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Antinociceptive and Antiinflammatory Effects of Methanolic Extract of *Benincasa hispida* Fruit Peel in Rodents

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The effect of methanolic extract of fruit peel of *Benincasa hispida* was investigated on chemical, thermal-induced pain as well as fresh egg albumin-induced inflammation and pentylenetetrazol (PTZ)-induced convulsion in rodents. The extract dose-dependently (0.25-1.5 g/kg) inhibited acetic acid-induced writhing, formalin-induced pain licking and hot plate-induced pain in mice. The extract significantly inhibited both the fresh egg albumin-induced inflammation in rats as well as pentylenetetrazol-induced convulsion in mice. These inhibitions were statistically significant ($p < 0.02$-0.001). It increased the latencies of both clonic and tonic convulsions and delayed their mortalities. Its ability to reduce both neurogenic and non-neurogenic pains may be related to its active constituents such as tannins, saponins, steroid and flavonoids.

Key Words: *Benincasa hispida* fruit peel, Antinociception, Anti-inflammatory, Convulsion, Neurogenic, Non-neurogenic

INTRODUCTION

Benincasa hispida (Thunb.) cogn (Cucurbitaceae)[1] fruit is widely used as a vegetable in India and tropical countries. In Oriya, it is called as panikakharu, in Hindi it is petha, in Bengali it is kumra, in Kanada it is rakhsa, in Tamil it is gummadi, in English it is white guard melon or ash guard[1]. It is a large climber, softly hairy all over. It is cultivated for its fruits throughout the plains of India and on the hills up to 4000 ft. It is an extensive climbing herb. The fruit is a broadly cylindrical or spheroidal gourd 1 to 1.5 ft long, with white flesh, containing numerous, much compressed and margined seeds. Generally the fruit is used as nutritive, tonic, laxative and diuretic. The seeds are said to possess anthelmintic properties. Its confectionery is alterrative, tonic, diuretic and restorative[1,2]. Many empirical applications have been used in India for centuries for various ailments such as G.I.T problem (dyspepsia), burning sensation heat disease, vermiguse, diabetes and urinary diseases[3,4], diuretic activity[5] and anticancer activity[6]. However the lehman or traditional people are

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using this plant-fruit for various rigorous disorder. Triterpenoids, flavonoids, glyconoids, saccharides and carotenes, vitamins, β-sitosterin and uromic acid[7,9] are the major constituent reported and isolated earlier.

Although several medicinal uses have been reported for *Benincasa hispida*, the plant fruit peel parts have not been examined for their central nervous system (CNS) effects. Therefore the present study is undertaken to evaluate the neuropharmacologiocal activities of the plant fruit peel extracts since it used in traditional medicine for the treatment of psychiatric disorder in parts of south Western Orissa (P.K. Panda, personal oral communication).

EXPERIMENTAL

B. hispida fruit was obtained from the local market in the month of Feb, 2006 and identified by the taxonomist Dr. S.P. Rath, Department of Botany, Utkal University. The preparation of the extract was carried out like after removing the stem and the seeds, the fruit peel was dried under shade Specimen vouchers (FPBH 014) were made and deposited at the herbarium of the Department of Pharmacology and Clinical Pharmacy, IGIPS. The dried fruit peel was pulverized by grinding using pestle and mortar. Then, 57 g of the fruit peel were subjected to exhaustive Soxhlet extraction in methanol (250 mL) for 72 h at 60 °C. This gave a mean yield of 16.6 ± 0.23 g w/w of extract. The extract was stored in -40 °C from where it was used when required.

Adult albino mice and rats (weighing 25-30 g and 165-200 g, respectively) were used in this study. All the animals were housed in a cross ventilated room (temperature 22 ± 2.5 ºC, 12 h light: 12 h dark cycle) and were fed with standard mash rodent pellet diet (Amrut, India) and the food was withdrawn 18-24 h before the experiment commenced, water was allowed *ad libitum*. Rearing up of animals in the experimental period and their up keeping during the entire experimental span conformed to ethical guidelines laid down by Institutional Animal Ethical Committee (IAEC) of IGIPS, India.

