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EVALUATION OF ANTI-ANXIETY ACTIVITY OF AQUEOUS EXTRACT OF *MOMORDICA DIOICA* FRUIT ON EXPERIMENTAL ANIMALS

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1. ABSTRACT

Aim and Objective: The aim of this study is to evaluate anxiolytic activity of *Momordica dioica* aqueous extract in experimental animal. **Methods:** The anxiolytic effects of the aqueous extract of *Momordica dioica* were evaluated in mice using the Elevated Plus Maze (EPM) test. Swiss albino mice weighing between 22-27grams, of either sex, will be randomly assigned into four groups, each containing six animals. Group 1 received vehicle control (normal saline, 0.05ml/10g,orally). Group 2 received standard drug diazepam (1mg/kg, orally). Group 3 received *Momordica dioica* fruit aqueous extract at a dose of 100mg/kg orally, and Group 4 received the extract 300 mg/kg orally for a duration of 21 days. All treatments were administered orally. Animals in Group 1,3 and 4 were pre-treated for 20 days, while the diazepam group will receive the treatment only on the 21st day, 30 minutes prior to testing. The Elevated Plus Maze were used to assess anxiolytic activity, measuring parameters such as : number of open arm entries,

number of closed arm entries and time spent in open arm and closed arms. **Result :** The administration of aqueous extract of *Momordica dioica* fruits at doses of 100mg/kg and 300mg/kg resulted in a notable decrease in both the number of entries and the time spent in the closed arms. Conversely, there was a significant increase in the entries and the time spent in the open arms compare to the control and standard groups. These findings indicate that the *Momordica dioica* fruit extract exhibits significant anti-anxiety effects. **Conclusion:** The results of the current study provide evidence that the aqueous extract of Momordica dioica fruit exhibits a dose dependent protective effect against anxiety.

KEYWORDS: Anxiety, *Momordica dioica* aqueous extract, EPM.

2. INTRODUCTION

Anxiety is generally described as a state involving emotions such as fear, nervousness, apprehension, panic, restlessness, and heightened tension or agitation. It can manifest through physical signs like trembling, dizziness or fainting, headache, and excessive sweating. In some cases it may also lead to increased blood pressure and alterations in physiological responses, including changes in the heart rate, muscle tension, and skin conductivity.[1] If anxiety symptoms interfere with daily life activities, it is referred to as an anxiety disorder.[1]

Anxiety impacts approximately one-eighth of the global population and has emerged as a significant focus of research in the field of psychopharmacology.[2] Anxiety is a state of excessive fear, characterized by motor tension sympathetic hyperactivity, feelings of apprehension, and increased vigilance.[3] Anxiety disorders are the most commonly occurring mental health conditions and are associated with a high burden of illness. Among them, specific or isolated phobias are the most frequent, effecting approximately 10.3% of individuals with in a 12 – month period.[4] While individuals with isolated phobias seldom pursue treatment, panic disorder with or without agoraphobia is the next most prevalent form, effecting 6.0% of the population. This is followed by social anxiety disorder (SAD or Social phobia) at 2.7%, and generalized anxiety disorder (GAD) at 2.2%. Women are 1.5 to 2 times more likely than men to be diagnosed with an anxiety disorder.[5]

Benzodiazepines most commonly used drugs for treating anxiety. Although they share the same class, their pharmacokinetic profiles vary significantly. While side effects are generally mild, long-time use even at standard therapeutic can lead to dependence, making careful usage monitoring important. The speed at which these drugs take effect is influenced by

factors such as the method of administration, formulation dissolution, absorption rate, and how quickly they reach the brain. Diazepam, for example, is quickly absorbed when taken orally and enters the brain rapidly, providing fast relief from anxiety.[6]

Several neurotransmitters play a role in the development of anxiety, including serotonin, dopamine, noradrenaline, GABA, corticotropin releasing factor (CRF), melanocyte stimulating hormone (MSH), as well as various neuropeptides and neurosteroids.[7]

