Original Article

Experimental Evaluation of Anti-Inflammatory activity of Punica granatum peel extract in Albino Rats

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ABSTRACT

Aim: The aim of present study is to evaluate Anti-inflammatory activity of Punica granatum. L peel extract in albino rats.

Materials and Methods: Albino rats of either sex weighing between 150 grams to 200 grams were divided into five groups of six animals (30) i.e control(C), standard (S), Test-1 (T1), Test-2(T2) & Test-3 (T3). Different groups of rats had received dose calculated according to body weight. C group had received 5ml/kg of 2% gum Acacia suspension orally. Diclofenac suspension in 2% gum Acacia was given to the standard (S) group in the dose of 5mg/kilogram body weight, orally. T1 group had received 100mg/kg, T2 group had received 200mg/kg & T3 group had received 400mg/kg body weight, orally. After half an hour of drug administration, sub-plantar injection of Carrageenin was administered by a tuberculin syringe into the right hind paw of all rats. Right hind paw was marked by a marker pen, at the level of lateral malleolous and the paw was dipped into the mercury of the plethysmograph and paw volume was measured by mercury displacement method immediately, and after 1 hour, 2 hour, 3 hours of the sub-plantar injection of carrageenin. The difference of paw volume recorded at the beginning of the experiment and at the end of 1 hour, 2hour & 3 hour of carrageenin administration. Percentage inhibition of inflammation was calculated by formula % inhibition = Vc-Vt/Vc X 100.

Result : Result will be recorded and results will be analyzed by appropriate statistical methods.

Conclusion: The present study revealed that Punica granatum L peel extract show anti-inflammatory activity in albino wistar rats.

Keywords: Anti-Inflammatory, punica granatum L, diclofenac suspension, albino rats

INTRODUCTION

Inflammation is a complex reaction in vascularised connective tissue, which is elicited by some exogenous & endogenous stimuli causing cell injury. The term inflammation is derived from the Greekword Phlegmasia.[1]

In simplest terms inflammation is defined as a protective response intended to eliminate the initial cause of cell inury as well as the necrotic cell & tissue resulting from the original insult. The classical signs of inflammation are: pain (Dolar), Heat (Calor), Redness (Ruber), Swelling (Tumor), Loss of function (Function laesa).[2,3]

The term inflammation is derived from the Latin word – Inflammare, means burn. Any form of injury to the human body can elicit a series of chemical changes in the injured area. The cardinal signs of inflammation are heat, redness, swelling, Pain, & loss of function. Inflammation results in the liberation of endogenous mediators like histamine, serotonin, bradykinin, prostaglandins etc.[4]

The drugs mainly in use to treat the inflammation in the present are the steroidal and non-steroidal anti-inflammatory drugs (NSAIDs). But since these drugs have quite bad adverse effects when used to treat chronic inflammatory disease, there is a thrive to search for drugs with more safety and efficacy.[5] Since time immemorial,

herbal treatment has been the mainstay of treatment of various diseases by the Romans, Greeks, Chinese and Indians. With the development of science and advent of newer technologies, newer molecules are being continuously developed and the herbal products or extracts are not given the due importance and recognition. A definite need is visualized for the introduction of an effective anti-inflammatory agent free from adverse affects.

Pomegranate (Punica granatum L), in addition to its ancient historical applications, has been used in several systems of medicine for a variety of diseases and disorders. Pomegranate juice is regarded as a polyphenol-rich juice with high antioxidant capacity. Pomegranate juice has been shown to exert significant antiatherogenic, antioxidant, anti-carcinogenic, and anti-inflammatory effects in several human and marine models. Pomegranate is potent antioxidant, anti-carcinogenic, and antiinflammatory properties other potential applications include male infertility, infant brain ischemia, Alzheimer's disease, obesity, arthritis.

