ฤทธิ์ต้านซึมเศร้าของสารสกัดโสม จี115 ในหนูเมาส์ที่ได้รับเอทานอล

นางสาววีรวรรณ บุญเลิศ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชวิทยา ภาควิชาเภสัชวิทยาและสรีรวิทยา
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2557

ANTIDEPRESSIVE EFFECT OF GINSENG EXTRACT G115 IN ETHANOL-TREATED MICE

Miss Weerawan Boonlert

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Pharmacology
Department of Pharmacology and Physiology
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Thesis Title	ANTIDEPRESSIVE EFFECT OF GINSENG EXTRACT G115 IN ETHANOL-TREATED MICE
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Field of Study	Pharmacology
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วีรวรรณ บุญเลิศ : ฤทธิ์ต้านซึมเศร้าของสารสกัดโสม จี115 ในหนูเมาส์ที่ได้รับเอทานอล (ANTIDEPRESSIVE EFFECT OF GINSENG EXTRACT G115 IN ETHANOL-TREATED MICE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: อ. ภญ. ดร.รัชนี รอดศิริ, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ. ดร.จริยา อำคา เวลบาท, 62 หน้า.

ผู้ป่วยที่ติดแอลกอฮอล์และเป็นโรคซึมเศร้าจากการลดลงของระดับ brain-derived neurotrophic factor (BDNF) และการเพิ่มขึ้นของระดับ cortisol การใช้ยาต้านซึมเศร้าในการรักษาผู้ติดแอลกอฮอล์ที่มีอาการซึมเศร้าร่วมด้วยนั้นมีประสิทธิภาพไม่แน่นอนและอาจเกิดปัญหาจากผลไม่พึงประสงค์และอันตรกิริยาของยา มีรายงานการศึกษาก่อนหน้าถึงฤทธิ์ต้านซึมเศร้าของสารสกัดจากโสมและ ginsenosides ในสัตว์ทดลอง งานวิจัยนี้จึงมีวัตถุประสงค์ที่จะทดสอบฤทธิ์และกลไกการต้านซึมเศร้าของสารสกัดโสม จี115 ในหนูเมาส์ที่ได้รับแอลกอฮอล์ หนูเมาส์กลุ่ม water-treated จะได้รับ sterile water (10 มล./กก.), amitriptyline (10 มก./กก.) หรือ สารสกัดโสม จี115 (100, 200, 400 หรือ 800 มก./กก.) โดยการป้อน 1 ชั่วโมงก่อนฉีด sterile water (10 มล./กก.) เข้าทางช่องท้อง ส่วนหนูเมาส์กลุ่ม ethanol-treated จะได้รับ sterile water (10 มล./กก.), amitriptyline (10 มก./กก.) หรือ สารสกัดโสม จี115 (100, 200, 400 หรือ 800 มก./กก.) โดยการป้อน 1 ชั่วโมงก่อนฉีดเอทานอล (3 ก./กก.) เข้าทางช่องท้อง ให้สารดังกล่าววันละ 1 ครั้งเป็นเวลา 8 วันติดต่อกัน ทำการทดสอบพฤติกรรมซึมเศร้าในหนูเมาส์โดยใช้ force swimming test และพฤติกรรมการเคลื่อนที่ 1 วันหลังจากการให้สารครั้งสุดท้าย จากนั้นเก็บสมองและเลือดเพื่อนำไปวัดระดับ BDNF ในสมองและระดับ corticosterone ในซีรัม ผลการทดลองพบว่าหนูเมาส์ที่ได้รับเอทานอล แสดงพฤติกรรมซึมเศร้าและพบการลดระดับของ BDNF ใน hippocampus และ prefrontal cortex แต่ไม่พบการเปลี่ยนแปลงระดับ corticosterone ในซีรัม ในหนูกลุ่มที่ได้รับน้ำพบว่าสารสกัดโสม จี 115 (100, 200 และ 800 มก./กก.) ลด immobility time ใน forced swimming test สารสกัดโสม จี115 (100 และ 200 มก./กก.) เพิ่มระดับ BDNF ใน hippocampus และสารสกัดโสม จี115 (800 มก./กก.) เพิ่มระดับ corticosterone ในซีรัมซึ่งแสดงว่าสารสกัดโสม จี115 ต้านซึมเศร้าในหนูปกติได้โดยการเพิ่มระดับ BDNF ใน hippocampus และระดับ corticosterone ในซีรัม ผลการทดลองในหนูเมาส์ที่ได้รับเอทานอล พบว่าสารสกัดโสม จี115 (100, 200 และ 800 มก./กก.) มีฤทธิ์ต้านซึมเศร้าใน forced swimming test นอกจากนี้พบว่า สารสกัดโสม จี115 (100, 200, 400 และ 800 มก./กก.) เพิ่มระดับ BDNF ใน hippocampus และสารสกัดโสม จี115 (200 และ 800 มก./กก.) เพิ่มระดับ BDNF ใน prefrontal cortex แต่สารสกัดโสม จี115 ไม่มีผลลดระดับ serum corticosterone ในหนูเมาส์ที่ได้รับเอทานอล งานวิจัยนี้เป็นครั้งแรกที่พบว่าสารสกัดโสม จี115 มีฤทธิ์ต้านซึมเศร้าในหนูที่ได้รับเอทานอล ซึ่งกลไกการต้านซึมเศร้าเกิดจากการเพิ่มระดับ BDNF ที่ hippocampus และ prefrontal cortex การวิจัยนี้ให้ข้อมูลทางวิทยาศาสตร์ที่สนับสนุนการใช้สารสกัดโสม จี115 สำหรับต้านซึมเศร้าที่เกิดจากการได้รับแอลกอฮอล์

