EFFECT OF DRIED FRUIT EXTRACT OF BENINCASA HISPIDA ON BRAIN BEHAVIOUR IN LABORATORY ANIMALS

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Abstract: The present investigation was aimed at determining the spectrum of neuropharmacological activity of different doses (100, 200, 400 mg/kg) of petroleum ether (BHP), methanolic (BHM) and aqueous extract (BHA) obtained from Benincasa hispida in laboratory animals. Benincasa hispida was studied for its effect on motor coordination, locomotor activity, cognitive behavior, anxiolytic activity, haloperidol induced catalepsy and anticonvulsant activity. The BHM and BHA 400 mg/kg showed significant (P< 0.01) decrease in locomotor activity and exploratory behavior but no effect on motor-coordination. Same doses showed significant (P< 0.01) increases in discrimination index in object recognition test. BHM 400 found to prolong haloperidol induced catalepsy in mice (P< 0.05). Moreover the BHM and BHA 400 mg/kg extracts also showed significant (P< 0.05) analgesic activity in hot plate method. The result points towards the potential activity of the Benincasa hispida as anxiolytic, analgesic and nootropic activity.

Key words: Benincasa hispida, Anxiolytic, Anticonvulsant,

INTRODUCTION

Numerous herbal medicines are recognized as active in the central nervous system and they have at least a hypothetical potential to affect chronic conditions such as anxiety, depression, headaches or epilepsy, that do not respond well to conventional treatments [1,2]. During the last two decades, pharmacotherapy with psychoactive drugs has been increasingly recognized as most effective in the management of anxiety, stress and psychosomatic disorders. However, the prolonged use of tranquilizers and psychotropic drugs leads to a variety of autonomic, endocrine, allergic, hematopoietic and neurological side effects. Moreover, such agents primarily relieve the symptoms and offer a palliative relief of a temporary nature [3]. Herbal medicine may provide good alternative for same with no or minimal side effect. The lack of effective and widely applicable pharmacological treatments in the modern therapy for the neurodegenerative disorders may explain a growing interest in the traditional medicines [4]. According to estimation of WHO, 70-80% of the world population relies on the traditional medicine, mostly plant drug for their primary healthcare needs [5].

Benincasa hispida (BH) is a widely used vegetable in India and other tropical countries and belongs to the family Cucurbitaceae [6]. In Ayurveda, BH is recommended for management of peptic ulcer, hemorrhages from internal organs, epilepsy and other nervous disorders [7,8]. BH was found to potentially inhibit the histamine release from the rat exudates cell induced by antigen-antibody reaction [9]. Alcoholic and petroleum ether extract of BH reported to have antiulcerogenic effect. BH probably has a CNS component in prevention of stress induced ulceration [10]. Aqueous extract of seeds of BH also showed immunopotentiator activity. The juice of BH was found to be effective against morphine
withdrawal symptoms [11]. Researcher also reported anxiolytic activity of BH [12].

The present investigation was aimed at determining the spectrum of neuropharmacological activity of different doses (100, 200, 400 mg/kg) of petroleum ether (BHP), methanolic (BHM) and aqueous extract (BHA) of Benincasa hispida in mice.

**MATERIALS AND METHODS**

**Plant material:** The plant material (fruits of Benincasa hispida) were collected from Pune region of Maharashtra, India and were authenticated by botanical survey of India.

**Preparation of extract:** Petroleum ether extract (BHP), methanolic extract (BHM) and aqueous extracts (BHA) were prepared by successive extraction method. The fruits of BH were dried in shade and coarsely powdered. The powder was successively extracted with petroleum ether followed by methanol in a Soxhlet apparatus. Powder remaining after methanolic extraction was subjected to aqueous extraction [13]. The aqueous extract was prepared by maceration with distilled water for 24 h. The extracts were concentrated under reduced pressure and were stored at 8–10°C throughout the study.

**Animals:** Swiss male albino mice (18-22g) were used. These mice were maintained at 25° C ± 2° C and 45-55% relative humidity and under standard environmental conditions (12:12 h L:D cycle). These mice had free access to food and water. They were deprived of food but not water 6 h before the drug administration. Institutional Animal Ethics Committee (IAEC) approved the protocol and entire study has carried out as per standard guideline of IAEC. All experiments were carried out between 12:00-16:00 hours.

