Future Journal of Pharmaceutical Sciences

RESEARCH

Open Access

Protective effect of methanolic extract of *Areca catechu* nut on ethanol withdrawal symptoms in mice

Vijayapandi Pandy[1,2*], Haritha Challa[1] and Preethi Byram[1]

Abstract

Background The purpose of the current study was to examine the potential impact of a methanolic extract of *Areca catechu* nut (MAN) on handling-induced convulsions (HIC), anxiety and anhedonia behaviour of alcohol-withdrawn mice. 30 female Swiss albino mice were divided into 5 groups, each with 6 animals. Group 1 (saline withdrawal) received saline during the 3-day alcohol/saline induction phase, while the other 4 groups (alcohol withdrawal) received 20% v/v ethanol (1.25 ml/100 g body weight, i.p.; 20% v/v ethanol was made from absolute ethanol with 79.9 ml saline + 0.1 ml fomepizole, an alcohol dehydrogenase inhibitor). Day four (test day) involved studying handling-induced convulsions; open field test (OFT), elevated plus maze test (EPM), marble burying test (MBT) for anxiety; 24-h sucrose preference test (SPT) for anhedonia in mice. On the test day, Group I and II (saline withdrawal and alcohol withdrawal) received oral treatments with 1% w/v sodium carboxyl methylcellulose 1 h prior to the behavioural testing. Group III received an injection of diazepam (1 mg/kg, i.p., 30 min prior) and Group IV and V were treated with two different doses of MAN (50 and 100 mg/kg, p.o.) 1 h prior to the behavioural test.

Results At doses of 50 and 100 mg/kg, p.o., the *Areca catechu* nut methanolic extract significantly reduced handling convulsions and anxiety, and had an anti-anhedonic effect using various evaluation criteria, such as convulsion score (HIC), no. of central and peripheral line crossings (OFT), % entries and time spent in open arms (EPM), no. of marbles buried (MBT), and sucrose intake ratio (SPT) in alcohol-withdrawn mice.

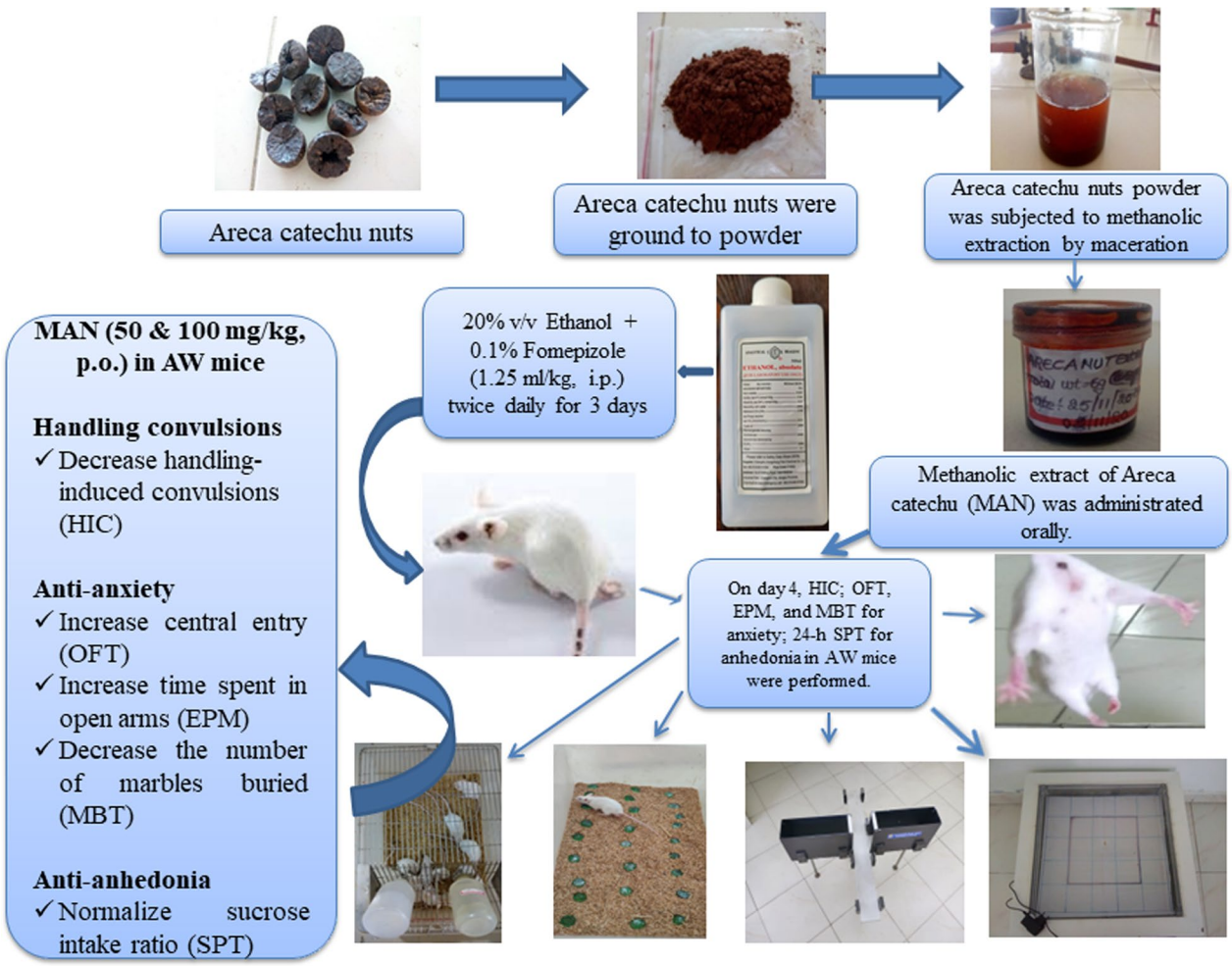
Conclusion In mice undergoing alcohol withdrawal, *Areca catechu* nut extract (MAN) greatly lessens handling-induced convulsions, anxiety and depression symptoms.

