Tropical Journal of Pharmaceutical Research February 2025; 24 (2): 195-202
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v24i2.7

Original Research Article

Therapeutic potential of Thai *Mucuna pruriens* (T-MP) seed aqueous extract on acute ethanol-induced behavioral and motor dysfunction

Jirayut Kaewmor, Sirinapa Rungruang, Kutcharin Phunikhom, Jintana Sattayasai, Charshawn Lahnwong*
Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

For correspondence:* ***Email:* *sarala@kku.ac.th*

Sent for review: 12 July 2024 *Revised accepted: 15 February 2025*

Abstract

Purpose: *To assess the therapeutic potentials of Thai Mucuna pruriens (T-MP) seed aqueous extract in mitigating acute ethanol-induced behavioral and motor dysfunction in mice.*
Methods: *The mice were orally administered either water or T-MP seed extract (600 mg/kg). One hour after this initial treatment, the mice were given either distilled water or 6 g/kg of 30 % (w/v) ethanol. Thirty minutes following the second treatment, the mice were subjected to behavioral and motor function tests, comprising of the exploratory test, rotarod test, footprint analysis, elevated plus maze (EPM), and tail suspension test (TST), respectively.*
Results: *Ethanol treatment significantly increased anxiety-like behaviors, as evidenced by the exploratory and EPM tests, and depressive behavior, as indicated by prolonged immobility time in the TST ($p < 0.05$). It also reduced the time spent on the rod in the rotarod test and heightened gait abnormalities observed in gait analysis, indicating impaired motor functions. Treatment with T-MP significantly alleviated these ethanol-induced behavioral and motor dysfunctions ($p < 0.05$).*
Conclusion: *Thai Mucuna pruriens seed extract effectively mitigates neurological and behavioral dysfunctions induced by acute ethanol intoxication in mice, highlighting its potential as a neuroprotective agent. Further studies are required to elucidate T-MP's mechanisms in combating symptoms of acute ethanol intoxication, which is crucial for advancing medical and neuropharmacological treatments.*

Keywords: *Mucuna pruriens, Acute ethanol, Behavior, Motor function*

Tropical Journal of Pharmaceutical Research is indexed by Scopus, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Worldwide, the brain's susceptibility to alcohol, particularly ethanol found in beverages such as beer, wine, and liquors, presents significant health concerns. In contrast, other forms of alcohol are either toxic metabolites or used for non-consumable purposes. The World Health Organization reports that alcohol is associated with 3.3 million deaths yearly, about 6 % of global fatalities [1]. Ethanol, a potent neurotoxin, is rapidly absorbed into the bloodstream and transported to the central nervous system. It readily crosses the blood-brain barrier, exerting a range of effects that vary depending on the dosage. Ethanol alters the activity of various neurotransmitters, among them being an increased effect of Gamma-Aminobutyric Acid (GABA) through binding to a specific allosteric site on the GABA-A receptor complex, and an

inhibition of NMDA receptor activity, reducing the excitatory effects of glutamate. It enhances dopamine release in the nucleus accumbens by inhibiting GABAergic interneurons in the ventral tegmental area, contributing to a positive reinforcement effect [2]. Furthermore, ethanol-induced disruptions in the brain's oxidative balance may result in inflammation and extensive neuronal damage, leading to a range of effects from euphoria to severe motor and cognitive deficits. These changes further impact neuronal function, contributing to psychomotor depressive disorders, impairments in information storage and logical reasoning, as well as motor incoordination [3]. *Mucuna pruriens* (L.) DC., an established Ayurvedic herb, contains several vital compounds, comprising of the levodopa, flavonoids, and polyphenols [4]. The seed extract demonstrates various therapeutic potentials, encompassing neuroprotection against Parkinson's disease, antioxidant activity, antidepressant and anxiolytic effects, as well as anticonvulsant properties [5]. A local variety of *Mucuna pruriens* found in Thailand, Thai *Mucuna pruriens* (L.) DC. var. *pruriens* (T-MP), has long been recognized in traditional medicine for its diuretic, aphrodisiac, and wound-healing properties. Additionally, T-MP extract has shown the potential to prevent reproductive impairment, enhance sexual function [6]. Building on previous findings that demonstrated the protective effects of T-MP seed extract against prolonged ethanol-induced mood disorders [7], this study hypothesizes that T-MP may also exhibit protective potential against acute ethanol-induced disturbances. This suggests that T-MP could alleviate not only long-term neurobehavioral deficits but also mitigate the immediate neurological impairments associated with acute ethanol. Accordingly, this study aims to investigate hangover symptoms caused by acute ethanol intoxication, along with the potential neuroprotective and behavioral benefits of T-MP seed extract on acute ethanol-induced behavioral and motor dysfunction in mice.

