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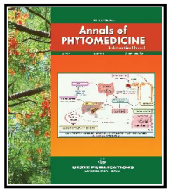

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# Protective effect of *Mucuna pruriens* (L.) DC. on ethanol withdrawal-induced behavioral changes in rats

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**Abstract**

Global alcohol addiction is a significant societal concern, and staying away from long-term use of alcohol results in the emergence of behavioural abnormalities. This study assesses the role of *Mucuna pruriens* (L.) DC. extract on the effects of ethanol withdrawal. Animals freely accessed ethanol for 21 days through a liquid diet. Administered *M. pruriens* extract (MPE) at dosages of 200 and 400 mg/kg during the alcohol-dependent period (15-21 days) or 30 min before measuring ethanol abstinence. The study examined the withdrawal consequences by evaluating depressive behaviour, locomotion, and anxiety. The administration of treatment to rats 30 min before the 24 h postethanol withdrawal evaluation had no impact on the scores of somatic and behavioral markers. During days 15 to 21, the administration of *M. pruriens* extract at doses of 200 and 400 mg/kg effectively decreased the heightened depressive behaviours, hyperlocomotion, and anxiety caused by ethanol withdrawal. *M. pruriens* extract lowered the high levels of corticosterone in their blood. *M. pruriens* extract may have helped ease ethanol withdrawal symptoms by lowering stress and stabilising the HPA (hypothalamic-pituitary-adrenocortical) axis activity.

## 1. Introduction

Alcohol is a highly prevalent and frequently misused substance. As per the WHO, there are 76.3 million people globally deal with alcohol use disorders and dependency, leading to a total of 1.8 million deaths annually (Sharma *et al*., 2021). Ethanol withdrawal syndrome manifests following a decrease or sudden discontinuation of chronic excessive alcohol consumption in individuals with alcohol addiction (Kayir and Uzbay, 2008). Withdrawal from ethanol induces several physical and mental symptoms linked to heightened excitability of the central nervous system, including irritation, nervousness, anxiousness, and depression (Connor *et al*., 2022). Several neurotransmitters, including GABA and NMDA, change after ethanol ingestion. Ethanol dependence leads to overexpression of NMDA receptors and downregulation of GABA, with dopamine activating reward circuits (Dharavath *et al*., 2023). Additionally, corticosterone, CRF, is elevated in ethanol withdrawal symptoms, leading to increased anxiety (Huang *et al*., 2009). The majority of drugs utilised for the treatment of alcoholism and promoting abstinence, such as benzodiazepines and naltrexone, are associated with significant adverse effects (Sachdeva, 2015; Leggio *et al*., 2008)

The widely utilised medicinal plant *M. pruriens* holds popularity and significance in ayurveda, an ancient holistic science practised in India for an extended period (Indira *et al*., 2023). This perennial legume possesses significant medicinal properties throughout its entire anatomy, and its derivatives are in considerable demand in both the Indian and global medicinal product industries. Prior studies have shown that *M. pruriens* demonstrates antidiabetic properties (Bhaskar *et al*., 2008), antidepressant effects (Galani and Rana, 2014), neuroprotective effects (Zahra *et al*., 2022), antioxidant effects (Susilowati and Purwati, 2021), and anxiolytic activity (Patil, 2021). The previous investigation also documented its ability to mitigate reproductive harm by reducing oxidative stress (Tangsrisakda *et al*., 2022). Multiple studies claim that *M. pruriens* contains L-dopa, serotonin, and 5-hydroxytryptamine. Brain dopamine activation is a prominent pharmacological effect. Dopamine governs the regulation of mood and motivation. Several studies have demonstrated that *M. pruriens* effectively reduces the symptoms of Parkinsonism (Shastrakar *et al*., 2023; Kasture *et al*., 2009). The current study examined the defensive characteristics of *M. pruriens* against the ethanol withdrawal symptoms in rats.

