Antidepressant-like activity of *Benincasa hispida* fruits in mice: Possible involvement of monoaminergic and GABAergic systems

Sir,

Depression is one of the major mental disorders affecting hundreds of millions of people all over the world. There is a constant need to identify newer antidepressants from plants. *Benincasa hispida* (*Cucurbitaceae*) fruits were selected for evaluating its antidepressant potential in mice. There is only one study showing the antidepressant-like effect of the methanolic extract (0.6 and 1 g/kg administered three times and only once, respectively) of *B. hispida* fruits in mouse forced swim test (FST).[1] The study showed the antidepressant-like effect of the methanolic extract in FST only and the mechanisms responsible for this activity have not been studied.

The fresh fruits of *B. hispida* were purchased from the local market of Hisar (Haryana, India) and authenticated as *B. hispida* (Thunb.) Cogn. (*Cucurbitaceae*) by Raw Materials Herbarium and Museum, NISCAIR, New Delhi, India (reference numbers NISCAIR/RHMD/Consult/-2010-11/1446/44 and 1448/46). After removing the outer skin and the seeds, the fruit pulp (1 kg) of *B. hispida* was mashed using an electric juicer to afford a soft mass. The pulp was macerated with methanol (1:4) for 7 days at room temperature with occasional stirring daily. On the day 8, the pulp was filtered and the filtrate was heated (below 55°C) and evaporated using a water bath till a dark brownish liquid was obtained. The yield of the extract was 5.5% w/w. The extract obtained was stored at 2–4°C in a refrigerator and dissolved in distilled water prior to the administration to the animals.[2]

Swiss male albino mice, weighing around 20–25 g, were purchased from Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Sciences (Hisar). Animals were housed under standard laboratory conditions with an alternating light and dark cycle of 12 h each. They had free access to food and water. The animals were acclimatized for at least 5 days before behavioral experiments. The study was carried out between 09:00 am and 5:00 pm. The experimental protocol was approved by IAEC and animal care was taken as per the guidelines of CPCSEA, Govt. of India.

Prazosin hydrochloride, (±)sulpiride, DL parachlorophenylalanine (p-CPA), baclofen (all from Sigma-Aldrich, St. Louis, MO, USA), imipramine hydrochloride, fluoxetine hydrochloride, and phenelzine (all from Ranbaxy Laboratories, Gurgaon, Haryana, India) were used in the present study. p-CPA was dissolved as reported earlier.[3] All other drugs were separately dissolved in normal saline. The mice were distributed into 25 groups and each group comprised a minimum of 6–10 mice. Methanolic extract (50, 100, and 200 mg/kg), imipramine (15 mg/kg), fluoxetine (20 mg/kg), and phenelzine (20 mg/kg) were administered orally for 14 successive days to separate groups

Table 1: Effect of the methanolic extract of *Benincasa hispida* on the immobility period of mice using the tail suspension test and forced swim test

Treatment for 14 days (p.o.)	Dose (per kg)	Immobility period in TST (s)	Immobility period in FST (s)
Vehicle (distilled water)	10 ml	166.1 ± 6.26	161 ± 7.58
Imipramine	15 mg	114.4 ± 6.63*	105.3 ± 5.53*
Fluoxetine	20 mg	93.9 ± 8.69*	89.6 ± 4.33*
Phenelzine	20 mg	117.2 ± 3.84*	106 ± 4.7*
Methanolic extract	50 mg	148 ± 8.09	157.4 ± 3.75
Methanolic extract	100 mg	106.7 ± 3.57*	81.6 ± 4.12*
Methanolic extract	200 mg	141 ± 4.06*	147.3 ± 5.38

N = 10 in each group except the 50 mg/kg methanolic extract group where n = 9. Separate groups of animals were employed for recording immobility periods in TST and FST. Values are in mean ± SEM. Data were analyzed by one-way ANOVA followed by Dunnett's t-test. F (6, 62) = 17.48; $P < 0.0001$ (for data of TST). F (6, 62) = 40.07; $P < 0.0001$ (for data of FST). *$P < 0.05$ when compared with the vehicle-treated group. FST - Forced swim test. TST - Tail suspension test

Table 2: Effect of the combination of methanolic extract of *Benincasa hispida* with sulpiride, baclofen, prazosin, and p-CPA on the immobility period of mice in the tail suspension test

