Evaluating the effect of α-pinene on motor activity, avoidance memory and lipid peroxidation in animal model of Parkinson disease in adult male rats

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Abstract

Background and objectives: Parkinson’s disease (PD) is a common neuropathologic disorder that is caused by degeneration of dopaminergic neurons of dense part of nigra. Oxidative stress has been found in the pathophysiology of PD. Since α-pinene has strong anti-oxidant effects, the purpose of this research was to study its effects on movement disorders and memory and lipid peroxidation in PD.

Methods: Thirty five male rats were divided in 5 groups: control, vehicle, PD (received injection of 6-hydroxydopamine (6-OHDA)) and Parkinson's groups receiving doses of 100 and 200 mg/kg via gavage for two weeks. Generating animal models for Parkinson was done by intracerebral injection of 6-OHDA in the left side of the brain in medial forebrain bundle (MFB). After the injection, the movement balance of the rats was measured by Rotarod. Memory test was done by shuttle box; their brain was extracted to analyze malondialdehyde (MDA) in striatum, hippocampus and blood.

Results: The results showed that Parkinson caused, movement disorder (p<0.01), avoidance memory reduction (p<0.001) and malondialdehyde accumulation in hippocampus (p<0.05) and striatum (p<0.001) tissues and in blood (p<0.001). Administration of 200 and 100 mg/kg α-pinene improved the movement disorder (p<0.05). Administration of both doses of 200 and 100mg/kg showed improvement in avoidance memory (p<0.001) and (p<0.01), respectively. Malondialdehyde showed reduction in striatum (p<0.001) and hippocampus (p<0.05, p<0.001), respectively in the treatment groups after administration of both doses. In the blood, the dose of 200 α-pinene significantly reduced MDA in the treatment groups.

Conclusion: The results of this research show that α-pinene could reduce the symptoms of PD in rats.

