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The effect of olive leaf methanolic extract on hippocampal antioxidant biomarkers in an animal model of Parkinson's disease

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ABSTRACT

Background and Objective: Parkinson's disease (PD) is a chronic neurodegenerative disease characterized by damages to striatal dopaminergic neurons that affects 1 to 2% of the population above 65 years of age. Olive leaf extract (OLE) is a powerful antioxidant that is considered as a source of various phenolic compounds. This study was conducted to evaluate the effects of methanolic OLE on hippocampal antioxidant biomarkers in an animal model of PD.

Materials and Methods: Intrastriatal 6-hydroxydopamine-lesioned rats were pretreated with OLE at doses of 50, 100 and 150 mg/kg/day for six weeks. At the end of treatment, the antioxidants activity in the hippocampal area was assayed.

Results: 6-hydroxydopamine–induced oxidative stress significantly decreased activity of superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase (GRX) and catalase (CAT) and treatment with OLE restored the activity of antioxidant enzymes SOD, GPX, GRX, and CAT and also decreased malondialdehyde (MDA) as an index of lipid peroxidation and increased glutathione (GSH) levels in the hippocampus.

Conclusion: These results suggest that OLE pretreatment reduces hippocampal oxidative biomarkers in an animal model of PD.

1. Introduction

Oxidative stress is as a result of enhanced formation of reactive oxygen species (ROS) and pathogenic factor in PD. ROS generally cause oxidative damage to proteins, lipids, and DNA and they are one of the most prominent factors related to neurodegeneration (1-3). The unilateral damage of the nigrostriatal dopaminergic system following intrastriatal injection of 6-OHDA leads to an increased oxidative stress in the brain (4). Natural polyphenol compounds with a neuroprotective role in the brain are known as abundant antioxidants (5). Mediterranean diets are natural sources of flavonoids and phenolic acids which can improve neurodegenerative disorders (6). Several recent studies have obviously shown that the free radical scavengers such as antioxidant factors can be useful in extending the permanence of dopaminergic neurons. The pharmacological attributes of the olive leaves have been discovered as main ingredients of medicine and a healthy diet due to their phenolic compounds and its antioxidant function can be used to reduce free radicals (7, 8). Several studies have shown olive leaf extract (OLE) has a diversity of biochemical tasks such as anti-ischemic (9), prohibition of LDL oxidation (10) and it prevents lipid peroxidation in rat model of stroke (11). In addition, Tyrosol which is one of essential elements of OLE has neuroprotective effect against dopaminergic cell death that could minimize oxidative stress and intensify antioxidant defensive system (12). Oral olive oil not only decreases brain edema, but also improves neurological losses after short-time middle cerebral artery obstruction (13). Many reports have shown that OLE has the ability to exert pharmacological effects (14), however, there are no researches done yet to evaluate its effect on hippocampal oxidative biomarkers in an animal model of Parkinson's disease. On this foundation, we designed this study to evaluate the neuroprotective effect of OLE on hippocampal antioxidant enzyme activities.

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2. Materials and Methods

2.1. Animals and experimental procedure

Male adult Wistar rats (220±20 g; n=42; Pasteur's Institute, Amole, Iran) were used in this study. The animals were kept in a colony room with a 12 h light/dark cycle (7:00–19:00 lights on) at 22±2°C. They had free access to food and tap water except during the time of experiments. The protocols for use and care of animals were approved by Ethics Committee of the University of Mazandaran (Babolsar, Iran) and according to NIH guidelines. Rats were randomly divided into six experimental groups: control group, vehicle group (gavaged by distilled water and received intrastriatal injection of saline), lesion group (gavaged by distilled water and received intrastriatal injection of 6-OHDA), and OLE-treated lesion groups. For modeling PD, the neurotoxin 6-OHDA was injected into the right striatum of anesthetized rats (ketamine 50 mg/kg and xylazine 4 mg/kg, *i.p.*) placed in stereotaxic apparatus (Stoelting, USA). According to Paxinos and Watson (1998)(15), stereotaxic coordinates for injection into the striatum were: + 1 mm anterior to bregma, + 2.5 mm lateral to the midline, and + 4.5 mm ventral to the dorsal surface of the skull (16). The 6-OHDA group received 2 µl of 0.9% normal saline containing 10 µg of 6-OHDA-HCl (Sigma Aldrich, USA) and 0.2% ascorbate. The OLE-treated lesion group received OLE at doses of 50, 100, and 150 mg/kg body weight for 3 weeks before and 3 weeks after injection of 6-OHDA.

