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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF PUNICA GRANATUM PEEL

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ABSTRACT

Extract of **Pomegranate peel** was used for the present project work. The Successive extract was subjected to preliminary phytochemical testing for the detection of major chemical groups. **Anti-** Ethanolic **Inflammatory activity** of plant was determined using **Carregeenan induced paw edema model**. Four groups of mice were prepared, each group having five mices. First group was control group. 0.1 ml of 1% Carregeenan was given to this group. Second group was given standard Indomethacin 5gm/kg. Third group was given drug 200 mg/Kg and fourth group was given 400 mg/Kg of *P.Granatum peel Extract*. Four readings of paw were taken using Plethysmometer. There was significant reduction in paw volume in Indomethacine standard group ($p<0.05$) as compared to control group at 4 hour readings. There was dose dependent decrease in paw volume in ethanolic extract treated groups. The ethanol extract (200and 400 mg/kg) significantly ($p<0.05$) and dose-dependently inhibited carrageenan-induced rat paw edema (75.39 % and 80.57 %, respectively) when compared with control group after 1 hour of carrageenan injection.

KEYWORDS: Carregeenan, Paw edema volume, Anti-inflammatory activity, Indomethacin, Punica granatum.

1. INTRODUCTION

1.1 Disease[1-2]

Inflammation is a normal, essential, and protective response to any noxious stimulus that may threaten the host and may vary from a localized reaction to a complex response involving the whole organism. Many substances called mediators are formed or realized either concurrently or in successive time sequences at the site of injury. Various cell sources are responsible to an etiological factor. Various cells containing potent mediators and in some instances, inhibitors of the inflammatory response. These cell sources may include neutrophils, basophiles, mast cells, platelets, macrophages and lymphocytes. The mediators of inflammation implicated in the inflammatory process and elaborated by the foregoing cells include histamine, serotonin, plasmakinins, lymphocytes and prostaglandins. The process of inflammation can be summarized as follows.

Initial injury to tissues causing release of mediators, histamine, serotonin, prostaglandins

- An acute transitory phase with enhanced capillary permeability and local vasodilation.
- A delayed sub-acute phase, most prominently characterized by infiltration ofleukocytes and phagocytic cells.
- A proliferative stage that lasts for a long time and is marked by tissue fibrosis and degradation.

1.2 Plant profile

1.2.1 Punica granatum[3]

The most heart-healthy juice contender is pomegranate juice. It seems to shield the arteries and heart. Small studies have shown that the juice improves blood flow and keeps the arteries from becoming stiff and thick. It may also slow the growth of plaque and buildup of cholesterol in the arterie. Punica granatum, also known as the pomegranate, is a shrub that bears a red fruit. The pomegranate fruit, which is classified as a berry, has a diameter of approximately 5–12 cm (2–5 inches). It has a stalk in the form of a flower, is red, spherical, and somewhat resembles a red apple. Its belong to family Punicaceae.

Fig. 01: Dry pomegranate peel.

Table 1: Taxonomical hierarchy.[4]

Kingdom	*Plantae*
Division	*Magnoliophyta*
Binomial name	Punica granatum
Class	*Magnoliopsida*
Subclass	*Rosidae*
Order	*Myrtales*
Family	*Punicaceae*
Subfamily	*Punicoideae*
Genus	*Punica L.*
Species	*Punica granatum*

Traditional use [5]

Flowers, leaves, bark of young shoots and roots, fruit peel, and pomegranate sauce have been traditionally used. All of the Punica Granatum L fruit's tannin-rich components exhibit fairly potent astringent properties. Several infusions or decoctions of the plant flowers have been used in traditional medicine to treat simple diarrhea, vaginal discharge, and also this extract accompanied with pomegranate peel have usually been gurgled to relieve pancreas inflammation of the pacreas. To treat gallbladder problems, Punica granatum L fruit juice is advised. Strong tannin found in the fruit is thought to be a bitter food. Its decoction seems to be effective in treating conditions including common diarrhoea, dysentery, and stomach issues. Although pomegranate seeds don't have very high tannin content, they are frequently used to treat women's vaginal discharge and wound healing. Due to the presence of alkaloid compounds, pomegranate root bark, whether fresh or dried, or ethanol preparations, are used to eliminate intestinal parasites.

Therapeutic use [6]

Extracts of all parts of the pomegranate fruit exhibit therapeutic properties and target arangeof diseases including cancer, cardiovascular disorders, diabetes, male infertility, Alzheimer's disease , aging, and AIDS.

2. MATERIAL AND METHODS

2.1 Collection of *Punica Granatum* plant- Fresh pomegranate fruits were collected from local area of Gwalior M.P. then peel was separated and dried.