EXPERIMENTAL

Animals

Male Mlac: ICR mice (weighing 25 – 35 g), aged between 6 – 8 weeks, were obtained from Khon Kaen University's Northeast Laboratory Animal Center, Thailand. The animals were housed in controlled conditions under a 12-hour light/dark cycle, at 22 ± 1 °C and relative humidity of 50 – 55 %. The mice received food and water ad libitum feeding. All animals received humane care, and all experiments followed the “Basic Principles for Animal Use” Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International and the study was approved by Khon Kaen University's Animal Research Ethics Committee (IACUC-KKU-42/65; Ref no. 660201.2.11/285 (48).

Plant material

The aqueous extract of T-MP seeds was generously provided by Professor Sitthichai Iamsaard, Department of Anatomy, Faculty of Medicine, Khon Kaen University, Thailand. The preparation according to the method described previously, the yield has been determined to be 16.29 % and has been verified to have antioxidant activity [6,8].

Acute ethanol treatment

Thirty-two male ICR mice were randomly divided into four groups (eight per group) through the peroral (PO) treatment: Group I: (Normal control, Con) mice received distilled water, Group II (Ethanol, EtOH) mice received 6 g/kg of 30 % w/v EtOH, Group III (Thai *M. pruriens*, T-MP) mice received T-MP seed extract (600 mg/kg), and Group IV (T-MP + EtOH) mice received a combination of 600 mg/kg T-MP extract and 6 g/kg of 30 % w/v EtOH. The treatment regimen consisted of administering distilled water or T-MP extract, followed by distilled water or ethanol one hour later. Behavioral assessments were performed 30 minutes after the second treatment and included the exploratory test, elevated plus maze (EPM), tail suspension test (TST), rotarod test, and gait analysis. The administration volume for all groups was standardized at 0.05 mL per 10 g of body weight. The doses of T-MP seed extract and ethanol were selected following previous studies [8,9].

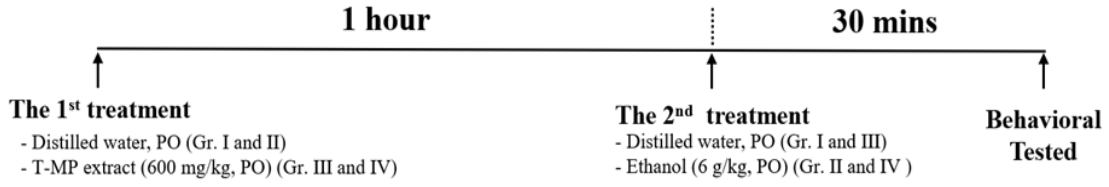

Figure 1: Experimental procedures to determine the effect of T-MP on acute ethanol exposure

Exploratory test

Rodent hyperactivity was assessed with a 40 × 40 cm white acrylic hole board surrounded by 25 cm high walls. The board was divided into 36 squares with black lines, each with a hole (1 cm in diameter and 1.5 cm deep) at the center. Over a 3-minute observation period, an observer recorded the duration of nose-poking behavior (defined as both eyes positioned within a hole), frequency of square crossings (requiring all four limbs to enter different squares), grooming (specifically, fur cleaning with the forepaws), and rearing (defined as standing on the hind legs or forelegs) [10].

Elevated plus maze (EPM)

EPM is a widely used test to evaluate anxiety-related behaviors in mice. It was constructed from wood, and it consisted of two opposing pairs of arms measuring 6 cm wide × 65 cm long, radiating from a central platform of 6 × 6 cm. The maze was elevated 50 cm above the ground. Each mouse was placed at the maze center facing an open arm, and its activity was observed for 5 minutes. The time used in open arms (in seconds) and the frequency of arm entries were documented. An increase in the time spent in the open arms and/or the frequency of open-arm entries was interpreted as indicative of reduced anxiety [11].