The aim of the present review is to discuss the cumulative evidence, which suggests that consumption of p omegranate possesses a diverse array of biological activities and may be helpful in the prevention of some inflammatory mediated diseases, including cancer. Analgesic and anti-inflammatory activity.[6]

Herbal medicine or phytomedicine is now attracting the world's attention as it enhances the health of the body systems without adverse side effects[7] Punica granatum L, commonly known as pomegranate, is a fruit bearing deciduous shrub or small tree, native to Asia and belongs to the family Punicaceae.[8]

Different parts of the plant such as bark, leaves, immature fruits and fruit rind (Peel) have medicinal significance.[9] Punica granatum is widely employed in various countries as a source of therapeutic agent against a variety of pathogenic microbes.[10]

It was utilized as a traditional remedy for thousands of years under the Ayurvedic system of medicine, with extracts from the rind of the fruit and bark of the tree being effective against diarrhea and dysentery.[11] Punica granatum L . is an ancient mystical fruit used in folkloric medicine as a treatment for many diseases such as diarrhea, parasitic worm infections, urinary tract infections, and kidney stones.[12]

Moreover, many studies indicate that Punica granatum.L can slow bacterial growth and inhibit bacterium-induced toxins.[13-16] Furthermore, Punica granatum.L peel extract (100 mg/kg) for 10 consecutive days had stimulated immune systems and enhanced cellular immunity in rabbits.[17] Several additional studies have demonstrated the therapeutic effects of Punica granatum.L fruit, peel, and juice as powerful antioxidants and anti-inflammatory substances that include polyphenols and tannins.[18-25] Punica Granatum. L also plays a role in protecting against inflammation.[26]

This study aims to evaluate anti-inflammatory activity of the peel extract of Punica granatum.L in normal rats as well as carrageenin induced rats.

MATERIALS AND METHODS

ANIMALS

Thirty Wistar rats of either sex weighing between 150 grams to 200 grams were selected from the animal house of the institution for the study. They were divided into five groups, control ©, standard (S), and test-1 (T1), test-2 (T2), and test-3 (T3). They were numbered 1-6 for each group and kept in separate cages and fed regularly in the animal house.

DRUGS

Preparation of extract:

Fresh pomegranates (1 kg) were collected from local market (Karimnagar, Telanagana, India). These were identified and Authenticated by the Department of Botany, SRR Govt College, Karimnagar. The Authenticated pomegranate fruit peel was used for the preparation of Extract.

In brief, pomegranate peels were collected and air dried in shade at room temperature. The dried fruit peel was crushed by using mortar and pestle and extraction was carried out in soxhlet apparatus using 50% ethanol. The extract was evaporated under reduced pressure and a brown mass was obtained.

Experimental design

The study was approved by Animal Ethics Committee (AEC) of the Institution and the experiments were carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest Government of India. The method employed here to study the anti-inflammatory activity is: Carrageenin induced rat paw oedema method.[28]

Procedure

Thirty Wistar rats of either sex weighing between 150 grams to 200 grams were selected from the animal house of the institution. They were starved over-night with water ad libitum prior to the day of experiment. The rats were divided into five groups of six animals each control

(C), standard(S), test-1 (T1), test-2 (T2) and test-3 (T3). They were weighed and numbered on their tails 1-6 for each group and kept in labeled cages separetely.

Prior to the experiment, Toxicity tests of the experimental drug had done. Also, the animals were given the experimental drug in graded doses to find out the effective dose. Accordingly 3 doses were chosen: T1 – Sub-effective dose, T2 effective dose & T3 Supra-effective dose. Different groups of rats received dose of drug calculated according to body weight of animals.

Control (C) group received 5ml of 2% gum Acacia suspension orally. Diclofenac suspension in 2% gum Acacia was given to the standard (S) group in the dose of 5 milligrams/kilogram body weight, orally.

Punica granatum peel extract suspension is adiministraed along with 2% gum Acacia was administered to test groups into three divided doses i.e., test-1 (T1) 100mg/kg, test-2 (T2) 200g/kg, and test-3 (T3) 400mg/kg body weight, orally. After half an hour of drug administration, sub-plantar injection of Carrageenin was administered by a tuberculin syringe into the right hind paw of all rats.

Right hind paw was marked by a marker pen, at the level of lateral mallelous and the paw was dipped into the mercury of the plethysmograph and paw volume was measured by mercury displacement method immediately, and after 1 hour, 2 hour, 3 hours of the sub-plantar injection of carrageenin.

The difference of paw volume recorded at the beginning of the experiment and at the end of the 3 hour carrageenin administration has been taken. The measured paw volume of T1, T2, T3 was compared to that of Control C & Standard S groups.

