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“A STUDY OF ANXIOLYTIC ACTIVITY OF MATRICARIA CHAMOMILE FLOWERS OF AQUEOUS EXTRACTION IN MICE”

G. Kiran*¹, K. Yaminisai¹, B. Naresh¹, M. Anand Babu¹, A. Samba Siva Rao¹, CH. Ashok Kumar¹

¹*Department of Pharmacology, A.M. Reddy Memorial College of Pharmacy, Narasaraopet, Guntur, Andhra Pradesh, India.

ABSTRACT

This study was performed to investigate the anxiolytic effects of aqueous extract of *Matricaria chamomile L* (AEMC) in mice using the elevated plus-maze model (EPM) and light dark model. The extract was administered orally in two different doses of 200mg/kg and 400mg/kg were able to increase the time spent and the number of arm entries in the open and closed arms of the elevated plus-maze, also increases the time spent by mice in the illuminated side of the light-dark test, dose of 200mg/kg and 400mg/kg showed more significant increase in comparison with control animals. This effect was comparable to that of the diazepam (5mg/kg p.o.). These results indicate that AEMC is an effective anxiolytic agent.

KEYWORDS

Anxiolytic-like effect, *Matricaria chamomilla L*, Elevated plus maze, Diazepam and Light-Dark box.

Author for Correspondence:

Kiran G,
Department of Pharmacology,
A.M. Reddy Memorial College of Pharmacy,
Narasaraopet, Guntur, Andhra Pradesh, India.

Email: kirn1987@gmail.com

INTRODUCTION

Anxiety is an emotion characterized by an unpleasant state of inner turmoil, often accompanied by nervous behavior, such as pacing back and forth, somatic complaints, and rumination¹. It is the subjectively unpleasant feelings of dread over anticipated events, such as the feeling of imminent death². Anxiety is not the same as fear, which is a response to a real or perceived immediate threat³, whereas anxiety is the expectation of future threat³. Anxiety is a feeling of uneasiness and worry, usually generalized and unfocused as an overreaction to a situation that is

only subjectively seen as menacing⁴. It is often accompanied by muscular tension³, restlessness, fatigue and problems in concentration. Anxiety can be appropriate, but when experienced regularly the individual may suffer from an anxiety disorder³.

People facing anxiety may withdraw from situations which have provoked anxiety in the past⁵. There are various types of anxiety. Existential anxiety can occur when a person faces angst, an existential crisis, or nihilistic feelings. People can also face mathematical anxiety, somatic anxiety, stage fright, or test anxiety.

AIM AND OBJECTIVE

Aim

The present study was taken up for elevating the anxiolytic activity of aqueous extract of the flowers of the *Matricaria chamomilla* L.

Objective

The objective of present study is,

1. To prepare aqueous extract by successive extraction technique of the flowers of *Matricaria chamomilla* L analyse them for the presence of phytoconstituents.
2. To establish pharmacological profile of the flowers of *Matricaria chamomilla* L.
3. To asses anxiolytic activity in mice by following models:
 1. Plus maze model
 2. Light-Dark Model

Introduction⁶⁻¹⁰

Chamomile or camomile is the common name for several daisy-like plants of the family Asteraceae that are commonly used to make herb infusions to serve various medicinal purposes. Popular uses of chamomile preparations include treating anti-anxiety, hay fever, inflammation, muscle spasms, menstrual disorders, insomnia, ulcers, gastrointestinal disorders, and haemorrhoids. Camomile tea is also used to treat skin conditions such as eczema, chickenpox and psoriasis.

Plant kingdom

Kingdom: plantae

Order: Asterales

Family: Asteraceae

Genus: Matricaria

Species: M.Chamomile

Synonym

Matricariarecutita

Biological source

It is the fully dried flower obtained from the plant *Matricaria chamomilla* belonging to the family Asteraceae.

Chemical constituents

Chamomile include: sesquiterpenes, terpenoids, flavonoids, coumarins such as herniarin and umbelliferone, phenylpropanoids such as chlorogenic acid and caffeic acid, flavones such as apigenin and luteolin, flavanols such as quercetin and rutin, and polyacetylenes.