Acetic acid induced writhing in mice: The abdominal constrictions resulting from intraperitoneal (i.p.) injection of acetic acid (3 %) consisting of the contraction of abdominal muscle together with a stretching of hind limbs, were carried out according to the reported procedures[9-11]. The animals were divided into four groups of 5 mice per group. Group I served as control while groups 2-4 were pre-treated with 500-1500 mg/kg, i.p. of *B. hispida* fruit peel extract. After 0.5 h, acetic acid was administered (i.p.). The numbers of writhing movements were counted for 0.5 h. Antinociception was expressed as the reduction of the number of abdominal constrictions between control animals treated with saline and mice pre-treated with the extract.

Formalin-induced paw licking in mice: The procedure was essentially similar to that described[12-14]. These animals were used to analyze the first phase of formalin-induced licking and 20 µL of 2.5 % formalin solution (0.9 % of formaldehyde)

made up in phosphate buffer solution (PES, Concentration: NaCl, 137 mM; KC1, 2.7 mM and phosphate buffer 10 mM) was injected subcutaneously under, the surface of the right hind paw. The amount of time spent licking the injected paw was timed and was considered as indicative of pain. The first of the nociceptive response normally peaked 5 min after formalin injection and the second phase 15-30 min after formalin injection, representing the neurogenic and inflammatory pain responses, respectively[14]. The animals were pretreated with *B. hispida* fruit peel extract (500-1000 fig/kg, i.p.) 0.5 h before being challenged with buffered formalin and the responses were observed for 0.5 h.

Thermally-induced pain in mice: The effect of extract on hot plate-induced pain was investigated in adult mice. The hot plate test was used to measure the response latencies according to the reported method[15]. In these experiments, the hot plate was maintained at 45 ± 1 °C. Animals were placed into a glass beaker of 50 cm diameter on the heated surface and the time(s), between placement and shaking or licking of the paws or jumping was recorded as the index of response latency. An automatic 30 s cut-off was used to prevent tissue damage. The animals were divided into four groups of 5 mice per cage. Group I served as a control and received only saline. Groups 2-4 were pre-treated with 500-1000 mg/kg i.p. of *B. hispida* fruit peel extract 0.5 h prior to the placement of the hot plate.

Fresh egg albumin-induced inflammation in rats: Increase in the rat hind paw linear circumference induced by subplantar injection of a phlogistic agent was used as the measure of acute inflammation[16]. The phlogistic agent employed in this study was fresh egg-albumin[17]. Adult albino rats of either sex were used after 24 h fast and deprived of water only during experiment. Inflammation of the hind paw was induced by injecting 0.1 mL of fresh egg-albumin into the sub plantar surface of the hind paw. The linear circumference of the injected paw was measured before and after of 1, 3, 5 h the administration of phlogistic agent. For routine drug testing, the increase in paw circumference 3 and 5 h after the administration of the phlogistic agent was adopted as the parameter for measuring inflammation[9,16-18]. Edema (inflammation) was assessed as the difference in paw circumference between the control and after of 1, 3, 5 h the administration of the phlogistic agent[19]. The drugs were administered i.p. to a group and orally to another group before 1 h inducing inflammation. Control rats received 1 mL of fresh egg albumin. The average (mean) edema was assessed by measuring with vernier callipers.

Pentylenetetrazol-induced convulsion in mice: Adult mice were used for this experiment. Before 12 h of experimentation, food was withdrawn but water remained available *ad libitum*. The animals were divided into four groups of 5 mice per group. Group I was given saline while groups 2-4 were pre-treated with 500-1000 mg/kg, i.p. of extract. After 0.5 h, pentylenetetrazol (PTZ) (110 mg/kg) was injected subcutaneously on the back of the neck of the animals. Latency of tonic convulsion and lethality during the following 24 h were assessed[20].

Statistical analysis: The results are expressed as the mean ± SEM and significance was determined by Student's t-test. A probability level of less than 5 % was considered significant.

RESULTS AND DISCUSSION

Acetic acid-induced writhing in mice: The extract (500-1500 mg/kg, i.p.) dose-dependently reduced acetic acid-induced abdominal constrictions and stretching of hind limbs. The reduction was significant (Table-1).

