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Original Research Article

Noni (*Morinda citrifolia* Linn.) Fruit Extract Protects Against Non-precipitated Alcohol Withdrawal Symptoms in Mice

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Abstract

Background: Noni (*Morinda citrifolia* Linn.) fruit has a long history of traditional use for treating several health conditions, including symptoms of major depression and anxiety.
Objectives: This study focused on evaluating the therapeutic potential of the methanolic extract of *Morinda citrifolia* Linn. fruit (MMC) and its ethyl acetate fraction (EAMMC) against alcohol withdrawal-induced symptoms like convulsion, anxiety, and anhedonia in mice using handling-induced convulsion (HIC), elevated plus maze (EPM), open field test (OFT), marble burying test (MBT), and sucrose preference test (SPT), respectively.
Materials and Methods: Swiss albino mice (male) weighing 30–35 g were used. Animals were grouped as saline withdrawn (SW) control alcohol withdrawn (AW), control, positive control (AW + diazepam 1 mg/kg i.p.), and MMC- and EAMMC-treated groups (AW + MMC 500 and 1,000; AW + 50 and 100 mg/kg p.o.), respectively. All test groups intraperitoneally received 1.25 mL/100 g b.w. of (20% v/v ethanol + 0.1% v/v 4-methylpyrazole) except the SW group, which received saline (1.25 mL/100 g, i.p.) instead of ethanol twice a day for 3 days. HIC, OFT, EPM, MBT, and SPT were performed subsequently.
Results: These results reveal that MMC (500 and 1,000 mg/kg p.o.) and EAMMC (50 and 100 mg/kg p.o.) attenuated the handling-induced seizures (HIS), anxiety, and anhedonia in AW mice by facilitating the benzodiazepine-$GABA_A$ergic and serotonergic (5-HT_{1A}) mediated mechanisms in the brain.
Conclusion: MMC and EAMMC protect against alcohol withdrawal symptoms such as handling seizures, anxiety, and anhedonia in mice.

Keywords

Alcohol withdrawal symptoms, *Morinda citrifolia*, handling-induced seizures, elevated plus maze, open field test, marble burying test, sucrose preference test, mice,

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Introduction

Alcohol withdrawal symptoms occur when someone who regularly drinks heavily suddenly stops. Adults are more commonly affected, and symptoms can range from moderate to severe, especially in those with certain medical conditions. Common signs include headache, anxiety, trembling, insomnia, mood swings, hallucinations, and delusions.[1] Gamma-aminobutyric acid (GABA) and glutamate are two key neurotransmitters that have been linked to alcohol withdrawal symptoms.[2] Alcohol disrupts the brain's neurochemical balance by impairing *N*-methyl-D-aspartate (NMDA) receptors, affecting memory, and inducing sedation. During alcohol withdrawal, glutamate release increases, activating NMDA receptors while GABA levels decrease, leading to a hyperactive central nervous system.[3] First-line treatment for alcohol

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withdrawal symptoms includes benzodiazepines (BZD) like chlordiazepoxide, diazepam, lorazepam, and oxazepam. These medications act on $GABA_A$ receptors in the brain to alleviate symptoms. However, their use can lead to dependence, which limits their long-term application in treating alcohol withdrawal.[3] This is a pressing factor that directs researchers to discover a novel drug for treating alcohol withdrawal.

The ethnopharmacological approach to drug discovery remains vibrant, with bioactive compounds from plants being an important source of therapeutic agents.[4] *Morinda citrifolia* Linn., commonly known as Noni, is part of the coffee family and is prevalent in the Asian Pacific islands.[5] This evergreen tree or shrub has been traditionally used in folk medicine to address various health concerns such as atherosclerosis, arthritis, diabetes, and addiction.[6–8] Scientific evidence supports its neuropharmacological activities, including anti-psychotic, anxiolytic, memory-enhancing, and anti-depressant effects.[9,10] Notably, Noni fruit extract shows anti-craving properties against substances like alcohol and heroin and has demonstrated anxiolytic and anti-depressant activities through BZD-$GABA_A$ergic and serotonergic mechanisms.[11] *In vitro* studies suggest that its methanolic extract exhibits significant anti-anxiety effects by acting as an agonist at $GABA_A$ receptors.[12] Rats given Noni juice for 15 days showed an anxiolytic effect in the elevated plus maze (EPM) test. *In vitro* studies have revealed that the ethyl acetate extract of Noni fruit inhibited monoamine oxidase enzymes A (MAO-A) by 78% and MAO-B by 49%.[12–14] Rutin and scopoletin are the main bioactive components in the methanolic extract of *M. citrifolia* Linn. fruit, responsible for its therapeutic activities. Previous studies estimated their quantities in the extract at 18.95 mg/mg for rutin and 1.66 mg/mg for scopoletin.[15]

Previous studies on *M. citrifolia* Linn. fruit's ethanolic extract have found high levels of rutin, scopoletin, and quercetin in the ethyl acetate fraction.[16] This study aims to explore the therapeutic potential of Noni fruit extract (MMC) and its fraction (EAMMC) against alcohol withdrawal symptoms, including seizures, anxiety, and anhedonia, in mice using various behavioral models.

