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
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 (\(\alpha_1\)-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, significant antidepressant-like activity in mice probably by inhibiting M\(\text{AO}-\)A, and through interaction with dopaminergic, \(\alpha_1\)-adrenergic, serotonergic, and GABA\(_B\)ergic systems. The extract of \(B.\) hispida may be further studied to find out the particular active component(s) responsible for its antidepressant-like activity.

**ACKNOWLEDGMENTS**

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**REFERENCES**

1. Rukmani R, Nidhiya IS, Nair S, Kumar A. Investigation of anxiolytic-like effect of antidepressant activity of \(B.\) hispida, methanolic extract. Indian J Pharmacol 2003;35:129. (35th Annual Conference of Indian Pharmacological Society, Gwalior, November 26-29, 2002; Abstracts of Research Papers - Part 1).
2. Kumar DA, Ramu P. Effect of methanolic extract of \(B.\) hispida against histamine and acetylcholine induced bronchospasm in guinea pigs. Indian J Pharmacol 2002;34:365-6.
3. Dhingra D, Kumar V. Evidences for the involvement of monoaminergic and GABAergic systems in antidepressant-like activity of garlic extract in mice. Indian J Pharmacol 2008;40:175-9.
4. Henry RJ, Cannon DC, Winkelman JW. Clinical chemistry, principles and

\[\text{Immobility period (s)} = 177.5 \pm 5.28 \text{ for Vehicle (distilled water; p.o.)}\]

\[\text{Immobility period (s)} = 106.7 \pm 3.57^a \text{ for Methanolic extract (p.o.)}\]

\[\text{Immobility period (s)} = 228 \pm 2.40^a \text{ for Vehicle (p.o.) + sulpiride (i.p.)}\]

\[\text{Immobility period (s)} = 135.5 \pm 4.16^b \text{ for Methanolic extract (p.o.) + sulpiride (i.p.)}\]

\[\text{Immobility period (s)} = 200.8 \pm 4.88^a \text{ for Vehicle (p.o.) + baclofen (i.p.)}\]

\[\text{Immobility period (s)} = 132.1 \pm 3.90^a \text{ for Methanolic extract (p.o.) + baclofen (i.p.)}\]

\[\text{Immobility period (s)} = 192.6 \pm 5.28^b \text{ for Vehicle (p.o.) + prazosin (i.p.)}\]

\[\text{Immobility period (s)} = 131.4 \pm 2.87^a \text{ for Methanolic extract (p.o.) + prazosin (i.p.)}\]

\[\text{Immobility period (s)} = 205.5 \pm 3.24^a \text{ for Vehicle (p.o.) + p-CPA (i.p.)}\]

\[\text{Immobility period (s)} = 152.6 \pm 8.73^b \text{ for Methanolic extract (p.o.) + p-CPA (i.p.)}\]

\(N = 10\) in each group; values are mean \(\pm\) 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). \(^aP < 0.05\) when compared with the vehicle-treated group. \(^bP < 0.05\) when compared with the methanolic extract-treated group.

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 \(\pm\) 15.92) of mice \((n = 6, \text{in each group})\) as compared to the control animals (666.17 \(\pm\) 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 M\(\text{AO}-\)A activities \((n = 7, 6, \text{in each group})\) were significantly reduced by 100 mg/kg of the methanolic extract (52.09 \(\pm\) 1.98 nmol/mg protein) and phenelzine (49.82 \(\pm\) 5.86 nmol/mg protein) as compared to the control (86.63 \(\pm\) 8.08 nmol/mg protein); hence, the extract might exert an antidepressant-like action by inhibiting the metabolism of monoamines.

