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The Hydro-Alcoholic Extract of Pomegranate Seed Has Anti-Inflammatory Effects on Formalin-Induced Inflammation in Rat Hind Paw

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Abstract: Several *in vivo* studies have documented that supplementation with pomegranate fruit extract inhibits inflammatory symptoms. In the present study, the anti-inflammatory properties of total hydro-alcoholic extract isolated from pomegranate seed were investigated in formalin-induced rat paw edema. The studied male Wistar albino rats (n = 30) were divided into 5 groups. Inflammation was created by injection of formalin (2.5%) intraplantarily. Then different doses of extract (100, 200, 400 mg kg^{-1}) were injected in 3 groups, indomethacin (5 mg kg^{-1}) was injected in 4th group and control group received 5 mL of normal saline intraperitoneally. About 1-3 h after injection of the extract, indomethacin and saline, the volume of paw was measured in all groups by plethysmometer. The results showed hydro-alcoholic extract of pomegranate seed has dose-dependent anti-inflammatory effect and the highest anti-inflammatory effect was observed in the dose of 400 mg kg^{-1}.

Key words: Pomegranate seed, anti-inflammatory, rat paw edema, formalin, indomethacin, Iran

INTRODUCTION

Punica granatum, pomegranate is a common fruit in Iran and Mediterranean that it is widely used for therapeutic formula, cosmetics and seasoning (Alper and Acar, 2004). It is usually used as antiparasitic and antipyretic agent (Mudzhiri, 1954). Other pharmacological properties of pomegranate include anti-oxidation, anti-tumor, anti-hepatotoxicity (hepatoprotection), anti-lipoperoxidation and anti-bacterial activities (Aviram *et al*., 2002; Khan *et al*., 2007; Lansky and Newman, 2007; Menezes *et al*., 2006). More important property of pomegranate is anti-inflammatory effect which has been investigated recently (Lee *et al*., 2009).

Inflammation is the complex biological response of tissues to harmful stimuli such as pathogens, damaged cells or irritants. Inflammation is a protective attempt by the organism to remove the noxious stimuli as well as initiate the healing process for the tissue. Inflammation is not a synonym for infection. Even in cases where inflammation is caused by infection, this two are not synonymous: infection is caused by an exogenous pathogen while inflammation is one of the responses of the organism to the pathogen (Cotran *et al*., 1998). In the absence of inflammation, wounds and infections would never heal and progressive destruction of the tissue would compromise the survival of the organism. However, chronic inflammation can also lead to a host of diseases such as hay fever, atherosclerosis and rheumatoid arthritis. So inflammation is normally closely regulated by the body (Cotran *et al*., 1998). Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues.

A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system and various cells within the injured tissue. Prolonged inflammation known as chronic inflammation, leads to a progressive shift in the type of cells which are present at the site of inflammation and is characterized by simultaneous destruction and heeling of the tissue from the inflammatory process (Cotran *et al*., 1998).

The process of acute inflammation is initiated by cells already present in all tissues, mainly resident macrophages, dendritic cells, histiocytes, Kuppfer's cells and mastocytes. At the onset of an infection, burn or other injuries, these cells undergo activation and release inflammatory mediators responsible for the clinical signs of inflammation. In addition to cell-derived mediators, several acellular biochemical cascade systems consisting

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of preformed plasma proteins act in parallel to initiate and propagate the inflammatory response. These include lysosome granules, histamine IFN-γ, IL-8, leukotriene B4, nitric oxide, prostaglandins, TNF-α and IL-1 (Cotran *et al*., 1998). The acute inflammatory response requires constant stimulation to be sustained. Inflammatory mediators have short half lives and are quickly degraded in the tissue. Hence, inflammation ceases once the stimulus has been removed (Cotran *et al*., 1998). Whereas, pomegranate fruit are popularly consumed throughout the world and its fruit and flower extracts are widely used for treatment of inflammatory disease in the traditional medicine of Asia and Europe in this study anti-inflammatory effect of hydro-alcoholic extract of pomegranate seed was investigated on rat paw edema (Shukla *et al*., 2008a).

