BENEFICIAL EFFECT OF *BENINCASA HISPIDA* METHANOLIC EXTRACT AGAINST NEUROLEPTIC DISORDERS

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Abstract: Neuroleptic activity of methanolic extract of *Benincasa hispida* was assessed using apomorphine induced cage climbing model and catalepsy test in mice. Methanolic extract showed significant reduction in apomorphine induced cage climbing activity at 600 mg/kg and catalepsy in mice. The phytochemical analysis of methanolic extract showed presence of phenolic compounds and triterpene saponins, which may be responsible for the neuroleptic activity.

Key words: *Benincasa hispida*, Methanolic extract, Neuroleptic disorders

INTRODUCTION

*Benincasa hispida* (Thunb), a member of Family Cucurbitaceae commonly known as Petha in Hindi and Waxgourd in English [1], is a long trailing or climbing gourd, cultivated in several warm countries. The fruit of this plant is reported to have several medicinal properties such as antiulcer [2], hypoglycemic [3], immunopotentiator [4], antihistaminics [5], anti-angiogenic [6], antioxidant [7] and angiotensin converting enzyme inhibitor [7]. In ethnobotonical literature *B. hispida* has been shown to inhibit mental instability, agitation and induces sound sleep [1, 8-10]. Fruit juice of *B. hispida* alone or with liquorice (*G. glabra*) is believed to be the best panacea for epilepsy, insanity and other nervous disorders [9]. Nevertheless, this important medicinal plant has never been tried for neuroleptic activity. Therefore, in present study an attempt is made to evaluate the neuroleptic potential of this plant.

MATERIALS AND METHODS

Plant material: Fresh fruit of *B. hispida* was purchased from the local market of Pune district, Maharashtra during June-July 2004 and was authenticated from Agharkar Research Institute, Pune.

Preparation of methanolic extract of *B. hispida* (MBH): The fruit was peeled, cut into small pieces, seeds were removed and weighed. 1.5 kg of fruit pulp was again macerated with 3000 ml of methanol and kept in a jar with tight cover for 7 days at room temperature. The methanolic extract was filtered and dried at 40 °C in rotary evaporator. The brownish dried mass thus obtain, was stored at 4 to 8 °C until further use.

Animals: Swiss albino mice (National Toxicology Centre, Pune) weighing 18 to 22 g were used. All animals were housed in conventional plastic cages in groups of 6 mice, in a room maintained at 24 ± 2 °C on a 12h-12h light/dark cycle. All the animals had free access to pelleted food (Chakan oil mills, Sangli) and water *ad libitum*. The experimental protocol was approved by Institutional Animal Ethical Committee of Poona College of Pharmacy, Pune as per the guidelines issued by Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Drugs: Apomorphine (Sigma) and Haloperidol (Serenace®, RPG Life Sciences) were purchased from respective source.

Phytochemical screening: MBH was tested (data
is not presented here) for presence of proteins, amino acids, alkaloids, flavonoids, glycosides, saponins and tannins using standard procedures [11].

**Acute toxicity study:** Male Swiss albino mice weighing 18 to 22 g, dosed intraperitoneally with MBH, were observed for any symptoms of toxicity for 48 hours. No toxicity was found upto 5000 mg/kg. Based on the results obtained from this study the doses (100, 300 and 600 mg/kg) for further pharmacological studies were selected.

**Experimental groups:** The animals were divided into 5 groups containing six animals in each group for each experiment. First group of animals was kept as control and treated with vehicle (distilled water). Groups II, III and IV were treated with 100 mg/kg, 300 mg/kg and 600 mg/kg of MBH (dissolved in distilled water) respectively. The V group of mice was given haloperidol (0.3 mg/kg) to study apomorphine induced climbing. All the injections were administered intraperitoneally.

**Apomorphine induced climbing behaviour in mice:** The procedure of Protais et al. [12] (apomorphine induced cage climbing) was used. Each animal was placed in a cylindrical wire mesh cage (height 12 cm, diameter 14 cm, and mesh size 3 mm) and the time of climbing the wall (time during which all four limbs were off the floor) was measured. Apomorphine (1.5 mg/kg, s.c) was injected in the animals of all groups after pretreatment with vehicle or drug. The time of climbing behavior was assessed for 3 minutes after 10 minutes of apomorphine (1.5 mg/kg, s.c) administration at 10 min intervals up to 40 min.

**Catalepsy (bar test) in mice:** In present study, catalepsy was measured by means of the bar test as suggested by Sanberg et al. [13]. An aluminium bar of 0.9 cm in diameter was placed 2.5 cm above the floor. Animal’s forepaws were gently put on the bar and the time spent in second with fore paws on the rod was noted at an interval of 5,15,30,60,90,120,180 and 300 min by an observer who did not know about the given drug treatment. The dose of haloperidol for catalepsy test was 1 mg/kg, i.p. If 5 min elapsed without movement, the test was interrupted. Between determinations, the mice were kept in their home cages. Individual animals were tested in a random order. The behavioral tests were carried daily between 10 am to 4 pm to avoid fluctuations due to circadian rhythm.

**Statistical Analysis:** Data was analyzed by Analysis of Variance (ANOVA) followed by Dunnett’s test. The difference was considered significant at 5% level.

**RESULTS AND DISCUSSION**

The unusual behaviour and experiences associated with schizophrenia (sometimes extended to psychosis in general) can be fully or largely explained by over activity in dopamine function in the brain [14,15]. Apomorphine is a non-selective dopamine D$_2$/D$_3$ receptor agonist that has been used for decades as one of many tests to screen for neuroleptic compounds [16]. Antagonism of apomorphine-induced effects was considered the best animal test to predict neuroleptic activity in patients [17, 18] and has proven robust and reproducible model sensitive to D$_3$ receptor antagonist for the prediction of therapeutic efficacy [19].