TABLE-1
EFFECT OF FRUIT PEEL OF *B. hispida* EXTRACT ON ACETIC ACID-INCLUDED WRITING IN MICE

Dose (mg/kg)	5 min	10 min	15 min	20 min	25 min	30 min	Total (min)
Control	14.0±0.12	72.0±0.34	106.0±1.04	90.0±0.01	64.0±0.62	65.0±0.4	411.0±0.22
500	9.0±0.30[b]	71.0±0.55[a]	70.0±0.76[b]	54.0±1.20[b]	45.0±0.45[b]	40.0±0.80[b]	289.0±0.67[b]
1000	0.0±0.0[b]	44.0±0.34[b]	54.0±0.65[b]	60.0±0.09[b]	42.0±0.25[b]	38.0±0.66[b]	238.0±0.45[b]
1500	2.0±0.06[b]	37.0±0.01[b]	38.0±0.62[b]	37.0±0.42[b]	26.0±0.31[b]	22.0±0.61[b]	162.0±0.21[b]

Significance relative to control: [a], $P < 02$; [b], $P < 0.001$. Values represent mean ± SEM (n = 5).

Formalin-induced paw licking in mice: The, extract pre-treated animals showed a significant ($p < 0.01$) dose related reduction of hind paw licking caused by formalin (Table-2).

TABLE-2
EFFECT OF FRUIT PEEL OF *B. hispida* EXTRACT ON FORMALIN-INDUCED PAIN IN MICE

Dose (mg/kg, i.p.)	Number of Paw licking (within 0.5 h)	Thermally-induced pain (s)
Control	38.40 ± 6.86	2.30 ± 0.09
500	25.60 ± 4.38[b]	3.00 ± 0.13[a]
750	16.20 ± 9.67[b]	3.60 ± 0.14[b]
1000	5.00 ± 5.59[c]	3.75 ± 0.21[b]

Significance relative to control: [a],$p < 0.02$; [b],$p < 0.01$; [c],$p < 0.001$. Data represent the mean ± SEM (n = 5).

Thermally-induced pain in mice: Pre-treatment of animals with *B. hispida* fruit peel extract (500-1000 mg/kg i.p.), elicited a dose-related increase in the latency response in the hot plate test. These increases in latency responses (analgesic effect) were statistically significant ($p < 0.02$, Table-2)

Fresh egg albumin-induced inflammation in rats: The extract showed significant antiinflammatory activity against acute inflammation (Table-3). It suppressed in a dose-related manner the increase in the rat paw edema caused by egg albumin. The inhibition by the extract was maximal after 1 h of administration of phlogistic agent.

TABLE-3
EFFECT OF FRUIT PEEL OF *B. hispida* ON FRESH EGG ALBUMIN-INDUCED INFLAMMATION IN RATS

Dose (mg/kg, i.p. administration)	Circumference of planter paw before administration of (0.1 mL) albumin	1 h	2 h	5 h
Control (0.01 mL fresh egg albumin)	3.38±0.16	10.56±0.10	6.20±0.22	4.90±0.22
250	3.20±0.14	8.74±0.22 (17.23)[a]	5.50±0.18 (11.29)[c]	4.50±0.31 (8.16)[d]
500	3.30±0.22	7.25±0.04 (31.34)[a]	5.10±0.15 (17.74)[b]	4.10±0.25 (16.33)[c]
1000	3.26±0.17	6.62±0.20 (37.31)	4.90±0.20 (20.97)[b]	3.46±0.21 (29.39)[b]
100 ASA	3.08±0.05	6.48±0.08 (38.64)[a]	4.30±0.26 (30.65)[a]	3.78±0.17 (22.85)[b]
Control (0.1 mL fresh egg albumin administration)	2.74±0.21	8.61±0.04	5.42±0.12	4.98±0.14
250	2.78±0.08	7.40±0.22 (14.06)[b]	5.26±0.10 (2.96)	4.70±0.12 (5.63)
500	2.90±0.11	6.30±0.14 (26.83)[a]	5.22±0.09 (3.70)	4.40±0.02 (11.65)[a]
1000	2.70±0.10	6.01±0.21 (30.2)[a]	4.08±0.03 (24.73)[a]	4.30±0.11 (13.66)[a]
100 ASA	2.83±0.02	5.64±0.13 (34.5)[a]	3.85±0.11 (28.97)[a]	3.90±0.21 (21.69)[a]

Significance relative to control: [a],$p < 0.001$; [b],$p < 0.01$; [c],$p < 0.05$; [d],$p < 0.04$; ASA, acetyl salicylic acid. Values are given as mean ± SEM (n = 5); numbers in parenthesis indicate percentage inhibition by the extract.

