

Analgesic and Anti-Inflammatory Activities of the Methanol Extract of *Elaeagnus oldhamii* Maxim. in Mice

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Abstract: We investigated possible mechanisms of analgesic and anti-inflammatory activities of the methanol extract from the leaf of *Elaeagnus oldhamii* Maxim. (EO_{MeOH}). EO_{MeOH} was evaluated for its analgesic activity in acetic acid-induced writhing response and formalin test, and anti-inflammatory effect was examined by λ -carrageenan-induced paw edema assay. We detected the activities of GPx, GRd and SOD in the liver, and the levels of inflammatory mediators including IL-1 β , IL-6, TNF- α , COX-2, MDA and NO in the edema paw to investigate the mechanism of action against inflammation. Total polyphenol, flavonoid and flavanol contents of EO_{MeOH} were detected to explore its antioxidant activities. Results showed that, in the analgesic test, EO_{MeOH} decreased acetic acid-induced writhing response and the licking time in the late phase of formalin test. In the anti-inflammatory test, EO_{MeOH} decreased paw edema at the 2nd, 3rd, 4th and 5th h after λ -carrageenan had been injected. EO_{MeOH} increased the activities of SOD and GPx in liver tissue and decreased MDA, NO, IL-1 β , IL-6, TNF- α and COX-2 levels in paw edema tissue at the 3rd h after λ -carrageenan-induced inflammatory reaction. EO_{MeOH} exhibited abundant polyphenol, flavonoid and flavanol contents. In HPLC fingerprint test of EO_{MeOH}, two index ingredients, ursolic acid and pomolic acid, were isolated from EO_{MeOH} and were exhibited in HPLC chromatographic analysis. The results demonstrated analgesic and anti-inflammatory effects of EO_{MeOH}. It was indicated that the anti-inflammatory mechanism of EO_{MeOH} may be due to declined levels of NO and MDA in the edema paw through increasing the activities of SOD, GPx and GRd in the liver. Additionally, EO_{MeOH} decreased IL-1 β , IL-6, TNF- α and COX-2 levels in the

edema paw. The results suggested its value in future development of herbal medicine for the treatment of inflammatory diseases.

Keywords: *Elaeagnus oldhamii* Maxim.; Anti-Inflammation; Analgesia; MDA; SOD; Interleukin-1 β ; Tumor Necrosis Factor- α .

Introduction

Inflammatory reaction, typically characterized by redness, swelling, heat and pain, is one of the most important host defense mechanisms against invading pathogens. Pro-inflammatory cytokines (e.g. TNF- α , IL-1 β and IL-6) are produced in large quantities by activated macrophages/monocytes, and would further stimulate active cellular responses by increasing prostaglandins (PGs) and reactive oxygen species (ROS, e.g. NO and O₂) (Cirino, 1998). Free radicals, in turn, attack the plasma membrane through lipid peroxidation to produce malondialdehyde (MDA). Thus, the inflammatory effect results in the accumulation of MDA (Janero, 1990).

Elaeagnus is a genus of about 90 species. The majority is native to temperate and subtropical regions in Asia, of which nine species can be found in Taiwan (Huang, 1998). Many species of *Elaeagnus* are considered as folk medicinal plants, e.g. *E. umbellata* (Ahmad and Mubasher, 2005), *E. angustifolia* (Ahmadiani *et al.*, 2000) and *E. pungens* (Yuebin *et al.*, 2009). *E. oldhamii* Maxim., a traditional herbal medicine mainly used for rheumatoid arthritis, has not yet been investigated in any research for its analgesic and anti-inflammatory effects.

Flavonoids and triterpenoids are isolated from several species of *Elaeagnus*, e.g. *E. unguis* (Fu and Wang, 2007), *E. bockii* Diels (Lou *et al.*, 2006) and *E. umbellata* (Fu and Wang, 2007). Many studies have indicated that flavonoids possess anti-inflammatory effects via scavenging ROS (Kim *et al.*, 2007; Hougee *et al.*, 2005), and triterpenoids possess anti-inflammatory effects via modulating inflammatory cytokines (Ringbom *et al.*, 1998; Yadav *et al.*, 2010).

In this study, we detected the total polyphenol, total flavonoid and total flavanol contents of *Elaeagnus oldhamii* *in vitro*. Additionally, two triterpenoid compounds, ursolic acid and pomolic acid, were isolated from EO_{MeOH} and were displayed in HPLC chromatographic analysis.

In this study we intended to investigate analgesic and anti-inflammatory effects of EO_{MeOH}. The analgesic activity was evaluated by acetic acid-induced writhing response and formalin test, while anti-inflammatory activity was determined using λ -carrageenan-induced mouse paw edema model. In order to evaluate the mechanisms of analgesic and anti-inflammatory effects of EO_{MeOH}, we also analyzed the levels of IL-1 β , IL-6, TNF- α , COX-2, MDA and NO in the edema paw tissue and antioxidant enzymes activities of SOD, GPx and GRd in the liver three hours after λ -carrageenan had been injected.

