Original Article

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Effect of Hydroalcoholic Extracts of Thyme on Movement Disorders, Depression and Pain Caused by the Injection of 6-Hydroxydopamine in Rat Model of Parkinson's

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ABSTRACT

BACKGROUND AND OBJECTIVE: Parkinson's disease is a common neuropathological disorder caused by degeneration of dopaminergic neurons in substantia nigra pars compacta. The aim of this study was to investigate the effect of hydroalcoholic extract of thyme on movement disorders, depression and pain resulting from injections of the neurotoxin 6-hydroxy dopamine in Parkinson models in male rats.

METHODS: In this study male Wistar rats weighing 250-300g were used and divided into five groups, eight rats per each group. The control group did not receive any lesion. Parkinson's Group (PD) received 6-OHDA (8 μg per rat in 2 μl saline) in the right anterior mid-brain (MFB). Third, fourth and fifth groups received 100, 200 and 400 mg/kg of thyme extracts through gavage feeding for 14 days. Then behavioral tests including forced swim test, hot plate test, Rotarod test, Open filed test and Catalepsy test were done.

RESULTS: Induction of PD by injection of 6-hydroxydopamine lead to depressive behavior, movement disorders and pain. Treatment of parkinsonian rats with thyme extract at concentrations of 100, 200 and 400 mg/kg significantly increased tolerance to pain. ($p<0.05$). Thyme extract at a concentration of 400 mg/kg significantly improved motor symptoms in Catalepsy test ($p<0.05$).

CONCLUSION: Thyme extract ameliorated pain and motor symptoms caused by Parkinson's in rats but had no effects on depression.

KEY WORDS: *Parkinson, thyme extract, depression, movement disorders.*

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Introduction

Parkinson disease (PD) is a neurodegenerative disease resulting from a gradual loss of dopamine nerve cells in the nucleus of substantia nigra, which is in the midbrain. The cause of death of dopamine cells in the nucleus of substantia nigra is not fully known (1). Factors such as oxidative stress, lipid peroxidation reduction, DNA damage and loss of glutathione and antioxidant enzymes cause degeneration of the dopaminergic neurons (1).

Parkinson's disease is complex cognitive disorders that included memory loss and impaired thinking and dementia. Movement disorders are well known in this disease, including tremor, stiffness, slowness of movement and imbalance (2). There are contradictory reports about the prevalence of mood disorders in Parkinson's disease.

Prevalence of depression in Parkinson patients have been reported about 2 to 76% (3-5). Depression symptoms overlap with symptoms of Parkinson's disease and often are considered synonymous to movement disorders in these patients. However, studies have shown that depression symptoms in patients will not be affected by physical symptoms and do not overlap with movement disorders (6). The destruction of dopaminergic neurons in the nigrostriatal region and reduction level of dopamine is associated with the development of Moody's behavior(5).

Pain, a common non-motor symptoms in Parkinson's disease disproportionately affects the quality of life of patients and it have been reported about 70 to 80% in Parkinson's disease (8 and 7). The pain symptoms of Parkinson's disease include, skeletal muscle pain (70%), dystonic pain (40%), published neuropathic pain (20%) of central neuropathic pain (10%) (9). In some patients, the pain is so severe and resistant to treatment that affects their mobility (10). Pain in these patients may occur before the onset of motor symptoms and motor symptoms in patients with pain may be more severe. In addition it was shown that the treatment of movement disorders by dopaminergic drugs reduce pain symptoms (7).

In the study by Cao et al, reported that the first week after injection of 6-hydroxy dopamine thermal and mechanical pain threshold in mice with Parkinson's disease decreases and continues to until the fifth week (11). Growing number of studies concerning the positive effects of some medicinal plants in the treatment of movement and cognitive disorders caused by Parkinson's disease in animal models and humans (11-13), However, few studies have been conducted in relation to pain and mood disorders. The positive effects of ellagic acid, phenolic compounds present in medicinal herbs is shown in one study that alleviate pain associated with Parkinson's model (14). In an experimental study, positive effects of Bacopamonnieri extract on depression and movement disorders caused by Parkinson's has been shown (15).

Thyme one Weed family Lamiaceae grows in different parts of the Mediterranean and some parts of Asia. In Iran, this plant is used in traditional medicine as an antispasmodic, carminative, anti-fungal, antiseptic, anti helmint, anti-rheumatoid and mucus(16). In addition, the antioxidant (17), antimicrobial (18), anti-inflammatory (19), anxiolytic (16), analgesic (20), antispasmodic (21) and against oxidative stress (22) and lowering blood pressure (23) effects of the plant in Laboratory studies have shown.