ภาควิชา เภสัชวิทยาและสรีรวิทยา ลายมือชื่อนิสิต .....

สาขาวิชา เภสัชวิทยา ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2557 ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

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WEERAWAN BOONLERT: ANTIDEPRESSIVE EFFECT OF GINSENG EXTRACT G115 IN ETHANOL-TREATED MICE. ADVISOR: RATCHANEE RODSIRI, Ph.D., CO-ADVISOR: ASST. PROF. JARIYA UMKA WELBAT, Ph.D., pp.

Comorbid alcoholism and depression results from the decrease of brain-derived neurotrophic factor (BDNF) levels and the increase of cortisol levels. Evidence for the efficacy of antidepressants used in co-morbid depression and alcoholism is inconsistent while there are high risks of adverse effect and drug interaction problems. Previous studies have been reported the antidepressive effect of ginseng extract and ginsenosides in rodents. The present study aimed to determine the antidepressive effect and antidepressive mechanisms of ginseng extract G115 in ethanol-treated mice. Mice in water-treated group were given either sterile water (10 ml/kg) , amitriptyline (10 mg/kg) or ginseng extract G115 (100, 200, 400 or 800 mg/kg) orally 1 hour before injecting sterile water (10 ml/kg i.p.). Mice in ethanol-treated group received either sterile water (10 ml/kg), amitriptyline (10 mg/kg) or ginseng extract G115 (100, 200, 400 or 800 mg/kg) orally 1 hour before injecting ethanol (3 g/kg i.p.). The treatments were given once daily for 8 consecutive days. Forced swimming test and locomotor activity test were performed 1-day after the last treatment. Brain and blood were collected for further analysis of brain BDNF levels and serum corticosterone levels. Repeated ethanol treatment caused depression-like behaviors in mice and decreased BDNF levels in the hippocampus and prefrontal cortex. However, ethanol treatment did not alter serum corticosterone levels. In water-treated mice, ginseng extract G115 (100, 200 and 800 mg/kg) decreased immobility time in forced swimming test. In addition, ginseng extract G115 (100 and 200 mg/kg) increased BDNF levels in the hippocampus and ginseng extract G115 (800 mg/kg) increased serum corticosterone levels indicating that ginseng extract G115 had anti-depressant-like effect in normal mice possibly due to the increases of hippocampal BDNF and serum corticosterone levels. In ethanol-treated mice, ginseng extract G115 (100, 200 and 800 mg/kg) showed the antidepressive effect in forced swimming test. Moreover, ginseng extract G115 (100, 200, 400 and 800 mg/kg) increased BDNF levels in the hippocampus and ginseng extract G115 (200 and 800 mg/kg) increased BDNF levels in the prefrontal cortex. However, ginseng extract G115 had no effect on serum corticosterone levels in ethanol-treated mice. In summary, this study demonstrated for the first time that ginseng extract G115 had antidepressive effect in ethanol-treated mice via the increase of BDNF levels in the hippocampus and prefrontal cortex. The results of this study provided scientific information of the potential and mechanism of ginseng extract G115 as an antidepressant to treat ethanol-induced depression.

