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Preliminary Phytochemical and Pharmacological Studies on the Rind of Pomegranate (*Punica granatum* L.)

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Abstract: The anti-inflammatory activity of an ethanolic extract of *Punica granatum* (pomegranate) rind on inflammation induced by carrageenan and cotton pellets implantation in rats; the effect of rind extract was also examined in aspirin and necrotizing agents-induced gastric mucosal damage in fasted rats. Oral administration of pomegranate rind at a dose of 500 mg kg^{-1} body weight showed a significant anti-inflammatory activity in rats. The extract exacerbated the intensity of gastric ulcers induced by aspirin. A preliminary phytochemical screening of rind of pomegranate revealed the presence of alkaloids, tannins, saponins sterols and/or triterpenes. The findings suggests that the rind extract possesses an anti-inflammatory activity through the inhibiting the prostaglandin biosynthesis in laboratory animals.

Key words: Pomegranate, *Punica granatum*, phytochemical tests, pharmacological activities, Arabian traditional medicine

INTRODUCTION

The pomegranate (*Punica granatum* L. Punicaceae) locally called Rumman, is one of the oldest known fruit species. Pomegranate grows wild in the Near East, Transcaucasia and in Asia Minor. The pomegranate has a long history of herbal use dating back more than 3000 years. In Saudi Arabian traditional medicine, it is regarded as a health giving fruit and almost all parts of the plant is used as home remedy to treat various ailments. The flowers are used in the treatment of dysentery, stomach ache and cough. If it is used along with the leaves and seeds, they expel worms[1]. The bark is known for its antibacterial, antiviral and astringent activity[2]. Seeds are demulcent and stomachic. The fruit is a mild astringent and refrigerant in some fevers and especially in biliousness[3]. The dried rind of the fruit is used in the treatment of amoebic dysentery, diarrhea and is a specific remedy for tapeworm infestation and certain inflammatory conditions[4]. The local herbalists made a decoction of the fruit rind and used it as a douch or wash after child birth and in cases of leucorrhea and other vaginal discharges and in mouth sores. The stem bark is emmenagogue and root bark is used to remove tapeworms. Keeping in view various medicinal uses in local folklore, the present study was undertaken to substantiate the claims of herbal practitioners.

MATERIALS AND METHODS

Plant material and extraction: Fresh pomegranate rind was shade dried. The pulverized rind was macerated in 96% ethanol for 36 h; the solvent was then evaporated by a rotavap *in vaccuo*. The extract was kept in refrigerator for biological studies.

Preliminary photochemical screening: A phytochemical analysis of the pomegranate rind was conducted for the detection of alkaloids, cardiac glycosides, flavonoids, tannins, anthraquinones, saponins, volatile oil. cyanogenic glycosides, glucosinolates, coumarins, sterols and/or triterpene[5].

Biological tests: Wistar albino rats roughly the same age (8-10 weeks), weighing 180-200 g obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh were used in these studies. The animals were kept in constant temperature (22±2°C), humidity (55%) and light-dark conditions (12/12 h light/dark). They were provided with Purina chow and free access to drinking water *ad libitum*.

Carrageenan-induced paw edema in rats: Experimental inflammation was induced according to the method described by Winter *et al.*[6] 0.05 mL of 1% carrageenan sodium salt (BDH) were injected into the right hind foot of each rat under the plantar aponeurosis. The test groups of rats were treated orally with ethanolic extract of pomegranate rind 500 mg kg^{-1} body weight orally 1 h before the carrageenan injection. At the same time, the control group was administered normal saline 5 mL kg^{-1} and the reference group was administered an aqueous solution of Phenylbutazone 100 mg kg^{-1} orally. The measurements of paw volumes were done by the

displacement technique using a plethysmometer (Apelex, France) immediately after and +3 h after the injection of carrageenan. The inhibitory activity was calculated according to the following formula:

$$\text{Percent inhibition} = 100\ [1 - (a-x/b-y)]$$

where, b is the mean paw volume of control rats after carrageenan injection and y before the injection whereas x is the mean paw volume of treated rats before injection and a is the mean paw volume after carrageenan injection.

Cotton pellet granuloma in rats: The method of Goldstein *et al.*[7] used with a few modifications. Sterilized cotton pellets weighing 30 mg each were introduced s.c. in the groin region of rats. The rats were treated orally with 500 mg kg^{-1} pomegranate rind extract once daily for four consecutive days. Animals in the control group received normal saline. Phenylbutazone 100 mg kg^{-1} (used as a standard drug) was given in other test group. On the fifth day, the animals were sacrificed with ether, the pellets were removed, freed from extraneous tissues, dried overnight at 60°C and weighed.