2.2. Rotational behavior

The rotations were evaluated by a method as described previously (17). Briefly, the animals were allowed to be adapted for 10 min in lab room and 1 min after the injection of apomorphine hydrochloride (0.5 mg/kg containing 0.1% ascorbic acid, *i.p.*), full clockwise and counterclockwise rotations were obtained in a cylindrical container with a diameter of 33 cm and a height of 35 cm for 1 h. Then, net number of rotations was calculated as positive scores minus negative ones.

2.3. Oxidative stress assessment

24 h after the last gavage, animals were killed for assessment of antioxidant enzymes activities in right hippocampal tissues. In this regard, 150 mg of hippocampal tissue was homogenized in 1 ml of buffer (0.32 mol/l sucrose, 1 mmol/l EDTA and 10 nmol/l Tris–HCl, pH 7.4) in a Teflon glass homogenizer. The homogenate was centrifuged at 13,600 g for 30 min, and the supernatant was collected and used for the measurement of hippocampal antioxidant enzymes activities (18).

2.3.1. Determination of MDA and protein content

MDA, which is a measure of lipid peroxidation and as a measure of thiobarbituric acid reactive substances (TBARS) formation, was determined by the method of Esterbauer (1990) (19). Tissue homogenates containing 1 mg protein was mixed with trichloroacetic acid (1 ml, 20%) and thiobarbituric acid (2 ml, 0.67%). Samples were incubated at 100°C for 1 h, samples were centrifuged and then precipitate was deleted. The absorbance of the supernatant was read at 532 nm using a blank containing all the reagents except tissue homogenates. Protein was measured using the dye binding method of Bradford (20) and bovine serum albumin (BSA) was used as a standard.

2.3.2. Measurement of catalase (CAT) activity

Catalase activity was assayed according to the method of Genet et al (2002) (21). Briefly, the mixture contained 50 mM sodium phosphate buffer pH 7.0, 10 mM hydrogen peroxide and 20 µl of tissue extract. The absorbance of the supernatant was measured at 240 nm for 5 min at 25 °C in the presence of the enzyme in spectrophotometer comparing with a blank containing all the reagents but not the homogenated tissue. One unit of enzyme was expressed as µmole of H_2O_2 consumed/min/mg protein.

2.3.3. Measurement of glutathione peroxidase (GPx) activity

GSH was measured as described before (22). Briefly, a reaction mixture containing 1 ml of 0.4 M phosphate buffer (pH 7.0), 0.4 mM EDTA, 1 ml of 5 mM NaN_3, 1 ml of 4 mM glutathione (GSH) and 200 µl of supernatant was preincubated at 37°C for 5 minutes. One unit of

the enzyme was as the amount of enzyme needed to oxidize 1 nmol GSH/min.

2.3.4. Measurement of superoxide dismutase (SOD) activity

The activity of total superoxide dismutase was assayed based on the method of Genet et al(2002) with some changes (21). The assay mixture contained 50 mM sodium phosphate buffer, 0.1 mM EDTA, 0.48 mM pyrogallol and 20 µl of tissue extract. Auto oxidation of pyrogallol was assayed at 240 nm for 1 min at 25°C against a blank containing all the reagents except the homogenated tissue. The activity of the enzyme was expressed as the amount of enzyme that leads to 50% inhibition of pyrogallol autoxidation.

2.3.5. Measurement of glutathione reductase (GR)

Glutathione reductase activity was measured by using the method of Romero et al (2000) (23). A reaction mixture contained 0.1 M potassium phosphate buffer, pH 7.0, 2.5 mM glutathione disulfide and 125 mM NADPH. The absorbance of the supernatant was monitored for 5 minutes at 25°C following the oxidation of NADPH at 340 nm against a blank. One unit of enzyme was expressed as 1 µmol of NADPH oxidized/min/mg protein.

