O perform the anxiolytic activity of ethanolic extract of Benincasa hispida (leaves) in experimental model (elevated plus maze) in mice

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Abstract

Objective: To perform the anxiolytic activity of ethanolic extract of Benincasa hispida (leaves) in experimental model (elevated plus maze) in mice.

Materials and methods: The leaves of Benincasa hispida was collected, dried and crushed. The leaves were extracted with ethanol by using maceration method of extraction. After the extraction phytochemical screening was performed. The ethanolic extract leaves of Benincasa hispida was studied at two dose level (200mg/kg and 400mg/kg, in oral administration) in mice by using elevated plus maze model. The mice are divided into four groups and each group contains 6 mice. Group-I mice were managed with water for 7 days. Group-II mice were managed with standard drug (Diazepam) 2mg/kg for 7 days. Group-III mice were managed with extract of B.hispida at a dose of 200mg/kg and Group-IV mice were managed with extract of B.hispida at a dose 400mg/kg for 7 days. Anxiolytic activity of ethanolic extract of B.hispida was estimated by using elevated plus maze model. In elevated plus maze model, ethanolic extract of B.hispida showed increased the percentage of time spent in open arm and percentage of open arm entries.

Results: Phytochemical analysis of B. hispida ethanolic extract revealed the presence of alkaloids, flavonoids, amino acids, proteins, and carbohydrates. Administration of Diazepam (2mg/kg i.p.) produced a significantly decrease the anxiety behaviour when compared with the control treated group. Administration of Benincasa hispida extract (200mg/kg & 400mg/kg) to mice significantly decrease the anxiety like behaviour when compared with the Diazepam treated group and it was found that the extract dose 400mg/kg show higher anxiolytic effect than 200mg/kg dose of extract of B.hispida.

Conclusion: The results suggest that the leaves of the Benincasa hispida possess significantly anti-anxiety activity.

Date of Submission: 26-05-2025   Date of acceptance: 07-06-2025

I.   INTRODUCTION:

Anxiety is a negative emotional state that is characterised by unease, discomfort, and worry or fear about a known threat. Anxietyis a some kindof common aspect of life. When it is excessive and disproportionate to the situation, treatment is required.The most frequent mental illnesses are anxiety disorders. It manifests our emotional, thought, behavior, and abnormalities in physiological activity. Examples of anxiety disorders include acute stressful disorder, obsessives-compulsive disorder, social anxiousness, fear of heights, generalised anxiety disorder, specific phobia, panic attack disorder, and post-traumatic stress disorder. For at least six months, anxiety is linked to impatience, restlessness, feeling tight or on edge, easily becoming weary, having issues focusing or going blank, troubles falling asleep, and annoyance. One of the most frequent mental and behavioral problems is anxiety. The terms anxiety and fear are frequently used despite not being interchangeable. Fear is a reasonable, present-focused, and quick reaction to a clearly recognised and specific threat, whereas anxiety is a prolonged future-oriented reaction that is largely focused on a scattered threat. [1] Anxiety is a mental illness which causes worrying, being afraid, and feeling uneasy. It is normal for a stressor.Anxiety is derived from the Latin word "Ango" which means "to torment or vex" (to annoy or to make somebody feel worried). It is a medical condition related to our physiological as well as psychological behaviourwhich affects number of characters like emotional, behavioural, cognitive and somatic.The majority of mental health challenges are anxiety disorders. They can be just as impairing but being less obvious than schizophrenia, depression, and bipolar illness. Anxiety disorder examination is constantly updated. If left untreated, anxiety is a complicated, continuous behavioural and physiological change in the body that can result in a wide range of central nervous system (CNS) problems.[2]

Fig. 1- Anxiety

Types of anxiety disorders: According to the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM IV) anxiety disorder are classified as follows:

1. Generalized anxiety disorder
2. Social anxiety disorder
3. Agoraphobia devoid of fear
4. OCD (obsessive-compulsive disorder)
5. Adrenal stress condition
6. Panic disorder with or without aversion to public places
7. Post-traumatic stress disorder, number seven
8. A disease of anxiety not otherwise specified
9. Particular phobia
10. Severe depression
11. Alcohol-related anxiety disorder
12. Discretionary mutism. [3]

Signs and symptoms: Feeling restless, Being easily fatigued, Headaches, muscle aches, stomach aches or unexplained pains, Sweating, Chest pain, Being out of control, Difficulty making eye contact to people they don't know, Having sleep problems, Difficulty in controlling to feeling of worry, Difficulty in concentrating, Racing heart.