Materials and Methods

Plant Material and Crude Extract Preparation

Elaeagnus oldhamii Maxim. was collected from the herbal garden in China Medical University, Taichung City, Taiwan. The plant was authenticated by Hsin-Fu Yen, Associate Researcher, National Museum of Natural Science, Taichung City, Taiwan.

Extraction and Isolation

The leaf of *E. oldhamii* Maxim. (750 g) was sliced into small pieces, dried and extracted with 7 liters of methanol for 48 h/cycle three times and concentrated under reduced pressure. The yield ratio of EO_{MeOH} extract was 15.0%. The dried crude extract was dissolved in 0.5% CMC solution prior to pharmacological testing.

Twenty grams of crude extract was loaded onto a silica gel column using *n*-hexane-ethyl acetate mobile phase. Ursolic acid (30 mg) and pomolic acid (20 mg) were purified using gradient *n*-hexane-ethyl acetate at a ratio of 65:35. Structures of ursolic acid and pomolic acid were confirmed by comparing ¹H and ¹³C NMR spectral data with the data given in literatures (Cheng *et al.*, 1983; Gohari *et al.*, 2009).

Chemicals and Drugs

λ-carrageenan, indomethacin and Griess reagent were purchased from Sigma-Aldrich Chemical Co. Formalin was purchased from Nihon Shiyaku Industry Ltd. SOD, GPx, GRd and MDA assay kits were purchased from Randox Laboratory Ltd. The enzyme immuno-metric assay kits for mice, IL-1β, IL-6, COX-2 and TNF-α were obtained from Assay Designs Inc. LC grade methanol was from Labscan (Ireland). All other reagents used were of analytical grade.

Chromatographic Analysis of EO_{MeOH}

The HPLC system consisted of a Waters 2695 Alliance LC with 2996 PDA. Chromatographic separation was performed on X-BridgeTM RP18 (25 cm × 4.6 mm I.D., 5 μm) with an injection volume of 10 μl. The mobile phase consisted of a mixture of acetonitrile-water in a ratio of 9:1. The flow rate was set at 1.0 ml/min.

Experimental Animals

Male ICR mice (20–25 g) were purchased from the Laboratory Animal Center of National Taiwan University, Taiwan. The mice were kept in the animal center of China Medical University at 22 ± 1°C, relative humidity 55 ± 5%, with light dark cycles of 12 hours (08:00 to 20:00 lights on) for one week before the experiment. Animals were provided with rodent diet and clean water *ad libitum*. All studies were conducted in accordance with the

National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. All tests were conducted under the guidelines of the International Association for the Study of Pain (Zimmermann, 1983).

Acute Toxicity Study

The acute toxicology test in mice was carried out by using the method of a previous study (Liao *et al.*, 2007). Male ICR mice (22–25 g) were divided into different groups and experimented with increased oral doses of EO_{MeOH} (2.5, 5 and 10 g/kg). The experimental mice were allowed for food *ad libitum*, and all of them were kept under regular observation for seven days for any mortality or behavioral changes.

Acetic Acid-Induced Writhing Response

The writhing test in mice was carried out following the method used by Koster *et al.* (1959). The writhes were induced by intraperitoneal injection of 1.0% acetic acid. EO_{MeOH} (0.1, 0.5 and 1.0 g/kg) and the positive control of indomethacin (10 mg/kg) were administered orally to separate groups of mice 60 min before the acetic acid injection. The number of writhes was counted 5 min after the acetic acid injection. The data collected would represent the total number of writhes observed in a duration of 10 min (5–15 min after the injection).

Formalin Test

Formalin test was conducted following the method used by Tjølsen *et al.* (1992), which began by injecting 20 μ l of 5% formalin subcutaneously into the right hind paws of mice. The time (in seconds) spent on licking and biting the injected paw was recorded as an indicator of pain response. Responses were measured for 5 min immediately after (early phase) and 20–30 min after the formalin injection (late phase). EO_{MeOH} (0.1, 0.5 and 1.0 g/kg) and indomethacin (10 mg/kg) were administered orally 60 min before the formalin injection. The λ -carrageenan control group received the same volume of 0.5% CMC by oral administration.

λ -Carrageenan-Induced Mouse Paw Edema

The anti-inflammatory activity of EO_{MeOH} was determined by the animal model of λ -carrageenan-induced edema (Posadas *et al.*, 2004). The basal volume of right hind paw was determined before the administration of any drug using a plethysmometer. 50 μ l of 1% λ -carrageenan suspended in saline was injected into the plantar side of right hind paw, and the paw volume was measured at the 1st, 2nd, 3rd, 4th and 5th h after the injection. The degree of swelling was evaluated by the delta volume ($a - b$), where “ a ” is the volume of right hind paw after λ -carrageenan treatment and “ b ” is the volume before λ -carrageenan treatment. EO_{MeOH} (0.1, 0.5 and 1.0 g/kg) and the positive control of indomethacin

(10 mg/kg) were administered orally 60 min before the λ -carrageenan injection. Mice in the λ -carrageenan control group were pretreated with equal volumes of 0.5% CMC.

