Effects of Strawberry (*Fragaria ananassa*) Extract on 6-Hydroxy Dopamine Induced Parkinson’s Disease Model in Male Rats

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Abstract
Introduction: Parkinson's disease is a prevalent neuropathological disorder. Oxidative stress is regarded as the main factor of this disease which causes death of neurons. Considering the protective antioxidative property of strawberry, the aim of the present study was to investigate effects of strawberry extract (SE) on motor and cognitive disorders, and lipid peroxidation in animal model of Parkinson.

Methods: 40 male wistar rats were divided into five groups: control, Parkinson and three treatment groups. To Parkinson, the rats received 6-OHDA toxin with dose of 8 µg in 2 µl of normal saline solution with 0.01% of ascorbic acid inside medial forebrain bundle (MFB) on the left side of the brain. The treatment groups received strawberry (*Fragaria ananassa*) hydroalcoholic extract with doses of 10, 25, and 50 mg/kg for 14 days. Then passive avoidance memory and movement tests were conducted on the rats one day after the last gavage. Then the brain was isolated to extract the brain tissue and malondialdehyde (MDA) test was conducted as a marker of lipid peroxidation. Results were presented as mean ± SD, one way analysis of variance, and Tukey's post-hoc test.

Results: Parkinson's disease induced significantly increased brain MDA (P<0.001) Further, treatment of Parkinson's disease with SE significantly decreased MDA in brain tissues, (P<0.001). Also, in the section on examining motor (P<0.001) and memory (P<0.05) tests doses of 25 and 50 extracts showed significant recovery effects.

Conclusion: Extract of strawberry with an antioxidant effect probably has neuroprotective effects against 6-OHDA toxin.

Keywords: Strawberry Extract, Memory, Movement, MDA, Parkinson, Rat

Introduction
After Alzheimer, Parkinson's disease is recognized as the second most common progressive neurodegenerative disorder which is characterized by severe motor disorders (1). Other symptoms include fatigue, depression, and memory impairment (2). Studies have shown that the incidence and the prevalence of the disease increases with age (3). One-way damage to the dopaminergic system of Substantia nigra following intra-steric injection of 6-Hydroxy dopamine is known as the common animal model for investigating Parkinson's disease, in which a gradual decrease in the amount of dopamine in the striatum is induced and an increase in the regulation of post-synaptic dopaminergic receptors will occur. These changes result in functional motion asymmetry, which is commonly measured by induction of a rotation to the same side (ipsilateral) and to the opposite side (contralateral) by dopaminergic agonists with direct (apomorphine) and indirect (amphetamine) effects (4). Factors such as oxidative stress, especially lipid peroxidation are among the most important reasons of destruction and degeneration of dopaminergic neurons.
Oxidative stress demolishes the dopaminergic neurons by disrupting the oxidative phosphorylation process and reducing the production of energy (5). Although a number of antioxidants are found in the plasma, the body’s immune system is unable to eliminate the released radicals in the body completely, hence there is a need to supply antioxidants from external sources which can be supplied through nutrients (6). It has been shown that the consumption of fruits and vegetables containing natural antioxidants can prevent many diseases, including cardiovascular diseases, brain diseases, and even different cancers (7). Strawberries contain minerals, vitamin C, folate and phenolic substances, which mostly are natural antioxidants and essential to health, especially the antioxidant and anti-inflammatory effects of phenolic compounds found in strawberry are well known. These compounds have direct and indirect antimicrobial, anti-allergic, and high-pressure preventing effects, and they also inhibit activity of certain physiological enzymes (8).

Ellagic acid is a polyphenolic acid found in fruits such as pomegranate, strawberry, raspberry and grapes (9). This molecule has various properties, including antioxidant effects (10). Recently, extensive efforts and coordination are being made to find a definite treatment for Parkinson's disease in order to stop the neuronal degeneration process during the disease (11). In this research, the main objective of the study was to evaluate the therapeutic effect of strawberry extract in motor, cognitive and malondialdehyde activity as an indicator of lipid peroxidation in an animal model of Parkinson's disease in adult male rats.