Keywords *Areca catechu* nut, Alcohol withdrawal, Handling-induced convulsions, Open field test, Elevated plus maze, Marble burying test, Sucrose preference test

10th International Conference of Laboratory Animal Scientists' Association (LASA), India

*Correspondence:
Vijayapandi Pandy
vijayapandi.pandy@mitwpu.edu.in; pandiphd@gmail.com
Full list of author information is available at the end of the article

Graphical Abstract



Background

Alcohol is the most frequently used and misused substance. According to World Health Organization research [1], drinking alcohol contributes to 3 million fatalities each year, as well as millions of impairments and ill health worldwide. Overall, 5.1% of the world's illness burden is attributable to hazardous alcohol usage. Alcohol abuse accounts for 7.1% and 2.2%, respectively, in men and women of the global burden of illness. Alcohol use accounts for 10% of all deaths in people aged 15 to 49, making it the top risk factor for early mortality and disability. Alcohol withdrawal syndrome (AWS) is a well-known condition that develops after heavy/constant drinking is abruptly stopped, whether on purpose or accidentally [2]. AWS is mediated by a number of neurochemical mechanisms, including (1) the alcohol-enhanced inhibitory effect of gamma-aminobutyric acid (GABA); (2) alcohol-mediated inhibition of *N*-methyl-D-aspartate (NMDA)-receptors, leading to their upregulation and increased responsiveness to the stimulating effect of glutamate (GLU); and (3) excessive availability of norepinephrine (NE) because of desensitization of alpha-2 auto receptors and augmentation of NE conversion from dopamine (DA). As a result, individuals experience the typical clinical symptoms of AWS, such as anxiety, irritability, agitation, tremors, and evidence of an excess of adrenaline, as well as withdrawal seizures and delirium tremens (DT) in more severe cases. The only gold standard method for the prevention and treatment of all stages of alcohol withdrawal syndrome is benzodiazepine (BZDP) medication. BZDPs do, however, have several disadvantages,

including cognitive decline and substantial neurological and medical side effects. Therefore, there is a need for new medications to treat AWS [3].

Areca catechu, often referred to as supari, is a type of dried, ripe nut that is grown in the tropical regions of South China, India, Malaysia, Sri Lanka, the Philippines Islands, and a small portion of East Africa. It belongs to the family Palmae or Arecaceae. In India and many other Asian nations, it is customary to chew areca nuts [4]. Alkaloids present in areca nuts such as arecoline, arecaidine, arecaine, guavacine, and guavacoline are primarily responsible for central nervous systems (CNS) activities like an antidepressant, and anxiolytic effects as well as a variety of parasympathetic effects. Areca nuts have historically been used to cure parasite illnesses, dyspepsia, gastrointestinal discomfort, abdominal pain, diarrhoea, oedema, and jaundice, and it has shown numerous pharmacological actions on the CNS, digestive, and cardiovascular systems [5]. Furthermore, Areca nut has been reported for antioxidant, antibacterial and antifungal, anti-inflammatory, analgesic, anti-allergic, antidiabetic, and antihyperlipidemic activities [5]. In the present study, a battery of behavioural tests on mice, including the HIC, OFT, EPM, MBT, and SPT were used to further explore *Areca catechu* nut's therapeutic potential to alleviate the AWS.

Materials and methods

Plant collection

The nuts of *Areca catechu* L. were collected from Nandigama, Krishna district, Andhra Pradesh, India. It was identified and authenticated by a botanist from the Department of botany and microbiology, Acharya Nagarjuna University, Guntur.

Preparation of extract

Areca catechu methanolic extract (MAN) was prepared by macerating 40 g of areca nut powder with 200 mL of 75%v/v methanol wrapped in aluminium foil for around 3 days with occasional shaking. The mixture was filtered using Whatman No. 1 membrane filter paper and vacuum-dried using a rotary evaporator at a temperature and speed of 45 °C and 180 rpm and determined the extract's yield in percentage.

Preparation of 20%v/v ethanol

0.1 ml of fomepizole (4-Methylpyrazole) was added to 20 ml of ethanol (99.9%v/v absolute alcohol), which was then diluted in 79.9 ml of normal saline.

Animals

We employed thirty female Swiss albino mice, which weighed between 25 and 30 g. Before the trial, the animals spent a week becoming acclimated to living at 24 ± 1 °C. Rodent pellet feed and purified water were made available freely to the animals. The experimental protocol was assessed and approved (Approval no. 03/IAEC/CLPT/2020-21) by the IAEC (Institutional Animal Ethics Committee) of Chalapathi Institute of Pharmaceutical Science, Guntur and followed the guidelines indicated by CPCSEA, New Delhi, India.

Drugs and chemicals

Fomepizole (4-Methylpyrazole) (Sigma-Aldrich), Sodium chloride injection I.P (0.9% w/v) (Otsuka Pharmaceutical India Private Limited), Ethanol (Changshu Hong sheng Fine Chemical Co., Ltd), and Diazepam injection I.P (Neon Laboratories Limited) were used.