## 2. Materials and Methods

### 2.1 Collection and identification of plant material

We collected the *Mucuna pruriens* (L.) DC (Fabaceae) plant seed from the Nawargaon (Sindewahi) area of Chandrapur district, Maharashtra, India. Vishal N. Patil, from the Department of Botany at Vidyabharti College Seloo in Wardha, Maharashtra, successfully identified and verified the seed. A voucher specimen, 04/ rgbacscbotany/2022-2023, was placed in the division's herbarium.

### 2.2 Preparation of extracts

The seeds were cleaned with water, dehydrated using absorbent material at approximately 26°C, crushed with manual grinders, and filtered to obtain a fine powder with a mesh number of 40. Afterwards, the product was dehydrated in an oven warmed to around 50°C for three days. The hydroalcoholic extract was prepared by combining distilled water and 95% ethanol (1:1, v/v). The mixture was then

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homogenised and sieved, and solid residues were removed. Finally, the extract was exposed to a thermal bath at 40°C for 24 h. The samples underwent freezing and subsequent lyophilization at 48°C at a pressure of 130 mmHg. This process lasted 24 h and produced the *M. pruriens* extract (MPE) in powder form (Tavares *et al*., 2020).

### 2.3 Phytochemical screening

Using our Institute's laboratory manual, the hydroalcoholic extract of *M. pruriens* was subjected to phytochemical screening tests to identify various phytoconstituents (Gokhale *et al*., 2016).

### 2.4 Animals

In a regulated setting, adult rats in a healthy state, weighing nearly 200 and 250 g and aged three to four months, were kept separately within a cage with unlimited access to water and food. The rats were kept in a controlled environment with a temperature of 25 ± 2°C and a light cycle of 12 h of light and dark, with the light turned on at 7:00 a.m. The Institutional Animal Ethical Committee approved the experimental protocols (535/PO/RcRcBt/S/02/CPCSEA/IPER/IAEC/2022-2023), which were strictly conducted in compliance with the directives of the Indian government's Committee for Control and Supervision of Experimental on animals.

### 2.5 Study design

We randomly allocated the rats into six groups, with six rats in one group, as follows: Group 1 consisted of rats who were given a modified liquid diet (MLD) (normal group). Group 2 received a liquid diet (MLD) and ethanol (control group). The following four groups were treated with a combination of liquid diet, ethanol, and *M. pruriens* extract (MPE), with varying doses and durations (Table 1). Based on prior research, we determined the dose of *M. pruriens* extract.

**Table 1: Treatment, dose, and route of administration**

| S.No. | Groups | Treatment, dose and route of administration |
|---|---|---|
| 1 | Group I (Normal) | MLD |
| 2 | Group II(Control) | MLD +Ethanol |
| 3 | Group III (Test 1) | MLD +Ethanol + MPE (200 mg/kg p.o.) on day 22 |
| 4 | Group IV (Test 2) | MLD +Ethanol + MPE (200 mg/kg p.o.) from day 15-22 |
| 5 | Group V (Test 3) | MLD +Ethanol + MPE (400 mg/kg p.o.) on day 22 |
| 6 | Group VI (Test 4) | MLD +Ethanol + MPE (4000 mg/kg p.o.) from day 15-22 |

Based on earlier research, the rats had a liquid meal, along with or without alcohol, and were offered no other food or water. Composition of a liquid diet consisting of 925 ml of cow milk, 25-75 ml of alcohol (ethyl alcohol 96.5%), 17 g of sugar, and 5,000 IU of vitamin A (Du *et al*., 2022). The animals were initially placed on a liquid diet, excluding alcohol, for 07 days. Following this, a liquid diet containing 2.4% ethanol was supplied for 3 days. Subsequently, the ethanol content was raised to 4.8% for 4 days, then 7.2% for 14 days. After being administered a diet containing 7.2% ethanol, the ethanol was cut off from the diet after 21 days of therapy. Normal group rats continued on a liquid diet, and other group rats were given a liquid diet containing the same number of calories as ethanol, with sucrose serving as a substitute for ethanol (Tian *et al*., 2022). A daily liquid diet was freshly made and served consistently (08.30 h). The rats' weight was documented daily, and the daily ethanol consumption was monitored and expressed as grams per kilogram. The protocol structure is divided into two parts: In the first, at the period of ethanol dependence (15-21 days), *M. pruriens* extract was administered orally at 200 and 400 mg/kg doses. In the second, rats were orally administered *M. pruriens* extract at 200 and 400 mg/kg doses for 30 min before measuring ethanol withdrawal. The rat's distinct cohorts (n = 6) were allocated to varying dosages. Simultaneously, appropriate controls that were treated with a vehicle were also maintained.