MDA Assay

MDA was evaluated according to the method of [Tatum *et al.* \(1990\)](#), which began by measuring thiobarbituric acid reacting substance (TBARS) to observe lipid peroxidation in paw edema tissue induced by λ -carrageenan injection. The absorbance of TBARS was recorded at 532 nm.

NO Assay

NO was measured according to the method used by [Moshage *et al.* \(1995\)](#). NO_3^- was converted into NO_2^- after enzymatic conversion caused by nitrate reductase. NO_2^- reacted with sulfanilic acid to produce diazonium ions and coupled with N-(1-naphthyl) ethylenediamine to form a chromophoric azo-derivative (purplish red) that could be detected by ELISA at 540 nm.

IL-1 β , IL-6 and TNF- α Assays

IL-1 β was measured by an enzyme-linked immunosorbent assay ([Ataoglu *et al.*, 2002](#)). Capture antibodies obtained from IL-1 β kit were added to a microtiter plate overnight before a second set of biotinylated antibodies were incubated with sample tissues and standard antigens. Streptavidin was added afterwards, and the color generated was recorded at 450 nm. IL-6 and TNF- α were detected using the same method as IL-1 β . Each sample was presented as pg/mg for the indication of cytokine concentration.

COX-2 Assay

COX-2 assay was carried out by measuring peroxidase activity of PGHS ([Petrovic and Murray, 2010](#)). Peroxidase activity of PGHS was determined by following the oxidation of N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD) at 37°C using arachidonate as the substrate. The color increased was recorded at 450 nm.

Antioxidant Enzyme Activity Measurements

SOD was measured according to the method used by [Vani *et al.* \(1990\)](#). Xanthine and xanthine oxidase (XOD) generated superoxide radicals reacted with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyl-tetrazolium chloride (I.N.T.) to form a red formazan dye, and the color generated was read at 540 nm.

GPx was measured according to the method used by [Ceballos-Picot *et al.* \(1992\)](#). GPx activity was measured by detecting the reducing state of glutamate reductase and NADPH.

The oxidation of NADPH to NADP⁺ was accompanied by a decrease in absorbance at 340 nm. One unit of activity was equal to the mM of NADPH oxidized/min per mg protein.

GRd was measured according to the method of Ursini *et al.* (1985). Glutathione reductase decreased glutathione (GSSG) in the presence of NADPH, which was oxidized to NADP⁺. The decrease in absorbance at 340 nm was measured. One unit of activity was equal to the mM of NADPH oxidized/min per mg protein.

Total Polyphenol Content (TPC)

Total phenolic compound was estimated using the Folin-Ciocalteu method (Ragazzi and Veronese, 1973). 20 μ l of EO_{MeOH} (1 mg/ml) was added to 200 μ l distilled water and 40 μ l of Folin-Ciocalteu phenol reagent. The blue complex produced was measured at 680 nm. Catechin was used as the standard for the calibration curve. TPC was presented as mg (+)-catechin equivalent/g of dry weight.

Total Flavonoid Content

Total flavonoid content of the crude extract was measured according to the method used by Chang *et al.*, (2002). 100 μ l of EO_{MeOH} (1 mg/ml) was added to 100 μ l of 2% AlCl₃ · 6H₂O, and the absorbance was read after 10 min of incubation at 430 nm. Rutin was used as the standard for the calibration curve. The total flavonoid content was presented as mg rutin equivalent/g of dry weight.

Total Flavanol Content

The total flavanol content was estimated using the p-dimethylamino cinnamaldehyde (DMACA) method (Arnous *et al.*, 2001). 40 μ l of EO_{MeOH} (1 mg/ml) was added to 200 μ l of DMACA solution, and the absorbance was read after 10 min of incubation at 640 nm. Catechin was used as the standard for the calibration curve. Results were presented as μ g catechin equivalent/mg dry weight.

Results

Chromatographic Analysis of EO_{MeOH}

HPLC chromatogram was established for EO_{MeOH} (Fig. 1). Two triterpenoid components were identified as pomolic acid (retention time, 14.7 min) and ursolic acid (17.5 min). The maximum absorbance was selected at 210 nm.

Acute Toxicity Study

Acute toxicity of EO_{MeOH} was evaluated at the dosages of 2.5, 5 and 10 g/kg administered orally to mice for 14 days. EO_{MeOH} did not cause any behavioral changes and no death was