**Chemicals and drugs:** Piracetam syrup, diazepam, pentazocin and haloperidol injection were purchased from the local market. Pentylenetetrazole was purchased from Loba Chemical, Mumbai, India.

**Acute toxicity test:** Healthy adult male albino mice (18-22g) were subjected to acute toxicity studies as per guidelines (AOT 425) suggested by the organization for economic co-operation and development (OECD-2001). The mice were observed continuously for 2 hours for behavioral and autonomic profiles and for any sign of toxicity or mortality up to a period of seven days [14].

**Effect on motor coordination:** The motor coordination was assessed using digital rota rod (INCO, Ambala, India). Mice were trained by placing them on a rotating rod (20 rev/min), twice daily for three consecutive days before the experiment. Thirty min interval was kept between two trails. Only those mice which have demonstrated their ability to remain on the rotating rod for at least 2 min were selected. These selected mice were divided into eleven groups with 6 animals in each group. The mice were then tested for motor coordination to record basal fall of time followed by BHP, BHM and BHA (100, 200, 400 mg/kg/p.o.). One hour following the administration of vehicle or drug, mice were placed again on the rotating rod and the fall off time per 300 sec was recorded. The difference between mean fall of time before and after drug treatment was considered for evaluation. Diazepam (2 mg/kg/i.p.) was used as a reference standard [15,16].

**Locomotor activity:** The locomotor activity was measured using a digital actophotometer (INCO, Ambala, India). Each mouse was placed individually in the actophotometer for 05 min and basal activity score was obtained. Subsequently animals were divided into eleven groups and treated with BHP, BHM and BHA (100, 200, 400 mg/kg/p.o.). 60 min after dosing; the mice were placed again in the actophotometer for recording the activity score as described earlier. The results were reported as mean change in the locomotor activity. Diazepam (2 mg/kg/i.p.) was used as a reference standard [17,18].

**Object recognition test:** The activity cage (INCO, Ambala, India), illuminated by a 40 W lamp suspended 50 cm above the apparatus was used for study. The object to be discriminated was also made of plywood in two different shapes of 10 cm height and colored black. One day before the test, mice were allowed to explore the box without any object for 02 min. On the day of test, in the first trial (T1) conducted 60 min after administration of vehicle (10 ml/kg), BHP, BHM and BHA (100, 200, 400 mg/kg/p.o.) and piracetam (150 mg/kg/i.p). Two identical objects were presented in opposite corners of the box and the time taken by each mouse to complete 20 s of object exploration was recorded (Exploration was considered as directing the nose at a distance...
less than 2 cm to the object and/or touching with nose). Second trial (T2) was performed 90 min after first (T1) and a new object replaced one of the objects presented in T1 and mice were left in the box for next 05 min. The time spent for exploring the familiar (F) and the new object (N) was recorded separately and discrimination index (D) was calculated as (N-F)/(N+F). The object was changed randomly and apparatus was cleaned with hydrogen peroxide after each trial to avoid place preference and the influence of olfactory stimuli respectively [16].

**Anxiolytic activity using elevated plus maze (EPM):** The elevated plus maze apparatus consisted of two open arms (30 x 5 cm) and two closed arms (30 x 5 x 20 cm) emanating from a common central platform (5 x 5 cm). Two pairs of identical arms were opposite to each other. Entire apparatus was elevated to a height of 50 cm above the floor level. Swiss albino male mice (25 ± 2 g) were used. Mice received vehicle, extracts or reference standard drug (Diazepam 2 mg/kg/i.p) as per treatment schedule 60 min before start of session. To start session mouse was placed at the center of maze, its head facing closed arm and allowed to explore maze for 5 min. During this 5 min time spent in open arm, percent entries in open and closed arm and total entries were recorded. An entry was defined as all four paws in the arm. The plus maze was carefully wiped with hydrogen peroxide and dried with sponge after each trial [19].

