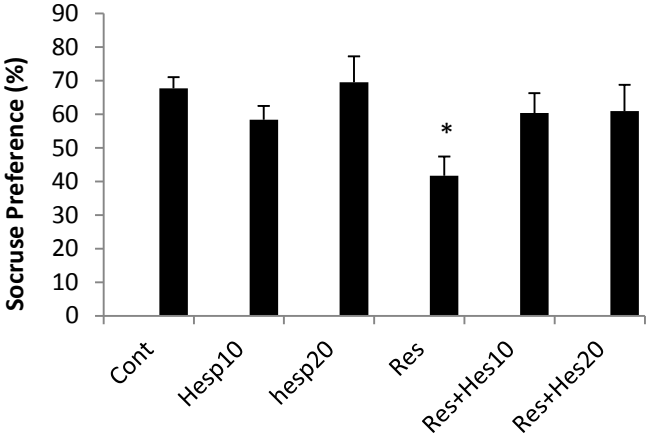

Figure. 6: SPT in experimental groups: The columns represent Socruse Preference percent ± standard deviation. * Significant with control group and hesperetin 20 mg/kg group (p<0.05). Con=control hes=hesperetin res=reserpine

In the FST, the reserpine receiving group (240.588±8.729) showed a significant increase (* P< 0.05) as compared to Control, Hesp10, hes20 (167±12.338, 162.9±18.378, 60.514±7.914) and Res+Hes 10, Res+Hes 20 groups (201.472±9.514 and 208.603±14.384), so Hes20 recipient group showed a significant decrease ($^{\$\$\$}$ P< 0.001) as compared to other groups (Figure 7).

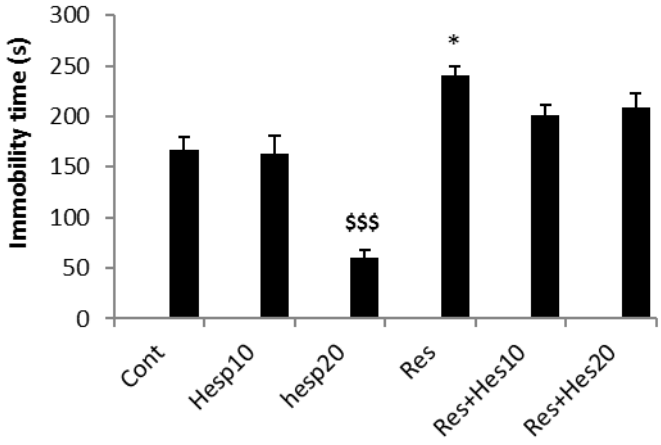

Figure. 7: Immobility time test in experimental groups of FST: The columns represent Immobility time ± standard deviation. * Significant with other groups ($p<0.05$) and $$$ Significant with other groups ($p<0.001$). Con=control hes=hesperetin res=reserpine.

4. Discussion

Hesperetin is a flavonoid in citrus peels such as lemon and grapefruit that has antioxidant, anti-inflammatory and neuroprotective properties. Hesperetin can cross the blood-brain barrier and be effective in treating diseases of the central nervous system, including depression (12). Kheradmanda and et al. showed that hesperetin in two doses of 10 and 20 mg/kg/body weight increases the activity of antioxidant enzymes and decreases malondialdehyde in the hippocampus therefore improves spatial memory (12). Ishola1 et al in a study on increased corticohippocampal memory due to hesperetin, concluded that hesperetin increases antioxidant effects, cholinergic and BDNF signaling, therefore improves impairments of spatial and non-spatial memory in scopolamine-induced Alzheimer's rats (14). Ikram et al concluded that hesperetin by regulating of Nrf2 / TLR4 / NF-κB signaling pathway, inhibits Aβ1-42-induced neuronal degradation in Alzheimer's rats and improves memory therefore can be used as an effective agent in the treatment of neurodegenerative diseases like Alzheimer (20).

Based on studies on depression, the role of reserpine in modeling depression (7), and properties of hesperetin(19,21,22), in this study we found out that hesperetin can reduce anxiety in depressed rats in OFT and EPMT and improve depression symptoms in the FST and SPT. But the central box spending, number of central entries in OFT and open arm time in EPMT did not reach the significant level. On the other hand, it is reported that in OFT, low dose of AMPH increases number of crossings and rearing behaviors in rats, while higher doses of this drug induce grooming and sniffing behaviors (23). It is possible that in other indexes that did not reach a significant level, there are other pathways involved in anti-depressant doses of hesperetin.

Conclusion

In accordance with behavioral tests, hesperetin can improve the symptoms of depression and anxiety induced by reserpine injection in male rats

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