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ANXIOLYTIC ACTIVITY OF ALCOHOLIC EXTRACT OF *MATRICARIA CHAMOMILLA* FLOWERS IN MICE

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ABSTRACT

Matricaria chamomilla (family Asteraceae) is a well-known medicinal herb traditionally valued for its calming and anxiety-relieving properties, attributed to bioactive constituents such as flavonoids, terpenoids and apigenin present in its flowers. The present study evaluated the anxiolytic potential of an alcoholic extract of the flowers of this plant in mice. The extract was prepared by hot continuous (Soxhlet) extraction of the dried flowers with ethanol. Acute oral toxicity, assessed up to a limit dose of 2000 mg per kilogram body weight, produced no mortality or abnormal behaviour, and two doses (100 and 400 mg per kilogram, orally) were accordingly selected for the pharmacological study, with diazepam (2 mg per kilogram) used as the reference standard. Anxiolytic activity was assessed using the staircase test and the elevated plus maze test in mice after seven days of treatment. In the staircase test, both doses of the extract significantly increased the number of steps climbed compared with control. In the elevated plus maze, the extract produced a significant increase in the number of entries into, and time spent in, the open arms, with a corresponding decrease in the closed-arm parameters, comparable to diazepam. Preliminary phytochemical screening of the extract indicated the presence of carbohydrates, flavonoids and saponins. These findings suggest that the alcoholic flower extract of *Matricaria chamomilla* possesses significant anxiolytic activity in mice, supporting its traditional use as a calming agent.

KEYWORDS: *Matricaria chamomilla*; Anxiolytic activity; Elevated plus maze; Staircase test; Flavonoids; Mice.

INTRODUCTION

Anxiety is a prolonged state of tension characterised by worry, nervousness and apprehension regarding uncertain, potentially negative future events,[1] and anxiety disorders are among the most prevalent categories of mental illness, with a substantial lifetime prevalence and considerable impact on quality of life, productivity and comorbidity.[2,3] Common presentations include generalised anxiety disorder, panic disorder, phobias, obsessive-compulsive disorder and post-traumatic stress disorder.[4] The neurobiology of anxiety involves dysregulation of gamma-aminobutyric acid (GABA), serotonin and noradrenaline;[5] benzodiazepines act via the GABA-A receptor complex,[6] but their long-term use is limited by sedation, tolerance and dependence, sustaining interest in safer, plant-derived alternatives.[7,8]

Matricaria chamomilla L. (Asteraceae), or German chamomile, has a long history of traditional use as a mild sedative. Apigenin, a flavonoid in its flowers, binds competitively to the central benzodiazepine receptor and produces anxiolytic activity without sedation at low doses;[9] the flowers also contain terpenoids, coumarins and flavonoid glycosides and possess anti-inflammatory, antioxidant, antimicrobial and gastroprotective activity.[10,11,12] However, systematic evaluation of the anxiolytic activity and acute toxicity of the flower extract remains limited. The present study therefore evaluated the anxiolytic activity of an alcoholic extract of *Matricaria chamomilla* flowers (ALEMC) in mice using the staircase test[13] and elevated plus maze test,[14] and correlated this activity with the extract's preliminary phytochemical profile.

MATERIALS AND METHODS

Plant material and extraction

The flowers of *Matricaria chamomilla* were collected during April-May from in and around Raichur, Karnataka, India, and authenticated by Dr. Pramod Kumar, Department of Pharmacognosy, V. L. College of Pharmacy, Raichur. The dried flowers were coarsely powdered and extracted with ethanol (Nice Chemicals, Cochin, India) by hot continuous (Soxhlet) extraction at room temperature (25 ± 2°C) for 10 days. The concentrated alcoholic extract of *Matricaria chamomilla* (ALEMC) was stored at 4°C, and a 2% w/v suspension in

gum acacia was freshly prepared for oral dosing; diazepam (Ranbaxy Laboratories Ltd., Mumbai, India) served as the reference standard.

