www.sphinxsai.com

PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.2, pp 1260-1263, April-June 2010

Evaluation of Anti-inflammatory and Membrane Stabilizing Properties of various extracts of *Punica granatum* L.(Lythraceae)

***G. Prakash Yoganandam[1], K.Ilango[2], Sucharita De[3]**

[1]Department of Pharmacognosy,

[2]Department of Pharmaceutical Chemistry,

[3]Department of Pharmaceutical Analysis,

SRM College of Pharmacy, SRM University, Kattankulathur – 603 203,

Kancheepuram Dist. Tamil Nadu, India.

Corres. author:glmsp2003@yahoo.co.in

ABSTRACT: The present study was undertaken to evaluate the efficiency of various extracts of fruit peel of *Punica granatum*.L (Fam: Lythraceae) for anti-inflammatory activity by simple, reliable, less toxic and less time consuming HRBC membrane stabilization method. Since HRBC membrane is similar to lysosomal membrane which influence in the process of inflammation. Water, ethanol, methanol and ethyl acetate extracts of peel of *P.granatum* were subjected to check the stabilization of HRBC membrane to predict the anti-inflammatory activity. Among the extract screened the methanol and ethyl acetate extracts exhibited better activity ($P<0.001$) than the other two extracts which may be due to the presence of higher phenolic content estimated by Folin-ciocalteu reagent. These observations will stimulate further research in the field of phytochemistry and also in clinical application of the phytochemical constituents of *P.granatum*.L.

Key words: *Punica granatum*.L, Anti-inflammatory, HRBC membrane stabilization, Folin-ciocalteu reagent.

INTRODUCTION

Plants are the main stay of medicine and credited with mystical and almost supernatural powers of healing. Mankind is almost totally dependent on plants for their basic requirements[1]. Herbs play a significant role especially in modern medicines. They are now being increasingly used in cosmetics, food, tea as well as alternative medicines. The growing interest in herb is based on the belief that the plants have a vast potential for use as a curative medicine and the basis that "natural" equal 'harmless' and; synthesis equal 'toxic' and 'dangerous'[2]. The fruit of *Punica granatum*.L 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, alzhemer's disease, male infertility, arthritis and obesity [3-5]. But most of the time especially in the use of juice extraction the peel of the fruit has been considered as a waste product since it is unpalatable. From the literature review, it was noticed that the peels of *P.granatum* possess various pharmacological activities and also considering the use of fruit in indigenous medicines, the present study was undertaken to evaluate its efficiency in the pain and inflammation.

MATERIALS AND METHODS

Preparation of various extracts of peels of *P.granatum*

The plant material was purchased from the local market and it was identified and authenticated by Prof. P. Jayaraman, Taxonomist, Plant Anatomy Research Centre (PARC), Chennai (No: PARC/2009/259/PG). From the collected fruits, the

peels were separated and dried in shade for one week. Then the shade dried peels were pulverized to get a coarse powder (sieve no: 60) by using a wearing blender. After dewaxing with n-Hexane the coarsely powered material was extracted successively with ethyl acetate, methanol and ethanol in a Soxhlet assembly. Each time before extracting with next solvent, the powdered material is dried in hot air oven below 50^0C. Finally it was extracted with water in a closed jar under cold maceration process. Each extract was concentrated by distilling the solvent and then evaporation to dryness on a water bath. The extract obtained with each solvent was weighed and its percentage yield was calculated in terms of dried weight of the plant material.

Preliminary phytochemical analysis

The peel extracts of *P.granatum* were subjected to various qualitative chemical tests for identification of its plant constituents by following standard procedure according to JB.Harborne[6]. The ethyl acetate, methanol, ethanol and aqueous extracts were subjected to thin layer chromatographic studies in different solvent system. The TLC plates were made with silica gel-G and activated. The extracts were spotted by means of micro pipette and dried, developed in the following solvent systems 1, 2, 3, 4 and 5 separately[7-8].

1. Benzene : chloroform (1:1)
2. n-Butanol : acetic acid : water (4:1:5)
3. Benzene : acetic acid : water (125:72:3)
4. Chloroform : methanol (9:1)
5. Toluene : ethyl acetate : formic acid (5:4:1)

The different spots developed in each solvent system were identified by means of iodine chamber, UV light and 5% alcoholic KOH. The R_f values were calculated and the best solvent system for separating the constituents was identified.