2.2 Authentication- Fruits of the selected plant's Punica *Granatum* collected from local market of purani chhawni area, Gwalior, M.P. Identified and authentication by **Prof. P.JAYARAMAN M.sc, Ph.d, Director, Plant Anatomy Research Center (PARC)** west Tambaram , Chennai -45 Reference : Nair, N.C & Henry, A.N. Flora of Tamil nadu, India and date of authentication 01/02/2021 , I : pg-167 .1983, Ibid II : - .1987 , ibid III .1989.

2.3 Chemical and Reagents: - Carrageenan, ethyl alcohol, acetone, chloroform, Mayer's reagent, Wagner's reagent, Dragendorff's reagent, Molisch's Reagent, Hager's reagent, Borntrager's Reagent, Benedict's Reagent.

2.4 Apparatus: - Plethysmometer, Soxhlet Apparatus, Electronic Balance, Heating Mantle, Petri Dish/ Glass rod, Funnel/ Measuring Cylinder.

2.5 Extraction process[7]

The hot continuous extraction technique is another name for extraction. Round bottom flask, syphon tube, distillation channel, condenser, cooling water inlet, cooling water exit, heat source, and thimble make up the Soxhlet extractor system. This procedure involves placing the sample in a porous bag or "thimble" made from a sturdy filter paper that is dried, powdered, and ground into fine particles. thimble chamber of the Soxhlet apparatus. This method is not suitable for thermolabile compounds as extended heating may lead to degradation of compounds. This method maintains a relatively high extraction temperature with heat from the distillation flask. A fine coarse powder was obtained

which was sieved through #40 to obtain uniformity. The powdered obtained was extracted in ethanol. To combat the crude medicine, petroleum ether or hexane were typically used to the powdered root. Defatting assists in removing the oily, fatty, and other low polar common components from the plants, making it easier to process them further with a yield of 8.87%. Two hundred grams of the powdered root was subjected to Soxhlet assembly in 60 % ethanol (200 g/1.5 L). The extract was then concentrated to dryness under pressure giving a dark greenish solid with a yield of 16.4 g (8.2%). The extracts were then made to powder under reduced pressure.

2.6 Qualitative analysis

Various biochemical components, such as reducing sugar, flavonoids, terpenoids, tannins, saponins, anthraquinones, glycosides, and alkaloids, among others, are analysed qualitatively.

2.6.1 Test for alkaloids[8,9]

Mayer's test: Extract treated with Meyer's reagent gives cream coloured precipitate which indicates the presence of Alkaloids**.**

Dragendorff's Test: Addition of Dragendorff's reagent (solution of Potassium Bismuth Iodide) to the extract gives reddish brown coloured precipitate.

2.6.2 Test for carbohydrates

Fehling's test: Extract on boiling with equal proportions of Fehling's A and Fehling's B solution gives yellow to brick red coloured precipitate.

Molisch's test: To the extract added few drops of Molisch's reagent and 1-2 ml conc. Sulphuric acid slowly through the sides of the test tube. When a violet ring forms at the junction, there are carbs present.

2.6.3 Test for proteins

Biuret Test: Extract on treatment with 4% Sodium hydroxide and 1% Copper sulphate solutiongives violet or pink colour.

2.6.4 Test for glycosides

Borntrager's Test: Extract when shaken with Benzene gently for few minutes. The organic layer on treatment with Ammonia, give rose pink color in Ammoniacal layer.

2.6.5 Test for saponins

Froth test: **20ml of distilled water was used to dilute the extracts, and this solution was agitated in a graduated cylinder for 15 minutes.**

2.7 Experimental animals

Studies were carried out using Wistar rats of either sex weighing 180–200 g. They were obtained from central animal facility of **Shri Ram College of Pharmacy, Banmore, M.P., India (SRCP/M.PHARMA/IAEC/65/19-20)** and was maintained in polypropylene cages on rodent pellet condition of controlled temperature (22±2^{o}C) and acclimatized to 12/12 h light/dark cycle. Free access to food and water was allowed until 2h before the experiment. The animals were maintained and cared for in accordance with accepted standards set by the "Commission for the purpose of control and supervision of experiments on animals (CPCSEA)". All experimentson animals were conducted according to the guidelines of establishment's ethical committee on animal experimentation.

The rats were randomly allocated into five groups of five rats each for the four Different experimental animal models. We have used Rat models for testing Anti-inflammatory.

2.8 Chemicals: Carrageenan, Standard drug (Indomethacin)

Table 2: Experimental design.