Keywords: lipid peroxidation, memory, movement, Parkinson’s disease, α-pinene

Introduction

Parkinson disease (PD) was first described by James Parkinson in 1817. This disease demonstrates a progressive neurodegeneration, which has already affected almost 1% of the population over 50 years old [1]. Disturbances in consciousness, memory, perception ability, and visual-spatial function decline are observed in these patients [2]. Parkinson is a degenerative disease of the central nervous system in which the substantia nigra cells in the midbrain gradually vanish and dopamine production gradually reduces [3]. The middle brain damage can cause disruptions in the dopamine content of striated objects, decrease in the density of...
dopamine receptors, decrease in neuronal activity, and increase in the activity of brain free radicals [4]. Degeneration of dopaminergic neurons in the midbrain and the sharp decline of dopamine at the back-middle of the dense part of the substantia nigra [3,5] cause debilitating and motor disorders such as bradykinesia, resting tremor, rigidity, and postural instability [6]. Several hypotheses have been suggested about the pathology and the death cause of pars compacta dopaminergic neurons in substantia nigra including oxidative stress, lipid peroxidation, reducing glutathione level, destruction of DNA, iron accumulation and increasing free radical formation [7]. Oxidative stress not only destroys dopaminergic neurons but also leads to cell death by impairing the oxidative phosphorylation process and reducing energy production [8]. Endogenous sources of oxidative stress include free radicals resulting from the metabolism of dopamine and melatonin. Reactive radicals of oxygen are produced constantly in the midbrain dopaminergic neurons by dopamine metabolism and monoamine oxidase B auto-oxidation enzyme [9]. The pharmacologic treatment of PD can be further divided into neuroprotective and symptomatic therapy. In practice, nearly all of the available treatments are symptomatic in nature and do not appear to slow or reverse the natural course of the disease. However, several potential neuroprotective agents for PD have shown some promise in animals and/or humans and are undergoing further investigations. Antioxidants with low molecular weight such as vitamins and protein molecules such as superoxide dismutase, glutathione peroxidase and glutathione can protect the body from creating oxidative stress induced by free radicals in the central nervous system dopaminergic neurons [10]. Studies have shown that plant phenols, such as flavonoids, phenolic acids and flavonolignan acids can act as effective antioxidants [11]. Dopamine has been introduced as a potential substrate in synaptic plasticity and memory mechanisms [12]. There is pharmacological evidence for the role of dopamine in learning and memory [13]. Both dopamine receptors (D1, D2) are involved in learning and memory processes [14]. It has been reported that dopamine receptors enhance the passive recognition [15] and improve cognitive performance in rats while they do not affect learning [16]. In recent years, a number of studies have described cognitive dysfunction in Parkinson's disease, which is significantly associated with their disability status. Cognitive dysfunction may include memory loss, difficulty in concentrating, slowed information processing, and cognitive problems in different tasks [17,18]. Memory disorder is one of the most common cognitive dysfunctions in Parkinson's disease. α-Pinene is an organic compound of the terpene class, one of two isomers of pinene. It is an alkene and contains a reactive four-membered ring. It is found in the oils of many species of coniferous trees. α-Pinene is naturally found in plants such as Prangos ferulacea [19], Hypericum richeri [20], Ferula gummosa [21], Teucrium stocksiainum [22] Salvia officinalis [23], Ferulago angulata [24], Originum majorana [25], and Salvia lachnocalyx [26]. α-Pinene is an isomer used in multiple reactions such as isomerization, oxidation, hydration, acetylation, etc. It is also used in the preparation of many terpenoids such as ocimene, terpinolene, terpinene hydrate, and camphor [26]. α-Pinene is the most important ingredient of turpentine that is used as a flavoring. It is an important interface of the aromatic compounds which is used as flavoring in salts, household sprays, disinfectants, and pesticides [27]. α-Pinene is the main ingredient of essential oils from various plants and has shown inhibitory effect of acetylcholinesterase activity [28]. It also has shown anti-depressant [29], anticonvulsants [30], antioxidant [31], antispasmodic [19], antibacterial [32], anti-inflammatory [33], anti-tumor properties [34]. In the present study, due to the antioxidant effects of α-pinene, the effect of its chronic administration on behavioral disorders and malondialdehyde (MDA) caused by the toxin 6-hydroxydopamine (6-OHDA) has been investigated for the first time.

**Experimental**

**Animals**

Thirty five adult male rats were used with the weight range of 200-250g, which were randomly
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divided into five groups of seven rats. All rats were maintained under the same conditions, including 21±2 °C, 12 h of light and 12 h of darkness. All tests were done based on the ethical protocols and standards of laboratory animal protection (No. 1394.2002).

Experimental Group
The control group: no surgery was performed (intact); vehicle group: stereotoxic injection surgery was performed on this group without neurotoxin 6-hydroxy-dopamine and they received 3% Tween 80 [35] for 14 days by gavage; Parkinson Group (PD); this group received 2 µL containing 8 micrograms of neurotoxin 6-hydroxy dopamine at MFB area by stereotoxic surgery; Parkinson group received a daily dose of 100 µg/Kg of α-pinene for 14 days by gavage administration. (α-pinene 100 mg/kg + PD) [35]; Parkinson group received a daily dose of 200 µg/Kg of α-pinene for 14 days by gavage administration. (α-pinene 200 mg/kg + PD) [35].

Stereotoxic injection surgery procedure
Initially, the rats were weighed and then anesthetized by intraperitoneal injection of 90 mg/kg ketamine hydrochloride (Alusan Co., Netherlands) and 10 mg/kg xylazine (Alusan Co., Netherlands). The rats were placed in stereo tax device and fixed by the mouthpiece and bars on the device. The skull dorsal hair was shaved and the animal’s scalp was disinfected by alcohol and a longitudinal cutting was created through the back of the head between the eyes to the dorsal surface between the ears. The crossbred tissues were removed from the external of the cranium in a way that bregma part was visible. Lambda and bregma points were placed equal on a level and the device index was set on. Then, (MFB) peculiarities were determined according to the extracted coordinates from the Atlas of brain surgery AP:-3/8 DV:-8/2, ML±1/6. In this study, the unilateral injection of 6-hydroxy-dopamine was used anterior-middle category (MFB) to create Parkinson’s disease [3].