Tail suspension test (TST)

The TST is a widely used method for evaluating behavioral despair, depression-like behavior, and learned helplessness in rodents, as well as for evaluating the efficacy of antidepressant treatments. In this test, each mouse was suspended by the tail in a plastic tail suspension box, with the tail secured to a swing suspended from above. The period of immobility, defined as the absence of escape-oriented behaviors, was recorded over a 5-minute duration. A longer immobility time is considered indicative of a more pronounced depressive-like [12].

Rotarod test

The rotarod test assesses the balance and motor coordination of rodents. Mice were acclimatized to the apparatus during one or two sessions before the test day. The test involved mice walking on a rotating rod, 25 cm off the ground and 2.5 cm in diameter, at 12 rotations per minute. Observers recorded the duration each mouse remained on the rod in 1 min, with a full minute indicating optimal motor coordination and balance, while shorter times indicated motor deficits [13].

Footprint analysis

Footprint analysis was employed to assess ataxia and gait abnormalities. Mice were allowed to walk down a 60-cm long, 7-cm wide runway enclosed by 10-cm high walls. Their forepaws and hind paws were painted blue and red, respectively, and a white paper was placed at the start and black at the end to encourage movement. The paper was then removed, and the footprint pattern, focusing on the middle six steps, was analyzed. The following parameters were measured in the six middle steps: forelimb stride length, hindlimb stride length, stride width, and overlap of left and right paws. An ataxic gait is characterized by a significant increase in stride lengths and width and increased distance between the overlap of the left and right paw [14].

Statistical analysis

Data analysis was conducted using IBM SPSS Statistics for Windows, Version 29.0 (IBM Corp., 2023), licensed to Khon Kaen University. All data are presented as the mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by a post-hoc Tukey's test was used to compare the effects of acute ethanol intake among experimental groups. A two-tailed Student's *t*-TEST was employed where appropriate.

A *p-value* of less than 0.05 was considered statistically significant.

RESULTS

Anxiolytic effects of T-MP seed extract against acute EtOH-induced anxiety in mice

Figure 2 illustrates the effects of T-MP on acute ethanol-cause anxiety-like symptoms in the exploratory test. EtOH treatment significantly increased locomotor activity, as evidenced by a marked increase in the number of crossings (Figure 2 A) and a concomitant decrease in rearings and nose pokes (Figure 2 B and C) compared with the control group. No significant differences were observed between the T-MP group and the control, indicating that T-MP did not exert any intrinsic effects on exploratory activity. However, co-administration of T-MP with EtOH significantly reduced the number of crossings, suggesting a potential mitigating effect of T-MP on EtOH-induced hyperactivity. Notably, the number of rearing and nose pokes in the T-

MP + EtOH group did not differ significantly from the control group (Figure 2 B and C).

Figure 3 illustrates the T-MP effect on acute ethanol intoxication in mice during an EPM test. The EtOH group exhibited heightened anxiety, characterized by a significant reduction in the time spent in the open arms (Figure 3 A), fewer open arm entries (Figure 3 B), and an increase in entry to the closed arm (Figure 3 C) compared to the control group. In contrast, T-MP treatment alone did not produce significant differences from the control group in any of the measured parameters. Moreover, the combination of T-MP with EtOH induced significantly longer times in the open arms (Figure 3 A) and fewer entries in the closed arms (Figure 3 C) compared to the EtOH group, suggesting a potential anxiolytic effect of T-MP under ethanol-induced anxiogenic conditions. However, no significant differences in the number of entries into the open arm between the T-MP and ethanol group and the controls (Figure 3 B).

Antidepressant-like activity of T-MP seed extract in response to acute-EtOH exposure

Figure 4 illustrates that acute EtOH exposure induced depressive-like behaviors in TST, as evidenced by a significant increase in immobility time compared to the control group. The T-MP-treated exhibited immobility times comparable to the control, indicating that T-MP alone did not influence depressive-like behaviors. Notably, co-administration of T-MP with EtOH resulted in a significant reduction in immobility time compared to the EtOH group, suggesting that T-MP may mitigate the depressive-like effects associated with acute EtOH exposure.