Group	Name of Group	Treatment	Dose
I	Control	Normal Saline	0.9ml/kg
II	Standard Drug	Indomethacin	5mg/kg
III	Test Group(A)	*P.Granatum Peel Extract*	200mg/kg.
IV	Test Group(B)	*P.Granatum peel Extract*	400mg/kg

3. RESULTS AND OBSERVATION

The plant material's phytochemicals were removed. The solvent, ethyl alcohol was used for the separation of chemical component.

3.1 Phytochemical screening[10-15]

Using a conventional screening test, the extract was checked for the presence of several elements. An established methodology was utilised to check for the presence of substances such as steroids, alkaloids, tannins, flavonoids, glycosides, etc

Table 3: Phytochemical screening.

S. NO	Test	Result
1	**Carbohydrates**	
I	Barfoed Test	+

II	Benedict Test	+
III	Fehling's test	+
IV	Molish Test	+
2	**Proteins**	
I	Biuret Test	+
II	Millon's Test	+
III	Ninhydrin Test	+
IV	Xanthoproteic Test	+
3	**Glycosides**	
I	Borntrager's Test	+
II	Killer-Killani test	+
III	Raymond's Test	+
4	**Saponins**	
I	Faom Test	_
II	Froth Test	_

(+) Indicates positive result, (–) Indicates negative result

3.2 Evaluation of Anti-Inflammatory Activity

- **Carrageenan induced paw edema in rats**

In the present study, anti-inflammatory activity was determined in wistar rats of either sex according to the method of Winter et al. All groups were injected with 1% carrageenan (in 1 % CMC) solution into the sub-plantar region of rat right hind paw. The first group is referred as Control Group as they received normal saline, whereas third and fourth groups received low dose (200mg/kg) and higher dose (400mg/kg) respectively of *punica granatum* through oral gavage. Second group served as standard, received Indomethacin (5mg/kg) through oral gavage. Before 1 hour of injecting of carrageenan the rats were treated with different doses of *punica granatum* Indomethacin and normal saline.The volume of paw oedema was measured by dislocation Displays the water column in an Orchid Scientific Plethysmometer after 1, 2, 3, and 4 hours after a carrageenan injection.

% of paw oedema inhibition is equal to Oc - Ot / Oc x 10. Oc represents the average increase in paw volume (average inflammation) of the rats in the control group at a given time.

O_t = was the average inflammation of the drug treated (i.e., plant extracts or test drug aspirin) rats at the same time. The difference in the initial 0h and volume at +1h indicate paw edema at 1h following carrageenan administration. Accordingly paw edema at +1, +2 +3, and +4 hours was calculated.

- **Plethysmometer**

A plethysmometer created for accurate and quick screening of tiny rodents for paw swelling or inflammation. The volume is determined by weight and specific gravity, based on the principles of Archimedes. The exact volume of the animal paw will be displaced when it is dipped into the water in the cell up to the pre-marked point on the paw. A finger restenables stable measurement to be made. It is designed to provide a highly useful tool in the measurement of small volume changes. This test is typically used to follow the evaluation of inflammatory response experimentally inducedin rodents and to screen potential anti-inflammatory or anti-oedema properties of pharmacological substances.

Fig. 2: Plethysmometer.

3.3 Evaluation

The average reaction time for each group that received therapy was calculated and contrasted with the results for each group before to treatment.Percentage increase in reaction time (I %), was derived, using the formula $I\% = \{(I_t - Io)/Io\} \times 100$, Where I_t = reaction time at time, t,

and Io = reaction time at time zero (0 h). The animals were subjected to the same test procedure at +1, +2, +3, +4 and +5 hr. after the administration of test/standard/control drug. Standard ($p<0.05$) as compared to control group at 4-hour readings. There was dose dependent decrease in paw volume in ethanolic extract treated groups. The Ethanolicextract (200mg/kg and 400mg/kg) significantly ($p<0.05$) and dose-dependently inhibited carrageenan- induced rat paw oedema (75.39 and 80.57 %, respectively) when compared with control group after 1 hour of carrageenan injection.

4. RESULTS

In carrageenan induced rat paw oedema there was significant reduction in paw volume in Indomethaci

Table 4: Effect of Ethanolic extract of punica granatum carrageenan induced rat paw oedema (Mean paw volume).

