



## Antihistaminic Effect of Various Extracts of *Punica granatum* Linn. Flower Buds

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### ABSTRACT

*Punica granatum* Linn. (Punicaceae) flower buds are used in the treatment of asthma traditionally, so the present work was undertaken to prove it scientifically using suitable animal models. Antihistaminic principles are useful in the treatment of asthma; hence, in present work antihistaminic activity of various extracts of *P. granatum* flower buds was checked using clonidine-induced catalepsy and haloperidol-induced catalepsy in Swiss albino mice at the dose of 50 and 100 mg/kg, p.o. The results showed that ethanol extracts (100 mg/kg, p.o.) are having significant antihistaminic activity amongst other extracts. Thus, it can be concluded that tannins from the flower buds of *P. granatum* may be responsible for antihistaminic activity and may have potential role in the treatment of asthma.

**Key words:** Antihistaminic, catalepsy, clonidine, haloperidol, *Punica granatum*

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### INTRODUCTION

*Punica granatum* Linn. (Punicaceae) tree is cultivated all over in India. The various parts root, bark, leaves, fruit, and flower buds are used in traditional medicine for treatment of asthma, diarrhea, dysentery, tuberculosis, antidotes for snakebite, and as anthelmintic and astringent.<sup>[1]</sup> The plant has antioxidant,<sup>[2]</sup> antiallergic,<sup>[3]</sup> antimicrobial,<sup>[4]</sup> antiplasmodial,<sup>[5]</sup> antidiabetic,<sup>[6]</sup> hepatoprotective,<sup>[7]</sup> and anticancer<sup>[8]</sup> activities. Catalepsy is a condition in which the animal maintains imposed posture for long time before regaining normal posture. Catalepsy is a sign of extra-pyramidal effect of drugs that inhibit dopaminergic transmission or increase histamine release

in brain. Clonidine, a  $\alpha_2$ -adrenoceptor agonist, induces dose-dependent catalepsy in mice, which is inhibited by histamine H<sub>1</sub> receptor antagonists but not by H<sub>2</sub> receptor antagonist.<sup>[9]</sup> They also showed that pretreatment with L-histidine, a precursor of histamine-potentiated clonidine-induced catalepsy in a dose dependent manner. Muley *et al.*, (1979) showed that intracerebroventricular injection of histamine in conscious mice induced catalepsy, which was inhibited by H<sub>1</sub> receptor antagonist but not by H<sub>2</sub> receptor antagonist.<sup>[10]</sup> It is known that clonidine releases histamine from mast cells.<sup>[11]</sup> Schwatz (1997) identified histamine-containing mast cells in brain.<sup>[12]</sup> Clonidine-induced release of histamine from mast cells is inhibited by  $\alpha_2$ -adrenoceptor blocker, prazocine.<sup>[13]</sup> Neuroleptic

agent also induced catalepsy, but by a different mechanism. Neuroleptic agents inhibit dopamine D<sub>2</sub> receptor in the substantia nigra.<sup>[14,15]</sup> Therefore, it was our objective to study the effect various extracts of *P. granatum* flower buds on clonidine-induced catalepsy, as it is used traditionally in the treatment of asthma.<sup>[16]</sup> Since catalepsy is a common extra-pyramidal side effect of neuroleptic agents and the effect of the plant on haloperidol-induced catalepsy is not known, we also studied their effect on haloperidol-induced catalepsy in mice.

## MATERIALS AND METHODS

### Plant material

Fresh flower buds of *P. granatum* was collected from Ahmednagar district and authenticated by Mr. S.C. Mujumdar, Deputy Director, Botanical survey of India, Koregaon Road, Pune. The herbarium of plant specimen has been deposited at B.S.I. Pune, the voucher no. BSBP1, vide letter no. BSI/WC/Tech/2007/795, dated 20<sup>th</sup> November 2007.

