Evaluation of Neuro-Pharmacological Activities of Methanolic Extract of *Benincasa hispida*

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**ABSTRACT**

*Benincasa hispida* (Thunb.) Cogn., is a fruit belonging to Cucurbitaceae family is widely used as a vegetable in India. It is used as an important ingredient in the ayurvedic formulation called Kusmada lehyam which is used in a variety of nervous disorders. In this work, the methanol extract of *Benincasa hispida* (MEBH) was found to have reducing uncharacterized CNS depression in various neurological animal models and reveals a variety of neuro pharmacological activity in mice. This study confirms that the influence of methanol extracts of *B.hispida* in psychopharmacology with animal model was investigated.

**Keywords:** Anti-depressant activity, Analgesic activity and Methanol extract of *B.Hispida*.

**INTRODUCTION**

Although modern medicines may be available, due to socio – economical, cultural and historical reasons, herbal medicines have maintained their importance. In India, herbal medicines have been the basis of treatment and cure for various diseases /physiological conditions in traditional methods practiced such as ayurveda, unani and siddha. Vegetables are good sources of carbohydrates, fat, protein and mineral salts. *Benincasa hispida* (Thunb.) Cogn (Syn. *Benicasa cerifera*) is a widely used vegetable in India and other tropical countries and belongs to the family Cucurbitaceae (Chopra et al., 1956). It is called Petha or Golkaddu in Hindi and White Gourd, Wax Gourd or Ash Gourd in English or kanari pusani in Tamil. It is a large climbing or trailing herb with stout, angular and hispid stems. Young fruit fleshy, succulent and hairy while the mature fruit has thickly deposited hairs with easily removable waxy bloom. The flesh of the fruit is white and spongy. In Ayurveda, *B. hispida* is recommended for management peptic ulcer, hemorrhages from internal organs, epilepsy and other nervous disorders (Warier, 1994; Sharma, 1984). Furthermore, Acid neutralizing and ulcer healing activities of *B. hispida* has also been described (CSIR, 2000). According to Raja Nirghantu (an ancient work therapeutics), medicine from *B. hispida* was prepared from old ripe fruits. The pulp was scraped into thin strips and the water juice that oozes out abundantly was collected and preserved (CSIR, 2000). In our earlier studies, the fresh juice of *B. hispida* showed significant anti-inflammatory activity in cotton pellet granuloma and carrageenan induced edema in rats (Grover and Rathi, 1994). The fresh juice was also effective in preventing morphine withdrawal in mice (Grover et al., 2000). Phytochemical screening of *B.hispida* using bioassay-guided separation has shown the presence of 4 triterpenes and 2 sterols together with a flavonoid C-glycoside, acylated glucose, and a benzyl glycoside (Yoshizumi et al., 1998). J.K.Grover et al has been reported on the anti-ulcerogenic activity of this plant against helicobacter pyroli. The fresh juice of *B.hispida* was found to show significant nesoprotective effect against mercuric chloride poisoning. (Dong MY et al, 1995). The fresh juice was also reported to have analgesic activity in mice with CNS depression activity with insignificant spinal depression action and in the same study the effect of fresh juice on various isolated tissues was also reported .(Ramesh M. et al.,1989). The isolated RIP (Ribosome inhibiting protein) from the fruit of *B.hispida* has shown to inhibit the replication of HIV-1 (Shih Cy et al., 1998). The Bioassay of separated two triterpenes (alinusenol and multiflurenol) of methanol extract of *B.hispida* was found to potentially inhibit histamine release from rat exudates cells induced by antigen—antibody reaction. (Yoshizumi S et al, 1998). The methanol extract of *B. hispida* was found to have potent anti histaminic effect in guinea pig model of histamine induced bronchoconstriction while the extract had no effect in acetyl choline induced bronchoconstriction. (Anil Kumar et al, 2002). The effect of methanol extracts of *B.hispida* in psychopharmacology with animal model was investigated in this paper.
MATERIALS AND METHODS

Chemicals and Pharmaceuticals
Acetic acid (S-d Fine Chemicals, India), Clozapine (Sun Pharmaceuticals), Carboxyl methyl cellulose (Sd fine chemicals, India), Diazepam (Ranbaxy, India), Haloperidol (Sigma USA), Imipramine (Torrent Pharmaceuticals), Methanol (Sd fine chemicals, India), Naloxone (Troikaa Pharmaceuticals Ltd), India. Saline (Wockhardt, Life science India), Water for Injection (TDPL, India).