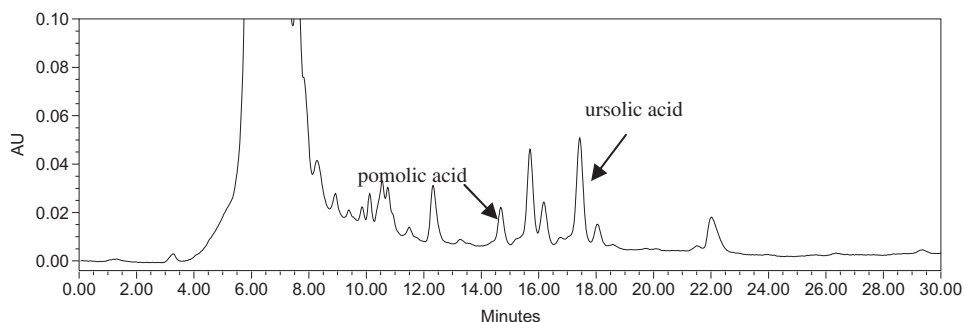


Figure 1. Chromatographic Analysis of EO_{MeOH}. HPLC assay was established for EO_{MeOH}. Two triterpenoid components were identified as pomolic acid (retention time, 14.7 min) and ursolic acid (retention time, 17.5 min).

observed. The oral LD₅₀ value of EO_{MeOH} was greater than 10 g/kg in mice. Therefore, EO_{MeOH} was considered to be a practically non-acute toxic substance.

Acetic Acid-Induced Writhing Response

Figure 2 shows acetic acid-induced writhing responses in mice. Both EO_{MeOH} (1 and 0.5 g/kg) and indomethacin (10 mg/kg) significantly reduced writhing responses induced

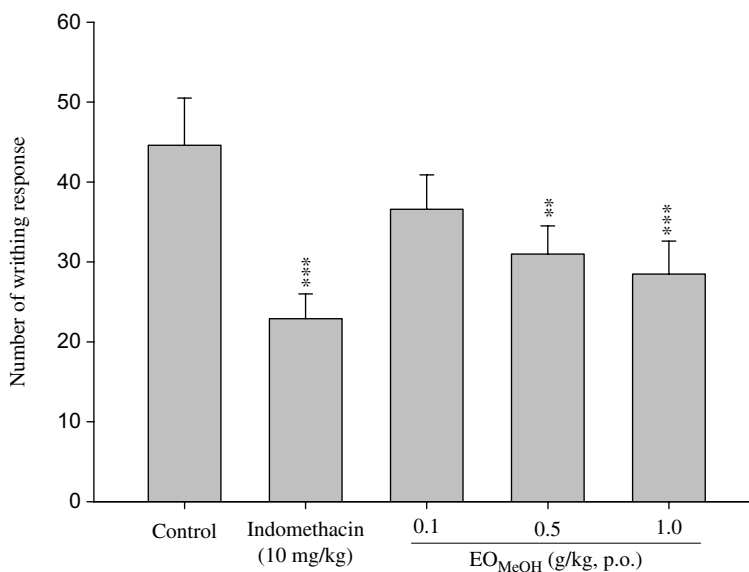
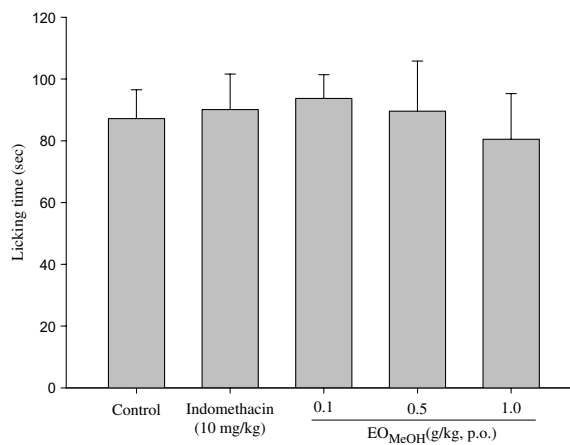


Figure 2. Analgesic effects of EO_{MeOH} and indomethacin on acetic acid-induced writhing response in mice. Each value represents mean \pm SEM ($n = 8$). The number of abdomen writhing response was counted over a period of 5–15 min after the acetic acid injection. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ when compared to the control group (one-way ANOVA followed by Scheffe's multiple range test).

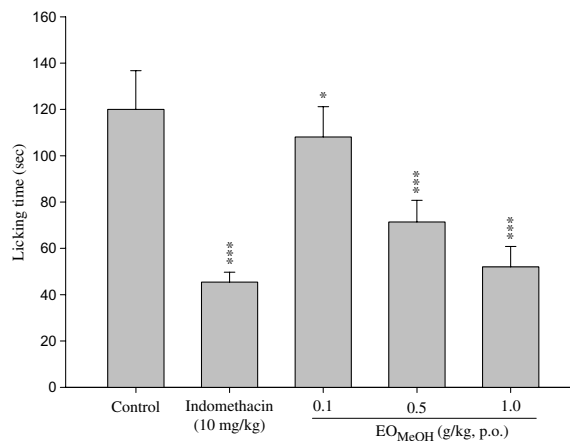
by acetic acid when compared to the control group, indicating analgesic activities of EO_{MeOH} and indomethacin.