Phytochemical analysis

The methanolic extract of *Areca catechu* nut (MAN) was weighed (0.5 g) and dissolved in 50 ml of methanol for the preparation of the test sample (10 mg/ml) for phytochemical analysis. Standard identification tests were carried out for the various phytoconstituents present in MAN. For alkaloids (Hager's test and Wagner's test), for flavonoids ($FeCl_3$ and lead acetate test), for glycosides (Bontrager's and Keller killani test), for steroids and lipids (Liberman Burchard and Salkowski test), for tannins ($FeCl_3$ and match stick test), for fixed and volatile oils (Spot and foam tests), and for proteins (ninhydrin and biurets tests) were used [6].

Experimental design

In this study, the effects of MAN on alcohol withdrawal-induced symptoms such as handling convulsions, anxiety, and anhedonia were examined. Ethanol solution (20%v/v) was prepared with fomepizole (0.1%v/v) to prolong the effects of alcohol withdrawal, such as anxiety and melancholy and it was injected intraperitoneally at 1.25 ml/100 g b.wt to all AW groups (Group II to V) twice daily for 3 days (i.e. dose 1 at 6 A.M and dose 2 at 4 P.M). Group I (saline withdrawal) received saline injection instead of ethanol following the same treatment schedule as other treatment groups. The experimental alcohol/saline withdrawal paradigm divided the animals into five classes (totalling five groups), with each class, made up of six mice. On the fourth day, Groups I and II (the saline-control and ethanol-control) received treatment with an oral dose of 1%w/v carboxymethyl cellulose (CMC). Group III (the standard group) received an intraperitoneal injection of diazepam at a dose of 1 mg/kg. Two separate doses (50 and 100 mg/kg) of methanolic extract of *Areca catechu* nut (MAN) were administered orally to Groups IV and V (the test groups). After one hour of oral treatment, we measured the alcohol

withdrawal symptoms such as handling convulsions, anxiety, and anhedonia using a battery of tests like HIC, OFT, EPM, MBT, and SPT in mice. The dose of MAN was selected based on an earlier study reported in the literature [7] and a pilot study performed in our laboratory. Dar and Khatoon (1997) reported the antidepressant effect of the ethanolic extract of *Areca catechu* in rodents and this study demonstrated a dose-dependent antidepressant effect of *Areca catechu* nut extract (4–80 mg/kg) without affecting locomotor activity [7]. Our pilot study also confirmed the reported activity, thereby, the present study fixed the doses at 50 and 100 mg/kg of MAN for alcohol withdrawal studies.

Handling-induced convulsions (HIC)

The mouse was first removed from its cage and checked for any indications of convulsions. If no convulsions occurred when picked up, the mouse was gently turned 360° clockwise and counter-clockwise using the thumb and fingers for around 5 s. A score of 0 meant there had been no convulsions, while a score of 5 meant there had been significant convulsions. Seizure scores are as follows: Score 0: Behavioural arrests (immobility), agitation, hair-raising, and quick breathing; Score 1: Lips and tongue movement, vibrissae movement, and salivation; Score 2: Head clonus and eye clonus; Score 3: Forelimb clonus and wet dog shake; Score 4: Clonic rearing; Score 5: Clonic rearing accompanied with lack of postural control and unrestrained jumping [8].

Open field test (OFT)

The OFT apparatus consists of a central compartment and a peripheral compartment. The central compartment has 9 squares measuring 10×10 cm each, while the peripheral compartment has 16 squares measuring 10×10 cm each. The overall length, width and height of the OFT box is $50 \times 50 \times 15$ cm. At starting the experiment, the animal was kept in the corner of the peripheral square of the OFT box and allowed to explore for 5 min, during which the number of square crossings and time spent in seconds in the central and peripheral compartments were recorded through a computer connected with Logitech HD webcam placed above the apparatus. The percentage number of square crossings and the percentage of time spent in the centre compartment were computed [9]. The experiments were done out under white light (150 lx).

Elevated plus maze (EPM)

EPM has two arms that are open (30×7.5 cm) and two arms that are closed ($30 \times 7.5 \times 30$ cm). The identical arms had a 7.5 cm centre square and were placed across from one another. A 40 cm elevation above the ground was used for the maze. Each animal was positioned in the plus maze's central square, facing one of the open arms. A PC connected to a Logitech HD webcam that was mounted above the equipment was used to record animal behaviour. The quantity and duration of entries in the open and closed arms during a 5-min period were noted [10]. The low red light was used for the EPM study.

Marble burying test (MBT)

Each mouse was kept in a separate plastic cage that measured $38 \times 21 \times 14$ cm and had a 5 cm depth of husk bedding inside. On the bedding, there were three rows and nine columns of twenty-seven little glass marbles, each measuring between 10 and 12 mm in diameter. After 5 min of exposure, the number of unburied marbles was counted. If the husk covered at least two-thirds (2/3) of the surface area of a marble, it was considered to be "buried" [11]. The marble burying behaviour of the animals represents a state of anxiety and anxiolytic drugs are known to reduce the total number of marbles buried.

Sucrose preference test (SPT)

Each mouse cage received two bottles, one containing normal drinking water and the other a 1%w/v sucrose solution, for 24 h. During this 24-h study, food was not provided. The test animals received the respective drugs just before the start of SPT and 12-h after the initiation of the study. The two bottles were interchanged after 12-h of the start of the study. The amount of sucrose solution and water consumed during 24-h was measured [12] and the sucrose consumption ratio was determined using the following formula.