6-Hydroxy-dopamine solution preparation
6-Hydroxy-dopamine (Sigma, USA) was prepared at a concentration of 8 µg per 2 µl of normal saline dissolved in 0.01% ascorbic acid. α-Pinne: α-pinene (Betagen, Iran) was administered by gavage method after dissolving in 3% tween 80.

Behavioral tests
Apomorphine-induced rotational behavior
Contralateral rotations of each animal were recorded after subcutaneous injection of apomorphine (0.5 mg/kg in normal saline containing 0.01% ascorbic acid) to confirm the dopamine depletion. Full spin were measured in a cylindrical proper place for 60 min in 10-min intervals [36].

Rotarod (motor coordination test)
This test aims to measure the motor balance and harmony in movement (motor efficiency and coordination). It measures the time (latency) it takes for the mouse to fall off the rod rotating at different speeds or under continuous acceleration. Briefly, the animals were placed on a rotarod device whose speed varied. The primery speed of the bar was 5 rpm. Then, the speed of rotation bars gradually increased within 300 seconds. The main standard for balance in all groups was 25 rpm. Then, the speed of rotation bars gradually increased within 300 seconds. The main standard for balance in all groups was 25 rpm. Rats became familiar with this device. Then, each animal was assessed 3 times and 45 minutes intervals between the sessions and the average time was calculated [37].

Passive avoidance memory test
This test was conducted using a shuttle box (5500-ST, Borj Sanaat Co., Iran), which contained two compartments, one was dark and the other light. Their bottom was covered by stainless steel wires with a diameter of 1 to 2 mm and at a distance of one centimeter. A slight shock was applied to animals' paw by an electric current generator (75 volt, 0.3 mA for 3 seconds) in the dark compartment only once. Initially, the animals were placed in the shuttle box with guillotine doors for 10 min in order to become familiar with the device (training) to move freely between the inside and outside of their enclosure.
Then, the animals were placed in the light box and their delay time to go to the dark box was recorded (learning). The guillotine door was closed as soon as the animal entered the dark compartment and the electric shock was applied to their feet. After 24 h, their delay time for entering the dark compartment was measured in seconds (no electric shock) as the passive avoidance memory. This operation was conducted for all rats in all groups.

Malondialdehyde (MDA) measurement
For (MDA) measurement striatum and hippocampus tissues of the brain were removed and blood samples were also taken from the tail. The MDA level of tissues was measured by spectrophotometric method, using the thio-barbituric acid (TBA) reagent, based on the response of a chromogenic reagent, (TBA) with MDA at 100 °C. Molecules of MDA would react with TBA to yield a complex dye. MDA concentration was measured at 532 nm.

Standard curve
Three mL of the 1% phosphoric acid solution was added to 0.5 mL of the standard solution with concentrations of 8, 10, 6, 4, 2, 1, 5.0 mM and the rest of the steps were performed as before and the absorption was recorded at 532 nm.

Statistical analysis
Data have been reported as mean ± SEM. The results were analyzed using SPSS software. ANOVA analysis was used to check the results in different groups and discrepancy between groups were considered significant if p<0.05.

