Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [6] May 2023 : 97-107

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Evaluation of Anxiolytic Activity of Ethanolic Extract of *Mentha Piperita* Leaves in a Rat Model

Atul Kumar Shukla[1], Vivek Gupta[1], Vimal Kumar Yadav[2], Ajay Kumar Shukla[2]
1. Shri Rawatpura Sarkar Institute of Pharmacy, Datia (M.P)
2. Institute of Pharmacy, Dr Rammanohar Lohia Avadh University Ayodhya U.P. 224001
Corresponding author:
Dr. Ajay Kumar Shukla
Email id: ashukla1007@gmail.com

ABSTRACT
This study aimed to evaluate the anxiolytic effects of Mentha Piperita Linn leaves' ethanol extract using various behavioral tests. The anxiolytic activity of the ethanol extract was assessed through the Elevated plus-maze, Hole-Board (Head Dipping), Light-Dark Exploration, and Open Field tests. The plant material was standardized by employing macroscopical features, phytochemical analysis, and ash value calculation methods. Phytochemical analysis revealed the presence of secondary metabolites such as alkaloids, glycosides, tannins, phenolic compounds, and flavonoids. Furthermore, the total ash value, water-soluble ash, and acid-insoluble ash values were found to be 4.9%, 10.24%, and 6.24% w/w, respectively. The extract exhibited highly significant (P < 0.001) anxiolytic activity at doses of 250 and 300 mg/kg. The results of the study indicate the presence of potent anxiolytic properties in the Mentha Piperita Linn leaves' ethanol extract. Further investigation, including compound isolation, is warranted.
Keywords: *Anxiolytic, Mentha Piperita, Ash value, Elevated plus-maze test, Hole-Board (Head Dipping), Light-Dark Exploration, Open Field test*

Received 19.03.2023 Revised 14.04.2023 Accepted 24.05.2023

INTRODUCTION

Anxiety is an emotion which is characterized by an unpleasant state of inner turmoil and includes feelings of dread over anticipated events.[1,2] It is often accompanied by nervous behavior such as pacing back and forth, somatic complaints, and rumination.[3] Anxiety is a feeling of uneasiness and worry, usually generalized and unfocused as an overreaction to a situation that is only subjectively seen as menacing.[4] It is often accompanied by muscular tension,[5] restlessness, fatigue, inability to catch one's breath, tightness in the abdominal region, nausea, and problems in concentration. Anxiety is closely related to fear,[6] which is a response to a real or perceived immediate threat (fight or flight response); anxiety involves the expectation of future threat including dread. People facing anxiety may withdraw from situations which have provoked anxiety in the past.[7] Though anxiety is a typical human response, when excessive or persisting beyond developmentally appropriate periods it may be diagnosed as an anxiety disorder.[8] There are multiple forms of anxiety disorder (such as generalized anxiety disorder and obsessive compulsive disorder) with specific clinical definitions. Part of the definition of an anxiety disorder, which distinguishes it from everyday anxiety, is that it is persistent, typically lasting 6 months or more although the criterion for duration is intended as a general guide with allowance for some degree of flexibility and is sometimes of shorter duration in children.[9]

For all types of anxiety disorder, cognitive behavioral therapy is the type of psychotherapy for which there is the strongest evidence and which receives the highest-level recommendation (Ia; A). Initial randomized controlled trials have confirmed the clinical efficacy of psychodynamic therapies, e.g., in social phobia.[10] Nonetheless, psychodynamic therapy receives evidence level IIa in the current German guidelines because of the incomplete state of the data from clinical trials, along with the recommendation that this type of psychotherapy should be offered if cognitive behavioral therapy has been ineffective or is unavailable, or if an informed patient expresses a preference for it.[11]The specifics of cognitive behavioral therapy vary depending on the particular anxiety disorder being treated, with the common element that the patient must make the experience that his or her situationally induced anxiety is

unfounded and the situation actually harmless. This is best achieved through exposure under the supervision of a therapis in the course of which the patient must experience habituation of the anxiety response, so that the central fear underlying it is refuted. Exposure in virtual reality is now increasingly a part of cognitive- behavioral therapeutic interventions.[12]

Exercise (eg, aerobic training, such as jogging 5 km three times a week) has been studied in PDA. However, it was found that exercise was less effective than clomipramine105 and no more effective than a control condition, relaxation. Thus, exercise can only be recommended as adjunctive treatment to standard treatments.[13]

Hypnosis, autogenic training, and biofeedback or complementary medicine methods such as acupuncture, osteopathy, or homeopathy are often recommended for the treatment of clinical anxiety. However, controlled studies fulfilling at least basic methodological standards are lacking. Antidepressants, such as selective serotonin reuptake inhibitors (SSRIs), are frequently used for this condition. If you have a mood or anxiety disorder in addition to your anxiety, medications used to treat those conditions may also help.