Formalin Test

In the first phase, no significant inhibitions were generated by the treatments of EO_{MeOH} and indomethacin (10 mg/kg) as compared with the control group (Fig. 3A). In the second phase, however, 1 and 0.5 g/kg of EO_{MeOH} and 10 mg/kg of indomethacin significantly



(A)



(B)

Figure 3. Effects of EO_{MeOH} and indomethacin on the (A) early phase and (B) late phase of formalin test in mice. Each value represents mean \pm SEM ($n = 8$). The time spent on licking and biting the injected paw was recorded separately at 0–5 min (first phase) and 20–30 min (second phase) as indicators of pain. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ when compared to the control group (one-way ANOVA followed by Scheffe's multiple range test).

reduced the time (in seconds) spent on licking and biting responses induced by formalin (Fig. 3B).

Effect of EO_{MeOH} on λ -Carrageenan-Induced Mouse Paw Edema

Figure 4 shows λ -carrageenan-induced paw edema. Indomethacin (10 mg/kg) abated paw edema as compared to the λ -carrageenan control group, similarly, EO_{MeOH} also significantly decreased λ -carrageenan-induced paw edema in the 2nd, 3rd, 4th and 5th h after the stimulus dose-dependently, indicating anti-inflammatory activities of EO_{MeOH} .

Effect of EO_{MeOH} on MDA Level

Figure 5 shows that MDA level in λ -carrageenan-induced edema paw was increased. However, 0.1, 0.5 and 1.0 g/kg of EO_{MeOH} and 10 mg/kg of indomethacin decreased MDA levels.

Effect of EO_{MeOH} on NO Level

Figure 6 indicates that NO level in λ -carrageenan-induced edema paw was obviously increased. However, NO levels were decreased by treatments with 0.1, 0.5 and 1.0 g/kg of EO_{MeOH} , similarly, 10 mg/kg of indomethacin also showed significant inhibition in the increase of NO level.

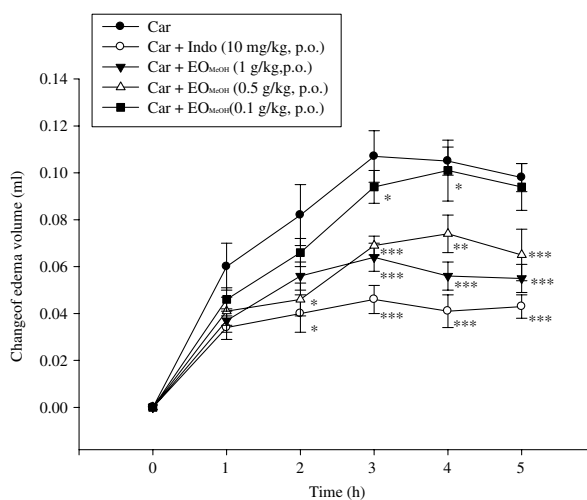


Figure 4. Effects of EO_{MeOH} and indomethacin (Indo) on λ -carrageenan-induced mouse hind paw edema. The volumes of edema paws were detected in the 1st, 2nd, 3rd, 4th and 5th h after λ -carrageenan injection. Each value represents mean \pm SEM ($n = 8$). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ when compared to the control group (one-way ANOVA followed by Scheffe's multiple range test).

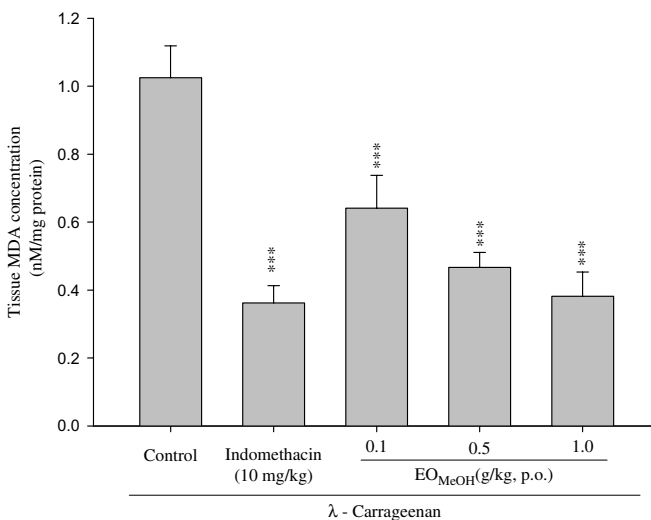


Figure 5. Effects of EO_{MeOH} and indomethacin on MDA concentrations in mouse edema paws. Each value represents mean±SEM ($n = 8$). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ when compared to the control group (one-way ANOVA followed by Scheffe's multiple range test).

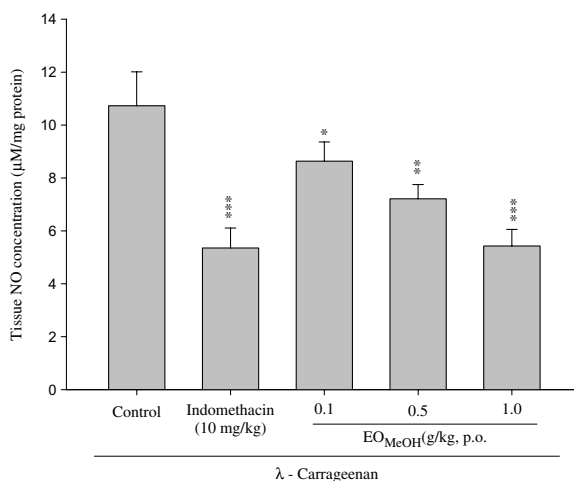


Figure 6. Effects of EO_{MeOH} and indomethacin on NO concentrations in mouse edema paws. Each value represents mean±SEM ($n = 8$). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ when compared to the control group (one-way ANOVA followed by Scheffe's multiple range test).

