

EVALUATION OF ANTI-NOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITY OF *PUNICA GRANATUM* SEED EXTRACT

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ABSTRACT

The plant *Punica granatum* of family Punicaceae is distributed throughout India and reputed to have numerous applications in traditional medicine system. In order to justify its folkloric use in nociception and inflammation, the study was performed.

In this study, the extraction of *Punica granatum* seed extract was carried out in aqueous media. In order to explore its potency, various experimental models of anti-nociceptive and anti-inflammatory activities were taken. The oral administration of the extract 100mg and 200mg per kg body weight showed significant pharmacological action. Furthermore the anti-ulcer activity was carried out with the help of Indomethacin induced ulceration model using Mesoprostol as standard drug and it showed no ulcerogenic effect in wistar albino rats.

Overall, the extract was found to be significant anti-nociceptive and anti-inflammatory activity with no ulcerogenic adverse effect.

KEY WORDS: anti-nociceptive, anti-inflammatory, *Punica granatum*

INTRODUCTION

A large number of analgesic and anti-inflammatory drugs are present in modern as well as ancient system of medicine, amongst which most of the drugs have gastric ulceration adverse effect after systemic administration. In the present scenario, it is a big challenge to explore such a novel anti-inflammatory drug, which has no ulcerogenic effect.

In present time, a large number of steroidal and non steroidal anti-inflammatory drugs are present in modern as well as ancient system of medicine, amongst which most of the drugs have gastric ulceration adverse effect. As a result, the search for other alternatives seems to be necessary and important to human beings.

The present study aims to explore the same for *Punica granatum* ripped seed extract. The plant *Punica granatum* of family Punicaceae known as 'Dadima' in Sanskrit and English name is 'Pomegranate'. The tree/shrub has been cultivated since ancient times throughout Asia, Africa and Europe². It is a shrub rarely a small tree and reputed to have numerous application in traditional medicine. Its different parts are seed, juice, peel, leaf, flower, bark and roots; each of these have different pharmacologic activity³. The values of many traditional uses of *Punica granatum* have been confirmed by experiments, like neuroprotective⁴ and anti-oxidant⁵. The stem and root bark is useful in the treatment of worm infection and flower decoction in throat infections⁶. It is also given for hematuria, hemorrhoids, hemoptysis¹ and in dysentery^{1,7}. Powdered and soaked peelings of pomegranate are used to treat external inflammation, sore throats, cuts and wounds in ancient natural remedy system of Arabia. In Mexican culture decoction of pomegranate flower is also used as mouth gargle to treat various oral and throat inflammation. During the preliminary phytochemical screening certain constituents like isopelletierine (alkaloid), estrone with tannins, ellagic acid, punicalagin, punicalin, strictinin A and granatin B have been seen, which are important to support different body functions^{8,9}. But, in order to justify its folkloric use in pain and inflammation, it was decided to investigate the property of aqueous extract of *Punica granatum* ripped seed in the present study.

MATERIALS AND METHODS

Fresh ripped fruits of *Punica granatum* were collected from the local supplier of Mathura and transported on the same day to the laboratory of university. The specimen was further authenticated by Dr. D. C. Saini, Scientist 'E', Birbal Sahni Institute of Palaeobotany, Lucknow, India (Registration no – 13363).

General surgical materials, anaesthetic agent (ether), sterilized cotton pellets, Indomethacin, Carrageenan were supplied by the department of pharmacology, Institute of Pharmaceutical Research GLA University, Mathura.

Preparation of aqueous extract of *Punica granatum*

Ripped seed of *Punica granatum* were dried in shade for one month duration. Dried seeds were crushed in semi solid mass. Then half a kilogram of the semisolid mass was tied in a thin muslin cloth and soaked in one liter of distilled water for 72 hrs at room temperature. The residue tied in the muslin cloth was discarded. Water was filtered and evaporated till dry residue was obtained. The dried mass was kept in a desiccator and was used, as and when required for the experiment.