### 2.6 Elevated plus maze

Anxiety in rats was evaluated through an elevated plus maze (EPM) using a previously reported experimental methodology. After a 24 h withdrawal period, rats were positioned in the centre of the EPM with the open arm directly in front of them. EPM determined variables like the duration spent in enclosed and open arms. The maze platform was wiped clean with a damp cloth after every test. Each rat is assessed for evaluation for five min (Siddique *et al*., 2022; Taksande *et al*., 2010).

### 2.7 Locomotor activity

The animals' spontaneous locomotor movement was documented for 5 min using an electronic actophotometer. Tracking the motion of an animal as it interrupts a light beam. The photocell was captured digitally. To record each of the following animals, the rectangular base of the actophotometer was thoroughly scrubbed. The observation is the average count for 5 min (Sutar *et al*., 2021; Mishra *et al*., 2020).

### 2.8 Forced swim test

A forced swim test (FST) was employed to assess the depressed behaviour in rats undergoing ethanol withdrawal. Each animal was made to swim against its will in a glass tank that was 46 cm × 20 cm and contained 30 cm of water. It was noted how long the immobility lasted. The decreased span of immobility was regarded as a reduction in depressive behaviour caused by ethanol abstinence. An impartial witness who had received proper training and was unaware of the therapies being administered recorded the duration of immobility, which serves as an indicator of depression (Slattery and Cryan, 2012).

### 2.9 Measurement of plasma ethanol and corticosterone concentrations

Blood samples were obtained from a single group of rats one hour before and 24 h after ethanol cessation. The samples were collected

from the tail vein using EDTA tubes and then radiating at 13000 × g for 12 min at 5°C to separate the plasma. After that, the plasma was kept at 20°C. The plasma samples, which had been separated, were subsequently utilised to measure the levels of ethanol and corticosterone.

Ethanol concentrations in plasma were measured using the nicotinamide adenine dinucleotide-alcohol dehydrogenase enzymatic evaluation. The levels of corticosterone in the plasma were determined using an HPLC system following the previously published method (Injamuri *et al*., 2022; Taksande *et al*., 2013; Sheikh *et al*., 2007)

### 2.10 Statistical analysis

The observation is shown as mean ± SEM. The data collected from the Student t-test was used to compare rats withdrawn from ethanol and those on a liquid diet. Using Dunnett's test, one-way ANOVA was used to examine the effects of different treatments. *$p$<0.01served as significant. Data was analysed with Prism 9 for Windows (9.5.1).

## 3. Results

### 3.1 Phytochemical analysis of plant extracts

The *M. pruriens* seed extract contains various chemical components, including flavonoids, alkaloids, tannins, glycosides, carbohydrates, and saponins (Table2).

### 3.2 Ethanol intake, body weight, ethanol level in blood

The rats in the ethanol-fed group (MLD + ethanol) and the groups treated with *M. pruriens* extract (200 and 400 mg/kg, p.o) had a daily ethanol intake ranging from 8.16 ± 0.318 to 12.49 ± 0.593 g/kg/day while being exposed to 7.2% (v/v) ethanol. Despite this, this distinction could not show statistical significance. The rats gained weight during the experiment. While ending the study, ethanol-fed and modified liquid diet groups gained 1.6% and 3.4%, respectively. The rats' body weights did not change significantly from the start of the trial (Table 3) (Student's t-test). Blood ethanol levels were 154.45 ± 4.58 mg/dl and 7.41 ± 01.29 mg/dl before and 24 h after ethanol abstinence, respectively. A drop in blood ethanol levels 24 hafter withdrawal was substantially linked to cessation signs.