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

Statistical analysis

One-way ANOVA (analysis of variance) was used to analyse the data, and then Sidak's multiple comparison test was performed. *P*-values under 0.05 were regarded as significant.

Results

Phytochemical analysis of MAN

The findings of the phytochemical examination revealed the presence of alkaloids, glycosides, steroids and lipids,

saponins, phenols, flavonoids, fixed oils, and volatile oils and the absence of carbohydrates and proteins.

Evaluation of the protective effect of methanolic extract of *Areca catechu* nut against alcohol withdrawal symptoms in mice

Handling-induced convulsions (HIC)

One-way ANOVA with post hoc Sidak's multiple comparison test revealed significant differences between the different treatment groups [$F(4,25) = 11.32$; $P < 0.0001$]. The alcohol withdrawal mice showed a high convulsion score when compared to saline withdrawal mice, whereas the reference drug diazepam-administered mice showed a low convulsion score when compared to alcohol withdrawal mice. Interestingly, MAN 50 mg/kg significantly lowered the convulsion score when compared with alcohol withdrawal mice (Fig. 1). This result implies the protective effect of MAN against alcohol withdrawal-induced handling seizures in mice.

Open field test (OFT)

One-way ANOVA with post hoc Sidak's multiple comparison test on (A) No. of line crossings in central (B) No. of line crossings in peripheral, (C) % No. of entries in central (D) Central time (E) Peripheral time (F) % central time revealed significant differences between the different treatment groups [$F(4,25) = 13.78$; $P < 0.0001$]; [$F(4,25) = 3.237$; $P < 0.0256$]; [$F(4,25) = 12.39$; $P < 0.0001$]; [$F(4,25) = 6.264$; $P < 0.0012$]; [$F(4,25) = 6.264$; $P < 0.017$]; [$F(4,25) = 6.206$; $P < 0.0013$], respectively. Alcohol withdrawal mice spent less time, had fewer entries, and crossed fewer lines in the central compartment of the open field test compared to saline withdrawal mice. The reference drug, the diazepam-treated group spent more time, had more entries and crossed more lines in the central compartment of the OFT when compared to alcohol withdrawal mice. MAN (50 and 100 mg/kg, p.o.) showed a similar effect as diazepam which indicates the anxiolytic effect of MAN in alcohol withdrawal mice (Fig. 2).

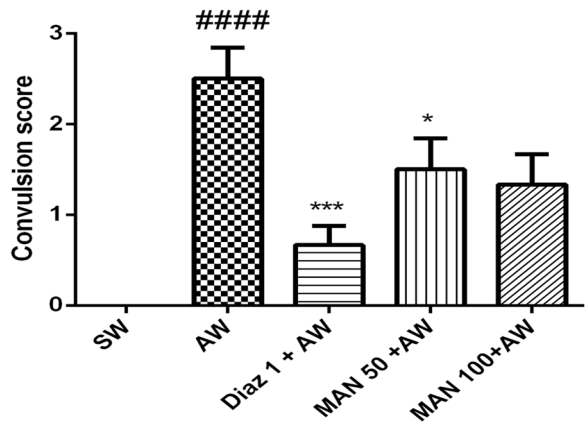


Fig. 1 Effect of MAN (50 and 100 mg/kg p.o.) on alcohol withdrawal-induced handling convulsion in mice. Convulsion scores are expressed as MEAN ± SEM ($n = 6$); $^{####}P < 0.0001$ when compared with the saline withdrawal group; $^{*}P < 0.05$ and $^{***}P < 0.001$ when compared with the alcohol withdrawal group

Elevated plus maze (EPM)

One-way ANOVA with post hoc Sidak's multiple comparison test A) % time spent in open arms, B) % no. of entries in open arm revealed significant differences between the different treatment groups [$F(4,25) = 16.06$; $P < 0.0001$]; [$F(4,25) = 26.47$; $P < 0.0001$], respectively. In the elevated plus maze test, alcohol withdrawal mice did not exhibit any anxiogenic activity in alcohol withdrawal mice in comparison with saline withdrawal mice. However, MAN at doses 50 and 100 mg/kg, p.o. administered mice spent more time and displayed more entries in the open arm than alcohol withdrawal mice as seen with the reference drug, diazepam-treated mice. This study highlights the anxiolytic effect of MAN in alcohol withdrawal mice (Fig. 3).

Marble burying test (MBT)

One-way ANOVA with post hoc Sidak's multiple comparison test revealed significant differences between the different treatment groups [$F(4,25) = 26.16$; $P < 0.0001$]. In the marble burying test, alcohol withdrawal mice buried more marbles than saline withdrawal mice which indicated a state of anxiety behaviour in alcohol withdrawal mice. The reference drug, diazepam-administered mice buried fewer marbles than alcohol withdrawal mice which implies the anxiolytic effect of diazepam in alcohol withdrawal mice. The test drug, MAN at 50 and 100 mg/kg, p.o. doses also buried fewer marbles than alcohol withdrawal mice as expressed in the diazepam-treated group which indicates the anxiolytic effect of MAN against alcohol withdrawal-induced anxiety behaviour in mice (Fig. 4).

Sucrose preference test (SPT)

In the sucrose preference test (SPT), the sucrose intake in alcohol withdrawal mice was decreased by 30% when compared with the saline withdrawal group which indicates the lack of feeling pleasurable effects (anhedonia) in alcohol withdrawal mice. Interestingly, MAN (50 and 100 mg/kg, p.o.) normalizes the sucrose intake in alcohol-withdrawn mice as seen with the reference drug diazepam which highlights the anti-anhedonia effect of MAN in the alcohol withdrawal mice (Fig. 5).

