

Original Research Article

A scientific validation of *In vitro* anti-inflammatory activity of *Punica granatum L.* by human red blood cell membrane stabilization

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ABSTRACT

Background: In recent years there has been growing interest in therapeutic use of natural products, especially those derived from plants. *P. granatum* is very common dietary ingredient in many parts of India and has remarkable biological and medicinal properties.

Methods: In the present study, the methanolic extract of fruit peels of *Punica granatum Linn.* (MEPG) were investigated for anti-inflammatory activity by simple, reliable, less toxic and less time consuming HRBC membrane stabilization method. The presentation of hypo tonicity induced HRBC membrane lysis was taken a measure of anti-inflammatory activity. Their activities were compared with standard drug diclofenac.

Results: The results of the study demonstrated that *P. granatum* contains active constituents, which possess anti-inflammatory activity which is probably related to the inhibition of prostaglandin synthesis.

Conclusions: It is concluded that methanolic extract of *P. granatum* fruit peel possesses significant anti-inflammatory activity and this is a possible rationale for its folkloric use as an anti-inflammatory agent.

Keywords: Anti-inflammatory, HRBC membrane stabilization, Methanolic extract, *Punica granatum Linn*

INTRODUCTION

The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine.¹ There is an increasing demand for the medicinal plants in developing countries like India. Attention needs to be given to assess the medicinal value of such plants to explore the potential drugs out of it.

Inflammation is the reaction of living tissues to injury, infection or irritation. Lysosomal enzymes released during inflammation produce a variety of disorders which leads to the tissue injury by damaging the macromolecules and lipid peroxidation of membranes which are assumed to be responsible for certain

pathological conditions as heart attacks, septic shocks and rheumatoid arthritis etc. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. Stabilization of lysosomal membrane is important in limiting the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release or by stabilizing the lysosomal membrane.²

HRBC or erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes.³ Stabilization of human red blood cell membrane (HRBC) by hypo tonicity induced membrane lysis can be taken as

an in vitro measure of anti-inflammatory activity of the drugs or plant extracts.

Most clinically important medicines belong to steroidal or non-steroidal anti-inflammatory chemical therapeutics for treatment of inflammation-related diseases. Though these have potent activity, long-term administration is required for treatment of chronic disease. Furthermore, these drugs have various and severe adverse effects. Therefore, naturally originated agents with very little side effects are desirable to substitute chemical therapeutics.⁴ Currently, the use of natural antioxidants present in food and other biological materials has attracted considerable interest due to their presumed safety, nutritional and therapeutic value. Although several plants have gained importance for the nutritional and therapeutic values, many remain to be scientifically investigated.⁵ One such plant is *Punica granatum* Linn. The fruit of *Punica granatum* Linn. is commonly known as pomegranate, belonging to the family Lythraceae has tremendous nutritional and medicinal values including treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, infant brain ischemia, Alzheimer's disease, male infertility, arthritis and obesity.⁶⁻⁸

P. granatum flowers, fruit peel and leaves have been used to treat mild pyrexia, visceral pain, gastritis and diarrhea.^{9,10} The methanol extract of *P. granatum* seed and dried peels have antidiarrheal activity and the rind extract has been shown to have gastroprotective activity through an antioxidant mechanism.^{11,12} Phytochemical analysis of *P. granatum* fruit peel extract revealed the presence of various biochemical compounds such as alkaloids, flavonoids, tannins, anthocyanes, triterpenoids, sterols and/or terpenes, quinones and saponin compounds.¹³ Since triterpenoids and flavonoids have remarkable anti-inflammatory activity, so our present work aims at evaluating the in vitro anti-inflammatory activity of methanolic extract of *P. granatum* fruit peel by HRBC membrane stabilization.

METHODS

Plant materials

The fruits of *P. granatum* Linn. were collected in the month of April 2015 from the local fruit market of Udaipur and authenticated by Botanist Dr. Kiran Tak, Department of Botany, Govt. Meera Girls College, Mohanlal Sukhadia University, Udaipur.

Preparation of plant extract

The preparation of the extract of the fruit of *P. granatum* Linn. was done in the Department of Pharmacology, Geetanjali Medical College and Hospital, Udaipur. The shaded dried and coarsely powdered fruit peel (200g) was extracted with analytical grade methanol at 60°C for 12 hrs using a Soxhlet apparatus. The extract was

concentrated to dryness under reduced pressure at 40±5°C until it became a brownish solid residue. The percentage of yield (w/w) is 26% respectively. Extract was stored as dried powder at 4°C.

Preliminary phytochemical screening

The methanolic extract was qualitatively tested for the presence of various phyto constituents using the following reagents and chemicals according to the methods described by Farouk et al.¹⁴

HRBC membrane stabilization method

The human red blood cell membrane stabilization method (HRBC) has been used as a method to study the invitro anti-inflammatory activity.¹⁵ Blood was collected from healthy human volunteer who was not taken any NSAIDs for 2 weeks prior to the experiment. Study was commenced with due per-mission of the Institutional ethics committee and written consent from the subjects. The collected blood was mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000rpm for 10 min and packed cells were washed three times with isosaline (0.85%, pH 7.2). The volume of the blood was measured and reconstituted as 10% v/v suspension with isosaline.^{16,17}

The assay mixture contains 1ml phosphate buffer [P^H 7.4, 0.15 M], 2ml hypo saline [0.36%], 0.5ml HRBC suspension [10% v/v] with 0.5ml of plant extracts and standard drug diclofenac sodium of various concentrations (50, 100, 250, 500, 1000, 2000µg/ml) and control (distilled water instead of hypo saline to produce 100% hemolysis) were incubated at 37°C for 30min and centrifuged respectively. The hemoglobin content in the suspension was estimated using spectrophotometer at 560 nm.¹⁸

The percentage of hemolysis of HRBC membrane can be calculated as follows:

$$\% \text{Hemolysis} = (\text{Optical density of Test sample} / \text{Optical density of control}) \times 100$$

The percentage of HRBC membrane stabilisation can be calculated as follows:

$$\% \text{Protection} = 100 - [(\text{Optical density of test sample} / \text{Optical density of control}) \times 100]$$

RESULTS

Phytochemical study

The preliminary phytochemical investigation of the MEPG revealed the presence of tannins, flavonoids, glycosides, saponins, triterpenoids and phenolic compounds.