Animals
For standard neuro-pharmacological experiments, Swiss albino male mice (18 – 25 g) and wistar male rats (100 – 150 g) were used. The animals were maintained in suitable nutritional and environmental conditions through out the experiments.

Ash gourd fruit
The basic plant material of Benincasa hispida fruit used for the investigation were purchased from the local market and its authencity was confirmed by Dept of Pharmacognosy, C.L.Baid Mehta College of pharmacy, Chennai, Tamilnadu, India.

Crude methanol extraction
The outer skin (cuticle) of the fruit Benincasa hispida was peeled. After cutting open the fruit, the seeds along with the endocarp were removed clean. The fruit pulp (mesocarp) was mashed using an electric juicer to afford a soft mass. The pulp was macerated with Methanol (in ratio of 1:4) for seven days at room temperature with occasional stirring daily. On the eighth day the pulp was filtered and the filtrate was condensed under reduced pressure till a strong brownish liquid was obtained. The yield (2.5% w/v) was stored at 0 – 4°C and protected from direct sunlight. The methonal extract of Beninasa hispida (MEBH) thus obtained was used for the pharmacological studies by dissolving each time with isotonic normal saline.

Drug Administration
All the prepared drug solution and the extract were administered by intraperitoneal route (I.P) at a dose volume of 0.1ml/10 g and 0.1 ml/100g to mice and rats respectively. The methanol extract of Benincasa Hispida (MEBH) was dissolved each time in saline. Imapramine, Naloxane and Diazepam were dissolved in saline, clozapine and haloperidol was made into suspension using 1% Carboxyl Methyl Cellulose (CMC).

Neuro -Pharmacological Investigations
In order to assess the antidepressant potential of Methanol Extract of Bennicas Hispida MEBH, the two well – established acute models of behavioral despair (unlearned helplessness) such as Forced swim test (FST) and Tail suspension Test (TST) were used. The following tests (all are unconditional paradigms) and it can be differentiated and described as a model of
- “Social anxiety” (assessed in social interaction test)
- “Novelty induced anxiety” (assessed in Marble burying test)
- Novel space induced anxiety (ass ed in open field test)

Forced swim test (Behavioral Despair Model)
The procedure is a modification of the techniques as described by Porsalt et al 1977. Each Mouse was placed individually in a transparent plastic cylinder (13.5 X 18.5 cm ) filled with water to a height of 12 cm .The temperature of water was maintained at 22 – 23 degree Celsius. Duration of immobility is defined as absence of active escape oriented behavior such as swimming, jumping, rearing, sniffing or driving was noted during the last 4 min of a single 6 min test in mice. The animals received MEBH / imipramine at 24, 4 and 1 hr before the behavioral assessment. Thrice dosing were done, as when administered acutely with in 24 hr before the swim test, it is common to observe changes in immobility (Nicole LSchramm et al, 2001) , as in humans, antidepressant effect of drugs is typically seen 2 – 3 weeks after the drug admnistration. (Hardman JG et al,1996)

Treatment groups
Mice were grouped in groups of 5- 9.
All the groups received the respective treatments (IP) 24, 4 and 1 hour before the test.
Group 1: Mice received saline as control.
Group 2: Mice received MEBH 200, 600 and 1000mg/kg respectively.
Group 5: Mice received fresh juice of B Hispida, 0.1ml/10g.
Group 6 and 7: Mice received imipramine 15 and 30mg /kg respectively as standard antidepressant reference. In another set of experiment, we wanted to see if the extract on a single acute dose would modify the behavior of mice in FST. As certain antidepressants flesinoxan and ipsapirone inhibited immobility when administered once whereas buspirinone failed to do so after single administration(Koek W et al., 1998). The antidepressants imipramine,Paroxetine produced moderate results after single administration and more effectively inhibited after three administration . (Koek W et al.,1998,Porsolt R et al 1978)

The procedure was same as described above, only the dosing regime was changed i.e., single administration of the agents 1h before the swim test and in addiction to the time spent (sec) in immobility, the observational parameter included were the time spent (sec) in swimming and climbing of the last 3 min of the 6 min test. (Lucki I et al, 1997)

Each group consists of 4–6 mice.
Group 1: Mice received saline
Group 2 – 4: Mice received MEBH 200, 600 and 1000 mg/kg respectively
Group 5: Mice received imipramine 30mg/kg.