Department:	Pharmacology and Physiology	Student's Signature	.....
Field of Study:	Pharmacology	Advisor's Signature	.....
Academic Year:	2014	Co-Advisor's Signature	.....

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ABBREVIATIONS

°C	=	degree celsius
<	=	less than
>	=	more than
µg	=	microgram (s)
µl	=	microliter (s)
%	=	percentage
±	=	plus-minus sign
x g	=	relative centrifugal force
5-HT	=	5-hydroxytryptamine
ACTH	=	adrenocorticotrophin
AM	=	Ante meridiem
ANOVA	=	analysis of variance
BDNF	=	brain-derived neurotrophic factor
BSA	=	bovine serum albumin
cAMP	=	3'-5'-cyclic adenosine monophosphate
cm	=	centimeter (s)
CNS	=	central nervous system
CRH	=	corticotrophin-releaseing hormone
DA	=	dopamine
ECL	=	enhanced chemiluminescence
ELISA	=	enzyme-linked immunosorbent assay
EtOH	=	ethanol
FST	=	forced swimming test
g	=	gram (s)
GABA	=	gamma-aminobutyric acid
GAPDH	=	glyceraldehyde-3-phosphate dehydrogenase
GTS	=	ginseng total saponin
HPA	=	hypothalamic-pituitary adrenal
HRP	=	horseradish peroxidase

i.p.	=	intraperitoneal injection
kg	=	kilogram (s)
mg	=	milligram (s)
ml	=	milliliter (s)
mRNA	=	messenger ribonucleic acid
n	=	number
NE	=	norepinephrine
ng	=	nanogram (s)
nm	=	nanometer (s)
NMDA	=	N-methyl-D-aspartate
pH	=	potential of hydrogen ion
PM	=	Post meridiem
p.o.	=	per os, oral administration
rpm	=	revolutions per minute
SDS-PAGE	=	sodium dodecyl sulfate polyacrylamide gel electrophoresis
S.E.M.	=	standard error of mean
TMB	=	Tetramethyl benzidine
U.S.	=	United States
VTA	=	ventral tegmental area
v/v	=	volume per volume

CHAPTER 1
INTRODUCTION

Background and Rationale

Alcohol use disorder is a serious social and public health problem in Thailand and worldwide. It was estimated that 28.6% of Thai adult population consumed alcohol in 2007 (1). Alcohol use disorder increases risks of several diseases including cardiovascular diseases (2), cancer (3), anxiety and depression (4). Psychiatric symptoms such as depression can occur during chronic alcohol exposure (5-7) and alcohol withdrawal state (8).

Co-occurring of depression and alcohol dependence has widely been reported (5-7). Depression in alcoholism causes an increase of alcohol drinking leading to the difficulty of alcoholic cessation (9) and increased of suicidal rate (10). Previous studies suggested that depression in alcoholism resulted from hippocampal volume reduction (11), decreased of brain-derived neurotrophic factor (BDNF) (12) and monoamine levels (13) as well as an increase of cortisol levels (14). Drugs used for the treatment of alcoholism which are approved by the U.S. Food and Drug Administration including disulfiram, naltrexone and acamprosate have not shown an efficacy in the treatment of co-occurring depression and alcoholism (15-17). Reports of their antidepressants efficacy in comorbid depression and alcoholism are inconsistent (18). Moreover, antidepressants have many side effects and drug interaction problems (19, 20). Therefore, herbal medicines with antidepressant-like property and fewer side effects are interesting for the treatment of comorbid depression and alcohol dependence.

Panax ginseng extract has been increasingly investigated for the treatment of various CNS disorders (21, 22). An antidepressant-like effect of ginseng extract and ginsenosides has been widely demonstrated in rodents (23-26). The mechanisms underlying an antidepressive effect of ginseng extract and ginsenosides involved increases of BDNF and monoamine levels (27, 28). Moreover, as an adaptogen ginseng extract and ginsenosides can increase corticosterone levels in normal rodents (29, 30) while decrease corticosterone levels in rodents with high corticosterone conditions (30, 31). Therefore, ginseng extract and ginsenosides treatment probably improve depression in alcoholism.