Animals and ethical approval

Healthy albino mice of either sex (20-25 g; Kedhar Bio Labs, Mahabubnagar) were acclimatised for 7 days under standard husbandry conditions (26 ± 2°C; 45-55% relative humidity; 12 h light/dark cycle) with free access to food and water *ad libitum*, and fasted overnight before dosing. The protocol was approved by the Institutional Animal Ethics Committee, V. L. College of Pharmacy, Raichur (Reg. No. 557/PO/Re/S/02/CPCSEA; approved 20 March 2023), in accordance with CPCSEA guidelines.

Acute toxicity, phytochemical screening.

Acute oral toxicity was determined by the fixed-dose procedure as per OECD Guideline No. 420[15] up to 2000 mg/kg; as no toxicity was observed, doses of 100 mg/kg (1/20th) and 400 mg/kg (1/5th) were selected for the pharmacological study. ALEMC was screened for carbohydrates, saponins, tannins and flavonoids by standard qualitative tests (Table 1).

Experimental design

Mice were divided into four groups of six (Group I: 2% gum acacia; Group II: diazepam 2 mg/kg; Group III: ALEMC 100 mg/kg; Group IV: ALEMC 400 mg/kg, all p.o.), dosed once daily for 7 days and tested one hour after dosing on day 8th.

Staircase test

Anxiolytic activity was assessed on a staircase apparatus (five steps, 2.5 × 10 × 7.5 cm) as described by Simiand et al.[13] Each mouse, used once, was placed with its back to the staircase, and the number of steps climbed (all four paws on a step) and rearings were recorded over 3 minutes.

Elevated plus maze test

The maze, comprising two open arms (16 × 5 cm) and two enclosed arms (16 × 5 × 12 cm) around a central platform (5 × 5 cm) elevated 25 cm, was used as described by Lister.[14] Each mouse was placed centrally facing an open arm, and entries into, and time spent in, the open arms, closed arms and central platform were recorded over 5 minutes.

Statistical analysis

Data (Mean ± SEM, n = 6) were analysed by one-way ANOVA followed by Dunnett's multiple comparison test; $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Results

Phytochemical screening and acute toxicity

ALEMC gave positive tests for carbohydrates (Molisch's, Benedict's, Fehling's) and saponins (foam test); the Shinoda test for flavonoids was positive, but the ammonia and vanillin-hydrochloric acid tests were negative (suggesting flavonols/flavones rather than catechin-type flavonoids), and the ferric chloride test for tannins was negative (Table 1). No mortality or behavioural abnormality was observed up to 2000 mg/kg, confirming a wide margin of safety.

Staircase test

ALEMC produced a dose-related increase in steps climbed, significant at 100 mg/kg (21.33 ± 2.10, $p < 0.05$) and with diazepam (25.17 ± 2.00, $p < 0.001$) versus control (14.17 ± 1.80), without significantly altering rearings (Table 2, Fig. 1).

Elevated plus maze

Both doses of ALEMC significantly increased open-arm entries and open-arm time while decreasing closed-arm entries and closed-arm time relative to control, comparable to diazepam ($p < 0.001$ for all comparisons); central-platform time increased significantly with ALEMC (128.50 ± 1.28 and 135.67 ± 3.02 s at 100 and 400 mg/kg) but changed little with diazepam (95.33 ± 1.54 s) relative to control (98.50 ± 1.91 s) (Table 3, Fig. 2).

Table 1: Preliminary Phytochemical Screening of Alcoholic Extract of *Matricaria Chamomilla* Flowers (ALEMC).

S. No.	Phytoconstituent	Test	Inference
1	Carbohydrates	Molisch's test	+
2	Carbohydrates	Barfoed's test	–
3	Carbohydrates	Benedict's test	+
4	Carbohydrates	Fehling's test	+
5	Saponins	Foam test	+
6	Tannins	Ferric chloride test	–
7	Flavonoids	Shinoda test	+
8	Flavonoids	Ammonia test	–
9	Flavonoids	Vanillin-hydrochloric acid test	–

+ = present; – = absent.

