ANTINOCICEPTIVE EFFECTS OF METHANOLIC EXTRACT OF BENINCASA HISPIDA (THUNB) CONG, FRUIT.

K.Hemamalini [1] and M.Vimal Kumar Varma [2]

[1] Dept of Pharmacology, Sri Sarada college of Pharmacy, Anantaram, Bhongir 508116 Andhra Pradesh, India

[2] Dept of Pharmaceutics, Mother Teresa college of Pharmacy, Ghatkesar, R.R. Dist 501301, Andhra Pradesh, India

Summary

Objective: To evaluate the analgesic effects of Benincasa hispida upon pain (antinociception).
Materials and methods: The antinociceptive activity of the methanolic extract of BH in 200, 400, and 600mg/kg was studied using acetic acid inducing writhing and hot plate method in mice for their antinociceptive.
Results: Methanolic extract of BH revealed significant antinociceptive activity with both the models. The methanolic extract of BH in doses of 200, 400 and 600 mg/kg showed percentage of protection from writhing was 46.9, 51.0 and 65.6 respectively. In the hot plate model the extract of BH in the above doses resulted in significantly and dose dependent prolongation of the response latency.
Discussion: Antinociception is through an opioid receptor antagonist in both the hot plate and abdominal writhing test revealed the results were positive and the mechanism of Benincasa hispida antinociception might involve opioid receptors and to local peritoneal receptors.
Conclusion: These results suggest that the methanolic extract of BH possesses antinociceptive activity.

Key words: Analgesic, hot plate, acetic acid.

Introduction

Benincasa hispida (Thunb) cong (cucurbitacecae) fruit is widely used as a vegetable in India and other tropical countries. The fruit Benincasa hispida is an important ingredient of "Kusmanda lehyam" (Ayurvedic medicine) which is used in India for centuries for various ailments such as gastrointestinal problems, respiratory diseases (cough, asthma)[1], heart diseases, vermifuge, diabetes mellitus and urinary diseases[2,3]. Though some scientific studies have been carried out reveal its anti-inflammatory activity[4], diuretic activity[5], anticancer[6], nootropic[7] and antidepressant activity[8]. The major constituents of this fruits are triterpenoids, flavonoids, glycosides, saccharides, carotenes, vitamins, βsitosterin and uronic acid[9-11]. However there is no report on analgesic activity of this plant but anti-inflammatory activity has been reported in carrageenin and formalin induced method. In the light of the above information the present investigation was under taken to evaluate the analgesic activity of Benincasa hispida fruit extract in mice.

Materials and methods

Plant material: Benincasa hispida fruit was obtained from the local market in the months of august/ September 2006 and identified by the Department of Botanist, of V.L.College of Pharmacy Raichur. The voucher specimen kept in our laboratory for future reference.

Extract preparation: After removing skin and the seeds, the fruit pulp was dried under shade. The coarsely powdered fruit pulp was extracted successively with petroleum ether 60-80 °C, and methanol in a soxhlet extractor for 24-32 hrs. On evaporation of methanol from the methanolic extract in vacuo, a residue was obtained (yield 4.02%W/W) and was stored in desiccators. For pharmacological experiments weighed amount of the methanolic extract was suspended in 2% W/V aqueous tragacanth solution.

Chemicals: Acetic acid (Ranbaxy laboratories Ltd) Aspirin (ASA), Pethidine and other standard laboratory chemicals were obtained from Sigma chemicals. All drugs were dissolved in normal saline prior to use.

Animal used: Adult male mice 20-25 g were used for all the antinociceptive experiments. The animals were maintained under standard laboratory conditions (light period of 12 h /day and temperature 27 °C ± 2 °C, with access to food and water adlibitum. The experimental procedures were carried out in strict compliance with the institutional animal ethics committee regulations. All experiments were performed in the morning according to the guidelines for the care of laboratory animals[12].

Acetic acid induced writhing

The antinociceptive activity of MEBH was assessed using writhing test (abdominal constriction test)[13]. Acetic acid solution (10ml/kg. 0.6%) was injected intraperitoneally, and the contraction of abdominal muscles together with stretching of the hind limbs was cumulatively counted over a period of 0.5h beginning 5 min after acetic acid injection. The extract was administered (200, 400 and 600 mg/kg, i.p) 0.5 h before the acetic acid injection. Antinociceptive activity was expressed as the percentage inhibition of abdominal constrictions between control animals and mice pre-treated (n =6) with the extract using the ratio (Control mean- Treated means) ×100/ control mean.

Hot plate test

The hot plate test was performed to measure response latencies according to the method previously described[14]. The hot plate was maintained at 55.0 ± 0.2°C and the animals were placed into the Perspex cylinder on the heated surface and the time (sec) to discomfort reaction (licking paws or jumping) was recorded as response latency, prior to and 30, 60, 120 and 150 min after administration of the extract (200, 400 and 600 mg/kg i.p). A latency period of 20 sec was defined as complete analgesia and the measurement was terminated if it exceeded the latency period in order to avoid injury.

Results

The results of the animal experiments are shown in Tables 1 and 2. The methanolic extract of Benincasa hispida (200, 400 and 600 mg/kg, i.p) suppressed the acetic acid induced writhing response significantly in a dose dependent manner. The results were found to be significant ($P<0.001$) in comparison to the control. Intraperitoneally administration of the extract resulted in significant dose dependent prolongation of the response latency in the hot plate test. The effect reached a peak at approximately 60 min after administration and then gradually decreased.

Table 1: Effects of the Methanolic extract of Benincasa hispida on acetic acid induced writhing response in mice.