Effect of EO_{MeOH} on TNF- α , IL-1 and IL-6 Levels

TNF- α , IL-1 β and IL-6 levels in λ -carrageenan-induced edema paws were remarkably raised. However 1 g/kg of EO_{MeOH} and 10 mg/kg of indomethacin decreased TNF- α levels as shown in Fig. 7. EO_{MeOH} at the dosages of 0.5 and 1.0 g/kg and indomethacin at the

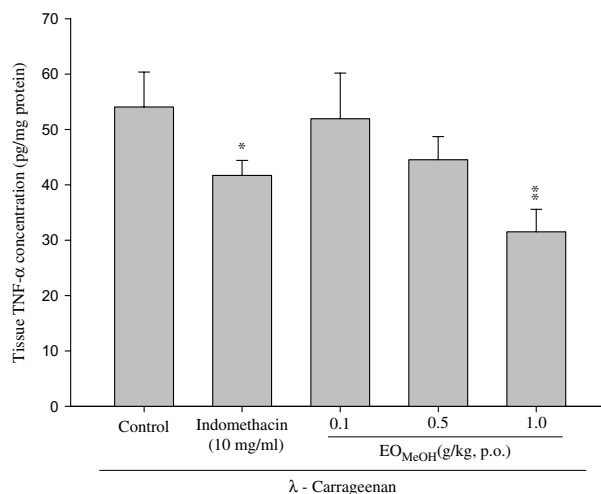


Figure 7. Effects of EO_{MeOH} and indomethacin on TNF- α concentrations in mouse edema paws. Each value represents mean \pm SEM ($n = 8$). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ when compared to the control group (one-way ANOVA followed by Scheffé's multiple range test).

dosage of 10 mg/kg decreased the level of IL-1 β (Fig. 8); while IL-6 levels were significantly decreased by treatments with 0.5 and 1.0 g/kg of EO_{MeOH} (Fig. 9).

Effect of EO_{MeOH} on COX-2 Level

COX-2 level in λ -carrageenan-induced edema paw was raised dramatically. However, COX-2 levels were decreased by treating with 0.5 and 1.0 g/kg of EO_{MeOH} as well as 10 mg/kg of indomethacin (Fig. 10).

Effect of EO_{MeOH} on the Activities of Antioxidant Enzymes

SOD and GPx activities were increased by treating with 0.5 and 1.0 g/kg of EO_{MeOH} and 10 mg/kg of indomethacin as compared with the λ -carrageenan control group. No such result was found in GRd activity assay as shown in Table 1.

Total Polyphenol, Flavonoid and Flavanol Contents

The total polyphenol, total flavonoid and total flavanol contents of EO_{MeOH} were $106.09 \pm 3.83 \mu\text{g}$, $43.60 \pm 2.77 \mu\text{g}$ and $17.93 \pm 0.77 \mu\text{g}$ of catechin equivalent/mg, respectively, as shown in Table 2.

Discussion

Herbal medicines with analgesic and anti-inflammatory effects have increased in the market in recent years for health maintenance and pathological treatments of chronic or recurring illness (Pereira *et al.*, 1999; Lin *et al.*, 2009).

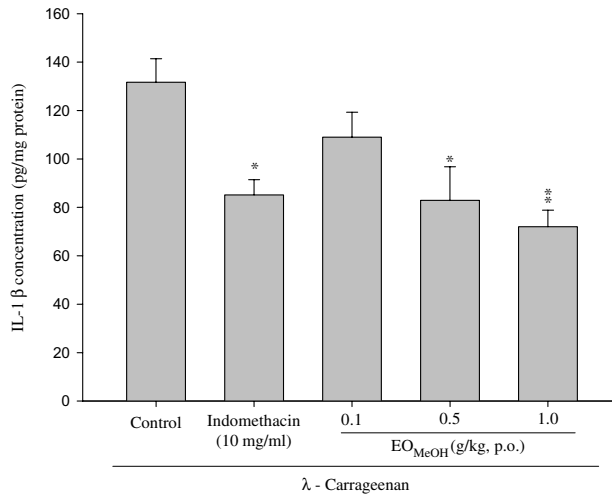


Figure 8. Effects of EO_{MeOH} and indomethacin on IL-1 β concentrations in mouse edema paws. Each value represents mean \pm SEM ($n = 8$). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ when compared to the control group (one-way ANOVA followed by Scheffe's multiple range test).

