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Original Article

Attenuation of LPS-Induced Oxidative Stress in the Rats' Brains by Olive Leaf Extract

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Abstract

Background and Aim: Today, oxidative stress and its association with neurological disorders have drawn researchers' attention. This study aimed to investigate the antioxidant effects of olive leaf extract (OLE) on oxidative stress induced by lipopolysaccharide (LPS) in rats' brains.

Materials and Methods: Twenty-eight adult male Sprague-Dawley rats were divided into four equal groups: control, LPS, and two OLE-pretreated plus LPS groups. The OLE-pretreated groups received OLE (40 and 60 mg/kg body weight as orally) and normal saline for 10 consecutive days. All groups, except the control group, received LPS (once; 5 mg/kg intraperitoneally) on the 11th day of treatment. The antioxidant status of the brain was measured using a lipid peroxidation marker (MDA) and the activities of antioxidant enzymes.

Results: Lipid peroxidation significantly increased in the LPS group in comparison with the control and OLE60+LPS groups. GSH content and glutathione peroxidase (GPx) activity significantly increased in the LPS group compared with the control and OLE60+LPS groups.

Conclusion: These results suggest that OLE partially attenuates LPS-induced oxidative stress in the rats' brains.

Keywords: Brain, Olive leaf extract, LPS, Oxidative stress

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Introduction

Recent studies suggest that the primary cause of many neurological diseases is the failure of mechanisms that control inflammatory responses in the brain (1). Large loads of pathogens, such as Gram-negative bacteria, can trigger neuroinflammation and immune responses via a component of the cell wall known as lipopolysaccharide (2, 3). LPS generates reactive oxygen species (ROS) and nitric oxide (NO) in macrophages. They are involved in oxidative as well as nitrosative stresses and endotoxic shock (4). LPS binds to CD14 and toll-like receptor 4, leading to the activation of transcription factors such as NF-κB. This factor plays a crucial role in inflammation by regulating the expression of genes that encode pro-inflammatory cytokines, adhesion molecules, and chemokines (5). NF-κB activates various enzymes, including NADPH oxidase, and produces reactive oxygen species (ROS). Superoxide creates other ROSs, and the overproduction

of these radicals can damage brain tissue (6). An imbalance between the production and elimination of free radicals leads to oxidative stress, and free radicals can affect important cellular structures such as DNA, proteins, carbohydrates, and lipids (7). Another function of LPS with regard to the brain is to activate the JNK (c-Jun N-terminal), which in turn activates the transcription factor C-JUN and can cause stress and cell death (apoptosis) by the activation of caspase-3 activity (8).

The beneficial impacts of antioxidants against oxidative stress to prevent brain damage such as Alzheimer's (AD) and Parkinson's diseases are widely known (9). Antioxidants are molecules with the ability to donate electrons to free radicals and scavenge ROS. One of the plants that contain antioxidants is olive tree, which is comprised of phenolic compounds in olive leaf extract (OLE) (10). Individual and combined phenolics (oleuropein, rutin, vanillin, and caffeic acid) exhibited remarkable levels of radical scavenging ability, concomitant with superoxide dismutase (SOD)-like activity (11). The data indicated that OLE could provide protective and remedial effects against stress situations, including oxidative stress and severe inflammatory responses (12). The recent data on OLE neuroprotection suggest a remedial role against AD (12). In our laboratory, oleuropein significantly reduced cognitive dysfunction via increasing antioxidant enzyme activities and decreasing lipid peroxidation (13). Given the promising antioxidant properties of OLE and deleterious effects of LPS in animal models, we decided to evaluate the pretreatment effects of OLE against oxidative stress induced by LPS in the rats' brains. Hence, the present study aimed to examine the pretreatment impacts of OLE on antioxidant enzyme activities and lipid peroxidation marker in the LPS-induced neurotoxicity of rats.