Results and Discussion
All animals tolerated the stereotaxic surgical procedures and no deaths were observed during the study. The results showed that rotation test of the Parkinson's groups significantly increased in contrast to the control group after the creation of MFB lesion due to infusion of 6-OHDA in the rats (p<0.001). In the Parkinson's groups which were treated with α-pinene 100, 200 mg/kg for 14 days, it was found that the treatment groups had less rotation [100, 200 mg/kg (p<0.001)]. Motor coordination in Parkinson group showed a significant decrease compared to the control group (p<0.01) and treatment with doses of 100 and 200 mg/kg of α-pinene could increase the motor coordination significantly compared with the Parkinson group (p <0.05) (figure 1). The passive avoidance memory significantly decreased in the group with Parkinson compared to the control group (p<0.001) (figure 2) furthermore, no perceptible difference was observed between the control group and the vehicle group. In addition, in all Parkinson's groups doses of 100 and 200 of α-pinene increased memory compared to the Parkinson group (p<0.01) and (p<0.001), respectively. Lipid peroxidation levels of experimental groups have been shown in figures 4 and 5. MDA level of hippocampal tissue homogenates increased significantly compared to the control group (p<0.05). In addition, no significant difference was observed between the control and the vehicle group. The results of fourteen-day prescribing α-pinene with two doses of 100 and 200 mg/kg reduced MDA in the hippocampus tissue compared to Parkinson group (p<0.05) and (p <0.001), respectively. On the other hand, MDA levels increased in Parkinson's group compared to the control group in the striatum tissue (p<0.001). Similarly, there was no significant difference between the control group and vehicle group. By comparing MDA measurement among Parkinson group that recieve α-pinene 100, 200mg/kg for 14 days, it was found that MDA had a significant reduction in the group receiving α-pinene in comparison with Parkinson group (p<0.001). For MDA measurement in the blood, only the dose of 200 mg/kg of α-pinene significantly decreased lipid peroxidation (p<0.001).

In the present study, the minimum amount of 6-OHDA was used to induce Parkinson's disease. The advantage of this model is that it is very similar to the early stages of Parkinson's disease in humans as well as minimizing or eliminating...
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**Figure 1.** Effect of 14-day gastric gavage of 100 and 200 mg/kg α-pinene on circling behavior in Parkinson's disease (PD). (mean ± SEM; One-way ANOVA and Tukey's test (n=7)); * shows significance between the control group and PD. # shows significant difference between PD and treated groups.

**Figure 2.** Effect of 14-day gastric gavage of 100 and 200 mg/kg of α-pinene on rotarod in Parkinson's disease (PD). (mean ± SEM; One-way ANOVA and Tukey's test (n=7)). * shows significance between the control group and PD. # shows significant difference between PD and treated groups.

**Figure 3.** Effect of 14-day gastric gavage of 100 and 200 mg/kg of α-pinene on memory in Parkinson's disease (PD). (mean ± SEM; One-way ANOVA and Tukey's test (n=7)). * shows significant between the control group and PD. # shows significant difference between PD and treated groups.
Figure 4. Effect of α-pinene on MDA levels in hippocampus tissue between control group, PD and PD groups orally receiving 100 and 200 mg/kg of α-pinene for 14 days. (mean ± SEM; One-way ANOVA and Tukey’s test (n=7) ). * shows significant difference between the control group and PD . # shows significant difference between PD and treated groups.

Figure 5. Effect of α-pinene on MDA levels in striatum tissue between control group, PD and PD groups orally receiving 100 and 200 mg/kg of α-pinene for 14 days. (mean ± SEM One-way ANOVA and Tukey’s test (n=7) ). * shows significant difference between the control group and PD . # shows significant difference between PD and treated groups.