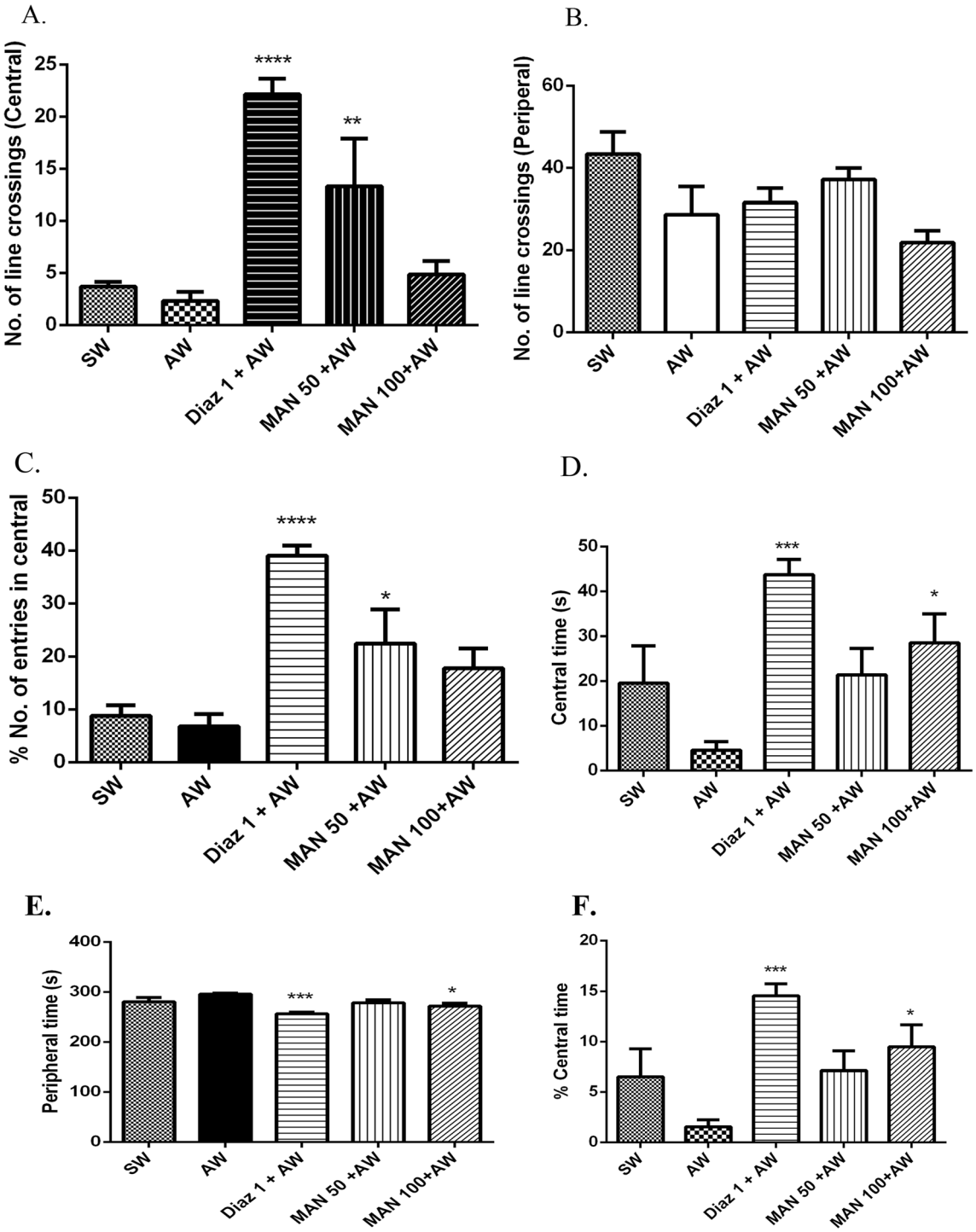


Fig. 2 Effect of MAN (50 and 100 mg/kg p.o.) on **A** % no. of line crossings in central, **B** % no. of line crossings in peripheral, **C** % no. of entries in central, **D** Central time, **E** Peripheral time, **F** % central time in the OFT mouse model. The data were expressed as MEAN ± SEM ($n = 6$); *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ when compared with the alcohol withdrawal group