Due to the effects of analgesic, anti-inflammatory and antioxidant reported for Thyme this extract seems to improve mental and physical disorders caused by Parkinson's disease, this study was performed to investigate the effect of hydroalcoholic extract of thyme on movement disorders, depression and pain caused by injections of the neurotoxin 6-hydroxy dopamine in Parkinson's disease model in male rats.

Methods

Animal grouping: In this experimental study, male Wistar rats with a weight range 200-250 grams were used. Animals were kept in standard conditions (temperature 21 ± 2 ° C and 12 hours of light and 12 hours dark cycle) with free access to food and water. Rats were divided randomly into 5 groups of 8 animals. The control group did not receive any lesion. Group with Parkinson's (PD) got injecting of neurotoxin 6-hydroxy dopamine-sided 8 mcg in the right anterior-medial brain (MFB)(11). Groups, third, fourth and fifth 7 days after induction of Parkinson model at doses of 100, 200 and 400 extracts of thyme were treated by intragastric administration for 14 days and then conduct tests were performed.

Creating models of Parkinson: rats kg were anesthetized by intraperitoneal injection of 90 mg/kg of ketamine hydrochloride and 10 mg/kg Xylazine. Then the rats were fixed on stereotaxic by mouthpiece and ear sticks and dorsal hairs on skull were shaved. By cotton alcoholic skin antiseptic head and a longitudinal section through the back of the head between the eyes to

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between the ears was created on the dorsal surface. Connective tissue on the surface of the skull was removed and the Bregma was seen. Bregma and lambda points were placed in an equal level and indicator on the device has been set. Then, according to the coordinates extracted from brain surgery atlas, coordinates MFB (8.3 AP:, 8.1±ML:, 3.8- DV:) was determined. Rat Parkinson's disease group induced by injecting 8 mcg neurotoxin 6-hydroxy dopamine (prepared in 2 ml saline containing 1% ascorbic acid) in right anterior medial brain (MFB).

Behavioral tests: 14 days after treatment of behavioral tests, including Katalpsy tests (Horizontal), motor coordination test, forced swim test, test pain, outdoor test was conducted for 3 days. Behavioral tests in lighting time (8:00 am - 6:00 pm) was performed. All behavioral tests were carried out by a person who was unaware of belonging to groups of mice.

Katalpsy test (Bars): In this study, the bars test were used to assess Katalpsy. Tools used in this test, were wooden bars with a podium. In the rat, height of bars from platform is 9 cm and diameter of bar is 0.9 cm. To perform the test, the animal was placed on the podium and two hands placed it gently on the horizontal bars, the length of time the animal was in this situation, was considered as the bars test time. Cut testing time was when animal hands off one or both of its hands from the bars Or moved his head to search. Obviously, the animal Katalpsy is more intense, more time spent in the applied state.

Motor coordination test: This test is performed to measure the amount of motor coordination and harmony in motion. For this purpose animals were placed on bars rotarod apparatus (Rotarod) that speed of its movement is variable, the initial speed of rotation was 5 rpm, and the speed of rotation of bar within 300 seconds (5 minutes) gradually increased until rpm 25. The main criterion for balance in all groups was the speed of 25 rpm. The test was already familiar to animals.Then each rat three times in one day and 45-minute intervals between sessions were tested and the average was calculated.

Forced swimming test: This test is one of the most prestigious and the most common animal tests for depression. An increase in the idle time consider as depression and reduction of depression consider as the effectiveness of antidepressants. The test method is Glass container with a length of 25 cm, width of 12 cm, height of 15 cm Is filled with 25°C water and animal from the height of 20 cm and placed gently into the water. The contract, cutting off movements of rat hands and feet considered as immobilization. All samples are timing by an individual. The forced swim test is ten minutes and the first two minutes is intended for the animal to comply with conditions and is not registered as immobility time but immobility time is measured for the next 8 minutes (3).

Pain test: using the tail flick threshold of pain was evaluated in animals in different groups. This method is the standard method for assessing pain in animal models, by radiating 50 to 55°C heat to the point at a distance of 8 cm from the tip of the tail of rats, or the tail flick latency was recorded from the thermal center.Thermal cut-off time set to 10 seconds and controlled to prevent tissue damage in the tail (2).