Table 2: Effect of ALEMC on Number of Steps Climbed and Rearings in the Staircase Test in Mice (mean ± SEM, n = 6).

Treatment	Dose	Steps climbed (3 min)	No. of rearings (3 min)
Normal control	2% gum acacia, p.o.	14.17 ± 1.80	2.67 ± 0.80
Diazepam	2 mg/kg, p.o.	25.17 ± 2.00***	2.50 ± 1.43 ns
ALEMC	100 mg/kg, p.o.	21.33 ± 2.10*	2.33 ± 0.92 ns
ALEMC	400 mg/kg, p.o.	19.00 ± 0.86 ns	2.17 ± 0.54 ns

*Values are mean ± SEM, n = 6 animals per group. *p<0.05, ***p<0.001 vs. normal control (one-way ANOVA followed by Dunnett's test); ns = not significant. ALEMC = alcoholic extract of Matricaria chamomilla flowers.*

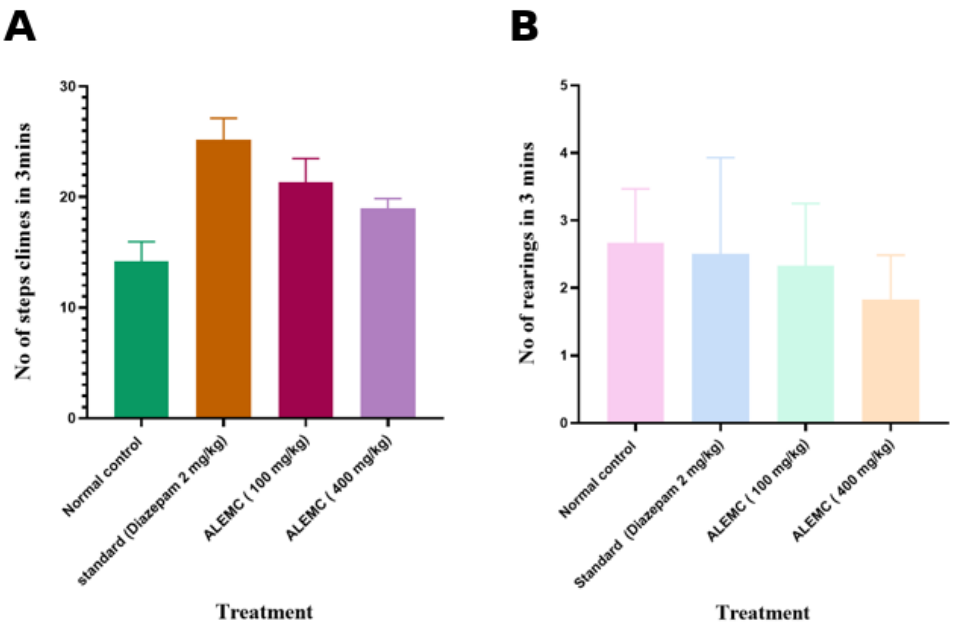

Fig. 1: Effect of ALEMC on the Staircase Test in Mice: (A) Number of Steps Climbed; (B) Number of Rearings (mean ± SEM).

Table 3: Effect of ALEMC on Number of Entries and Time Spent in the Elevated Plus Maze Test in Mice (mean ± SEM, n = 6).

Treatment	No. of entries (5 min)		Time spent, seconds (5 min)		
	Open arm	Closed arm	Open arm	Closed arm	Central platform
Normal control (2% gum acacia)	6.83 ± 0.48	16.33 ± 0.76	36.67 ± 1.17	161.83 ± 1.85	98.50 ± 1.91
Diazepam (2 mg/kg)	15.00 ± 0.73***	12.17 ± 0.70***	127.00 ± 1.80***	88.83 ± 2.24***	95.33 ± 1.54***

ALEMC (100 mg/kg)	13.00 ± 0.57***	9.33 ± 0.55***	97.67 ± 1.62***	48.67 ± 2.01***	128.50 ± 1.28***
ALEMC (400 mg/kg)	15.00 ± 0.57***	9.83 ± 0.60***	124.20 ± 2.60***	69.50 ± 1.62***	135.67 ± 3.02***

