บทความวิจัย

ผลของสารสกัดจากใบกระท่อมในสัตว์ทดลองที่ชักนำให้มีการเสพติดสุรา
Effects of the Extract from Kratom (Mitragyna specioca) Leaves on Alcohol-induced Dependent Animals

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บทคัดย่อ

ผู้ป่วยโรคพิษสุราเรื้อรัง (alcoholism) ส่วนใหญ่มักประสบปัญหาจากกลุ่มอาการถอนแอลกอฮอล์ โดยเฉพาะการทำงานมากเกินกว่าปกติของระบบประสาทส่วนกลาง กลุ่มยาต้านอาการซึมเศร้าที่มีผลเพิ่มระดับซีโรโทนินในระบบประสาทส่วนกลางพบว่าสามารถนำมาใช้รักษาอาการเหล่านี้ได้ผล และจากการศึกษาที่ผ่านมาพบว่าพืชกระท่อมมีสรรพคุณคล้ายกลุ่มยาต้านอาการซึมเศร้า ดังนั้นการศึกษาครั้งนี้จึงมีวัตถุประสงค์เพื่อศึกษาผลของสารสกัดอัลคาลอยด์จากพืชกระท่อมต่ออาการถอนเอทานอล หลังจากกสลบหนูขาวใหญ่สายพันธุ์วิสต้าเพื่อฝัง electrode และชักนำให้ติดเอทานอลแล้ว ในวันที่ถอนเอทานอลสัตว์ทดลองจะได้รับสารสกัดอัลคาลอยด์จากพืชกระท่อมก่อนการถอนเอทานอล หลังจากนั้นจะทำการบันทึกคลื่นไฟฟ้าสมอง เพื่อวิเคราะห์ช่วงเวลาที่ตื่นตัว และวิเคราะห์ช่วงความถี่ theta ซึ่งจะบ่งบอกถึงสภาวะ CNS hyperexcitation ผลการศึกษาพบว่าการถอนเอทานอลมีผลเพิ่มช่วงเวลาตื่นตัวและเพิ่มพลังงานในช่วงความถี่ theta ส่วนสารสกัดหยาบอัลคาลอยด์จากพืชกระท่อมมีผลบรรเทาการเพิ่มขึ้นของพลังงานในช่วงความถี่ theta ที่ถูกชักนำโดยการถอนเอทานอลได้

คำสำคัญ: อาการถอนเอทานอล กระท่อม คลื่นไฟฟ้าสมอง

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จากงานประชุมวิชาการและเสนอผลงานวิจัยมหาวิทยาลัยทักษิณ ครั้งที่ 21 ประจำปี 2554

Abstract

Most of alcoholics have been found to suffer ethanol withdrawal symptoms especially central nervous system (CNS) and behavioral hyperactivity when they give up ethanol consumption. Antidepressants that increase serotonin levels in the CNS were successfully used to treat these symptoms. Previously, Mitragyna speciosa korth. (MS) exhibited antidepressant-like activity, so this study was aimed to study effects of MS alkaloid extracts on ethanol withdrawal. Male Wistar rats were anesthetized for electrode implantation and rendered dependent on ethanol. Then, ethanol was removed to induce ethanol withdrawal symptoms. Crude MS alkaloid extract was given to animals before ethanol withdrawal. Electroencephalography (EEG) was used to record brain activity. Total awake period and theta band wave during withdrawal peroid were analyzed to indicate the state of CNS hyperexcitation. The results showed that total awake period and EEG power of theta wave from frontal and parietal cortices were increased by ethanol withdrawal. However, the increase of theta powers were attenuated by crude MS alkaloid extract.

Keywords: Ethanol withdrawal, Mitragyna speciosa, EEG

INTRODUCTION

Hyperexcitability of the central nervous system (CNS) has been a major problem that underlies most of ethanol withdrawal symptoms. It has been regularly found when ethanol consumption is reduced or completely stops [1] Consequently, many behaviors emerge as result of cortical excitation. Overactivation of NMDA-type glutamate receptors [2] and suppression of $GABA_A$ receptor activity [3] were found during early phase of ethanol withdrawal. Central monoaminergic systems also play important roles in the development of some ethanol withdrawal symptoms. Serotonergic dysfunction has been observed during ethanol withdrawal period [4-5] Some of previous studies found that serotonin levels were decreased in various brain regions of rat brain during chronic ethanol consumption and ethanol withdrawal [6-7] There might be a correlation between decreased serotonergic activity and ethanol withdrawal symptom. Thus, enhancement of serotonergic function by selective serotonin reuptake inhibitors (SSRIs) could be effective in treatment of ethanol withdrawal.