Etiology Anxiety disorder may be caused by various interactions of biological, psychological and social factors.

Biological factors: Genetics, Neurotransmitter disorder, Sickness, Drugs, Dietary habits.

Psychological factors: Behavioural characteristics, Poor self-worth, Unfavorable feelings, Perceptions of surroundings.

Social factors: Negative circumstances in life, a lack of interaction with others, Stress in the workplace, a lack of social abilities, Events of nature. [4]

II. MATERIAL AND METHODS:

Collection of plant materials: Based on its traditional and phytochemical profile, the plant was chosen for the research work and collected from herbal garden of Patel College of Pharmacy, MPU, Bhopal, M.P. Ratibad, Bhopal (M.P.)

Plant authentication: The plant was authenticated by India, Department of Botany Prof. Nawal Kishor Dubey, Banaras Hindu University Varanasi. For future use, a voucher specimen with the number Cucurbita, 2023/3 was placed in the BHU Varanasi herbarium.

Glassware and Chemicals: Glassware: Beaker, Glass rod, Test tube holder, Test tube, Measuring cylinder, Weighing balance, Test tube stand, Petridish, Desicators etc. Chemicals: Mayer reagent, Wagner reagent, hager reagent, Millon's reagent, Ninhydrin solution, Molisch's reagent, Borfoed's reagent, HCL, magnesium turning, Zinc dust, Pyridine, Nitroprusside, Sulphuric acid, Ferric chloride, Gelatin, Ammonia, Biuret reagent, Sulphur powder etc. **Preparation of the extract (Maceration Method):** The plant parts (leaves) that had been gathered were washed with distilled water and left to dry in the shade over newspaper. It tooks approx. 25-30 days for completely dry. The dried leaves was pulverized into coarse powder and kept in a tightly closed container for further use. Dried leaves are weighed 100g and transferred into a round bottom flask. The solvent (ethanol) was then poured over coarsely powder material in round bottom flask until the leaves completely soaked (about 250-500ml solvent used). After that the flask was closed with aluminium foil and set aside. A maceration extraction with ethanol was performing from the BH leaf for 72 hours at room temperature with occasional stirring. After 72 hours the ethanolic extract was filtered, vacuum-concentrated, dried in a hot air oven at 40 to 50 °C, and stored in a closed container for future use. [5]

Experimental Animals: Swiss albino mice of either sex, weighing 24-30 g, were procured from animal house of Patel College of Pharmacy, MPU, Bhopal, M.P. Ratibad, Bhopal (M.P.), have CPCSEA registration no. 1698/PO/a/13/CPCSEA & 10/05/2013 under the usual conditions of 25±2°C temp, 45–55 % relative humidity,

and a 12–12 cycle of light and dark. Water was available at all times, and the animals were fed regular rat pellets. The animals were given 48 hours to acclimatize to the laboratory setting before the trial began. Each study included six-mouse groups, ranging in age from 4-6 weeks. All research were completed with consent from the Patel College of Pharmacy, MPU, Bhopal, M.P. Ratibad, Bhopal (M.P.) Institutional Animal Ethics Committee (IAEC). [6]