### Extraction

Dried and coarsely powdered flower buds of *P. granatum* were subjected to successive solvent extraction in Soxhlet extractor using petroleum ether, chloroform, and ethanol as solvent, and the marc left was refluxed with water. All the extracts were vacuum dried to produce PEE (7.3%),

CLE (5.5%), EE (56%), and AQE (16%) respectively.

### Animals

Male albino mice (Swiss strain) weighing 22-25 g were housed under standard laboratory conditions, in groups of six each. The animal had free access to food and water. The ethical committee of the institute approved the protocol of the study.

### Drugs and Chemicals

The following drugs and chemicals were used. Drugs: clonidine (Unichem, India), haloperidol (Sunpharma, India), pheniramine maleate (Pfizer Ltd.) purchased from commercial source. Chemicals: petroleum ether AR (60-80°C) (PCL, India), chloroform AR (PCL, India), ethanol AR (PCL, India), and Tween 80 AR (PCL, India).

### Preliminary phytochemical study

The preliminary phytochemical study of various extracts of *P. granatum* was performed as per Khandelwal.<sup>[17]</sup>

### Effect on clonidine-induced catalepsy

The bar test was used to study the effect of various extracts on clonidine-induced catalepsy.<sup>[18]</sup> Clonidine (1 mg/kg, s.c.) was injected to mice ( $n=6$ ) pretreated 60 min before with vehicle (Tween 80 in distilled water) (5 ml/kg, p.o.), petroleum ether, ethyl acetate, ethanol, and aqueous extracts of *P. granatum* (50 and 100 mg/kg, p.o., each) or standard drug pheniramine maleate (10 mg/kg, i.p.). The

**Table 1: Effect of various extracts of flower buds of *P. granatum* on clonidine induced catalepsy in mice**

Treatment	Dose	Duration of catalepsy (sec) at Mean $\pm$ SEM							
		0 min.	15 min.	30 min.	60 min.	90 min.	120 min.	150 min.	180 min.
Vehicle (tween 80 in distilled water)	0.13 ml (p.o.)	2.815 $\pm$	58.0 $\pm$	59.75 $\pm$	100.50 $\pm$	140.38 $\pm$	80.3 $\pm$	76.91 $\pm$	57.46 $\pm$
		0.3889	1.337	0.717	12.576	12.579	12.644	16.714	1.626
Pheniramine maleate	10 (mg /kg, i.p)	2.51** $\pm$	26.74** $\pm$	34.51** $\pm$	38.71** $\pm$	41.45** $\pm$	36.3** $\pm$	22.47** $\pm$	20.41** $\pm$
		0.4778	4.340	5.853	5.766	6.45	5.54	4.816	3.148
Petroleum ether extract	50(mg /kg, p.o.)	16.95 $\pm$	32.98 $\pm$	39.10 $\pm$	75.50 $\pm$	94.00 $\pm$	65.07 $\pm$	46.30 $\pm$	30.69 $\pm$
		1.69	1.25	0.58	9.26	8.045	1.46	1.32	1.39
		16.45 $\pm$	34.28 $\pm$	42.305 $\pm$	67.72 $\pm$	94.05 $\pm$	62.08 $\pm$	49.62 $\pm$	19.95 $\pm$
Chloroform extract	50 (mg /kg, p.o.)	1.04	1.033	1.19	5.11	8.24	1.92	1.39	0.98
		0.97 $\pm$	34.85 $\pm$	44.83 $\pm$	58.44 $\pm$	107.36 $\pm$	60.715 $\pm$	45.150 $\pm$	17.00 $\pm$
		0.034	0.76	0.75	0.85	16.885	2.234	2.23	1.231
Ethanol extract	50 (mg /kg, p.o.)	1.4 $\pm$	32.43 $\pm$	42.58 $\pm$	54.005 $\pm$	127.78 $\pm$	62.84 $\pm$	45.97 $\pm$	22.405 $\pm$
		0.119	1.34	0.67	1.097	15.89	2.90	1.56	0.677
		4.62* $\pm$	26.29* $\pm$	35.91* $\pm$	38.59* $\pm$	46.83* $\pm$	40.76* $\pm$	32.85* $\pm$	19.37* $\pm$
Aqueous extract	50 (mg /kg, p.o.)	0.38	1.19	1.33	1.65	1.25	1.85	1.08	0.65
		12.56** $\pm$	22.26** $\pm$	36.85** $\pm$	38.37** $\pm$	44.01** $\pm$	35.53** $\pm$	26.57** $\pm$	19.33** $\pm$
		0.49	1.49	0.92	0.70	1.008	0.95	1.09	1.97
Aqueous extract	100 (mg /kg, p.o.)	6.43* $\pm$	31.34* $\pm$	39.88* $\pm$	44.22* $\pm$	53.79* $\pm$	47.7* $\pm$	38.19* $\pm$	24.54* $\pm$
		0.53	1.108	0.4202	0.88	0.63	0.74	0.54	0.58
		5.89* $\pm$	33.45* $\pm$	42.08* $\pm$	45.94* $\pm$	57.20* $\pm$	46.99* $\pm$	37.06* $\pm$	27.22* $\pm$
		0.73	1.158	0.99	1.27	1.354	0.855	1.182	0.569

