

Comparative Anti-Inflammatory Activity of Aril Extracts of *Punica granatum* Fruits [†]

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Abstract: A substance or treatment with an anti-inflammatory property is one that reduces inflammation or swelling. The main objective of this study is to evaluate the anti-inflammatory activity of pomegranate aril extract on rat paw. The anti-inflammatory activity of pomegranate was tested on rats by employing the induced carrageenan rat paw edema method. Various concentrations of the arils and the aril mixture (1:1) were prepared by dissolving in hydroalcohol and alcohol to obtain final concentrations of 100 mg/kg, 200 mg/kg, and 400 mg/kg to be tested against the organisms. The effectivity of granatin B in aqueous and alcoholic extracts of the arils of *Punica granatum* was calculated by measuring the increase in paw volume and the percent of inhibition by comparing with the control and the standard drug.

Keywords: anti-inflammatory; pomegranate arils; carrageenan rat paw; edema method; granatin B; percent inhibition

1. Introduction

Pomegranate (*Punica granatum* L. (Punicaceae); the common name is derived from the Latin words *ponus* and *granatus*) is a delicious seeded fruit consumed worldwide. The fruit is native to Afghanistan, Iran, China, and the Indian sub-continent. From the west of Persia (modern-day Iran), pomegranate cultivation has spread throughout the Mediterranean region to the Turkish European borders and American southwest, California, and Mexico [1]. Pomegranate peels are characterized by an interior network of membranes comprising almost 26–30% of the total fruit weight and are characterized by good amounts of phenolic compounds, including flavonoids (anthocyanins, catechins, and other complex flavonoids) and hydrolysable tannins (punicalin, pedunculagin, punicalagin, gallic and ellagic acid, granatin B, strictinin A). These compounds are concentrated in pomegranate peel and juice, which account for 92% of the antioxidant activity associated with the fruit [1]. Anti-inflammatory drugs make up about half of all analgesics, remedying pain by reducing inflammation, as opposed to opioids, which affect the CNS to block pain signaling to the brain. Pomegranate has dietary, as well as medicinal use. It has been widely used in Traditional Indian Medicines (TIM) worldwide for the treatment of different types of diseases (Olapour et al., 2010) [1]. Furthermore, it has antioxidant activities, which include radical scavenging ability, ferrous ion chelating, and ferric ion-reducing antioxidant power, preventing oxidation and reducing the effect of oxidizing agents [2]. The chemical composition and pharmacological properties of *Punica granatum* L. (Punicaceae) have been studied in this article. In past years, various studies have been done on the antioxidant, anti-carcinogenic, anti-inflammatory, anti-atherosclerotic, and anthelmintic properties of pomegranate constituents, focusing on the treatment and prevention of cancer, cardiovascular

diseases CVS disease, diabetes, dental problems, erectile dysfunction, bacterial infections, which lead to antibiotic resistance, and skin damage due to various forms of radiation such as UV. Other uses include neonatal brain ischemia and male infertility. Pomegranate is well reported for its medicinal properties. *Punica granatum* fruits have been used to treat inflammatory disorders and wounds. In this study, rats were used. The rats were randomly divided into five groups, including saline water as a control and indomethacin as the standard drug. Pomegranate is a widely-used plant having medicinal activity. In this review, we have mainly focused on the already published data to study the effect of the alcoholic and hydroalcoholic extracts of the arils of pomegranate (PME) and done a comparison with other literature studies on *Punica granatum* (Lythraceae).

2. Chemical Constituents

When the fatty acid composition of the seeds was examined, it was found that it contained:

1. punicalic acid, 4-methyl lauric acid,
2. 1,3 dimethyl stearic acid, sterols (stigmasterol, sitosterol),
3. phospholipids (phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol)
4. mono-, di- and tri-glycerides and free fatty acids (Santagati et al., 1984; Sergeeva, 1973).

The anti-inflammatory components of pomegranate seeds, i.e., punicalagin, punicalin, strictinin A, and granatin B (Figure 1), significantly reduce the production of nitric oxide and PGE2 by inhibiting the expression of proinflammatory proteins [3].