Estimation of total phenolic content

Each of the 100µl of extract was taken in to 25ml volumetric flask, to which 10ml of water and 1.5ml of Folin-ciocalteu reagent were added. The mixture was kept for 5 minutes and 4ml of 20% w/v sodium carbonate solution was added and the volume was made up to 25ml with double distilled water. The mixture was kept for 30 minutes until blue color develops. The resulting solution was measured at 765nm using UV-visible spectrometer (Shimadzu, UV-1601, Japan). The % of total phenolic content was calculated by using gallic acid as standard[9].

HRBC membrane stabilization method

The anti-inflammatory activity of various extracts of peel of *P.granatum* was assessed by *in vitro* HRBC membrane stabilization method. Blood was collected from healthy volunteers. The collected blood was mixed with equal volume of Alsever's solution (Dextrose 2%, Sodium citrate 0.8%, Citric acid 0.05%, Sodium chloride 0.42% and Distilled water 100 ml) and centrifuged with isosaline. To 1 ml of HRBC suspension equal volume of test drug in three different concentrations was added. All the assay mixtures were incubated at 37^0C for 30 minutes and centrifuged. The haemoglobin content in the supernatant solution was estimated by using spectrophotometer at 560nm. The percentage of haemolysis was calculated by using the following formula,[10-11]

Percentage of haemolysis $= \frac{OD\ of\ test}{OD\ of\ control} X100$

The percentage of HRBC membrane stabilization or protection was calculated by using the following formula,

Percentage protection $= 100 - \frac{OD\ of\ test}{OD\ of\ control} X100$

RESULTS

The extractive values were found to be 4.09 % w/w, 6.76 % w/w, 14.4% w/w and 19.70% w/w for ethyl acetate, methanol, ethanol and water respectively. The preliminary phytochemical screening of these extracts revealed the presence of carbohydrates, glycosides, saponins, tannins and flavonoids (Table 1). The TLC analysis of various extracts of peel of *P.granatum* under various solvent systems 1-5 shows the presence of solutes and R_f values were calculated. In that solvent system benzene, acetic acid, water in the ratio of 125:72:3 shows good separation and gave many numbers of compounds when compared to the rest of the solvent syatems. The total phenolic content of extracts were 195µg/ml, 220µg /ml, 310µg /ml and 495µg /ml for water, ethanol, ethyl acetate and methanol respectively determined by Folin-ciocalteu method using gallic acid as reference standard. The order of phenolic content of each extracts were methanol > ethyl acetate> ethanol>water.

Anti-inflammatory activity

The lysosomal enzyme released during inflammation produces a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The non-steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. Since HRBC membrane is similar to lysosomal membrane, the study was undertaken to check the stability of HRBC membrane by these four extracts to

predict the anti-inflammatory activity. The various extracts at the concentration of 100, 200 and 300 µg/ml were incubated separately with HRBC solution and the % of haemolysis was compared with standard drug Diclofenac sodium at the same concentration. The methanol and ethyl acetate extracts at a concentration of 300 µg/ml exhibited significant % protection ($p<0.001$) when compared with control. These results may be attributes due to the presence of high phenolic content in the two extracts (Table 2).

Table No.1. Preliminary phytochemical screening of various extracts of fruit peel of *P.granatum*.L

S. No	Constituents	Water	Ethanol	Methanol	Ethyl acetate
1	Carbohydrate	+	+	+	-
2	Phytostrol	-	-	-	-
3	Fixed oil	-	-	-	-
4	Alkaloid	-	-	-	-
5	Glycoside	-	+	+	-
6	Saponin	+	-	-	-
7	Flavonoid	-	-	+	+
8	Tannin	-	+	+	+

(+) = Present, (-) = Absent.