Methods

During the study, 40 wistar rats weighing 200-250 grams (from Jundishapur University of Medical Sciences in Ahwaz) were used. Animals were kept in 12-hours of light and 12-hours of darkness, the temperature was set to (21 ± 2°C), and they had free access to water and adequate food. The rats were kept in individual cages and randomly divided into the following groups.

1- Control group: No injuries were induced to the animals in this group (control).

2. Parkinson's group: Animals in this group received 2 μl (containing 8 μg) of 6-hydroxy dopamine neurotoxin in the MFB region (PD).

3- Three Parkinsonian treated groups, who received 10, 25 and 50mg/kg of strawberry extract (as gavage) for 14 days (PD+SE10, PD+SE25 and PD+SE50).

After separating the green leaf from the top of the strawberries, they were cut into thin layers and laid on a clean cloth, then they were exposed to the room's air in order to become waterless. The dried layers were then powdered using a grinding machine. After preparing an alcohol (70%) solution, based on the amount of extract required, the strawberry powder was weighed and then mixed with the alcohol. The prepared solution was stirred frequently over the following 2-3 days, then the extract was filtered using a thin clean cloth and a glass funnel. The filtered solution was then placed inside a beaker and then it was treated in a water bath (at 50° C). 1-2 days later, within the condensation of the extract, it was spread on a clean glass surface and exposed to air inside the room to dry. After it became dry, the extract was collected using a spatula (12).

For stereotaxic surgery method, first, the rats were weighed and anesthetized by intraperitoneal (IP) injection of 90 mg/kg of ketamine hydrochloride and 10 mg/kg of xylazine per kg of body weight (both drugs had been produced by Alosfan Company in the Netherlands). Afterwards, the animals were placed in the stereotaxic device, on which they were fixed using a mouthpiece and intramedullary rods. The hair at the dorsal skull was also shaved. The head skin was disinfected using a piece of soft cotton and a glass funnel. The filtered solution was placed inside a beaker and then it was treated in a water bath (at 50° C). 1-2 days later, within the condensation of the extract, it was spread on a clean glass surface and exposed to air inside the room to dry. After it became dry, the extract was collected using a spatula (12).

Then, according to the coordinates extracted from Atlas of Neurosurgical Techniques: Brain, the MFB coordinate (with coordinates of -4.8; a relative to Bregma point ±1.6, ML, - 8.2; DV) was determined. In this study, to make a PD animal model, the unilateral
injection of 6-OHDA into the MFB was used (13). 6-hydroxy-dopamine (American Sigma Company) was prepared with the concentration of 8 μg in 2 ml of normal saline containing 0.01% ascorbic acid. The rotational behavior of the rats was tested by injecting 2.5 mg/kg of apomorphine hydrochloride. Complete rotations (in a cylindrical chamber) were measured for 60 min in 10 min intervals. For testing 3 and 9 centimeters platforms, rat’s right hand was placed on a platform at a height of 3 centimeters. If the animal did not step its hand off from the platform for at least 10 seconds, half a score would have been recorded. Then the animal's left hand was placed on a platform at a height of 3 cm. If the animal did not step its hand off from the platform for at least 10 seconds, then another half score would have been recorded. The right hand of the animal was placed on the platform at a height of 9 cm so that the rest of the body did not touch the platform. If the animal did not remove its hand from the platform for at least 10 seconds, one score would have been recorded. Then the animal's left hand was placed on the platform at the height of 9 cm, so that the rest of the body did not contact the platform. If the animal had no hand removed from the platform for at least 10 seconds, it would again have been recorded as one score (14). For walking test: The animals were put on a flat surface, if they started walking, they would score zero, if it did not move or started moving with the touch of a hand, they would score 0.5, and within the platforms test, it was measured as a muscular hardness test (14).