n = six in each group. \*\* $P < 0.001$ , \* $P < 0.05$  significant compared with control group

dosages were selected based on acute toxicity studies (data not shown). The forepaws of mice were placed on horizontal bar (1 cm in diameter, 3 cm above the table) and the time required to remove the paws from bar was noted for each animal and the duration of catalepsy was measured at 0, 15, 30, 60, 90, 120, 150, and 180 min.

#### Effect on haloperidol-induced catalepsy

The same Bar test was used using haloperidol.<sup>[18]</sup> Haloperidol (1 mg/kg, i.p.) was injected to mice ( $n=6$ ) pretreated 60 min before with vehicle (Tween 80 in distilled water) (5 ml/kg, p.o.), petroleum ether, ethyl acetate, ethanol, and aqueous extracts of *P. granatum* (50 and 100 mg/kg, p.o., each). The duration of catalepsy was measured at 0, 15, 30, 60, 90, 120, 150, and 180 min.

#### Statistical analysis of data

Data are presented as mean  $\pm$  SEM. Statistical comparison between groups were analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's test. Prism Graph pad 3 was used for statistical analysis. \*\* $P < 0.001$ , \* $P < 0.05$ , compared with the control group.

## RESULTS

### Preliminary phytochemical study

The ethanol extract of *P. granatum* flower buds showed the presence of tannins.

### Clonidine-induced catalepsy

The result showed that ethanol extract significantly inhibited clonidine-induced catalepsy than other extracts. The results are compared with pheniramine maleate. Other extract were found to be non-significant [Table 1].

### Haloperidol-induced catalepsy

None of the extracts inhibited haloperidol-induced catalepsy [Table 2].

## DISCUSSION

Several drugs are known to induce catalepsy in animals. The neuroleptic agents induce catalepsy by inducing dopamine