Many drugs have been applied to prevent against the ethanol withdrawal syndrome. Most common drugs popularly used to treat ethanol withdrawal syndrome are anxiolytic [8] and antidepressant groups. However, the use of anxiolytics such as benzodiazepines has been found to develop dependence and sedative actions by themselves [9-10]

Mitragyna speciosa Korth. (MS) is a native tropical plant mainly found in Southeast Asian countries particularly in Thailand and Malaysia. This plant belongs to the family Rubiaeceae. It is traditionally used to

treat diarrhea, fever, asthma and coughing. In addition, it was also used to substitute for opium or morphine in the treatment program. Farmers and laborers often chew fresh leave or smoke the dry leaves of MS to increase work efficiency and tolerance of hard work [11] Over 22 indole alkaloids from the MS have been isolated including mitragynine a main constituent (~66.2% based on the crude extract) [12] Most studies have focused on pure mitragynine and its antinociceptive action on the brain trough μ- and δ-opioid receptor [13-14] In addition, it was found that noradrenergic and serotonergic systems were involved in the antinociceptive effect of mitragynine. Moreover, 7-hydroxymitragynine, a minor constituent, possess antinociceptive effects with high affinity for opioid receptors and its potency was 30- and 17-fold higher than those of mitragynine and morphine, respectively. [15-16]

Previously, antidepressive activity of the MS extracts was demonstrated by using behavioral studies. Its action may be partially mediated through stimulations of serotonergic [17-18] and HPA axis pathways [19] These findings have brought the idea of MS application for ethanol withdrawal treatment. Until recently, a pilot study in our laboratory has already shown that aqueous extract from the MS attenuated some ethanol withdrawal induced-specific behaviors. [18]

This study utilized EEG recording technique that would allow quantifying the severity levels of ethanol withdrawal and the effectiveness of treatment. Sleep disturbance has been found during ethanol withdrawal [20] EEG signals were also used for analysis of wakefulness period to evaluate severity of ethanol withdrawal and treatment.

Materials and Methods

Extraction and isolation of alkaloids from the plant were described in a previous study [21] The solution of the MS alkaloid extract was prepared by dissolving the extract in co-solvent (Tween80: propylene glycol: H_2O at a 1:4:4 ratio) and adjusted to desired concentrations with distilled water. Adult male Wistar rats (300-350 g) were anesthetized with intramuscular injection of 60 mg/kg Zoletil® 100 (Virbac, Thailand Co. Ltd.). Stainless steel screw electrodes were stereotaxically implanted in the frontal cortex (AP; +3, ML; 3) and the parietal cortex (AP; -4, ML; 4) on the left side skull (Fig. 1 A – C). Electrodes placed at midline overlying the cerebellum were used as reference and ground electrodes. All electrodes were secured in place with acrylic resin (Unifast trad, Japan).

To induce ethanol-dependent, 7-10 days after surgery, the animals were housed individually and given a modified liquid diet (MLD) containing ethanol, at a progressively increasing concentrations (from 2.4 % for 3 days, to 4.8 % for 4 days, to 7.2 % for 21 days) to induce dependence as previous described [22] Then, animals were divided into 3 groups; 1) ethanol non-withdrawal, 2) ethanol withdrawal and 3) ethanol

withdrawal with 60 mg/kg MS alkaloid extract. On the testing day, the ethanol containing MLD was replaced with isocaloric ethanol-free MLD to induce withdrawal. Before the testing day, animals were acclimatized with recording condition for 4 times. The dimension of recording chamber was 40x40x40 cm^3.

Fifteen minutes before ethanol withdrawal, ethanol dependent animals received oral administration with either vehicle (control) or MS alkaloid. Then, EEG signals of individual rats were recorded for 8-h period. EEG signals were amplified with a low-pass 60 Hz, high-pass 0.1s and digitized at 400 Hz by a PowerLab/4SP system (AD Instruments) with 12-bit A/D, and stored in a PC through the Chart program software. The EEG signals were processed through 1.25 – 45 Hz bandpass filter. The digitized EEG data were segmented into 1024-point (50% overlap) and the signals were converted to power spectra by the fast Fourier transform algorithm (Hanning window cosine transform). Then the power spectra of 2.56-sec sweeps in each 5-min length of selected period of artifact-free signals were averaged to give the power spectra of the period. Awake periods were summed to reflect arousal levels. EEG power of theta wave (4 – 8 Hz) was also analyzed to represent CNS hyperactivity. It has been found that theta power was produced during hyperac tive period. The powers of theta band (4-8 Hz) of each group were averaged and expressed as mean ± S.E.M.

One-way analysis of variance (ANOVA) followed by Student-Newman-Keuls method was used for statistical analysis. The differences were considered statistically significant at $p < 0.05$.