PLAN OF WORK

MATERIAL AND METHODS¹¹⁻²⁰

Plant materials

The plant *Matricaria chamomilla* L, belongs to the family Asteraceae. The description, history, cultivation and constituents of which have been already described. The plant is largely found in Andhra Pradesh. The flowers of the plant are collected from the local area of Guntur district .The plant was authenticated by Dr. Sathyanarayana Raju (M.Sc.,M.Phil.,Ph.D.), plant taxonomist, Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna Sagar Guntur-522510,A.P.

Preparation of extract

The flowers of *Matricaria chamomile* were washed and separate the petals and dried under the shade. Coarse powder of all the flowers was made and extracted by water for 24 hrs at room temperature. The extract was then analyzed by qualitative tests and was found to contain sesqiterpens, flavanoids, terpinoids, and coumarin.

Animals

Male albino mice weighing 20 -25g of were used for the study. The animals were housed in solid-bottomed polypropylene cages and acclimatized to animal conditions. The mice were fed with commercial mice diet and water adlibation. The experiments were designed and conducted in

accordance with ethical forms approved by Committee for the purpose of control and supervision on Experiments on Animals (CPSCEA) and Institutional Animal Ethical Committee (ICEA).

Drug treatment

Albino mice's were divided into four groups of 5 animals each.

Group I - Received Control (2% saline)

Group II - Received Standard drug(diazepam-5mg/kg i.p)

Group III - Test-Received low dose(200mg/kg p.o)

Group IV - Test-Received high dose(400mg/kg p.o)

Acute toxicity studies

Acute toxicity tests were performed in mice. All animals were fasted overnight before treatment and were given food 1 h after AEMC treatment. A single high dose (400 mg/kg), as recommended by the OECD guidelines was administered orally to mice. General behavior was also observed at 1, 3 and 24 h after administration. The number of animals that died after administration was recorded daily for 14days.

DETERMINATION OF ANXIOLYTIC ACTIVITY BY VARIOUS METHODS

1. Elevated plus maze method.
2. Light Dark method.

Elevated plus maze method

Elevated plus-maze test

The elevated plus-maze comprised two open (30 cm×5 cm×0.25 cm) and two enclosed (30 cm×5 cm×15 cm) arms that radiated from a central platform (5 cm×5 cm) to form a plus sign. The maze was constructed of black painted wood.

A slight raised edge on the open arms (0.25 cm) provided additional grip for the animals.

The plus-maze was elevated to a height of 40 cm above floor level by a single central support. The experiment was conducted during the dark phase of the light cycle (9:00-14:00 h).

The trial was started by placing an animal on the central platform of the maze facing an open arm. The number of entries into, and the time spent in,

each of the two types of arm, were counted during a 5 min test period were used as indices of anxiety.

A mouse was considered to have entered an arm when all four paws were on the arm. The apparatus was cleaned thoroughly between trials with damp and dry towels.

Light Dark method

Light dark test

The apparatus consisted of two 20 cm×10 cm×14 cm plastic boxes: one was dark and the other was transparent. The mice were allowed to move from one box to the other through an open door between the two boxes. A 100W bulb placed 30 cm above the floor of the transparent box was the only light source in the room. A mouse was put into the light box facing the hole. The transitions between the light and the dark box and time spent in the light box were recorded for 5 min immediately after the mouse stepped into the dark box. The apparatus was cleaned thoroughly between trials.

STATISTICAL ANALYSIS

Results are expressed as mean. All data are subjected to analysis of graphical methods are followed by mean.

Experiment part

Effect of AEMC on Elevated plus maze

In EPM saline treated animals the time spent and entries in the open and closed arms, were compared with AEMC extract at the dose of 200mg/kg and 400mg/kg and also Diazepam (5mg/kg) showed significant increase in the time spent in the open arms.

AEMC extract at the dose of 200mg/kg and 400mg/kg and also Diazepam (5mg/kg) showed significant increase in the time spent in the closed arm.

