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Anti-inflammatory Effect of Hydro alcoholic Extract of the *Olea europaea l.* Fruit in Carrageenan-Induced Paw Edema in Rats.

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ABSTRACT

Gastrointestinal complications caused by using non steroidal anti-inflammatory drugs (NSAIDs) stimulated researchers to pay more attention to certain herbs with anti-inflammatory effect. Olive has always been considered as an antihypertensive, anti atherosclerotic c, laxative, refrigerant and antioxidant drug in traditional medicine. This study aimed to investigate the anti-inflammatory activity of the *Olea europaea l. fruit* hydro alcoholic extract. The hydro alcoholic extract of Olive fruit was administered to male Wistar rats. Carrageenan-induced paw edema was used to evaluate the anti-inflammatory activity. The hydro alcoholic extract of *Olea europaea l. Fruits* (200, 300, 400, 500 mg/kg) or vehicle were administered intra peritoneally 30 minutes before the sub plantar injection of carrageenan. Intra peritoneal Aspirin (300 mg/kg) was used as standard drug. Doses of 200 and 300 mg/kg of the extract had a lower effect on reduction of paw's edema in comparison to aspirin in all measure hours; whereas, doses of 400 and 500 mg/kg of the extract, in comparison to aspirin, did not show any significant difference in reducing edema ($p < 0.05$). Since there was no significant difference between doses of 400 and 500 mg/kg of the extract, the dose of 400 mg/kg was chosen as the best dose. These results indicated that the hydro alcoholic extract of Olive fruit has a potent anti-inflammatory action and confirmed that the extract contains an effective anti-inflammatory substance(s)

Keywords: *Olea europaea l.*, Aspirin, Carrageenan, Inflammation, Plethysmo meter, Rat

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INTRODUCTION

Inflammation is a common complication of many diseases by which immunity system is weakened. This process may result in infectious problems and also can postpone the disease process. Today, common chemical drugs, including different types of gluco corticoids, have helped us to take effective strides to alleviate inflammation. However, their serious side effects are unavoidable. Therefore, new studies suggest using complementary medicine, especially herbal therapy, as an inexpensive way with minimum side effects [1].

Medicinal plants are believed to be important sources of new chemical substances with potential therapeutic efficacy. Considering that the most important anti-inflammatory drugs (e.g. salicylic acid) were originally derived from plant sources, the study of traditionally used plant species should be seen as a useful research strategy in the search of new drugs. [3]. Since ancient days, olive has been used as a medicinal plant to treat many diseases. Olive (*Olea europaea* L.) is from family of Oleaceae [5]. Olive has been widely studied, due to extensive use of it as a food in the world.

Olive leaves are important because they have secondary metabolites such as oleacein and oleuropein. They are known as an old herb for its positive effects on metabolism caused by phenol compounds in olive leaves [6].

Olive fruits contain sterol and triterpene dialcohol compounds and long-chain aliphatic alcohols [7]. The most important effective materials of olive fruits are fixed oil, oleuropein glycosides, hydroxy tyrosol and oleosin [2, 8].

Grey et al. (2005) suggest that the component 'oleocanthal' in virgin olive oil has a similar anti-inflammatory effect as ibuprofen. Despite its structural difference from ibuprofen, it uses a similar mechanism, i.e. preventing cyclo-oxygenase enzyme, to alleviate inflammation [9].

Bogani et al. showed the effect of post-meal intake of virgin olive oil. In their study, blood samples were analyzed 1, 2, and 6 hours after the consumption of olive oil. Results showed a decreased level of inflammatory factors TXB₂ and LTB₄ [10].

In this study, the effect of hydro alcoholic extract of olive fruit on carrageenan -induced inflammation was studied. If the effect has been demonstrated it would be a good beginning for conducting further studies on animal samples and finally on certain human samples, observing certain standards and rules, to be used as an anti-inflammatory drug.

METHODOLOGY

Plant Material and Preparation of the Extract

Plants were collected from dezful. *Olea europaea* L. was identified by Ahvaz Jundishapur University of Medical Sciences (AJUMS), Ahvaz, Iran, and voucher samples were preserved for reference in the herbarium of School of Pharmacy, Ahvaz, Iran (A141600100FP). To prepare hydro alcoholic extract of fruits, powdered fruits (300g) were macerated by 2000 mL of 70% ethanol (v/v) for 72 hours. The extract was then shaken and filtered, and the solvent was removed in a vacuum evaporator to obtain semisolid extract and then was placed in an oven in 60°C for 72 hours [1].

Drugs

Aspirin and carrageenan were purchased from Sobhan Pharmaceutical Co (Tehran, Iran) and Sigma Chemical Co (St. Louis, MO, USA), respectively The hydro alcoholic extract of olive fruit and drugs were diluted in saline.