The narrow safety margin of benzodiazepine where the therapeutic anxiolytic effects closely border unwanted side effects has led researchers to explore new compounds in the search for anxiolytic drugs with fewer adverse effects.[8] The discovery of the anxiolytic properties of non-benzodiazepines azapirone agents, which function as partial agonist at 5HT1A receptors, has highlighted the therapeutic potential of these compounds in treating anxiety and mood disorders, drawing increased attention to the role of the 5 – HT1A receptor.[9] Hence, there is a need for a novel agent with strong therapeutic efficacy and improved patient compliance for the treatment of anxiety disorders.[10]

3. METHODOLOGY

3.1 EXPERIMENTAL ANIMALS

Healthy Swiss albino mice (22 – 27 grams) of either sex were used for the experiment were procured from the animal house of Srinivas College of Pharmacy, Mangalore. They were maintained under standard conditions (temperature 22 ± 2, relative humidity 60 ± 5% and 12 h light / dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water ad libitum. The Institutional Animal Ethics Committee approved the experimental protocol (Approval no SCP/IAEC/27/JUN/2025/268). All the animals received human care according to criteria outlined in the “Guide for the Care and Use of Laboratory Animals” prepared by “National Academy of Sciences” and published by the “National Institute of Health”. The animals were acclimatized for at least one week before use. All the procedures were performed in accordance with Institutional Animal Ethics Committee constituted as per the direction of the CPCSEA, under ministry of animal welfare division, Government of India, New Delhi, India.

3.2 ETHICAL CONSIDERATIONS

Every possible effort was taken to reduce animal suffering and limit the number of animals used in the experiments. The animals were treated humanely, all the procedures were carried out in the strict compliance with the guidelines approved by the Institutional Animal Committee (IAEC), as regulated by the CPCSTA, in accordance with the principles established by Government of India for the care and use of Laboratory animals. The study protocol was approved by IAEC, Srinivas College of Pharmacy, Valachil, Mangalore (Ref no. SCP/IAEC/27/JUN/2025-268).

3.3 CHEMICALS AND INSTRUMENTS

- HCL (Hydrochloric acid)
- NaOH (Sodium Hydroxide)
- $FeCl_3$ (Ferric Chloride)
- CCl_4 (Chloroform)
- Oral Feeding Needle
- Electronic Weighing Balance
- Stop Watch
- Elevated Plus Maze

3.4 COLLECTION AND AUTHENTIFICATION OF PLANT MATERIAL

The fruits of *Momordica dioica* used for the present studies were collected from the local market of Mangalore on June 2025. It was authenticated by Dr. Siddaraju. M. N, Assistant Professor and Research Guide, Dept. of Botany, University College Mangalore.

Preparation of Aqueous Extract of *Momordica dioica* Fruit: The fruit was shade dried and ground into a coarse powder. The powder was soaked in distilled water for 12 hours at 25 with continuous stirring. The extract was then filtered and centrifuged (10,000 rpm, 10 minutes, at room temperature) to remove any residual or fibrous material. The extract was lyophilized and referred to as AEMD (aqueous extract of Momordica dioica).[11]

Fig. No. 1: Aqueous extract of *Momordica dioica* fruit.

3.5 Preliminary Qualitative Phytochemical Analysis.[12]

Table 1: Preliminary Phytochemical analysis.

Sunoo	Phytochemicals	Test	Observation	Inference
1	Alkaloids	Wagner's Test	Reddish brown precipitate	Presence of Alkaloids
2	Alkaloids	Mayer's Test	White or creamy precipitate	Presence of Alkaloids
3	Saponins	Froth's Test	Foams formation	Presence of Saponin
4	Flavonoids	Shinoda Test	Pink colour	Presence of Flavonoids
5	Flavonoids	Sulphuric acid Test	Light yellow colour	Presence of Flavonoids
6	Carbohydrates	Fehling's Test	Formation of Brick red ppt	Presence of Carbohydrates

Fig.No:2 Phytochemical analysis of aqueous extract of *Momordica dioica* fruit.