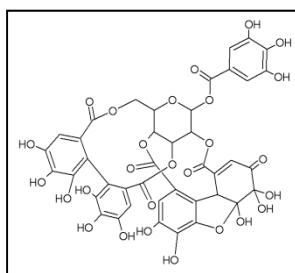


Figure 1. Granatin B

3. Materials and Methods

3.1. Preparation of Extracts

3.1.1. Hydroalcoholic Extract

Fruit peel, pulp, and arils were carefully separated. Maceration was used for the preparation of the extract. Three hundred grams of shade-dried arils were powdered, to which 3 L of ethanol: water (3:1) were added. The mixture was shaken at the appropriate temperature for 72 h. The extract was filtered and concentrated using a rotary device (at 60 RPM and 45 °C) and then dried at the appropriate temperature.

3.1.2. Alcoholic Extract

Fruit peel, pulp, and arils were carefully separated. Maceration was used for the preparation of the extract. Three hundred grams of shade-dried arils were powdered, to which 3 L of ethanol were added. The mixture was shaken at the appropriate temperature for 72 h. The extract was filtered and concentrated using a rotary device (at 60 RPM and 45°C) and then dried at the appropriate temperature [4]. The wound-healing activities of pomegranate (*Punica granatum* (Lythraceae)) peel, arils, and pulp were evaluated.

3.2. Drugs and Chemicals

Carrageenan (Type V, C3799), acetic acid 1%, pomegranate seed aril hydroalcoholic and alcoholic extracts (400 mg/kg), acacia suspension (2% *w/v*), and indomethacin 5 mg/kg were used.

4. Evaluation of In Vivo Anti-Inflammatory Activity

4.1. Experimental Animals

Rats were used for the experiments. They were kept in polypropylene cages under standard laboratory conditions at 24 °C. The rats were provided with food and water. The animals were quarantined and acclimatized to laboratory conditions for 7 days prior to study initiation, and they were also observed for general health and suitability for testing during this period.

4.2. Pharmacological Screening

4.2.1. Carrageenan-Induced Rat Paw Edema

The anti-inflammatory activity of the test compounds was evaluated in rats employing the standard rat paw edema method. The animals were fasted overnight and were divided into control, standard, and different test groups. The test compounds were administered by the oral route, giving a suspension at doses of 2.5, 5, and 10 mg/kg of rat weight. The animals in the standard group received indomethacin at the dose of 5 mg/kg by the oral route, and rats in the control group received the vehicle solution without test compounds. One hour after the test drugs' administration, rats in all groups were administered 0.1 mL of 1% carrageenan in the sub-plantar region of the right hind paw. The paw volumes (Figure 2) were measured before and 3 h after the administration of carrageenan using a digital plethysmometer (Ugo Basile, Italy). The percentage inhibition of paw volume for treated groups was calculated by comparing with the mean paw volume of the control group.

4.2.2. Statistical Analysis

The results obtained are expressed as the mean $\pm$ S.E.M. The data were analyzed by using ANOVA followed by Dunnett's *t*-test to determine the level of significance. A value of $p < 0.05$ was considered to be significant [4].

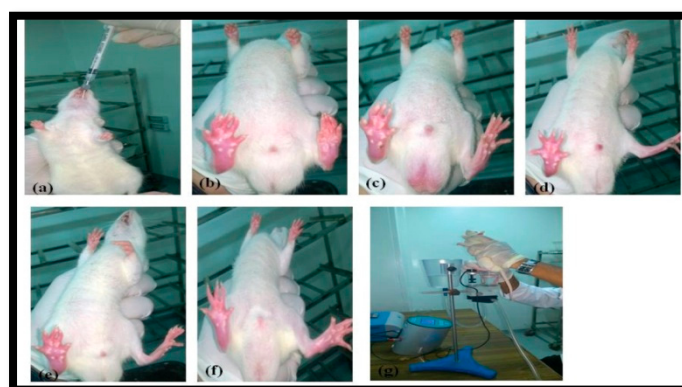


Figure 2. *In vivo* anti-inflammatory activity in rats.

Table 1. Acute anti-inflammatory activity of the test compound in the carrageenan-induced rat paw edema model.

Group	Test Material (Dose) mg/kg	Mean Increase in Paw Volume		
		1 hour	2 hour	3 hour
1	Control	1.29	1.73	1.9
2	HA 100	1.11	1.28	1.68
3	HA 200	1.22	1.38	1.5
4	HA 400	1.69	1.75	1.82
5	A 100	1.06	1.38	1.66
6	A 200	1.13	1.38	1.51
7	A 400	1.19	1.24	1.5
8	Standard (Indomethacin 5 mg/kg)	0.95	1.09	1.03

Values are the mean $\pm$ S.E.M. ($n=6$). $*p<0.001$ (one-way ANOVA and Dunnett's t -test), significantly different from control. Figures in parentheses are the % inhibition of paw edema in both the pomegranate-treated and indomethacin-treated groups.

