นิพนธ์ต้นฉบับ . Original Article

ผลของสารสกัดด้วยน้ำจากเชื้อรา *Polycephalomyces Nipponicus* ต่ออารมณ์และการประสานงานการเคลื่อนไหวในหนูถีบจักรที่ได้รับเอทานอลเฉียบพลัน

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Effects of *Polycephalomyces Nipponicus* Aqueous Extract on Mood and Motor Coordination in Acute Ethanol-Treated Mice

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บทคัดย่อ

หลักการและวัตถุประสงค์: การดื่มเอทานอลทำให้การประสานงานของกล้ามเนื้อและการตัดสินใจผิดพลาด ซึ่งเป็นสาเหตุของการเกิดอุบัติเหตุและเสียชีวิต ในปัจจุบันนักวิทยาศาสตร์ได้ให้ความสนใจในพืชสมุนไพรที่มีสารกลุ่มโพลีฟีนอล ซึ่งมีศักยภาพในการลดผลกระทบที่เป็นพิษและอาการเมาค้างจากการบริโภคเอทานอลเฉียบพลัน *Polycephalomyces nipponicus* (*P. nipponicus*) ซึ่งเป็นเชื้อราที่ทำให้เกิดโรคในแมลงได้แสดงศักยภาพที่โดดเด่นในฐานะแหล่งของสารต้านอนุมูลอิสระและสารต้านจุลชีพตามธรรมชาติ และมีปริมาณฟีนอลและฟลาโวนอยด์สูง ดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อประเมินผลของสารสกัดจาก *P. nipponicus* ต่อผลของเอทานอลแบบเฉียบพลันในการการป้องกันเหนี่ยวนำให้เกิดความผิดปกติทางอารมณ์และความบกพร่องทางการเคลื่อนไหวในหนูถีบจักร

วิธีการศึกษา: หนูถีบจักรเพศผู้ สายพันธุ์ ICR ถูกแบ่งออกเป็น 6 กลุ่ม กลุ่มละ 8 ตัว ได้แก่ กลุ่มควบคุม กลุ่มที่ได้รับเอทานอล (2 กรัม/กก.) กลุ่มที่ได้รับสารสกัดจาก *P. nipponicus* (200, 600 มก./กก.) และกลุ่มที่ได้รับสารสกัดจาก *P. nipponicus* (200, 600 มก./กก.) ก่อนได้รับเอทานอล โดยหนูถูกป้อนครั้งแรกด้วยน้ำหรือสารสกัด *P. nipponicus* (200 หรือ 600 มก./กก.) หลังจากนั้นหนึ่งชั่วโมง หนูถูกป้อนครั้งที่สองด้วยน้ำหรือเอทานอล (2 กรัม/กก.) หลังจากการป้อนครั้งที่ 2 30 นาที หนูจะถูกทดสอบด้วย exploratory test, tail suspension test (TST), rotarod test และ footprint analysis ตามลำดับ

ผลการศึกษา: การได้รับเอทานอลทำให้จำนวนการเดินข้ามช่อง (crossings) การยืนบนขาหลัง (rearing) และจุ่มจมูก (nose-poking) ลดลงอย่างมีนัยสำคัญในการทดสอบพฤติกรรมการสำรวจ (exploratory test) ซึ่งบ่งชี้ถึงพฤติกรรมคล้ายกับอาการวิตกกังวล และเพิ่มระยะเวลาการอยู่นิ่ง (immobility time) ในการทดสอบ TST ซึ่งบ่งชี้ถึงพฤติกรรมคล้ายภาวะซึมเศร้า การทดสอบ rotarod แสดงให้เห็นว่าระยะเวลาการทรงตัวบน rod (time on rod) ลดลงและพบความผิดปกติของการเดินจากการวิเคราะห์รอยเท้าหนูในกลุ่มที่ได้รับเอทานอลซึ่งแสดงถึงความบกพร่องของการทำงานของกล้ามเนื้อ การรักษาด้วย *P. nipponicus* โดยเฉพาะในขนาด 600 มก./กก. ช่วยบรรเทาผลของเอทานอลในการเหนี่ยวนำความผิดปกติทางอารมณ์และความบกพร่องทางการเคลื่อนไหวได้อย่างมีนัยสำคัญ