3.6 PREPARATION OF STOCK SOLUTION OF THE EXTRACT FOR DOSING

The aqueous extract of *Momordica dioica* was weighed and dissolved in distilled water. Each time fresh preparation of the extract was prepared before administration. The extract was administered post orally at volume of 100/kg (lower dose) and 300/kg (higher dose) for test 1 and 2 animals.

Dose Selection: Dose of 100mg/kg and 300mg/kg body weight was chosen as per previous works.[13]

3.7 ANXIOLYTIC ACTIVITY

Preparation of animals

The animal chosen per the study were healthy, free from illness, injury, or disease, and were kept in their cages for a minimum of five days before dosing to ensure acclimatization to the laboratory conditions. Only animals in good health, weigh in between 22 – 30 g, were selected and maintained under standard laboratory conditions.

Preparation and administration of doses: All the doses were prepared using distilled water and given orally. In every case, the concentration was adjusted to 1ml/100g of body weight. The test substances were administered as a single dose via oral gavage following a fasting period of 3 – 4 hours.

OBSERVATIONS

Following dosing, the animals were first monitored within the initial 30 minutes and then at regular intervals during the first 24 hours. Additional assessments included absorbing changes in skin, sur, eyes, and mucous membranes, as well as evaluating respiratory, circulatory, autonomic, and central nervous system functions, along with somatomotor activity and behavioural patterns. Special attention was given to detecting signs of tremors and convulsions.

ANXIOLYTIC MODELS

Elevated Plus Maze Test.[14]

Purpose and rationale

This rodent anxiety model has been widely employed to assess new anxiolytic compounds and to explore the psychological and neurochemical mechanisms underlying anxiety.

This test is designed to specifically identify anxiolytic and anxiogenic drugs. Anxiolytic agents reduce anxiety and thereby increase the time spent exploring the open arms, whereas anxiogenic agents produce the revers effect. The main parameters recorded are the percentage of entries into the open arms and the percentage of total time spent in the open arms compare to the combined time in the both open and closed arms.

Procedure

Before beginning the experiment, the mice were handled daily to minimize the stress. Two hours after the oral administration of the test drugs, and 30 min following oral injection of diazepam, each animal was positioned at the centre of the maze, facing one of the open arms.

Subsequently, the number of entries and the duration spent in both the open and closed arms were noted over a period of 5 minutes, with and arm entry defined as the moment when all four pass of the animal are inside the arm.

Following parameters measured

1.Number of open and closed arm entries.

2. Percentage time spent in open and closed arm.Type equation here.

After each trial, the apparatus was thoroughly cleaned to remove any olfactory cues that could influence the behaviour of the subsequent animal. The experiment was preferably carried out in a sound – attenuated room, with observations recorded from and adjacent room using a web camera (CyberPix S – 300) connected to a computer system.

EXPERIMENTAL DESIGN

The Swiss albino mice (22 – 30g) of either sex was randomly divided into four groups of six animals each. The different groups were assigned as below.

Group I: Vehicle control (0.05ml/10g of normal saline) orally.

Group II: Standard group (Diazepam-1mg/kg) (p.o).[15]

Group III: Test group (MDAE) (100mg/kg, p.o).[13]

Group IV: Test group (MDAE) (300 mg/kg, p.o).[13]

TREATMENT

The treatment had been administered orally. All animals, except those receiving diazepam, were pre-treated for 20 days. On the 21st day, the treatments were given 30 minutes before the evaluation.

Evaluation

The above – mentioned parameters of the standard and test compounds had been carefully evaluated and compared to determine the anxiolytic activity of the test compound.

Statistical Analysis

The results were expressed as Mean ± SEM. Multiple comparisons were analysed using one way ANOVA, followed by multiple comparison tests. A "P" value of 0.05 or lower was considered statistically significant for all analyses.

ANOVA (Analysis of variance)

In statistics, analysis of variance (ANOVA) refers to a set of statistical models and related procedures that divide the observed variance into components attributed to different explanatory factors. In its basic form, ANOVA provides a statistical method to determine whether the means of multiple groups are equal, thereby extending Dunnett's multiple comparison test to situations involving more than two groups.