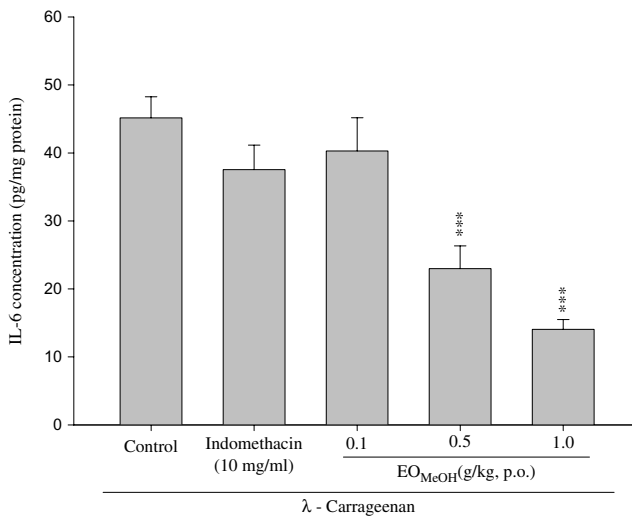


Figure 9. Effects of EO_{MeOH} and indomethacin on IL-6 concentrations in mouse edema paws. Each value represents mean \pm SEM ($n = 8$). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ when compared to the control group (one-way ANOVA followed by Scheffe's multiple range test).

The most important transmission pathways for inflammatory pain induced by acetic acid comprise of peripheral polymodal nociceptors that are sensitive to protons such as ASICs (Alexandre-Moreora *et al.*, 1999) and to algogen substances such as bradykinin, serotonin, cytokines and prostaglandin (Verma *et al.*, 2005).

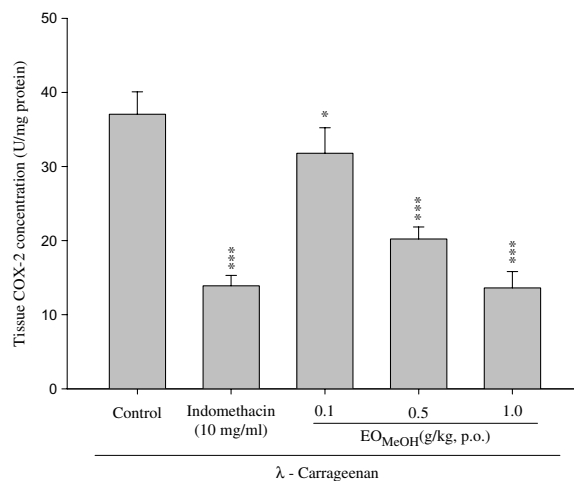


Figure 10. Effects of EO_{MeOH} and indomethacin on COX-2 concentrations in mouse edema paws. Each value represents mean ± SEM ($n = 8$). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ when compared to the control group (one-way ANOVA followed by Scheffe's multiple range test).

Table 1. Effects of EO_{MeOH} and Indomethacin on the Liver

Group	SOD (U/mg protein)	GPx (U/mg protein)	GRd (U/mg protein)
Car	25.87 ± 3.80	1.184 ± 0.266	0.024 ± 0.003
Car + Indomethacin (10 mg/kg, p.o.)	36.02 ± 5.77*	1.239 ± 0.213	0.018 ± 0.005
Car + EO _{MeOH} (0.1 g/kg, p.o.)	34.17 ± 3.69	1.350 ± 0.167*	0.017 ± 0.004
Car + EO _{MeOH} (0.5 g/kg, p.o.)	35.64 ± 3.23*	1.395 ± 0.123*	0.017 ± 0.003
Car + EO _{MeOH} (1.0 g/kg, p.o.)	38.57 ± 3.06***	1.482 ± 0.128***	0.016 ± 0.003

Notes: SOD, GPx, and GRd activities were increased as compared with the λ-carrageenan control group. Each value represents mean ± SEM ($n = 8$). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ when compared to the control group (one-way ANOVA followed by Scheffe's multiple range test).

Table 2. Total Polyphenol, Flavonoid and Flavanol Contents in EO_{MeOH}

Species	Total Polyphenol (μg catechin/mg)	Total Flavonoid (μg rutin/mg)	Total Flavanol (μg catechin/mg)
EO _{MeOH}	106.09 ± 3.83	43.60 ± 2.77	17.93 ± 0.77

Note: Each value represents mean ± SEM ($n = 8$).

In the acetic acid-induced writhing response test, 1 and 0.5 g/kg of EO_{MeOH} and 10 mg/kg of indomethacin showed significant decreases in acetic acid-induced abdominal writhing response when compared with the control group.

The formalin-induced nociception model was described by Dubuisson and Dennis (1977), and the test has been used to study analgesic drugs in rodents, mainly in mice. This

test provokes two distinct phases of painful sensitization. The first phase occurs in the first 5 min after the formalin injection (neurogenic nociceptive response) and is characterized by the direct stimulation of nociceptors present on afferent C, and in part, $A\delta$ fibers (glutamate, bradykinin, and substance P release). The second phase occurs between 15 to 30 min after the formalin injection (inflammatory nociceptive response) and is related to inflammatory mediator releases such as nitric oxide, adenosine, bradykinin, histamine, prostaglandin and serotonin (Tjølsen *et al.*, 1992). Centrally acting drugs such as narcotics and morphine, inhibit both phases, while peripherally acting drugs, such as NSAIDs and corticosteroids, only inhibit the second phase. EO_{MeOH} was able to diminish nociceptive response in the second phase of the formalin test, which signified that the anti-nociceptive effect of EO_{MeOH} was due to its peripheral action.