Materials and Methods

Drugs

The MMC (*M. citrifolia* Linn. fruit's standardized methanolic extract) was obtained from Sunpure Extracts Pvt. Ltd., New Delhi. Sodium carboxymethyl cellulose (CMC) and fomepizole were obtained from Sigma–Aldrich, St. Louis, MO. Ethanol 20% v/v was prepared using normal saline from absolute ethanol (Changshu Hongsheng Fine Chemicals Company Ltd., Jiangsu Province, China), with the addition of 4-methylpyrazole (fomepizole) 0.1% v/v. Diazepam, in the form of injection (LORI®; Neon Laboratories Ltd., India), was used as a reference drug.

Preparation of the Ethyl Acetate Fraction of the Methanolic Extract of Morinda citrifolia Linn. Fruits (EAMMC)

A 250 mL separating funnel was used to process 5 g of methanolic extract of Noni fruit (MMC) with 100 mL of water and 100 mL of ethyl acetate. The funnel was gently shaken for 2 h and left undisturbed for another 2 h to separate the layers. The organic and aqueous layers were collected into separate beakers. An additional 100 mL of the aqueous layer was mixed with ethyl acetate, agitated, and left to separate for 45 min. The organic layer was then evaporated using a rotary evaporator at 100 pascals, 50°C, and 200 rpm for 3 h. The semisolid EAMMC was collected in a Petri dish and air-dried for 3 days, resulting in a yield of 62.6% w/w (3.13 g from 5 g).

Phytochemical Screening

Phytochemical screening for secondary metabolite identification was conducted using standard methods. A 1 mg/mL EAMMC solution was prepared by dissolving 10 mg of EAMMC in methanol and adjusting it to 10 mL. Key tests included: Wagner's test for alkaloids, Biuret test for proteins and amino acids, Liebermann–Burchard test for steroids, Foam test for saponins, Shinoda test for flavonoids, Salkowski test for terpenoids, Sudan red III test for fixed and volatile oils, and ferric chloride test for tannins.[17]

Thin-layer Chromatography (TLC)

A standard TLC protocol was followed,[18] using EAMMC (1 mg/mL) and rutin hydrate (0.25 mg/mL) samples in methanol. Methanol served as the mobile phase, with visualization achieved using various spraying agents: (a) dilute NaOH (yellow spot); (b) zinc chloride in HCl (red/orange spot); (c) 10% NaCl solution (yellow spot). The retardation factor (R_f) was calculated as the distance traveled by the solute divided by the distance traveled by the solvent front.[18]

High-performance Thin-layer Chromatography (HPTLC)

The HPTLC methodology is a flexible and automated form of TLC that improves separation efficiency and detection limits, often serving as an alternative to gas chromatography (GC) and high-performance liquid chromatography (HPLC).[19] In this study, HPTLC was used to identify flavonoids in EAMMC, with rutin hydrate as the reference drug. The Elite Luminous AETRON instrument employed a mobile phase of toluene, ethyl acetate, and formic acid in a 5:4:1 ratio. The chromatogram was developed for 15 min, dried at room temperature, and scanned at 257 and 359 nm.

Animals

Male Swiss albino mice (25–30 g) were housed in groups of six in polycarbonate cages under standard laboratory conditions (temperature 24°C ± 1°C, 12-h light–dark cycle). They were acclimatized for 1 week before the experiment and provided with adequate food and water. Proper animal care was ensured to minimize stress, with environmental enrichment included. The experimental protocol received approval from the Institutional Animal Ethics Committee (approval no. 04/IAEC/CLPT/2020-21 and 16/IAEC/CLPT/2020-2021), and animal well-being followed the Committee for the Control and Supervision of Experiments on Animals (CCSEA) guidelines in India.

Preparation of Drug Solutions

20 mL of ethanol (99.9% v/v) was mixed with 79.9 mL of normal saline and 0.1 mL of fomepizole, then administered intraperitoneally at a dose of 1.25 mL/100 g b.w.[20] MMC/EAMMC was prepared in a 1% w/v CMC solution and given orally. Diazepam was diluted to 0.1 mg/mL in normal saline and injected intraperitoneally at 1 mL per 100 g b.w.[20]

MMC Study

Five groups of animals were segregated (six mice/group): Group I served as saline withdrawn (SW); Group II served as alcohol withdrawn (AW); Group III served as standard (AW + diazepam 1 mg/kg i.p.); Groups IV and V served as test groups (AW + MMC 500 mg/kg p.o.) and (AW + MMC 1,000 mg/kg p.o.), respectively.

Procedure

All test groups received a 20% v/v ethanol solution (1.25 mL/100 g, i.p.), and the SW group received saline (1.25 mL/100 g, i.p.) twice daily for 3 days. On day four, Groups I and II were given CMC (10 mL/kg, p.o.), Group III received diazepam (1 mg/kg, i.p.), Group IV got MMC (500 mg/kg, p.o.), and Group V received MMC (1,000 mg/kg, p.o.). AW symptoms were assessed 14 h later using tests such as handling-induced convulsions (HICs), EPM, open field test (OFT), and marble burying test (MBT). A 24-h sucrose preference test (SPT) was followed, with test drugs administered before and 12 h after SPT initiation. Sucrose intake was measured 24 h after the SPT started.