Animals

Healthy adult albino mice of either sex weighing 25-30 gm were used for the anti-nociceptive activity and healthy wistar albino rats of either sex (150-200 gm) were taken for anti-inflammatory activity. The animals were kept under standard conditions with 12 hrs light and 12 hrs dark cycles. Temperature was maintained at 22°C (±3°C). They were fasted for overnight before experimentation and water ad libitum. The study was conducted in the research laboratory of Institute of Pharmaceutical Research GLA University, Mathura, after obtaining ethical clearance from Institutional animal ethical committee (IAEC).

Anti-Nociceptive Activity

The anti-nociceptive activity of the aqueous extract of *Punica granatum* seed was studied in mice by Tail immersion method.

Tail immersion method

Swiss albino mice of either sex were utilized for this experiment. The animals were divided in to four groups, namely control, standard, extract treated at lower dose (100mg/kg body weight) and extract treated at higher dose (200mg/kg body weight) groups. The lower 5cm of tail was marked. This marked part of tail was immersed in water bath containing warm water at 55 ± 1°C. The reaction time of tail withdrawal was determined before and periodically after each administration. A cut off time of 15 seconds was fixed to avoid the tail injury. The reaction time was observed at intervals of 30, 60, 90, and 120 minutes of drug administration.

Anti – Inflammatory Activity

Carrageenan induced paw oedema model¹⁰

The crude plant extract were screened for anti-inflammatory activity by the Carrageenan induced rat's paw oedema method. Male albino

rats (150-200gm) were housed in groups of six in each. They were fasted over night before experimentation and water ad libitum. The extract was suspended in water and administered orally at the dose of 100 and 200mg/kg body weight. Paw volumes were measured by Plathysmograph.

Cotton pellet induced granuloma method¹¹

The cotton pellet induced granuloma model in rat shows good correlation between degree of activity and clinical usefulness in rheumatoid arthritis and other inflammatory diseases. The animals were divided in to four groups of six animals each (n = 6). After shaving the fur, the rats were anaesthetized and 20mg of sterile cotton pellets were implanted subcutaneously in to the both sides of the groin region of each rat. The extract at the dose level of 100mg/kg and 200mg/kg body weight administered orally to the treated group and Indomethacin at the dose level of 10mg/kg body weight was administered orally to the standard group and control vehicle as 0.3 ml of normal saline (0.9% w/v NaCl) was administered orally for 7 consecutive days from the day of cotton pellet implantation. On the 8th day the animals were anaesthetized by diethylether and cotton pellet were removed by surgically and made free from extraneous tissue. The pellets were then incubated at 37°C for 24 hrs and finally dried at 60°C to constant weight. Increment in the dry weight of the pellets was taken as measure of granuloma formation.

Anti-Ulcer Activity¹²

Indomethacin induced ulcer in rats

Six groups of albino rats weighing 150 – 250gm were used. Each group contains six animals. The test drug was administered orally 10 min. prior to oral Indomethacin in a dose of 20mg/kg. Six hrs later, the rats were sacrificed in ether anesthesia and their stomach removed. The stomachs were opened along the greater curvature, then washed in warm water and examined under a 3 fold magnifier. The lengths of the longest diameter of the lesions were measured and scored for each animal. The mean count for each group was calculated.

RESULT AND DISCUSSION

The aqueous extract of *Punica granatum* was shown to possess anti-nociceptive activity in the different models of nociception. In the present study, *Punica granatum* aqueous extract (100mg/kg) increases reaction time in tail immersion tests suggesting its central analgesic activity. The acetic acid induced writhing is widely used for the evaluation of peripheral anti-nociceptive activity.

In order to investigate the anti-inflammatory activity, two models, namely Carrageenan induced paw edema and cotton plate induced granuloma were taken. The extract showed significant activity in both the models. Carrageenan induced rat paw edema is a prototype of exudative phase whereas cotton pellet induced granuloma is a prototype of proliferative phase of inflammation. The anti-

ulcerogenic property of the extract was studied using Indomethacin induced ulceration in rats model.

CONCLUSION

In the present study, the aqueous extract of *Punica granatum* seed was shown to possess Anti-nociceptive as well as Anti-inflammatory activity in the models.