Animals

Male Wistar rats weighing 150 to 180 g were obtained from a random bred colony in the animal house of AJUMS. Animals were housed in standard cage with 12-hour light/ dark cycle and air temperature was maintained at 22°C ± 2°C. Experiments reported in this study were performed in accordance with local guidelines for the care of laboratory animals of AJUMS.

Experimental Groups

Acute edema was induced in the right hind paw of rats by injecting 100 µL of 1% carrageenan solution after 30 minutes of vehicle or olive fruit extract (200, 300,400 and 500 mg/kg, i.p.). The doses of Olive fruit extract were selected based on pilot experiments in our laboratory. The reference drug, Aspirin (300 mg/kg, i.p.), were also used for comparison [1]. Carrageenan was injected under the plantar region of right hind paw, and the volume was measured using a plethysmo meter (UGO Basile, Italy) at hours one, two, three, four, and five of carrageenan challenge. Inflammation was expressed as change in paw volume [23].

Statistical Analysis

All experimental results are given as means ± for six animals per group. Curves were constructed by plotting the change in paw edema as a function of time

The following formula was used to calculate rat paw edema in percentage:

$$\% \text{ Relative Paw Edema} = \frac{V_2 - V_1}{V_1} \times 100$$

V1: Rat paw size before the injection of carrageenan

V2: Rat paw size after the injection of carrageenan

RESULTS

There was a significant difference between anti-inflammatory effect of aspirin and that of normal saline in all examined hours (p<0.05) (figure 1). The anti-inflammatory effect of 200 and 300 mg/kg doses of hydroalcoholic extract of olive fruit was not significantly different from that of normal saline in none of the examined hours (figure 2).

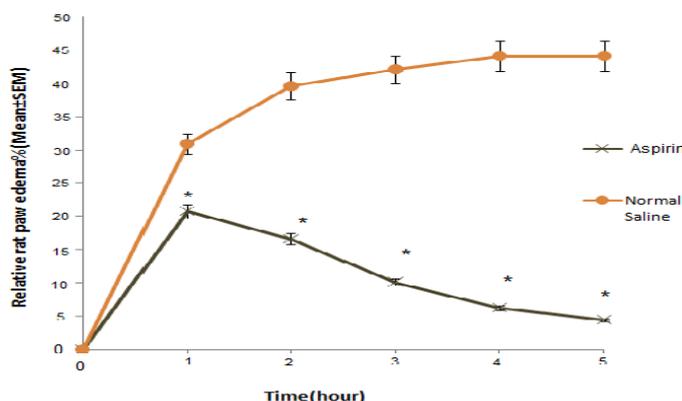


Figure 1: comparing the anti-inflammatory effect of Normal saline (5 ml/kg) receiver group with the aspirin receiver group (300mg/kg) on the carageenan induced edema in the rats hind paw.

The difference of the anti-inflammatory effect Normal saline with the Aspirin is significant (p<0.05)

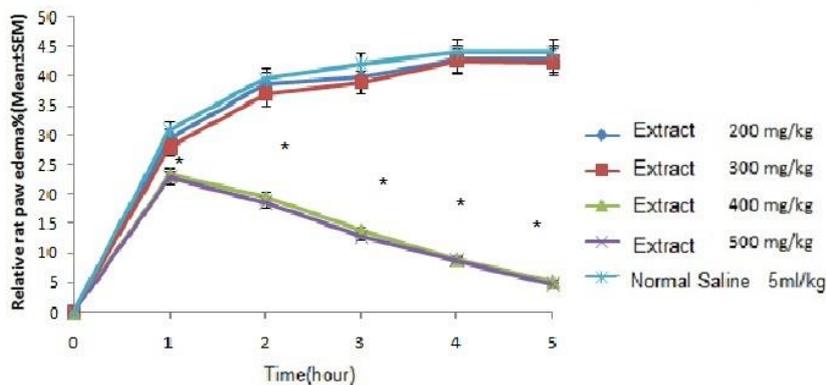


Figure 2: comparing the anti-inflammatory effect of doses 200, 300, 400 & 500mg/kg of hydroalcoholic extract of Olive fruit with normal saline 5 mg/kg on the carrageenan induced edema in rats hind paw

*: The difference of the anti-inflammatory effect of doses 400, 500 mg/kg with the Aspirin is significant ($p < 0.05$):

In all examined hours, 400 and 500 mg/kg doses of hydro alcoholic extract of olive fruit had significantly greater anti-inflammatory effect than physiological serum after injecting carrageenan ($P < 0.05$) (figure 2). In addition, the anti-inflammatory effect of 200 and 300 mg/kg doses of hydro alcoholic extract of olive was significantly different from that of aspirin ($p < 0.05$) (figure 3); while, there was no significant difference between the anti-inflammatory effect of 400 , 500 mg/kg doses of the hydro alcoholic extract of olive fruit and aspirin (figure 3).