EAMMC Study

The EAMMC experimental study consisted of two parts: (a) EAMMC's effect on animal behavior in SW mice, and (b) its effect on animal behavior in AW mice. In the SW study, 24 mice were divided into four groups (six per group). The groups received: (a) oral CMC solution (1% w/v, control), (b) intraperitoneal diazepam (1 mg/kg, reference), and (c and d) oral EAMMC at doses of 50 and 100 mg/kg. All groups received intraperitoneal normal saline (12.5 mL/kg) twice daily for 3 days. On the fourth day, the treatment was administered according to their group. Anxiolytic activity was evaluated using anxiety models: the OFT and the EPM after 30 min to 1-h post-administration.

In the AW behavioral studies, 30 mice were divided into five groups (six mice per group): SW control (Group I), AW control (Group II), 1 mg/kg diazepam (i.p.) + AW (Group III), EAMMC test-1 (50 mg/kg) + AW (Group IV), and EAMMC test-2 (100 mg/kg) + AW (Group V). All groups, except the saline group, received a 20% v/v ethanol solution intraperitoneally for 3 days, while the saline group received saline injections instead. The ethanol solution was prepared from 99.9% absolute ethanol with the alcohol dehydrogenase inhibitor 4-methylpyrazole (0.1% v/v).[20] On the fourth day, we administered 1% w/v CMC for the SW and ethanol-withdrawn control groups; EAMMC at oral doses of 50 and 100 mg/kg for the test groups; and at a dose of (1 mg/kg, i.p.) diazepam for the standard group after 1 h or 30 min of oral or intraperitoneal administration of drugs. A battery of animal behavioral alcohol withdrawal studies, such as OFT, EPM, MBT, and finally, SPT in mice, was carried out.

HICs

The mouse was removed from its cage and examined for convulsions. It was gently rotated for 5 s. Convulsions were scored as follows: Score 0: Motionless with excitement and rapid breathing; Score 1: Vibrissae movements, salivation, and mouth movements; Score 2: Clonus (head and eyes); Score 3: Wet dog shakes and forelimb clonus; Score 4: Clonic rearing; Score 5: Loss of postural control and uncontrollable jumping with clonus.[20]

OFT

This experiment investigates the fear and anxiety behaviors of rodents in an OFT. The OFT apparatus has a central compartment of 9 squares and a peripheral compartment of 16 squares, each measuring 10 × 10 cm. Each rodent is placed in a corner of the peripheral compartment for 5 min, with the floor cleaned with 10% v/v ethanol to eliminate olfactory cues. A Logitech HD webcam records the behavior for later analysis by a blinded observer. Key measurements include the time spent in, and the number of entries to, both the central and peripheral areas. The drug's anxiolytic effect is indicated by an increased entries and the time in the central zone.[21]

EPM Test

The EPM in mice is a validated model for studying pharmacological substances with anxiolytic properties.[11] The

EPM consists of two opposing open arms (30 × 7.5 cm) and two enclosed arms (30 × 7.5 × 30 cm), connected to a central platform (7.5 × 7.5 cm) raised 40 cm from the ground, made from black-painted Plexiglas. Mice are placed in the center facing an open arm, and entries and time spent in both open and closed arms are recorded over 5 min. Ethanol (10% v/v) wipes the maze between trials. An entry is defined by the mouse crossing its four paws over the line between an arm and the center. Anxiolytic drugs typically increase both the number of entries into the open arms and the time spent there, whereas entries into the closed arms primarily reflect general locomotor activity.[11]

MBT

The MBT measures anxiety in rodents by observing their response to a new object.[22] Anxiolytic drugs typically reduce the number of marbles buried. The MBT apparatus, measuring 17.5 × 10 × 5.5 inches, contained 5 cm of husk bedding and a 9 × 3 grid of twenty-seven 1.4 cm dark glass marbles. Mice were allowed to explore for 5 min before being removed. Successful burying was defined as marbles covered to a depth of two-thirds.[22]

SPT

After MBT, animals underwent a 24-h SPT, receiving test drugs just before the procedure. Two bottles were placed in the home cage, one with water and one with a freshly prepared 1% w/v sucrose solution. After 12 h, the test drugs were administered again, and the positions of the bottles were swapped.[23] Food was withheld during the SPT, and consumption of sucrose and water was measured in mL/kg body weight.[23] The sucrose consumption ratio was calculated using the specified formula.

$$\text{Sucrose consumption ratio} = \frac{\text{Sucrose intake/kg body weight}}{\text{Water intake/kg body weight} + \text{Sucrose intake/kg body}}$$

Statistical Analysis

The experimental data are represented as means ± standard error of the mean (SEM). GraphPad Prism 6 was used to assess the statistical analysis. The data were analyzed by one-way analysis of variance (ANOVA) with a *post hoc* Sidak's multiple comparison test. A probability value of <.05 was considered significant.

Results and Discussion

MMC Study

Handling-induced Convulsions Test

One-way ANOVA with *post hoc* Sidak's multiple comparison test revealed significant differences between the different treatment groups [F (4,25) = 11.85; $p < .0001$]. The *post hoc* multiple comparison test demonstrated a significant increase in the convulsion score of AW mice when compared with SW. MMC (1,000 mg/kg, p.o.) and diazepam (1 mg/kg, p.o.) markedly decreased the convulsion score in AW animals. The graphical presentation of the results of HIC is depicted in Figure 1A.