**Table 2: Phytochemical analysis of extracts**

| Test | Observation | Result |
|---|---|---|
| Shinoda test (Test for flavonoids) | Pink colour | + Ve |
| Dragendorff'stest (Test for alkaloids) | Red colour | + Ve |
| Ferric chloride test (Test for tannins) | Greenish to black | + Ve |
| Killer killani test (Test forglycoside) | Blue colour | + Ve |
| Molisch's test (Carbohydrates) | A purple ring at the junction | + Ve |
| Foam test (Test for saponins) | Foam appearance | + Ve |
| + Ve = present | | |

**Table 3: Weight variation during the study**

| Alterations in body weight | | | |
|---|---|---|---|
| **Groups** | **Start of the investigation** | **End of the investigation** | **Body weight changes** |
| Normal group | 218 ± 5.45 | 224 ± 5.24 | 3.4% |
| Ethanol fed group | 226 ± 4.35 | 229 ± 4.35 | 1.6%, |

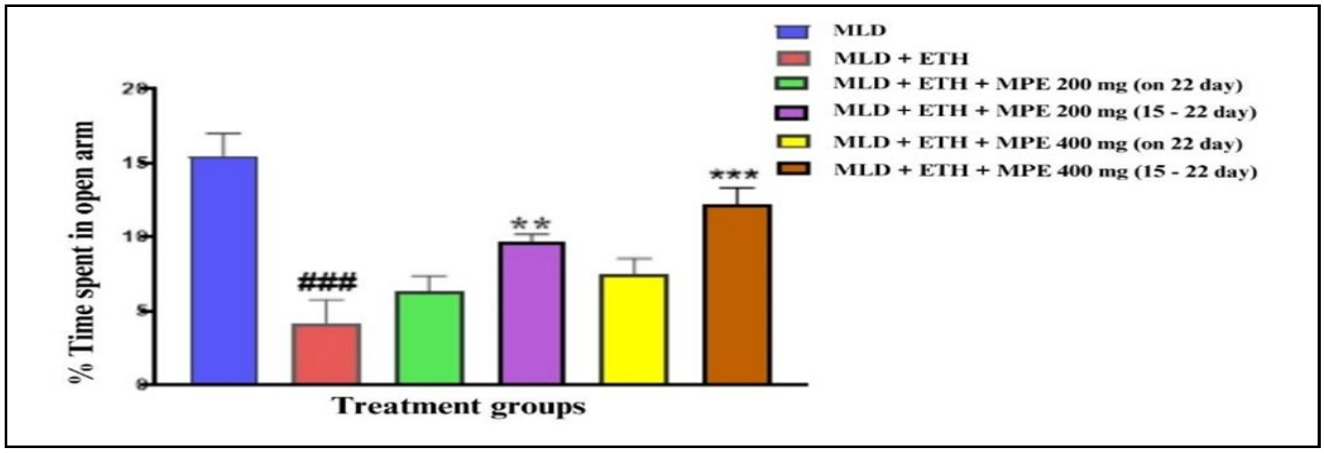


**Figure 1: The effect of different treatments of *M. pruriens* extract on % time spent in open arm in ethanol withdrawal induces anxiety. Every bar denotes the mean ± SEM (n = 6), ###$p$<0.001 when compared against the modified liquid diet group. **$p$<0.01 and ***$p$<0.001 when compared against ethanol-fed groups (MLD + Ethanol). Dunnett's test proceeds with a one-way ANOVA.**

