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EVALUTION OF ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF *PUNICA GRANATUM* LINN

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ABSTRACT

The aqueous-ethanolic (50%) extracts of fruit rind (PGR), flower (PGF), and leaves (PGL) of Punica granatum were examined for its oral anti-inflammatory and analgesic activities at the doses of 150, 250 and 500 mg/kg body weight. Oral pretreatment with the dried extracts of P. granatum produced statistically significant and dose dependent inhibition of edema induced by carrageenan at all doses when compared to the control groups. The highest activity was shown in the PGR that at 500 mg/kg p.o. inhibited inflammation by 82.14%. (79 % for indomethacin at 10 mg/kg). On the contrary, the aqueous-ethanolic (50 %) extracts of PGF and PGL exhibited 71.42% and 67.85% inhibition, respectively, at 500 mg/kg dose. The extracts at tested doses were found to possess analgesic activity in mice against tail-flick method. These results indicated that extracts of P. granatum possessed significant anti-inflammatory and analgesic activities suggesting its potential as an anti-inflammatory agent for use in the treatment of various inflammatory diseases in traditional medicine.

Keywords: *Punica granatum, Anti-inflammatory, Analgesic & In vivo*

Introduction

Punica granatum Linn. (Punicaceae), commonly called pomegranate, is a shrub that grows well in the warm valleys and outer hills of the Himalayas and is cultivated throughout the India. It has long been esteemed as food and medicine and as a diet in convalescence after diarrhoea [1].

The pomegranate tree, *P. granatum*, possesses a vast ethnomedical history and represents a phytochemical reservoir of heuristic medicinal value. In traditional medicine of India, the plant has been used for treating various inflammatory and infectious diseases. The unripe fruit lessens inflammation and is utilized to treat keratitis and as a good appetizer and tonic [2]. The ripe fruit is tonic, astringent, laxative, diuretic, used in brain diseases, chest troubles, bronchitis and earache. Juice of fruit is prescribed is prescribed to cure edema, leprosy, cough, emesis, anorexia and inflammation of liver [3]. Bark and fruit rind are administered orally to prevent dysentery, diarrhoea, piles, bronchitis and bilious affection. A decoction of the dried rind of the fruit is taken for the relief of stomachache and dysentery. The juice of the leaves and young fruits is drunk to cure dysentery. The powdered flower buds are given internally to relieve bronchitis, diarrhoea and dysentery of children. A decoction of the flowers is gargled to reduced oral and throat inflammation [4].

The biological activities viz. antibacterial [5], antifungal [6], anthlemintic [7], antifertility [8], antioxidant [9], antidiabetic [10], antiatherogenic [11] and antiulcer [12] of

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the various extracts of different parts of this plant have also been reported.

The need for safer and effective analgesic and anti-inflammatory drugs and the lack of enough scientific data to support the claims made in ancient literature prompted the present study. Moreover alkaloids, flavonoids and polyphenols are reported to have several biological properties. Flavonoids, a group of polyphenolic compounds found in vegetables and fruits, are beneficial for prevention of inflammatory [13], cardiovascular and other diseases [14].

P. granatum is reported to contain alkaloids, flavonoids, several polyphenolic compounds (such as delphinidin, cyanidin and pelargonidin) and hydrolyzable tannins (such as punicalin, pedunculagin, punicalagin, gallic and ellagic acid esters of glucose) which possesses strong anti-inflammatory and antioxidant properties [15].

Thus, the present study was undertaken to work out whether the extract of plant exerts an anti-inflammatory and analgesic action. Since, the fruit rind, flowers and leaves of the *P. granatum* contained important phytoconstituents; it was used for the pharmacological investigations.

Material and methods

Plant material:

The fruit rind and flowers of *P. granatum* were purchased from Khari Baoli market, Delhi and fresh leaves of *P. granatum* were collected from Herbal garden of Jamia Hamdard, and authenticated by Dr. M.P. Sharma, Taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi. A voucher of the specimen is deposited in the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi. The experimental study was approved by the Institutional Animal Ethical Committee of Jamia Hamdard, New Delhi.