Materials and Methods

Materials

Olive leaf extract was purified in our laboratory according to the method described previously (16). LPS (*Salmonella typhosa* cell wall, 98% purity) was prepared from Sigma Chemical Company (Sigma, St. Louis, MO). The superoxide dismutase (SOD), glutathione peroxidase (GPX), malondialdehyde (MDA), catalase (CAT), GSH content, and Bradford protein kits were purchased from Kiazist (Kiazist Life Sciences, Iran) Company.

Experimental Design

A total of 28 adult male Sprague-Dawley rats (weighing 200 ± 20 g) were kept in a room with controlled conditions for laboratory animals, including a temperature of 23 ± 2°C, the humidity of 50%, 12/12-h light/dark cycles, and free access to food and water. All the rats were handled humanely according to the recommendations of the Animal Care Committee of Lorestan University (Khorramabad, Iran) with the Approval Number: (LU. ACRI.2020.35).

All the experimental procedures were conducted between 08.00 and 10.00 a.m. to prevent circadian rhythm alterations during daytime. The rats were divided into four equal groups (n = 7 rats per group) and treated daily for 10 consecutive days as follows: the LPS group received 1.5 ml normal saline orally at 8.00 am daily. The OLE-pretreated groups received OLE (40 and 60 mg/kg body weight orally at 8.00 am daily). OLE dosage was based on the previous report (14). OLE was dissolved in sterile physiologic saline solution prior to the administration daily. On the 11th day of the treatment, the entire groups except the control group received one dose of *Salmonella typhosa* cell wall suspension (5 mg/kg BW) intraperitoneally (12-14). The animals were administrated while they were conscious following careful handling to avoid any stressful impact. The weight of the rats was measured at the end of the experiment, and there was no remarkable distinction among the groups. One day after the LPS injection, the rats were sacrificed using xylazine and ketamine anesthesia. Anesthesia was achieved intraperitoneally by a combination of ketamin (Alfasan, Woerden, Holland, 70 mg/kg BW) and xylazine hydrochloride (Alfasan, Woerden, Holland, 5 mg/kg BW) (15). The brain was entirely washed by phosphate buffer and dissected by a scalpel. Subsequently, the brain samples were reserved at -70°C for later biochemical analysis.

Tissue Preparation for Biochemical Analysis

Almost one g of the brain hippocampal area was taken to specify antioxidant status in the rats. The samples were manually homogenized in cold phosphate buffer

(0.1 M, pH 7.4) containing 5 mM EDTA on liquid nitrogen, and debris was removed by centrifugation at 2000 RPM for 10 min (16). Supernatants were recovered and used for the evaluation of GPx, SOD, CAT activities, GSH, and MDA concentrations, as well as protein measurement.

Measurement of Total Protein

Bradford protein assay kits (Kiazist Life Sciences, Iran) were used to measure the concentration of protein in the samples.

Measurement of Malondialdehyde (MDA) Concentration

MDA is a product of lipid peroxidation induced by oxidative injuries. MDA concentration was calculated in the samples with the MDA kit, according to the manufacturer's instructions (Kiazist, life sciences, Iran). In this method, malondialdehyde makes a complex with thiobarbituric acid. Absorbance was followed at 532 nm. The results were expressed as nmol MDA per mg of tissue protein (nmol/mg of tissue protein).

Measurement of the GPx Activity

GPx catalyzes the decrease of various hydroperoxides and lipid hydroperoxides to H2O and lipid alcohols through the oxidation of reduced GSH into its disulfide form (GSSG). The activity of GPx in brain homogenate was examined using a GPx assay kit (Kiazist Life Sciences, Iran). In this method, in the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is instantly changed to the decreased form with the attendant oxidation of NADPH to NADP+. The change in absorbance was evaluated spectrophotometrically against blank at 340 nm, and the activity of GPx was expressed as U/mg of tissue protein.