During extraction, the average percentage yield of aqueous extract of *Punica granatum* seed was found to be 3% w/v. The extract showed significant analgesic property in different models of nociception namely, Tail immersion and Acetic acid induced writhing model. On the other hand, the extract also exhibited optimal anti-inflammatory activity thirty minutes after administration. Further the ulcerogenic activity of the extract was assessed on wistar albino rats. The extract was found to be non – ulcerogenic in the experimental rats and safe even at the dose of 200mg/kg body weight.

In conclusion, it can be said that the aqueous extract of the ripped seed extract of *Punica granatum* has significant anti-nociceptive and anti-inflammatory activity without ulcerogenic effect. Further researches are also being carried out in our department to explore the phyto-chemistry and other pharmacological activities.

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Table 1- Effect of the aqueous extract of *Punica granatum* on Tail Immersion.

Treatment/Dose	Reaction Time in Seconds				
	0 Min	30 Min	60 Min	90 Min	120 Min
Control	3.35±0.278	3.42±0.085	3.52±0.179	3.14±0.184	3.65±0.137
Pentazocine (10 mg/kg s.c)	3.36±0.116	7.22±0.087**	9.47±0.167**	8.29±0.129**	7.68±0.165**
Extract (100 mg/kg p.o)	3.45±0.126	4.07±0.202*	5.61±0.213**	5.76±0.148**	4.36±0.210
Extract (200 mg/kg p.o)	3.53±0.098	4.67±0.203**	7.293±0.196**	6.31±0.132**	6.05±0.218**

Values are mean ± SEM; n=6 animals in each group. *P<0.05 and **P<0.01 when compared to control (by using one way ANOVA with Dunnett t- test).

Table 2- Effect of *Punica granatum* seed extract on Rats left hind paw edema induced by Carrageenan.

Group	Treatment (per kg body weight)	Mean Paw volume in ml (Mean ± SEM)			
		1 st h	2 nd h	3 rd h	4 th h
Group 1	Carrageenan control	0.41±0.013	0.60±0.025	0.72±0.012	0.58±0.027
Group 2	Extract 100 mg	0.37±0.008*	0.54±0.012**	0.60±0.012**	0.51±0.015*
Group 3	Extract 200 mg	0.36±0.009**	0.51±0.014**	0.54±0.009**	0.39±0.007**
Group 4	Indomethacin 10 mg	0.31±0.007**	0.44±0.007**	0.47±0.004**	0.35±0.004**

Values are mean ± SEM; n=6 animals in each group. *P<0.05 and **P<0.01 when compared to control (by using one way ANOVA with Dunnett t- test).

Table 3- Effect of the aqueous extract of *Punica granatum* on Cotton pellet induced granuloma.

Group	Treatment (per kg b. wt)	Mean wt of the granuloma in mg (Mean ± SEM)	% change in granuloma wt.
Group 1	Vehicle control	68.6±2.16	-
Group 2	Extract 100mg	65.5±1.87*	4.5
Group 3	Extract 200mg	52.0±1.79**	24.2
Group 4	Indomethacin 10mg	36.2±2.04**	47.2

Values are mean ± SEM; n=6 animals in each group. *P<0.05 and **P<0.01 when compared to control (by using one way ANOVA with Dunnett t- test).

Table 4:- Effect of the aqueous extract of *Punica granatum* ripped seed on acetic acid induced writhing (swiss albino mice).

Group	Treatment	No of writhings
Group 1	Vehicle control	17.5±0.46
Group 2	Extract 100mg/kg b.wt	14.3±0.30
Group 3	Extract 200mg/kg b.wt	10.8±0.61
Group 4	Standard	7.53±0.51

Table 5- Ulcerogenic effect of the aqueous extract of *Punica granatum* seed on Rats stomach.

Group	Treatment(mg/kg body wt)	Ulcerogenic activity (No. of ulcers)
Group 1	Vehicle control	Nil
Group 2	Extract 100mg	Nil
Group 3	Extract 200mg	Nil
Group 4	Indomethacin 20mg	09±0.77

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