Anti-inflammatory activity

Methanolic extract of *P. granatum* was exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte

membrane is analogous to the lysosomal membrane and its stabilization indicates that the extract may also well stabilize lysosomal membranes membrane is shown in Table 1. It possesses significant activity comparable with that of the standard diclofenac sodium.^{21,22}

Table 1: Effect of *P. granatum* and standard drug on HRBC membrane hemolysis and membrane stabilization.

Conc. (µg/ml)	% Hemolysis of <i>P. granatum</i>	% Stabilisation of <i>P. granatum</i>	% hemolysis of diclofenac sodium	% stabilisation of diclofenac sodium
50	36.54	63.38	35.32	64.77
100	24.16	75.85	23.12	76.92
250	18.72	81.15	16.37	83.65
500	15.65	84.43	12.58	87.41
1000	9.44	90.52	7.26	92.65
2000	5.38	94.59	2.85	97.12

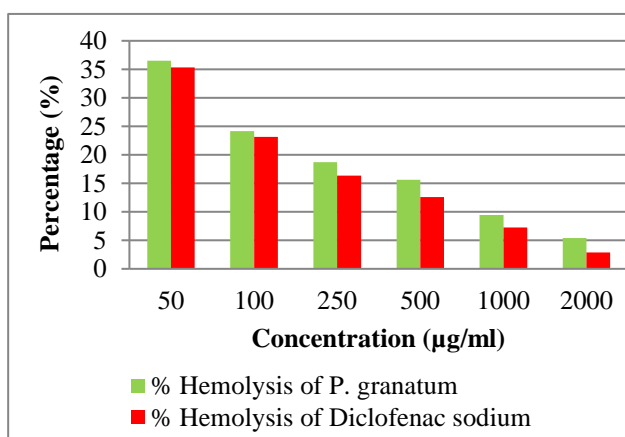


Figure 1: Effect of *P. granatum* (MEPG) on HRBC membrane hemolysis.

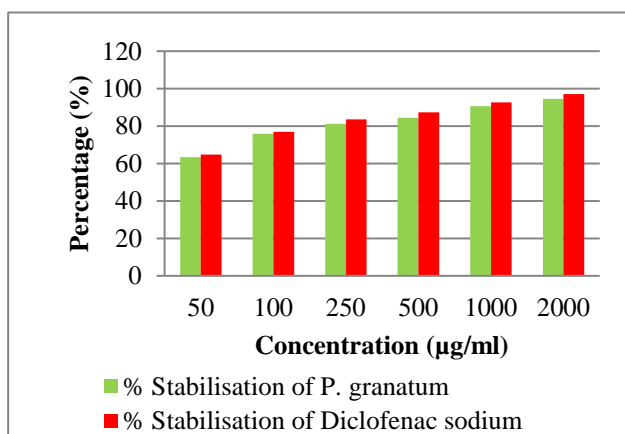


Figure 2: Effect of *P. granatum* (MEPG) on HRBC membrane stabilization.

In vitro anti-inflammatory activity of methanol extract of *P. granatum* (MEPG) were concentration dependent, the maximum protection of 94.59% was seen at the concentration of 2000µg/ml. All results were compared

with standard diclofenac sodium which showed 97.12% protection at the concentration of 2000µg/ml is shown in Figure 1 and 2.

DISCUSSION

The lysosomal enzymes released during inflammation produce a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The main action of anti-inflammatory agents is the inhibition of cyclooxygenase enzyme which is responsible for conversion of arachidonic acid to prostaglandins.¹⁹ The non-steroidal drugs (NSAIDs) act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membranes by means of inhibiting the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes (cyclooxygenase) and proteases, which cause further tissue inflammation and damage upon extra cellular release or by stabilizing the lysosomal membrane.²⁰ Furthermore, chronic use of these drugs have various and severe adverse effects.

Therefore, naturally originated agents with very little side effects are desirable to substitute chemical therapeutics. The investigation is based on the need for newer anti-inflammatory agents from natural source with potent activity and lesser side effects as substitutes for chemical therapeutics. Realizing the fact this study was carried out to evaluate the *in vitro* anti-inflammatory activity of methanolic extract of *P. granatum* the fruit peel (MEPG) in this direction. Results of the study is obtained that the methanolic extract of *P. granatum* was exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane in concentration dependent manner It is due to the presence of active principles such as flavonoids and triterpenoids may responsible for this activity. Hence, *P. granatum* can be used as a potent anti-inflammatory agent. Nevertheless, further studies needed to substantiate these findings.

CONCLUSION

In conclusion, the present study has shown that the fruit peel of *P. granatum* have membrane stabilization effect by inhibition of hypo tonicity induced lysis of erythrocyte membrane. Hence, it implies the anti-inflammatory and analgesic properties mediated by prostaglandin synthesis inhibition. Membrane stabilization may contribute to the anti-inflammatory effect. Further research on isolation of the active principle for anti-inflammatory activity has to be worked out in future studies.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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