Some medications for health anxiety come with serious risks and side effects. It's important to review your treatment options with your doctors thoroughly.Although controlled studies on the usefulness of self-help groups are lacking, patients should be encouraged to participate if appropriate.[14]

Research in the area of herbal psychopharmacology has increased markedly over the past decades. To date however, a comprehensive review of herbal antidepressant, anxiolytic and hypnotic psychopharmacology and applications in depression, anxiety and insomnia has been absent. A search of MEDLINE (PubMed), CINAHL, PsycINFO, and the Cochrane Library databases were conducted (up to February 21st 2011) on commonly used psychotropic herbal medicines. Analysis of evidence levels was conducted, as were effect sizes (Cohen's d) where data were available. [15]. *Mentha Piperita* Linn, commonly known as peppermint, is a significant medicinal herb from the family Laminaceae, which has been used for food, medicine, and cosmetics since ancient times. Peppermint leaves have been found to provide relief from common cold symptoms and decrease symptoms of irritable bowel syndrome, including digestive problems such as nausea, vomiting, diarrhea, flatulence, and dyspepsia. Peppermint leaves contain phenolic constituents such as rosmarinic acid and several flavonoids, including eriocitrin, luteolin, and hesperidin. The essential oil of peppermint contains menthol and menthone as its primary volatile components. Peppermint has been found to exhibit significant antimicrobial and antiviral activities, strong antioxidant and antitumor actions, and potential antiallergenic properties in vitro. Animal model studies have demonstrated a relaxation effect on gastrointestinal tissue, as well as analgesic and anesthetic effects on the central and peripheral nervous system, immunomodulating actions, and chemopreventive potential. Our research aimed to assess the anti-anxiety activity in experimental rats by using *Mentha Piperita* Linn leaves [16, 17].

MATERIAL AND METHODS

Collection of Plant

The entire plant was procured from a rural area in Datia and validated by a botanist. The leaves parts of plant separated and was then thoroughly washed and cleaned with water, dehydrated under shaded conditions at room temperature, and subsequently subjected to evaluation using various parameters. For the study, the leaves of *Mentha piperita* were collected from different locations in and around Madhya Pradesh.

Preparation of extract

To prepare the sample, one kilogram of *Mentha piperita* leaves were crushed into coarse powder and defatted using Soxhlet's extractor with petroleum ether (65°-85°C). The residue obtained was then extracted with ethanol. The collected liquid ethanol extract was further evaporated by using rotator evaporator. The ethanol extract was storied in desiccators for further analysis. The phytochemical tests were done [18-23].

Phytochemical Parameters [18-23].

Determination of Loss on Drying

To determine the loss on drying, 5-6 grams of powder were precisely weighed and placed into a tared vanishing dish. The sample was then dehydrated for 4 hours at 110°C. After cooling, the sample was dehydrated and weighed at hourly intervals until a constant weight was obtained. The calculation of loss on drying was based on the moisture content present in the sample and was determined using the following formula:

$$Loss\ on\ Drying\ = \frac{\text{weight of powder after drying in geight of empty crucible}}{\text{Initial weight of the powder in g}} x\ 100$$

Ash Values

Total ash

To determine the total ash value, 2 grams of pulverized air-dried powder were accurately weighed and placed in a lighted crucible (typically platinum or silica) in an even layer. The crucible was then gradually heated up to 600°C until it turned white, indicating the absence of carbon. The material was cooled in a desiccator and weighed. If the ash contained carbon, it was not considered for further analysis. In such cases, the crucible was cooled and the deposit was moistened with 2 ml of water or ammonium nitrate solution. It was dried on a water bath and burned again to constant mass. The residue was allowed to cool in a desiccator for 35 minutes and weighed again. The total ash value was calculated as a percentage of the weight of the dried material using the following formula:

$$Total\ as\quad value = \frac{\text{weight of empty crucible}}{\text{weight of drug taken}} x\ 100$$

Acid insoluble ash

In a container, the total ash was mixed with 25 mL of hydrochloric acid (HCl) and covered with a glass plate. The crucible was gently immersed in a water bath for 5-7 minutes. After that, the glass plate was rinsed with 5 mL of warm water and the resulting solution was poured into the container. The insoluble material was collected on an ash-less mesh and washed with warm water until the residue became neutral. The insoluble matter was then transferred to a new crucible from the filter paper. The crucible containing the material was placed on a hot plate and burnt until a constant weight was obtained. The excess was allowed to cool in a desiccator for 30 minutes and then weighed immediately. The acid-insoluble ash was calculated as a percentage with respect to the dehydrated plant material.

Water soluble ash

25 mL of purified water was added to a silica crucible containing total ash and heated for 5 minutes. The insoluble matter was then transferred to a sintered glass crucible and washed with hot water. The remaining insoluble matter was then moved to a new crucible and heated at 450°C for 15 minutes. The excess was allowed to cool for 30-40 minutes in a desiccator, and then weighed immediately. The weight of the deposit was subtracted from the weight of the total ash. The percentage of water-soluble ash was calculated with respect to the dehydrated plant material.

Alcohol extractive value

Approximately 5.0 g of coarsely powdered air-dried material was accurately weighed and transferred into a conical flask with a stopper. The powder was macerated with 100 mL of ethanol for 6 h with occasional shaking. After 18 h, the mixture was filtered quickly, and care was taken to avoid the loss of any solvent. Then, 25 mL of the filtrate was transferred to a flat-bottomed tarred dish and evaporated to dryness. The resulting extract was dehydrated for 6 h at 105°C, cooled in a desiccator for 30 min, and weighed immediately. The percentage of the extractive in terms of the air-dried powdered medicinal material was determined.

Water extractive value

Approximately 5.0 g of the drug substance was mixed with 100 mL of chloroform and allowed to macerate for 24 hours in a closed flask, with intermittent shaking for the first 6 hours, followed by an additional 18-hour rest. The resulting solution was rapidly filtered, and 25 mL of the filtrate was evaporated to dryness in a tared flat-bottomed dish, dehydrated at 105°C, and weighed. The percentage of the water-soluble extractives with respect to the dehydrated powder of the drug material was then calculated.

Qualitative Phytochemical Analysis

The dried ethanolic extracts were subjected to various color reactions to identify the nature of the phytoconstituents [17-23].