OFT

A one-way ANOVA with Sidak's multiple-comparison *post hoc* test on the number of line crossings in the central compartment showed no significant differences between the treatment groups, F (4, 24) = 2.185, p = .1012. The graphical findings of the OFT are displayed in Figure 1B.

EPM

One-way ANOVA with Sidak's multiple comparison *post hoc* test revealed significant differences among the different drug-treated groups on % time spent [F (4,24) = 6.702; p = .0009], respectively. The graphical results of EPM are depicted in Figure 1C.

MBT

One-way ANOVA with Sidak's multiple comparison *post hoc* test revealed significant differences among the different drug-treated groups [F (4,24) = 15.90; $p < .0001$]. The graphical representation of the results of MBT is shown in Figure 1D.

SPT

The sucrose consumption ratio was drastically decreased in the AW group when compared to the SW group, which indicates the state of anhedonia in AW mice. Interestingly, MMC at both doses (500 and 1,000 mg/kg, p.o.) normalized the sucrose consumption ratio in AW mice, as observed with diazepam (1 mg/kg, i.p.), the reference drug (Figure 1E).

EAMMC Study

EAMMC Phytochemical Analysis

Phytochemical components such as flavonoids, phenolics, alkaloids, glycosides, sugars, fixed oils, tannins, terpenoids, steroids, and saponins were detected in the EAMMC plant extract using standard phytochemical identification tests.

TLC

The values for the R_f reference standard, rutin, and EAMMC were determined using different spraying agents as 0.65 and 0.67 (using dilute NaOH); 0.66 and 0.69 (ZnCl + HCl); 0.61 and 0.60 (10% aqueous NaCl), respectively.

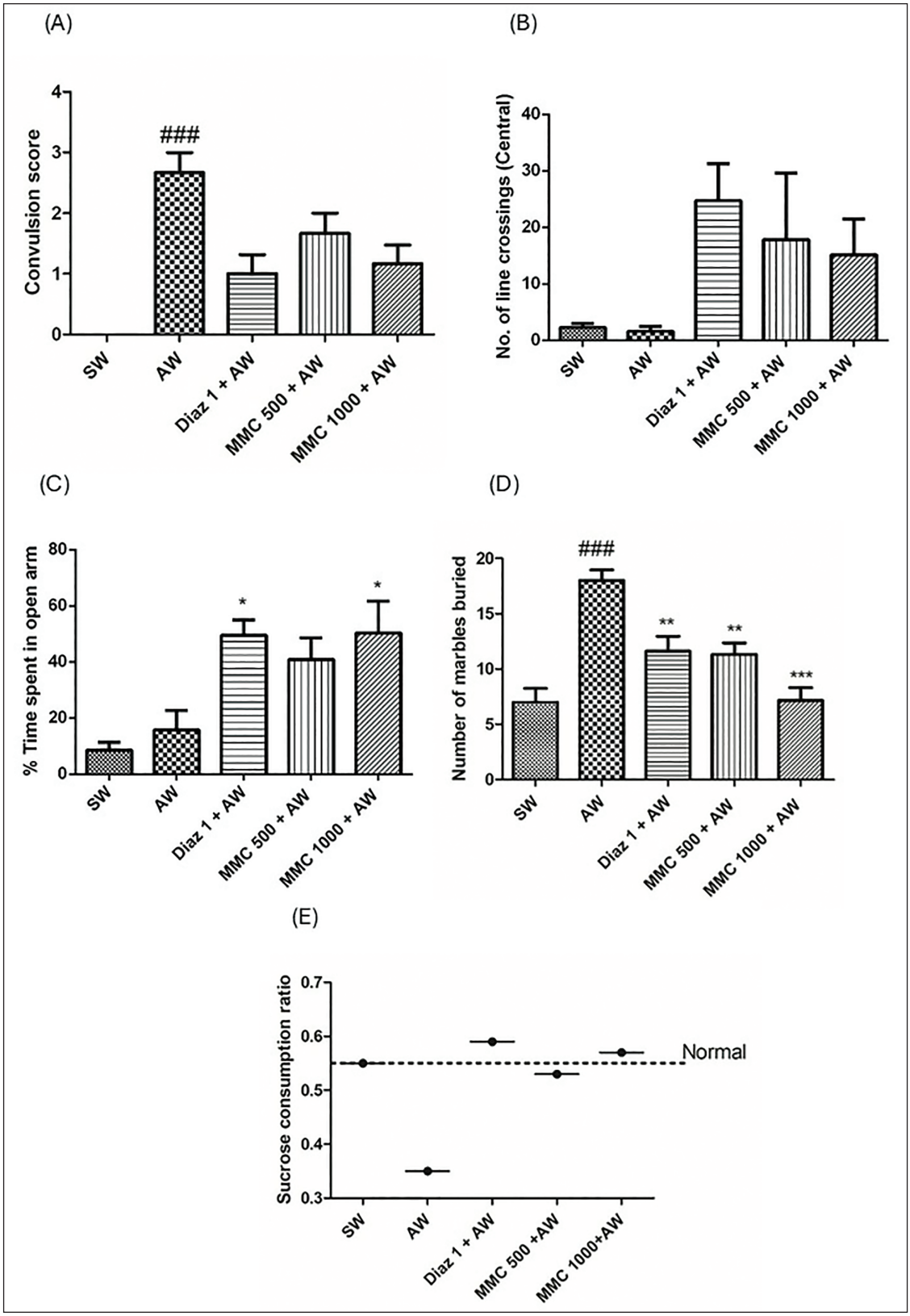


Figure 1. Effect of *Morinda citrifolia* Linn. Fruit Extract (MMC) (500 and 1,000 mg/kg p.o.) on Alcohol Withdrawn (AW)-induced (A) Handling Seizures. (B) Anxiety in Open Field Test. (C) Anxiety in Elevated Plus Maze Test. (D) Anxiety in Marble Burying Test. (E) Anhedonia in Sucrose Preference Test in Mice.

