

Short Report

## Effect of Oleuropein on Tissue Myeloperoxidase Activity in Experimental Spinal Cord Trauma

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### ABSTRACT

**Background:** Neutrophil infiltration plays an important role in inflammatory reactions following spinal cord injury (SCI) and these cells cause substantial secondary tissue damage. The purpose of this study was to determine the effect of oleuropein (OE) on myeloperoxidase (MPO) activity as an index of neutrophil infiltration. **Methods:** Rats were randomly divided into four groups of 7 rats each as follows: sham-operated group, trauma group, and OE treatment groups (20 mg/kg, i.p., immediately and 1 hour after SCI). Spinal cord samples were taken 24 hours after injury and studied for determination of MPO activity. **Results:** The results showed that MPO activity was significantly decreased in OE-treated rats. **Conclusion:** On the basis of our findings, we propose that OE may be effective in protecting rat spinal cord from secondary damage by modulating of neutrophil infiltration. *Iran. Biomed. J. 15 (4): 164-167, 2011*

**Keywords:** Oleuropein (OE), Neutrophil infiltration, Myeloperoxidase

### INTRODUCTION

Neurological damages after traumatic spinal cord injury (SCI) result from both primary mechanical injury and secondary degeneration process. The outcome of SCI depends on the extent of secondary damage mediated by a series of cellular, molecular and biochemical cascades including calcium ion influx, oxygen free radical-induced lipid peroxidation, inflammatory reaction, autoimmune response, vascular events, and apoptosis [1]. Secondary injury appears to be susceptible to pharmacological interventions including the use of free radical scavengers and anti-inflammatory agents. Neutrophils play a major role in inflammation by releasing reactive oxygen species, pro-inflammatory factors and histolytic enzymes, which lead to severe and irreversible secondary tissue damage [2, 3].

Olive oil is a rich source of phenolic components (such as oleuropein), which have many beneficial health effects in human [4]. On the other hand, hydrolysis of oleuropein (OE) results in the formation of other phenolics including hydroxytyrosol and tyrosol [5]. Experimental studies attributed the

beneficial effects of OE and its derivatives such as hydroxytyrosol to a variety of biological activities, including free radical scavenging/antioxidant actions, anti-inflammatory effects, anti-carcinogenic properties, and anti-microbial activities [6-8]. Olive oil phenols have some of protective effects against brain hypoxia-reoxygenation [9, 10], cerebral ischemia [11, 12], brain damage after hypoxia-reoxygenation in diabetic rats [13] and ageing [14]. Although the exact neuroprotective mechanism of olive oil phenols is unclear, the anti-oxidative and anti-inflammatory effects of these phenols are considered to be the main mechanisms leading to this neuroprotective effect.

In the present study, we investigated biochemically the potential anti-inflammatory effect of oleuropein against neutrophil infiltration in the spinal cord after experimental contusion injury.

### MATERIALS AND METHODS

**Animals.** Male adult Sprague-Dawley rats (250-300 g, the Pasteur Institute of Iran, Tehran) were used in this study. The animals were kept under standard

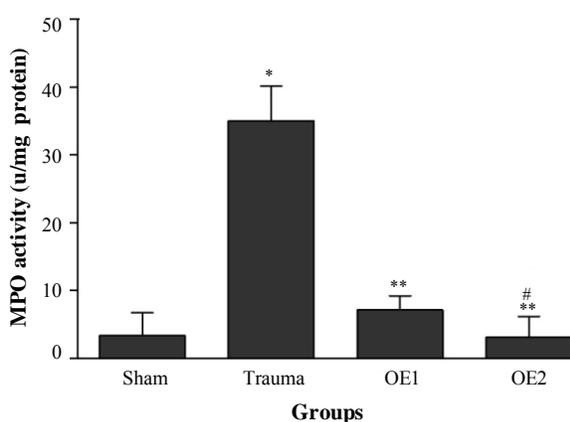
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conditions according to the Guidelines of the University's Animal Care Codes to minimize the animals' suffering.