In the present study, standard ginseng extract G115 are used to investigate its antidepressive effect in ethanol-treated mice using foced swimming test. Antidepressive mechanisms of ginseng extract G115 including an alteration of serum corticosterone levels and an increase of BDNF levels in the hippocampus and prefrontal cortex were also examined.

Research Questions

1. Does ginseng extract G115 produce an antidepressive effect in normal and ethanol-treated mice?
2. What are the antidepressive mechanisms of ginseng extract G115 in normal and ethanol-treated mice?

Objectives

1. To determine the antidepressive effect of ginseng extract G115 in normal and ethanol-treated mice.
2. To elucidate the antidepressive mechanisms of ginseng extract G115 in normal and ethanol-treated mice.

Hypothesis

Ginseng extract G115 has an antidepressive effect in normal and ethanol-treated mice due to increases of BDNF levels in the hippocampus and prefrontal cortex and alterations of blood corticosterone levels.

Expected Benefits

This research would provide a scientific information of the potential use of ginseng extract G115 as an antidepressant agent for treatment of ethanol-induced depression.

CHAPTER 2
LITERATURE REVIEWS

2.1 Alcohol use disorder

Alcohol use disorder (i.e. alcohol abuse and alcohol dependence) is a serious social and public health problem in Thailand and worldwide. It was estimated that 28.6% of Thai adult population consumed alcohol in 2007 survey and the prevalence tends to increase (1). Alcohol use disorder increases risks of cardiovascular diseases (2), cancer (3), anxiety, depression (4), death from traffic accidents (32), fight, assault and suicides (33). In terms of economic problems, alcohol use disorder causes an increase of medical treatment cost from alcoholic illness (34) and a decrease of productivity of addicts and patients (32). Alcohol dependence or alcoholism is a condition caused by prolonged and usually intense consumption of alcohol resulting in psychological and/or physiological dependence on alcohol consumption (35). In addition, cessation of chronic alcohol exposure causes alcohol withdrawal symptoms such as tremor, autonomic excitability, seizure, hallucination and delirium (36-38).

Like other drugs of abuse, alcohol acutely activates brain reward pathway. Brain reward pathway is a part of mesolimbic dopamine system which dopamine cell bodies arise from the ventral tegmental area (VTA) and project their axons to the nucleus accumbens (Figure 2.1) (39). Alcohol acutely inhibits NMDA receptor (40), activates $GABA_A$ receptor (41) and increases dopamine levels in brain reward pathway (42, 43). Chronic alcohol exposure causes neuroadaptation including up-regulation of NMDA receptor (44, 45), down-regulation of $GABA_A$ receptor (46) and decreased of dopamine levels in brain reward pathway (47). Therefore, alcohol intake in this state is needed to balance neuroadaptation effects and consequently caused alcohol dependence (Figure 2.2) (35). Cessation of alcohol intake in alcohol addicts results in alcohol withdrawal symptoms due to the unbalance of brain neurotransmitter functions (35). Convulsion is caused by an increase of glutamate function and decrease of $GABA_A$ function (48). Anxiety results from decreases of $GABA_A$ function (49), brain serotonin levels and 5-HT_{1A} receptor mRNA levels (50, 51). In addition, a decrease of dopamine levels in brain reward system causes alcohol craving (52, 53).

Depression can also occur during chronic alcohol exposure (5-7) and alcohol withdrawal state (8).

