

Effects of *Ferulago angulata* Extract Against Oxidative Stress Induced by 6-hydroxydopamine in Rats

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Abstract

Background: Parkinson's disease is one of the prevalent degenerative diseases of the neural system. Oxidative stress, which has been recognized as the most important factor of Parkinson's disease, plays a main role in the death of neurons in this disease. Antioxidants have a proved role in the prevention of oxidative stress.

Objective: Considering the presence of evidence regarding antioxidant property of *Ferulago angulata*, effect of oral administration of hydroalcoholic extract of this plant was investigated on parameters of the oxidative damage caused by Parkinson's disease in adult rats.

Methods: In this research, 40 Wistar rats with the average weight of 200 to 250 g were randomly assigned to 5 groups, each with 8 rats. Also, to develop Parkinson's disease animal model, 2 µl of 6-hydroxydopamine drug was injected into medial forebrain bundle on the left side of the animals and the treatment groups received *Ferulago angulata* extract with doses of 400, 200, and 100 mg/kg for 2 weeks as gavage. Then, the brain of the animals was isolated and parameters of Malondialdehyde and Thiol were measured.

Results: Results showed that Parkinson's disease induced significantly increased brain peroxidation lipid, which is one of the factors of oxidative stress, and reduced Thiol level ($p < 0.001$). Further, treatment of Parkinson's disease with *Ferulago angulata* extract significantly decreased peroxidation lipid in striatum and hippocampus and increased Thiol level ($p < 0.001$).

Conclusion: oral administration of hydroalcoholic extract of *Ferulago angulata* probably reduces oxidative damage and also reduces Malondialdehyde and increases Thiol in the treated groups due to its strong antioxidant property.

Keywords: *Ferulago angulata*, Malondialdehyde, Parkinson's disease, Thiol groups

Introduction

Parkinson's disease (PD) which is one of the prevalent neurodegenerative disorders is the most prevalent neuropathological disorder that is induced by degeneration of dopaminergic neurons of the dense part of substantia nigra and their terminals in striatum. Destruction of these neurons reduces dopamine neurotransmitter in this area [1, 2]. Reduction of dopamine leads to disorders such as bradykinesia, vibration, catalepsy, and posture instability [3]. Oxidative stress forms free radicals which are created in basal ganglia under pathologic conditions, lead to progressive damage and degeneration of nuclear neurons of substantia nigra, and induce Parkinson's disease [4, 5]. Other factors such as increase of lipid peroxidation, DNA destruction, and accumulation of iron play some role in the degeneration of dopaminergic neurons and Parkinson's disease [6]. Endogenous sources of oxidative stress include free radicals resulting from dopamine metabolism. Active radicals of oxygen are continually produced by Monoaminoxidase B enzyme or dopamine autoxidation in the dopaminergic neurons of medial brain due to metabolism of dopamine [7]. Oxidative stress is induced in brain when production of free radicals is higher than the ability of antioxidant system to delete additional reactive oxygen species (ROS)³. Natural chemical compounds with strong antioxidant activity are found in high concentrations in some plants [8]. Role of many plants has recently attracted the attention of human societies owing to their antioxidant property in treatment of neurodegenerative diseases. Studies have shown that plant phenols such as flavonoids and phenolic acids can act as effective antioxidants for molecular level [9]. Secondary metabolites derived from plants such as phenol and total

flavonoid have a strong potential to clean free radicals and the plants which are enriched with antioxidant compounds can protect cells against oxidative damage [10, 11]. *Ferulago angulata* (FA) plant belongs to *Apiaceae* family and is a natural and native plant of mountainous regions of west of Iran [12]. Its extract which contains phenolic compounds and has antioxidant and anti-diabetic effects is considered one of important herbs in Iran [13-15]. According to studies by Soudfian et al. (2011), analysis of aerial parts of different species of ferulago by ethanol has led to identification of its several components, the most important of which are cis-ocimene, α -pinene, β -germacrene, σ -terpinene, trans- β -ocimene, germacrene D, limonene, bornyl acetate, myrcene, camphene, allo-neo-ocimene, β -pinene, bicyclogermacrene and sophrosyne, respectively [13]. Phenolic compounds, due to having antioxidant activity, are capable of inhibiting free radicals; thus, they could be effective in preventing many diseases like cancer, cardiovascular and nervous diseases [16]. This plant is applied as an inhibitor of acetylcholine [17]. It has also antimicrobial, antioxidant [18], and cytotoxic [19] properties., it is used as a flavor and strong tranquilizer of digestive system, and also for its antihelmentic property [20, 21]. Studying oxidative stress is done using different indices, one of which is the end product of peroxidation lipid or Malondialdehyde (MDA). This aldehyde is produced in a biomarker organism for the measurement of oxidative stress level. One of the other indices of studying oxidative stress is Thiol index. The most important role of Thiol in in vivo studies is its performance as an intracellular and extracellular reductive buffer which indicates that effect of Thiol bands as a defensive antioxidant mechanism has been