The experimental protocols for care and use of experimental animals described in the present study were approved and guided by the Animals Ethical Committee of the Prince of Songkla University, Hat Yai, Songkhla, Thailand.

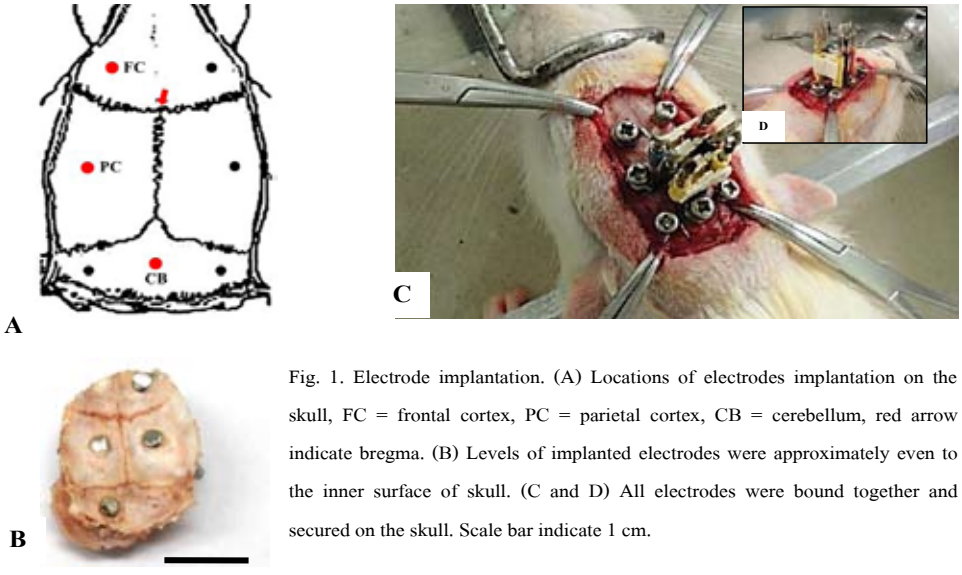


Fig. 1. Electrode implantation. (A) Locations of electrodes implantation on the skull, FC = frontal cortex, PC = parietal cortex, CB = cerebellum, red arrow indicate bregma. (B) Levels of implanted electrodes were approximately even to the inner surface of skull. (C and D) All electrodes were bound together and secured on the skull. Scale bar indicate 1 cm.

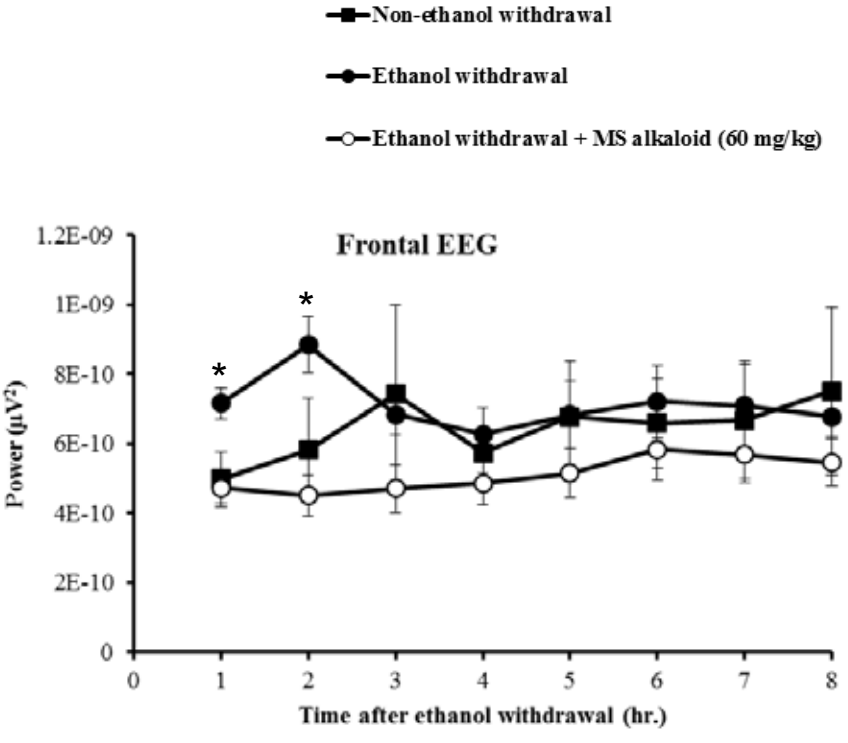
RESULTS AND DISCUSSION

Effects of ethanol withdrawal and treatment with MS alkaloid (60 mg/kg) were analyzed from EEG signal recorded for 8-h period following ethanol withdrawal. Theta powers were increased in ethanol withdrawal group compared to those of non-ethanol withdrawal in frontal ($F(23, 119) = 2.59$, $p < 0.001$) (Fig. 2 A) and parietal cortices ($F(23, 119) = 10.27$, $p < 0.001$) (Fig. 2 B). However, these increases were significantly prevented by the MS alkaloid.