Social Interaction Test

Rats were familiarized with each pair (cage mates) to the arena for a period of 8 in for 2 consecutive days. On the third day, the rats were grouped together and then administrated with drug/extract. After administration, the rats were again replaced to the original cage with its cage mate. After 30 min of administration of the drug /extract ,each rat of equal weight was randomly assigned to an undamilar partener in the test arena and observed for the social interaction time (sec) in which the rats spent in sniffing the partener,climbing over and crawling under the partner , facial grooming , genital investigation and following and walking around the partner. Aggressive behavior was not considered as social interaction. Also, passive social contact was not counted as social interaction. i.e If the animals were beside each other for more than 10 sec and not actively interacting , the scoring was discontinued until the movement was resumed. A total of 4-6 pairs were used for the experiment.(Dunn RW et al ,1989)

Each group consists of 4 -6 of either sex.
Group 1: Rats received saline as control.
Group2 – 4: Rats received MEBH 50, 200 and 1000mg/kg respectively.
Group5 – 6: Rats received diazepam 0.5 and 2 mg/kg as standard antianxiety drug.

Marble Burying Test

Marble burying test was carried out as described elsewhere (Broekkamp CL et al 1986) with slight modification. After 30 min administration of extract/drug in their bodies, Mice were individually placed in cage (26x19x13cm) in which 10 marbles (dia 15mm) were placed evenly in 2 rows on a 5cm saw dust bed. After 1 h exposure the mice were removed and the number of unburied marbles was counted. Marbles were considered as buried if they were at least 1/3 covered with bedding.

Each group consists of 5 -6 mice of either sex.
Group 1: Mice received saline as control
Group 2 – 4: Mice received MEBH 200, 600 and 1000 mg/kg respectively.
Group 5: Mice received fresh juice of B.Hispida, 0.1ml/10g
Group 6 and 7: Mice received diazepam 2 and 4 mg/kg respectively as standard antianxiety agent
Group 8: Mice received haloperidol 0.5mg/kg as typical antipsychotic reference drug.
Group 9: Mice received clozapine 10 mg/kg as typical antipsychotic reference drug
Group 10: Mice received imipramine 30mg/kg as antidepressant reference drug.

Open field Test

Open field test was performed as described by pertovaara et al, 1990 with a slight modification in the apparatus which is of a square clear Plexiglass box (26 x 26 cm) open at the bottom . The box was placed on a filter paper which had 4 quadrants marked lightly with pencil . The centre of the floor (15 X15 sq cm) was marked with pencil. The center of the floor (15 X 15 sq cm) was marked with a pencil. Similar size of a paper had marking which was with the observer enabling him /her to trace the path of the mice traveled. After 30 min of the administration of the agents, each mouse was placed inside the chamber, first10 min was allowed for the animal to explore and the last 10 in the following observation made, the number of rearing, the distance traveled (cm), the time (sec)
spent in the center, the number of quadrants crossed and the number of defecation. Each time a
new animal was placed on a newly marked filter paper.
Each group consists of 5-6 mice.
Group 1: Mice received saline as control
Group 2 – 4: Mice received MEBH 200, 600 and 1000mg/kg respectively.
Group 5: Mice received diazepam 0.5 mg/kg as standard anti-anxiety agent.
Group 6: Mice received imipramine 10mg/kg as standard antidepressant agent.

Tolerance test for Analgesic activity
Acetic acid writhing method was employed. MEBH (200 and 400 mg/Kg i.p )was administrated to
ice thrice at every 4h interval.30 min after admistration of the extract ,mice were injected each time
with 0.1ml/10g of 0.6%of acetic acid (Ramasamy S et al , 1997). The number of writhing was
noted for a period 20 min after admistration of acetic acid.
Each group consists of 6 male mice
Group 1: Mice received of saline as control
Group 2 -3: Mice received MEBH 200,400mg/kg respectively.