Note: All data expressed as mean ± standard error of the mean (SEM) (n = 5–6); ###p < .001 when compared with the saline withdrawn (SW) group; ***p < .001, **p < .01, and *p < .05 when compared with the AW group.

HPTLC

The flavonoid rutin was detected in EAMMC using the standard HPTLC method. The chromatogram showed two bandwidths with a particular volume of the compound. The bandwidth of 21 showed 2 bands, and the volume was 47.06. The bandwidth of 20 showed 2 bands, and the volume was 3.03. The R_f values of rutin and EAMMC were computed as 0.868 and 0.850, respectively.

Impact of EAMMC on Anxiety in SW Mice

EPM Test

Significant [F (3,19) = 12.63; $p < .0001$] differences were observed in open arms (% time spent) based on one-way ANOVA (Figure 2A). The Sidak's multiple comparison tests indicated that EAMMC exhibited an anxiolytic effect at both tested doses (50 and 100 mg/kg, p.o.) by significantly increasing the % time spent, similar to diazepam (the reference drug) at 1 mg/kg, i.p. in SW mice.

OFT

Significant results [F (3,19) = 52.68; $p < .0001$] were noted for the central time (%). The results of Sidak's multiple comparison tests (*post hoc*) demonstrated that EAMMC (50 and 100 mg/kg, p.o.) significantly increased % times spent in the central compartment, as shown for the standard anxiolytic drug (diazepam-treated group) that infers the anti-anxiety activity of EAMMC in SW mice (Figure 2B).

Effect of EAMMC on Non-precipitated AW Symptoms, Anxiety, and Depression in Mice

Handling-induced Convulsions

One-way ANOVA results revealed significant differences between the different treatment groups [F (4,22) = 8.556; $p = .0003$]. Sidak's multiple comparison *post hoc* test results demonstrated a significant increase in convulsion scores in alcohol-withdrawn animals, and EAMMC pre-treatment significantly decreased the handling-induced seizures (HIS), as shown for the diazepam-treated group, which implies the protective effect of EAMMC against HIS in AW mice (Figure 3A).

EPM Test

Significant results [F (4,22) = 12.90; $p < .0001$] were noted for % open arm time spent. Sidak's multiple comparison test (*post hoc*) revealed that EAMMC-treated AW groups (50 and 100 mg/kg, p.o.) showed a significant increase in the % time spent (open arms) when compared with the AW-control group. This result indicates the anti-anxiety effect of EAMMC in AW mice (Figure 3B).