Animals

Male albino mice (22-25 gm) were housed into groups of five at an ambient temp of 25±1°C at an ambient temp of 25±1°C. Animals had free access to food (Hindustan Lever, India) and water. Animals were deprived of food but not water 4h before all acute experiments. All experiments were carried out during the light period (08:00-16:00 h). The Institutional Animal Ethical Committee approved the protocol of all the experimental studies. The above extracts and fractions were subjected to the following acute models of anxiety based on exploratory behaviour.

Induction of Anxiety

Meta-Chlorophenylpiperazine (MCPP) was dissolved in saline and injected SC at a volume of 1 ml/kg.

Anxiolytic models

Elevated plus-maze test

Principle

This method is based on the principle that exposure of animals to an elevated open maze evokes an approach- avoidance conflict and fear and elevation causes greater fear and avoidance conflict

Apparatus

Elevated plus-maze was a wooden, cross shaped maze, consisting of four arms arranged in the shape of a plus sign. Two of the arms have no side or end walls (open arms; 16×5 cm). The other two arms have side and end walls, but are open on the top (closed arms; 16×5×12 cm). At the intersection of 4 arms, there is a square platform of 5×5 cm. The maze was elevated to a height of 25 cm.

Procedure:

Prior to beginning examination, mice were taken care of day by day to decrease stress. Two hours after oral organization of test medications and 30 min after intraperitoneal organization of diazepam, creature was set in focal point of labyrinth, confronting one of encased arms. Thereafter, number of passages and time spent in open and shut arms were recorded amid next 5 min. arm passage being characterized when each of the four paws are in arm [24].

Following parameters measured

1. Number of open and shut arm sections.
2. Percent time spent in open and shut arm.

At end of every trial mechanical assembly was wiped perfect with a specific end goal to dispense with any olfactory pieces of information, which may alter conduct of next creature.

Hole-Board (Head Dipping) Test [25]

Principle

Apparatus

Hole-board apparatus was used to assess the anxiolytic behavior of mice. The apparatus consists of a wooden box (40 x 40 x 25 cm) with 16 holes (each of diameter 3 cm) evenly distributed on the floor Head dipping behavior is a measure of anxiety is the principle of this method

Procedure

Two hours after oral organization of test medications and 30 min after intraperitoneal organization of diazepam, creature was set separately at one side of device and watched for 5min.

The taking after parameters were measured

1) Number of head plunges
2) Number of line intersection

At end of every trial contraption was wiped perfect with a specific end goal to dispense with any olfactory intimation, which may adjust conduct of next creature. The methodology was led ideally in stable weakened room, with perceptions produced using nearby room by means of web camera appended to PC framework.

Light-Dark Exploration Test [25]

Principle The principle is based on the concept of natural aversion of rodents to brightly lit areas.

Apparatus

The light-dark apparatus consists of two compartment chambers (40×60×20 cm) comprising of a brightly illuminated area (40×40 cm) and a dark area (40×20 cm) separated by a wall with a round hole (7 cm diameter).

Procedure:

The mechanical assembly comprised of light and dull chamber isolated by little parcel containing 13 cm long x 5 cm high opening which isolates dim chamber from light chamber. In two chambered framework, where animals can uninhibitedly move between brilliantly lit open field and dim corner, they indicate more intersections between two chambers and more locomotor action after treatment with anxiolytics. quantities of intersections in the middle of light and dull locales were recorded. Movements through allotment and time spent in dim and light chamber were numberd. Male mice were put into confine. The animals were dealt with 30 min before trial with test medications or vehicle intraperitoneally and after that watched for 10 min, gatherings of 6 animals are utilized for each dose72. Onaive &Martin have performed aneuropharmacological and physiological acceptance utilizing light dull test apparatus. The taking after behavioral were measured:

1) The number of sections in dull and light chamber.
2) Time spent in minutes in dull and light chambers.

The technique was led ideally in stable weakened room, with perceptions produced using nearby room by means of web camera connected to PC framework.

Open Field Test [25]

Principle

Induction of anxiety state by light source in a novel environment is the principle of this method.

Apparatus

The open-field apparatus was made of plywood and consisted of squares (61×61 cm). The entire apparatus was painted black except for 6mm thick white lines which divided the floor in to 16 squares. Open field was lighted by a 40 W bulb from a height of about 100 cm. The entire room except the open field was kept dark.

Procedure

This test uses behavioral changes in rodents presented to novel situations and is utilized to affirm that watched upper impact is not because of incitement of general engine movement. Different sorts of open field mechanical assembly have been utilized to test mice. The open field test was completed on dim floor subdivided into 16 a balance of in wooden box (100 x 100 x 30 cm). A focal square was attracted center of open field. focal square is utilized on the grounds that a few mice strains have high locomotor movement and cross lines of test load commonly amid test session. Additionally, focal square has adequate space encompassing it to offer intending to focal area as being unmistakable from external areas.

Mice were put into one of four edges of open field and permitted to investigate mechanical assembly for 5 minutes. Following 5 minutes test, mice were returned in their home pens and open field was cleaned with 701 % ethyl liquor and allowed to dry between tests.

To survey procedure of habituation to oddity of coliseum, mice were presented to mechanical assembly for 5 minutes on 2 continuous days.