Opioid Antagonism Test
Mice were admi
nistrate
ed with naloxone (0.01, 0.1 and 1mg/kg) and 15 min later MEBH 400mg/kg
was administrated to the respective treated mice. After 30 min 0.1mg/10g of 0.6% acetic acid was
administrated and the number of writhing was noted for 20 min.
Each group consists of 3 -6 male mice.(Viswanathan S et al, 1990)
Group 1: Mice received naloxone 0.01 mg/kg and MEBH 400mg/kg
Group 2: Mice received naloxoane 0.1 mg/kg and MEBH 400 mg/kg
Group 3: Mice received naloxone 1mg/kg and MEBH 400 mg/kg.

Statistical Analysis
Data analysis was performed making use one way of analysis of variance (ANOVA) followed by
dunnett’s t test or Dunnet’s multiple comparison test t two tailed unpaired test.

RESULT
Forced swim test
In this test, MEBH when administered acutely (i.e.thrice before the test), 600 and 1000mg/kg
Produced significant reduction in immobility 75.0 ± 22.9 (P< 0.01): 51.4 ± 11.9(P < 0.001) secs
respectively (Fig1A).While imipramine at 15 mg/kg did not produce any significant activity. At 30
mg/kg imipramine showed significant reduction in immobility (66.8± 13.9 sec: P< 0.01) When
administrated only once before the experiment. MEBH at 600–1000 mg/kg significantly in a dose
dependent manner are increasing the struggle time and reducing the immobility (Fig 1B). Also
imipramine at 30mg/kg also produced significant reduction (p< 0.001) in immobility 62.3±10.1 sec
that is consistent with previous findings.
Open field Test
In this test (Fig 2-7), MEBH at 1000mg/kg produced significant reduction in total distance travelled, spent time in centre, number of rearing, number of quadrants explored and number of defecation. While 200 and 600mg/kg of MEBH and Diazepam did not produce any significant changes in all the parameters when compared to control .Imipramine at 30mg/kg in all the parameters did not produce any result. In the sense, the animal seems to be heavily sedated; there was no movement at all (results not enclosed). Imipramine at 10mg/kg significantly reduced total distance travelled, number of rearing, quadrants explored. MEBH (200 – 1000mg/kg) in a dose dependent manner produced significant reduction in immobility (Fig 3) when the mice were subjected to tail suspension test. Imipramine also produced significant reduction in immobility.
Fig. 4: Reduction of Time Spent in centre (Time in Sec)

Fig. 5: Reduction of Number of Quadrants explored

Fig. 6: Reduction of Number of Defecation
Marble Burying Test

In this test, MEBH treated mice at 200, 600 and 1000mg buried fewer glass marbles significantly 2.8±0.7 (p<0.05);3.2 ±1.3 (P<0.02):8.1±0.9 (P<0.001) respectively when compared to control Fig 8). Even Fresh juice also produced significant (P<0.05) reduction in marble burying behaviour 2.8±0.5 when compared to control 0.9±0.2. All the standard drugs haloperidol (0.5mg/kg), clozapine (10mg/kg), diazepam (2 and 4mg/kg) and imipramine (30mg/kg) all produced significant (p<0.001) reduction in marble burying.

Social Interaction Test

In this test, MEBH (50, 200 and 1000 mg/kg) a dose dependent manner significantly reduced social interaction time in rats when compared to control (Fig 9) Reference drug diazepam (0.5 and 2 mg/kg) also produced significant (P<0.01) reduction.
Tolerance Test
In tolerance test for analgesia induced by MEBH (200 and 400 mg/kg i.p), the extract (administered every 4h interval) did not increase the number of writhing induced by 0.6%v/v of acetic acid. In the fact when compared to writhing produced at the 1 interval ii and iii interval showed reduced number of writhing at both the dose levels of MEBH as shown in Fig 10.

![Fig. 10: Tolerance Test](image)

Opioid Antagonism Test
In opioid antagonism test (fig 11), naloxone (0.01 -1mg/kg) failed to antagonize the analgesic effect of MEBH at 400 mg/kg i.e, the number of writhing did not increase. MeBH and naloxane 1mg/kg produced drastic reduction in number of writhing, which may be due to the fact that higher doses opioid antagonist itself produces analgesic activity.