Measurement of the GSH Content

The total level of GSH was determined using the 5,5′-dithiobis-(2-nitrobenzoic) acid (DTNB) method according to the Kiazist kit (Kiazist Life Sciences, Iran) instructions. Classical Ellman reagent known as DTNB reacts with reactive sulfhydryl groups and results in a DNTB-sulfhydryl complex which is detectable at 405 nm. The results were demonstrated as nano mol per milligram of tissue protein.

Measurement of the SOD activity

SOD scavenges the superoxide anion (O2−) by catalyzing the dismutation of this free radical to oxygen and hydrogen peroxide. The activity of SOD in brain homogenate was examined using a SOD assay kit (Kiazist Life Sciences, Iran). This method uses xanthine and xanthine oxidase to generate superoxide radicals and the SOD enzyme converts anion superoxide into H2O and oxygen. In this kit, a blue colorant called resazurin is used, and in the proximity of anion superoxide (O2-) resorufin becomes pink. Eventually, the products can be measured for absorbance at 570 nm (colorimetric method), and the activity of SOD is expressed as U/mg of tissue protein.

Measurement of CAT activity

Catalase (CAT) is an antioxidant enzyme, which promotes the depletion of H2O2 to H2O and O2. CAT activity was evaluated using CAT assay kits (Kiazist, life sciences, Iran) according to its manufacturer's instructions. In this method, catalase produces formaldehyde in the presence of methanol, and formaldehyde reacts with Purpald to produce a purple color. The reaction began by adding H2O2 and the change in the absorbance was followed at 495 nm. The CAT activity was expressed as milliunit per milligram of tissue protein (mu/mg of tissue protein).

Statistical Analysis

Statistical analysis was carried out using the statistical package GraphPad PRISM version 5 (GraphPad Software, San Diego, CA, USA). The data were evaluated for normal and homogeneous variances using Levene's statistic test. All the results have been presented as Mean + SEM. The statistical distinction of the data was performed between all the groups by one-way analysis of variance (ANOVA) with Tukey's post-hoc analysis. A calculated P value of less than 0.05 was considered statistically significant.

All mice were treated humanely and in compliance with the recommendations of Animal Care Committee for the Lorestan University (Khorramabad, Iran) with approval number: LU. ACRI.2020.35.

Results and Discussion

The concentration of MDA (Mean ± SEM) in LPS groups significantly increased compared with the control group ($P<0.05$). Moreover, the concentration of MDA remarkably decreased in the rats treated with

OLE 60 mg/kg compared with the LPS groups (P<0.05, Fig. 1).

The mean values (± SEM) of the antioxidant enzyme activities (GPX, SOD, and CAT) and GSH content in the brain homogenates have been presented in Figures 2, 3, 4, and 5. The GPX activity, as the main antioxidant

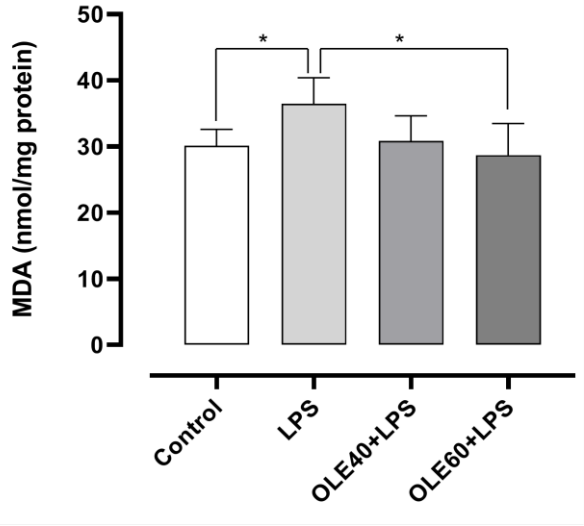

Figure 1. Effects of LPS and OLE pretreatment on the MDA concentration in the control and treated rats. Values represent the Mean ± SEM of MDA (nmol/mg protein of brain tissue). Control, lipopolysaccharide (LPS), olive leaf extract (OLE) 40 mg/kg+LPS, OLE60 mg/kg+LPS. *Indicates significant differences among LPS with the