The taking after behavioral were measured:

1.) Ambulation
2.) Rearing
3.) Self preparing
4.) Activity in focus
5.) Fecal hanging

Anxiety disorders are highly prevalent, chronic, and disabling conditions that impose enormous health and economic costs both on individuals and on society. Medicinal plants are an invaluable source of bioactive metabolites that can be useful as new pharmacological treatment. Within the disorders that affect the central nervous system, anxiety is one of the most frequently diagnosed conditions worldwide. Generalized anxiety disorder is a well-defined condition characterized by excessive, uncontrollable, and persistent worry about everyday internal and external events. Generalized anxiety disorder is usually accompanied by psychological and somatic complaints, such as autonomic arousal, restlessness, fatigue, problems with concentrating, irritability, and sleep problems (insomnia, difficulty to fall or stay asleep, and poor quality sleep). These clinical symptoms have a huge impact on individual's interpersonal relationships, work performance, and mental and physical health.

Phytomedicine is term, which comprise of dynamic substance constituents show in different parts of plant having particular pharmacological activity on body. These phytomedicines are likewise called as phytoconstituents, which are being utilized persistently for long time or decades or hundreds of years in different diverse courses from those of ordinary therapeutic endorsing.

Research improvement in field of phytomedicines of phytotherapy has experienced different issues, for example, absence of patent assurance differing qualities. Natural cures which are prominent in conventional utilize, producers are permitted to submit important bibliographic information as proof for inspecting their prior licenses of right. Else it must be considered as hesitant concession by permitting powers so as to audit of permit additional confirmation may be needed.

Medicinal plants are considered colossal producers of bioactive therapeutics agents. The genus *Mentha* possesses commercial values owing to its aromatic species. *Mentha piperita* is a hybrid mint, a cross between water mint and spearmint. It is an herbaceous rhizomatous perennial plant. The leaves of *Mentha piperita* were collected from nearby botanical garden. The extract of leaves of peppermint was prepared by Soxhlet extraction method using ethanol as a solvent. The phytochemicals present in the leaves of the plant sample extracted were studied. The solvent used for the extraction was ethanol. The methanolic extraction of peppermint showed the presence of phenols and tannins, flavonoids, carbohydrates, glycosides and alkaloids.

RESULTS AND DISCUSSION

Morphological characters of plant material

The various morphological characters like colour, odour, taste, size, shape, etc. has been studied for all three plant materials i.e leaves of *Mentha piperita*. All the plant parts are greenish in colour with Characteristic odour. The taste of leaveas is mint in taste. The compiled results of macroscopical study are shown in table below.

Table: 1 Macroscopical features of plant materials

S. No	Parameters	Parts of plant
1	Colour	Greenish
2	Odour	Aromatic
3	Taste	Mint
4	Size of leaves	2.5-3.5cm
5	Shape	Ovalate

Extractive Values

For ethanol solutions, the extractive values of the plant were assessed.

Table: 2 Extractive Values of the plant extract

S.NO	Name of The Plant Yield	% w/w
1	*Leaves extract of Mentha piperita*	14.52

Phytochemical parameters

Ash Values:

Water soluble ash of Leaves extract of *Mentha piperita* was discovered to be 4.46 %.

Extractive Values:

Extractive value (water and ethanol soluble) of Leaves extract of *Mentha piperita* were discovered to be 14.52 %.

Loss on Drying:

The loss on drying of extract of *Mentha piperita* was discovered to be 4.46 % w/w. All the compiled results are shown in table below.

Table: 3 Loss on Drying and Foreign Organic Matter

Crude drugs	Loss on drying (% w/w)	Foreign matter (% w/w)*
Extract of Mentha piperita	4.46	1.28

Table: 4 Total Ash, Acid Insoluble Ash and Water Soluble Ash Values

Crude drugs	Total ash value* % w/w	Water soluble ash* % w/w	Acid insoluble ash value* % w/w
Leaves extract of Mentha piperita	4.9	10.24	6.24

Phytochemical Screening

Plant material concentrates have shown that saponins, tannins, glycosides, and sugars are available. The concentrates not entirely set in stone to be absent any and all proteins. As indicated by this examination, the ethanolic separate has more parts. A preliminary study has reported that the leaves extract contained large number of bioactive secondary molecules like phenols, alkaloids, tannins, glycosides, carbohydrates, flavonoids. The presence of these components in this species is an indication that it may have some medicinal potential. Moreover, the restorative activities of the two unmistakable concentrates might be because of the presence of a few phytoconstituents.

Table: 5 Phytochemical screening for extract of Leaves extract of *Mentha piperita*

S.N.	Chemical Tests	Ethanolic extract of *Mentha piperita*
1	Steroids and Triterpenoids:	-
2	Saponins:	-
3	Alkaloids:	+
4	Glycosides:	+
5	Tannins and Phenolic compounds:	+
6	Flavonoids:	+
7	Proteins:	-
8	Carbohydrates:	-

Pharmacological Study

Elevated Plus Maze Test:

Animals treated with each of the three dosages (Table below) indicated reduction in number of passages in shut arm of raised in addition to model which was critical when contrasted and control. Additionally, animals treated with diazepam (1 mg/kg), obviously, indicated critical reduction in number of passages at open arm of raised in addition to model furthermore demonstrated increment in number of sections in open arm of hoisted in addition to model which was noteworthy when contrasted and control. Additionally, animals treated with standard drug(1 mg/kg), not surprisingly, indicated huge diminishing in number of passages at open arm of lifted in addition to labyrinth model. Animals treated with moderate and high dosage (250 and 300 mg/kg) demonstrates more noteworthy increment in no of sections and time spent at open arm of lifted in addition to model when contrasted and low measurements.