2.3.6. Estimation of GSH (reduced) content

The concentration of glutathione was measured following the method of Fukuzawa (1976) (24). Supernatant (200 µg/l) was added to 1 ml of 0.25 M sodium phosphate buffer (pH 7.4) followed by the addition of 130 µg /l DTNB [5,5′-dithio-bis (2-nitrobenzoic acid)] to form TNB (5-thio-2-nitrobenzoicacid) 0.04%. The mixture was brought to a final volume of 1.5 ml with distilled water and absorbance was read at 412 nm and results were defined as µg GSH/ µg protein.

2.4. Statistical analyses

All data were presented as mean ± standard error. Statistical analysis of data was performed using one-way analysis of variance followed by Tukey's post-hoc test. Results were considered statistically significant when $p<0.05$.

3. Results

There was no significant difference between the groups regarding rotational behavior before the surgery. In contrast, 6-OHDA-lesioned group showed significant contralateral rotations versus control ($p<0.001$) and OLE treatment significantly reduced this behavior relative to lesion group ($p<0.001$) (Fig. 1). The lipid peroxidation is a marker for oxidative stress (16). Measurement of oxidative stress-related markers indicated that treated lesion groups showed a significantly decreased level of MDA ($p< 0.05$) and increased GSH levels ($p<0.001$) (Table 1) in the hippocampal area of rats compared to the lesion group. Lesion group showed a significantly lower hippocampal CAT, GPX, GRX, and SOD activities versus control group ($p<0.001$), whereas a significant increase of the antioxidant enzymes activities were observed in treated lesion groups (100 and 150 mg/kg of OLE) as compared to lesion group ($p<0.01$) (Table 2).

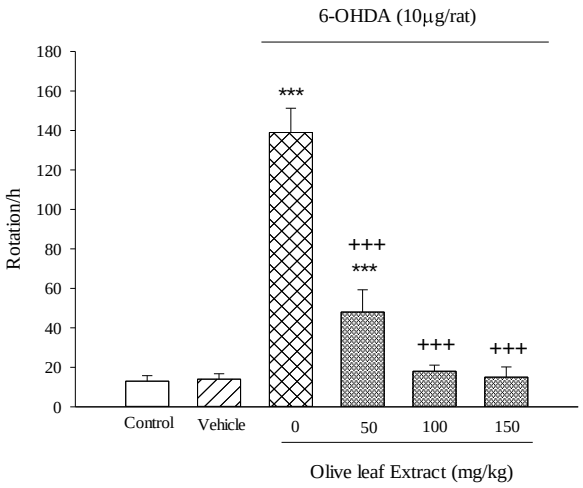

Fig. 1. The effect of OLE on the net number of apomorphine induced rotations/ h after intrastriatal 6-OHDA injection. Values are expressed as mean ± SEM. n = 7 for each group.
*** $p<0.001$ (vs. control); +++$p<0.001$ (vs. lesion)

Table 1. The effect of OLE on hippocampual level of antioxidant biomarkers 3 weeks after intrastriatal 6-hydroxydopamine (6-OHDA) injection

Groups	MDA (µg/mg protein)	GSH (µg/mg protein)
Control	66.06 ± 1.58	0.41 ±0 .0.01
Vehicle	67.46 ± 3.48	0.37 ± 0.02
6-OHDA	121.85± 0.83 ***	0.18± 0.01 ***
50 m/kg OLE + 6-OHDA	84.05 ± 0.29***+++	0.22 ± 0.003 ***
100 m/kg OLE + 6-OHDA	72.86 ± 3.21 +++	0.36± 0.006 +++
150 m/kg OLE + 6-OHDA	67.71 ± 2.39 +++	0.40 ± 0.01 +++

Values are expressed as mean ± SEM. n = 7 for each group.
*** P<0.001, ** P< 0.01
* P<0.05 (vs. control); +++ P<0.001, ++ P<0.01, + P< 0.05 (vs. lesion)