Passive avoidance memory test was conducted using a shuttle box (ST-5500 model), consisting of two boxes (one dark and the other illuminated), with stainless steel wires covering its floor (with a diameter of 1-2 mm and 1 cm interval), and supplying a mild 75-volt, 0.3 mill ampere A/C electric shock waveform which was applied for 3 seconds to the dark box which was only once applied to the metatarsal area of the animal. Each rat was first placed inside a shuttle box with an open guillotine lid to circulate freely inside and outside the compartment for a period of 10 minutes in order to familiarize itself with the device (training). The animal was then placed in the illuminated box and the animal's lag time to go to the dark box (learning) was recorded. Upon arrival, the guillotine lid of the dark box was closed and an electric shock was applied to the palm of the foot of the rat. 24 hours later (one day after), the delay time (seconds) took for the rats to arrive to the dark chamber (which previously had shocking device, but this time there was no shock) was measured as passive avoidance memory. This procedure was performed for all rats in all investigated groups (15).

In MDA Assays test, the groups with eight rats were used. Tissues of striatum and hippocampus were homogenized separately with the specified amount of KCL 1.5 %. 0.5 ml of the homogenized solution was removed and 2.5 ml of the TCA solution 3% was added and kept in the water bath at 37°C for 10 min. Then, it was centrifuged at 3000 rpm for 10 min. 0.5 ml of the supernatant solution was removed after the centrifuge and 3 ml of phosphoric acid solution 0.1 and 1 ml of TBA 0.67 were added to each of them and put in boiling water for 45 min. The tubes were cooled in the ice container and 4 ml of butanol was added to each one. After vortex mixture, it was centrifuged at 2000 rpm for 20 min and finally absorption with the wavelength of 532 nm was read. After putting the numbers obtained from a spectrophotometer and absorption in standard curve line equation, concentration of MDA was evaluated (13). The data were expressed as mean ± SD. The significance level was determined by one-way ANOVA applying Tukey’s post-hoc test (SPSS, 18). A value of P ≤ 0.05 was considered significant.

Results
After subcutaneous injection of Apomorphine to the control group and Parkinsonian rats (after MFB lesion two weeks after surgery), a significant contralateral rotation was identified in Parkinsonian rats (P<0.001). Administration of strawberry extract to Parkinsonian rats at doses 25 mg/kg and 50 mg/kg significantly decreased the rotations with (P<0.01) and (P<0.001), respectively, compared to the Parkinson's group (Fig. 1). During another part of the study, muscular stiffness in the Parkinson's group showed a significant increase (P <0.001) in comparison to the control group and it was also found that administration of 25 mg/kg and 50 mg/kg of strawberry extract significantly reduced muscle stiffness (P <0.001).
Comparison between the mean time of initial entrance latency to the dark room (passive avoidance memory) in Figure 3 shows that the initial delay significantly decreased in the Parkinsonian group compared to the healthy control group (p <0.001). The chart also shows that the administration of 10, 25, and 50 mg/kg, of strawberry extract increased the doses of 25 and 50 mg/kg (P<0.05) and the memory increased significantly (P <0.05).

The comparison between mean value of MDA in control and Parkinsonian groups showed a significant increase in MDA concentrations in cerebellum, cortex, hippocampus (p <0.001) and striatum (P <0.05) in Parkinsonian groups in contrast to control group. The MDA level decreased significantly in treatment groups by administration of strawberry extract in 25 and 50 mg/kg doses in hippocampus tissue (P <0.001), and the cerebral cortex (P <0.01) and (P <0.05), MDA levels decreased by administration of 10, 25 and 50 mg/kg strawberry extract with the amount of (P <0.01) and (P <0.001) (P <0.05) in striatum and (P <0.001) (P <0.05) and (P <0.001) in the cerebellum, respectively (Fig. 4A-D).

**Figure 1.** Effect of 14 days oral administration of 10, 25, 50 mg/kg of strawberry extract (SE) on rotation in animal model of Parkinson’s disease. Results were presented as mean ± SD. Analysis of variance (one way ANOVA) test post hoc Tukey (n =8), * on columns significant difference between the control group and the # symbol on the columns significant difference with PD group.
**Figure 2.** Effect of 14 days oral administration of 10, 25, 50 mg/kg of strawberry extract (SE) on rigidity in animal model of Parkinson's disease. Results were presented as mean ± SD Analysis of variance (one way ANOVA) test post hoc Tukey (n=8), * on columns significant difference between the control group and the # symbol on the columns significant difference with PD group.