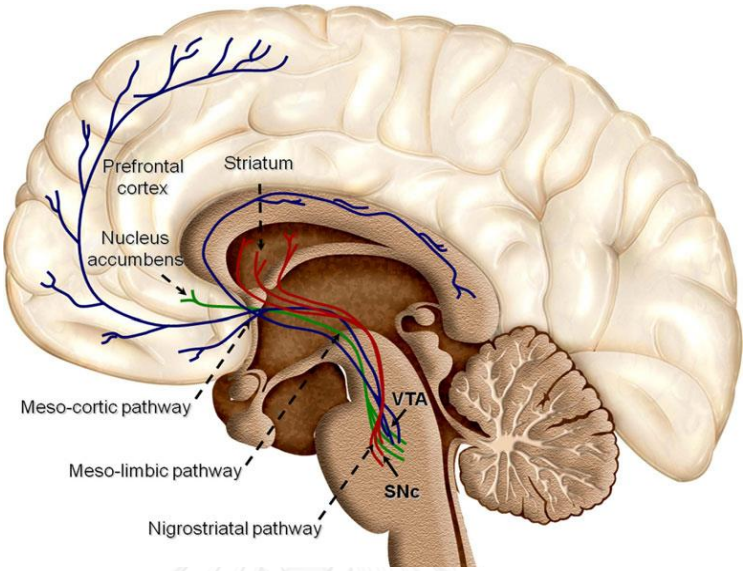


Figure 2.1 Brain reward pathway is a part of mesolimbic dopamine system which dopamine cell bodies arise from the ventral tegmental area (VTA) and project their axons to the nucleus accumbens (39).

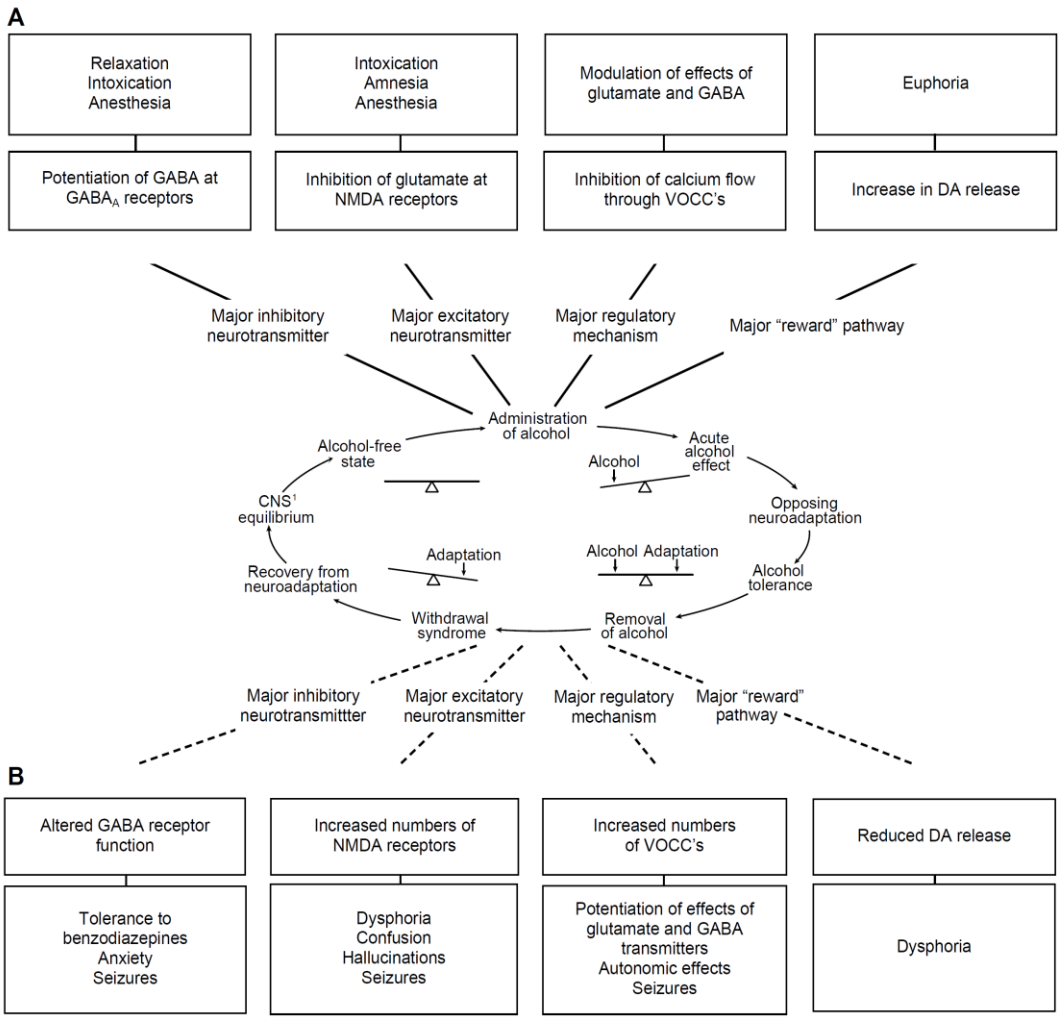


Figure 2.2 (A) Effects of acute alcohol exposure on GABA, glutamate, voltage-operated calcium channels (VOCC's) functions and brain reward pathway and related-clinical effects of alcohol intoxication.