reduced. Reduction of Thiol level indicates reduction of antioxidant capacity of the brain [22]. Peroxidation of lipids changes chemical characteristics and physical structure of the cell membrane and reduces function. It also has a role in the pathogenesis of diseases such as neurodegenerative diseases including Parkinson's disease [23]. Different molecules like aldehyde are created by peroxidation of lipids, one of which is MDA that is one of the indices of oxidative stress caused by Parkinson's disease [24]. Thus in the current study, neuroprotective (neuronal protection) effects of *Ferulago angulata* extract (FAE) were investigated in animal model of Parkinson's disease through studying the effect on oxidative stress induced by 6-hydroxydopamine in rats.

Materials and Methods

Animals and experimental groups

In this research, Wistar rats with the weight range of 200-250 g from the animal house of Ahvaz University of Medical Sciences were used for reproduction and maintenance. The animals were kept in a bright-dark place for 12 h at $21\pm 2^{\circ}\text{C}$ with free access to compressed food of Animal Feed Company, Shahreza, Isfahan, and piped, treated water of Izeh city, and were maintained in groups (each with four rats) inside standard cages in Animal center of Islamic Azad University, Izeh Branch. Protocol of this research was prepared based on the international regulations regarding laboratory animals and enacted in ethics committee of the University. The animals were randomly divided into the following groups:

- 1-Control group without inducing Parkinson's disease and treatment
- 2-Parkinson's group without treatment
- 3-Parkinson's group treated with 100 mg/kg of FAE with gastric gavage (i.g)

4-Parkinson's group with 200 mg/kg of FAE with gastric gavage (i.g)

5-Parkinson's group treated with 400 mg/kg of FAE with gastric gavage (i.g) [25].

Parkinson's experimental model

The animals were anaesthetized by the intraperitoneal injection of 90 mg/kg hydrochloride ketamin + 10 mg/kg xylazine and then heads of the rats were fixed in stereotaxy for the brain surgery. Afterward, 2 μl of 6-hydroxydopamine (6-OHDA) neurotoxic drug purchased from American Sigma Company was injected into medial forebrain bundle (MFB) on the left side of the brain of the animals and the treatment groups received *Ferulago angulata* with different doses for 2 weeks as gavage after three-day recovery. Then, the brain of the animals was isolated and parameters of MDA and Thiol were measured [26].

Preparation *Ferulago angulata* extract

Ferulago angulata plant was collected in early spring from the surrounding of Izeh city, Iran, and then its leaves were separated and dried in open air under shade for 2 weeks. After drying, the leaves were finely powdered (with diameter of less than 0.4 mm) with an electric grinder and *Ferulago angulata* powder was rinsed in ethanol 72% for 3 days at room temperature. *Ferulago angulata* powder and ethanol mix were stirred occasionally and then alcohol and powder mix were finely filtered to obtain its extract. The obtained extract was distilled in vacuum to evaporate its alcohol completely. Finally, FAE was obtained (yield 29%) as a brown powder after full evaporation [27].

Malondialdehyde (MDA) Assays

In this test, the groups with 8 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 Thiobarbituric acid (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 [28].

Standard curve

Standard curve had to be first drawn and it was necessary to prepare MDA standard solution and measure wavelengths with the spectrophotometer (Figure 1). 0.5 ml of the

standard solution with concentrations of 0.5, 1, 2, 4, 6, 8, and 10 was removed and then 3 ml of the phosphoric acid 1% solution was added and other stages were performed like the above stages. Finally, absorption was read with the wavelength of 532 nm [28].

Total sulphydryl (–SH) groups assay

Total SH groups were measured using DTNB as the reagent. This reagent reacts with the –SH groups to produce a yellow coloured complex which has peak absorbance at 412 nm (Ellman, 1959). Briefly, 1 ml Tris–EDTA buffer (pH = 8.6) was added to 50 µL homogenate in 2 ml cuvettes and sample absorbance was read at 412 nm against Tris–EDTA buffer alone (A_1). Then, 20 µL DTNB reagent (10 mM in methanol) was added to the mixture and after 15 min (stored in room temperature) the sample absorbance was read again (A_2). The absorbance of DTNB reagent was also read as a blank (B). Total thiol concentration (mM) was calculated from the following equation: Total thiol concentration (mM) = $(A_2 - A_1 - B) \times 1.07 / 0.05 \times 13.6$. [29].