**Figure 3.** Effect of 14 days oral administration of 10, 25, 50 mg/kg of strawberry extract (SE) on passive avoidance memory in animal model of Parkinson's disease. Results were presented as mean ± SD. Analysis of variance (one way ANOVA) test post hoc Tukey (n=8), * on columns significant difference between the control group and the # symbol on the columns significant difference with PD group.
Figure 4. Effect of 14 days oral administration of 10,25,50 mg/kg of strawberry extract (SE) on MDA concentration of hippocampal tissue (A), striatum (B), cortex (C) and cerebellum (D) in Parkinson's Animal Model. Results were presented as mean ± SD. One-way ANOVA and post hoc Tukey test, the * sign on the columns showed a significant difference with the control group and the # sign on the columns, with a significant difference with the PD group.

Discussion

Processes involved in oxidation and production of free radicals have multiple effects on various chronic and degenerative diseases, including Alzheimer's and Parkinson's diseases (16, 17). 6-Hydroxydopamine toxin induces motor and cognitive disorders such as memory impairment in Parkinson's disease by inducing oxidative stress (13). The results of the previous studies indicated that plasma lipid peroxidation may be a risk factor for Parkinson's disease (18). In this study, using 6-hydroxy dopamine toxin for creating a Parkinson's model showed increased lipid peroxidation in various brain tissues, and impaired movement and memory. In brain degenerative diseases, using chemical compounds with antioxidant activity which are found in plants is very beneficial. Biologically, phenolic and anthocyanin compounds are two large and heterogeneous groups known in fruits and vegetables with antioxidant activity. Phenolic compounds are plants' secondary metabolites which are expanded in the herbaceous field. In strawberries (as a potent antioxidant fruit), phenolic compounds including P-Comaric acid, ellagid acid, and flavonoids such as quercetin, camphorol and myristin are found (19). The results of this study showed that the administration of different doses of strawberry extract (10, 25 and 50) mg/kg resulted in a significant treatment of 6-hydroxydopamine toxin induced motor disorders, including rotation, muscle stiffness and memory impairment, compared to the untreated group. Also, it reduced levels of malondialdehyde as a lipid peroxidation index in multiple brain tissues. In support of the findings, neuronal protection effects of strawberry fruits in neurodegenerative diseases are hoping (20). The imbalance between free radical production and antioxidant defense system plays the main role in the pathogenesis of neurodegenerative
and neurological diseases such as Parkinson's, trauma, Alzheimer's and Stroke (21). Due to their antioxidant activity, phenolic compounds can inhibit free radicals, and therefore they can be effective in preventing many diseases, such as cancer, cardiovascular and neurological diseases (22, 23). The effects of neuronal protection of many polyphenols are depended on their ability to cross the blood-brain barrier, which directly purge the pathological concentration of reactive species of oxygen and nitrogen (24). Ellagic acid is an important compound in strawberries with analgesic and pro-inflammatory cytokine agents' inhibitory effects (25, 26). It has antioxidant, antifibrosis, and anticarcinogenic properties (26). Also, the inhibitory activity of Nitric Oxide radicals has been shown by anthocyanin extracts found in L. Morus nigra (mulberry), Fragaria vesca L. (strawberry), and blackberry (Morus alba L. var. Nigra) (27).

Conclusion
According to the previous studies and the results of this study, evidence suggests that aqueous alcoholic extract of strawberry could reduce and improve the motor and cognitive impairments caused Parkinson's disease by its antioxidant role, which is well shown by decreasing the concentration of lipid peroxidation in brain tissues. Further studies are necessary to clarify the neuroprotective mechanisms of strawberry and it is necessary to study the effective materials and biochemical and molecular tests.

Ethical issues
No applicable.

Authors’ contributions
All authors equally contributed to the writing and revision of this paper.

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References
12. Rafieirad M, Zangenehnezhad Z. Effects of oral ferulago angulate hydroalcoholic extract on lipid peroxidation induced by ischemia hypoperfusion in the