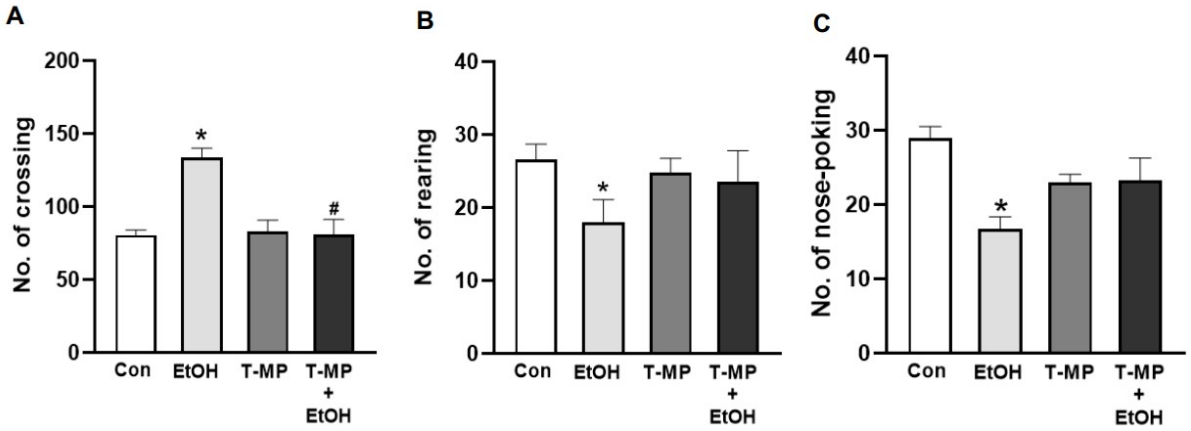

Figure 2: Effect of acute ethanol intoxication on exploratory behavior in mice. (A) The number of crossings, (B) number of rearing, and (C) number of nose-poking. Data are presented as the mean ± SEM. (*p < 0.05 vs control group, and $^{\#}p$ < 0.05 vs EtOH group)

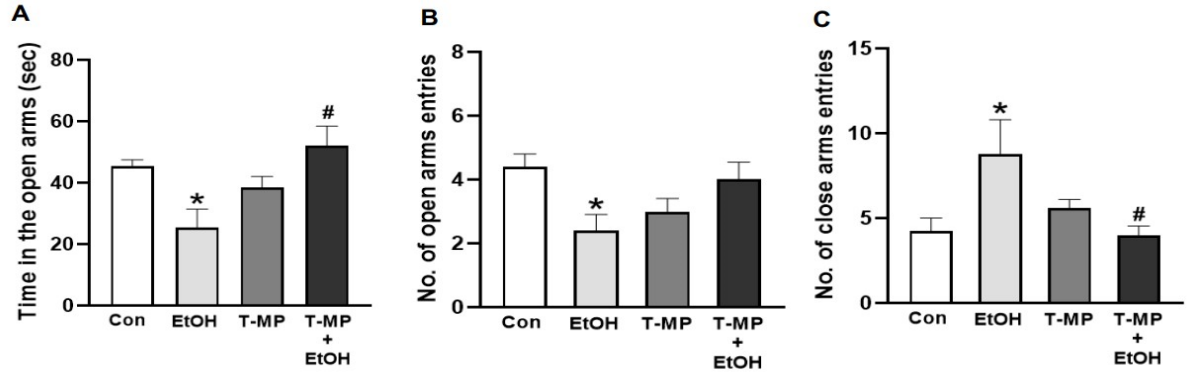

Figure 3: Effect of acute ethanol intoxication on Elevated plus maze in mice. (A) Time in the open arms (sec), (B) The number of open arm entries, and (C) The number of closed arm entries. Data are presented as mean ± SEM. (*p < 0.05 vs control group, and $^{\#}p$ < 0.05 vs EtOH group)