สรุป: ผลการศึกษาชี้ให้เห็นว่าสารสกัดจาก *P. nipponicus* สามารถบรรเทาผลของเอทานอลแบบเฉียบพลันในการเหนี่ยวนำพฤติกรรมคล้ายกับอาการวิตกกังวลและภาวะซึมเศร้า รวมถึงความบกพร่องทางการเคลื่อนไหวได้

คำสำคัญ: *Polycepholomyces nipponicus*, เอทานอล, ความผิดปกติทางอารมณ์, ความบกพร่องทางการเคลื่อนไหว, หนูถีบจักร

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Abstract

Background and Objective: Acute ethanol drinking leads to impaired motor coordination and judgment, which is one of the common causes of many accidents and deaths. Presently, many scientists have attracted medicinal plants containing polyphenols that have shown potential in alleviating the toxic and hangover effects of acute ethanol consumption. *Polycephalomyces nipponicus* (*P. nipponicus*), an insect pathogenic fungus has demonstrated notable potential as a source of natural antioxidants and antimicrobial compounds and contains high total phenolic and flavonoid content. Therefore, the present study aimed to evaluate the effects of *P. nipponicus* extract on acute protective effects of ethanol-induced mood disorders and motor impairments in mice.

Methods: Male ICR mice were divided into 6 groups of 8 animals per group.: normal control, ethanol (2 g/kg), *P. nipponicus* extract (200, 600 mg/kg), and pretreated *P. nipponicus*, extract groups (200, 600 mg/kg). Before receiving ethanol, Mice were orally administered either water or *P. nipponicus* extract 200 or 600 mg/kg. One hour after, the mice were given either water or ethanol (2 g/kg). Thirty minutes following the second treatment, mice were subjected to exploratory test, tail suspension test (TST), rotarod test and footprint analysis.

Results: Ethanol treatment significantly decreased number of crossings, rearing and nose-poking in exploratory test indicating anxiety-like behavior, and increased immobility time in tail suspension test (TST) indicating depressive-like behavior. And then the rotarod test showed a decrease of time on the rod and an increase in gait abnormalities as observed in gait analysis, revealing the impairment of motor functions. *P. nipponicus* treatment, especially at 600 mg/kg, significantly alleviated the effects of ethanol in inducing mood disorders and motor impairments.

Conclusion: The results suggested that *P. nipponicus* extract could mitigate the acute effects of ethanol in inducing anxiety and depressive-like behaviors and motor impairments.

Keywords: *Polycephalomyces nipponicus*, ethanol, mood disorder, motor impairment, mice

Introduction

Ethanol is the most commonly used recreational beverage and drug of abuse among the adult population, ethanol-related death is the third leading cause of death for more than 3.3 million global deaths annually[1]. Ethanol uses disorders linked to substance abuse, acute anxiety, sleeplessness, and depressive episodes[2]. These conditions can severely impact the individual daily life, functioning and sometime serious enough to require treatment. It has been known that ethanol can affect several neurotransmitter systems within the brain and disrupting the delicate balance between inhibitory and excitatory neurotransmitters[3]. In addition, acute ethanol administration also affects the brain's cellular oxidative status and causes unbalance cellular oxidative homeostasis[4].

Presently, many scientists have attracted medicinal plants containing polyphenols that have shown potential in alleviating the toxic and hangover effects of acute ethanol consumption[5] . Many studies suggest that polyphenols found in medicinal plants, with theirs antioxidant properties, may reduce oxidative stress and also increase the activities of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), which are enzymes critical for metabolizing ethanol in the liver[6,7]

P. nipponicus, an insect pathogenic fungus found in the Northeastern area of Thailand, has demonstrated notable potential as a source of natural antioxidants and antimicrobial compounds, and contains high total phenolic and flavonoid content[8]. Recent studies exhibited that *P. nipponicus* has been suggested to possess preventative properties against breast cancer[9] and antibacterial activity[10]. However, the effect of *P. nipponicus* on ethanol consumption has not been studied. So, this study aimed to investigate the potential of *P. nipponicus* extract in protecting against mood disorders and motor impairment in mice acutely treated with ethanol.