Table: 6 Elevated Plus Maze Test

Group No	Treatment	Dose (mg/kg)	Number of entries		Time spent in second	
			Open Arm	Close Arm	Open Arm	Close Arm
1	Control	Normal saline	7	11	50	150
2	Inducing	5	6	12	30	170
3	Standard drug	10	11	6	80	120
4	Leaves extracts of *Mentha piperita*	250	9	9	40	160
5	Leaves extracts of *Mentha piperita*	300	8	7	80	120

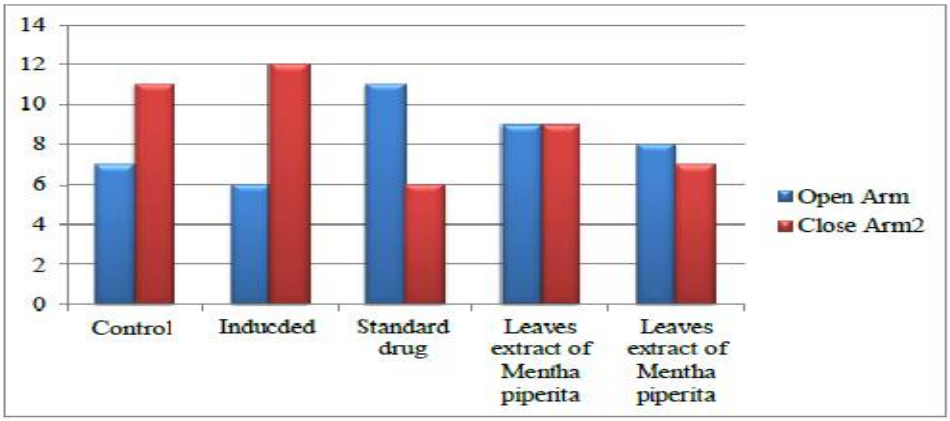

Figure: 4 Graph of Number of entries (Elevated plus Maze Test)

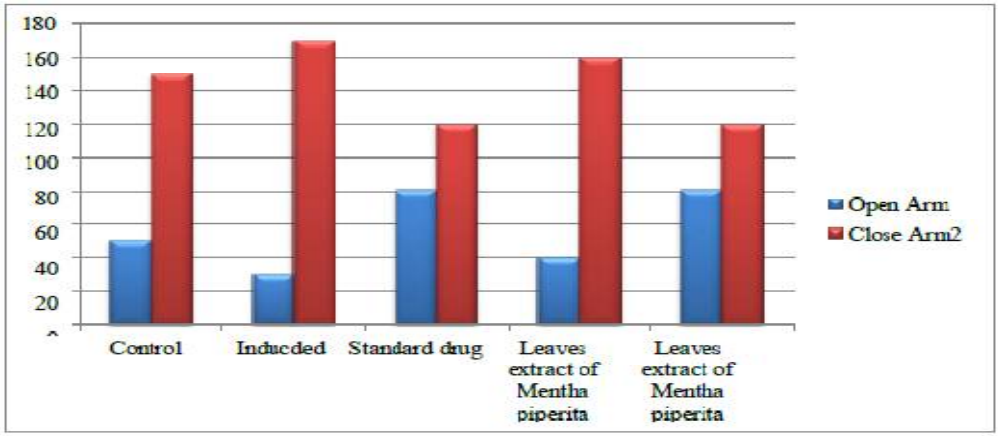

Figure: 5 Graph of Time spent in sec (Elevated plus Maze Test

Light/Dark test

Light dark Test has been depicted as basic conduct display in mice to identify mixes with anxiolytic impacts. Mice and rats have a tendency to investigate novel environment, however to withdraw from aversive properties of splendidly lit open field. In two chambered framework, where animals can

uninhibitedly move between splendidly lit open field and dull corner, they demonstrate more intersections between two chambers and more locomotor action after treatment with anxiolytics. Quantities of passages and time spent in dim and light chambers are recorded. All animals treated with three measurements of indicated expanded number of passages in dim chamber and with expansion in number of sections in time in light chamber when contrasted and controls individually.

Table: 7 Light/Dark test

Group No	Treatment	Dose (mg/kg)	Number of entries		Time spent in min	
			Dark	Light	Dark	Light
1	Control	10	4	2	6	1
2	Inducing	10	8	10	8	0
3	Standard drug	10	12	6	4	1
4	Leaves extracts of Mentha piperita	250	7	3	5	2
5	Leaves extracts of Mentha piperita	300	11	4	3	2

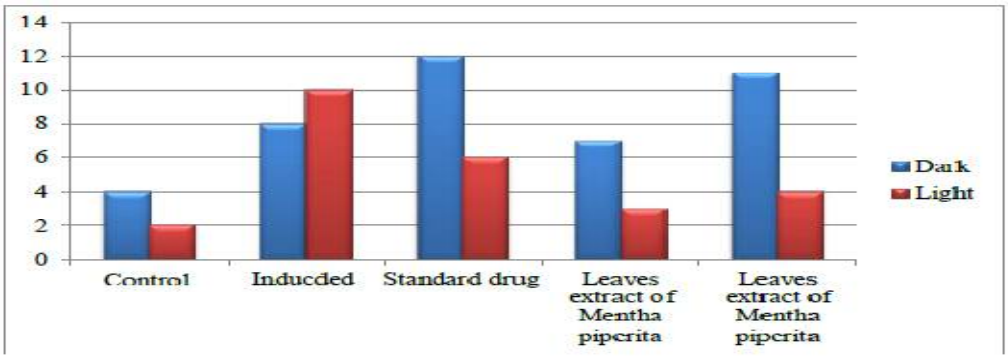

Figure: 6 Graph of Number of entries (Light/Dark test)