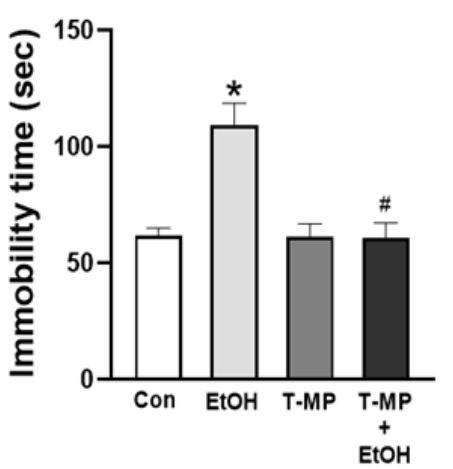

Figure 4: Effect of acute ethanol intoxication on tail suspension test in mice, represented the immobility time (sec). Data are presented as mean ± SEM. (*p < 0.05 vs control group, and $^{\#}p$ < 0.05 vs EtOH group

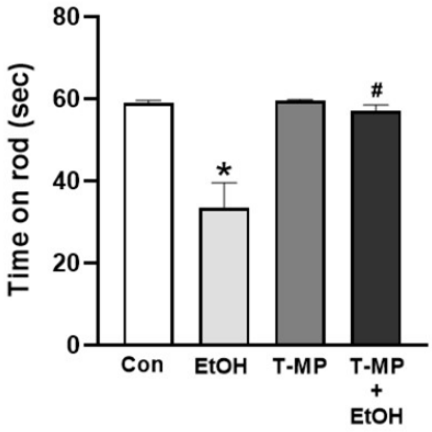

Figure 5: Effect of acute ethanol intoxication on the rotarod test in mice. The figure represented the Time on rod (sec). Data are presented as mean ± SEM. (*p < 0.05 vs control group, and $^{\#}p$ < 0.05 vs EtOH group)

T-MP seed extract ameliorates acute EtOH-induced motor coordination impairment

In the rotarod test (Figure 5), control mice demonstrated normal coordination, maintaining balance on the rotarod for more than 60 seconds. In contrast, EtOH-intoxicated mice exhibited significant motor incoordination, resulting in a marked reduction in time spent on the rod compared to the control group. The T-MP-treated group did not differ significantly from the control, indicating that T-MP alone did not impair motor function. Interestingly, Co-administration of T-MP with EtOH significantly improved motor coordination, as evidenced by increased time on the rod compared to the EtOH group.

The footprint analysis (Figure 6), EtOH exposure resulted in gait abnormalities, characterized by increased stride lengths of the forelimbs and hindlimbs (Figure 6 C), increased hindlimb stride width (Figure 6 D), and an increased overlap between left and right limbs (Figure 6 E) compared to the control group, indicating impaired motor coordination. T-MP treatment alone did not significantly differ from the control group, suggesting no inherent effect on gait. In contrast, the step patterns of animals treated with T-MP with EtOH showed significant improvement in hindlimb stride width compared to the EtOH group (Figure 6 D), suggesting the beneficial effect of T-MP on ethanol-induced motor dysfunction. However, no significant differences in stride length or limb overlap were observed between the T-MP + EtOH-treated mice and the control group (Figure 6 C and E).

DISCUSSION

The findings demonstrate that acute ethanol intoxication (6 g/kg of 30 % w/v) significantly enhanced anxiety-like behaviors, as indicated by increased locomotor activity and decreased exploratory actions, such as nose-poking and rearing, in an exploratory test. These changes suggest heightened nervousness and reduced curiosity. In the EPM test, ethanol exposure significantly reduced the time spent in the open arms and the number of entries, further indicating anxiety-related behaviors. In the TST, ethanol exposure resulted in a marked increase in immobility time, reflecting depressive-like states. The Motor function was also impaired, as shown by reduced endurance on the rotarod test and deteriorated gait characteristics during footprint analysis. These results suggest that ethanol effectively simulates hangover symptoms, encompassing both motor dysfunction and mood disturbances, including anxiety and depression. Treatment with T-MP seed extract exhibited potent anxiolytic and antidepressant properties, fully ameliorating the behavioral and motor deficits observed. This recovery underscores the therapeutic potential of T-MP in mitigating the adverse effects of acute ethanol exposure, likely due to bioactive components, such as phenolic compounds, which may counteract oxidative stress and restore normal neurological function.