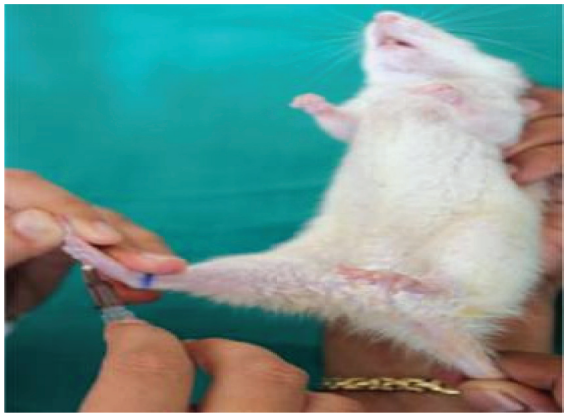

Figure 1: Sub-Plantar injection of rat hind paw

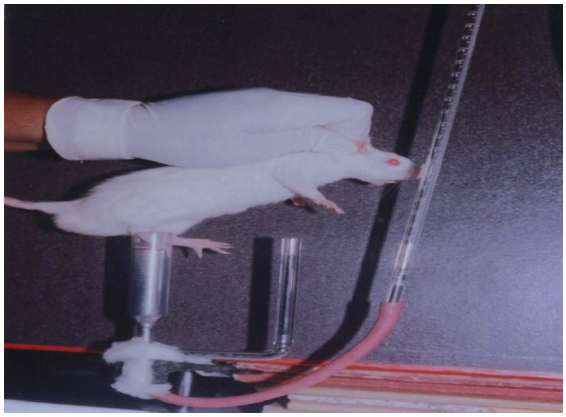

Figure 2: Showing dipping of rat right hind paw into the mercury

Percentage inhibition of inflammation was calculated by using formula

% inhibition = Vc-Vt/Vc X 100

Where,

Vc = mean volume of the paw oedema in control animals.

Vt = mean volume of the paw oedema in the drug treated group.

OBSERVATIONS AND RESULTS

In the present study, the anti-inflammatory effect of punica granatum was compared with standard non-steroidal anti-inflammatory drug - Diclofenac and control groups in laboratory Wistar rats.

The study was carried out by: Carrageenin induced rat paw oedema Method: In this, anti-inflammatory effect ofPunica granatum was compared with Diclofenac and control group on rat paw oedema.

The doses administered in this method were 5 ml of 2% gum acacia as control (C), 5mg/kg body weight of diclofenac as standard (S), 100mg/kg body weight as test-1 (T1), 200mg/kg body weight as test-2 (T2) and 400mg/kg body weight as test-3 (T3) of Punica granatum as test.

Statistically, the average of six animals, rat paw oedema volume in ml measured by mercury displacement in the plethysmograph in control group was 1.41±0.20, in Diclofenac group was 1.00±0.19, and in Punica granatum groups i.e., T1 was 1.06±0.0675, T2 was 1.02±0.1074, and T3 was 1.01±0.0509 (Table & Graph).

Table 1: Effect of Drugs on Carrageenin induced by Rat Paw Oedema

	Mean Volume			Percentage Inhibition		
Groups	After 1hrs	After 2hrs	After 3 Hrs	After 1hr	After 2 Hrs	After 3hrs
Control – C (2% Gum Acacia)	1.46 ±0.32	1.35±0.16	1.41±0.20	0%	0%	0 %
Standard – S (Diclofenac 100mg/kg)	1.02±0.22	1.02±0.22	1.00±0.19	28.64%	29.45%	31.03%
Test T1 (Punica granatum 100mg/kg)	1.10±0.13	1.06±0.15	1.06±0.0675*.	19.08%	22.58%	24.08%
Test T2 (Punica granatum 200mg/kg)	1.06±0.10	1.04±0.10	1.02±0.1074*	23.71%	25.36%	26.53%
Test T3 (Punica granatum 400mg/kg)	1.04±0.09	1.03±0.18	1.02±0.0509**	24.65%	27.91%	29.65%

