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Research Article

Evaluation of Anti-inflammatory Action of Ethanolic Extract of Peels of *Punica granatum* In Experimental Model

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ABSTRACT

The present study was planned to investigate the anti-inflammatory activity of ethanolic extracts of fruit peel of *P. granatum* and compared it with standard drug Ibuprofen. Ethanolic extracts of fruit peel of *P. granatum* was prepared using Soxhlet apparatus. Wistar albino male rats weighing 150 to 200 gm of were used in this study. The acute anti-inflammatory property of the extracts was screened by using the carrageenan induced paw edema model in rats. The percent inhibition of paw edema and was noted. Fruit peel extracts of *P. granatum* exhibited a significant reduction in carrageenan induced paw edema when compared to control. Acute anti-inflammatory activity of the standard drug Ibuprofen treatment was better than all the extracts, but there was no significant difference between the activities at higher doses of plant extracts and standard drug. No toxicity was observed even when extracts were administered at 10 times of highest dose used in this study. The results obtained indicate that fruit peel of *P. granatum* has favorable anti-inflammatory activity and thus support the traditional use of fruit peel of *P. granatum* as anti-inflammatory agent.

INTRODUCTION

Nature has provided a complete store-house of remedies to cure all ailments of mankind. This is where, nature provides us drugs in the form of herbs, plants and algae's to cure the incurable diseases without any toxic effect¹. Research on medicinal plants is an important fact of biochemical research in India because of several reasons. Inflammation is a disorder involving

localized increases in the number of leukocytes and a variety of complex mediator molecules. Prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses involved in inflammation. Their biosynthesis has also been implicated in the pathophysiology of cardiovascular diseases, cancer, colonic adenomas and Alzheimer's disease². Medicinal plants are believed to be an important source of new

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chemical substances with potential therapeutic effects. The research into plants with alleged folkloric use as pain relievers, anti-inflammatory agents, should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs. Because existing synthetic molecule like non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors that increase the incidence of adverse cardiovascular thrombotic effects. So, in order to overcome, there is need to focus on the scientific exploration of herbal drugs having fewer side effects³. According to an all India ethnobiological survey carried out by the Ministry of Environment and Forests, Government of India, there are over 8000 species of plants being used by the people of India. A WHO consultation had drafted "Guidelines for the Assessment of Herbal Medicines" in 1991. Their objective had been to assist national regulatory authorities, scientific organizations and manufacturers to undertake an assessment of the documentation for herbal medicinal products⁴. Inflammation is one of the body defense mechanism to get rid of causative agent which causes cell injury and further cell necrosis or it is protective response by the host body to inflammatory stimulus. Acute inflammation is a short-term process, usually appearing within a few minutes or hours and ceasing upon the removal of the injurious stimulus. It is characterized by five cardinal signs; the acronym that may be used for this is "PRISH" for Pain, Redness, Immobility (loss of function), Swelling and Heat. The traditional names for signs of inflammation come from Latin⁵. The pomegranate (*Punica granatum* L.) is a member of the Punicaceae family, characterized by the presence of red flowers and large, sweet fruits. Moreover, the fruit is delimited by a membrane, the pericarp (peel), which contains the arils (the external part of the seed)⁶. Each seed is encased in a sac containing transparent juice. Thin

membranes extend from the pericarp into the fruit, forming a latticework in which the arils are suspended. The pomegranate originated in the Middle East, from where it was widely distributed throughout the Mediterranean region, later spreading east and west to China, India, Mexico, California, and the American Southwest⁷. The presence of several bioactive compounds, mainly polyphenols, in pomegranate has led to its incorporation into the functional food category, where it is used for its numerous therapeutic properties. These include antiviral, bactericidal, antifungal, immune modulation, stomachic, laxative, styptic, diuretic, and anthelmintic properties⁸.

MATERIAL & METHOD:

Selection, identification and authentication of plant material

The fruits of *Punica granatum* was collected from East region of Uttar Pradesh. The initial identification was based on its organoleptic and morphological characteristics, and the verification was conducted by botanist of Botanical Survey of India, Pune.

Extraction of plant material

Fruit peel of *P. granatum* was carefully removed, cleaned, shade dried at room temperature to avoid degradation of phytoconstituent. After drying, peels were reduced to coarse powder using a grinding mill. Based upon the extensive literature survey, we decide to use the ethanol as a solvent for extraction of phytoconstituents from plant material for further study⁹. Dried pomegranate peels (500 g) were powdered and extracted with analytical grade ethanol in a Soxhlet apparatus for 12 h; the extract was concentrated to dryness under reduced pressure at $40 \pm 5^\circ\text{C}$ until it became a



brownish solid residue¹⁰. Extracts were stored as dried powder at 4°C.