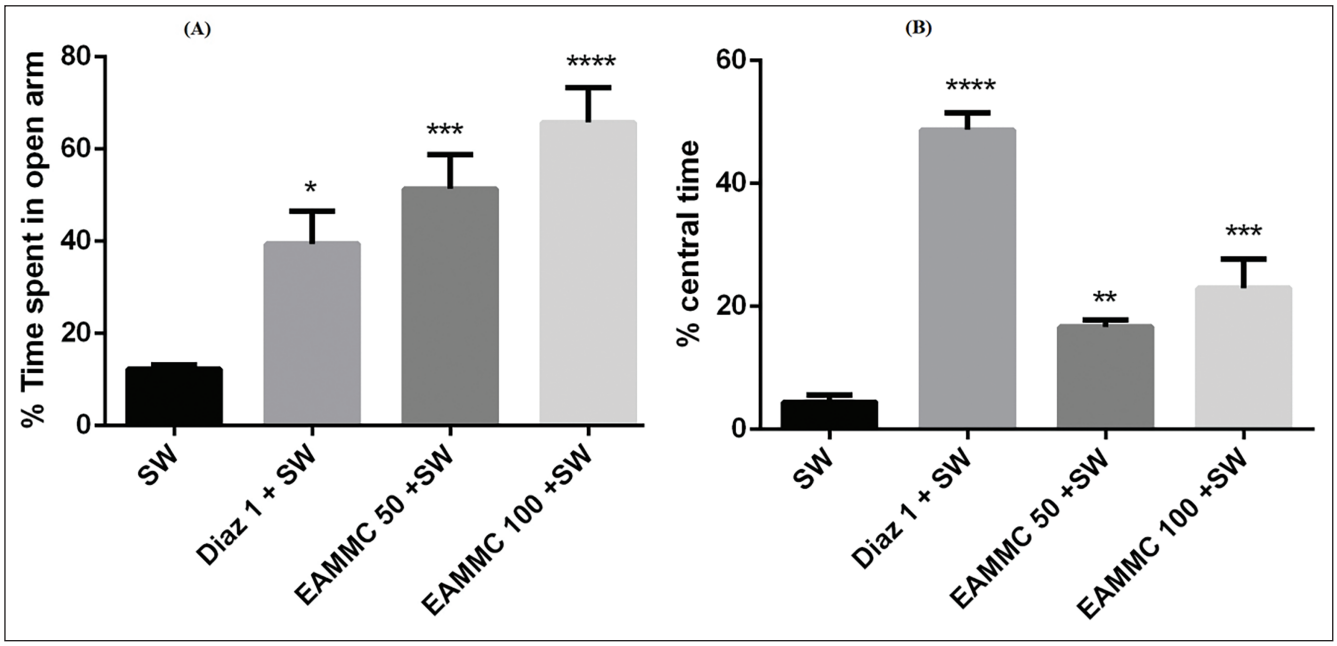


Figure 2. Effect of Oral Doses of 50 and 100 mg/kg of Ethyl Acetate Fraction *Morinda citrifolia* Linn. Fruit Extract (EAMMC) on Saline Withdrawn (SW)-induced Anxiety in (A) Elevated Plus Maze Test. (B) Open Field Test.

Note: The data are expressed as mean ± standard error of the mean (SEM) ($n = 6$); ****$p < .0001$, ***$p < .001$, **$p < .01$, and *$p < .05$ when compared with the SW group.

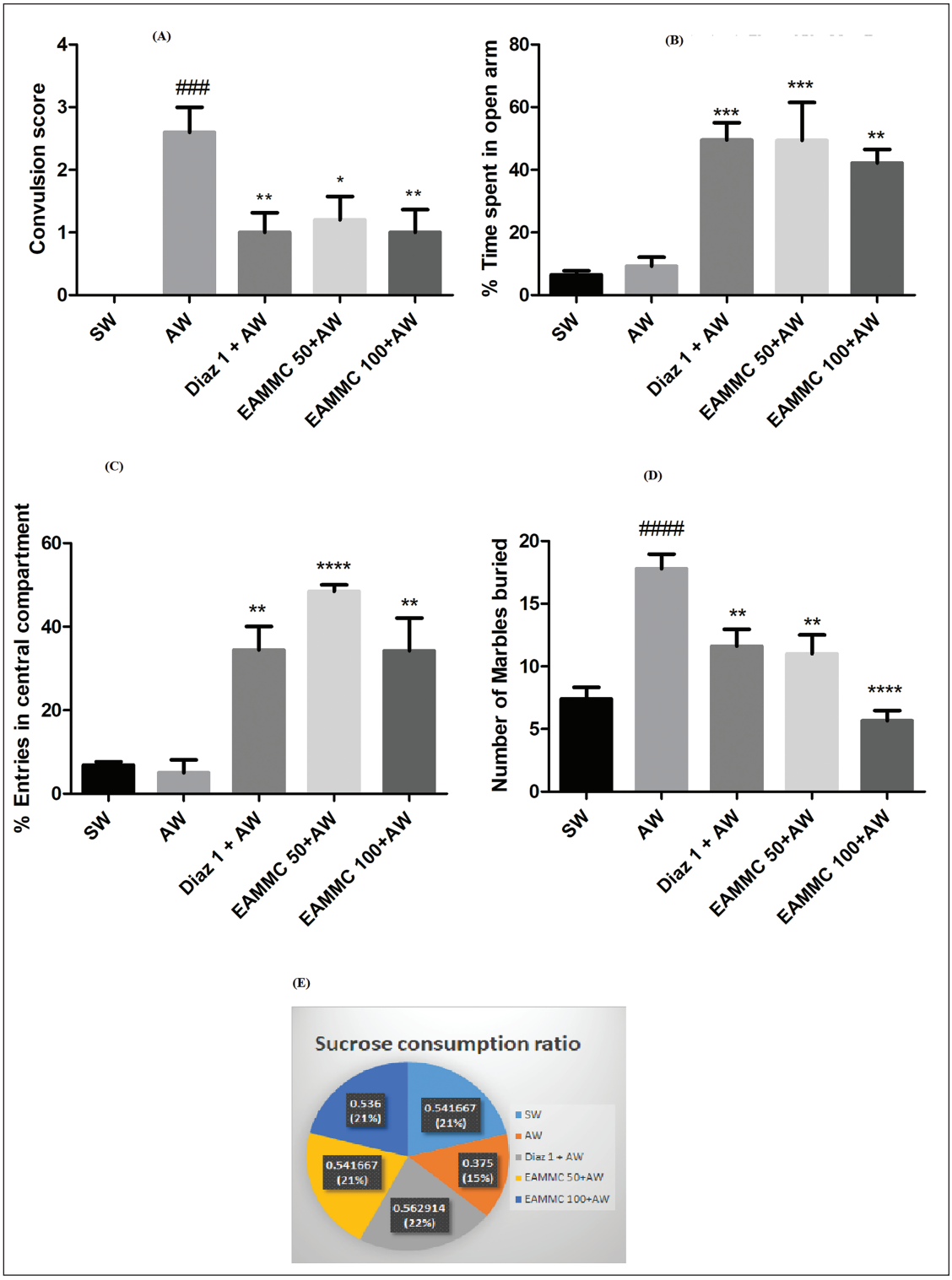


Figure 3. Effect of Ethyl Acetate Fraction (EAMMC) (50 and 100 mg/kg p.o.) on Alcohol Withdrawal (AW)-induced (A) Handling Seizures. (B) Anxiety in Elevated Plus Maze Test. (C) Anxiety in Open Field Test. (D) Anxiety in Marble Burying Test. (E) Anhedonia in Sucrose Preference Test in Mice.