MATERIALS AND METHODS

Preparation of the extract: Powdered dry plant materials were extracted through maceration in ethanol for 72 h. Then the extract was concentrated using rotary vacuum to get the solid mass.

Animals: For this study, about 30 male Wistar albino rats (average weight: 200 g) were used. Animals were kept in a clean holding room on a 12 h light and dark cycle with relative humidity 45-55% and tempretur 23±2°C. During the experimentation, all rats were fed with concentrated food pellets (Pars Khurakdam Shushtar, Iran) and water *ad libitum*. The animals were randomly divided into 5 groups (n = 6) (Castardo *et al*., 2008; Maleki *et al*., 2001). At first, the volume of intact rats paw was measured in all groups by plethysmometer (UGO Basile 7140).

Then formalin (2.5%) was administered intraplantary in rats paw. After 10 min, volume of rats paw was measured. Then the pomegranate seeds extract (100, 200, 400 mg kg^{-1}) was administered (IP) to the 1st-3rd groups. The 4th group received indomethacin (5 mg kg^{-1}, IP) and control group received normal saline (5 mg kg^{-1}, IP). About 1-3 h after injection, the volume of rat paw was measured in all groups (Castardo *et al*., 2008; Maleki *et al*., 2001). In this study because in time zero, the paw volumes of the animals are not equal we used the following equation for calculation of the percent of resulted edema as follows:

$$\text{Relative paw edema (\%)} = \frac{V_2 - V_1}{V_1} \times 100$$

Where:

V_1 = The animal paw volume before injection of irritant
V_2 = The paw volume from the 1st to 3rd h after the injection so the irritant

Statistical analysis: Results of the experiment were statistically analyzed using one-way ANOVA and Tukey tests.

RESULTS

The results of this study showed that after 1 h of injection, there was no significant differences between 5 groups ($p>0.05$). But after 2 and 3 h, minimum of inflammatory was seen in 3rd group (400 mg kg^{-1}) that this showed significant difference in comparison to the 1st group (100 mg kg^{-1}), 2nd group (200 mg kg^{-1}) and 5th group (saline) ($p<0.05$). Finally, it was shown that there was no significant differences between 3rd group (400 mg kg^{-1}) and 4th group (indomethacin 5 mg kg^{-1}) ($p>0.05$).

DISCUSSION

Inflammation, the first physiological defense system in the human body can protect against injuries caused by physical wounds, poisons, etc. This defense system also called short-term inflammation can destroy infectious microorganisms, eliminate irritants and maintain normal physiological a regular physiology, i.e., asthma and rheumatoid arthritis (Lee *et al*., 2009).

The currency and wide of treatment inflammatory is being used of NSAIDs drug. But whereas the majority of the NSAIDs have gastrointestinal irritation therefore, use of plant that has anti-inflammatory effect without side effect can be good replacement for this drug.

The health promoting effects of plant constituents and extracts are being increasingly studied and their consumption is on the rise in the western world. Although, several studies have reported the effectiveness of different herbal preparations or fruit extracts for the treatment and prevention of disease (Shukla *et al*., 2008a).

Pomegranate has been used for centuries to confer health benefits in a medicine; dietary supplements containing pomegranate extract are becoming popular in the Western world for the treatment and prevention of arthritis and other inflammatory disease. More recently, standardized extracts of pomegranate fruit have been shown to possess anti-inflammatory and cartilage sparing effects in vitro (Ahmed *et al*., 2005).