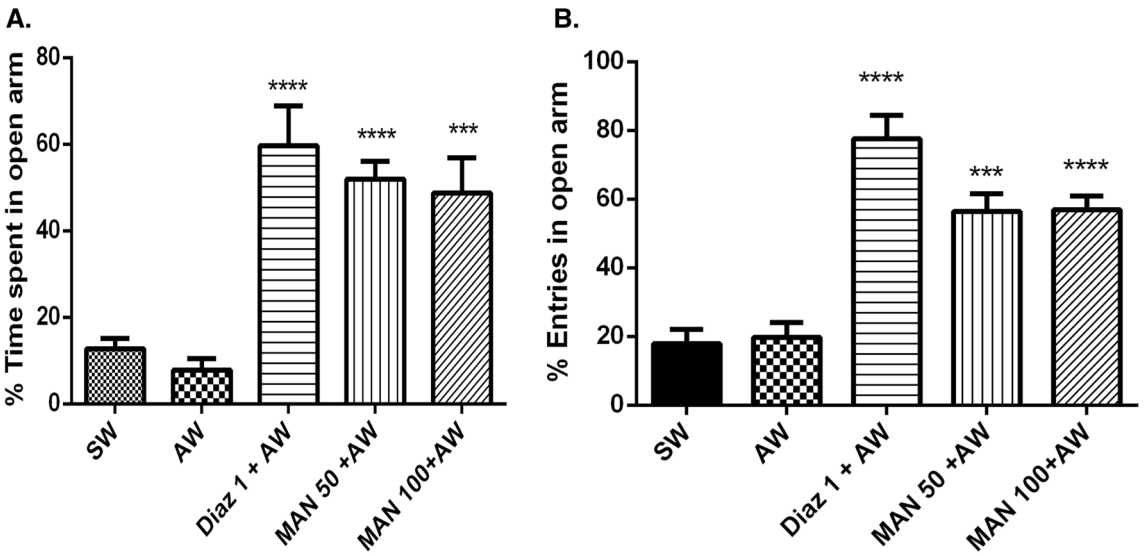


Fig. 3 Effect of MAN (50 and 100 mg/kg p.o.) on **A** % time spent in open arm, **B** % no. of entries in open arm in the EPM mouse model. The data were expressed as MEAN ± SEM ($n = 6$); ***$P < 0.001$ and ****$P < 0.0001$ when compared with the alcohol withdrawal group

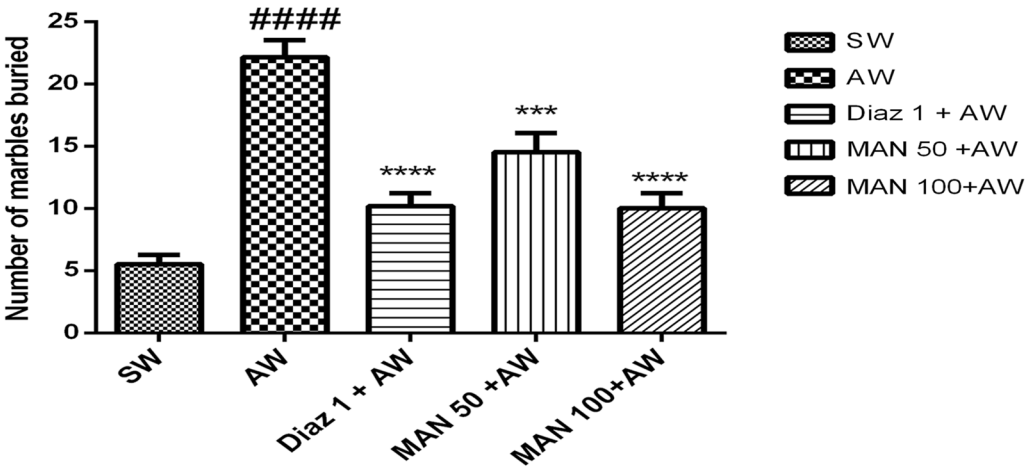


Fig. 4 Effect of MAN (50 and 100 mg/kg p.o.) on alcohol withdrawal-induced marble burying in mice. No. of marbles buried was expressed as MEAN ± SEM ($n = 6$); ####$P < 0.0001$ when compared with the saline withdrawal group; ***$P < 0.001$ and ****$P < 0.0001$ when compared with the alcohol withdrawal group

Discussion

The current study discovered that the *Areca catechu* nut methanolic extract greatly reduced alcohol withdrawal-induced handling convulsions, anxiety, and anhedonia in mice. One or more neurotransmitters and their receptors, notably GABA, serotonin, adenosine, and adrenergic receptors, are said to malfunction during alcohol withdrawal to cause withdrawal symptoms [13]. Abuse of alcohol reduces the quantity of GABA needed to keep the inhibitory and excitatory pathways of the brain in balance. During alcohol withdrawal, alcohol is no longer available to trigger the $GABA_A$ receptors which leads to diminished GABAergic inhibitory activity in the brain as a result of handling convulsions, anxiety and anhedonia in alcohol withdrawal mice. In the present study, we used 0.1% v/v of 4-methyl pyrazole, also known as Fomepizole for 20%v/v ethanol solution preparation to enhance ethanol bioavailability by inhibiting ethanol metabolism by blocking alcohol dehydrogenase enzymes (8000-fold higher affinity for alcohol dehydrogenase than ethanol does) thereby reinforcing the properties of ethanol is prolonged [8].

Dopamine's inhibitory actions mediated through D2, D3, and D4 receptors (coupled with G-protein-coupled receptors (GPCRs) Gi/o-proteins which inhibit adenylyl cyclase) are strengthened by ethanol [14]. On the

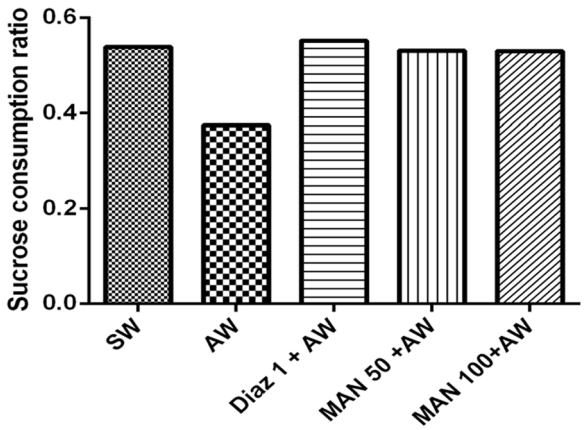


Fig. 5 Effect of MAN (50 and 100 mg/kg p.o.) on alcohol withdrawal-induced sucrose preference in mice