Table No.2 Anti-inflammatory activity of various extracts of fruit peel of *P.granatum*.L by HRBC membrane stabilization method

S. No	Name of the drug	Concentration		
		100µg/ml	200µg/ml	300µg/ml
1	Water extract	46.76±2.03(49.24)	41.18±1.06(58.82)	36.26±2.05(69.74)
2	Ethanolic extract	39.12±1.02(60.88)	34.55±1.19(65.45)	30.76±0.06(69.24)
3	Methanolic extract	08.75±0.03(91.25)*	05.16±1.11(94.84)*	00.74±2.04(99.26)*
4	Ethyl acetate extract	12.27±0.62(87.73)*	04.26±0.98(95.84)*	02.87±1.54(97.13)*
5	Diclofenac sodium	2.87±1.20(97.13)*	2.38±0.90(97.62)*	0.97±0.54(99.03)*

n=3, Values are given ± SEM. The values given within the brackets are % protection of HRBC membrane. *P<0.001 considered as significant.

DISCUSSION

Pain is a symptom of many disease requiring treatment with analgesics, it is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Pain can also be elicited by inflammation. Progress has been made in elucidating the role of various endogenous substances such as prostaglandins and peptides in the inflammatory process. Most of the so called non-steroidal anti-inflammatory agents have analgesic activity. Some plant active constituents also possess anti-inflammatory activity[12-13]. In the present investigation an authenticated fruit of *P.granatum* was extracted with ethyl acetate, methanol, ethanol and water successively after defatted with n-Hexane. Using these extracts the anti-inflammatory activities have been performed by *in vitro* HRBC membrane stabilization method. Among the extracts screened for pharmacological activity, the methanol and ethyl acetate extracts showed better results may be due to the presence of higher phenolic content than the other two extracts. The preliminary phytochemical investigation of the peel extracts shown the presence of tannins, flavonoids, glycosides, saponins and phenolic compounds. The TLC analysis under the solvent system benzene, acetic acid and water (125:72:3) shows two distinct spots. These observations will stimulate further research in the field of phytochemistry and also in the clinical application of phytochemical constituents of *P.granatum*.

REFERENCES

1. Kirtikar K.R., and Basu B.D., Indian Medicinal Plants.Vol-I, 2nd edn, International Book Distributors, Dehra Dun, India.2005, XVII-XXII.
2. Vinod D.Rangari., Pharmacognosy and Phytochemistry, 1st edn, Part-I, Career Publications, Nashik.2002, 1-8.
3. Ram P. Rastogi., Mehrotra B.N., Compendium of Indian Medicinal Plants. Vol-I(1960-69), CDRI Lucknow and Publication & Information Directorate, New Delhi 1995, 335.
4. Asolkar L.V., Kakkar K.K., Chakre O.J., Glossary of Indian Medicinal Plants with Active Principles. Part-I, Publication & Information Directorate, New Delhi, 1992.
5. Gamble J.S., Flora of Presidency of Madras. Part-IV, Secretary of state for India in council, London.1921.
6. Harborne J.B., Phytochemical Methods, Chapman and Hall, London, New York, 1928,132-133.
7. Anonymous., Quality control methods for medicinal plant materials, WHO, Geneva. AITBS Publishers & Distributors, New Delhi, 2004, 22-28.
8. Beckett A.H., Stenlake J.B., Practical Pharmaceutical Chemistry. Part-2, 4th Edn , CBS Publishers & Distributors, New Delhi, 2004,115-127.
9. Ashvin V.D., and Mishra S.H., *Invitro* anti-oxidant activity of adoptogenic homeopathic formulations, Pharmacognosy magazine, 2007, 3(10), 124-128.
10. Varadarasu M., Mounisamy S., Kavimani V., Balu S., Darlin Quine., Evaluation of anti-inflammatory and membrane stabilizing properties of ethanol extract of *Cansjera rheedii* J. Gmelin (Opiliaceae), Iranian Journal of Pharmacology and Therapeutics, 2007, 235-237.
11. James O., Nnacheta O.P., Wara H.S., Aliyu U.R., Invitro and invivo studies on the anti oxidative activities, membrane stabilization and cytotoxicity of water spinach from ipogi ponds (Nigeria), Int J of Pharmtech Research., Vol 1(3),474-482.
12. Manohara K.P., Raveendra Reddy P., Nandeesh R., Vijay kumar S., Evaluation of Anti- inflammatory activity of various extracts of *Tagetes erecta* Linn. Herbal Heritage, 2009,1(2),58-63.

*****