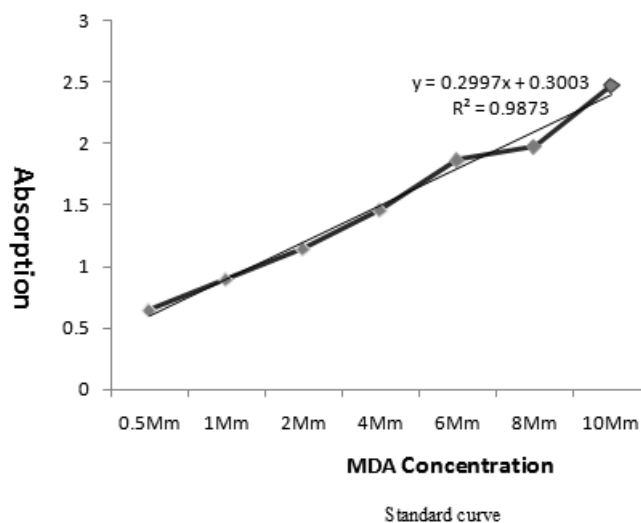


Figure 1- The Standard Curve of MDA was constructed Over the Concentration Range of 0–10 µM

Statistical Analysis

Data of this research were presented as mean \pm SEM and then analyzed using suitable statistical methods in Excel and SPSS software by one way ANOVA and LSD test methods. Difference of the results between different groups was significant with minimum $p < 0.05$.

Results

In this research, all the animals properly tolerated stereotaxic surgery and no mortality was observed during the study. The results of this study showed that the amount of MDA as lipid peroxidation index significantly increased in Parkinson's group compared with the control group in striatum and hippocampus tissues after MFB lesion due to the injection of 6-OHDA ($p < 0.001$). At the next, comparison of MDA measurement between the Parkinson's group without treatment and treatment Parkinson's group which orally received the extract of *Ferulago angulata* with doses of 200 and 400 mg/kg for 14 days specified that MDA was significantly reduced in the group receiving FAE ($p < 0.05$), while no significant reduction was found in the group receiving

100 mg/kg of the extract of *Ferulago angulata* in this tissue compared with the Parkinson's group. Comparison of MDA measurement between the Parkinson's group and Parkinson's groups which received extract of *Ferulago angulata*, 200 and 400 mg/kg led to finding a significant reduction in the striatum tissue ($p < 0.01$), while it was not effective for the amount of MDA in this tissue in the group receiving FAE (100 mg/kg) (Figures 2 and 4).

In another section of this research, it was found that Thiol level was significantly decreased in Parkinson's group compared with the control in hippocampus tissue after MFB lesion in rats, and decreased significantly in striatum ($p < 0.001$). Comparison between the Parkinson's group and treatment Parkinson's groups which orally received extract of *Ferulago angulata*, 100, 200 and 400 mg/kg for 14 days demonstrated that Thiol level significantly increased in hippocampus tissue in the groups receiving *Ferulago angulata* compared with Parkinson's group ($p < 0.05$) and significant increase was also found in Thiol level in striatum tissue ($p < 0.001$) (Figures 3 and 5).

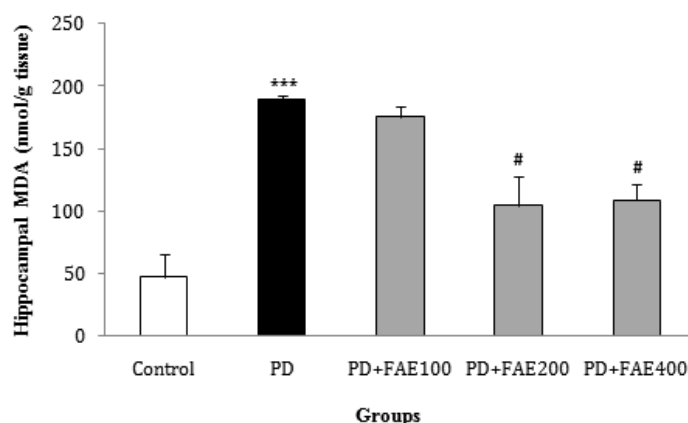


Figure 2- Effect of *Ferulago angulata* on MDA levels in hippocampus tissue between Control group, PD and PD groups orally receiving 100, 200 and 400 mg/kg FAE for 14 days. Values are expressed as mean \pm SEM ($n = 8$).
 *** $p < 0.001$ vs. control rats, # $p < 0.05$ vs. PD-treated rats