λ -carrageenan-induced paw edema, an *in vivo* model for inflammation, is frequently used to evaluate the anti-edematous effect of natural products. Previous studies have indicated that edematous effect reaches the maximum 3 h after the λ -carrageenan stimulus. In our study, 1 and 0.5 g/kg of EO_{MeOH} and 10 mg/kg of indomethacin showed significant anti-inflammatory effects on mouse paw edema at the 2nd, 3rd, 4th and 5th h after λ -carrageenan had been injected.

It is well known that COX-2 expression is at its maximal in the late phase of λ -carrageenan-induced paw edema (Seibert *et al.*, 1994). EO_{MeOH} showed significant inhibition in the increase of COX-2 expression in λ -carrageenan-induced paw edema, which may be related to prostaglandin synthesis inhibition, similar to the description of anti-inflammatory mechanism of indomethacin (Di *et al.*, 1971).

L-arginine-NO pathway plays an important role in λ -carrageenan-induced inflammatory response (Salvemini *et al.*, 1996); free radicals, prostaglandins and NO would be released 1–6 h after the injection (Dudhgaonkar *et al.*, 2006). However, the level of NO was decreased significantly by treatment with EO_{MeOH} as well as indomethacin.

Inflammation induced by λ -carrageenan involves neutrophil migration, plasma exudation and production of mediators, such as IL-1 β , IL-6 and TNF- α . These mediators are able to increase leukocytes, such as neutrophils as shown in several experimental models (Salvemini *et al.*, 1996).

A putative mechanism associated with the anti-inflammatory effect of EO_{MeOH} may be the inhibition of inflammatory mediators of which some involvements have been examined in the present study. The levels of TNF- α , IL-1 β and IL-6 in λ -carrageenan-induced paw edema were decreased by treatment with EO_{MeOH}.

Inflammation would result in the accumulation of MDA. MDA is a reactive aldehyde, which causes toxic stress in cells and forms covalent protein adducts referred to as advanced lipoxidation end products (ALE). Enhancing the levels of glutathione and SOD then reduce the MDA production (Hsieh *et al.*, 2011). Therefore, endogenous glutathione plays an important role against λ -carrageenan-induced local inflammation (Cuzzocrea *et al.*, 1999; Lai *et al.*, 2010).

In this study, SOD and GPx activities increased in the liver with the treatment of EO_{MeOH}. Conversely, MDA was decreased significantly by the treatment with 0.1, 0.5 and

1.0 g/kg of EO_{MeOH}. Therefore, we assumed the suppression of MDA production may be due to the increase in SOD and GPx activities.

Elaeagnus oldhamii Maxim. showed antinociceptive and anti-inflammatory activities in this study. The anti-inflammatory mechanisms of EO_{MeOH} may be related to the decreases in MDA and NO levels in the edema paw via increasing the activities of SOD and GPx in the liver and decreasing the levels of IL-1 β , IL-6, TNF- α and COX-2 in the edema paw.

Flavonoids have also been reported to be good antioxidative and anti-inflammatory agents (Nijveldt *et al.*, 2001; Meotti *et al.*, 2006; Yadav *et al.*, 2010). Various types of flavonoids have been isolated from the genus of *Elaeagnus* and have been reported to possess different biological activities. For example, *E. angustifolia* is used in Iranian folk medicine for its anti-inflammatory and analgesic effects (Ahmad and Mubasher, 2005; Ahmadiani *et al.*, 2000; Fu and Wang, 2007).

Abundant flavonoid contents were detected in total flavonoid test and flavonoid composition identification of EO_{MeOH} *in vitro*. Two triterpenoid compounds, ursolic acid and pomolic acid, were isolated from EO_{MeOH} and were exhibited in HPLC chromatographic analysis. Furthermore, both ursolic acid and pomolic acid have been previously demonstrated to possess anti-inflammatory activities (Ringbom *et al.*, 1998; Schinella *et al.*, 2008).

This study demonstrated that *Elaeagnus oldhamii* Maxim. exhibited antinociceptive and anti-inflammatory activities. The anti-inflammatory mechanisms of EO_{MeOH} against λ -carrageenan induced paw edema involved two possible pathways. The first pathway was likely associated to decreases in the levels of MDA and NO in the edema paw via increasing the activities of SOD, GPx and GRd in the liver. The other pathway alleviated the levels of inflammatory factors, such as IL-1 β , IL-6, TNF- α and COX-2 in the edema paw induced by λ -carrageenan. In conclusion, this study not only evidenced antinociceptive and anti-inflammatory effects of EO_{MeOH} but also provided possible supporting mechanisms for its inflammatory pain mitigating activities.

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