

ANTINOCICEPTIVE AND ANTI-PYRETIC ACTIVITY OF *BENINCASA HISPIDA* (THUNB.) COGN. IN WISTAR ALBINO RATS

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ABSTRACT

The seeds of *Benincasa hispida* (Thunb) COGN. (Family: Cucurbitaceae) was extracted with ethanol and was used to study acute toxicity, antinociceptive and anti-pyretic effects. Brewer's yeast (15%) was used to induce pyrexia in rats. The extract was non lethal to the rats up to the dose of 5000 mg/kg b.w. At doses of 250 and 500 mg/kg b.w, the extract significantly ($P<0.05$) increased the antinociceptive effective in a dose dependent manner in rats. Similarly, at doses of 250 and 500 mg/kg b.w the extract significantly ($P<0.05$) decreased yeast-induced pyrexia in rats. These results indicate that ethanolic extract of *Benincasa hispida* possesses potent antinociceptive and antipyretic effects and thus pharmacologically justifying its folkloric use in the management of fever and pain conditions.

Key words: *Benincasa hispida*, Brewer's yeast, antinociceptive, anti-pyretic effects, acute toxicity.

INTRODUCTION

Plants continue to serve as possible sources for new drugs and chemicals. These can be extremely useful as lead structure for synthetic modification and optimization of bioactivity. Widespread use of botanicals as medicinal products in developing countries and herbal products are also becoming a part of the integrative health care systems of industrialized nations nowadays. A scientific evaluation of herbs according to their traditional and folk uses in treatment of various disease curing can be included in "complementary alternatives system of medicines" (CAM). Safety and efficacy of natural herbal product is therefore a cause of concern to promote and rationalize their use.

Benincasa hispida (Thunb) COGN. is employed traditionally to treat disorders such as dry-cough, fever, urethral discharges, biliousness, thirst. It acts as brain tonic and also possesses anti-helminthic property. In China, it is used in the treatment of appendicitis. Oil from seeds is soporific, good for the brain and liver and effective in the treatment of syphilis. Seed ash is a prized remedy for gonorrhoea; ash is applied to painful wounds and swellings (Nadkarni's Indian Materia Medica, 1995; Kirtikar and Basu, 1985). The present study was carried out to scientifically prove some of the folkloric use of this plant in conditions of pain and fever by exploring antinociceptive and antipyretic effects.

MATERIAL AND METHODS

Collection and authentication of plant material

The seeds of *Benincasa hispida* were collected in the

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month of December 2006 and the seeds was authenticated by Dr.Marimuthu, Professor, Department of Botany, Government Arts and Science College, Salem and the specimen of *Benincasa hispida* bearing reference number 106/col./219 was kept in the museum of Vinayaka Mission's College of Pharmacy, Salem for future reference.

Preparation of extract

The seeds of *Benincasa hispida* were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve No 40 and stored in an airtight container for further use.

Drugs and Chemicals

Petroleum Ether (60-80°C),
Chloroform,
Acetone,
Alcohol (95% v/v),
Brewer's yeast (15%),
Diclofenac (Lupin Pharmaceuticals Ltd. Mumbai),
Aspirin (Ricketts and Collman Pharmaceuticals Ltd. Mumbai).

Other chemical were of analytical grade supplied by the respective manufacturers.

Extraction procedure

The dried powdered seeds of *Benincasa hispida* were defatted with petroleum ether (60-80°C) in a Soxhlet apparatus. The defatted powder material thus obtained was further extracted with chloroform, acetone, ethanol and water. The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator. The extractive values are represented in table 1.

Experimental animals

Studies were carried out using male Wistar Albino rats (150-200 g). They were procured from Sri Venkateswara Enterprises, Bangalore, India. The animals were grouped and housed in polyacrylic cages (38 x 23 x10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25±2°C) with dark and light cycle (12/12 h). The animals were fed with standard pellet diet supplied by Hindustan Lever Ltd., Bangalore, India and fresh water *ad libitum*. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. All procedures described were reviewed and approved by the University Animal Ethical Committee.

Preliminary phytochemical screening

The ethanolic extract of *Benincasa hispida* were tested for the presence of carbohydrates, glycosides, alkaloids, phytosterols, fixed oils, gums and mucilages, saponins, proteins and free amino acids, phenolic compounds, tannins and flavonoids.