The environment test: movement disorders, anxiety and depression in laboratory animals are measured by the environment test. Outdoor encapsulate is the glass with dimensions of 80 x 80 cm and height of 60 cm, which is equally divided into 16 squares and located in the middle of the quiet room. For testing at least one hour before the experiment, the rats were placed in the test chamber. One day before the test individual animals were placed in the machine for 10 minutes to become familiar with it. The next day, each animal was placed in the center of the square for 10 minutes of animal behavior, including frequency of being in the center and the sidelines measured.

Statistical analysis: Statistical analysis was performed using SPSS 18 software. In order to determine significant differences between groups ANOVA (One way ANOVA) was used and Tukey test was used to compare the mean direction and $p<0.05$ was considered significant.

Results

6-hydroxy-dopamine injection to rats significantly increased duration of immobilization compared to controls ($p<0.05$, Fig 1). Parkinson's treated with thyme extract at concentrations of 100, 200 and 400 mg/ kg had no significant effect on the duration of immobility in the forced swim test. 6-hydroxy-dopamine injection to rats reduced significantly the frequency of the center and the periphery being compared to the control group ($p<0.05$, Fig 2).

Parkinson's treated with thyme extract at concentrations of 100, 200 and 400 mg/kg had no significant effect on the number of times being on the periphery and the center. 6-hydroxy-dopamine injection to rats significantly increased the duration of the test rod compared to the control group ($p<0.05$, Fig 3).

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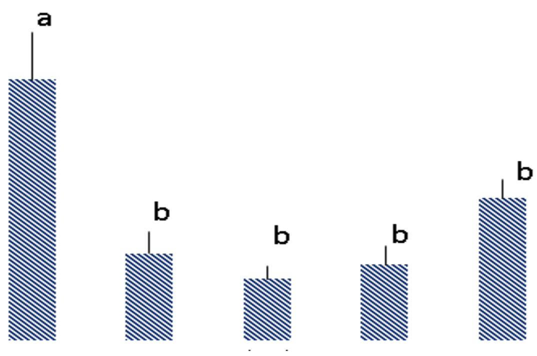

Figure 1. The effect of different doses of thyme extract on duration of immobilization in forced swim test.

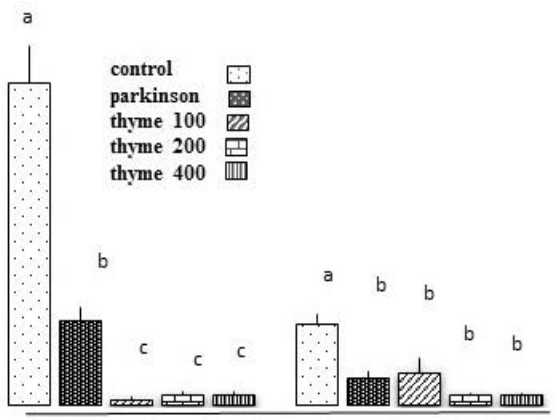

Figure 2. Effect of different doses of thyme extract on duration of being in the periphery and in the center of in the environment test a indicate significant difference with b and c in the level of p<0.05, b indicate significant difference with a and c in the level of p<0.05.

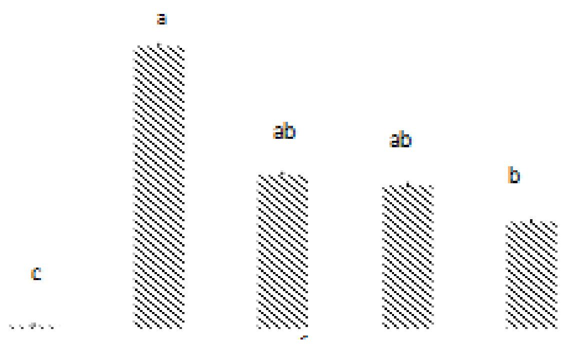

Figure 3. The effect of different doses of thyme extract on the time of test bars in Katalpsy test (Horizontal a indicate significant difference with b and c in the level of p<0.05, b indicate significant difference with a and c in the level of p<0.05, ab indicate no significant differences with a and b

Parkinson's treated with thyme extract at a concentration of 400 mg kg significantly reduced duration of the test bars compared to PD (p<0.05). In the groups receiving the extract at concentrations of 100 and 200 the duration of the test bars was less than Parkinson's, but the difference was not significant. According to the results of the pain response in control rats was significantly longer than rats with Parkinson's (p<0.05, Fig 4). Parkinson's treated with thyme extract at concentrations of 100, 200 and 400 significantly increased response to pain (p<0.05). The results showed no significant difference between the control group and Parkinson's in terms of balancing time in rotarod test (Fig 5). Parkinson's treated with thyme extract at concentration of 400 mg/kg significantly increased time balancing out (p<0.05)