Figure 6. Effect of α-pinene on MDA levels in blood between control group, PD and PD groups orally receiving 100 and 200 mg/kg of α-pinene for 14 days. (mean ± SEM One-way ANOVA and Tukey’s test (n=7) ). * shows significant difference between the control group and PD . # shows significant difference between PD and treated groups.
non-specific effects of neurotoxin on other systems. The antioxidative treatment in the early stages of Parkinson's disease has been examined in the clinic. One treatment is to use antioxidants to reduce the oxidative stress and protect dopaminergic neurons. Biological antioxidants play a vital role in protecting cells against oxidative stress caused by free radicals. The results of the present study showed that the chronic consumption of α-pinene improved movement disorders in rats with Parkinson's disease. The results of rotation testing in Parkinson's group compared with the control group demonstrated stricture and devastation of dopaminergic neurons, while rotation in the groups receiving α-pinene were much lower than that in the lesion group, which can display prevention from the demolition of dopaminergic cells and reduction of motor coordination following this destruction with the gavage of α-pinene. Learning and passive memory avoidance tests were performed under the same conditions in the shuttle box for all groups. Increasing the delay time for the first entry to the dark room and the total time for spending in the light room and decreasing the total time for spending in the dark room indicated the improvement of passive avoidance memory by α-pinene. In addition, our results showed that the level of malondialdehyde (MDA) in the hippocampus and striatum of Parkinson group receiving α-pinene significantly reduced and it possibly prevented the progression of the disease. Some previous researches have also investigated the effects of plant extracts on animal models of PD. For example, oral prescription of ginseng extract has led to stopping cell damage of the substantia nigra and decreasing the dysfunction in Parkinson rats [40]. In another study which was carried out on Ginkgo biloba, it was found that the leaf extract of this plant reduced the behavioral disorders resulting from injuries caused by 6-hydroxydopamine [41]. Studies have shown that the unilateral damage of nigrostriatum dopaminergic system by injecting striatal 6-hydroxydopamine decreases the level of dopamine and up-regulation of postsynaptic dopaminergic receptors located on the affected side of striatal neurons [42]. Salar et al. have reported that the aqueous extract of barberry could reduce the behavioral symptoms of Parkinson by inhibiting the acetylcholinesterase enzyme in the brain. In previous studies, barberry extract had an antioxidant property and prevented the production of lipid peroxidation and MAO-A activity. As a result, the amount of dopamine and monoamines increased in brain. MAO inhibitors increase the amount of dopamine and norepinephrine in the nerve synapse and they have antidepressant effects [43]. It has been reported that oral administration of rosemary leaf extract induced neuroprotective effects on the hippocampus and prevented memory impairment caused by neurotoxin 6-hydroxydopamine. Therefore, it might be considered to be used in improving memory disorders in Parkinson patients [4]. There are several reports showing that oxidative stress is involved in the pathogenesis of Parkinson disease by producing free radicals and weakening the brain antioxidant system [10,44]. Oxidative stresses cause apoptosis and loss of dopamine cells [44,45]. Findings show that the use of plant extracts with antioxidant substances can improve the cognitive and motor symptoms of Parkinson disease [46]. α-Pinene is a single ring monoterpenoid [47]. Some terpenoids act as serotonin reuptake inhibitors and increase the norepinephrine and dopamine activity (like monoamine oxidase inhibitors) [48-50]. It was also reported that taking α-pinene at a dose of 100 mg/kg prevents damage to gastric mucosa walls and protects the gastric mucosa against acidification, accumulation of bacteria, and mechanical forces resulting from proteolytic digestion [51]. Various pharmacological effects have been reported for α-pinene, including anti-microbial, anti-inflammatory, analgesic, antioxidant, memory-enhancing, anti-anxiety and neuronal protection properties [52]. Previous studies have shown that α-pinene and many monoterpenoids have anti-acetyl-cholinesterase activity [53,54]. In addition, such plants contain monoterpenes that are useful for the treatment of memory disorders, including Alzheimer's disease [55]. Findings have shown that some plants possess terpenoids such as
carvacrol and pinene and it has also been reported that carvacrol, α-pinen, β-pinene, and β-caryophyllene are able to increase GABA evoked current responses. [56]. It seems that the antioxidant properties of α-pinene plays an important role in its protective effects which suggest it to be used as an adjuvant treatment in patients with Parkinson's disease. Antioxidant effect of α-pine was confirmed in the present study by measuring oxidative stress parameters such as malondialdehyde (lipid peroxidation) in brain tissues and blood. α-Pinene enhances the memory probably due to its anti-acetylcholine esterase activity. On the other hand, monoterpenes increase dopamine by interfering with monoamine systems and MAO inhibition. Terpenoids might adjust GABAergic system modulation, which cause extra movements in the weakened Parkinson patients. In order to treat Parkinson, dopamine must be increased and acetylcholine must be decreased. The dual function of α-pinene might improve Parkinson's symptoms and impaired memory. Based on the results of this study, consumption of α-pinene might protect dopaminergic neurons against 6-OHDA-induced lesions, and possibly could have a protective effect against Parkinson's disease.

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Declaration of interest
The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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