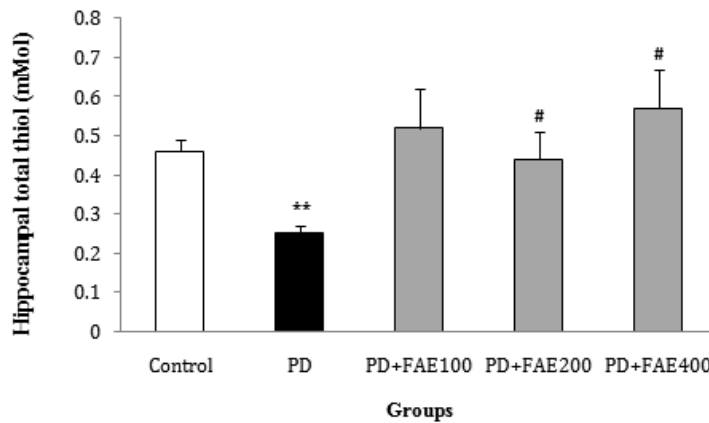


Figure 3- Effect of *Ferulago angulata* on thiol levels in hippocampus tissue between Control group, PD and PD groups orally receiving 100, 200 and 400 mg/kg FAE for 14 days. Values are expressed as mean \pm SEM (n = 8).
**p < 0.01 vs. control rats, #p < 0.05 vs. PD-treated rats

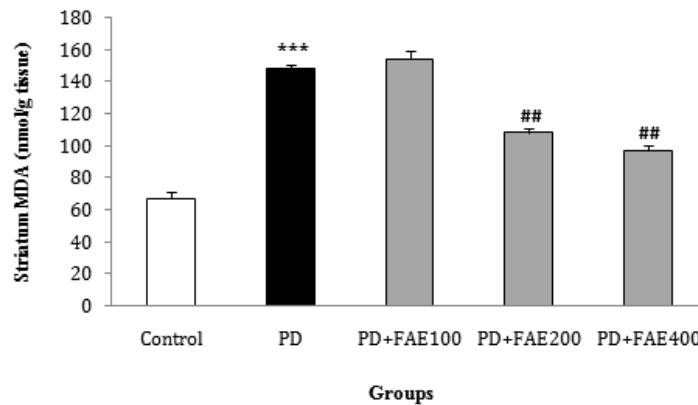


Figure 4- Effect of *Ferulago angulata* on MDA levels in striatum tissue between Control group, PD and PD groups orally receiving 100, 200 and 400 mg/kg FAE for 14 days. Values are expressed as mean \pm SEM (n = 8).
***p < 0.001 vs. control rats, ##p < 0.01 vs. PD-treated rats

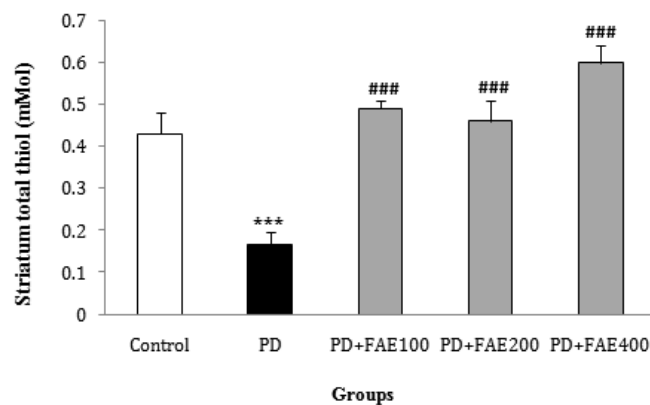


Figure 5- Effect of *Ferulago angulata* on thiol levels in striatum tissue between Control group, PD and PD groups orally receiving 100, 200 and 400 mg/kg FAE for 14 days. Values are expressed as mean \pm SEM (n = 8).
***p < 0.001 vs. control rats, ***p < 0.001 vs. PD-treated rats