Figure 1: Soxhlet extraction of peels of *Punica granatum*

Drugs and chemicals

The necessary chemicals utilized during this research work have been carrageenan, and Ibuprofen was used as standard drugs.

Equipment

Plethysmometer, Soxhlet Apparatus, Digital Balance, Desiccator, Hot Air oven.

Experimental Animals

From the Animal House, Saraswati Higher Education and Technical College of Pharmacy, Gahni, Ayar, Varansi, albino rats of either sex weighing 150–200g were acquired. The animals were kept in ideal conditions, with a 12-hour light/dark cycle and room temperatures of $25 \pm 1^\circ\text{C}$. They were fed a regular mouse pellet diet and given access to unlimited water, with the relative humidity being kept at 44–56%. Rats are denied food one hour before the experiment¹¹.

Phytochemical Screening

Extracts was subjected to phytochemical analysis to identify the different phytoconstituents found in the plant were put through a standard qualitative chemical examination to determine the type of phytochemical components they contained. The existence and nonexistence of various phytochemical constituents, like Alkaloids, Carbohydrates, Glycosides, Saponins, Amino acids and Proteins, Flavones and Flavonones, Tannins and Phenolics, Steroids, Fixed oils were determined using standard established techniques¹².

Toxicity Study

Acute toxicity was evaluated *in vivo* in male wistar rats. According to the guidelines of OECD for testing of chemicals 425. Three different doses of 50, 300, and 2000 mg/kg were administered orally through gastric gavage to three different groups of rats (n=6 per group) for the determination of the

lethal dose that kills 50% of the test animals in a group (LD50)¹³. Rats were weighed before, during, and after the procedure to measure any changes in body weights. Rats were also monitored to observe any possible changes in their behaviors after dosing at least once during the first 30 min and periodically through 24 h. Special attention was given during the early 4 h and daily for 14 days. Rats were monitored to observe any signs of toxicity including changes in skin and eyes, breathing issue, seizures, or death.

Experimental Design:

Rats were kept in different 5 groups; n=6.

Group 1: Normal saline.

Group 2: Carragenan + *Punica granatum* (100 mg/kg)

Group 3: Carragenan + *Punica granatum* (200 mg/kg).

Group 4: Carragenan + *Punica granatum* (400 mg/kg).

Group 5: Carragenan + Ibuprofen (100 mg/kg)

Assessment of anti-inflammatory activity

Carrageenan-induced paw edema in rats

The study of the anti-inflammatory activity of fruit peel of *P. granatum* was assessed using the carrageenan-induced paw edema in rats. Animals were divided into five groups of six animals each. Control, Test and standard drug were given orally. After 30 minutes of oral administration of the drugs, animals of all the groups were injected with 0.1 ml of 1% suspension of carrageenan in 0.9% normal saline, under the plantar aponeurosis of the right hind paw. Group 1 received normal saline solution as vehicle orally and was considered as control. Groups 2, 3 and 4 received PEPG orally at a dose of 100, 200 and 400 mg/kg respectively. Group 5 received Ibuprofen 100 mg/kg and served as the standard control¹⁴. All the groups received the same volume of preparations. The paw volume from each rat from all groups was measured at 1h, 2h, 3h and 4 h after carrageenan injection using Plethysmograph. Acute anti-inflammatory activity was expressed as the percentage inhibition of paw volume between control animals and mice pretreated (n=6) with the extract or standard drug using the ratio:

$$\frac{(\text{Control mean} - \text{Treated mean})}{\text{Control mean}} \times 100$$



Figure 2: Administration of Carragenan in rat paw for induce inflammation



Figure 3: Administration of oral dose of standard or test drug.

Statistical analysis

All values were expressed in Mean $\pm$ SD. * significant p-value (<0.05) with one-way ANOVA followed by post-hoc test Bonferroni when compared with control.

RESULTS

Phytochemical screening

The ethanol extract of pomegranate peels showed positive detection to all phytochemical classes tested such as phenols, flavonoids, anthocyanins, quinones, tannins, saponins, steroids, triterpenoid, and alkaloids respectively.