**Table 2: Effect of various extracts of flower buds of *P. granatum* on Haloperidol induced catalepsy in mice**

Treatment	Dose (mg /kg, p.o.)	Duration of catalepsy (sec) at Mean $\pm$ SEM							
		0 min.	15 min.	30 min.	60 min.	90 min.	120 min.	150 min.	180 min.
Vehicle (tween 80 in distilled water)	-	3.365 $\pm$ 0.7413	38.38 $\pm$ 5.571	56.58 $\pm$ 2.100	60.37 $\pm$ 01247	90.385 $\pm$ 13.262	60.26 $\pm$ 0.090	52.81 $\pm$ 0.96	48.50 $\pm$ 2.15
Petroleum ether extract	50	2.141 $\pm$ 0.432	35.71 $\pm$ 2.99	60.25 $\pm$ 1.93	64.005 $\pm$ 1.93	107.92 $\pm$ 10.425	58.48 $\pm$ 0.94	53.85 $\pm$ 1.619	47.815 $\pm$ 1.258
	100	4.03 $\pm$ 0.42	36.94 $\pm$ 1.258	61.19 $\pm$ 1.02	66.62 $\pm$ 2.33	92.83 $\pm$ 14.504	60.85 $\pm$ 1.087	52.37 $\pm$ 0.83	46.50 $\pm$ 1.53
Chloroform extract	50	2.44 $\pm$ 0.43	36.19 $\pm$ 1.12	56.95 $\pm$ 1.90	60.87 $\pm$ 1.79	161.27 $\pm$ 1.79	61.275 $\pm$ 1.573	52.74 $\pm$ 1.37	42.16 $\pm$ 1.023
	100	3.87 $\pm$ 0.48	40.265 $\pm$ 1.40	54.35 $\pm$ 2.47	63.64 $\pm$ 1.44	96.29 $\pm$ 13.67	71.86 $\pm$ 6.22	55.47 $\pm$ 1.47	45.81 $\pm$ 1.286
Ethanol extract	50	4.26 $\pm$ 0.48	42.96 $\pm$ 1.76	54.09 $\pm$ 1.49	63.15 $\pm$ 1.47	100.04 $\pm$ 8.25	60.29 $\pm$ 4.69	32.85 $\pm$ 1.94	44.64 $\pm$ 1.27
	100	4.33 $\pm$ 0.40	44.62 $\pm$ 1.60	53.89 $\pm$ 2.079	62.69 $\pm$ 1.205	91.205 $\pm$ 10.436	67.03 $\pm$ 4.27	54.015 $\pm$ 1.512	43.39 $\pm$ 1.214
Aqueous extract	50	4.95 $\pm$ 0.50	41.75 $\pm$ 1.52	54.21 $\pm$ 1.621	62.32 $\pm$ 1.103	100.33 $\pm$ 8.298	75.45 $\pm$ 6.916	356.93 $\pm$ 1.35	43.06 $\pm$ 0.98
	100	3.17 $\pm$ 0.454	39.82 $\pm$ 1.137	56.39 $\pm$ 3.98	62.63 $\pm$ 1.036	91.47 $\pm$ 12.895	67.305 $\pm$ 5.05	55.97 $\pm$ 1.18	43.21 $\pm$ 0.64

n = six, in each

D<sub>2</sub> receptor in the substantia nigra.<sup>[14]</sup> Chopra and Dandiya (1975) have studied the relative role of acetylcholine and histamine in perphenazine-induced catalepsy and suggested that anticholinergic activity of antidepressant might be due to an increase in dopamine content in brain or their ability to inhibit release of acetylcholine.<sup>[19]</sup> They also showed that different stages of catalepsy appear to be directly correlated with brain histamine content. Uvnas (1969) studied the mast cell degranulation and its correlation with the release of histamine after administration of mast cell degranulating agent (Compound 48/80).<sup>[20]</sup> Lakdawala *et al.* (1980) have shown that clonidine releases histamine from mast cell in a similar manner to a selective liberator like compound 48/80.<sup>[11]</sup>

The observation of this study indicated that the ethanol and aqueous extracts of *P. granatum* flower buds inhibited clonidine-induced catalepsy and not inhibited haloperidol-induced catalepsy. From the present study, we can conclude that the cataleptic effect of clonidine in the mouse is mediated by histamine release from mast cells and the clonidine-induced catalepsy was inhibited by ethanol extract of *P. granatum* flower buds. The effect of these extracts on clonidine-induced catalepsy is probably due to their mast cell-stabilizing property, and the plant does not have activity on dopaminergic transmission. So it can be concluded that ethanol extract containing tannins may be responsible for antihistaminic activity and may be used in the treatment of asthma.

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