Table 2. Acute anti-inflammatory activity against carrageenan-induced rat paw edema expressed as: percent of inhibition of edema formation.

Group	Test Material (Dose)	Mean Percent Inhibitions in Paw Volume (%)		
		1 hour	2 hour	3 hour
1	Control	0	0	0
2	HA 100	13.95	26.01	11.58
3	HA 200	5.43	20.23	21.05
4	HA 400	−31.01	−1.16	4.21
5	A 100	17.83	20.23	12.63
6	A 200	12.40	20.23	20.53
7	A 400	7.75	28.32	21.05
8	Standard (Indomethacin 5 mg/kg)	26.36	36.99	45.79

5. Discussion

Inflammation is associated with the pathophysiology of various clinical conditions such as arthritis and osteoarthritis; where acute inflammation is a beneficial host defensive response to tissue damage or any injurious stimuli [5]. NSAIDs are used for treatment of acute and chronic inflammatory conditions, but have gastrointestinal irritation; therefore, the use of plants that have anti-inflammatory effects without side effects can be good replacements for this drug class. In the present study, the anti-inflammatory and analgesic activities of pomegranate seed extract were investigated, using acute models of inflammation induced by formalin and by the acetic acid writhing test; showing that the pomegranate seed extract possessed significant anti-inflammatory, antiedematogenic and analgesic effects on rats with acute inflammatory paw edema and mice injected i.p. with acetic acid. Therefore, the results of the study are an indication that pomegranate seed extracts can be effective in acute inflammatory painful disorders, most probably via inhibition of soluble proinflammatory mediators TNF- α , interleukins (e.g., IL-6 and IL-8), and bioactive lipids such as eicosanoids (e.g., prostaglandinE2 and lipoxygenase-derived products) [6–16].

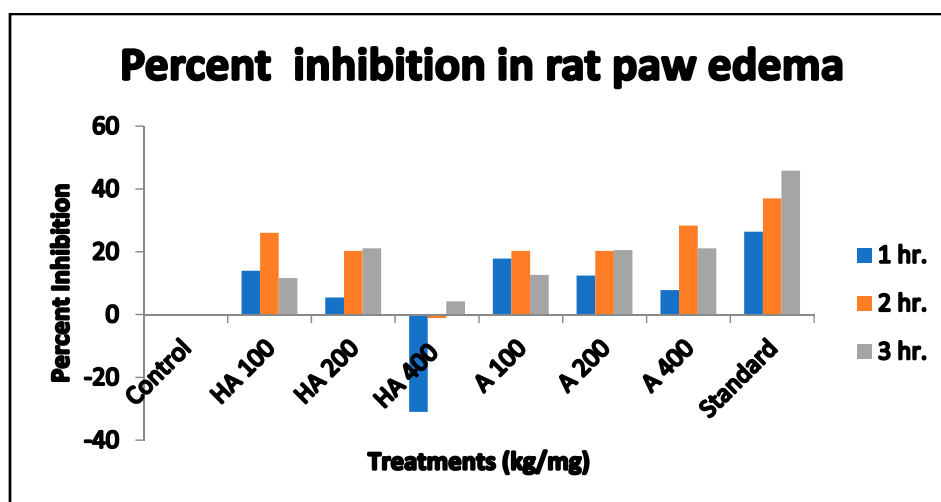


Figure 3. Comparison of anti-inflammatory activity between indomethacin and Granatin B.

6. Conclusions

Pomegranate aril ethanolic and aqueous extracts have shown good promise for anti-inflammatory activity. The hydroalcoholic and alcoholic extracts contain Granatin B, which has shown (Figure 3) (Tables 1 and 2) significant anti-inflammatory activity compared with the standard drug indomethacin. Pomegranate is a potent anti-inflammatory compared with other standard drugs. In addition, anti-carcinogenic and anti-oxidant, and anti-bacterial properties have been used as a therapy or adjunct for the prevention and treatment of cancer and CVS disease. There is the possibility that pomegranate extracts may also have an effect on other disease processes, such as Alzheimer's disease, osteoarthritis, neonatal brain injury, male infertility, and obesity.

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