(B) Neuroadaptations during alcohol tolerance and effects of alcohol withdrawal on GABA, glutamate, voltage-operated calcium channels (VOCC's) functions and brain reward pathway and related-clinical effects of alcohol withdrawal (35).

2.2 Comorbidity of alcohol dependence and depression

Alcoholism often occurs with psychiatric disorders such as anxiety (54) and depression (5-7). Co-occurring of major depressive episode and alcohol dependence was strongly related with odd ratio of 3.7 (55). Depression in alcoholism causes an increase of alcohol drinking leading to the difficulty of alcohol cessation (9) and an increase of suicidal rate (10). Moreover, depression during alcohol withdrawal period causes an alcohol craving and a relapse of alcohol intake (56).

Previous studies reported pathological similarities between depression and alcohol dependence. Hippocampal volume decreased in both alcoholic (11) and depressed patients (57). Moreover, alcohol induced depressive-like behavior in rats and decreased monoamine levels in the hippocampus and frontal cortex (13). Furthermore, basal cortisol level in alcoholics are higher than healthy volunteers due to decrease of feedback control (14). In addition, BDNF level significantly decreased in depression (58), suicidal depression (59) and alcohol-dependent patients (60).

2.3 The involvement of BDNF in alcohol dependence and depression

Brain-derived neurotrophic factor (BDNF) has a major role in neurogenesis, growth and survival of neurons in the prefrontal cortex and limbic system (61). BDNF deficiency causes prefrontal cortex, hippocampal and amygdala cell atrophy leading to abnormalities of mood, learning and memory and depressive disorder (Figure 2.3) (61-63). The decrease of serum BDNF levels was found in alcohol dependence (60) and comorbid depression and alcohol-dependent patients (64). Chronic daily high dose alcohol exposure induced depressive-like behaviors and decreased hippocampal BDNF levels in rats (12). In addition, chronic binge alcohol drinking decreased BDNF levels and neuronal survival and differentiation leading to depressive-like behaviors in rats while the selective BDNF TrkB receptor agonist, 7,8-dihydroflavone, inhibited alcohol-induced depression and increased BDNF levels and neurogenesis (65).

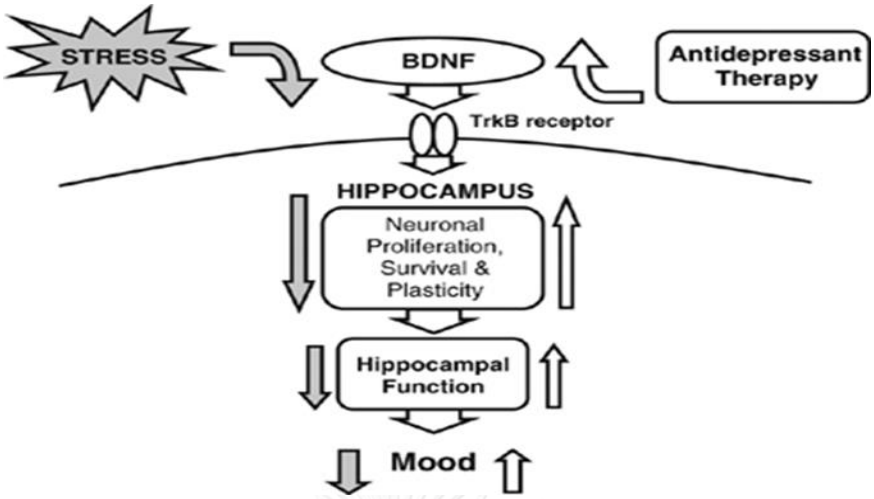


Figure 2.3 Effects of stress and antidepressant therapy on hippocampal BDNF expression and hippocampal function. BDNF binds to tyrosine kinase receptor B (TrkB) in the hippocampus resulting in neuronal proliferation, survival and plasticity. Stress decreases BDNF levels leading to an abnormality of hippocampal function and depressed mood. On the other hand, antidepressant therapy improves hippocampal function and mood by an increase of BDNF levels (63).