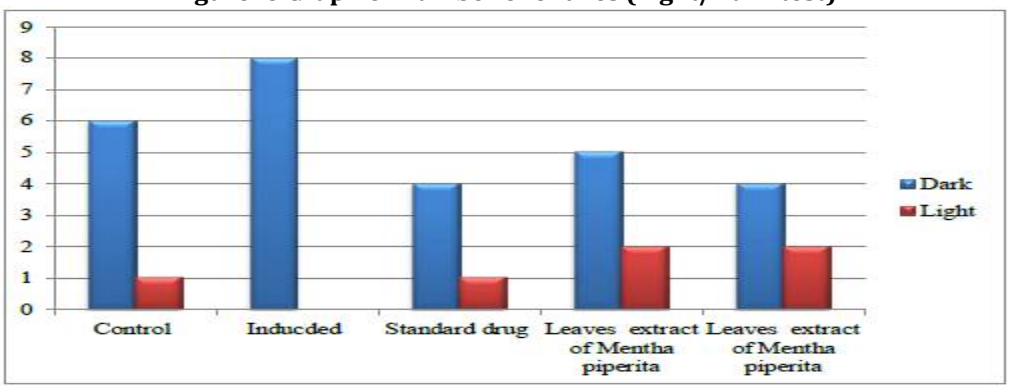

Figure: 7 Graph of Time spent in min (Light/Dark test)

Open field apparatus test

Open Field test was proposed as model to test for anxiolytic movement. Taking after behavioral perspectives were noted

i. Rearing: number of times creature remained on its rear appendages

ii. Self prepping: number of times creature prepared facial locale, and licked/washed/scratched different parts of its body

iii. Action in focus: number of focal squares crossed by creature.

Table: 8 Open field apparatus test

Group No	Treatment	Dose (mg/kg)	Activity in Centre (N)	Rearing (N)	Self Grooming (N)
1	Control	10	3	8	5
2	Inducing	10	8	5	6
3	Standard drug	10	5	11	2
4	Leaves extracts of *Mentha piperita*	250	4	10	4
5	Leaves extracts of *Mentha piperita*	300	3	9	3

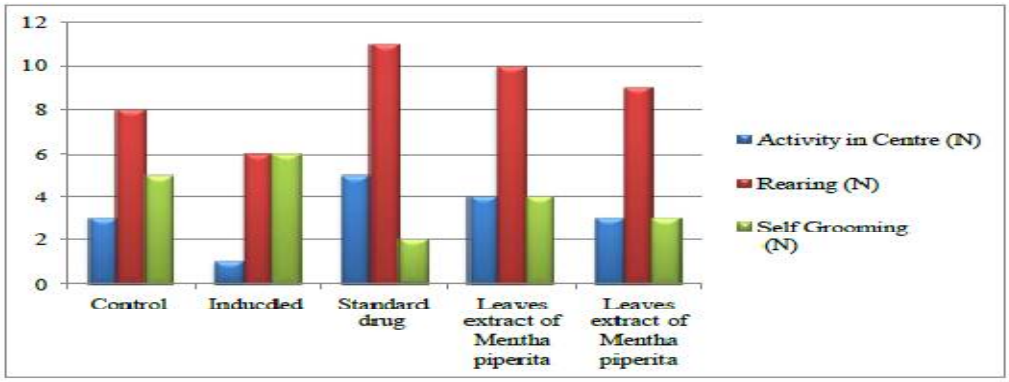

Figure: 8 Graph of Open field apparatus test

Hole Board Apparatus test

Hole Board test as straightforward conduct show in mice to distinguish mixes with anxiolytic impacts. They utilized open field with gaps on base into which animals could jab their noses. opening board test gives straightforward strategy to measuring reaction of creature to new environment and is broadly used to survey emotionality, uneasiness and/or reactions to push in animals. It has been demonstrated that head-plunging conduct was delicate to changes in enthusiastic condition of creature, and proposed that outflow of anxiolytic state in animals may be reflected by expansion in head plunging conduct all out locomotor action and number and length of time of head-dippings were recorded. head plunge was scored if both eyes vanished into gap. No. of head dipping were recorded and recorded data is tabulated in the table below.

Table: 9 Hole Board Apparatus test

Group No	**Treatment**	**Dose (mg/kg)**	No. of head dipping
1	Control	10	25
2	Inducing	10	10
3	Standard drug	10	60
4	Leaves extracts of *Mentha piperita*	250	35
5	Leaves extracts of *Mentha piperita*	300	45