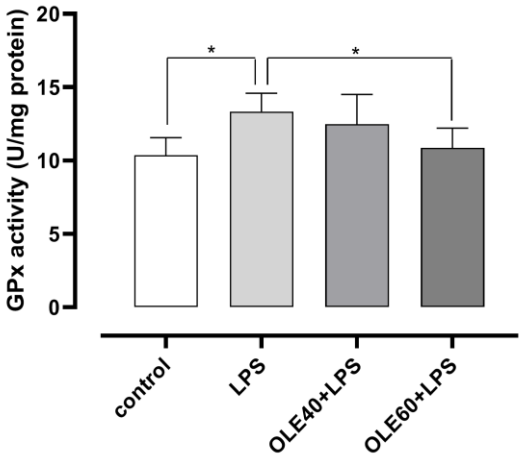

Figure 2. Effects of LPS and OLE pretreatment on the GPx activity in the control and treated rats. Values represent the Mean ± SEM of GPx (U/mg protein of brain tissue). Control, lipopolysaccharide (LPS), olive leaf extract (OLE) 40 mg/kg+LPS, OLE 60 mg/kg+LPS. *Indicates significant differences among LPS with the control and OLE 60+LPS groups P<0.05.

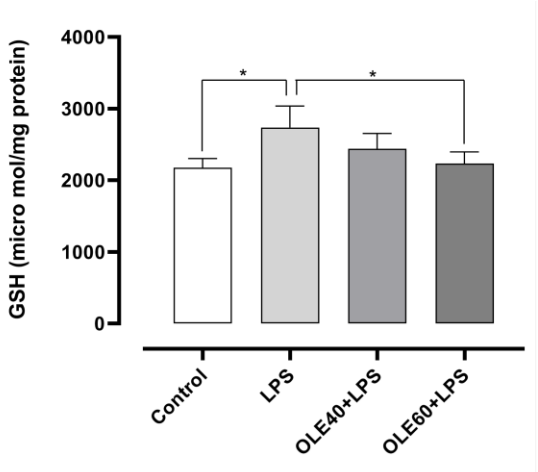

Figure 3. Effects of LPS and OLE pretreatments on the GSH content in the control and treated rats. Values represent the Mean±SEM of GSH (micro mol/mg protein of brain tissue). Control, lipopolysaccharide (LPS), olive leaf extract (OLE) 40 mg/kg+LPS, OLE 60 mg/kg+LPS. *Indicates significant differences among LPS with the control and OLE 60+LPS groups P<0.05.

enzyme, significantly increased in the brain of the LPS group in comparison with the control and OLE60+LPS groups (P<0.05, Fig. 2). This enhancement might serve as a compensatory mechanism to suppress oxidative

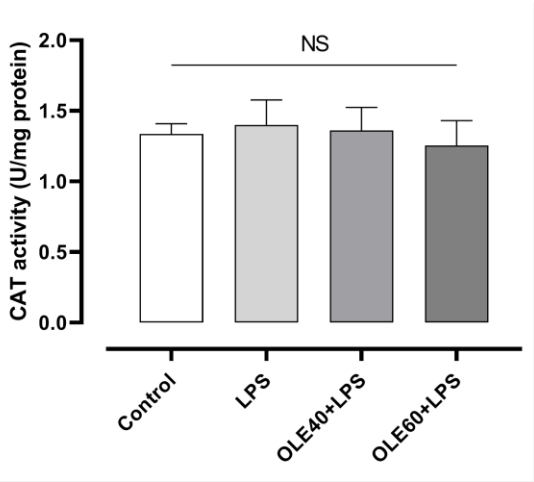

Figure 4. Effects of LPS and OLE pretreatment on the CAT activity in the control and treated rats. Values represent the Mean ± SEM of CAT (U/mg protein of brain tissue). Control, lipopolysaccharide (LPS), olive leaf extract (OLE) 40 mg/kg+LPS, OLE 60 mg/kg+LPS. NS. There was no significant difference among the groups (P>0.05).

stress. The level of GSH content also significantly increased in the brain of the LPS group in comparison with the control and OLE 60+LPS groups (P<0.05, Fig. 3).