As with previous reports, the data showed that acute exposure to ethanol produced a dose-dependent influence on animal locomotion such that lower doses amplified and higher doses diminished movement [15]. It also affected changes in rearing behavior and was associated with learning and memory deficits as well as ataxia and motor incoordination.

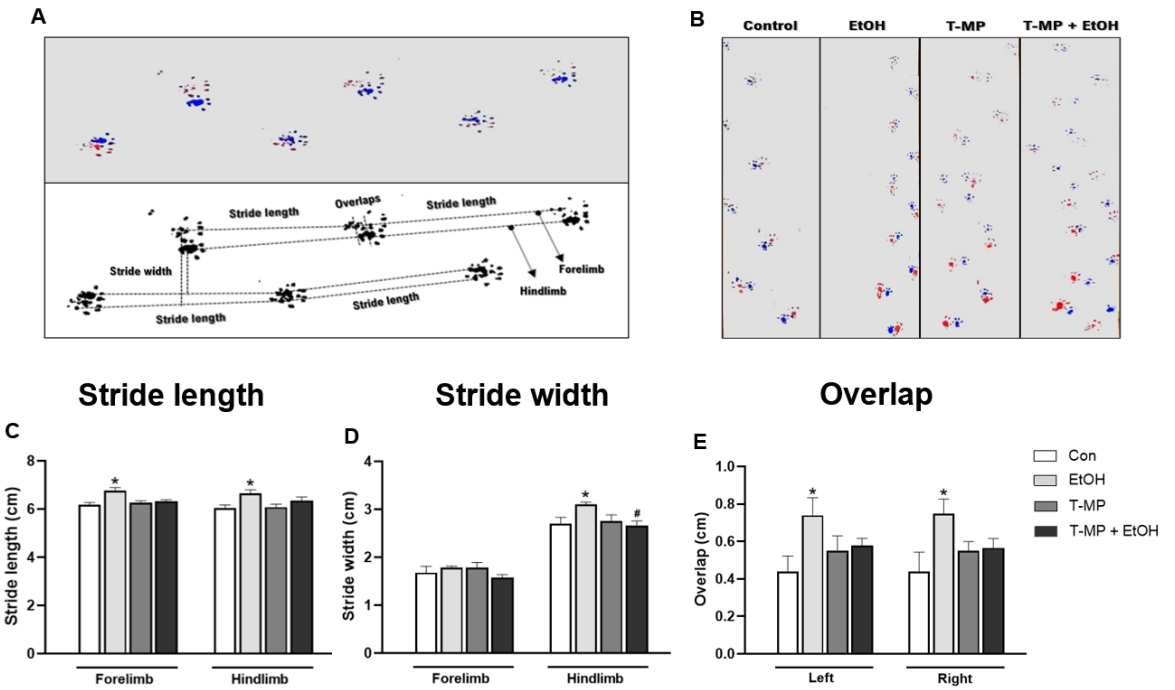

Figure 6: Acute ethanol intoxication effected on footprint analysis in mice. (A) Parameters were observed in the footprint analysis, with dotted lines indicating the direction of progression. (B) Representative examples of mouse footprints were recorded on paper from each group. (C) Forelimb and hindlimb stride length. (D) Forelimb and hindlimb stride width (E) Overlap between left and right limbs. Data are presented as mean ± SEM. (*$p < 0.05$ vs control group, and $^{\#}p < 0.05$ vs EtOH group)

Ethanol disrupts synaptic plasticity by potentiating inhibitory GABA-A receptor activity and attenuating the effects of glutamate on NMDA receptors, resulting in anxiety-related effects. Depressive symptoms, including sedation and loss of the righting reflex, become particularly pronounced 30 minutes after ethanol administration, highlighting ethanol's substantial neurological impact [16]. Additionally, ethanol impairs the brain's oxidative balance, leading to increased oxidative stress characterized by elevated reactive oxygen species (ROS) generation [3].