The Diazepam showed a significant in elevated plus-maze.

Elevated plus maze apparatus

Effect of AEMC on Light and dark model In animals treated with two doses of AEMC (200 and 400 mg/kg) and diazepam (5mg/kg) showed reduced time spent but increase in number of entries in dark chamber and with concomitant increase in

time and number of entries in light chamber when compared with controls. Animals treated with low and high dose (200 and 400 mg/kg) shows more significant results when compared with control.

DISCUSSION

The medicinal plant is Matricaria chamomile is collected from the medicinal plant garden of A.M Reddy Memorial College of Pharmacy, Petlurivaripalam, Narasaraopet, Guntur (d.t). This plant authentication was done in department of botany in Acharya Nagarjuna University.

In order to test the anxiolytic activity we have taken the reference or standard drug diazepam.

We selected the 20 mice for control, test and standard samples with regular intervals and taken the wash out period three days for each type of sample. Inject the sample to the mice through intraperitoneal route and oral dose in certain doses at regular intervals of time for every 5 min and note the entries by using elevated plus maze and light/dark box. Compare the response of control, standard drug. Observe the responses by plotting the graph. So the standard response is increases compare to control, the standard response has more significant value (Table No.1-4, Graph No.1-4).

Table No.1: Open arm entries

S.No	Treatment (groups)	Open arm entries					mean
		1	2	3	4	5	
1	Control-saline	5	6	8	6	7	6.4
2	Standard-diazepam 5mg/kg	12	11	9	10	11	10.6
3	Test-200 mg/kg	8	7	6	7	6	6.8
4	Test-400mg/kg	9	8	9	8	9	8.6

Table No.2: Closed arm entries

S.No	Treatment	closed arm entries					Mean
		1	2	3	4	5	
1	Control-saline	10	9	9	10	11	9.8
2	Standard- diazepam 5mg/kg	3	2	2	1	3	2.2
3	Test-200 mg/kg	8	7	7	8	9	7.8
4	Test-400mg/kg	6	5	5	4	4	4.8

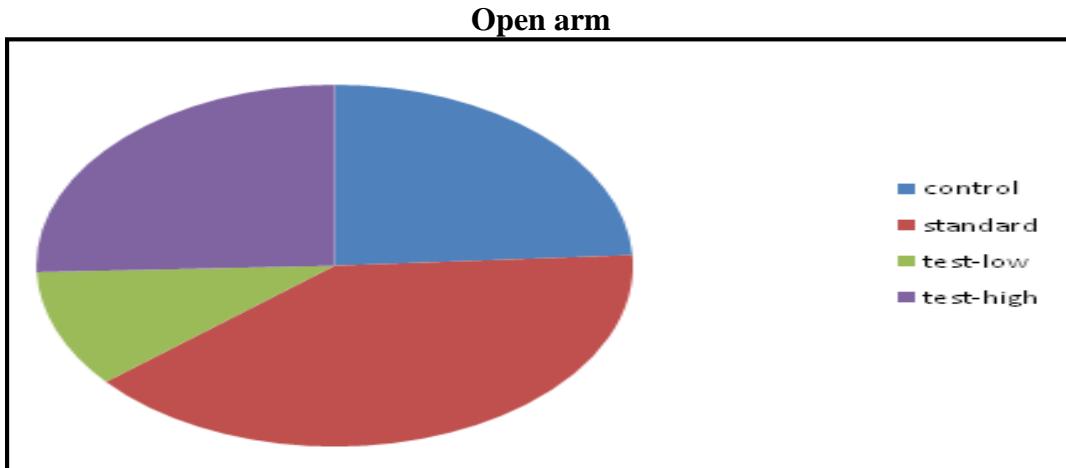
Table No.3: Light box entries

S.No	Treatment(groups)	Light box entries					Mean
		1	2	3	4	5	
1	Control-saline	5	4	3	3	2	3.4
2	Standard-diazepam 5mg/kg	15	14	13	14	15	14.2
3	Test-200 mg/kg	5	6	4	6	5	5.2
4	Test-400mg/kg	9	8	7	8	9	7.8