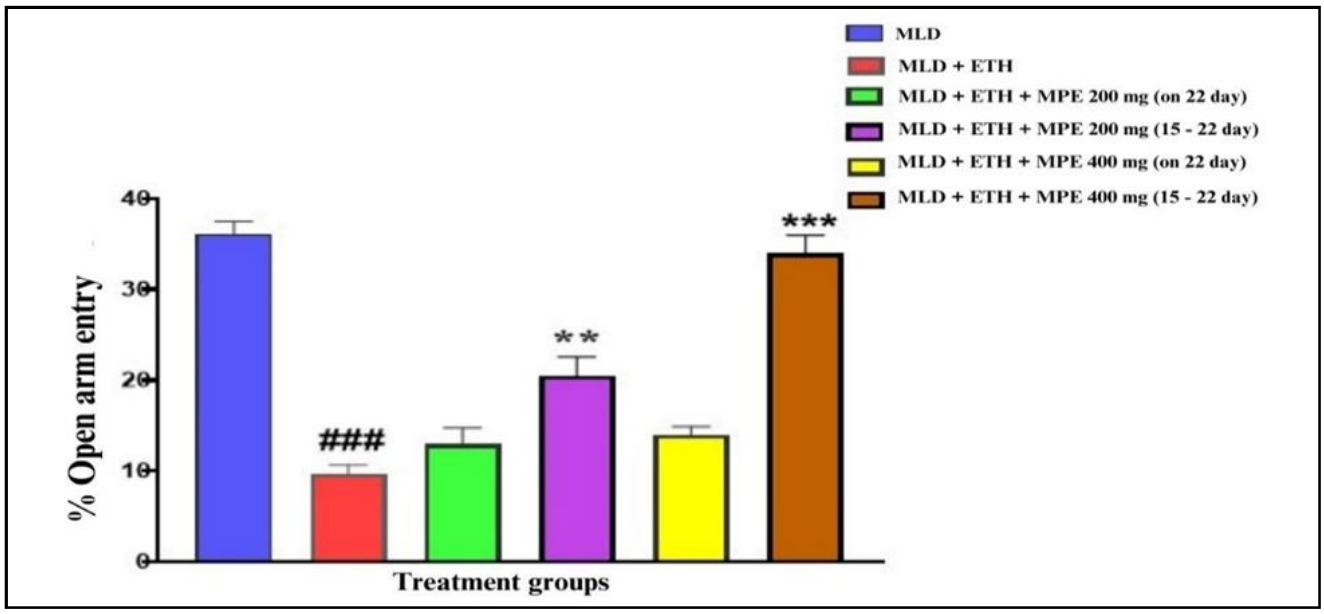


**Figure 2: The effect of different treatments of *M. pruriens* extract on % open arm entry in ethanol withdrawal induces anxiety. Every bar denotes the mean ± SEM (n = 6), ###$p$<0.001 when compared against the modified liquid diet group. **$p$<0.01 and ***$p$<0.001 when compared against ethanol-fed groups (MLD + Ethanol). Dunnett's test proceeds with a one-way ANOVA.**

### 3.3 Effect of MPE on anxiety behaviour

In contrast to the regular group on a liquid diet, the group fed ethanol (LD + Ethanol) exhibited a reduction in the number of entries and the duration spent in the open arms following 24 h of ethanol withdrawal. The treatment group received oral administration of *M. pruriens* extract at 200 and 400 mg/kg as part of the treatment. At 24 h of alcohol withdrawal, the group that received chronic therapy during the dependent period (from 15 to 21 days) exhibited an increase in time spent (Figure 1) and the number of entrances into the open arms (Figure 2). However, a single dose of therapy 30 min before 24 h of withdrawal does not affect anxiety-like behaviour.

### 3.4 Effect of MPE on locomotor activity

After 24 h of abstaining from ethanol, rats fed with ethanol exhibited hyperlocomotion activity compared to the normal group. Chronic therapy with *M. pruriens* extract (200 and 400 mg/kg, oral) for 15-21 days significantly reduced locomotor hyperactivity in the 24 h of alcohol withdrawal. However, acute treatment with a single dose 30 min before 24 h of alcohol abstinence does not affect hyperlocomotor activity (Figure 3).