Note: All data expressed as mean ± standard error of the mean (SEM) (n = 5–6); and ###p < .001 when compared with the saline withdrawn (SW) group; ***p < .001, **p < .01, and *p < .05 when compared with the AW group.

OFT

Significant differences [F (4,22) = 15.46; $p < .0001$] by one-way ANOVA were observed for the number of entries (%) in central. Sidak's multiple comparison test (*post hoc*) demonstrated that EAMMC-treated AW groups at oral doses (50 and 100 mg/kg) exhibited a significant rise in the % central entries when compared with the AW-control group. These results corroborate with the standard anxiolytic drug, diazepam; thereby, EAMMC demonstrates an anxiolytic effect in AW mice (Figure 3C).

MBT

Significant [F (4,22) = 16.91; $p < .0001$] differences were observed between the different treatment groups by one-way ANOVA. The Sidak's multiple comparison test (*post hoc*) results demonstrated a significant increase in the number of marbles buried in AW animals, whereas EAMMC pre-treated animals showed a significant decrease in the number of marbles buried, as shown for the reference drug (diazepam)-treated group, which implies the anti-anxiety effect of EAMMC in AW mice (Figure 3D).

SPT

The anhedonic effect in the AW group was noted by a drastic reduction in the intake of sucrose in the AW group. EAMMC and diazepam pre-treatment normalize the sucrose intake as seen with SW control, thereby EAMMC showed a reversal effect on AW-induced anhedonia in mice (Figure 3E).

Conclusion

The present study results suggest that the methanolic extract of *M. citrifolia* Linn. fruit (MMC) and its bioactive fraction (EAMMC) exhibit an anxiolytic effect in OFT, by increasing time spent in the central compartment; in EPM, by increasing time spent in open arms; in MBT, by reducing the number of marbles buried in AW mice. This study revealed that the anxiogenic effect in AW mice is demonstrated only in the MBT. The anti-anhedonic effect of MMC and EAMMC in AW mice is shown in the SPT by normalizing sucrose intake, which is drastically reduced in AW mice. Both MMC and EAMMC also exhibit protection from handling convulsions in AW mice. This study postulates the involvement of benzodiazepine-$GABA_A$ergic and serotonergic ($5\text{-}HT_{1A}$) mediated mechanisms for Noni's protective effect against AW symptoms. Further preclinical research is warranted to elucidate the actual mechanism of action of Noni (*M. citrifolia* Linn.) fruit for its protective effect against AW symptoms.

Abbreviations

AW: Alcohol withdrawn; **BZD:** Benzodiazepines; **CMC:** Sodium carboxymethyl cellulose; **CPP:** Conditioned place preference; **EAMMC:** Ethyl acetate fraction; **EPM:** Elevated plus maze; **GABA:** Gamma-aminobutyric acid; **HIC:** Handling-induced convulsion; **HPTLC:** High-performance thin-layer chromatography; **MAO:** Monoamine oxidase enzymes; **MBT:** Marble burying test; **MMC:** Methanolic extract of *Morinda citrifolia* Linn. fruit; **NMDA:** *N*-methyl-D-aspartate; **OFT:** Open field test; **SPT:** Sucrose preference test; **SW:** Saline withdrawn; **TLC:** Thin-layer chromatography.

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Authors' Contribution

A.B. and R.D. carried out the experiments and were involved in data analysis and drafting of the manuscript. J.C. was involved in the critical revision of the manuscript. V.P. conceived, designed, performed data analysis, and drafted the manuscript. This article has been duly read and approved by all authors.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

The Institutional Animal Ethics Committee of Chalapathi Institute of Pharmaceutical Sciences, Guntur evaluated and approved the experimental protocol (approval no. 04/IAEC/CLPT/2020-21 and 16/IAEC/CLPT/2020-2021). The animals' well-being was taken care of according to the CCSEA guidelines, India.

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Informed Consent

Human volunteers are not involved in the present study. This animal study has obtained prior approval from IAEC.

References

1. Newman RK, Stobart Gallagher MA, and Gomez AE. Alcohol withdrawal. In: *StatPearls* [Internet]. Florida: StatPearls Publishing, Treasure Island. 2022.
2. Brousse G, Arnaud B, Vorspan F, et al. Alteration of glutamate/GABA balance during acute alcohol withdrawal in emergency department: a prospective analysis. *Alcohol Alcohol* 2012; 47(5): 501–508. DOI: 10.1093/alcalc/ags078