Although the CAT and SOD activities in OLE-treated

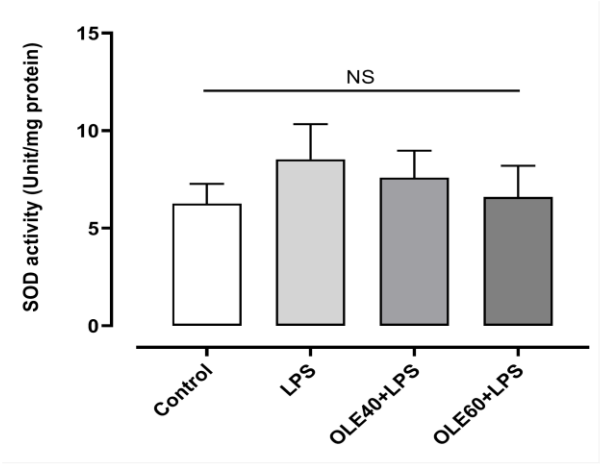

Figure 5. Effects of LPS and OLE pretreatment on the SOD activity in the control and treated rats. Values represent the Mean ± SEM of SOD (U/mg protein of brain tissue). Control, lipopolysaccharide (LPS), olive leaf extract (OLE) 40 mg/kg+LPS, OLE 60 mg/kg+LPS. NS. There was no significant difference among the groups (P>0.05)

rats were lower than those of the LPS group, these reductions were not statistically significant (P>0.05, Figs. 4, 5).

It was found in the present study that olive leaf extract (OLE) treatment could partially protect the brain tissue against oxidative stress induced by lipopolysaccharide in a rat model. The neuroprotective impact of OLE is associated with the suppression of oxidative stress indicated by the reduction of lipid peroxidation (MDA) level in the hippocampal area of the rats.

As previously mentioned, the failure to adequately regulate and control inflammatory responses in the brain is a major cause of various neurological diseases (1). Previous studies have shown that LPS injection could induce oxidative stress (19). In the brain tissue, TLR4 and other TLR receptors are found on neurons, and microglia. Moreover, astrocytes detect LPS and lead to the onset of inflammation as well as oxidative stress (20). It has been indicated that animals can respond to LPS stimuli depending on age, species, the stimulus source, dose, method of use, and duration (21). For example, a single dose of LPS in a rat model at approximately 1, 2, 4, and 6 hours after injection twice increased IL-1β and TNF-α levels in the cerebral cortex and hippocampus of mice (13). Another study showed that the systemic injection of LPS at a dose of 5 mg/kg in rats increased inflammatory factors such as TNFα and transmitted inflammation to the brain tissue eventually leading to neurotoxicity (14). In the nervous system, ROS can activate the expression of apoptotic genes such as Bax and Bcl-2 genes, and an elevated Bax/Bcl-2 ratio can trigger apoptosis. In this regard, it was shown that LPS could activate caspase-3 in the brain of mice that subsequently resulted in the induction of automatic cell death or apoptosis (22). Brain oxidative stress can damage neurons, and this damage can lead to neurological diseases such as Alzheimer's, Parkinson's, and neurogenic disorders such as bipolar disorder, schizophrenia, depression, epilepsy, and anxiety (23-27). Oxidative stress leads to motor cognitive impairment and learning as well as behavioral disorders (28, 29).