Materials and methods

Animals

In this study, male outbred Mlac: ICR mice, 6-8 weeks, weighing 25-35 g, were purchased from the Northeast Laboratory Animal Center at Khon Kaen University, Thailand. The animal has received ad libitum access to standard rodent chow and water under a 12-hour light: 12-hour dark cycle (lights on at 06.00 am) with controlled temperature and humidity. Mice were allowed to acclimate in a designated room under standard housing conditions for 1 week before beginning the experiment. All animal experiments were strictly in accordance with international ethics. The experimental protocols were also approved by the Animal Research Ethics Committee, Khon Kaen University, Thailand; record number IACUC-KKU 53/2565, reference number 660201.2.11/366.

Plant extraction

The mycelia of ***P. nipponicus*** isolate Cod-MK1201 were identified and extracted as previously described[10]. In summary, the fungal mycelium of the ***P. nipponicus*** isolate was collected, dried at 50 °C overnight, and then ground into powder with a mortar and pestle. The dried powdered mycelium had been mixed with sterile distilled water (100 mg/ml), sonicated, centrifuged, and filtered through a 0.2-μm filter before use.

Chemicals and reagents

The absolute ethanol (Merck, Germany) and blue and red inks (Horse Blue Endorsing Ink, Thailand) were used as a chemical for foot printing. Absolute ethanol was diluted with distilled water to make 30% W/V solution and fed the animal with a dose of 0.05 ml per 10 g (2g/kg) of mice body weight[11].

Experimental protocol

Male ICR mice were divided randomly into six groups, with eight mice in each group. Animals were received 2 treatments, with one hour apart, by forced feeding. The treatments were either distilled water-distilled water (Control), 2 g/kg ethanol+ distilled water, 200 mg/kg *P. nipponicus* extract ± distilled water, 600 mg/kg *P. nipponicus* extract -distilled water, 200 mg/kg *P. nipponicus* extract ± 2 g/kg ethanol or 600 mg/kg *P. nipponicus* extract±2 g/kg ethanol. Thirty minutes after the second treatment, assays were performed, including exploratory test, tail suspension test, rotarod test and footprint analysis.

Exploratory test

The exploratory test was used to evaluate anxiety-like behavior. If the mice show these more behaviors, then they are less anxious. On the other hand, if the mice don't show or less show these behaviors, then it is more anxious. The exploratory test apparatus was a white acrylic hole board (78 × 78 cm; walls 29 cm high). Black lines divide the floor into 16 squares with a hole in each square (1 cm in diameter). Mice were placed in the middle of the apparatus and allowed to adapt to the test environment for 3 min. During the next 3 min, the number of squares crossed (with all four limbs), nose-pokes (both eyes disappearing in the hole) into the holes and rearing (to rise on the hind or fore legs) were recorded[12]. The apparatus was cleaned with 70% ethanol after finishing each test.

Tail suspension test (TST)

The tail-suspension test was used for assessment of depressive-like behavior. The total amount of immobility time is measured for each mice and considered as an index of "depression-like" behavior. The tail suspension box was made of plastic sheet (55 height * 15 width * 20 cm depth). The mice were wrapping with adhesive tape around the tail in a constant position (three quarters of the distance from the base of the tail) at the suspension bar. The total amount of immobility times was recorded for 5 min interval (interval from 1 min)[13].

Rotarod test

Rota-rod test was used to assess motor coordination and balance in mice. The AccuRotor Rota Rod (Accuscan Instruments, Columbus, OH) was used for fixed-speed rota-rod tests. Mice were placed with the forepaws on the bar (diameter 2.5 cm) which are turning at 12 revolutions per minute and recorded the time that animal remained on the bar. Any mice that stay on the rod for the full 60-sec trial is allocated a maximum value of 60 sec for analysis. In the analysis, the mean latency to fall for each mouse was analyzed. The bar was cleansed with 70 % ethanol after each test[14].