n = 6 in each group *P<0.05 – significant, ** P<0.001 Highly significant

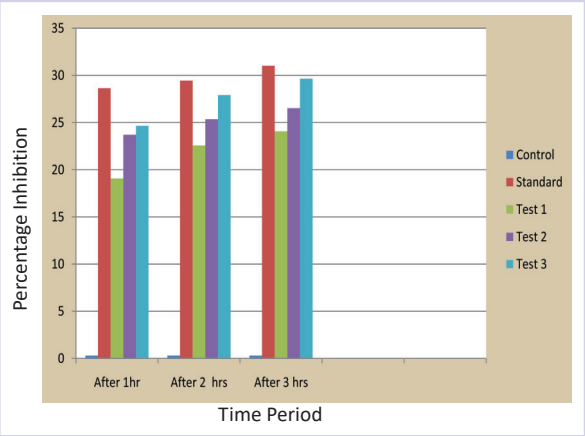

Figure 1: Carrageenin Induced Rat Paw Oedema Ercentage Inhibition

The percentage inhibition of rat paw oedema for Diclofenac was 31.03%, and for Punica granatum peel extract as T1 was 24.08%, T2 was 26.53%, and T3 was 29.65%.

The statistical "P" values of rat paw oedema in the carrageenin induced rat paw oedema method was significant for Diclofenac as standard (P<0.001), mild significant as test-1 (P<0.01), significant in test-2 and test-3 (P<0.001) groups of animals.

DISCUSSION

In the present study, the anti-inflammatory activity of Punica granatum was compared with non-steroidal anti-inflammatory drug Diclofenac and control group of animals were done by using Wistar rats. The experimental models employed for the present study were:

In this method carrageenin is used as phlogistic agents in inducing inflammation of rat paw oedema in rats. It is a known fact that in oedema there is accumulation excess fluid in the intercellular spaces. This phenomenon was applied to study the anti-inflammatory activity of an agent in rat paw oedema. Here, carrageenin was used as phlogistic agent to induce the inflammation. Inflammation, thus, produced in rat paw is equated with the rheumatoid arthritis in man.

To test the variance and statistical significance the Student "t" test was used for comparison between each group and to time interval.

In all groups swelling was seen after administration of carrageenin with the mean increase in paw volume immediately. The percentage inhibition of paw oedema in carrageenin induced rat paw oedema method in Punica granatum i.e., test-1 (T1) 24.08%, test-2 (T2) 26.53%, test-3 (T3) 29.65%, and Diclofenac (S) 31.03%. The antiinflammatory activity demonstrated by Punica granatum was mild significant when compared to diclofenac.

The statistical "P" value of rat paw oedema were highly significant in Diclofenac and mild significant in Punica granatum T1 group of animals and significant in Punica granatum T2 and T3 groups.

CONCLUSION

Inflammation is a series of events occurring in orderly sequence in the tissues in response to injurious stimulus. It can be evoked by a wide variety of noxious agents (e.g., infections, antibodies, physical injuries). The ability to mount an inflammatory response is essential for survival in the face of environmental pathogens and injury in some situations and diseases, the inflammatory response may be exaggerated and sustained without apparent benefit and even with severe adverse consequences.

No matter what the initiating stimulus, the classic inflammatory symptoms include calor (warmth), dolor (pain), rubor (redness), and tumor (swelling). The goal of anti-inflammatory drugs is to reduce any of these classical symptoms. Hence, a definite need is visualized for the induction of an effective anti-inflammatory agent free from adverse effects.[29] Punica granatum in the doses 100mg/kg, 200mg/kg and 400mg/kg and Diclofenac in

5mg/kg has shown anti-inflammatory activity when compared to control group. The percentage inhibition of paw oedema in carrageenin induced rat paw oedema method in Punica granatum i.e., test-1 (T1) 24.08%, test-2 (T2) 26.53%, test-3 (T3) 29.65%, and in Diclofenac (S) 31.03%.

Punica granatum has dose dependent anti-inflammatory activity in carrageenin induced rat paw oedema. The anti-inflammatory effect of Punica granatum is significant & is similar to anti-inflammatory activity of Diclofenac in carrageenin induced rat paw oedema.

Punica granatum 100mg/kg showed mild anti-inflammatory activity where as 200mg/kg & 400mg/kg showed significant dose dependend anti-inflammatory activity while standarad drug Diclofenac is highly effective in reducing carrageenin induced rat paw oedema.

Results of pomegranate showed a favorable anti-inflammatory activity could be used for prevention and treatment of several inflammatory painful conditions as osteoarthritis, rheumatoid arthritis, and lung pleurisy. Furthermore, clinical trials needed for investigating anti-inflammatory effects of pomegranate to signify its therapeutic uses on human clinically.

CONFLICT OF INTEREST :

The authors declared no conflict of interest.

FUNDING : None

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