The effect of methanolic extract demonstrated a significant reduction in apomorphine-induced climbing behavior at 600 mg/kg dose. The lower doses of MBH failed to show such effect as compared to control group. Standard drug, haloperidol caused significant (P < 0.01) reduction in the time spent in climbing. These results suggested that lower doses of MBH contain extremely low concentration of neuroleptic phytochemical(s) and may not be sufficient to exhibit neuroleptic effects. However, a higher dose (600 mg) of methanolic extract contain sufficient amount of neuroleptic phytoconstituent(s), hence could show such activity.

Neuroleptic drugs invariably possess extrapyramidal symptoms (EPS) as their major side effects, which are characterized by muscle rigidity, stiffness, tremors and a strange gait. These symptoms are known to
occur due to blockade of dopamine receptors in the nigrostriatal pathway of the basal ganglia [20]. When a D2 receptor antagonist is administered to the animal, it shows immobile posture for an extended period of time when placed in an unnatural position [21]. This immobility (called catalepsy) is caused by the blockade of dopamine receptors within the neostriatum and is considered to be a predictor of extrapyramidal symptoms inducing potential in humans [22,23]. The typical catalepsy test consists of placing an animal into an unusual posture and recording the time taken to correct this posture. The time is regarded as an index of the intensity of the catalepsy [13]. The low doses of MBH (100 mg/kg and 300 mg/kg) showed no catalepsy in mice. However, the neuroleptic dose of methanolic extract (600 mg/kg), exhibited significant (P < 0.001) catalepsy from 15 min onwards of administration (Table 1). Haloperidol (1 mg/kg, i.p.) also showed catalepsy for all time intervals. These results suggested the blockade of D2 receptor as primary mechanism of neuroleptic action of methanolic extract.

Preliminary phytochemical study showed presence of proteins, amino acids, saponins, phenols and tannins in the juice and methanolic extract (unpublished data). The analysis also showed presence of triterpene saponins (unpublished data), which may be responsible for the dopamine blocking activity as saponin is known to act on the central dopaminergic system [24-26]. Polysylasaponins form plants, which possess dopamine and serotonin receptor antagonist properties, have been prescribed for hundreds of years to treat psychotic illnesses in Korean traditional medicine [27]. Likewise, many plant products that possess saponin as one of their phytoconstituent, have demonstrated neuroleptic activity against apomorphine climbing behavior [25,28,29]. Triterpene present in other plants showed moderate to strong degree of neuroleptic activity [30]. Zhu and Li [31] demonstrated that bodirin, a triterpene constituent from plant origin, binds to D2 receptors. Yoshizumi et al. [5] reported two triterpenes (alnusenol and multiflorenol) in the methanolic extract of B. hispida. The neuroleptic activity might also be contributed by phenolic compounds of methanolic extract of B. hispida as the latter in other plants too, demonstrated the similar results [32]. Further, phenolic metabolites of typical antipsychotic drugs were shown to have high selectivity for the D2 receptor [33,34] and caused catalepsy [35]. Therefore, it is concluded from the study that neuroleptic activity shown by methanolic extract of B. hispida may be by the virtue of D2 receptor antagonism which might be contributed by saponins, triterpines and/or phenolic phytochemicals. However, further isolation and purification of phytochemical are necessary and work in this respect is in process.

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REFERENCES


Table 1: Effect of methanolic extract of B. hispida on catalepsy in mice

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Control</th>
<th>MBH (100)</th>
<th>MBH (300)</th>
<th>MBH (600)</th>
<th>Halo (1)</th>
</tr>
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<tr>
<td>0</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
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<td>0.00 ± 0.00</td>
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<td>5</td>
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<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
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<td>23.33 ± 4.25</td>
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<td>15</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>58.50 ± 5.30*</td>
<td>145.00 ± 1.00*</td>
</tr>
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<td>30</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>51.00 ± 3.43*</td>
<td>255.00 ± 17.44*</td>
</tr>
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<td>60</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>46.25 ± 4.50*</td>
<td>300.00 ± 0.00*</td>
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<tr>
<td>90</td>
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<td>11.33 ± 0.94</td>
<td>11.00 ± 0.57</td>
<td>47.50 ± 2.00*</td>
<td>300.00 ± 0.00*</td>
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<td>120</td>
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<td>5.33 ± 0.23</td>
<td>5.50 ± 1.20</td>
<td>51.25 ± 1.95*</td>
<td>300.00 ± 0.00*</td>
</tr>
<tr>
<td>180</td>
<td>1.00 ± 0.13</td>
<td>0.00 ± 0.00</td>
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<td>123.75 ± 4.52*</td>
<td>260.00 ± 28.28*</td>
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<tr>
<td>240</td>
<td>1.00 ± 0.16</td>
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<td>12.67 ± 1.24</td>
<td>53.75 ± 1.95*</td>
<td>233.33 ± 31.18*</td>
</tr>
<tr>
<td>300</td>
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<td>5.50 ± 0.86</td>
<td>6.33 ± 0.94</td>
<td>58.75 ± 5.36*</td>
<td>233.33 ± 31.18*</td>
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</table>

n=6, Figures in the bracket indicate dose in mg/kg. MBH - Methanolic extract of Benincasa hispida, Halo- Haloperidol. *p < 0.001