other hand, an enhanced serotonin 5-HT2A receptor activity may also be proposed as a factor for the withdrawal symptoms. It has been demonstrated that alcohol withdrawal mice showed increased susceptibility to audiogenic, electrical, and chemical-induced seizures in 5-HT2C null mutant mice. Moreover, mCPP a 5-HT2C/1B receptor agonist attenuated and SB 242,084 selective 5-HT2C receptor antagonist aggravated the alcohol withdrawal symptoms in D2 mice (have less brain 5-HT level). These findings confirmed the involvement of 5-HT2c receptors in alcohol withdrawal symptoms [15]. Withdrawal from chronic ethanol consumption is known to induce anhedonic and anxiogenic effects as a consequence of hypoactivity in benzodiazepine-$GABA_A$ergic inhibitory pathways in the brain. The current study offers vital information about the use of methanolic extract from *Areca catechu* nut can reduce alcohol withdrawal-induced anxiety and anhedonia in mice. Earlier studies showed that the methanolic extract from MAN has both anxiolytic and antidepressant effects in normal mice [16]. Similarly, Arecoline, a prime bioactive alkaloid present in MAN demonstrated anxiolytic-like activity as seen with areca nut water extract in the zebrafish model [17]. Arecaidine and guvacine, the other bioactive principles of *Areca catechu* nut demonstrated an inhibitory effect on GABA uptake in the cat's central nervous system. Moreover, arecaidine at a larger dose (1 g/kg, s.c.) attenuated the lethal effect of the GABA-A antagonist, bicuculline [18]. These study results highlighted the $GABA_A$ergic facilitatory effect of MAN could stem from its bioactive alkaloidal compounds. Therefore, it has been postulated that the protective effect of MAN against alcohol withdrawal-induced anhedonia and anxiety in mice could be mediated through benzodiazepine-$GABA_A$ergic-mediated mechanisms.

Conclusion

According to this study, the methanolic extract of *Areca catechu* nut (MAN) at an oral dose of 50 and 100 mg/kg normalized sucrose intake in alcohol-withdrawn mice which demonstrates the anti-anhedonic effect of MAN against alcohol withdrawal-induced anhedonia. Moreover, MAN (50 and 100 mg/kg, p.o.) by enhancing time spent in the central compartment of OFT; time spent in EPM; and attenuating the number of marbles buried in MBT exhibits the attenuation effect on anxiety symptoms in alcohol-withdrawn mice. Furthermore, MAN inhibits handling seizures in alcohol-withdrawn mice. The protective effect of MAN against alcohol withdrawal symptoms could be mediated through benzodiazepine-$GABA_A$ergic mechanisms that would be brought by distinct *Areca catechu* nut alkaloidal components such as arecoline, arecaidine, guavacine, and guavacoline. Further preclinical studies are required to investigate the therapeutic potential of these alkaloidal compounds for novel drug discovery in the treatment of alcohol withdrawal. A study in this direction is currently in progress in our laboratory to elucidate molecular mechanisms involved in its protective effect against alcohol withdrawal.

Abbreviations

MAN	*Areca catechu* Nut's methanolic extract
HIC	Handling-induced convulsions
OFT	Open field test
EPM	Elevated plus maze
MBT	Marble burying test
SPT	Sucrose preference test
AWS	Alcohol withdrawal syndrome
GABA	Gamma-amino butyric acid
NMDA	*N*-Methyl-D-aspartate
GLU	Glutamate
NE	Norepinephrine
DA	Dopamine
DT	Delirium tremens
BZDP	Benzodiazepine
CNS	Central nervous system
AW	Alcohol withdrawal
IAEC	Institutional Animal Ethics Committee
ANOVA	Analysis of variance
GPCRs	G-protein-coupled receptors

Acknowledgements

The authors are thankful to Prof. Rama Rao Nadendla, the Principal of Chalapathi Institute of Pharmaceutical Sciences, Guntur, and Sri YV Anjaneyulu, the President of Chalapathi Educational Society, Lam, Guntur for providing the necessary facilities to perform this study.

Significance statement

Areca catechu nut commonly known as Betel nut is consumed traditionally along with Piper betle leaf in Asia pacific countries and in Indian and Chinese traditional medicine for centuries. This study highlighted the protective effect of *Areca catechu* nut against alcohol withdrawal symptoms in animal models for the first time. This article attracts wider readers from different disciplines because *Areca catechu* nut is prevalently used throughout the world and scientists are looking for a new therapeutic outcome from this plant.

Author contributions
VP conceived, designed, analysed the data, supervised, drafted, and critically reviewed the manuscript; HC performed the experiments and drafted the manuscript; PB critically revised the manuscript. All authors read and approved the final manuscript.

Funding
The author VP would like to express his sincere gratitude to DST-SERB, India for the research grant (Sanction No. CRG/2018/000813). The funders had no role in the design and conduct of the study, nor the decision to prepare and submit the manuscript for publication.

Availability of data and materials
All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
All animal procedures were conducted in accordance with the standards set forth in the guidelines for the care and use of experimental animals by the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), India. The Institutional Animal Ethics Committee of Chalapathi Institute of Pharmaceutical Sciences, Guntur approved all experimental procedures (IAEC approval no. 03/IAEC/CLPT/2020-21).

Consent for publication
Not applicable.