Aspirin-induced gastric ulcers: Aspirin was suspended in 1% carboxymethylcellulose in water and administered p.o. at dose of 30 mg kg^{-1} (0.5 mL/100 g) to rats fasted for 36 h[8]. Rind extract was administered orally 500 mg kg^{-1} body weight 30 min before aspirin. The rats were killed 6 h after aspirin administration.

Gastric lesions induced by necrotizing agents (*Cytoprotection studies*): The experiments were done on Wistar male rats fasted for 36 h with access to drinking water *ad libitum*. The following necrotizing agents were administered orally in a volume of 1 mL: 80% ethanol and 0.6 M HCl.[9] The rind extract in the dose of (500 mg kg^{-1}, body weight) was administered orally 30 min before the necrotizing agents treatment.

RESULTS AND DISCUSSION

The preliminary qualitative phytochemical screening of the pomegranate rind revealed the presence of alkaloids, tannins, saponins, sterols and/or triterpenes. The ethanolic extract was found to suppress carrageenan-induced rat paw edema significantly. The extract also exhibited the ability to significantly reduce the granulation mass formation in the animals (Table 1). Administration of aspirin resulted in the production of gastric mucosal damage mainly in the glandular segment of the stomach. Pretreatment of the animals with rind extract was found to exacerbate the gastric damage (Table 2). Gastric lesions induced by known necrotizing agents like (80% ethanol and 0.6 M HCl) were grouped in varying sized patches, usually parallel to the major axis of the stomach. Oral administration of the pomegranate rind extract failed to inhibit the lesion, although the reduction in the intensity of the lesions was found to be insignificant (Table 3).

The results of the present investigation revealed that the oral administration of an ethanolic extract of pomegranate rind possesses a anti-inflammatory effect which was evidenced by the significant reduction in paw edema and cotton-pellet granuloma methods. However, the effect was less when compared with phenylbutazone. Inflammation, a dynamic process considered as a protective mechanism, leads to a chronic inflammatory state when degranulated[10,11]. During the condition of inflammation associated with pain and fever, arachidonic acid is liberated from the phospholipids fraction of cell membranes and then enzymatically transformed to prostaglandins which sensitize blood vessels to the effect of mediators such as bradykinin, 5-HT and histamines that increase permeability[12,13]. It was also observed that the extract significantly reduced the granuloma formation in rats. Multiplication of small blood vessels as well as proliferation of fibroblasts are the characteristic features at the repair phase of inflammation. Such proliferating cells penetrate the exudates, producing a highly vascularized reddened mass known as granulation tissue[14]. The extract effectively reduced the cotton-pellet-induced granuloma suggesting its activity in the proliferative phase of the inflammation.

The pharmacological properties of the rind extract resemble those of the Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) which are known to possess anti-inflammatory activity. One of the major mechanisms involved in the anti-inflammatory activity of NSAID is due to inhibition of prostaglandin (PG) biosynthesis[15]. In the

Table 1: Effect of ethanolic extract of *Punica granatum* rind on carrageenan induced paw edema and cotton pellets granuloma in albino rats

Treatment (n = 6)	Dose orally (mg kg^{-1})	Paw volume after carrageenan administration mean increase in paw volume±SEN 3 h	% Inhibition	Cotton pellets granuloma formation increase in pellets wt (mg)	% Inhibition
Control carrageenan only.	—	0.91±0.03	53.49±2.83		
Punica granatum rind extract + carrageenan	500	0.72±0.04**	20.82**	41.55±1.56**	23.00
Oxyphenylbutazone + carrageenan	100	0.34±0.041***	62.00**	23.80±3.00***	56.00

**p<0.01 Students t-test, ***p<0.001 Students t-test

Table 2: Effect of ethanolic extract of *Punica granatum* rind on the gastric mucosal damage induced by Aspirin

Treatment	Dose (mg kg^{-1})	Ulcer index
Aspirin control (N = 6)	—	23.00±3.00
Punica granatum rind extract	500	25.33±1.69

Table 3: Effect of ethanolic extract of *Punica granatum* rind on the gastric lesions induced by necrotizing agents

	Ulcer index mean±SE	
Procedure	Control	Extract 500 (mgkg^{-1}), (p.o.)
80% Ethanol (n = 6)	6.60±0.51	6.00±0.58
0.6 M HCl (n = 6)	6.33±0.67	6.00±0.58

present study, the extract was shown to potentiate aspirin-induced gastric mucosal damage by inhibiting the PG synthesis[16]. On the basis of this finding, it may be suggested that the extract produced anti-inflammatory and ulcer promoting activities by inhibiting PG synthesis like other NSAIDs. However, further studies are needed to conform or rule out this hypothesis. On the other hand, the rind extract also failed to protect gastric mucosa against the necrotic agents (80% ethanol and 0.6 M HCl)-induced gastric lesion. This finding further confirms the ability of extract to inhibit PG synthesis[17].