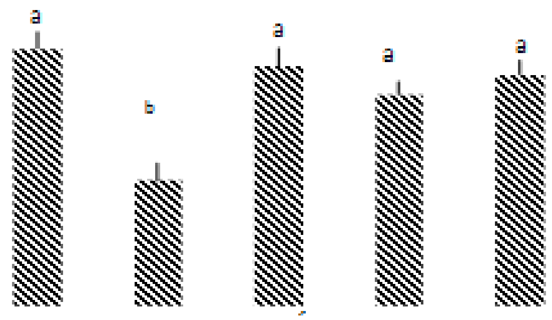

Figure 4. The effect of different doses of thyme extract the response to pain in the hot plate test a indicate significant difference with b in the level of p<0.05

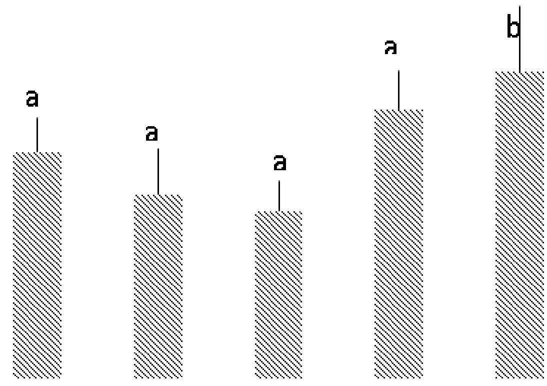

Figure 5. Effect of different doses of extracts of thyme over balancing time in rotarod test.

Discussion

According to the results of this study Parkinson's model by injecting 6-hydroxydopamine in rats was with depressive behavior in forced swim test, the movement disorders in bars test and environmental test and pain in the hot plate test. Induced depression, movement disorders and pain following injection of 6-hydroxy dopamine in rats have also been reported in previous studies (11, 14, 20, 24). In this study, treatment by thyme extract had no significant effect on the depression in the forced swim test and movement disorders in open

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page test. Thyme extract at a concentration of 400 mg kg significantly improved movement disorders in the test bars. Treatment by Thyme extract at concentrations of 100, 200 and 400 mg kg significantly increased pain tolerance.

In general, movement disorders in Parkinson's disease caused as a result of imbalance of dopamine, acetylcholine in the basal ganglia of the brain that caused by reduction of the dopamine production and high activity of cholinergic neurons (25). It has been reported that thyme extract inhibits contractions induced by acetylcholine (21), in addition anticholinergic and anti-acetylcholinesterase activity of phenolic compounds such as rosmarinic acid, ferulic acid, cinnamic acid and Quinic acid that abound in the extracts of thyme, has been shown in previous studies (15, 16, 27). Ethanolic thyme extract analgesic effects on pain induced by hot plate, acetic acid and formalin attributed to its anticholinergic activity(28).

So it seems that the effects of thyme on relieving pain and movement disorders caused by Parkinson's disease due to anticholinergic activity of extract and its compounds. In recent decades, the role of inflammatory processes in the destruction of neurons of patients with Parkinson's Nigrostriatal resulting in the development of pain and movement disorders has been shown (7). The presence of activated microglia and elevated levels of inflammatory mediators in the striatum of patients with Parkinson's have been reported (29).

Given that the anti-inflammatory effects of extracts of thyme and phytochemical compounds have been reported in previous studies (19, 30), It seems that thyme extract through inflammatory mechanisms of damage to dopaminergic neurons and thus prevents the occurrence of movement disorders and pain. Studies show the role of oxidative stress in damaging dopaminergic neurons and is followed by physical and mental disorders (31,32). Increasment of lipid peroxidation in SNC area in patients with Parkinson's as a result of increased iron ions (33), impaired activity of mitochondrial complex 1 (34), increased production of nitric oxide (35) and decreased levels of antioxidant enzymes (36). Due to the antioxidant effects of thyme extract, in vivo and in vitro in a number of studies have shown (17, 22, 23, 37) antioxidant mechanisms may also be involved in the effects seen in this study. PD model by injecting 6-hydroxydopamine induced depression, movement disorders and pain.

Treated Parkinson by thyme extract at concentrations of 100, 200 and 400 were significantly increased tolerance to pain.

Thyme extract at a concentration of 400 milligrams per kilogram improved movement symptoms in the Katalpsy test the screen test was not significant effect on movement symptoms, which may be due to the use of gavage and thus reduce the effectiveness of the extract.

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