3. Hood SD, Norman A, Hince DA, et al. Benzodiazepine dependence and its treatment with low dose flumazenil: benzodiazepine dependence and its treatment. *Br J Clin Pharmacol* 2014; 77(2): 285–294. DOI: 10.1111/bcp.12023
4. Pandy V, Bonam SR, Raya AR, et al. *Morinda citrifolia* Linn. (Noni) fruit, antioxidant effects, and neuroprotection. *In: Oxidative stress and dietary antioxidants in neurological diseases*. London, UK: Academic Press; 2020. pp. 291–307. DOI: 10.1016/b978-0-12-817780-8.00019-0
5. Pandy V and Khan Y. Noni (*Morinda citrifolia* Linn.) fruit juice attenuates the rewarding effect of ethanol in conditioned place preference in mice. *Exp Anim* 2016; 65(4): 437–445. DOI: 10.1538/expanim.16-0018
6. Khan Y and Pandy V. Methanolic extract of *Morinda citrifolia* L. (Noni) unripe fruit attenuates ethanol-induced conditioned place preferences in mice. *Front Pharmacol* 2016; 7: 352. DOI: 10.3389/fphar.2016.00352
7. Khan Y and Pandy V. *Morinda citrifolia* Linn. (Noni) fruit extract attenuates ethanol seeking behavior in mouse runway paradigm. *J Res Pharm* 2020; 24(5): 632–639. DOI: 10.35333/jrp.2020.218
8. Narasingam M, Pandy V, and Mohamed Z. Noni (*Morinda citrifolia* L.) fruit extract attenuates the rewarding effect of heroin in conditioned place preference but not withdrawal in rodents. *Exp Anim* 2016; 65(2): 157–164. DOI: 10.1538/expanim.15-0088
9. Pandy V, Khan Y, Yarlagadda DP, et al. *Morinda citrifolia* Linn. fruit extract mitigates heroin seeking behavior in mice. *Acta Neurobiol Exp* 2021; 81(4): 328–334. DOI: 10.55782/ane2021.031
10. Pandy V, Wai YC, Amira Roslan NF, et al. Methanolic extract of *Morinda citrifolia* Linn. unripe fruit attenuates methamphetamine-induced conditioned place preferences in mice. *Biomed Pharmacother* 2018; 107: 368–373. DOI: 10.1016/j.biopha.2018.08.008
11. Narasingam M, Vijeepallam K, Mohamed Z, et al. Anxiolytic- and antidepressant-like activities of a methanolic extract of *Morinda citrifolia* Linn. (Noni) fruit in mice: involvement of benzodiazepine-GABAAergic, serotonergic and adrenergic systems. *Biomed Pharmacother* 2017; 96: 944–952. DOI: 10.1016/j.biopha.2017.11.148
12. Deng S, West BJ, Palu AK, et al. Noni as an anxiolytic and sedative: a mechanism involving its gamma-aminobutyric acidergic effects. *Phytomedicine* 2007; 14(7–8): 517–522. DOI: 10.1016/j.phymed.2007.04.005
13. Kalandakanond-Thongsong S and Charoenphandhu J. Anxiolytic-like effects of Noni juice (*Morinda citrifolia* L.) on the respective changes of neurotransmitters in rat brain in the elevated plus-maze test. *Thai J Vet Med* 2012; 42(3): 275–280.
14. Deng S and West B. Antidepressant effects of Noni fruit and its active principles. *Asian J Med Sci* 2011; 3(2): 79–83.
15. Pandy V, Narasingam M, Kunasegaran T, et al. Effect of Noni (*Morinda citrifolia* Linn.) fruit and its bioactive principles scopoletin and rutin on rat vas deferens contractility: an ex vivo study. *Sci World J* 2014; 2014: 909586.
16. Pachauri SD, Tota S, Khandelwal K, et al. Protective effect of fruits of *Morinda citrifolia* L. on scopolamine-induced memory impairment in mice: a behavioral, biochemical and cerebral blood flow study. *J Ethnopharmacol* 2012; 139(1): 34–41.
17. Deore S, Khadabadi SS, and Baviskar BA. *Pharmacognosy and phytochemistry: a comprehensive approach*. 2nd ed. Hyderabad, India: PharmaMed Press; 2019.
18. Cai L. Thin layer chromatography. *In: Current protocols essential laboratory techniques*. vol. 8. New Jersey, USA: Wiley; 2014. pp. 6.3.1–6.3.18.
19. Sethi PD. *Sethi's HPTLC high-performance thin layer chromatography quantitative analysis of pharmaceutical formulations*. 2nd ed. Delhi, India: CBS Publications; 2013.
20. Farook JM, Krazem A, Lewis B, et al. Acamprosate attenuates the handling induced convulsions during alcohol withdrawal in Swiss Webster mice. *Physiol Behav* 2008; 95(1–2): 267–270. DOI: 10.1016/j.physbeh.2008.05.020
21. Wscieklica T, de Barros Viana M, Le Sueur Maluf L, et al. Alcohol consumption increases locomotion in an open field and induces Fos-immunoreactivity in reward and approach/withdrawal-related neurocircuitries. *Alcohol* 2016; 50: 73–82. DOI: 10.1016/j.alcohol.2015.11.005
22. Thomas A, Burant A, Bui N, et al. Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. *Psychopharmacology* 2009; 204(2): 361–373. DOI: 10.1007/s00213-009-1466-y
23. Lee KM, Coehlo M, McGregor HA, et al. Binge alcohol drinking elicits persistent negative affect in mice. *Behav Brain Res* 2015; 291: 385–398. DOI: 10.1016/j.bbr.2015.05.055