Antinociceptive study (Vongtau *et al.*, 2004)

The study involved the use of analgesiometer (Model 7200, Ugo Basile, Italy), which exerts force at a constantly- increasing rate on the rat paw. The force is monitored by a pointer moving along a linear scale and the rat's response is taken to be the point at which its struggles or its paw slips off the plinth of the instrument. The animals were divided into four groups of six rats each. Groups I received normal saline 10 ml/kg and served as control, group II and group III received the extract at doses of 250 and 500 mg/kg *i.p.*, respectively and group IV was administered diclofenac sodium (20 mg/kg *b.w*) and served as standard. Thirty minutes after respective treatment, readings were taken at intervals of 0 min., 30 min., 60 min., 90 min. and 120 min.

Anti-pyretic activity

Anti-pyretic activity of *Benincasa hispida* was carried out using the methods of Al-Ghamdi (2001). Rats were weighed and randomized into four groups of six rats per group. The baseline body temperatures of the rats were taken by inserting a digital thermometer into their anal cavities for about 2 min. The steady temperature readings obtained were recorded as the pre-treatment temperatures. Pyrexia was induced in the rats by the administration of 1 ml/kg *b.w* of 15% brewer's yeast suspension subcutaneously and 24 h later of yeast administration, the anal temperatures were measured again. Rats that did not show a minimum increase of 0.5°C were discarded for the study. Twenty four rats selected were grouped into four groups and immediately treated as follows: Normal saline 10 ml/kg *b.w* were administered to group I, while group II were treated with ethanolic extract of seeds of *Benincasa hispida* (250 mg/kg *b.w*), group III were treated with ethanolic extract of seeds of *Benincasa hispida* (500

mg/kg *b.w*) and group IV were treated with 100 mg aspirin/kg *b.w*, *i.p.* Anal temperatures were then measured by using digital thermometer and after every thirty minutes interval for each rat up to 2 h.

STATISTICAL ANALYSIS

Results were expressed as Mean ± Standard Error of Mean (SEM). The data was analyzed using student's t-test and P<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The dried seeds of *Benincasa hispida* (Thunb.) COGN. was extracted with solvents of increasing polarity by Soxhlet apparatus. The percentage yield of the seeds of *Benincasa hispida* (Thunb.) COGN. was found to be 3.2%, 1.3%, 1.1%, 7.2% and 4.1% with petroleum ether, acetone, chloroform, alcohol and water, respectively (table 1). The percentage yield of the ethanolic extract of *Benincasa hispida* (Thunb.) COGN. was found to be greater (7.2%) than extracts with other solvents.

The preliminary phytochemical screening of the dried seed extract showed the presence of carbohydrates, phenolic compounds, amino acids and proteins, flavonoids and sterols. In acute toxicity study, the ethanolic extract of *Benincasa hispida* (Thunb.) COGN. seeds were found to be safe and no mortality was observed at a dose as high as 5 g/kg.

The data presented in tables 2 and 3 suggests that the methanolic extract of *Benincasa hispida* (Thunb.) COGN. possesses antinociceptive and antipyretic activities. This study, which employed mechanically-induced pain, revealed significant and dose-dependent increase in pain threshold by the extract compared to control in the rats. The effect was also time-related (table 2).

Pain and inflammation is associated with many pathophysiology of various clinical conditions like arthritis, cancer and vascular diseases (Weitzman *et al.*, 1990, Suffness *et al.*, 1991, Mukherjee, 2003). A number of natural products are used in various traditional medical systems to treat relief of symptoms from pain and inflammation. The ethanolic extract *Benincasa hispida* demonstrated significant antinociceptive activity at two different dose levels.

In the present study, *Benincasa hispida* (Thunb.) COGN. produced analgesic activity in a dose dependent manner and significant effect was observed at 500 mg/kg. Generally, plants showing the antipyretic effect also possess analgesic activity (Dewan *et al.*, 2000). A number of flavonoids have been reported to produce analgesic activity. Also, there are few reports on the role of tannins in analgesic activity (Hossinzadeh *et al.*, 2002). Hence, in

the present study the analgesic activity of *Benincasa hispida* (Thunb.) COGN. may be attributed to the presence of flavonoids.

The infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediators (cytokines like interleukin 1 β , α , β and TNF- α) which increase the synthesis of PGE2 near pre-optic hypothalamus area thereby triggering the hypothalamus to elevate the body temperature (Spacer and Breder, 1994). The extract produced a significant reduction in yeast induced pyrexia in rats dose-dependently and its effect is comparable to that of the standard anti-pyretic drug (Aspirin) used in this study. Pyrexia is a result of secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states.

The results of ethanolic extract of seeds of *Benincasa hispida* on yeast induced pyrexia in rats are represented in Table 3. Ethanolic extract of *Benincasa hispida* produced significant ($P < 0.05$) antipyretic effect in dose dependent manner. The appreciable antipyretic effect noticed at 500 mg/kg was comparable to standard drug Aspirin. Normal saline treated rats did not show any decrease in elevated body temperature.

Based on these results, we conclude that ethanolic extract of seeds *Benincasa hispida* (Thunb.) COGN. possesses analgesic effect, and that its action on nociception may be different from that of aspirin and decrease in elevated body temperature proves the extract of *Benincasa hispida* possesses antipyretic effect. Hence, proves the traditional use by traditional medicine practitioners as analgesic and antipyretic remedies.

Table 1: Data Showing the Extractive Values of Seeds of *Benincasa hispida* (Thunb.) COGN

Plant Name	Part used	Method of extraction	Yield in percentage				
			Petroleum ether	chloroform	Acetone	Alcohol	Aqueous
<i>Benincasa hispida</i>	Dried seeds	Continuous hot percolation	3.2	1.3	1.1	7.2	4.1

Table 2: Effect of ethanolic extract of seeds of *Benincasa hispida* (Thunb.) COGN. on antinociceptive activity using analgesiometer

Group	Dose (mg/kg)	Time (min.)				
		0	30	60	90	120
I	Saline (10 ml/kg)	118.5 \pm 16.2	137.06 \pm 27.2	106.4 \pm 18.8	146.00 \pm 7.0	113.0 \pm 10.4
II	EESBH (250 mg/kg)	110.25 \pm 12.4	225.67 \pm 20.6*	300.67 \pm 24.4*	245.0 \pm 24.3*	190.5 \pm 15.6*
III	EESBH (500 mg/kg)	95.50 \pm 12.4	277 \pm 10.62*	325.33 \pm 24.06*	269.5 \pm 9.0*	247 \pm 20.5*
IV	Diclofenac sodium (20 mg/kg)	110.1 \pm 10.5*	280.0 \pm 10.2*	330.25 \pm 8.6*	380.0 \pm 8.54*	408.1 \pm 7.23*

Values are expressed as Mean \pm SEM, n = 6.

* Significantly different from the control at $P < 0.05$

EESBH represents ethanolic extract of seeds of *Benincasa hispida* (Thunb.) COGN.

Table 3: Effect of ethanolic extract of seeds of *Benincasa hispida* (Thunb.) COGN. on induced hyper-pyrexia in rats

Group	Treatment	Dose (mg/gm)	Base Line Temp. °C	Time (h)				
				0.0h	0.5h	1.0h	1.5h	2.0h
I	Normal Saline	10 ml	37.0 \pm 0.85	40.0 \pm 0.89	40.0 \pm 0.89	40.0 \pm 0.89	39.50 \pm 0.59	39.4 \pm 0.66
II	EESBH	250.0	37.0 \pm 0.64	40.1 \pm 0.72 ^{ns}	39.2 \pm 0.13 ^{ns}	39.0 \pm 0.34*	38.2 \pm 0.62	37.9 \pm 0.27*
III	EESBH	250.0	37.0 \pm 0.64	40.1 \pm 0.72 ^{ns}	39.2 \pm 0.13 ^{ns}	39.0 \pm 0.34*	38.2 \pm 0.62	37.5 \pm 0.27*
IV	Aspirin	100.0	37.5 \pm 0.86	39.8 \pm 0.54 ^{ns}	39.4 \pm 0.23 ^{ns}	38.7 \pm 0.27*	37.8 \pm 0.25*	37.3 \pm 0.22*

Values are expressed as Mean \pm SEM, n = 6.

* Significantly different from the control at $P < 0.05$

^{ns} represents non-significant

EESBH represents ethanolic extract of seeds of *Benincasa hispida* (Thunb.) COGN.

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