2.4 The involvement of HPA axis activation in alcohol dependence and depression

The hypothalamic-pituitary adrenal (HPA) axis is the body system involved the nervous, endocrine and immune systems (66). Activation of HPA axis causes the release of corticotrophin-releaseing hormone (CRH) from the hypothalamus. CRH then activates the pituitary causing adrenocorticotrophin (ACTH) release. ACTH activates adrenal cortex to release cortisol (in human) or corticosterone (in rodents) (Figure 2.4) (67). There are two types of corticosteroid receptors in rat brain; type 1 mineralocorticoid-like receptors or corticosterone-preferring receptors and type 2 glucocorticoid receptors (68, 69). Type 1 corticosterone-preferring receptors are found in the hippocampus and act in positive feedback mechanism. The activation of type 1 corticosterone-preferring receptors causes corticosterone release. Type 2 glucocorticoid receptors are commonly found in neurons and other organs and involve in the negative feedback of the HPA axis (67-69).

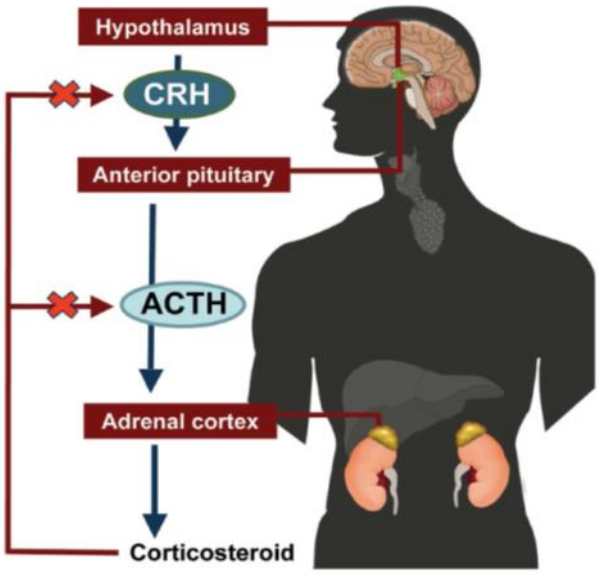


Figure 2.4 Hypothalamic–pituitary–adrenal (HPA) axis (67)

Several factors including stress, chronic medical illness-induced stress, depression and alcohol dependence can activate HPA axis (70-72). Moreover, a decrease of glucocorticoid receptor has been reported in depression, and consequently, causes an impairment of negative control and an increase of cortisol levels in these patients (70). Similarly, alcoholic patients and heavy alcohol consumption have been reported to impair negative feedback control of the HPA axis (14) and activate HPA axis activity (72) leading to an increase of cortisol secretion. In addition, an elevation of cortisol levels was found in alcoholics during withdrawal period due to HPA axis activation (73). Majumdar *et al.* (74) reported an increase of plasma cortisol level in comorbid alcoholic with depression, additionally, plasma cortisol levels and depression rating were highly correlated. In animal model, acute alcohol treatment activated HPA axis causing an increase of plasma ACTH and corticosteroid levels in adult rats (75, 76). CRH level remained increased during alcohol withdrawal period and related to an increase immobility time in forced swimming test (77). *In vitro* study showed that corticosterone can induce neuronal apoptosis (78). Neuronal apoptosis in the prefrontal cortex and limbic system can cause an impairment of an emotional control and depressive symptoms (79).

To summarize, a decrease of BDNF levels and an increase of corticosteroid levels as well as an impairment of HPA axis negative feedback control have been reported in depression, alcoholism and comorbid depression and alcohol dependence. The decrease of BDNF levels affects neuronal survival and plasticity while excessive cortisol causes neuronal apoptosis. Targeting BDNF and cortisol levels are one of the therapeutic approach for the treatment of comorbid depression and alcohol dependence.

2.5 Treatment of comorbidity of alcohol dependence and depression

Drugs used to treat alcohol dependence approved by the U.S. Food and Drug Administration include disulfiram, naltrexone and acamprosate (80). These drugs however have not shown an efficacy in the treatment of co-occurring depression and alcoholism. Disulfiram, an acetaldehyde dehydrogenase inhibitor, had no effect in neuropsychiatric disorders such as schizophrenia, bipolar disorder, anxiety and depression (81). In addition, disulfiram had no antidepressive effect in comorbid alcoholism and depression (15). Naltrexone, an opioid receptor antagonist, did not improve mood in depressive patients with and without alcohol dependence (15, 16). Although acamprosate was reported an antidepressant-like effect in mice (82), it did not improve depressive symptoms in alcoholic with depression patients (17).