Footprint analysis

Footprint analysis was used to assess ataxia and gait abnormalities in mice. By turning on a lamp at the beginning and inserting a dark box at the end of a 60 cm long, 7 cm wide (with 10 cm walls) runner lined with white paper. The fore and hind paws of mice were dipped in red and blue harmless paints, respectively, to record their footprints. The middle 6 steps were analyzed for fore/hindlimb stride length (FSL and HSL) and fore/hindlimb stride width (FSW and HSW)[15] (Figure 1).

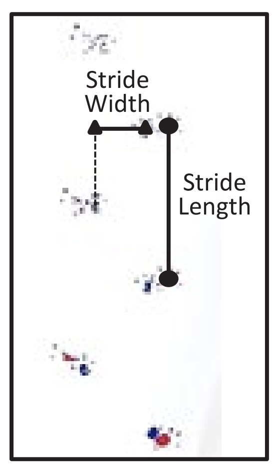

Figure 1 Measurement of stride length and stride width in footprint analysis.

Statistical analysis

The data were presented as mean ± standard error of mean (SEM). Data were analyzed by one-way ANOVA followed by Tukey test. Results were considered significant when the p-value of less than 0.05 was considered statistically significant. Graph Pad Prism software version 9.5 was used to evaluate all results.

Results

Effect of *P. nipponicus* extract on ethanol-induced anxiety-like behavior.

The effects of *P. nipponicus* extract on ethanol-induced anxiety-like behavior in the exploratory test were shown in Figure 2. In the exploratory test, the normal control group showed normal locomotor activity. At 30 min after forced fed with 2 g/kg ethanol, mice showed anxiety-like behavior as seen by a significant ($p < 0.05$) decrease in the number of crossings, rearing and nose-poking when compared to the control group. Mice forced fed with *P. nipponicus* alone showed no significant differences in all parameters observed when compared to the control. Pretreatment with *P. nipponicus* 1h before ethanol, especially at 600 mg/kg, could significantly reduce the effects of ethanol on either number of crossings, rearing and nose-poking, and all parameters were comparable to the ethanol group.

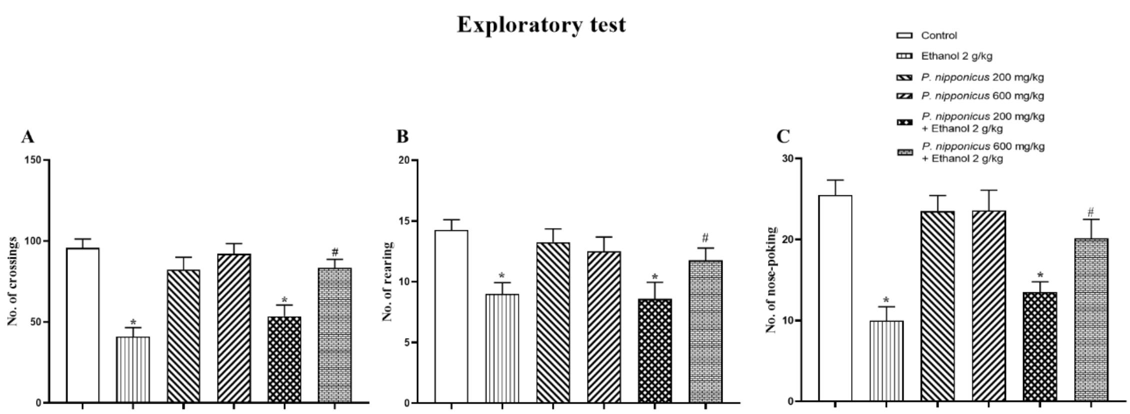

Figure 2 The effects of *P. nipponicus* extract on ethanol-induced anxiety-like behavior in exploratory test; A: number of crossings, B: number of rearing and C: number of nose-poking, in acute ethanol-treated mice. Data were expressed as mean ± SEM (n = 8). * $p < 0.05$ as compared to the control, # $p < 0.05$ as compared to the ethanol alone. The statistical analysis was performed by one-way ANOVA followed by Tukey test.

Effect of *P. nipponicus* extract on ethanol-induced depressive-like behavior.