**Analgesic activity:** The analgesic effect was studied using digital hot plate (INCO, Ambala, India) method wherein the reaction time (paw licking, jumping or any other sign of discomfort) was recorded at 0, 60, and 120 min after administration of vehicle (10 ml/kg/p.o.) and BHP, BHM and BHA (100, 200, 400 mg/kg/p.o.) The temperature of the plate was maintained at 55°C ± 01°C. A cut off reaction time of 30 s was chosen in order to avoid injury. Pentazocin (30 mg/kg/i.p) was used as a reference standard [15, 18].

**Haloperidol induced catalepsy:** Mice were divided into four groups. The control group received vehicle (10 ml/kg/p.o.) whereas the other group received BHP, BHM and BHA (100, 200 and 400 mg/kg/p.o.) 60 min before haloperidol (1 mg/kg/i.p). After the treatment, the forepaws of the mice were placed on rod of 1.0 cm diameter set at 3.0 cm from top. Duration for which the mice retains the forepaws on the elevated rod was noted down at 0, 15, 30, 60, 90 and 120 min. the cut off time was 300 sec. The animals were tested twice at each time interval and only the greater duration of time was recorded. Between measurements, the mice were returned to their home cages [20, 21].

**Pentylenetetrazole (PTZ) induced seizure:** Clonic seizures were induced 60 min after respective drug treatment in mice by administering pentylenetetrazole (80 mg/kg/s.c). The latency to the onset of seizures in non-protected mice and lethality during the following 24 h was recorded and compared with those of control mice to assess the anticonvulsant activity of the extract. Clonazepam (0.1 mg/kg/i.p.) was used as a reference standard [22,23].

**Maximal electroshock (MES) induced seizures:** Tonic clonic convulsions were induced 60 min after the respective drug treatment by giving maximal electroshock seizures (MES) (40mA for 0.2sec) using an electroconvulsiometer (INCO, Ambala, India) via crocodile ear clip 60 min after administration of either vehicle (10 ml/kg/i.p.) , BHP, BHM and BHA (100, 200 and 400 mg/kg/p.o.) or Phenytoin (20 mg/kg/i.p). The number of animals protected from tonic hind limb extension seizure (abolition of tonic hind limb extension within 10 sec after delivery of the electroshock was considered as protected mice.) and duration of tonic hind limb extension seizure was determined in each dose group [23,24].

**Statistical analysis:** The results are expressed as mean ± SEM. Comparison between the groups was made by one way analysis of variance (ANOVA) followed by Dunnett’s test.

**RESULTS**

**Acute oral toxicity test:** All extracts found to be safe at dose used and all mice were free of any toxicity up to the dose of 2 gm/kg.

**Effect on motor coordination:** All doses of BHP, BHM and BHA (100, 200 and 400 mg/kg/p.o.) were found to be statistically insignificant in reducing the time of fall. BHP and BHM (400 mg/kg/p.o.) showed non significant reduction in time of fall. Diazepam (2 mg/kg/i.p.) showed significant (P<0.01) reduction in time of fall (Data not presented).

**Locomotor Activity:** BHP and BHM in a dose of 400 mg/kg produced significant (P<0.05) reduction in mean change in locomotor activity as compared to control. Other doses did not produce any significant
Table 1: Anxiolytic activity of BHP, BHM, BHA and diazepam on Elevated Plus Maze. Results are expressed as mean ± SEM (n = 6). Data was analysed by one way analysis of variance (ANOVA) followed by Dunnetts test. *P<0.05, **P<0.01.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% of open arm entries</th>
<th>Time spent in open arm (Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (10 ml/kg/p.o.)</td>
<td>35.16 ± 1.13</td>
<td>21.50 ± 1.05</td>
</tr>
<tr>
<td>BHP (100 mg/kg/p.o.)</td>
<td>30.00 ± 1.36</td>
<td>20.83 ± 1.77</td>
</tr>
<tr>
<td>BHP (200 mg/kg/p.o.)</td>
<td>39.16 ± 2.56</td>
<td>22.50 ± 1.17</td>
</tr>
<tr>
<td>BHP (400 mg/kg/p.o.)</td>
<td>38.83 ± 2.56</td>
<td>25.00 ± 2.43</td>
</tr>
<tr>
<td>BHM (100 mg/kg/p.o.)</td>
<td>43.16 ± 1.07</td>
<td>38.66 ± 1.05**</td>
</tr>
<tr>
<td>BHM (400 mg/kg/p.o.)</td>
<td>48.33 ± 2.47**</td>
<td>42.00 ± 0.85**</td>
</tr>
<tr>
<td>BHA (100 mg/kg/p.o.)</td>
<td>40.16 ± 2.3</td>
<td>36.50 ± 2.07</td>
</tr>
<tr>
<td>BHA (200 mg/kg/p.o.)</td>
<td>42.00 ± 1.29</td>
<td>37.00 ± 1.15**</td>
</tr>
<tr>
<td>BHA (400 mg/kg/p.o.)</td>
<td>48.00 ± 1.59**</td>
<td>43.50 ± 0.99**</td>
</tr>
<tr>
<td>Diazepam (2 mg/kg/ip.)</td>
<td>67.5 ± 4.56**</td>
<td>81.83 ± 1.83**</td>
</tr>
</tbody>
</table>