Table 1: Preliminary phytochemical analysis of extract



Sr. No.	Phytochemical	Tests	Result
1	Tannins	FeCl ₃ test	+
2	Saponins	Foam test	+
3	Alkaloids	Mayer's test	+
4	Flavonoids	Shinoda test	+
5	Phenols	Fecl ₃ test	+
6	Triterpenoids	Libermann - Buchard test	+
7	Steroids	Liebermann Burchard test	+
8	Glycosides	Salkwaski's test	+

+: Positive -: Negative

Acute Toxicity Test:

Acute *in vivo* administration of ethanolic pomegranate peel extract shows no signs of toxicity

During the 14 days of the experiment, even with a high dose of 2000 mg/kg, no significant change in rats' weight compared with the control group was noticed ($p > 0.05$). Moreover, no signs of toxicity or deaths were reported. The LD₅₀ value was indeterminable even at the highest EPPE dose,

which indicates that EPPE is non-toxic at doses of expected efficacy. Results show that the extract is non-toxic even at relatively high concentrations.

Effect of fruit peel of *P. granatum* on carrageenan induced paw edema

The ethanolic extract of fruit peel of *P. granatum* in higher doses showed a significant dose-dependent reduction in the volume of paw edema when compared with control at the end of 2h, 3h and 4 h while in lower doses at the end of 4 h. EPPE 200 and EPPE 400 showed significant reduction (34.54%, 48.72% and 51.81% respectively) in paw edema of respectively at the end of 4 h as compared to control group. The maximum reduction was observed at a dose of 400 mg/kg of fruit peel extract of *P. granatum* among all extracts. Standard drug Ibuprofen showed 51.81% reduction in paw edema which was greater than that showed by all extracts. There was no significant difference between the higher dose of both plant extracts and standard drug at the end of 4 h. [Table 2 and 3]

Table 2: Effect of oral administration of fruit peel extract of *p. granatum* and Ibuprofen on carrageenan-induced paw edema in rats. (n=6)

Groups	Treatment (mg/kg)	Paw Edema (ml)				
		Initial	1hr	2hr	3hr	4hr
Group 1	Normal	0.77 ±1.07	0.84±1.63	0.88±0.79	0.88±0.79	1.10±0.15
Group 2	100 mg/kg	0.74 ±1.04	0.80±0.64	0.82±0.67	0.92 ±0.15*	0.88±1.3**
Group 3	200 mg/kg	0.71 ±0.82	0.75±0.50*	0.74±0.54*	0.72±0.50**	0.64±0.67**
Group 4	400 mg/kg	0.68±0.78	0.70±0.46	0.67±0.50	0.65±0.52	0.60±0.62
Group 5	Ibuprofen	0.68±0.48*	0.63±0.49**	0.60±0.28**	0.53±0.66**	0.52±0.61**

Results were expressed in Mean ± SD. * significant p-value (<0.05) with one-way ANOVA followed by post-hoc test Bonferroni when compared with control. **Significant p-value

(<0.001) with one-way ANOVA followed by post-hoc test Bonferroni when compared with control. EPPE: Ethanolic Pomegranate Peel Extract.

Table 3: Effect of oral administration of fruit peel extract of *p. granatum* and Ibuprofen on percentage reduction of carrageenan-induced paw edema in rats. (n=6)

Groups	Paw Edema (% inhibition)
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	Treatment (mg/kg)	1hr	2hr	3hr	4hr
Group 1	Normal	-	-	-	-
Group 2	100 mg/kg	3.9	4.76	6.81	16.36*
Group 3	200 mg/kg	7.79	10.71*	15.91*	34.54**
Group 4	400 mg/kg	10.05	12.80	20.42**	48.72**
Group 5	Ibuprofen	12.99*	25**	31.81**	51.81**

* significant p-value (<0.05) with one-way ANOVA followed by posthoc test Bonferroni when compared with control. ** Significant p

value (<0.001) with one-way ANOVA followed by post-hoc test Bonferroni when compared with control. PEPG: Fruit peel extract of *P. granatum*.