Table No.4: Dark box entries

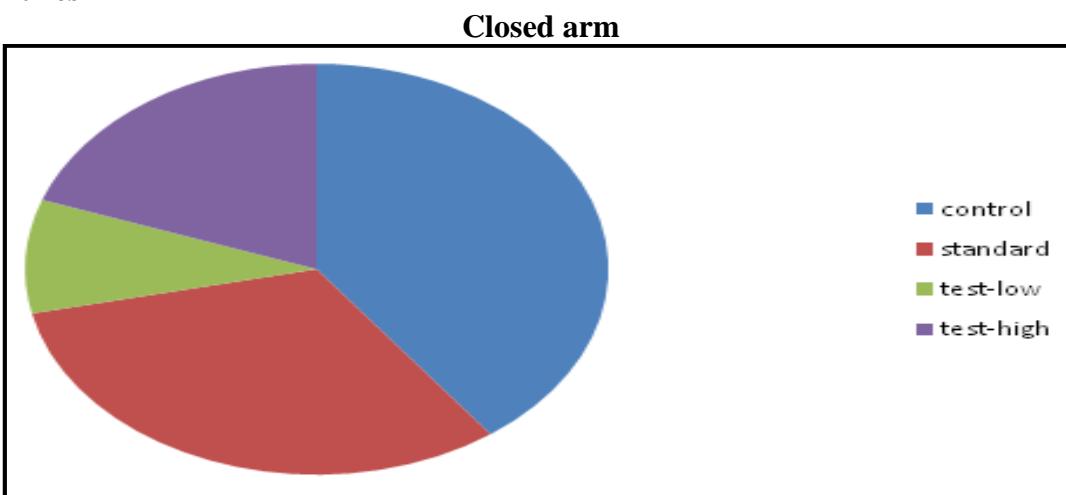
S.No	Treatment(groups)	Dark box entries					Mean
		1	2	3	4	5	
1	Control-saline	12	13	15	15	15	14
2	Standard-diazepam 5mg/kg	3	4	3	3	3	3.2
3	Test-200 mg/kg	9	8	9	10	9	9
4	Test-400mg/kg	7	6	6	5	7	6.2