![Fig. 11: Opioid Antagonism Test](image)

DISCUSSION
Depression is a complex behavioural disorder and the treatment or pathophysiology of this disease is also complex which reflected by the various receptors, agonist and antagonist involved in producing anti-immobility activity in FST which is indicative of potential antidepressant activity. The FST developed by Posrsolt and colleagues in rat (Porsolt RD et al, 1977) and subsequently in mouse (Porsolt Rd et al 2000) was widely used for assessing antidepressant potential (Borsini F et al, 1998). Forced swim is a non-escapable stressful situation where in there is initial vigorous
activity and then shows signs of immobility (despair), which is quantified as a symptom of behavioural despair. (Lucki et al, 1997).

Antidepressant modify the balance between activity and immobility in this model in favour of activity at the expense of energy (Porsolt Rd et al.,1977 ,1978, Cryan JF et al., 2002). For reasons unknown in mice, one exposure is sufficient to generate a stable immobility that can be effectively countered by antidepressant as compared to rats, which needs a pre-trial exposure (Borsini et al., 1998,JF Cryan et al 2002). Though porsolt swim test has higher validity, the major drawback is its unreliability in detecting SSRI (Lucki I et al.,1997), which is most widely antidepressant.(Dubini A et al,1997). Thus the modified FST reveals catecholaminergic agents decrease immobility with corresponding increase in climbing behaviour whereas 5HT related compounds (SSRI) decrease immobility by increasing swimming behaviour.(Lucki et al.,1997,.Cryan JF et al ,2000). In humans too noradrenergic drugs (reboxetine) tend to drive or motivate where as fluxotline (serratogenic) tend to improve mood.(Cryan JF et al.,2000,. Dubini A et al.,1997, Charney DS et al.,1998.)

Imipramine is a compound that blocks NE reuptake and also 5-HT in the pre-synaptic neurons (Brilley M et al, 1993). Therefore, we used this test agent namely imipramine top validate FST, as fluoxetine seems to be less efficacious in mice(Sanchez C et al.,1997). From the results in both the models of forced swim test (thrice and single administration of MEBH), it can be suggested that the extract of B.hispida possess significant anti depressant activity.

Open field immobility or open field test (OFT) is often employed to measure the general activity level in ice by taking into account various parameters to ascertain if the active behaviour in FST is not attributable to impaired mobility and to observe the anxiolytic activity of the extract if any in this behavioural model of despair (Nicole L Schramm et al 2001). The parameters are also suggested to be most reliable indicator for the magnitude, rate of intrinsic oscillation of habituation in OFT. (Kauppila T et al.,1991). Contrary from the results in FST, Mice administered with MEBH (0.6 - 1g/kg) in a dose dependent manner decreased the total distance travelled. Thus, the reduced immobility or antidepressant effect of MEBH is not attributable to a possible stimulant effect of the extract. In fact MEBH appear to have sedative effect in OFT paradigm.

Rearing is a stress related response that elicits the release of NE in central and peripheral nervous system and it represents the state of activity. From the results it can be presumed that the extract does not have much effect in the non-adnergeni system. Only MEBH 1g/kg significantly decreased the rearing behaviour when compared with control. No possible explanation can be given as 200 mg/kg actually increased the number of rearing.

Mice in general prefer or feel safer in the perimeter regions of the open field chamber, close to the walls. More venturs into the centre of the chamber are therefore interpreted as decrease in anexy (Nicole L Schramm et al, 2001). Again MEBH produced variable results i.e .200mg/kg produced increase in time spent in centre while MEBH 1000mg/kg produced significant reduction in time spent in centre. Thus, MEBH is producing both anti – anxiety and anxiogenic or sedative effect in this paradigm.

The number of bolus was used as indicator of emotionality. Anxiety related emotionality, which is supported to correlate positively to defecation mostly mediated by increased sympathetic activity (Kauppila T et al,1991). The results suggest that the extract acts as anxiolytic agent.