Preparation of plant extracts:

The drugs were shade dried, powdered and extracted by refluxing with aqueous-ethanol (50% v/v) on boiling water bath for 6 h. The extract was filtered and residue was re-extracted in the same manner four times. All the filtrate were combined together and recovery of the solvent under reduced pressure yielded solid mass.

Experimental animals:

Wistar albino rats (150-180 g each) and Swiss albino mice (25-30 g each) of either sex, maintained under standard animal housing conditions, were used for anti-inflammatory and analgesic activity respectively. The animals were allowed standard laboratory feed and water *ad libitum*. Study was performed according to the Guidelines of Institutional Animal Ethics Committee (IAEC) of Jamia Hamdard, New Delhi. India.

Anti-inflammatory activity:

Carrageenan induced paw edema is the simplest and most widely used model for the studying the anti-inflammatory activity [16]. The acute hind paw was produced by injecting 0.1 ml of carrageenan (prepared as 1% suspension in sterile normal saline) locally into the planter aponeurosis of the right hind paw of rats. PGR, PGF and PGL extracts (150, 250 and 500 mg/kg, p.o., dissolved in 0.5% w/v sodium carboxy methyl cellulose in distilled water) were administered orally to nine different groups while the other two groups served as control and received vehicle, (1 ml/kg, p.o.) and standard drug, indomethacin

(10 mg/kg, p.o.), respectively. PGR, PGF and PGL extracts and indomethacin were administered 1 h prior to the injection of carrageenan. The rat pedal volume up to the ankle joint was measured using Plethysmometer at 0 (just before) and 3 h after the injection of carrageenan. Increase in paw edema was considered as the difference between 0 and 3 h. Percent inhibition of edema volume between treated and control was calculated as follows:

$$\% \text{ inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where *Vc* and *Vt* represent mean increase in paw volume in control and treated groups respectively.

Analgesic activity:

Analgesic activity was evaluated by tail immersion method [17]. The aqueous-ethanolic (50%) extracts of PGR, PGF and PGL of *P. granatum* (150, 250 and 500 mg/kg) were administered orally as suspension 0.5% w/v sodium carboxy methylcellulose. The lower 5 cm portion of the tail was gently immersed into thermostatically controlled water at 55 ± 0.5 °C. The time in second for tail withdrawal from the water was taken as the reaction time with a cut of time of immersion, set at 10 seconds for both control as well as treated groups of animals. The reaction time was measured before and after 3 h interval of the administration of extract and standard drugs.

Statistical analysis:

The experimental results were expressed as the mean ± standard error of mean (SEM) and the statistic found to be dose dependent. However, significance of the results of

anti-inflammatory and analgesic activity was calculated by ANOVA followed by Dunnett's multiple comparison *t*-test and paired student's *t*-test respectively.

Table 1: Anti-inflammatory and analgesic activities of aqueous-ethanolic (50%) extract of PGR, PGF and PGL.

Treatment	Anti-inflammatory activity[ab]			Analgesic activity[c]		
	Dose (mg/kg)	Paw volume at 3 h	% inhibition	Normal at 0 h	Post Treatment at 3 h	% analgesia
Control	Vehicle	0.28 ± 0.011	---	---	---	---
PGR	150	0.13 ± 0.007$^{*, \dagger}$	57.14	1.05±0.053	1.33±0.08**	26.66
	250	0.10 ± 0.011$^{*, \dagger}$	66.66	1.15 ± 0.06	1.70 ± 0.07**	48.10
	500	0.05 ± 0.006$^{*, \dagger}$	82.14	1.34±0.029	2.38±0.07**	77.61
PGF	150	0.15 ± 0.008$^{*, \dagger}$	46.42	1.29±0.035	1.55±0.06*	20.15
	250	0.12 ± 0.007$^{*, \dagger}$	57.14	1.22±0.055	1.66±0.05*	36.06
	500	0.08 ± 0.007$^{*, ns}$	71.42	1.11±0.056	1.71±0.10**	54.05
PGL	150	0.16 ± 0.007$^{*, \dagger}$	42.85	1.02±0.034	1.22±0.03**	19.60
	250	0.13 ± 0.004$^{*, \dagger}$	53.57	1.40±0.133	1.70±0.15**	21.42
	500	0.09 ± 0.011$^{*, ns}$	67.85	1.41±0.104	2.12±0.13**	50.35
Standard	10	0.06 ± 0.008*	79.0	1.48±0.126	2.36±0.26**	59.49

[a] Compared with respect to control, values are mean ± SEM (n=6), $^{*}p<0.01$,
[b] Compared with respect to standard, $^{\dagger}p<0.01$, $^{ns}p>0.05$. Data were analyzed by ANOVA followed Dunnett's multiple comparison *t*-test.
[c] Compared with respect to normal (0 h), values are mean ± SEM (n=6), $^{*}p<0.001$, $^{**}p<0.0001$. Data were analyzed by paired student's *t*-test.