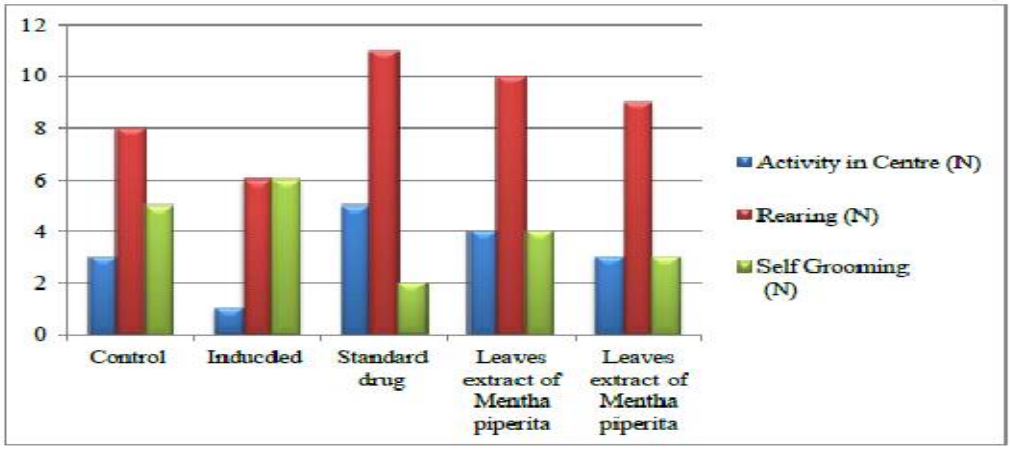

Figure: 9 Graph of Hole Board Apparatus test

Natural products, including chamomile, have been used as alternative medicines or functional food for centuries worldwide. Herbal formulations have been utilized for human well-being, including physical endurance, mental functions, and non-specific resistance of the body, and are commonly referred to as adaptogens. Stress is a prevalent factor in modern society, and it is essential to keep stress under control to maintain normal body functioning. Stress perturbs the body's homeostasis, and if it becomes extreme, the organism's survival is threatened due to a deficit in homeostatic mechanisms.

The aim of this study was to develop a formulation for treating anxiety induced by MCCP in rats using the leaves extract of *Mentha piperita*. The results of the study are as follows:

The morphological characteristics, such as color, odor, taste, size, and shape, of all three plant materials, i.e., leaves of *Mentha piperita*, were studied. The plant parts were greenish in color with an aromatic odor.

The extractive value of the plant in ethanol solutions was found to be 14.52% w/w, and the water-soluble ash was 4.46%. The loss on drying of the leaves extract of *Mentha piperita* was found to be 4.46% w/w. Table 5.3 summarizes all the results compiled.

The animals were divided into five groups: the control group (group 1), the induced group (group 2), the standard group (group 3), the treatment group with Leaves extract of *Mentha piperita* Dose (250mg/kg) (group 4), and the treatment group with Leaves extract of *Mentha piperita* Dose (300mg/kg) (group 5). A preliminary study indicated that the leaves extract contained a large number of bioactive secondary molecules such as phenols, alkaloids, tannins, glycosides, carbohydrates, and flavonoids [26-30]. The presence of these components in this species suggests its medicinal potential. The findings of the study clearly indicate the presence of significant anxiolytic properties in the ethanol extract of *Mentha piperita*, which requires further investigation, including compound isolation

The present study aimed to investigate the anxiolytic effects of ethanolic extract of *Mentha piperita* using Elevated plus-maze, Hole-Board (Head Dipping), Light-Dark Exploration, and Open Field tests, while the phytochemical parameters were employed to assess standardization of extract. The behavioral effects of *Mentha piperita* were compared to those of diazepam, a known anxiolytic drug, to determine its efficacy. In the light/dark test, anxiety was induced by the novelty of the environment, and the anxiolytic effect was measured by assessing the number of transitions and time spent in the light chamber. Our findings showed that the extract (250 mg/kg) significantly increased time spent in the light chamber, indicating its anxiolytic potential. Our results demonstrated that the ethanolic extract (200 mg/kg) increased head dipping, supporting the anxiolytic-like effect observed in the light/dark test [26-30].

CONCLUSION

The present study provides scientific evidence supporting the traditional use of *Mentha piperita* for anxiety treatment. Despite the long-standing use of *Mentha piperita* for various ailments, its anxiolytic activity has not been scientifically evaluated until now. Our results indicate that the extract of *Mentha piperita* had significant anxiolytic effects on mice, as observed in the light/dark test and the hole board, and were comparable to those of a standard drug. Future studies will investigate the underlying neurobiological mechanisms of action and potential interactions of *Mentha piperita* with classical neurotransmitters. Furthermore, the isolation and identification of the phytoconstituent(s) responsible for the observed central effects will be a focus of future research.

ACKNOWLEDGMENTS

The authors express their gratitude to Dr. Vivek Kumar Gupta for his guidance and to Shri Rawatpura Sarkar Institute of Pharmacy, Datia (M.P) for providing all necessary facilities for conducting this research.

REFERENCES

1. Davison GC (2008). Abnormal Psychology. Toronto: Veronica Visentin. p. 154.
2. Miceli M, Castelfranchi C (2014-11-27). Expectancy and emotion. OUP Oxford. ISBN 978-0- 19-150927-8.
3. Bouras N, Holt G (2007). Psychiatric and Behavioral Disorders in Intellectual and Developmental Disabilities (2nd ed.). Cambridge University Press. ISBN 9781139461306.
4. American Psychiatric Association (2013). Diagnostic and Statistical Manual of Mental Disorders (Fifth ed.). Arlington, VA: American Psychiatric Publishing. p. 189
5. Chand, SP; Marwaha, R (2022), "article-17728", Anxiety, Treasure Island (FL):
6. Robinson, Oliver J; Pike, Alexandra C; Cornwell, Brian; Grillon, Christian (June 29, 2019). "The translational neural circuitry of anxiety". Journal of Neurology, Neurosurgery, and Psychiatry. BMJ. 90 (12): jnnp–2019–321400
7. Leichsenring F, Salzer S, Beutel ME, et al. Psychodynamic therapy and cognitive-behavioral therapy in social anxiety disorder: a multicenter randomized controlled trial. Am J PSychiatry. 2013; 170:759–767.
8. Gloster AT, Wittchen HU, Einsle F, et al. Psychological treatment for panic disorder with agoraphobia: a randomized controlled trial to examine the role of therapist-guided exposure in situ in CBT. J Consult Clin Psychol. 2011; 79:406–420.
9. Diemer J, Mühlberger A, Pauli P, Zwanzger P. Virtual reality exposure in anxiety disorders: impact on psychophysiological reactivity. World J Biol Psychiatry. 2014; 15:427–442.
10. Broocks A., Bandelow B., Pekrun G., et al Comparison of aerobic exercise, clomipramine, and placebo in the treatment of panic disorder. Am J Psychiatry. 1998; 155(5):603–609.
11. Sarris, J., Panossian, A., Schweitzer, I., Stough, C., & Scholey, A. (2011). Herbal medicine for depression, anxiety and insomnia: a review of psychopharmacology and clinical evidence. European neuropsychopharmacology: the journal of the European College of Neuropsychopharmacology, 21(12), 841–860.
12. McKay DL, Blumberg JB. A review of the bioactivity and potential health benefits of peppermint tea (Mentha piperita L.). Phytother Res. 2006; 20(8):619-33.