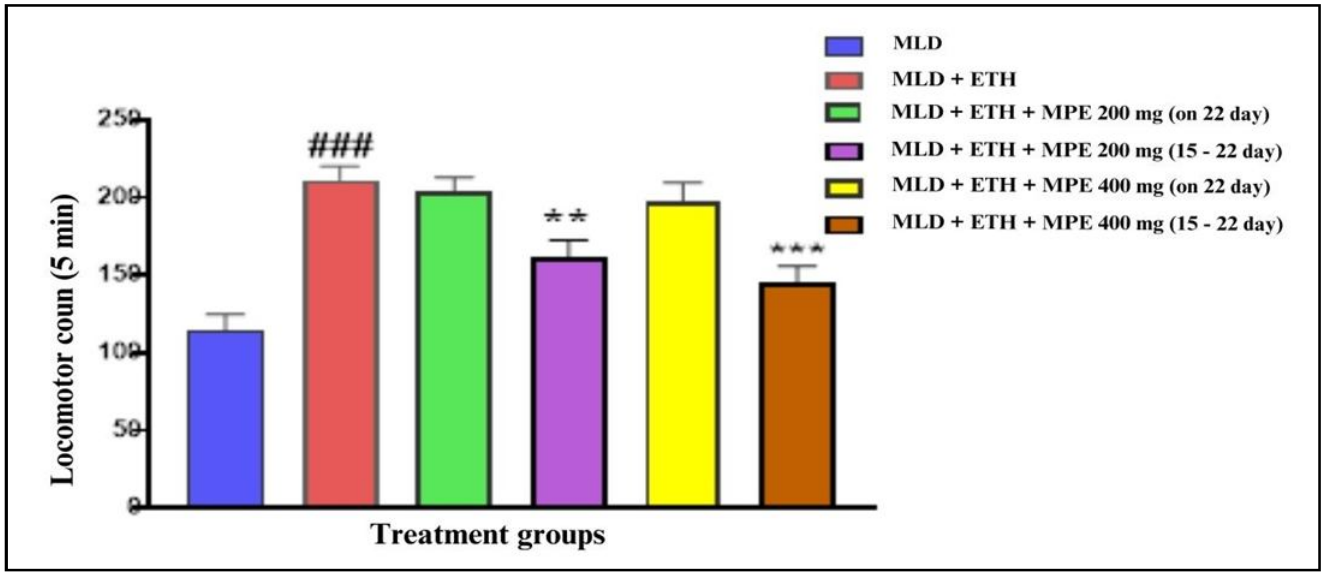


**Figure 3: The effect of different treatments of *M. pruriens* extract on ethanol withdrawal induces locomotor hyperactivity. Every bar denotes the mean ± SEM (n = 6), ###$p$<0.001 when compared against the modified liquid diet group. **$p$<0.01 and ***$p$<0.001 when compared against ethanol-fed groups (MLD + Ethanol). Dunnett's test proceeds with a one-way ANOVA.**

### 3.5 Effect of MPE on depressive behaviour

Figure 4 shows that after 15-21 days of chronic therapy with MPE (200 and 400 mg/kg) compared to the control, there is a significant (*$p$<0.05) reduction in immobility time dose-dependently. Similarly, acute treatment with a single dose 30 min before 24 h of ethanol withdrawal (200 and 400 mg/kg, p.o.) had no antidepressant effects.