Group of animals:
A vehicle (distilled water) was given to the animals in Group 1 (the vehicle-treated control) one hour before they were put through the raised plus maze model. The animals in Group 2 (standard treatment) received medication 30 minutes prior to exposure to raised plus maze models, administer diazepam (2 mg/kg i.p.). (Test group) Groups 3 and 4 the animal were administerd Ethanolic extract of *Benincasa hispida* of different dose such as 200mg/kg and 400mg/kg p.o before one hour subjecting to elevated plus maze models. [7] Anti anxiety activity of Ethanolic extract of *Benincasa hispida* leaves:

Elevated Plus Maze model: The validity of this test for assessing anxiety in rodents is well established. In the plus-maze device, 2 open arms (16x5cm) and 2 closed arms (16x5x12cm) extend from a single central platform (55cm). The whole maze is elevated to a 25 centimetre height above the ground. Four groups of six animals each were formed from the animals. EEBH was administered orally to Groups III and IV at doses of 200 and 400 mg/kg, respectively, whereas Group-I was given distilled water orally as a control, Group-II received diazepam 2 mg/kg intraperitoneally as a standard. An hour after receiving the prescribed amount of the vehicle, standard, and extract, each mouse was placed in the middle of the maze, facing one of the open arms. During the five-minute session, the quantity and length of time spend to the open and closed arms were noted. The animal has to place all four paws on the arm in order to enter it. The maze was meticulously cleaned using wet tissue paper (10% ethanol solution) following each test. [8]

Statistical analysis: The data was express as Mean±SD and was analyse using one-way ANOVA followed by Dunnet's test (*$P<0.05$, **$P<0.01$ was considered as statistically significant). Results were considered significant with P values below 0.05.

III. RESULT AND DISCUSSION:

Phytochemical analysis: The phytochemical examination of ethanolic extract of B.hispida, which identified the presence of triterpenes, glycosides, sterols, flavonoids, and carbohydrates.

Sr. No.	Components	Ethanol extract
1.	Glycosides	-
2.	Flavonoid	+
3.	Tannins	+
4.	Alkaloids	+
5.	Terpenoids	+
6.	Carbohydrates	+
7.	Amino Acid	-

Table 1: Preliminary phytochemical analysis of *B.hispida* leaves.

Elevated Plus Maze: In regard to the results, in the extract-treated groups, more mice entered open arms and spent more time there whereas significantly fewer mice entered and remained longer in joined arms. The ethanolic extract of B. hispida had anxiolytic effects at a dose 200 mg/kg and 400 mg/kg that were comparable to diazepam 2 mg/kg effects and control group. The dose of ethanolic extract of *Benincasa hispida* at 400mg/kg produced more anxiolytic effect than ethanolic extract of 200mg/kg of dose.

| Sr. No.	Group	No. of enteries to open arm
1.	Control	9.01±1.12
2.	Standard	27.98±1.15**
3.	DEE 150mg/kg	14.69±1.07*
4.	DEE 300mg/kg	19.05±1.41**

Table 2: Effect of *Benincasa hispida*(L). extract on no. of entries in open arm in Elevated plus maze.
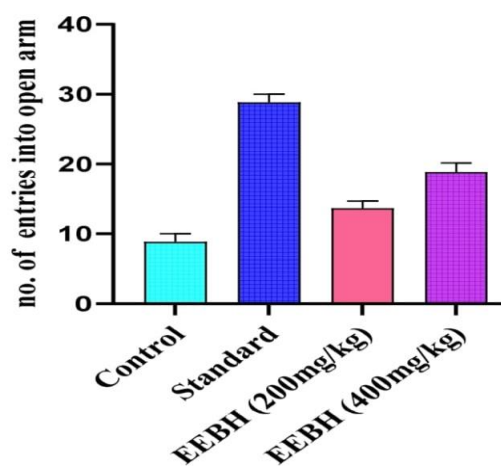
Graph 1:- Effect of *Benincasa hispida* (L). extract on no.of entries to open arm in Elevated plus maze.