\begin{table}[h]
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\begin{tabular}{|l|l|l|}
\hline
\textbf{Treatment for 14 days} & \textbf{Dose (per kg)} & \textbf{Immobility period (s)} \\
\hline
Vehicle (distilled water; p.o.) & 10 ml & 177.5 \(\pm\) 5.28 \\
Methanolic extract (p.o.) & 100 mg & 106.7 \(\pm\) 3.57\(^a\) \\
Vehicle (p.o.) + sulpiride (i.p.) & 10 ml + 50mg & 228 \(\pm\) 2.40\(^a\) \\
Methanolic extract (p.o.) + sulpiride (i.p.) & 100 mg + 50 mg & 135.5 \(\pm\) 4.16\(^b\) \\
Vehicle (p.o.) + baclofen (i.p.) & 10 ml + 10 mg & 200.8 \(\pm\) 4.88\(^a\) \\
Methanolic extract (p.o.) + baclofen (i.p.) & 100 mg +10 mg & 132.1 \(\pm\) 3.90\(^a\) \\
Vehicle (p.o.) + prazosin (i.p.) & 10 ml + 62.5 \(\mu\)g & 192.6 \(\pm\) 5.28\(^b\) \\
Methanolic extract (p.o.) + prazosin (i.p.) & 100 mg + 62.5 \(\mu\)g & 131.4 \(\pm\) 2.87\(^a\) \\
Vehicle (p.o.) + p-CPA (i.p.) & 10 ml + 100 mg & 205.5 \(\pm\) 3.24\(^a\) \\
Methanolic extract (p.o.) + p-CPA (i.p.) & 100 mg +100 mg & 152.6 \(\pm\) 8.73\(^b\) \\
\hline
\end{tabular}
\end{table}
The stem bark of Erythrina mysorensis (Gamb. (Fabaceae) was collected from the regions of Shimoga District, Karnataka, India, and authenticated by a plant taxonomist. Coarse powder was used for this study. The preliminary phytochemical screening of the extracts revealed the presence of secondary metabolites such as glycosides, alkaloids, flavonoids, tannins, triterpenoids and saponins.

All the extracts were distilled, dried, and alcohol in a Soxhlet apparatus, finally with chloroform.

Erythrina mysorensis is a small tree with few or no prickles. Literature survey reveals that there are no scientific reports regarding anti-epileptic activity of Erythrina mysorensis.

Hence this study was undertaken to evaluate anticonvulsant activity of extract of Erythrina mysorensis.

The maximal electro shocks (MES) induced epileptic seizures in animals represent grand mal type of epilepsy. All the animals received maximal electro shock (150 mA, 60 Hz for 2 s). The animals were observed individually for 30 min from the time of electric shock for different phases of epileptic seizures.

The chloroform extract in higher dose significantly (P < 0.001) reduced hind limb extensor phase of convulsion as compared to vehicle-treated animals [Table 1]. In PTZ-induced convulsion, prevented the death of animals, and increased the time of onset of convulsion, decreased duration of convulsion, prevented the death of animals, and increased the time of onset of convulsion, decreased duration of convulsion (P < 0.01) and percentage protection of seizure or mortality on comparison with the reference standard diazepam 4 mg/kg [Table 2].

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Epilepsy is a common neurological disorder characterized by recurrent seizures and can be associated with long-term physical, cognitive, psychological, and emotional sequelae.

Anticonvulsant activity of extract of Erythrina mysorensis.

There are many mechanisms by which seizures can develop in either normal or pathologic brains. Three common mechanisms include: (i) diminution of inhibitory mechanism (especially mediated by GABA), (ii) enhancement of the excitatory synaptic mechanism (especially those mediated by NMDA), and (iii) enhancement of endogenous neuronal burst firing (usually by enhancing voltage dependent calcium currents).

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5. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology: 5th ed. Philadelphia: Churchill Livingstone; 2003. P. 535-49.

6. Nakagawa Y, Ishima T, Ishibashi Y, Tsuji M, Takashima T. Involvement of GABA_b receptor systems in experimental depression: Baclofen but not bicuculline exacerbates helplessness in rats. Brain Res 1996;741:240-5.