Table 2. The effect of OLE on hippocampual level of antioxidant biomarkers 3 weeks after intrastriatal 6-hydroxydopamine (6-OHDA)

Table 2: The effect of OLE on hippocampual level of antioxidant biomarkers 3 weeks after intrastriatal 6-hydroxydopamine (6-OHDA) injection				
Groups	**GPX (U/mg protein)**	**GRX (U/mg protein)**	**SOD (U/mg protein)**	**CAT (U/mg protein)**
Control	28.43 ± 0.25	41.60 ± 1.9	67.19 ± 0.96	110.83 ± 14.63
Vehicle	25.62 ± 0.22	37.45 ± 2.00	63.29 ± 1.35	110.01 ± 7.33
6-OHDA	6.32 ± 0.20 ***	18.14 ± 1.02 ***	6.54 ± 1.97 ***	8.73 ± 113 ***
50 m/kg OLE + 6-OHDA	8.01 ± 0.39 ***+	22.73 ± 0.33 ***	23.42 ± 2.68***+++	14.81 ± 0.16 ***
100 m/kg OLE + 6-OHDA	17.71 ± 0.49 ***+++	36.98 ± 0.64 +++	48.10 ± 1.72***+++	68.18 ± 3.80 ***+++
150 m/kg OLE + 6-OHDA	24.55 ± 0.30 ***+++	40.47 ± 2.6 +++	63.66 ± 1.54 +++	79.29 ± 5.92 +++

Values are expressed as mean ± SEM. n = 7 for each group. *** P<0.001, ** P< 0.01, * P<0.05 (vs. control); +++ P<0.001, ++ P<0.01, + P< 0.05 (vs. lesion)

4. Discussion

In this study, OLE treatment of 6-OHDA group reduced oxidative stress in hippocampal area of parkinsonian rats by potentiation of antioxidant defensive system. It has been shown that OLE treatment could decrease contralateral turning in treated lesion groups at doses of 100 and 150 mg/kg as compared to lesion group. A lower rotational behavior in our study may be attributed to the potential of OLE in neuroprotective effect against dopaminergic cell death. Injection of 6-OHDA by unilateral lesioning of the dopaminergic neurons in the striatum destroys striatal dopaminergic neurons for modeling PD in rodents (14).

The toxicity of 6-OHDA is attributed to the production of oxygen radical species through autoxidation and causes cell death (23). Also some studies suggest that 6-OHDA increased lipid peroxidation (estimated by MDA level), along with reduced activities of antioxidant enzymes (GSH, SOD, GPX, GRX and CAT) in brain (19). Our results indicate the protective effect of OLE at doses of 50,100, and 150 mg/kg body weight that can lead to significant improvement of GSH, reduction in lipid peroxidation and improvement of SOD, GPX, GRX and CAT enzyme activities in 6-OHDA - induced oxidative stress in hippocampal area.

In consistent with our findings, oleuropein administration for 10 days at a dose of 15 mg/kg to male rats could reduce spatial memory deficits and increase antioxidant status by injecting an anesthetic drugs in the hippocampus (24). In addition, olive polyphenol effects could confer a protective potential against alcohol-induced oxidative stress (25). Moreover, bioactive flavonoid compounds from OLE as hydroxytyrosol are capable to restores insulin signaling in Alzheimer's disease (26). These agents can be administered as an adjuvant medication to lower the required dose of prescribed drugs (3). Lee et al. (2010) suggested that OLE has superoxide dismutase (SOD)-like activity which may be probably due to radical scavenging abilities (27). On the other hand, pre-treatment with hydroxyl tyrosol that is present in OLE may be responsible for the induction of Nrf2-dependent gene expression. The transcription factor Nrf2 is an important molecular switch that orchestrates the gene expression of antioxidant enzymes (6). OLE could alter the balance between the scavenging capacity of antioxidant defense system and oxidant-pr. These results suggest that OLE pretreatment reduces hippocampal oxidative biomarkers in an animal model of PD.

Acknowledgments

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