4. RESULTS

Preliminary phytochemical screening

Results of preliminary phytochemical investigation of aqueous extract of MDA extract are shown in table 2.

Table 2: Preliminary phytochemical screening of MDAE.[16]

Sl. No	Test	Result
1.	Alkaloids	+ve
2.	Saponins	+ve
3.	Flavonoids	+ve
4.	Glycosides	+ve
5.	Carbohydrates	+ve
6.	Steroids	-ve

Screening of MDAE for its anxiolytic activity

In present study, anxiolytic model namely Elevated plus maze model were employed.

Elevated plus maze model: Elevated plus maze (EPM) has been suggested as a model for evaluating anxiolytic activity. It is specifically designed to identify anxiolytic drugs, as these compounds reduce anxiety and promote increased exploration time in the open arms.

Group No	Drug Treatment	Dose	Number of entries (mean ± SEM)		Time spent in sec. (mean ± SEM)	
			Open arm	Closed arm	Open arm	Closed arm
1	Control	1ml/kg	3.500±0.428	18.667±0.494	87.667±4.169	172.667±4.624
2	Standard (Diazepam)	1mg/kg	19.333±0.494***	6.500±0.428***	204.167±4.527***	56.000±4.000***
3	Test 1 (Lower dose)	100mg/kg	10.333±0.715**	13.000±0.365**	134.667±2.679**	129.167±3.859**
4	Test 2 (Higher dose)	300mg/kg	17.500±0.428***	8.000±0.365***	195.333±2.777***	65.500±5.220***

All the results are expressed in terms of Mean± SEM, n=6 animals in each groups; Statistical Significance was determined by ANOVA followed by Dunnett's test. **p<0.01, ***p<0.001 statistically significant compare to control group.

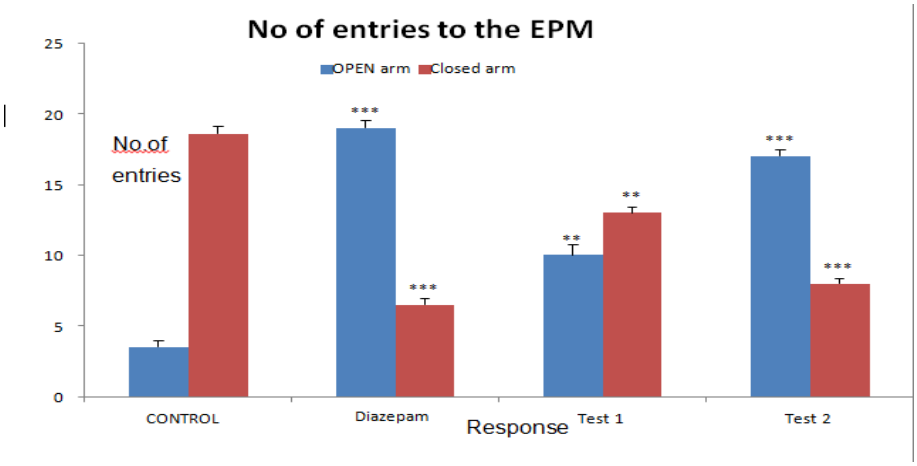

Fig.No:3 Comparative profile of No. of entries in open and closed arm in EPM after oral administration of 100mg/kg and 300mg/kg of MDAE.