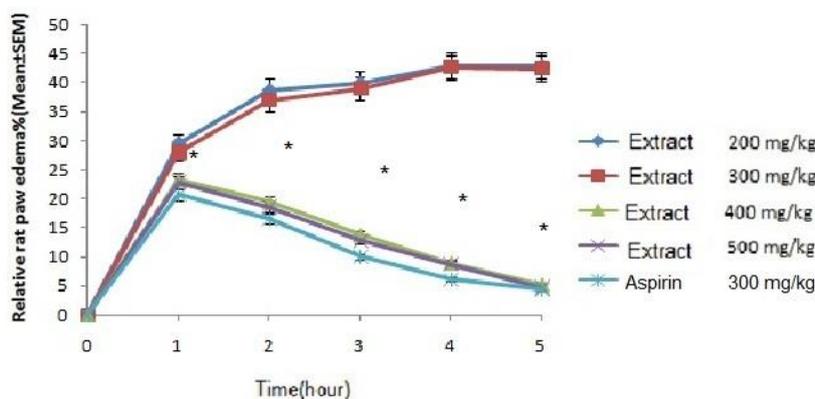


Figure 3: comparing the anti-inflammatory effect of doses 200, 300, 400 & 500 mg/kg of Olive fruit hydroalcoholic extract with aspirin 300mg/kg on the carageenan induced edema resulted in the rats hind paw.

* :The difference of the anti-inflammatory effect of doses 200 ,300 mg/kg with the Aspirin is significant ($p < 0.05$):

On the other hand, comparing the effect of 200, 300, 400 and 500 mg/kg doses of hydroalcoholic extract of olive fruit indicated that the doses 400 and 500 mg/kg significantly reduced inflammation in all examined hours ($p < 0.05$). Also , there was no significant difference between the anti-inflammatory effect of 400 and 500 mg/kg doses of the hydroalcoholic extract of olive fruit .

DISCUSSION

In this study, 1% carrageenan-induced edema model was used to analyze the anti-inflammatory effect of the hydro alcoholic extract of olive fruit. This model is broadly used in analyzing inflammatory processes and also in screening anti-inflammatory factors. The inflammatory responses induced by carrageenan 1% have two phases. The first phase occurs within 2-3 hours after injection, whereas the second one occurs three hours after injecting [16, 17].

In this study, the hydro alcoholic extract of olive fruit with doses of 200, 300, 400, and 500 mg/kg was injected intra peritoneally into the rats, half an hour before injecting carrageenan. Then the anti-inflammatory effect of various doses was compared with negative control group (normal saline, 5 ml/kg) and positive control group (aspirin, 300 mg/kg). According to the results, the anti-inflammatory of hydro alcoholic extract of olive fruit increased depending on the dose.

The anti-inflammatory effect of hydro alcoholic extract of olive fruit is due to its certain compounds such as oleuropein and its derivative, hydroxytyrosol, and other derivatives produced through its hydrolysis. Oleuropein in the blood samples decreases inflammatory mediators *TXB2* and *LTB4* [18]. Oleuropein is an invitro strong antioxidant in comparison with a hydrophilic tocopherol analogue. Oleuropein collects superoxide anions and hydroxyl radicals [19].

Despite its structural difference from ibuprofen, oleocanthal compound uses a similar mechanism, i.e. preventing cyclo-oxygenase enzyme, to alleviate inflammation [9].

Since aspirin is a non-selective inhibitor for synthesis of prostaglandins that are the main mediators of carrageenan-induced inflammation in the second phase of it, then lack of significant difference between the two aspirin and receivers of 400 and 500 mg/kg dosages of hydro alcoholic extract of the olive fruit can be attributed to the possible effect of hydro alcoholic extract of the olive fruit on inhibition of prostaglandins synthesis. Yet, since the hydro alcoholic extract of the olive fruit is used here, the possible effect of other parts of the extract on inflammatory mediators cannot be denied.

Olive oil is a source of at least 30 phenolic compounds. The major phenolic compounds in olive oil are oleuropein, hydroxytyrosol and tyrosol. Recently there has been a surge in the number of publications that has investigated their biological properties. The phenolic compounds present in olive oil are strong antioxidants and radical scavengers. Olive "waste water" also possesses compounds which are strong antioxidant and radical scavengers. [20,22]. Because of its polyphenol compounds, olive fruits show antioxidant and anti-inflammatory activities [21,22]. Therefore, both antioxidant and anti-inflammatory properties of the hydro alcoholic extract of the olive fruit act more effectively and will be helpful especially in decreasing the chronic inflammation. This is because in chronic inflammatory cases, the damage caused by active species of oxidant will exacerbate the damages.

In general, in this study, the hydro alcoholic extract of the olive fruit has anti-inflammatory and dose-dependent effects. Its best anti-inflammatory effect, in comparison to aspirin, is found by using dose 400 mg/kg of the extract. All animals receiving the extract were survived during the trial without any side effect; however, analyzing the side effects of the plant needs further studies.

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