Sr. No.	Group	Time spent in open arm
1.	Control	84.21±1.86
2.	Standard	147.7±4.16**
3.	DEE 150mg/kg	97.33±4.85*
4.	DEE 300mg/kg	116.85±14.41**

Table 3: Effect of *Benincasa hispida* (L). extract on time spent to open arm in Elevtaed plus maze.
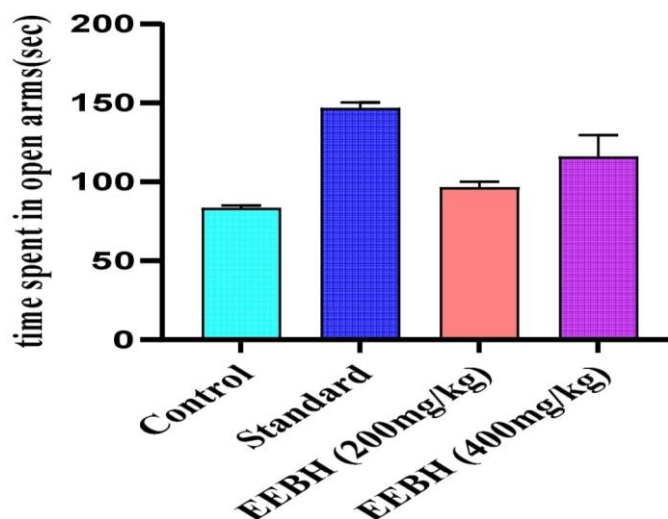
Graph 2:- Effect of *Benincasa hispida* (L). extract on time spent in open arm in Elevated plus maze.

IV. DISCUSSION:

Alkaloids, flavonoids, proteins, and saponin chemicals are all confirmed by the phytochemical screening. In order to demonstrate the efficacy of novel medications, investigate their mechanisms of action, or assess the pathophysiological events associated with anxiety, numerous animal models have been designed to examine anxiolytic activity. The preferred paradigm for testing anxiolytic medications on both rats and mice is the Elevated Plus Maze. B.hispida was tested for its ability to reduce anxiety using the elevated plus-maze (EPM) models. The percentage of entry into the open arms and the amount of time spent in the open arms are the main metrics in the EPM. When the medication enhance open arm entries with no changing the total number of arm entries, it may have an anxiolytic effect. Similar to the effects seen after taking the reference anxiolytic medication diazepam, mice were treated with extracts in the current investigation significantly enhance the number of entry and the amount of time spent in While recognising that there are many pharmaceuticals on the market for treating anxiety, including alprazolam, hydroxyzine, buspirone, citalopram, and diazepam, the majority of these medications have a number of adverse reactions, including sedation, dizziness, impairment of the psychomotor and cognitive functions, confusion (specially in the elderly), increase hunger, & changes in sexual function. There are medicinal herbs that may relieve anxiety that have less negative side effects. The result of present study showed that Benincasa hispida leaves of ethanolic extract possesses anxiolytic activity.

However, the exact mechanism by which this extract reduces anxiety remains undetermined, phytochemicals like the flavonoids that make up the extract may be important. Flavonoids are of special importance because studies have shown that they have anxiolytic properties. The results from study demonstrate that B.hispida leaf extract significantly reduces anxiety in animal models. In comparison to common medications like diazepam, it has anxiety reducing properties. Even when present at quite high amounts, the extract is non-toxic. The presence of flavonoids is probably what causes the anti-anxiety activity. To characterise and investigate the biological activity of the chemicals found in the extract, more research is being done.

V. CONCLUSION:

B.hispida ethanolic extract has anti-anxiety effects. However, more research is needed to pinpoint the phytoconstituents that give rise to the plant apparent anxiolytic effects and to explain how they work in the brain. Additionally, the allopathic drugs employed in its therapy are linked to side effects that worsen human health. Researchers are now concentrating on herbal plants that have therapeutic properties as a result of this. These medicinal plants contain a variety of phytochemicals, including alkaloids, tannins, flavonoids, phenols, saponins, and others; these substances can be isolated and used to promote health. Consequently, the medicinal plants with anxiolytic effect that are not only secure but also reasonably priced are explored here.

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