Treatment group	MPV 1st hour	MPV 2nd hour	MPV 3rd hour	MPV 4th hour
Control	0.88±0.09.	0.86±0.09.	0.8±0.21	0.79±0.95
Standard drug	0.31±0.96*	0.29±0.08*	0.2±0.10*	0.17±0.10*
P.Granatum(200)	0.56±0.07	0.48±0.10	0.36±0.15*	0.25±0.52*
P.Granatum(400)	0.41±0.09	0.32±0.08*	0.28±0.08*	0.2±0.48*

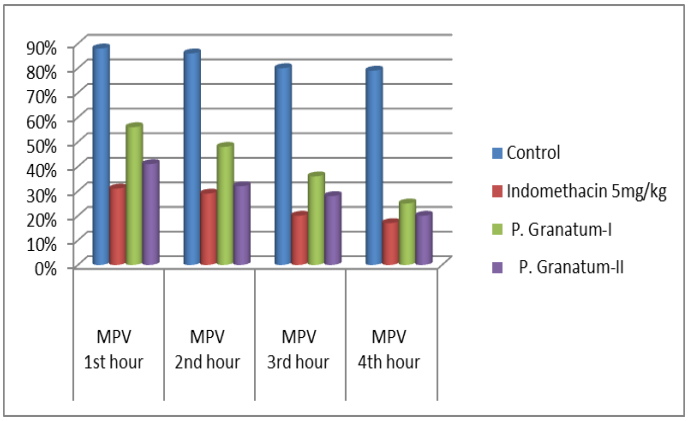

Fig. 3: Mean Paw volume (MPV).

Table 5: Effect of Ethanolic extract of *punica granatum carrageenan* induced rat paw edema (Percentage of inhibition).

Treatment group	(%) inhibition 1st hour	(%) inhibition 2nd hour	(%) inhibition 3rd hour	(%)inhibition 4th hour
Control	12%	14%	20%	22%
Standard drug	69.00%	71.00 %	80.00%	86.00 %
P.Granatum (200mg/kg)	44.33%	52.19 %	64.96 %	75.39 %
P.Granatum (400mg/kg)	59.34 %	68.62 %	72.20%	80.57

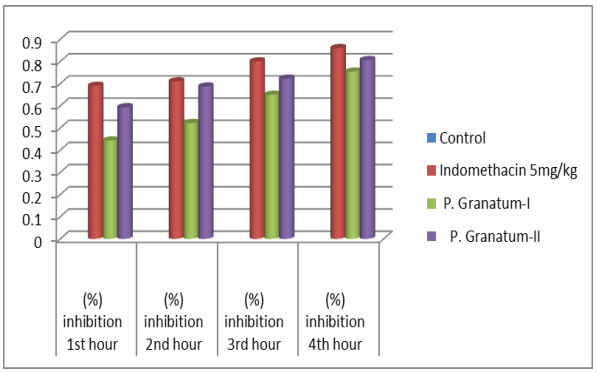

Fig. 4: Effect of Ethanolic extract of *punica granatum* carrageenan induced rat paw edema (Percentage of inhibition).

5. DISCUSSION

This method was chosen for the present study as edema induced by carrageenan is the most popular and prominent experimental model in the discovery of new anti- inflammatory drugs. The carrageenan induced rat paw edema is considered as the most suitable anti-inflammatory drugs both steroidal and nonsteroidal since it involves several mediators. In this study, an anti-inflammatory effect of ethanolic extract of *Punica Granatum* is investigated after sub-plantar injection of carrageenan in rat paw. This study showed that the crude extracts (ethanol) of *Punica Granatum* possess anti-inflammatory activities in rat model. The observation that the Ethanolic extract of *Punica Granatum* exhibited greater anti-inflammatory activity, more of the active principle responsible for the anti-inflammatory activity might be present in higher concentration in the ethanolic extract. One of the primary indicators of inflammation is the presence of edoema. In addition, it is a method that been frequently used to assess the anti edematous effect of natural products. Furthermore, carrageenan is devoid of apparent systemic effects and exhibits a high degree of reproducibility. Carrageenan injections locally cause two distinct phases of inflammatory responses. Though it is difficult to speculate the time of on set for anti-inflammatory activity, ethanol extract of the dried peel of *Punica Granatum* for anti- inflammatory activity showed rapid onset, i.e. starting from 1h. The duration, however, seems to be fairly long. To get full pictures of their pharmacokinetic profiles, further investigation should be undertaken. Both extracts and fractions of *Punica Granatum* showed persistent anti- inflammatory activity during the four hours observation time. This study may give hint about the onset and duration of action of the extracts.

6. CONCLUSION

The drug also has a significant anti-inflammatory activity; this study has shown that ethanol extract of *Punica Granatum* does possess prominent anti-inflammatory activities with high doses of the extract being more active. The results, thus, might support the traditional use of this plant in inflammatory process. Further investigation, however, should be pursued after isolation and characterization of the active principle in order to come up with the active compound responsible for the anti-inflammatory properties of the plant. Moreover, other studies should be performed to confirm the exact mechanism and anti-inflammatory activity of the plant in chronic inflammatory models.

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