13. Mahendran G, Rahman LU. (2020). Ethnomedicinal, phytochemical and pharmacological updates on Peppermint (Mentha × piperita L.)-A review. Phytother Res. 2020; 34(9):2088-2139.
14. Bajaj, J., Dave V., Sharma S., Shukla A., Chakole R.D. Pharmacognostical and phytochemical studies on achyranthes aspera. World Journal of Pharmacy and Pharmaceutical Sciences. 2012; 1(4):1316-1331.
15. Mehta, S., Garg, A., Garg, S., Kumar, M., Shukla, A. Concise report on Standardization of herbal drugs and its products. Advance Pharmaceutical Journal. 2018; 3(3):83-89.
16. Mahto, B.K., Patel R., Bapna R., Shukla A.K. (2022). Development and Standardization of a Poly Herbal Formulation. The Scientific Temper. 13(2):118-125.
17. Tiwari, R., Shukla, A.K. (2020). Plant metabolites and their role in health benefits: A brief review. Advance Pharmaceutical Journal. 5(2):47-53.
18. Shahnawaz, M., Goswami, S., Shukla, A. K. (2019). Preliminary assessment of Calotropis gigantea leaves extract for in vitro antidiabetic activity. Advance Pharmaceutical Journal. 4(5):128-24.
19. Graeff FG, Zangrossi Jr H. (2002). Animal models of anxiety disorders. In: D'Haenen H, den Boer JA, Willner P. Biological Psychiatry. London: John Wiley & Sons Ltd. p. 96-103.
20. Lepicard EM, Joubert C, Hagneau I, Perez-Diaz F, Chapouthier G. Differences in anxiety-related behavior and response to diazepam in BALB/cByJ and C57BL/6 J strains of mice. PharmacolBiochemBehav. 2000; 67:739-48.
21. Takeda H, Tsuji M, Matsumiya T. Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. Eur J Pharmaco. 1998;l3(Suppl 50):21-9.
22. Dunham NW, Miya TSA. "A note on a simple apparatus for detecting neurological deficit in rats and mice," J Am Pharm Assoc. 1957; 46(3):208–9.
23. Amos S, Adzu B, Binda L, Wambebe C, Gamaniel K. Neuropharmacological effect of the aqueous extract of Sphaeranthussenegalensis in mice. J Ethnopharmacol. 2001; 78(1):33-7.
24. Sangeeta, S., Tanavade, S.M.T., Nilofer, Naikwade., Dhanyakumar, D.C. (2012). In-vitro anticancer activity of ethanolic and aqueous extracts of Peristrophe bivalvis merrill. *Research J. Pharm. and Tech*; 1324-1327.
25. Bajaj, J., Dave, V., Sharma, S., Shukla, A., Chakole, R.D. (2012). Pharmacognostical and phytochemical studies on Achyranthes aspera. *World Journal of Pharmacy and Pharmaceutical Sciences*; 1(4):1316-1331.
26. Gupta, M, Lodhi, S, Shukla, A. (2015). Preliminary phytochemical analysis and in-vitro anti-helminthic activity of Martynia annua Linn and Permotrema reticulatum. *Asian Journal of Biomaterial Research*; 1(2):72-74.
27. Mahto, B.K., Patel, R., Bapna, R., Shukla, A.K. (2022). Assessment of antioxidant, anticancer activity of standardized poly-herbal capsule formulation. *Neuro Quantology*; 20(9):4187-4203.
28. Razali, S., Firus, Khan., A., Khatib, A., Ahmed, Q., Abdul, Wahab., R., Zakaria, Z. (2021). An In-vitro anticancer activity evaluation of Neolamarckia cadamba (roxb.) bosser leaves' extract and its metabolite profile. *Front. Pharmacol*; 1-4.
29. Shahnawaz, M., Goswami, S., Shukla, A. K. (2019). Preliminary assessment of Calotropis gigantea leaves extract for in-vitro antidiabetic activity. *Advance Pharmaceutical Journal*; 4(5):128-132.
30. Tiwari, R., Shukla, A.K. (2020). Plant metabolites and their role in health benefits: A brief review. *Advance Pharmaceutical Journal*; 5(2):47-53.

CITATION OF THIS ARTICLE

Atul K S, Vivek G, Vimal K Y, Ajay K S. Evaluation of Anxiolytic Activity of Ethanolic Extract of *Mentha Piperita* Leaves in a Rat Model. Bull. Env. Pharmacol. Life Sci., Vol 12[6] May 2023: 97-107.