ANTIAMOEBIC ACTIVITY OF RIND AND FLOWERS OF *PUNICA GRANATUM* LINN

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Abstract

The antiamoebic activity of water soluble fractions of the rind and flowers of *Punica granatum* has been assessed. The *in vitro* and *in vivo* studies, carried out on a virulent strain of *Entamoeba histolytica*, exhibited encouraging results.

Introduction

In the annals of medical history, many descriptions of diarrhoeal diseases have been available since the earliest times. Chinese and Hebrew writings point to the occurrence of diarrhoea and dysentery. Hippocrates observed that dysentery was more prevalent during the warm season [1].

Kartulis [2] established a clear correlation between tropic diarrhoea and amoeba. Bacillary and amoebic dysenteries were first clearly distinguished by Councilman and Lafleur [3]. Krause and Pasquale [4] differentiated pathogenic and non-pathogenic strains of amoeba, but true amoebiasis was described for the first time by Aleman in 1611 [5].

The rind of *Punica granatum* is reported to possess anti bacterial properties against intestinal pathogenic bacilli and is useful in gastritis, diarrhoea and dysentery [6-8]. Antifungal activity has also been reported on some plant pathogenic fungi [9-11] but not on human pathogenic fungi. No scientific investigations on the antiamoebic activity of *P. granatum* have been undertaken, hence the present study.

Experimental Section

Dried rind and flowers of *Punica granatum* (vernacular: Anar) belonging to the family *punicaceae* were procured from Hamdard (Wakf) Laboratories, Delhi and were taxonomically identified in the Department of Botany, Hamdard University. Voucher specimens were deposited in the department's herbarium.

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These samples were powdered separately using an iron pestle and mortar. The fine powder of rind (10.0g) and flowers (2.5g) were soaked separately in 250ml of distilled water at 40°C. Both were kept overnight at room temperature (32°C to 36°C), to acquire the aqueous extract of the drugs. After 24 hours, this was filtered through Whatman filter paper No. 1.

In vitro Screening of Potential Amoebicidal Drug

Antiamoebic studies were carried out *in vitro* following Vinayak and Prakash [12]. A virulent strain of *Entamoeba histolytica*, isolated in the All India Institute of Medical Sciences, New Delhi, was used for testing. The strain was maintained on a diphasic egg medium with ringer-calf serum as overlayer (Boeck and Drohlav's media).

E. histolytica was grown in all liquid Liver Marmite Serum (L.M.S.) medium [13] and, after 48 hours, the culture was harvested. The harvested culture was then mixed with a fresh medium, washed twice with normal saline (0.85% sodium chloride pH-7.2) and then the washed amoeba culture was again mixed with a fresh medium in sterile conditions. The count of amoebae was taken and adjusted to about five trophozoites per 0.05ml of the medium approximately. The medium was dispensed in 10ml sterile test tubes at the rate of 2ml/tube. To each of these tubes, approximately 10mg of rice starch (sterile) was added. Both the aqueous extracts of the drugs were sterilized by sitz filter and the different dilutions of the extracts were made in sterile distilled water. The various drug dilutions were then added to the test tubes containing

the medium at the rate of 2ml/tube and the tubes were incubated at 37°C for 48 hours. For the comparative evaluation, Metronidazole powder (1-B hydroxyethyle-2 methyle 5-nitroimedazole) May and Baker Ltd. Co., Bombay, India, was used in each experiment side by side. In the controlled tubes, instead of using the antiamoebic drug, 2ml of the distilled water (sterile) was added.

Test for Intestinal Amoebiasis (Experimental Infection)

For the screening of luminal amoebicides, which possess activity against intestinal amoebiasis, twenty - four infection free albino rats (one week after weaning) weighing 30 to 35g were selected. After performing laprotomy, under ether anaesthesia, the albino rats were given approximately 2,50,000 trophozoites (48-hour old culture, washed and suspended in normal saline 0.85% sodium chloride pH-7.2, was used for inoculation), intracaecally. The abdominal wall was sutured with fine nylon and the skin with fine silk thread. Tincture of iodine was applied as an antiseptic. After 24 hours, the rats were divided into four groups of six. The rats of the first group were given aqueous extracts of the rind of *P. granatum* orally at 10ml/10g p.o; the second group of rats were given aqueous extract of flowers of *P. granatum* orally 2.5mg/10g p.o; the third group of rats were given control drug Metronidazole solution 0.5mg/10g p.o; and the fourth group of rats were given vehicle (sterile distilled water) 1ml/10g, p.o. only.

The rats were fed with their regular diet during the treatment which continued for ten days. The fresh contents of the various portions of the intestine were examined microscopically for motile amoebae, particular attention was paid to those of the caecum or those containing mucous.

Results and Discussion

In vitro testing: Both the aqueous extracts showed encouraging antiamoebic activity against *E. histolytica*. Dilutions less than 10mg/ml of rind of *P. granatum* and 1mg/ml flowers of *P. granatum* could not inhibit the growth of *E. histolytica* (Table 1). Good results were obtained with the 40mg/ml and 20 mg/ml rind of *P. granatum* and 5mg/ml, 4mg/ml and 2mg/ml flowers of *P. granatum* drug dilutions, and Metronidazole (control drug) showed amoebicidal activity up to 4 µg/ml dilution *in vitro*.

In vivo testing: The fourth group of rats (the control group) revealed caeca smaller in size than those of rats in the treated groups i.e. the first and second group. The caecal and intestine walls were thicker while many rats showed ulcerated patches. The contents were mostly of mucous nature and on microscopical examination revealed myriads of motile amoebae. The scrappings of the caecal and intestine wall also revealed large numbers of motile trophozoites.

The Metronidazole treated rats (control group) revealed caeca of normal size. The caecal and intestinal walls were normal without any ulcerations. Contents were without mucous, and on microscopic examination showed no motile amoebae. The scrappings of the caecal and intestinal wall did not reveal any motile trophozoites either.

P. granatum (extract of rind and flowers) treated rats revealed normal caeca and intestines, with no thickening or ulcerations or any other lesions. The contents were of a much less mucous nature and motile amoeba were not present. The caecal and intestinal scrappings did not reveal any motile trophozoites. No toxic signs were shown by the rats during the medication. *In vivo* studies of both aqueous extracts at the concentrations of 10mg/10g body weight of rats (rind of *P. granatum*) and 25mg/10g body weight of rats, (flowers of *P. granatum*) completely inhibited the growth of *E. histolytica* in albino rats. The effects compared well with those of Metronidazole at the concentrations used.

Thus, it can be seen that the tested drug revealed good results both *in vitro* and *in vivo*, warranting further studies for the isolation of the active principle responsible for antiamoebic action.

Table 1: *In vitro* antiamoebic studies of *Punica granatum* (Rind and Flowers)

Name of the drugs	Conc. per ml	Motile trophozoites seen
Aqueous extracts of *PUNICA GRANATUM* (rind)	40 mg/ml	-
	20 mg/ml	-
	10 mg/ml	-
	5 mg/ml	+
	2 mg/ml	+
	1 mg/ml	+
Aqueous extracts of *PUNICA GRANATUM* (Flowers)	4 mg/ml	-
	2 mg/ml	-
	1 mg/ml	-
	0.5 mg/ml	+
	0.2 mg/ml	+
	0.1 mg/ml	+
Aqueous solution of Metronidazole Powder	10 µg/ml	-
	5 µg/ml	-
	4 µg/ml	+
	3 µg/ml	+
	2 µg/ml	+
	1 µg/ml	+

Note:
(-) Indicates no motile trophozoites seen.
(+) Indicates one or more motile trophozoites seen.

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