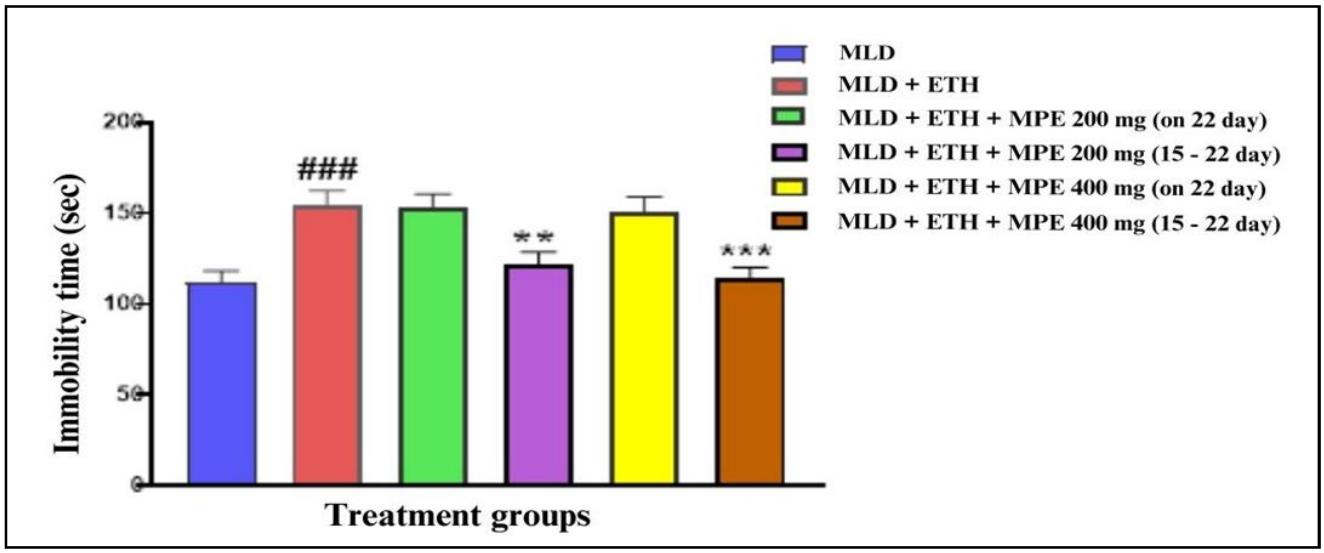


**Figure 4: The effect of different treatments of *M. pruriens* extract on ethanol withdrawal induces depression. Every bar denotes the mean ± SEM (n = 6), ###$p$<0.001 when compared against the modified liquid diet group. **$p$<0.01 and ***$p$<0.001 when compared against ethanol-fed groups (MLD + Ethanol). Dunnett's test proceeds with a one-way ANOVA.**

### 3.6 Effect of MPE on plasma corticosterone level

The plasma corticosterone level is elevated following 24 h of ethanol abstinence compared to the level before withdrawal. The administration of 200 and 400 mg/kg of chronic therapy for 15-20 days effectively reduced the high level of corticosterone in the blood rats experiencing ethanol withdrawal. Despite acute treatment with a single dose 30 min before 24 h of alcohol abstinence, it does not affect corticosterone levels (Table 4).

**Table 4: Different treatments of *M. pruriens* extract on ethanol withdrawal induce elevated plasma corticosterone levels. value shown as mean ± SEM (n=6), *$p$<0.05, in comparison against ethanol-fed groups (MLD + Ethanol)**

| Corticosterone in blood (ng/ml) | | |
|---|---|---|
| **Treatment groups** | **Before withdrawal** | **24 h after withdrawal** |
| MLD + Ethanol (1 ml/kg ip) | 58 ± 6.34 | 157 ± 4.51# |
| MLD + Ethanol + MPE (200 mg/kg p.o.) on day 22 | 55 ± 5.64 | 147 ± 6.11 |
| MLD + Ethanol + (MPE 200 mg/kg p.o) from day 15-22 | 47 ± 3.69 | 101 ± 8.41* |
| MLD + Ethanol + MPE (400 mg/kg p.o.) on day 22 | 50 ± 7.72 | 161 ± 8.81 |
| MLD + Ethanol + MPE (400 mg/kg p.o.) from day 15-22 | 42 ± 7.72 | 94 ± 6.37* |