*Values are mean ± SEM, n = 6 animals per group. ***p<0.001 vs. normal control (one-way ANOVA followed by Dunnett's test). ALEMC = Ajlcoholic extract of Matricaria chamomilla flowers.*

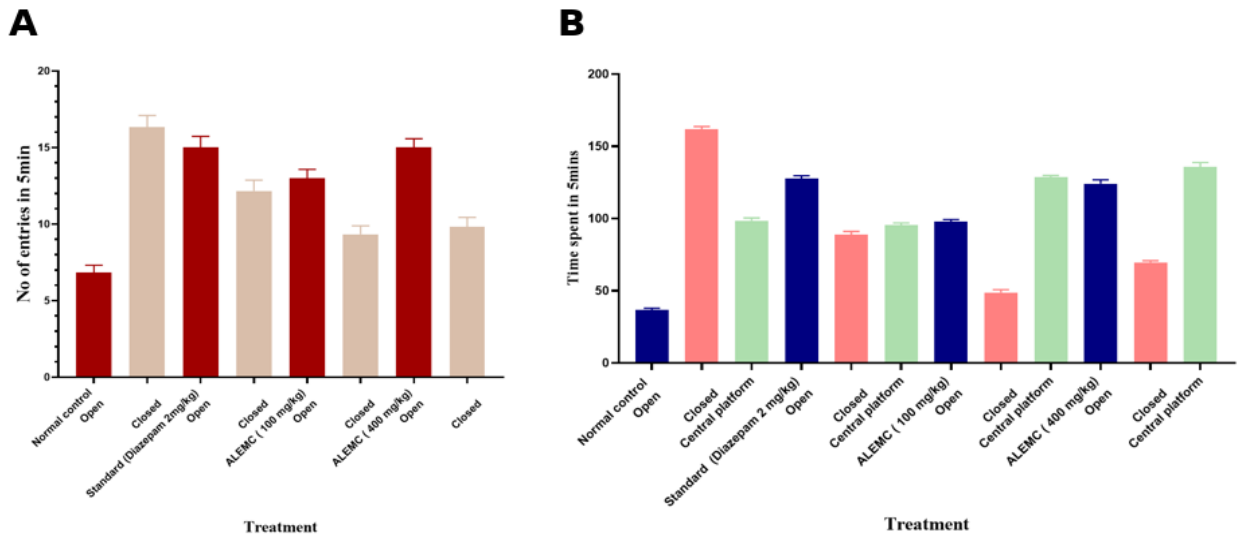

Fig. 2: Effect of ALEMC on the Elevated Plus Maze Test in Mice: (A) Entries into Open and Closed Arms; (B) Time Spent in Open Arm, Closed Arm and Central Platform (mean ± SEM).

DISCUSSION

The staircase test reflects exploratory activity (steps climbed) and anxiety-related rearing.[13] ALEMC increased step-climbing dose-dependently, comparable to diazepam, without significantly affecting rearing. In the elevated plus maze, increased open-arm exploration and reduced closed-arm activity indicate anxiolysis mediated via the GABA-A-benzodiazepine receptor complex;[14] ALEMC produced this pattern at both doses, with the lower dose (100 mg/kg) closer to diazepam, suggesting the effect is not strictly dose-dependent.

Flavonoids such as apigenin are well documented to interact with the central benzodiazepine receptor[9] and are the most plausible contributors to the anxiolytic activity of ALEMC observed here, with saponins possibly contributing additively via GABAergic modulation. Together with the absence of toxicity up to 2000 mg/kg, these findings support the traditional use of *Matricaria chamomilla* as a calming agent and warrant further mechanistic study.

CONCLUSION

ALEMC was well tolerated in mice up to 2000 mg/kg and produced significant anxiolytic activity in both the staircase and elevated plus maze tests, near similar and comparable in several respects to diazepam. Preliminary phytochemical screening revealed carbohydrates, saponins and flavonoids, which may collectively underlie this activity.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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