Comparative Anti-inflammatory Activity of Arils Extracts of *Punica granatum* Fruits

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Abstract:

Anti-inflammatory is the property of a substance or treatment that reduces inflammation or swelling.The main objective of this study is to evaluate Anti-inflammatory activity of pomegranate arills extract on Rat's Paw. Anti-inflammatory activity of pomegranate was tested on rats by employing induced Carrageenan rat Paw Edema Method. Various concentrations of the arils and arils mixture (1:1) prepared by dissolving in Hydroalcohol and alcohol to obtain a final concentration of 100mg/kg, 200mg/kg, 400mg/kg against the test organisms. The effectivity of Granatin B to aqueous and alcoholic extracts arils of *Punicagranatum* calculated by measuring the increase in paw volume and percent inhibition by comparing with control And Standard drug.

Keywords: Anti-inflammatory, pomegranate aril's, Carrageenan rat Paw, Edema Method, Granatin B, percent inhibition

Introduction

Pomegranate (*Punica granatum* L. (*Punicaceae*); the common name is derived from Latin words ponus and granatus), a seeded, is a delicious 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 stretched through 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 total fruit weight and are characterized by 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 analgesics, remedying pain by reducing inflammation as opposed to opioids, which affect the CNS to block pain signaling to the brain. It has edible as well as medicinal use. It has been widely used in TIM worldwide for the treatment of different types of diseases (Olapour et al., 2010)[1].Also it gives antioxidant activities, which includes radical scavenging ability, ferrous ion chelating and ferric ion reducing antioxidant power, prevent oxidation and reduces 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 the various studies have been done on the antioxidant, anti-carcinogenic, and anti-inflammatory, Anti-atherosclerotic, Anthelmintic properties of pomegranate constituents, focusing on treatment and prevention of cancer, CVS disease, diabetes, dental problems, erectile dysfunction, bacterial infections will leads to antibiotic resistance, and skin damage due to various radiations such as UV. Other uses include neonatal brain ischemia, 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 control; Indomethacin as 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 alcoholic and hydroalcoholic extract of Arills of pomegranate (PME) and done comparisn with other literatures on Punica granatum (Lythraceae).

Chemical Constituents:

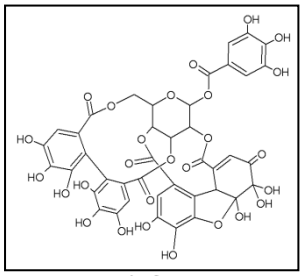

Fig No. 01Granatin B

When fatty acid composition of the seeds were examined it was found that it contains

1. punicic acid, 4-methyl lauric acid,
2. 1,3 dimethyl stearic acid, sterols (stigmasterol, sitosterol),
3. phospholipids (phosphatidyletanolamine, phosphatidylcholine, phosphatidylinositol)
4. mono, di- and triglycerides and free fatty acids were detected (Santagati et al., 1984; Sergeeva, 1973).

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

Materials and Methods:

Preparation of Extract:

Hydroalcoholic extract:

Fruit peel, pulp, and **arils** were carefully separated. Macerationwas used for the preparation of the extract. 300 g shade dried arils was powdered, to which 3 L ethanol:water (3:1) was added. The mixture was shaken at appropraite temperature for 72 hrs. Thenextract was filtered and concentrated using a rotary device (at 60 RPM and 45°C), and then dried at appropraite temperature.

Alcoholic extract:

Fruit peel, pulp, and **arils** were carefully separated. Maceration was used for thepreparation of the extract. 300 g shade arils was powdered, to which 3 L ethanolwas added. The mixture was shaken at appropraite temperature for 72 hrs. Then extract was filtered and concentrated using a rotary device (at 60 RPM and 45°C), and then dried at appropraite temperature[4].Evaluation of wound healing activities of pomegranate (Punicagranatum - Lythraceae) peel, arils and pulp.

Drugs and Chemicals:

Carrageenan (Type V, C3799), acetic acid 1 %, Pomegranate seed arils hydroalcoholic and alcoholic extract (400 mg /kg), acacia suspension (2% w/v) and Indomethacin 5mg/kg were used.

Evaluation of *in vivo* anti-inflammatory activity

Experimental animals

The rats were used for the experiments. They were kept in polypropylene cages under standard laboratory conditions at 24 ^{o} C. The rats were provided with their diet 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.

Pharmacological screening

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 oral route as giving suspension at the doseof 2.5,5, 10mg/kg of rat . The animals in the standard group, received indomethacin at the dose of 5mg/kg by oral route, and rats in the control group received the vehicle solution without test compounds. One hour after test drugs administration, rats in all the groups were administered with 0.1 ml of 1% carrageenan in the sub plantar region of right hind paw. The paw volumes were measured before and after3 hrs after the administration of carrageenan using digital plethysmometer (Ugo Basile, Italy). The percentage inhibition of paw volume for treated groups was calculated by comparing with mean paw volume of control group.