Results

Anti-inflammatory activity:

Table 1 indicated that aqueous-ethanolic (50%) extracts of different parts of *P. granatum* showed anti-inflammatory effects in carrageenan induced paw edema when compared with the control animals injected with carrageenan only. The extracts showed significant anti-inflammatory activity at all tested dose levels. The mean increase in paw edema volume was about 0.28 ± 0.011 in the vehicle-treated control rats. PGR, PGF and PGL extracts (150, 250 and 500 mg/kg, p.o.) significantly ($p<0.01$) reduced the mean paw edema volume at 3 h after carrageenan injection. *P. granatum* extracts exhibited anti-inflammatory activity in dose dependent manner with the percentage inhibition of paw edema of 57.14, 66.66 and 82.14% (PGR), 46.42, 57.14 and 71.42% (PGF) and 42.85, 53.57 and 67.85% (PGL) at 150, 250

and 500 mg/kg, respectively, as compared with the control group. However, on comparison with standard drug, PGR, PGF and PGL extracts at dose of 500 mg/kg showed no significant difference ($p>0.05$) in percentage inhibition. Standard drug indomethacin showed an inhibition of 79% (Table 1). Inhibition of carrageenan-induced edema by the extracts of *P. granatum* indicates the presence of active molecules in the plant.

Analgesic activity:

PGR, PGF and PGL extracts were subjected for analgesic activity. The results are presented as percentage inhibition and tabulated in Table 1. PGR, PGF and PGL extracts exhibited analgesic activity in dose dependent manner with the percentage analgesia of 26.66, 48.10 and 77.61% (PGR), 20.15, 36.06 and 54.05% (PGF) and 19.60, 21.42 and 50.35% (PGL) at 150, 250 and 500 mg/kg, p.o., respectively, as compared with the control group. Highest percentage analgesia, i.e., 77.61% was shown by PGR extract at dose of 500 mg/kg, whereas, standard drug indomethacin showed percentage analgesia of 59.49%.

Discussion

The present study demonstrates the potent anti-inflammatory and analgesic activity of aqueous-ethanolic extract (50%) of different parts of *P. granatum* (PGR, PGF and PGL), indicating the possibility of use of *P. granatum* as the cheaper, safer and potent anti-inflammatory therapeutic agent. *P. granatum* extracts inhibited carrageenan-induced edema in albino wistar rats. Carrageenan is a mixture of polysaccharides composed of sulfated galactose units and derived from Irish Seamoss (*Chondrus crispus*). The delta type galactan elicited an inflammatory response. The development of the edema in paw of the rat after the injection of carrageenan has been described as a biphasic event. The initial phase of the edema has been attributed to the release of histamin, serotonin and kinin-like substance, and the second accelerated the phase of swelling to release prostaglandin-like substance [18].

In the present study, fruit rind extract showed maximum anti-inflammatory activity followed by flowers and leaves, indicating the presence of active constituents in the drug. Further studies are in progress to isolate the active constituents, which can be used as a lead molecule. The extract that shows maximum anti-inflammatory activity also possesses maximum analgesic effects in dose dependent manners.

Results reported in the present work constitute a scientific basis to justify and support the use of this traditional plant for the treatment of inflammation in traditional medicine. Hence, in the present study it can be concluded that the strong anti-inflammatory and analgesic activity of aqueous-ethanol (50%) extract of PGR, PGF and PGL is probably due to the fact that the extracts contain the polar bio-active components responsible for these activities. Significant anti-inflammatory profile needs further studies to identify the possible mechanism of action as well as establishing the therapeutic value in the treatment of inflammatory diseases.

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