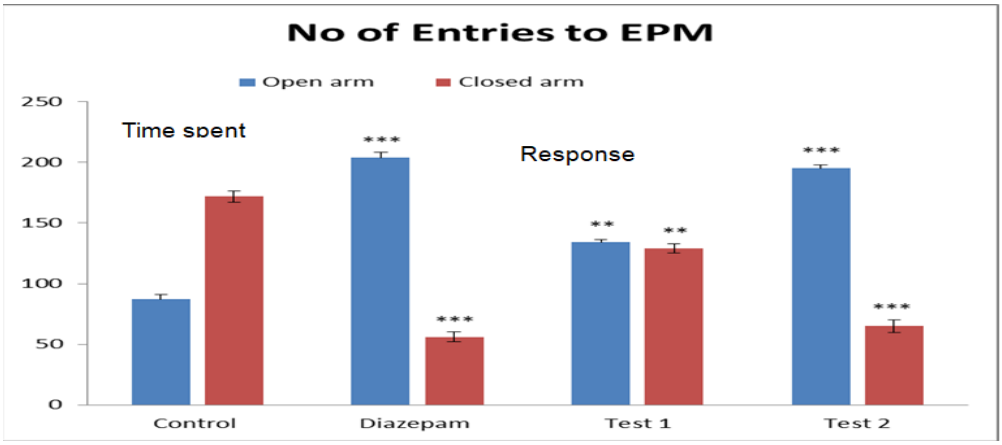

Fig.No:4 Comparative profile of time spent in open and closed arm in EPM after oral administration of 100mg/kg and 300mg/kg of MDAE.

DISCUSSION

This study aimed to assess the anxiolytic activity of MDAE using established anxiolytic models. Anxiety is an adoptive response that occurs when an individual faces danger or treat, with accompanying behavioural and physiological changes preparing individual to respond effectively. Among the most commonly used animal models for evaluating potential anxiolytic agents is the elevated plus maze.[17]

Elevated plus maze (EPM) is regarded as an etiologically valid animal model for anxiety, as it incorporates natural fear inducing stimuli, including the apprehension of a new, brightly lit open space and the challenge of balancing on a narrow-elevated platform. It is well established that anxiolytic agents enhance both the frequency of entries into, and the time spent in, the open arms of the EPM.[18]

In the EPM test, animal administered a single dose of MDAE at 100mg/kg and 300mg/kg exhibited a highly significant increase in the number of open arm entries compare to the control group. Additionally, a moderately significant reduction in closed arm entries was observed in comparison to the control.

The effects of MDAE on the EPM test, were almost equivalent to that of 1mg/kg diazepam. In the present study, the anxiolytic activity of the Momordica dioica aqueous extract was observed at dose of 100mg/kg and 300mg/kg in mice. These observations clearly indicate that Momordica dioica exerts an anxiolytic activity.

Mechanism of action by which MDAE shows anxiolytic activity may be similar to that of diazepam [that acts via the gamma - aminobutyric acid (GABA)a receptor complex]. MDAE exerts anxiolytic activity due the presence of flavonoids.[19] We observed that following oral administration of MDAE demonstrated significant (compare to control treated group) results which indicate it as a significant anxiolytic effect in all these paradigms.

Previous studies on the chemical component and pharmacological properties of plants indicate that those containing alkaloids, flavonoids, carbohydrates, glycosides, and tannins may exhibit activity against various central nervous system (CNS) disorder.

The anxiolytic effect of MDAE may attributed to the combined action of its phytochemical constituents. Findings from this study indicate that the aqueous extract of Momordica dioica exhibit anxiolytic activity. Therefore, MDAE holds promise for potential clinical use in

treating anxiety disorders. However, additional research is needed to identify the precise mechanism involved and to isolate the active compounds responsible.

CONCLUSION

In conclusion, the findings of the current study demonstrate that the extract of Momordica dioica exhibits a significant anxiolytic effect in Swiss albino mice, as evidenced by results from the elevated plus maze model.

The results obtained were both satisfactory and conclusive, successfully meeting the objectives of the study. In summary, the current findings suggest that administration of MDAE to mice produced anxiolytic effects, thereby supporting traditional claims about its anxiolytic potential. Preliminary phytochemical analysis of the MDAE extract confirmed the presence of various chemical constituents, including flavonoids, glycosides, alkaloids, carbohydrates, saponins, and tannins.

Although the precise mechanism responsible for the anxiolytic activity is not yet fully understood, it is likely associated with the active compounds present in the extract. Therefore, additional research is required to determine the specific chemical constituent contributing to the observed anxiolytic effects as the exact component responsible have yet to be identified.

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