## 4. Discussion

The findings of our investigation showed that the *M. pruriens* extract effectively suppressed ethanol withdrawal signs in rats. The administration of alcohol with a liquid diet is an appropriate and commonly used model for studying ethanol consumption in rats. Similar to prior research, our recent findings indicate that rats exposed to a daily dosage of around 8-13 g/kg/day for 15 days develop physical dependence (Uzbay *et al*., 2000). The current study found high blood ethanol levels (154.45 ± 4.58 mg/dl) before ethanol withdrawal. This suggests they consumed sufficient ethanol (8.16 ± 0.318to 12.49 ± 0.593 g/kg) to induce withdrawal signs.

Our laboratory's pilot investigations, together with previously released studies, have shown that the most pronounced behavioural and biochemical symptoms of ethanol withdrawal occur 24 h after the cessation of ethanol consumption. The study found that abstaining from ethanol resulted in increased locomotor activity, behavioural desperation, and behaviour resembling anxiety in the elevated plus maze test. Prior studies have demonstrated that the symptoms of anxiety and depression experienced during ethanol withdrawal in rodents contribute to the emergence of psychological dependency (Taksande *et al*., 2010). Negative reinforcement during ethanol withdrawal is considered acrucial factor contributing to ethanol dependency and reinstatement. Likewise, dysphoria, which is a sense of unease or dissatisfaction, frequently occurs after the cessation of addictive substances. This has been observed in various rodent models used to study depression (Frankowska *et al*., 2010). It has been documented that the negative moral state that occurs during abstinence

from ethanol is associated with the continuation of addiction (*i.e.*, ethanol self-administration to avoid withdrawal) (Koob, 2008).

*M. pruriens'* many pharmacological characteristics have prompted substantial research. Steroid glycosides, alkaloids, flavonoids, tannins, and others are phytochemicals. *M. pruriens* is also precursor of dopamine L-Dopa. This drug has anti-inflammatory, antistress, immunomodulatory, Parkinson's disease, anxiolytic and antidepressant benefits in rats without toxicity, according to several studies (Lampariello *et al*., 2012). The present investigation examined the influence of acute and chronic treatment with *M. pruriens* extract on hyperlocomotion, anxiety, and depressive signs in withdrawal. Our study found that chronic *M. pruriens* extract (200 and 400 mg/kg) administration (days 15-21) during ethanol exposure significantly prevented anxiety and depression-like behaviour in rats. Acute treatment with a single dosage of *M. pruriens* extract (200 and 400 mg/kg) before 30 min of the pre-withdrawal test found no effect on withdrawal symptoms. The disturbance in the HPA (hypothalamic-pituitary-adrenocortical axis) is a widely recognised occurrence during periods of stress. In rodents, an elevation in plasma corticosterone levels and humans, an increase in cortisol levels has been used to indicate stress-induced withdrawal and its severity (Sinha, 2022; Jyothilekshmi *et al*., 2022; Kotagale *et al*., 2018). Significantly, rodents that experienced ethanol withdrawal showed a notable increase in corticosterone levels, mitigated by administering *M. pruriens* extract. *M. pruriens* extract may have reduced withdrawal signs in rats by interacting with the central stress regulating circuit. The anti-stress impact and stability of HPA axis activity caused by *M. pruriens* extract may have contributed to its ability to suppress ethanol withdrawal symptoms.

## 5. Conclusion

The findings of our study demonstrated beyond a possibility that the administration of *M. pruriens* extract over an extended period had a beneficial effect on the symptoms of ethanol withdrawal like anxiety and depression. Based on the findings, the extract of *M. pruriens* may have the capability to prevent alcohol dependence and may also have the potential to minimise the likelihood of relapse. It is necessary to conduct additional research to identify and isolate the active components and evaluate their effectiveness in rodents.The study's findings offer compelling data that supports the potential of *M. pruriens* extract as an alternative treatment approach for addressing issues related to dependency on alcohol.

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## Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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