In addition, ethanol withdrawal significantly increased total awake period ($F(2, 14) = 21.28$, $p < 0.001$) and treatment with MS alkaloid did not prevent this effect (Fig. 3).

This study showed CNS hyperactivity in terms of theta power and total awake period increases following ethanol withdrawal. Attenuation of ethanol withdrawal-induced increase of theta power may indicate effectiveness of MS alkaloid treatment. By the way, this MS alkaloid did not affect the induced awake period. Previously, this MS alkaloid was found to mediate CNS effect at least partly via serotonin enhancement.[23-24] Thus, the MS alkaloid may promote wakefulness and could not reduce total awake period-induced by ethanol withdrawal.

However, this extract reduced CNS hyperactivity during ethanol withdrawal period. Although wakeful ness was not attenuated by the MS alkaloid extract, this treatment may be considered at least mentally effective.



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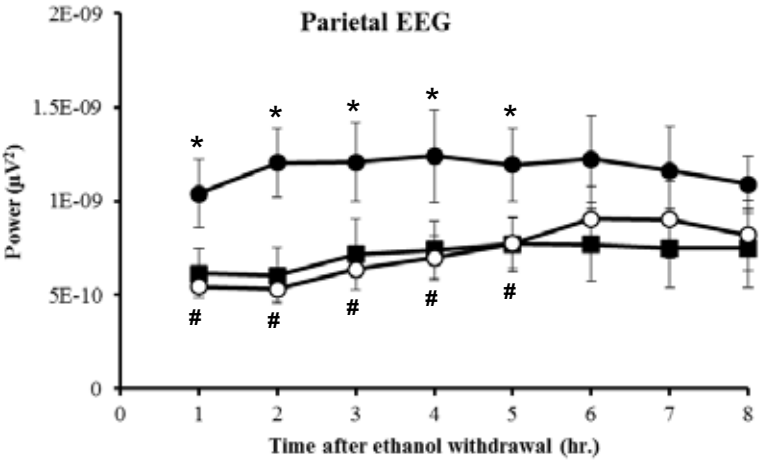

Fig. 2. Theta power from frontal (A) and parietal (B) cortices following ethanol withdrawal. Values (mean ± S.E.M.) were calculated from non-ethanol withdrawal, ethanol withdrawal and ethanol withdrawal + MS alkaloid (60 mg/kg) * = non-ethanol withdrawal vs ethanol withdrawal and # = ethanol withdrawal and ethanol withdrawal + MS alkaloid (60 mg/kg).

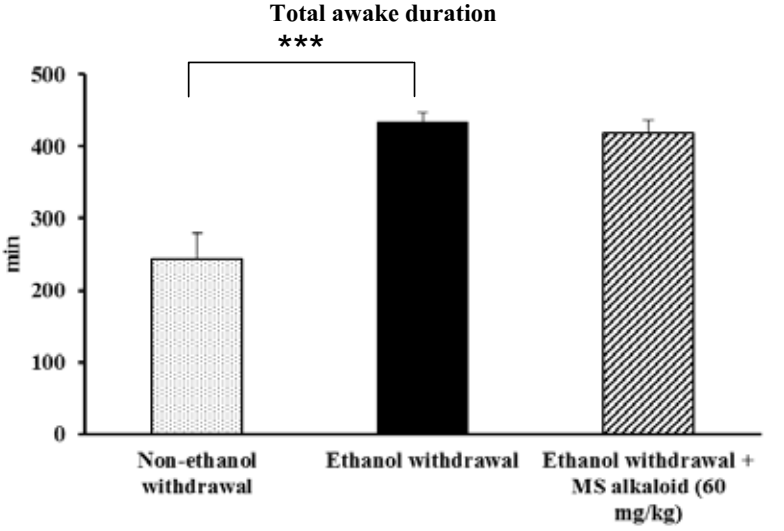

Fig. 3. Summation of awake period following ethanol withdrawal. Values (mean ± S.E.M.) were calculated from non-ethanol withdrawal, ethanol withdrawal and ethanol withdrawal + MS alkaloid (60 mg/kg). *** = $p < 0.001$

CONCLUSIONS

Theta powers from frontal and parietal cortices were increased by ethanol withdrawal. Treatment with the MS alkaloid extract at 60 mg/kg was found to reverse the increase of these theta powers. These findings suggest therapeutic potential of this plant for treatment of alcoholism.

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