Group	Dose Mg/kg. i.p	No. of writhing movements	Percentage of protection
Normal saline	8ml/kg	80.30 ± 0.95	-
BH	200	42.67± 4.18**	46.9
BH	400	39.33 ± 6.46**	51.0
BH	600	27.66± 3.48*	65.6
Aspirin	100	16.33 ± 6.70**	79.7
One way ANOVA	df	4,25	
	P	<0.001	

n = 6 in each group, *P< 0.01, **P<0.001 compared to control.

Table 2: Effect of Benincasa hispida extract on hot plate reaction time in mice

Group	Dose mg/kg, i.p	Time in minutes				
		Pre	30	60	120	150
Saline		5.60 ±0.38	5.48 ±0.55	5.45±0.52	6.20±0.61	5.30±0.67
BH	200	6.18±0.68	6.82±0.32	7.50±0.58	7.08±0.27	7.38±0.71
BH	400	5.73±0.28	7.75±0.33	8.13±0.55	8.00±0.32	7.42±0.92
BH	600	5.72±0.18	8.83±0.28	9.42±0.76	8.85±0.32	8.97±0.60
Pethidine		5.93±0.58	7.50±0.57	7.18±0.65	7.50±0.20	7.12±0.41

n = 6 in each group, mean ± SD (sec) df- 4,25, P<0.05.

Discussion

Administration of Benincasa hispida extract showed significant antinociceptive activity in the hot plate and acetic acid induced writhing tests (Table 1 and 2). These results indicate that the plant extract possesses centrally and peripherally mediated antinociceptive properties[15]. The hot plate method is one of the most common tests for evaluating the analgesic efficacy of drugs/ compounds in rodents[16]. However, care must be taken for drugs /compounds those produce false positive results by modifying the behavior of the rodents. The potency of antinociception was less than Pethidine and aspirin at similar doses. The inhibition of antinociception is through an opioid receptor antagonist in both the hot plate and abdominal writhing test revealed the results were positive and the mechanism of Benincasa hispida antinociception might involve opioid receptors and to local peritoneal receptors. On preliminary phytochemical screening the methanolic extract of Benincasa hispida was found to contain flavonoids compounds. Flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception[17]. Hence the presence of flavonoids may be contributory to analgesic activities of methanolic extract.

Further studies may reveal the exact mechanisms of action responsible for the analgesic activity of Benincasa hispida.

References

1. Anil kumar D, Ramu P: Effect of methanolic extract of Benincasa hispida against histamine and acetylcholine induced brochospasm in guinea pigs, Indian J Pharmacol. 2002, 34, 365-6.
2. Sivarajan VV, Balachandran I, Editors: Ayurvedic drugs and their plant sources, 1st Edn. New delhi, Oxford and IBH Publishing 1994.
3. Aslokar LV, Kakkar KK, Charkre OJ: Glossary of Indian medicinal plants with active principles, Part 1, 1st Edn. New Delhi, CSIR, 1992, 119.
4. Gover JK, Rathiss: Anti inflammatory activity of fresh juice of Benincasa hispida, Indian J Pharmacol. 1994, 26, 66.
5. Dong MY, Lumz Yin QH, Feng WM, XVJX, XVWM: Study of Benincasa hispida contents effective for protection of kidney, Jiangsu. J. Agricultural Sciences. 1995, 1 (3), 46-55.
6. Kumar A, Rama II: Antiulcer properties of methanolic extract of Benincasa hispida cogn, Indian drugs. 2002, 39, 9-13.

7. Kumar A, Nirmala V: Nootropic activity of methanol extract of Benincasa hispida fruit, Indian J Pharmacol. 2003, 35, 130.
8. Rukumani R, Nidya ISR, Suresh Nair, Anil kumar: Investigation of anxiolytic like effect of antidepressant activity of Benincasa hispida methanol extract, Indian J Pharmacol. 2003, 35, 129-30.
9. Nadkarni AK, In. Indian material medica, Popular Prakashan, Bombay, India 1976, 185-6.
10. Wollen Weber E, Faure R, Gaydou EM: A rare triterpene as major constitute of the "wax" on fruits of Benincasa hispida, Indian drugs. 1991, 28(10), 458-460.
11. Yashizumi S, Murakam T, Kadoya M, Matsuda H, Yamahara J, Yoshikava M: Hiatamine release inhibitors from was guard the fruits of Benincasa hispida (thunb). Yakugaku zassi. 1998, 118 (5), 188-192.
12. Zimmermann M: Ethical guidelines for investigation of experimental pain in conscious animals, Pain.1983, 16, 109-10.
13. Collier HDJ, Dinnin LC, Johnson CA, Schneider C: The abdominal response and its suppression by analgesic drugs in the mouse, Br J Pharmacol. 1968, 32, 295-310.
14. Hosseinzadeh H, Ramezani M, Salmani G: Antinociceptive, anti-inflammatory and acute toxicity effects of Zataria Multiflora Boiss extracts in mice and rats, J Ethanopharmacol. 2000, 73, 379-85.
15. Zakaria MNM, Islam MW, Radhakrishnan R, Chen HB, Kamil M., Al-Gifri AN, et.al: Antiniciceptive and anti-inflammatory properties of caralluma Arabica, J Ethanopharmacol. 2001, 76, 155-8
16. Carter RB: Differentiating analgesic and non-analgesic drug activities on rat hot plate effect on behavioral end point, Pain. 1991, 47, 211-20.
17. Rajnarayana K, Reddy MS, Chaluvadi MR, Krishna DR: Bioflavonoids classification, Pharmacological biochemical effects and therapeutic potential, Indian J Pharmacol. 2001, 33, 2-16.