Pentylenetetrazol-induced convulsion and mortality in mice: The results of the effect of extract pretreatment on pentylenetetrazol. Induced convulsion in mice is as shown in Table-4. The extract significantly ($p < 0.05$) increased the latency of convulsions and decreased mortality and the number of convulsive events.

TABLE-4
EFFECT OF FRUIT PEEL OF *B. hispida* EXTRACT ON PTZ-INDUCED CONVULSIONS AND MORTALITY IN MICE

Dose (mg/kg, i.p.)	Latency of clonic convulsion (min)	Latency of tonic convulsion (min)	Convulsion (100 %)	Mortality (100 %)
Control	1.48±0.34	2.98±0.28	100	100
500	2.55±0.19[a]	6.65±1.23[b]	100	Nil
750	3.12±0.42[b]	7.12±0.89[b]	100	Nil
1000	8.65±1.27[c]	16.05±1.04[d]	100	Nil

Significance relative to control: [a],$p < 0.1$; [b],$p < 0.05$; [c],$p < 0.001$: [d],$p < 0.001$. Data represent mean latency ± SEM (n = 5).

The extract caused dose and time-dependent antinociception against chemical and thermal-induced nociception (pain) in mice. Acetic acid causes inflammatory pain by inducing capillary permeability[21] formalin exhibits neurogenic and inflammatory pain[15,22] while hot plate-induced pain indicates narcotic involvement[9,23]. The extract showed significant effect in these three types of pain induction suggests that its analgesic effect may in part be related to its antiinflammatory neurogenic and narcotic properties. The extract progressively reduced edema of the rat hind paw induced by fresh egg albumin. This dose-related effect, was comparable to that of acetyl salicylic acid, a cyclo-oxygenase inhibitor[24]. Flavonoids which are some of the constituents of the extract have antiinflammatory property[25,26]. Due to a primary stimulus, two mechanisms contribute to the development of edema caused by increased vascular permeability. One induced by local release or formation of various autocoids and another induced neurogenically by stimulation of primary sensory neurons and subsequent mediator (substance P) release from peripheral endings of these fibers[21,27,28]. The neurogenic component plays an important role in maintaining the non-nerogenic plasma extravasation since the stimulation of peripheral neurons and subsequent release of substance p from peripheral sensory endings causes further release of histamine from mast cells[29]. It therefore means that the possible specific action of this extract in blocking the neurogenic component of the stimulated vascular permeability can stop, the series of pathogenetic events locally evoked by noxious stimuli. Therefore, the antiinflammatory properties of this extract strongly support the evidence of a major antiedematous component.

Two mechanisms have been proposed for the mode of pentylenetetrazol-induced convulsion. It is proposed that pentylenetetrazol induces convulsion by either inhibiting γ-amino butyric acid (GABA) pathway in CNS[30,31] or by increasing the central nor adrenergic activity[1,32]. However, drugs or substances that inhibit-induced convulsion, exert their effects either by increasing γ-amino butyric acid-receptor-chloride channel complex[33] or by decreasing the central noradrenergic activity[32]. That the extract produced a significant dose-dependent protective effect against pentylenetetrazol-induced convulsion in mice, may in part be due to its involvement in γ-amino butyric acid-ergic or noradrenergic pathways.

In conclusion, although the exact mechanism of antinociceptive properties of the extract is not fully understood, it may not be unrelated to the present study which involves suppression of capillary permeability through neurogenic and non-neurogenic pathways as well its narcotic potential, γ-amino butyric acid-ergic increase and noradrenergic central depletion. However, further work is advocated to elucidate the exact mechanism of the action.

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