The data underscores the idea that T-MP seed extract may modulate the behavioral effects of acute ethanol intake, potentially through its potent antioxidant properties. The results also demonstrate that even a single dose of ethanol causes motor dysfunction, as evidenced by performance deficits in the rotarod test. These impairments are likely due to ethanol's adverse effects on cerebellar function and neuronal activity within the cerebellar cortex [17]. Furthermore, ethanol exposure resulted in depressive-like behaviors, characterized by increased immobility time, which were significantly mitigated by T-MP treatment, indicating its antidepressant-like properties, and interestingly The higher doses of ethanol have been shown to exacerbate depressive symptoms, including sedation and ptosis [16]. The study also confirmed that acute high-dose ethanol exposure leads to ataxia and gait disturbances, reinforcing the concept that ethanol intake causes cerebellar ataxia, manifesting as impaired motor coordination and balance.

This study demonstrated that the T-MP seed extract effectively mitigated anxiety-like and depressive-like behaviors, motor incoordination, and gait abnormalities caused by acute ethanol exposure. These results align with prior findings on the anxiolytic, antidepressant, and antioxidant properties of T-MP seeds [5,7]. Notably, the extract's ability to reduce oxidative stress—a key contributor to ethanol-induced brain damage and inflammation—was particularly significant. Ethanol-induced oxidative stress is known to impair neuronal function and behavior, highlighting the role of T-MP in restoring oxidative balance in the brain [3]. The protective effects of T-MP are attributed to its high phenolic content, including isoflavones, flavonoids, and tannins, which are recognized for their neuroprotective properties [4]. Furthermore, glutathione, a key component of T-MP seeds, likely contributed to maintaining neuronal health by neutralizing oxidative damage caused by reactive oxygen species such as superoxide anions and hydrogen peroxide [18]. These findings underscore the therapeutic potential of T-MP phenolic compounds in counteracting the detrimental effects of ethanol on neurological and

behavioral functions. However, to fully understand the intricate mechanisms behind these protective effects, further detailed study is necessary.

Overall, this study highlights the properties through which T-MP exerts a protective effect against hangover symptoms induced by acute ethanol intoxication. The phenolic compounds present in T-MP, known for their potent antioxidant activities, play a key role in alleviating oxidative stress, a major contributor to ethanol hangover symptoms. This study, therefore, underscores the potential of T-MP phenolic components in counteracting the negative effects of high ethanol doses, including anxiety- and depressive-like behaviors as well as motor dysfunctions affecting neurological and behavioral functions. However, to fully elucidate the complex mechanisms by which these phenolic compounds provide protection, further detailed investigations are warranted.

CONCLUSION

Administration of 30 % w/v ethanol at 6 g/kg induced anxiety-like behaviors in the exploratory and EPM tests, as well as depressive-like states in TST. Motor coordination deficits were evident in the rotarod and footprint analysis tests, highlighting the extensive neurological and behavioral effects of ethanol exposure. Administration of T-MP seed extract counteracted these effects, highlighting its potential in preventing ethanol hangover symptoms. Further studies are required to elucidate T-MP's mechanisms in combating symptoms of acute ethanol intoxication, which is crucial for advancing medical and neuropharmacological treatments.

DECLARATIONS

Acknowledgement/Funding

The author wishes to express gratitude to Professor Dr. Sitthichai Iamsaard from the Department of Anatomy, Faculty of Medicine at Khon Kaen University, for generously providing the T-MP seed extract specimen used in this study.

Financial support for this project was provided by the Faculty of Medicine at Khon Kean University, Thailand, under grant number IN65244. Additionally, Jirayut Kaewmor received funding through a Postgraduate Study Support Grant from Faculty of Medicine at Khon Kean University, Thailand.

Ethical approval

This study was approved ethically by the Institutional Animal Care and Use Committee (IACUC) at Khon Kaen University, Thailand.

Availability of data and materials

The datasets utilized and/or analyzed in the current study are available from the corresponding author upon reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

The authors affirm that this study was carried out by the listed authors, and all liabilities related to claims concerning the content of this article will be assumed by them. The study was conceptualized and designed by CL, JS, and KP. Data collection and experimentation were conducted by JK and SR. Data analysis was performed by JK, JS, and CL. The manuscript was written and revised by JK, JS, and CL. All authors read and approved the final draft of the manuscript for publication.