Table 2: Analgesic activity of BHP, BHM, BHA and Pentazocin using hot plate analgesiometer. Results are expressed as mean ± SEM (n = 6). Data was analysed by one way analysis of variance (ANOVA) followed by Dunnetts test. *P<0.05, **P<0.01.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Reaction time (Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Vehicle (10 ml/kg/p.o.)</td>
<td>4.54 ± 0.147</td>
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<tr>
<td>BHP (100 mg/kg/p.o.)</td>
<td>4.46 ± 0.114</td>
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<tr>
<td>BHP (200 mg/kg/p.o.)</td>
<td>4.58 ± 0.116</td>
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<tr>
<td>BHP (400 mg/kg/p.o.)</td>
<td>4.53 ± 0.152</td>
</tr>
<tr>
<td>BHM (100 mg/kg/p.o.)</td>
<td>4.55 ± 0.164</td>
</tr>
<tr>
<td>BHM (200 mg/kg/p.o.)</td>
<td>4.30 ± 0.103</td>
</tr>
<tr>
<td>BHM (400 mg/kg/p.o.)</td>
<td>4.41 ± 0.140</td>
</tr>
<tr>
<td>BHA (100 mg/kg/p.o.)</td>
<td>4.50 ± 0.173</td>
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<td>BHA (200 mg/kg/p.o.)</td>
<td>4.30 ± 0.106</td>
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<tr>
<td>BHA (400 mg/kg/p.o.)</td>
<td>4.58 ± 0.135</td>
</tr>
<tr>
<td>Pentazocin (30 mg/kg/ip.)</td>
<td>4.53 ± 0.143</td>
</tr>
</tbody>
</table>

Fig. 1: Effect of BHP, BHM, BHA and Diazepam on motor performance in mice using actophotometer apparatus. Results are expressed as mean ± SEM. (n = 6). Data was analyzed by one way analysis of variance (ANOVA) followed by Dunnetts test. **P<0.01.

Fig. 2: Effect of BHP, BHM, BHA and Piracetam on discrimination index in object recognition test in mice. Results are expressed as mean ± SEM. (n = 6). Data was analyzed by one way analysis of variance (ANOVA) followed by Dunnetts test. *P<0.05, **P<0.01.
effect in locomotor activity. Diazepam (2 mg/kg/i.p.) showed significant reduction in mean change in locomotor activity (Fig. 1).

**Object recognition test:** BHM and BHA (400 mg/kg/p.o.) treated mice showed significant increase in discrimination index (P<0.01) when compared against vehicle treated mice. BHP 400mg/kg and BHM 200 mg/kg was less significant in this regard. Piracetam (150 mg/kg/i.p.) was also significant (P<0.01) as compared with vehicle treated animals (Fig. 2).

**Anxiolytic activity using elevated plus maze:** BHM and BHA 400mg/kg significantly increased the % of open arm entries and time spent in open arm, compared with the vehicle treated group. Diazepam showed significant (P<0.01) increase in the % of open arm entries and time spent in open arm (Table 1).