The effects of *P. nipponicus* extract on ethanol-induced depressive-like behavior in TST were shown in Figure 3. Mice treated with *P. nipponicus* extract had immobility time comparable to the control, in which suggested that *P. nipponicus* at the doses 200 or 600 mg/kg did not have any anti-depressant activity. At 30 min after forced fed with ethanol, mice showed depressive-like behavior as seen by a significant increase in immobility time when compared to the control. Pretreatment with *P. nipponicus* 1h before ethanol, especially at 600 mg/kg, could significantly reduce the effects of ethanol as seen by an increase in the immobility time to the level comparable to the ethanol group.

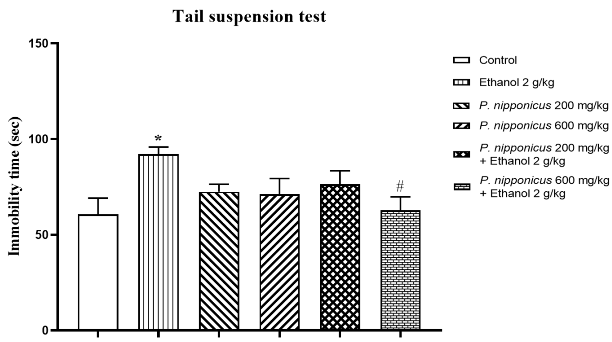

Figure 3 The effects of *P. nipponicus* extract on ethanol-induced depressive-like behavior in TST. Data were expressed as mean ± SEM (n = 8). * $p < 0.05$ as compared to the control, # $p < 0.05$ as compared to the ethanol alone. Statistical analysis was performed by one-way ANOVA followed by Tukey test.

Effect of *P. nipponicus* extract on ethanol-induced motor coordination impairment.

In the rotarod test, the control mice showed normal coordination and maintained a balance on the rod for over 60 sec. Mice, forced fed with ethanol 2 g/kg, showed motor coordination impairment as seen by a significant reduction in time on rod when compared to the control (Figure 4). Mice that received 200 or 600 mg/kg *P. nipponicus* extract had normal coordination and could stay on the rod for over 60 sec, suggesting that *P. nipponicus* alone did not impair motor function. And mice that received 200 or 600 mg/kg *P. nipponicus* with ethanol 2 g/kg exhibited improved coordination by significantly increasing the time on the rod compared to the ethanol group.

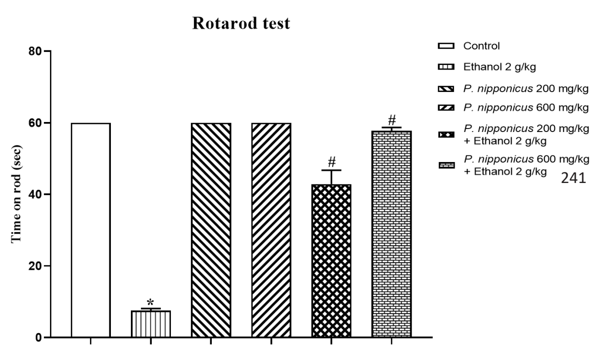

Figure 4 Effect of *P. nipponicus* extract on ethanol-induced motor coordination impairment on rotarod test. Data were expressed as mean ± SEM (n = 8). * $p < 0.05$ as compared to the control, # $p < 0.05$ as compared to the ethanol alone. Statistical analysis was performed by one-way ANOVA followed by Tukey test.

Figure 5 showed footprint patterns, gait patterns (Figure 5A) and hindlimb footprint patterns (Figure 5B), of the control and ethanol-treated mice. Footprint analysis showed that the control mice walk with a narrow-based stance with close proximity forelimb and hindlimb footprints with 6.30 ± 0.48 and 6.18 ± 0.49 cm of FSL and HSL, respectively. No significant different could be seen in either FSL, HSL, FSW or HSW among all treatments. However, animals treated with 2 g/kg ethanol, walked on their whole paw while the control used only the front parts of the paws (Figure 5B). Pretreatment with either 200 or 600 mg/kg *P. nipponicus* 1h before ethanol could reverse the effects of ethanol on hindlimb footprint patterns.