REFERENCES

1. *Singal AK, Bataller R, Ahn J, Kamath PS, Shah VH. ACG clinical guideline: alcoholic liver disease. Am J Gastroenterol 2018; 113(2): 175–194.*
2. *Clapp P, Bhave SV, Hoffman PL. How adaptation of the brain to alcohol leads to dependence. Alcohol Res Health 2008; 31(4): 310–339.*
3. *Baliño P, Romero-Cano R, Sánchez-Andrés JV, Valls V, Aragón CG, Muriach M. Effects of acute ethanol administration on brain oxidative status: the role of acetaldehyde. Alcohol Clin Exp Res 2019; 43(8): 1672–1681.*
4. *Upadhyay P. Phytochemistry and pharmacological activity of Mucuna pruriens: A review. Int J Green Pharm 2017; 11(02).*
5. *Patil RA. Anxiolytic, antidepressant and anticonvulsant activity of Mucuna pruriens seeds. JMPAS 2021; 10(4): 3479–3483.*
6. *Iamsaard S, Arun S, Burawat J, Yannasithinon S, Tongpan S, Bunsueb S, Lapyuneyong N, Choowong-In P, Tangsrisakda N, Chaimontri C, et al. Evaluation of antioxidant capacity and reproductive toxicity of aqueous extract of Thai Mucuna pruriens seeds. J Integ Med 2020; 18(3): 265–273.*
7. *Kaewmor J, Rungruang S, Phunikhom K, Sattayasai J, Lahnwong C. Anti-anxiety and anti-depressive effects of*

Thai Mucuna pruriens seed aqueous extract against ethanol withdrawal syndrome in mice. Phytomed Plus 2024; 4(4): 100630.

8. *Choowong-In P, Sattayasai J, Boonchoong P, Poodendaen C, Wu AT, Tangsrisakda N, Sawatpanich T, Arun S, Uabundit N, Iamsaard S. Protective effects of Thai Mucuna pruriens (L.) DC. var. pruriens seeds on sexual behaviors and essential reproductive markers in chronic unpredictable mild stress mice. J Tradit Complement Med 2022; 12(4): 402–413.*
9. *Supawat A, Srisuwan S, Sattayasai J. Oral glutamate intake reduces acute and chronic effects of ethanol in rodents. Trop J Pharm Res 2016; 15(7): 1493–1498.*
10. *Johansson AK. Novelty seeking and harm avoidance in relation to alcohol drinking in intact rats and following axon-sparing lesions to the amygdala and ventral striatum. Alcohol Alcoholism 2002; 37(2): 147–156.*
11. *Polissidis A, Zelelak S, Nikita M, Alexakos P, Stasinopoulou M, Kakazanis ZI, Kostomitsopoulos N. Assessing the exploratory and anxiety-related behaviors of mice. Do different caging systems affect the outcome of behavioral tests? Physiol Behav 2017; 177: 68–73.*
12. *Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacol 1985; 85: 367–370.*
13. *Soares PM, Patrocínio MCA, Assreuy AMS, Siqueira RCL, Lima NM, Arruda MDOV, Escudeiro SDS, de Carvalho KM, Sousa FCF, Viana GSB, et al. Aminophylline (a theophylline–ethylenediamine complex) blocks ethanol behavioral effects in mice. Behavioural Pharmacol 2009; 20(4): 297–302.*
14. *Karadayian AG, Cutrera RA. Alcohol hangover: type and time-extension of motor function impairments. Behav Brain Res 2013; 247: 165–173.*
15. *López-Cruz L, Salamone JD, Correa M. The impact of caffeine on the behavioral effects of ethanol related to abuse and addiction: a review of animal studies. J Caffeine Res 2013; 3(1): 9–21.*
16. *Papanicolaou J, Fennessy MR. The acute effect of ethanol on behaviour, body temperature, and brain histamine in mice. Psychopharmacol 1980; 72(1): 73–77.*
17. *Matsunaga W, Shinoe T, Hirono M. GAD65 deficient mice are susceptible to ethanol-induced impairment of motor coordination and facilitation of cerebellar neuronal firing. PLoS One 2023; 18(5): e0286031.*
18. *Dhanasekaran M, Tharakan B, Manyam BV. Anti-Parkinson drug - Mucuna pruriens shows antioxidant and metal chelating activity: antioxidant and metal chelating activity of Mucuna pruriens. Phytother Res 2008; 22(1): 6–11.*