**Analgesic activity:** BHM 200 and 400 mg/kg demonstrated significant increase in reaction time in hot plate analgesic activity. Pentazocin (30 mg/kg/i.p.) showed significant (P<0.01) analgesic activity by prolonging the reaction time in hot plate method (Table 2).

**Haloperidol induced catalepsy:** In haloperidol-induced catalepsy, maximum catalepsy was noted at 90 min and 120 min. BHM (400mg/kg) treatment showed marked potentiation of catalepsy from 90 min to 120 min. The BHP and BHA did not show any significant potentiation at all doses.

**Pentylenetetrazole induced seizure (PTZ):** The onset of convulsion in vehicle treated control mice were 191.32 ± 03.02 sec, BHM and BHA showed non significantly delayed this onset of convulsion.

**Maximal electroshock induced seizures (MES):** Maximal electroshock induced seizure was not affected by the pre treatment of BHP, BHM and BHA. However, standard phenytoin showed significant (P<0.01) anticonvulsant activity.

**DISCUSSION**

Mental disorders are characterized by abnormalities in cognition, emotion or mood, or the highest integrative aspects of behavior [25]. To date, the available efficacies of drugs treatment for these conditions are still very limited due to their adverse side effect [26]. Numerous strategies have been developed in order to increase the efficiency of treatment and decrease side effects. Therefore, the investigations of novel pharmacotherapy from medicinal plants to prevent psychiatric illnesses and cognitive impairment have significantly progressed [27,28] and obtained very much concentration. BH is claimed to be useful in various nervous disorder in Indian traditional system of medicine but yet not documented scientifically in this regard.

The rota-rod test was used to assess the exploratory activity. Similar performance observed in mice treated with all doses of BHP, BHM and BHA in relation to the control group. This suggests absence of impaired motor coordination, equilibrium or exploratory behavior due to treatment with BH. BHP and BHM at 400 mg/kg/p.o. showed significant reduction in locomotor activity suggesting sedative property of drug. Moreover the drug which reported to have inhibitory effect on locomotor activity also reported for anxiolytic activity e.g. benzodiazepine, barbiturate etc [29]. BHM and BHA 400mg/kg demonstrated anxiolytic activity in elevated plus maze model.

Results obtained on the elevated plus maze after treatment with BHM and BHA reveal anxiolytic activity, since increases in open-arm parameters are the most representative indices of anxiolytic activity [30]. Time spent on the central platform appears to be related to decision making and/or risk assessment, and the total arm entries is a contaminated measure reflecting changes in anxiety or in general activity [31].

The anticonvulsant property was evaluated by experimental procedures widely used to investigate antiepileptic drugs, with high predictive value for detection of clinically effective drugs [32]. It has been stated that, MES induced hind limb extension can be prevented by inhibition of voltage dependent Na+ channels or blocking glutamatergic excitation mediated by NMDA receptors. On the other hand, PTZ induced seizures can be prevented either by reducing T- type current or by facilitation of GABA–A receptor mediated inhibitory neurotransmission [33]. All doses of BI were found statistically insignificant in anticonvulsant activity against PTZ and MES model. BHP and BHM showed non significant effect in PTZ induced convulsion, suggest GABA mediated behavior which can be correlated to its anxiolytic activity and mean change in locomotor activity.

In the haloperidol-induced catalepsy, the lower doses of BHM and all doses of BHP and BHA had no effect on the catalepsy but the BHM (400 mg/kg/ p.o.) potentiated and prolonged the catalepsy. These
changes can be correlated with a decrease in brain levels of dopamine. The BHP and BHA had no significant effect on the brain DA concentration but BHM at the higher dose showed significant reduction in DA level [34].

The increase in the discrimination index by BHM and BHA has proved that the plant possesses nootropic activity. The BHM and BHA demonstrated analgesic activity by prolonging reaction time. The analgesic activity may be central or peripheral. Moreover further investigation is required to explore mechanism of action of BI for analgesic activity.

CONCLUSION

Benincasa hispida showed inhibition of locomotor activity, nootropic, anxiolytic and analgesic activity. Moreover Benincasa hispida also showed potentiation of haloperidol induced catalepsy. BHM 400 mg/kg showed significant activity in this regard.

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REFERENCE