**Contusive SCI using the weight-dropping technique.** The animals were anesthetized with ketamine (75 mg/kg i.p.) and xylazine (10 mg/kg i.p.). Laminectomy was performed at T9 level vertebra. The dorsal surface of the cord was then subjected to weight-drop impact using a 10-g weight dropped from a height of 2.5 cm in order to produce contusive SCI [15]. Following the surgery, the recovery of the animals was assisted by administering lactated Ringer's solution (12-25 ml) subcutaneously immediately after surgery and cefazolin (50 µg/kg, Jaber Ibn Hayan, Tehran). The urinary bladders were pressed three times a day. The rats were randomly allocated in four groups, each containing 7 rats: (i) sham- operated group, underwent laminectomy alone; (ii) trauma group, which underwent laminectomy followed by SCI and received saline (vehicle); (iii and iv) OE treatment groups, which underwent laminectomy followed by SCI and received a 20- mg/kg single dose of OE (Sigma, USA) i.p. immediately (OE1) and 1 hour (OE2) after trauma.

**Biochemical analysis.** Twenty four hours after SCI, the rats were euthanized and 1.5-cm traumatized spinal cord sample was removed for biochemical analysis. The obtained samples were thoroughly cleaned of blood and the meninges were carefully removed. Then, the tissue samples were immediately frozen and stored in a -70°C freezer for assays of tissue myeloperoxidase (MPO) activity, an indicator of neutrophil infiltration, as previously described by Mullane [16]. The spinal cord samples were homogenized in a solution containing 0.5% (w/v) hexadecyltrimethyl ammonium bromide dissolved in 10 mM potassium phosphate buffer (pH 7) and centrifuged at 20,000 ×g at 4°C for 30 min. An aliquot of the supernatant was then allowed to react with a solution of 1.6 mM tetramethylbenzidine and 0.1 mM H<sub>2</sub>O<sub>2</sub>. The absorbance of the supernatant was measured by spectrophotometry at 650 nm. The MPO activity was expressed as units of MPO/mg of proteins.

**Statistical analysis.** Statistical analysis was carried out using SPSS package. Results were presented as mean values (± SEM). The Kolmogorov-Smirnov test was used in order to evaluate the normality of the data. Also, the Tukey's multiple comparison tests and the analysis of the variance were used in order to compare each two groups and compare the data among the groups, respectively. A value of  $P < 0.05$  was considered significant.



**Fig. 1.** Effects of oleuropein on myeloperoxidase (MPO) activity. The histogram shows the activity of MPO at 24 hours after SCI. MPO activity was expressed as units of MPO/mg of proteins. \* $P < 0.05$  versus sham; \*\* $P < 0.05$  versus trauma; # $P > 0.05$  versus OE1 group.

## RESULTS AND DISCUSSION

The histogram of the MPO activity for all groups at 24 hours post-injury has been shown in Figure 1. Induction of SCI in trauma group produced a significant elevation ( $P < 0.05$ ) in MPO activity compared to the sham-operated group. The MPO activity in OE treatment groups was significantly lower than trauma group ( $P < 0.05$ ), while the differences between OE1 and OE2 were not significant ( $P > 0.05$ ).

Post-traumatic inflammation is characterized in part by the accumulation of activated leukocytes, especially neutrophils which are the first leukocytes to arrive within the traumatized spinal tissue, peak at 24 hours post-injury [17].

Some evidences suggested that these cells play an important role in the pathogenesis of secondary degeneration of SCI by releasing of inflammatory mediators, including cytokines, chemokines, proteases, and free radicals, which can cause neuronal and glial toxicity [17]. Tissue MPO, a well-known oxidative enzyme, is a exclusive indicator of the extent of post-traumatic neutrophil infiltration [18]. On the other hand, MPO generates hypochlorous acid that damages nearby tissues. It is well-documented that a decrease in MPO activity correlates with reduction in traumatic spinal cord damage and better functional outcome after SCI in rats [19]. In the present study, we have observed that the MPO activity reduced significantly in OE-treated rats when compared with non-treated rats. Although the most famous and widely renowned properties of olive phenolics have long been attributed to the antioxidant and free radical scavenging effects, emerging evidences have shown the anti-inflammatory

effects of these phenolics [20, 21]. In this regard, it has been documented that OE strongly inhibited the enzyme MPO in the inflamed tissue [20]. Plasma levels of the pro-inflammatory cytokines were also significantly reduced by OE in mice subjected to collagen-induced arthritis [22]. Visioli and colleagues [23] reported that OE inhibits the respiratory burst of neutrophils and hypochlorous acid-derived radicals. Moreover, other study has shown that olive oil polyphenols inhibit endothelial-leukocyte adhesion molecule expression [24].

Finally, our results showed that administration of OE immediately and 1 hour after SCI significantly attenuated MPO activity. This finding indicates a reduction of the neutrophil influx in the injured spinal tissue and possibly neuroprotective effects of OE after SCI.

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