According to current reports, several parts of pomegranate tree, Punica granatum consist of juice, peel, leaf, flower, bark, roots, especially its fruit possesses anti-inflammatory effect for the reason that it have phenol compounds. This compound contains significantly high level of hydrolysabel tannins such as punicalin, punicalagin, pedunculagin, punigluconin, strictinin A and

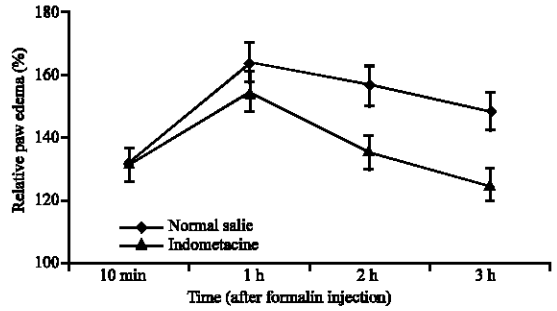

Fig. 1: Comparison of reduction of formalin- induce paw edema in rat between indomethacin (5 mg kg^{-1}, IP) and normal saline (5 mg kg^{-1}, IP)

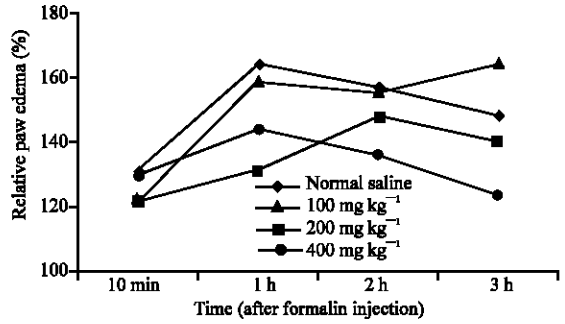

Fig. 2: Comparison of reduction of formalin-induce paw edema in rat between different doses of hydro-alcoholic extract pomegranate seed (100, 200, 400 mg kg^{-1}, IP) and saline (5 mg kg^{-1}, IP)

granatin B. Among them, granatin B more strongly inhibited COX-2. It (COX-2) plays an important physiological role in inflammation (Lee *et al*., 2009; Dudonne *et al*., 2009; Lansky and Newman, 2007; Shukla *et al*., 2008b). We used *in vivo* models to confirm the anti-inflammatory activity of hydro-alcoholic extract of pomegranate seed. Results of the present study also highlight that anti-inflammatory effects of hydro-alcoholic extract of pomegranate seed in rat paw edema is dose-dependent and the best anti-inflammatory effect was observed in the dose of 400 mg kg^{-1}. In Fig. 1, we have compared the results of indomethacin and control groups. In Fig. 2, the results of all three dose groups have been compared with normal saline group. Except 3rd h in dose of 100 mg kg^{-1} of the extract, the both other doses of the extract (200 and 400 mg kg^{-1}) had significant differences, $p< 0.05$ and 0.01, respectively. In Fig. 3, the results of all three dose groups have been compared with indomethacin group. The difference of the group received 400 mg kg^{-1} with the indomethacin group was insignificant ($p> 0.05$). But other two doses (100 and 200 mg kg^{-1}) had significant difference in comparison to the indomethacin group ($p< 0.05$).

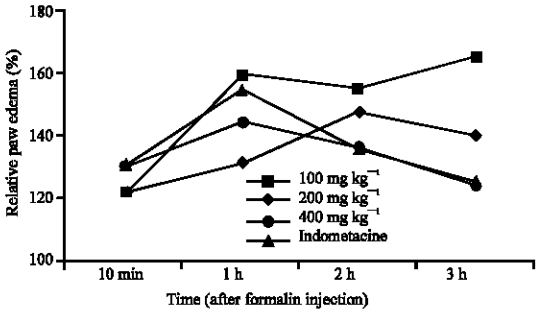

Fig. 3: Comparison of reduction of formalin- induce paw edema in rat between different doses of hydro-alcoholic extract pomegranate seed (100, 200, 400 mg kg^{-1}, IP) and indomethacin (5 mg kg^{-1}, IP)

CONCLUSION

These results of the present study showed that the best anti-inflammatory effect of hydro-alcoholic extract of pomegranate seed in rat paw edema was observed in the dose of 400 mg kg^{-1}. Taken together, we suggest that hydro-alcoholic extract of pomegranate seed could be used for treatment of inflammation. Although, we suggest more studies for investigating the molecular bases of these effects.

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