In MBT, Anxiolytic drugs have been proposed to decrease the number of marbles buried (Broekkamp CL et al,1986.). However the validity of MBT as an isomorphic model of anxiety is not clear (Njung’e K et al, 1991).The interpretation that the rodents have less anxiety when they are burying foreign objects is based on the assumption that animals would consider marbles startling by virtue of their novelty. Alternatively it has been also suggested that the burying behaviour would be rewarding or that compulsive, Hence it has been also considered as animal model for obsessive compulsive disorder (OCD) (Abe M et al, 1998), because animals bury marbles in a familiar setting that have nor been paired with aversive stimuli, display the behaviour regardless satiety condition and continue to show the behaviour even after repeated exposure.

It is also interesting to note that SSRI used for OCD was most sensitive in this model (Njung’e et al 1991b). Nevertheless, MBT is one of the few models that show sensitivity to majority of the anti-depressant. MEBH in a dose dependent manner significantly reduced the marble behaviour activity. Even fresh juice showed significantly activity. The fruit is used in traditional systems in various neurological diseases (Sivarajab VV et al,1994). It would be tempting to suggest that the fruit has potential use in OCD disorders as one of the isolated compound in our lab was inositol from the methanol extract and it has shown that inositol has potential use in OCD (Einat H et al,2001,2002). However, the reference drugs used in this experiment namely imipramine, diazepam and clozapine, which are all ineffectve in treating OCD, produced significant activity.
Therefore it is suggested that the dose levels of the reference drugs in this test to be too high so as to produce high sedation activity with muscle tone impairment. The amount of the time, two unfamiliar rats actively interacted with each other in a familiar environment is measured in social interaction test. Therefore the partner is the only exogenic stimuli. This paradigm along with elevated plus maze differential potential antiloytic agent with anxioselective with anxiodeleective mechanism of action independent of GABA – benzodiazepine receptor complex (Robert W et al,1989). From the results the extract reveals anxiogenic effect, even the reference drug used (diapzem 0.5mg/kg) also produced anxiogenic effect, which can be suggested that the sedative effects predominates and interferes with the result. MEBH produced significant antidepressant activity in both models of acute depression (FST AND TST) by prolongation of escape – oriented behaviour rather by a generalized motor stimulant effect, which is all suggestive of anti-depressant activity .Although these models do not include the symptomology identical to human depression , these test appear as suitable models to detect anti-depressants. Though these models are valid for screening anti-depressant potential (Borsini F et al, 1998) , it has been mentioned by several authors (P Wilner,1990) that many a time false positive response is elicited after acute administration of antidepressant. Hence further investigations are needed to validate the claimed of MEBH as a antidepressant.

Pharmacological based models such as reserpine induced depression and use of various agonists and antagonist behavioural based models such as learned helplessness and chronic stress on repeated administration could be carried out in future. Moreover as the extract showed anti anxiety effect in MBT and OFT, to confirm the anti-anxiety potential of the anti-depressant nature of the extract it would in best interest to carry out guinea pig induced vocalization (Cryan JF et al,2002), which is supposed the most predictable and sensitive test for detecting anti-anxiety nature of antidepressant compounds exclusively. In a separate set of experiments relating to the analgesic characterization of MEBH ,the extract did not produce any tolerance nor the analgesic effect and was antagonized by nalozone ( an opioid antagonist ) in acetic acid induced writhing model suggesting that that the analgesic property of MEBH is mediated by certain central non –opioid mechanism.

CONCLUSION
The present study is the methanol extract of Benincasa hispida (MEBH) was found to have reduction of uncharactized CNS depression in various neurological animal models. The present study reveals a wide spectrum of neuro pharmacological activity in mice .The most prominent being the antidepressant activity in forced swim test. While exploring the anti-anxiety profile of the extract in open field test in mice and social interaction test in rats ,the results were inconclusive indicating to some degree of anxiogenic or no anti anxiety effect in these models which is true to certain antidepressants used clinically. The extract in marble burying test revealed the potential use in obsessive – compulsive disorders whereas here also SSRI agents have been shown good activity in these models. In summary, the study revealed potential antidepressant activity. Therefore, further pharmacological studies could be targeted in revealing the effect of the extract in serratogenic systems. The study also for the first time showed that MEBH produces analgesic activity not mediated by opioid mechanism. Nevertheless, bioassay guided separation could also be performed to find out the active constituents of this plant. In end the study further reiterates the importance of India traditional system knowledge to be used as a lead molecule in finding out newer drugs for complicated psychiatric disorders.

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