Open arm entries



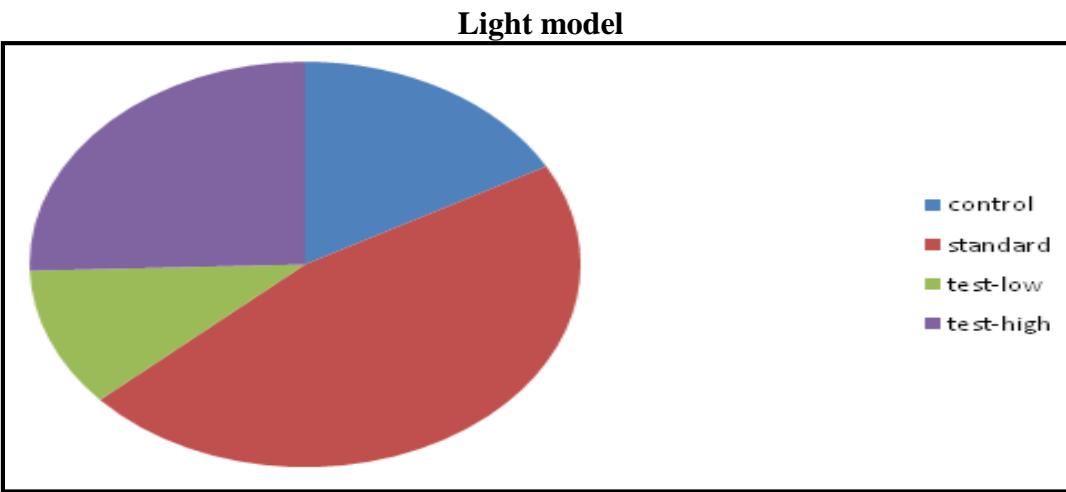
Graph No.1

Closed arm entries

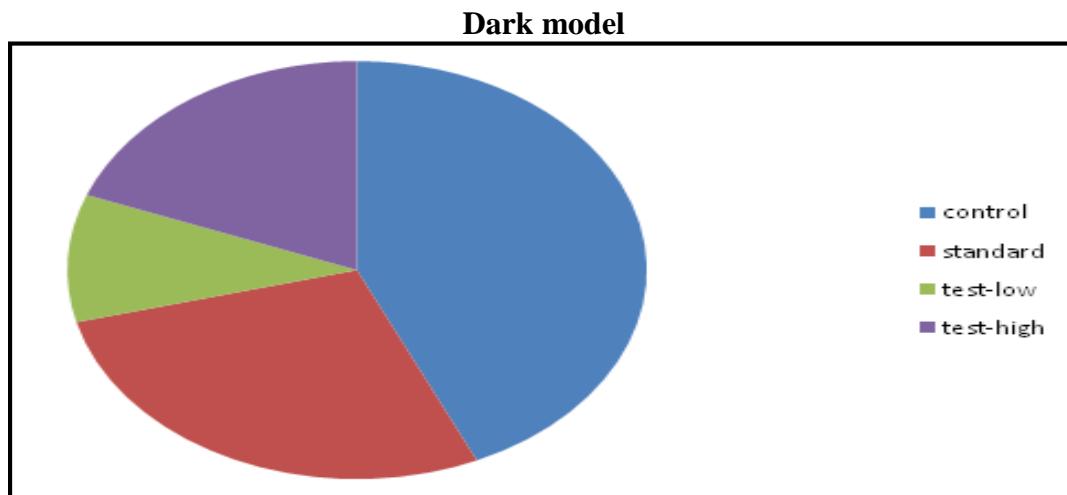


Graph No.2

Light and dark model



Graph No.3



Graph No.4
Plant profile



Figure No.1



Figure No.2

Instrument description

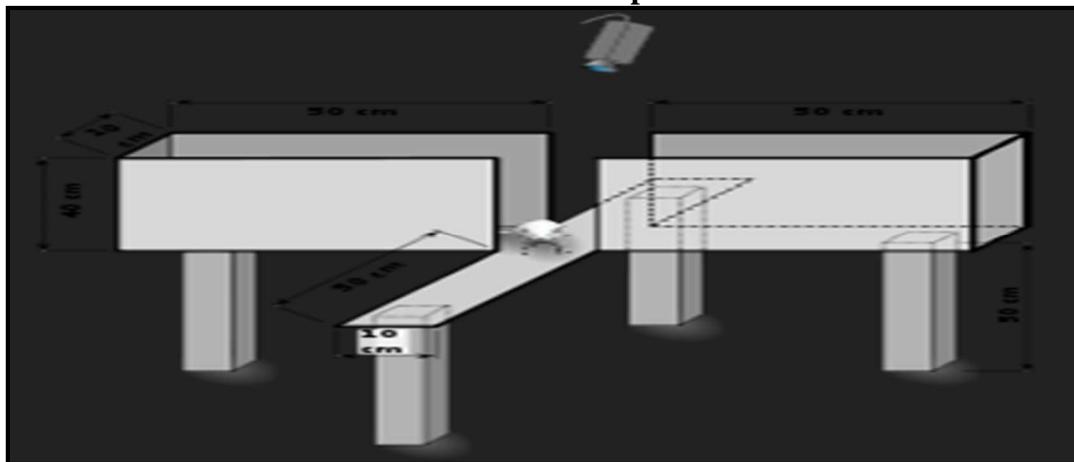


Figure No.3



Figure No.4

CONCLUSION

In pharmacological screening method, the *Matricaria Chamomile* flower extraction when administered in mice shown less potent anxiolytic activity when compared to the standard drug, by using elevated plus maze and light/dark box.

The phytochemical study it was proved that flavanoides, sesquiterpens, coumarin, terpinoids, are present.

From the study it was shown that the Aqueous extract low and high doses shown more significant response when compare with control.

And it was proved that *Matricaria Chamomile* shows fewer side effects.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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