Plant material
It was identified, authenticated, and approved by Dr. P. Satyanarayana Raju, Ph.D., from the Department of botany and microbiology, Acharya Nagarjuna University, Guntur.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Department of Pharmacology, Chalapathi Institute of Pharmaceutical Sciences, Chalapathi Nagar, Lam, Guntur, Andhra Pradesh, India. [2]Present Address: School of Pharmacy, Dr. Vishwanath Karad MIT World Peace University (MIT-WPU), 124 Paud Road, Kothrud, Pune, Maharashtra 411038, India.

Received: 12 November 2022 Accepted: 19 January 2023
Published online: 02 February 2023

References

1. WHO World Health Organization (2022) https://www.who.int/news-room/fact-sheets/detail/alcohol. Accessed 9 May 2022.
2. Gupta A, Khan H, Kaur A, Singh TG (2021) Novel targets explored in the treatment of alcohol withdrawal syndrome. CNS Neurol Disord Drug Targets 20(2):158–173. https://doi.org/10.2174/1871527319999201118155721
3. Wolf C, Curry A, Nacht J, Simpson SA (2020) Management of alcohol withdrawal in the emergency department: current perspectives. Open Access Emerg Med 12:53–65. https://doi.org/10.2147/OAEM.S235288
4. Ansari A, Mahmood T, Bagga P, Ahsan F, Shamim A, Ahmad S, Shariq M, Parveen S (2020) *Areca catechu*: a phytopharmacological legwork. Food Front 2021:1–21. https://doi.org/10.1002/fft2.70
5. Salehi B, Konovalov DA, Fru P, Kapewangolo P, Peron G, Ksenija MS, Cardoso SM, Pereira OR, Nigam M, Nicola S, Pignata G, Rapposelli S, Sestito S, Anil Kumar NV, de la Luz C-G, Segura-Carretero A, Mishra P, A, Sharifi-Rad M, Cho WC, Taheri Y, Setzer WN, Sharifi-Rad J, (2020) *Areca catechu*-From farm to food and biomedical applications. Phytother Res 34(9):2140–2158. https://doi.org/10.1002/ptr.6665
6. Neamah SR, Mohsin DH, Kamil ZH (2021) Phytochemical screening and antibacterial effect of methanol extracts of *Suaeda aegyptiaca* leaves on *Staphylococcus aureus, Staphylococcus epidermis, Escherichia coli,* and *Pseudomonas aeruginosa*. Arch Razi Inst 76(5):1343–1349. https://doi.org/10.22092/ari.2021.356133.1784
7. Dar A, Khatoon S (1997) Antidepressant effects of ethanol extract of *Areca catechu* in rodents. Phytother Res 11:174–176. https://doi.org/10.1002/(SICI)1099-1573(199703)11:2%3c174::AID-PTR65%3e3.0.CO;2-B
8. Farook JM, Krazem A, Lewis B, Morrell DJ, Littleton JM, Barron S (2008) Acamprosate attenuates the handling induced convulsions during alcohol withdrawal in Swiss Webster mice. Physiol Behav 95(1–2):267–270. https://doi.org/10.1016/j.physbeh.2008.05.020
9. Seibenhener ML, Wooten MC (2015) Use of the open field maze to measure locomotor and anxiety-like behavior in mice. J Vis Exp 96:e52434. https://doi.org/10.3791/52434
10. Narasingham M, Vijeepallam K, Mohamed Z, Pandy V (2017) Anxiolytic- and antidepressant-like activities of a methanolic extract of *Morinda citrifolia* Linn. (noni) fruit in mice: involvement of benzodiazepine-$GABA_A$ergic, serotonergic and adrenergic systems. Biomed Pharmacother 96:944–952. https://doi.org/10.1016/j.biopha.2017.11.148
11. Kedia S, Chattarji S (2014) Marble burying as a test of the delayed anxiogenic effects of acute immobilisation stress in mice. J NeuroSci Methods 233:150–154. https://doi.org/10.1016/j.jneumeth.2014.06.012
12. Liu MY, Yin CY, Zhu LJ, Zhu XH, Xu C, Luo CX, Chen H, Zhu DY, Zhou QG (2018) Sucrose preference test for measurement of stress-induced anhedonia in mice. Nat Protoc 13(7):1686–1698. https://doi.org/10.1038/s41596-018-0011-z
13. Di Chiara G, Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci U S A 85(14):5274–5278. https://doi.org/10.1073/pnas.85.14.5274
14. Landolt HP, Gillin JC (2001) Sleep abnormalities during abstinence in alcohol-dependent patients Aetiology and management. CNS Drugs 15(5):413–425. https://doi.org/10.2165/00023210-200115050-00006
15. Kari JB, Matthew TR, Laura MR, Ken S, Kathleen G, Mark SB (2004) Serotonin 5-HT2 receptors and alcohol: reward, withdrawal and discrimination. Alcohol Clin Exp Res 28(2):211–216. https://doi.org/10.1097/01.alc.0000113423.40075.a3
16. Ruckmani A, Vinayak M, Kavitha KN (2014) Anxiolytic and anti-depressant activity of *Areca catechu* Linn. in mice. WJPR 3(10):1367–1376
17. Serikuly N, Alpyshov E, Wang D, Wang J, Yang L et al (2021) Effects of acute and chronic arecoline in adult zebrafish: Anxiolytic-like activity, elevated brain monoamines and the potential role of microglia. Prog Neuropsychopharmacol Biol Psychiatry 10(104):109977. https://doi.org/10.1016/j.pnpbp.2020.109977
18. Patil PR, Rakesh SU, Dhabale PN, Burade KB (2009) Pharmacological activities of *Areca catechu* Linn.—a review. J Pharm Res 2(4):683–687

Publisher's Note