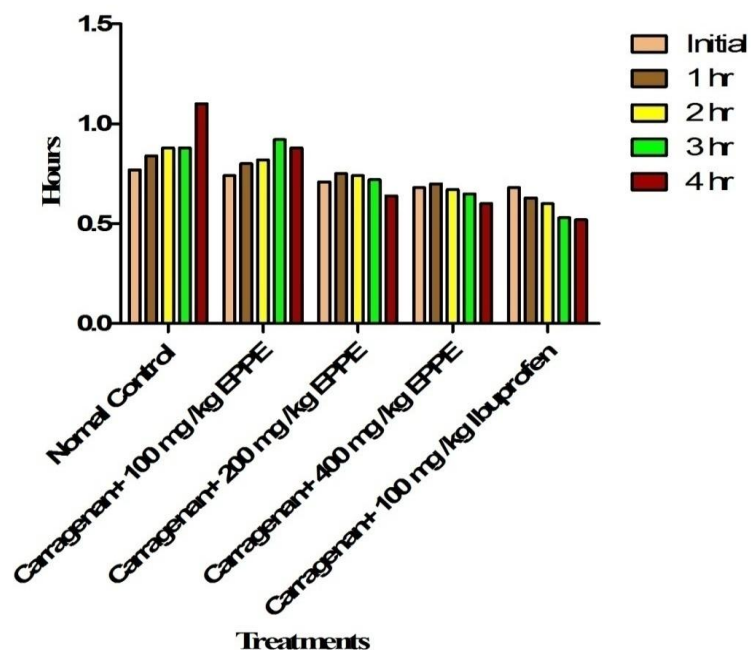


Figure 4: Rat paw edema in Normal & Carragenan treated rats.



Figure 05: Measurement of Paw volume with the use of Plethysmometer

Effect of EPPE on Carrageenan-induced paw edema



DISCUSSION

In the present study, anti-inflammatory activities of fruit peel extracts of *P. granatum* were investigated by experimental animal models.

Acute anti-inflammatory activity of fruit peel of *P. granatum* was assessed using the carrageenan-induced paw edema in rats. Carrageenan is a widely used irritant or a phlogistic agent. Injection of carrageenan under the plantar aponeurosis of the hind paw of rat produces edema by local inflammation. The carrageenan induced inflammation model is a test for evaluation of acute anti-inflammatory activity¹⁵. Fruit peel extracts of *P. granatum* in both doses produced significant anti-edematogenic effect on paw edema induced by carrageenan. The higher doses of both extracts exhibited better anti-inflammatory effect. The inflammation induced by carrageenan injection is biphasic in nature¹⁶. The first phase observed during the first hour is attributed to the release of serotonin, histamine and kinin while the second phase is related to the release of prostaglandin^{17, 18}. Fruit peel extracts of *P. granatum* are effective after first hour, suggesting that the extracts inhibit the second phase of carrageenan induced inflammation. In the present study, administration of fruit peel extracts of *P. granatum* exhibited inhibition of inflammation close to the inhibitory effect of Ibuprofen in a dose dependent manner¹⁹. This suggests that fruit peel extracts have potential to inhibit the chronic inflammation. Fruit peel of *Punica granatum* possess anti-inflammatory effect which could be due to the presence of phytochemicals such as Tannins and Flavonoids²⁰. Qualitative phytochemical screening showed that content of Flavonoids in fruit peel are same but tannins are more in fruit peel. Flavonoids also have anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation. Gallic acid is the major tannin found in both fruit Peel but the other tannins like Gallagylidilacton and Granatin B is found in fruit peel of *P. granatum*. Among tannins, Granatin B more strongly

inhibited COX-2 and this could be the reason for better activity of fruit peel extract of *P. granatum*

CONCLUSION

In the present study, administration of fruit peel extracts of *P. granatum* exhibited inhibition of inflammation close to the inhibitory effect of Ibuprofen in a dose dependent manner. This suggests that fruit peel extracts have potential to inhibit the chronic inflammation. The effects of ethanolic peel extract at dose (100, 200 and 400mg/kg, p.o.) of *Punica granatum* on Wistar albino rats for the assessment of anti-inflammatory activity. The current research revealed that EPPE (200 and 400mg/kg,p.o.) possessed significant ($P<0.05$) anti-inflammatory activities in a dose-dependent manner. Furthermore EPPE (200 and 400mg/kg p.o) were effective as standard drug (Ibuprofen 100 mg/kg, p.o). Fruit peel of *Punica granatum* possess anti-inflammatory effect which could be due to the presence of phytochemicals such as Tannins and Flavonoids. All these methods were performed under highly supervised conditions and by proper handling of experimental animals having been granted permission by IAEC. This study will be supportive in the future for the improvement of a novel herbal formulation for the diagnosis and treatment of related complications.

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