Treatment for 14 days	Dose (per kg)	Immobility period (s)
Vehicle (distilled water; p.o.)	10 ml	177.5 ± 5.28
Methanolic extract (p.o.)	100 mg	106.7 ± 3.57[a]
Vehicle (p.o.) + sulpiride (i.p.)	10 ml + 50mg	228 ± 2.40[a]
Methanolic extract (p.o.) + sulpiride (i.p.)	100 mg + 50 mg	135.5 ± 4.16[b]
Vehicle (p.o.) + baclofen (i.p.)	10 ml + 10 mg	200.8 ± 4.88[a]
Methanolic extract (p.o.) + baclofen (i.p.)	100 mg +10 mg	132.1 ± 3.90[b]
Vehicle (p.o.) + prazosin (i.p.)	10 ml + 62.5 µg	192.6 ± 5.28[a]
Methanolic extract (p.o.) + prazosin (i.p.)	100 mg + 62.5 µg	131.4 ± 2.87[b]
Vehicle (p.o.) + p-CPA (i.p.)	10 ml + 100 mg	205.5 ± 3.24[a]
Methanolic extract (p.o.) + p-CPA (i.p.)	100 mg + 100 mg	152.6 ± 8.73[b]

N = 10 in each group; values are mean ± SEM. Data were analyzed by one-way ANOVA followed by Dunnett's t-test. F (4, 45) = 22.159; $P < 0.001$ (for vehicle-treated groups). F (4, 45) = 10.068; $P < 0.001$ (for extract-treated groups). [a]$P < 0.05$ when compared with the vehicle-treated group. [b]$P < 0.05$ when compared with the methanolic extract-treated group.

of mice. The antidepressant-like activity was evaluated 60 min after the drug administration on day 14 by employing FST and tail suspension test (TST).[3] Probable mechanisms of action of the extract were investigated by the co-administration of prazosin (α_1-adrenoceptor antagonist), sulpiride (D_2-receptor antagonist), p-CPA (a serotonin synthesis inhibitor), and baclofen ($GABA_B$ agonist) separately in mice pretreated with the most effective dose of the extract for 14 successive days, followed by behavioral testing in TST. After behavioral testing in FST, mice were sacrificed and the brain MAO-A activity was assessed spectrophotometrically (Perkin-Elmer) at a wavelength of 280 nm. Total protein was estimated in the brain homogenate by using the total protein kit (Crest Biosystems, Goa, India) using a colorimeter (Photochem, India).[3,4] Locomotor activities of control and test animals were recorded for a period of 10 min using a Photoactometer (INCO, Ambala, Haryana, India).

The methanolic extract (100 mg/kg, p.o.) significantly decreased the immobility periods in both TST and FST, indicating a significant antidepressant-like activity. The efficacy of the extract was found to be comparable to imipramine, fluoxetine, and phenelzine [Table 1]. The extract did not show any significant change in the locomotor function (637 ± 15.92) of mice (n = 6, in each group) as compared to the control animals (666.17 ± 14.33). It confirms our hypothesis that the antidepressant-like effect of the extract is specific and not false positive. Our investigation also demonstrated that the antidepressant-like effect of the extract was significantly reversed by pretreatment of the animals with sulpiride, baclofen, prazosin, and p-CPA when tested in TST [Table 2]. This suggested that the extract might produce an antidepressant-like effect by increasing the levels of norepinephrine, dopamine, and serotonin, and decreasing the levels of GABA. Drugs which enhance the levels of these monoamines have been used as antidepressant drugs.[5] A decrease in $GABA_B$ neurotransmission may contribute to the action of antidepressants.[6] Furthermore, brain MAO-A activities (n = 7, in each group) were significantly reduced by 100 mg/kg of the methanolic extract (52.09 ± 1.98 nmol/mg protein) and phenelzine (49.82 ± 5.86 nmol/mg protein) as compared to the control (86.63 ± 8.08 nmol/mg protein); hence, the extract might exert an antidepressant-like action by inhibiting the metabolism of monoamines.

Thus, the methanolic extract of *B. hispida* showed significant antidepressant-like activity in mice probably by inhibiting MAO-A, and through interaction with dopaminergic, α_1-adrenergic, serotoninergic, and GABAergic systems. The extract of *B. hispida* may be further studied to find out the particular active component(s) responsible for its antidepressant-like activity.

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