Discussion

In this research, effect of unilateral injection of 6-OHDA neurotoxic drug into MFB and its resulting oxidative stress after lesion and treatment with hydroalcoholic extract of *Ferulago angulata* were studied. The findings in this research showed that Parkinson's animal model in rats by the injection of 6-OHDA into MFB through reducing antioxidant substances in brain caused death of dopaminergic neurons inside the substantia nigra and striatum and oral administration of different amounts of FAE for two weeks was effective for the rats with Parkinson's disease, which led to reduction of MDA and increase of Thiol group, and prevented the disease progress. In this study, MFB lesion was used, because developing Parkinson's by the injection of 6-OHDA neurotoxic drug into MFB is more similar to the pathology of this disease in humans than other models and its symptoms are closer to human Parkinson's disease [30]. Based on the conducted studies, Parkinson's disease is accompanied by neuron oxidative degeneration and substantia nigra is specifically susceptible to oxidative damage due to oxidative content (dopamine and neuromelanin) and relatively low antioxidant content [2]. The evident characteristic of this disease is pathologically slow and gradual degeneration of dopaminergic neurons of substantia nigra [5,31]. Studies have represented that unilateral damage of dopaminergic system reduced dopamine level and increases regulation of dopaminergic postsynaptic receptors located in striatum neurons of the damaged side through the injection of 6-OHDA [32]. Oxidative stress weakens antioxidant systems of the brain by producing free radicals and other reactive oxygen species (ROS) and the brain is

damaged by its destructive effects [33]. According to the conducted studies, free radicals and other ROS are important factors for degenerative disorders including Parkinson's disease, Alzheimer's disease, and some hepatic diseases and cardiovascular disorders [34]. ROS production and inducing oxidative stress are the known factors for the functional disorder of Parkinson's disease which oxidize lipids and proteins in the central nervous system [33]. In Parkinson's disease, striatum and hippocampus are more susceptible to damage caused by oxidative stress and free radicals than other parts of the brain [33]. In this disease, concentration of fatty acids has been reduced in substantia nigra, while MDA which is oxidative stress index is increased and also Thiol are decreased. Reduction of Thiol level indicates reduced antioxidant capacity of the brain [35]. One of the factors in the level of proteins which plays an effective role in its antioxidant properties is Thiol group and reduction of this group can be a good indicator for oxidative injuries [36]. Glutathione is one of the Thiol groups which plays an essential role in the protection of cells against degeneration caused by peroxidase hydrogen and oxygen species and these active oxygen species result from the disorder in dopamine metabolism. This disorder in dopamine metabolism reduces glutathione in nerve ending and degenerative diseases [37]. Many studies have shown that 6-OHDA neurotoxic drug creates its toxicity by creating free radicals [38]. This toxic substance is rapidly oxidized under physiological conditions and is converted into hydrogen peroxide which is one of the most destructive free radicals for live cells [39]. Many natural and artificial antioxidants are accessible which can be used for the treatment of Parkinson's disease [40]. Plants have

recently attracted the attention of human communities as natural antioxidants. Recent studies have demonstrated that *Ferulago angulata* contains phenol compounds [12]. It has been also recognized as one of the native and medicinal plants for the treatment of diseases [41]. This plant is also used for treating digestive diseases and chronic ulcer due to snakebite and headache [41]. In addition, antioxidant and antidiabetic effects of *Ferulago angulata* have been reported [42]. Aryan Poor et al. (2006) showed that antioxidants had a protective effect on Parkinson's disease [43]. Results of Salar et al. (2010) showed that hydraulic extract of plants improved Parkinson's disease in male rats due to antioxidant property [44]. Balooch Nejad et al. (2011) reported that intraperitoneal injection of antioxidants had a protective effect on 6-OHDA in Parkinson's experimental model [45]. Rafiei Rad et al. (2014) reported that different doses of FA was able to reduce MDA as a lipid peroxidation in ischemia [25]. Studies showed that FAE led to the reduction of MDA [46]. In other studies conducted by Mansouri et al. (2013), it was found that level of Thiol groups was significantly reduced after treatment with different doses of Gallic acid

by MFB lesion due to administration of 6-OHDA in rats [29]. The present results in this research were in line with those of the above studies and, considering findings of others and results of this research, it can be suggested that extract of *Ferulago angulata* has an antioxidant property and its consumption significantly increases antioxidant capacity. According to the conducted studies, antioxidant activity of *Ferulago angulata* may be due to the presence of phenols, particularly polyphenol compounds which are used as a natural antioxidant in food and pharmaceutical industries [12].

Conclusion

In this study, for the first time, it was shown that treatment with different doses of FAE could reduce MDA and increase level of Thiol in Parkinson's animal model.

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