Antioxidants such as olive leaf extract have the ability to improve the conditions created by LPS and other antioxidant agents. As a solution to counteract the adverse impact of oxidative stress, antioxidant-based treatment can reduce the disorders related to oxidative stress (30). Recent studies have revealed that many of these complications are reduced upon supplementation with special dietary antioxidants such as vitamins E, C, and k (31-32). The use of polyphenols such as flavonoids has been reported with the same advantages (33). As mentioned previously, olive leaf extract is a phenolic compound whose antioxidant properties enable it to have beneficial effects, including vasodilatory, hypotensive, anti-inflammatory, anti-rheumatic, diuretic, anti-atherogenic, and antipyretic activities (34-40). Olive leaf extract has both the ability to prevent the production and elimination of free radicals. Prevention of the production of free radicals by olive leaf extract might be due to its potential to chelate metal ions such as copper and iron, which catalyze free radical production reactions (41). Other protective properties of oleuropein and its metabolite hydroxytyrosol include the elimination of superoxide anions and the inhibition of respiratory bursts of neutrophils as well as hypochlorous acid-derived free radicals, which may do this through the hydroxyl group (42).

We previously investigated the effects of olive leaf extract on antioxidant enzymes in the testicular tissue, liver, stomach, and kidney in animal models. The results of these studies showed that olive leaf extract

could act as an antioxidant in preventing oxidative stress and renal failure induced by LPS, and liver as well as stomach damage in ethanolic rats. (43-46). Olive leaf extract also showed remarkable neuroprotective effects in several studies and experimental models, and thus might have potential healing effects in neurodegenerative diseases. Recently, olive leaf extract indicated the ability to reduce or even prevent the accumulation of amyloid-beta (Aβ), a causative agent of Alzheimer's disease in our laboratory (47). Moreover, the neuroprotective effect of olive leaf extract was shown in the hippocampal CA1 region of the brain against oxidative stress induced by anesthetics and colchicine in a previous report (46).

GPx is the main antioxidant enzyme that fragments H_2O_2 and prohibits ROS production (16, 17). According to previous reports, GSH as a cofactor of glutathione peroxidase has an important function in the cellular protection system against lipid peroxidation. Glutathione is found most of the time in its reduced form because the enzyme that returns it from its oxidized form (in cellular oxidative conditions), glutathione reductase, is unceasingly active (48). The results of this study indicated that GPx activity and GSH content increased in the LPS group as a compensatory mechanism to suppress oxidative stress compared with the control group. In this setting, the administration of olive leaf extract (60 mg/kg as orally) for 10 consecutive days reduced GPx activity and GSH content in olive leaf extract-treated rats compared with the LPS group indicating the antioxidant effects of OLE by scavenging ROS. In fact, olive leaf extract can chelate metal ions, which catalyze free radical generation reactions, scavenge the peroxyl radicals, and break peroxidative chain reactions that produce stable structures (49, 50). Lower MDA concentrations in the OLE 60 plus LPS group showed that olive leaf extract was able to prevent lipid peroxidation in the rats' brains. However, CAT and SOD activities were insignificantly lower in the OLE groups against the LPS group.

Conclusion

The data presented in this study provide convincing evidence that the decrease in the MDA level after a short period of olive treatment is likely to be the result of alterations in antioxidant responses and detrimental effects of oxidative stress in the rats' brains. Therefore, restoration of antioxidant status after treatment of rats with the extract might be due to the antioxidant features of olive as a good candidate to decline oxidative stress in complications induced by LPS. Although pretreatment with two doses of olive leaf extract reduced oxidative stress induced by LPS in comparison with non-treated rats, the optimal inhibition of oxidative stress results was observed in the rats pretreated with 60 mg/kg of olive leaf extract. Thus, it seems that there is a dose-dependency of olive leaf extract. According to our results, it could be concluded that a dose of 60 mg/kg of OLE could prevent oxidative stress against LPS effects.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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