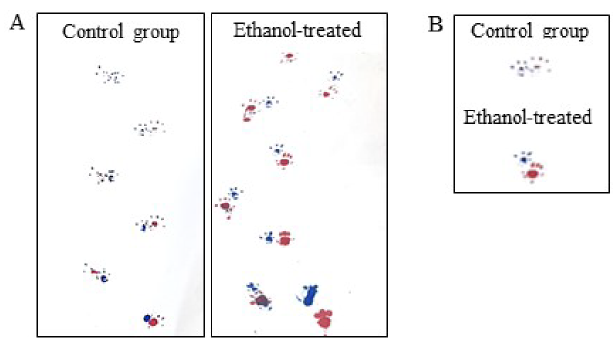

Figure 5 Footprint patterns of the control and ethanol-treated mice. The forelimb and hind were colored red and blue, respectively. A: Gait patterns, B: Hindlimb footprint patterns.

Discussion

The results of this study demonstrate that pretreatment with *P. nipponicus* extract could reduce the effect of acute ethanol treatment in inducing mood disorders, including anxiety- and depressive-like behaviors, and motor impairments.

In this study, it is clearly seen that the animals treated with 2 g/kg ethanol showed anxiety- and depressive-like behaviors and motor impairments. In motor assessment, although the significant change in stride length and width could not be seen, the abnormal walking patterns were observed in ethanol group. Ethanol-treated mice walked on their whole paws and produced toe dragging which were not seen in the controls, as reported earlier[16,17]. Acute ethanol consumption disrupts the delicate balance between inhibitory and excitatory neurotransmission in favor of inhibitory influences by facilitates the GABAergic transmission, and inhibits glutamatergic function[18]. Previous studies showed that motor impairment and depressive effect of ethanol might cause by enhancing the inhibitory action of GABA through GABA-A receptor and inhibition of ionotropic glutamate receptors including NMDA, AMPA and kainate types, with NMDA type receptors being the highest affinity targets for ethanol in the CNS[16,17,19]. In addition to the effects of ethanol to inhibitory GABAergic and excitatory glutamatergic systems, many other neurotransmitters/neuromodulators were reported to be molecular targets of ethanol, including dopamine and corticotropin-releasing factor[20]. Recently, it has been reported that acute and/or chronic ethanol exposure during adolescence disturbs the homeostasis of the adenosine modulation system in the brain, contributing to hazardous symptoms related to anxiety and depression[21]. Ethanol also induces oxidative stress in motor areas of the brain through the action of the enzyme CYP2E1 and increases toxic acetaldehyde level as byproduct of ethanol metabolism. These pathways result in the impairment of psychomotor functioning, including drowsiness, ataxia, and motor incoordination[22,23].

It has been shown that *P. nipponicus* mycelia had strong DPPH and ABTS radicals scavenging activities with quite high total phenolic and flavonoid contents[24]. In addition, it has been reported recently that 2 compounds, cordytropolone and leptosphaerone A, were presented in the culture broth of the fungus *P. nipponicus*[25]. It is interesting to note that cordytropolone isolated from *Polycephalomyces phaothaiensis* exhibit anti-inflammatory effect and reduce the production of many pro-inflammatory cytokines, including PGE2, IL-6, and TNF-α production, as well as iNOS, COX2, and NF-κB protein expression[26]. It might be possible that the active polyphenolic compounds and cordytropolone in *P. nipponicus* extract help reduce the oxidative stress, inflammatory cytokines which then help restore homeostasis of neurotransmitters/neuromodulators in the brain and reduce ethanol-induced mood disorders and motor impairments.

Various natural compounds, especially polyphenols from plants were reported to be able to increase hepatic metabolism of ethanol by activate the activities of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) enzymes[27]. It might be interesting to see whether *P. nipponicus* extract has any effect on these enzyme activities.

Conclusion

The results of this study suggest that *P. nipponicus* extract has the potential to reduce the effect of acute ethanol administration in inducing either mood disorders and motor impairments. However, the exact mechanisms need to be further investigated.

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