Statistical Analysis

The results obtained were expressed as mean ± S.E.M. The data was 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]

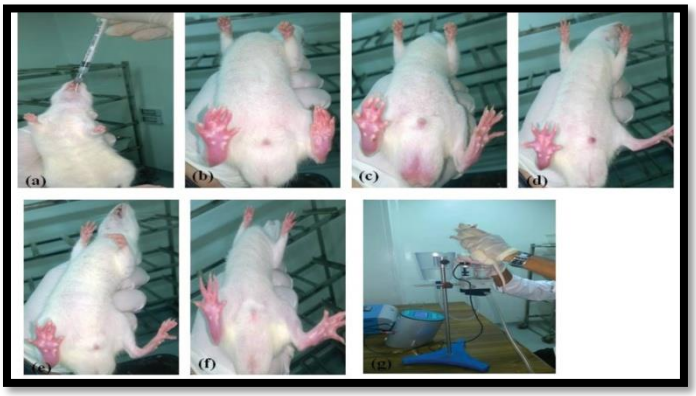

Fig No.02 *In Vivo* anti-inflammatory activity in rats

Table 1: Acute Anti-inflammatory Activity of the test compound in the Carrageenin-induced Rat Paw Edema Model

Group	Test Material (dose) mg/kg	Mean increase in paw volume		
		1 hr.	2 hr.	3 hr.
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 5mg/kg)	0.95	1.09	1.03

Values are mean ± SEM. (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 groups of pomegranate treated and Indomethacin treated groups.

Table 2; Acute Anti-inflammatory Activity Against Carrageenin-induced Rat Paw Edema expressed as: Percent Of Inhibition of Edema Formation At time(9 Hr)

Group	Test Material (dose)	Mean Percent inhibitions in paw volume (%)		
		1 hr.	2 hr.	3 hr.
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 5mg/kg)	26.36	36.99	45.79

Discussion:

Inflammation is associated with the pathophysiology of various clinical conditions such as arthritis and oesarthritis; where acute inflammation is a beneficial host defensive response to tissue damage or any injurious stimuli [5]. NSAIDs used for treatment of acute and chronic inflammatory conditions, but have gastrointestinal irritation therefore, the use of plants that having anti-inflammatory effects without side effects can be good replacement for this drug class. In the present study, the anti-inflammatory and analgesic activities of pomegranate seed extract was investigated, using acute models of inflammation induced by formalin and by acetic acid writhing test ; showed that the pomegranate seed extract possess a significant anti-inflammatory, antidematogenic and analgesic effects on rats with acute inflammatory paw edema and mice injected i.p acetic acid Therefore, the results 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), bioactive lipids such as eicosanoids (e.g. prostaglandin E2 and lipoxygenase derived products, which are strongly

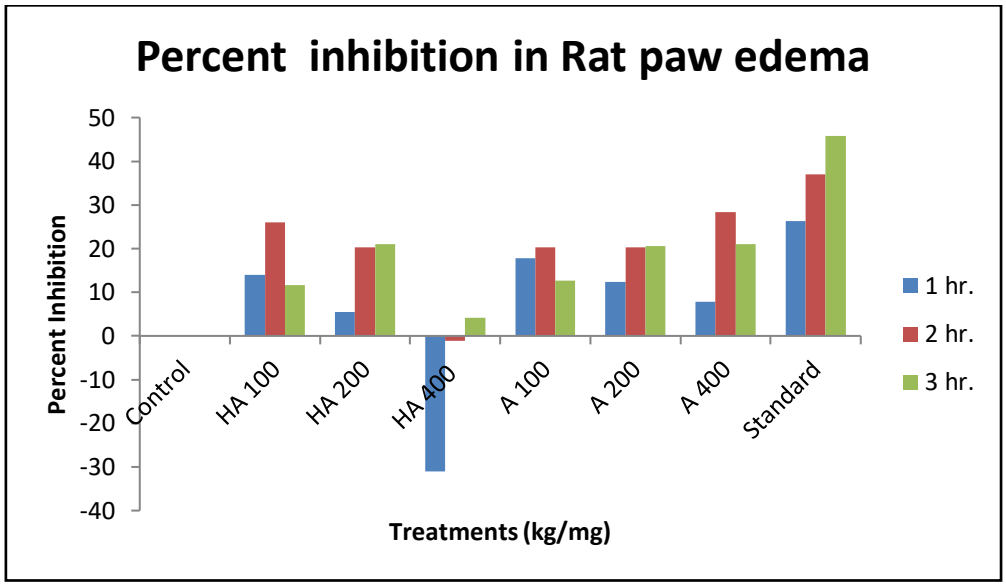

Fig No 03: Comparison of Anti-Inflammatory Activity between Indomethacin and Granatin B

Conclusion:

Pomegranate arils ethanolic and aqueous extracts has been giving good promising for anti-inflammatory activity. The hydroalcoholic and alcoholic extracts has granatin B which showed significant anti-inflammatory activity compared with the standard drug indomethacin. Pomegranate is a potent for Anti-inflammatory as comparing with other standard drugs. In addition,anti-carcinogenic and anti-oxidant, Anti-Bacterial properties have been used as a therapy or adjunct for prevention and treatment of cancer and CVS disease. 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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