The chemical constituents responsible for the pharmacological activity of the extract are not known. The rind of pomegranate was found to contain alkaloids, tannins, saponins, sterols and triterpenes all of these components were found to have antiphlogistic potential. Further studies are required to isolate the active constituents, responsible for the anti-inflammatory activity and to substantiate its use in Arabian traditional medicine for various inflammatory conditions.

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REFERENCES

1. Bown, D., 1995. Encyclopaedia of Herbs and Their Uses. Dorling Kindersley, London.
2. Mouhajir, F., J.B. Hudson, M. Rejdali and G.H.N. Towers, 2001. Multiple antiviral activities of endemic medicinal plants used by Berber peoples of Morocco. Pharmaceut. Biol., 39: 364-374.
3. Tabasum, S., S. Ahmed, N. Akhlaq and K. Rabman, 2001. Estimation of tannins in different food products. Intl. J. Agric. Biol., 3: 529-530.
4. Russ, R.G., S. Selvasubramanian and S. Jayasundar, 2001. Immuno-modulatory activity of *Punica granatum* in rabbits-a preliminary study. J. Ethnopharmacol., 78: 85-87.
5. Farnsworth, N.R., 1966. Biological and phytochemical screening of plants. J. Pharm. Sci., 55: 225-272.
6. Winter, C.A., E.A. Risley and G.W. Nuss, 1962. Carrageenan-induced oedema in hind paw of rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. BioI. Med., 111: 544-547.
7. Goldstein, S.A., I. Shemano, R. Daweo and J.M. Betler, 1967. Cotton pellet granuloma pouch method for evaluation of anti-inflammatory activity. Arch. Intl. Pharmacodyn. Therap., 165: 294-301.
8. Bhargawa, K.P., Gupta, M.B. and K.K. Tangari, 1973. Mechanism of ulcerogenic activity of indomethacin and oxyphenbutazone. Dur. J. Pharmacol. 2: 191-195.
9. Valcavi, U., R. Caponi and A. Brambilla, 1982. Gastric anti-secretory, anti-ulcer cytoprotective properties of 9-hydroxy-19,20-bis-nor-prostanoic acid in experimental animals. Arzneim.-Forsch. (Drug Res.) 32: 657-663.
10. DiRosa, M., G.P. Vela and D.A. Willoughby (Eds.), 1974. Effect of Non-steroidal anti-inflammatory drug in leucocyte migration in future trends inflammation. Picin Medical Books Podova, pp: 143-152.
11. Vingar, R., J.F. Traux, J.L. Selph P.R. Johnstron, A.L. Vinable and K.K. McKenzie, 1987. Pathway to carrageenan-induced inflammation in the hind limb of the rat. Fed. Proc., 46: 118-126.
12. Al-Rehaily, A.J., K.E.H. EI-Tahir, J.S. Mossa and S. Rafatul1ah, 2001. Pharmacological studies of various extract and the major constituents, Lupeol, obtain from hexane extract of *Ticlea nobilis* in rodents. Natural Product Sci., 7: 76-82.
13. Al-Rehaily, A.J., 2001. Chemical and biological evaluation of essential oil of *Teclea nobilis* leaf. Pak. J. Biol. Sci., 4: 166-168.
14. Ahmed, M., H.A. Shikha, S.K Sadhu, M.T. Rahman and B.K Datta, 2001. Analgesic, diuretic and anti-inflammatory principal from *Scoparia dulcis*. Pharmazie, 56: 657-60.
15. Vane, J.R., 1971. Inhibition of prostaglandin in synthesis as a mechanism of action for aspirin-like drugs. Nature, 231: 232-235.
16. Rafatullah, S., Tariq, M., J.S. Mossa, M.A. Al-Yahya, M.S. Al-Said and A.M. Ageel, 1993. Protective effect of *Swertia chirata* against indomethacin and other ulcerogenic agent-induced gastric ulcers. Drugs Exptl. Clin. Res., 19: 69-73.
17. Al-Yahya, M.A., J.S. Mossa, A.M. Ageel and S. Rafatullah, 1994. Pharmacological and safety evaluation studies in *Lepidium sativum* L., seeds. Phytomedicine, 1: 155-159.