Tricyclic antidepressants and serotonergic antidepressants have been increasingly investigated their efficacy in comorbid depression and alcoholism (18, 83). Getachew *et al*. (13, 84) showed that desipramine and imipramine significantly decreased the immobility time in alcohol-induced depression rats using forced swimming test. De Vry *et al.* (85) also reported the acute and subacute antidepressive effects of imipramine and fluoxetine in the rat forced swimming test. In addition, acute treatment of imipramine and fluoxetine decreased alcohol intake in cAA rat model of alcoholism. In clinical study, Mason *et al*. (86) found that desipramine significantly reduced depressive symptoms and risk of drinking relapse in alcoholics with depression. However, McGrath *et al*. (87) showed that although imipramine significantly decreased depressive symptoms, it did not decrease risk of drinking relapse in depressed alcoholic patients. Cornelius *et al*. (83) found that long-

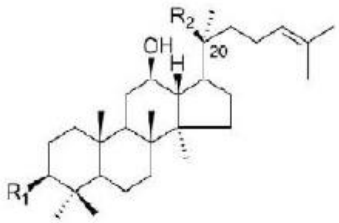
term treatment of fluoxetine significantly improved depressive symptoms and decreased alcohol consumption in comorbid alcoholic with severe major depression. A 1-year follow-up study also reported the continuous efficacy of fluoxetine for the treatment of depression and a decrease of alcohol consumption in alcoholic patients (88). Roy (89) and Moak *et al*. (90) reported that long-term treatment of sertraline significantly improved depressive symptoms but did not decrease alcohol drinking in alcoholic with depression. In contrast, Hernandez-Avila *et al*. (91) showed that nefazodone did not significantly improved depressive symptoms but decreased alcohol drinking in alcoholic with depression. In summary, although antidepressants are likely to decrease depressive symptoms in comorbid alcoholism with depression, their effect on alcohol consumption is inconsistent. Moreover, antidepressants have many side effects and drug interaction problems. Therefore, herbal medicines with antidepressant-like property and fewer side effects are interesting for treating patients with comorbid depression and alcohol dependence.

2.6 Panax ginseng

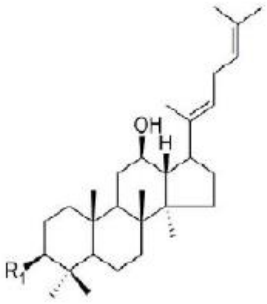
Panax ginseng is a perennial umbel plant, one stalk with compound palmate leaves**,** red berries, yellowish white roots (92) and human-like shape (Figure 2.5) (93). *Panax ginseng* extract has been increasingly investigated for the treatment of various CNS disorders (21, 22) such as parkinson's disease (94), learning and memory deficit (95), convulsion (22, 96), anxiety (21, 97, 98) and depression (24, 25, 99). Ginsenosides, the active compounds of *Panax ginseng* extract, can be classified into two categories according to the number and position of sugar molecules on the sterol chemical structure which are protopanaxadiol (R1, Ra1, Ra2, Rb1, Rb2, Rb3, Rc, Rd, Rg3, Rh2, Rk1, Rp1, Rs1, Rs2) and protopanaxatriol (Re, Rf, Rg1, Rg2, Rh1) (Figure 2.6) (100, 101). Several rare types of ginsenosides have also been identified such as the ocotillol saponin (pseudoginsenoside) and the oleanic acid (ginsenoside Ro) (101, 102). Standard ginseng extract G115 used in the present study contains 4% of total ginsenosides. Previous studies using high-performance liquid chromatography (HPLC) reported that ginseng extract G115 composes of ginsenosides Rb1, Rb2, Rc, Rd, Re, Rg1 and Rg2 (102).

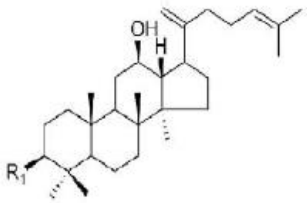
Figure 2.5 *Panax ginseng* root (93)

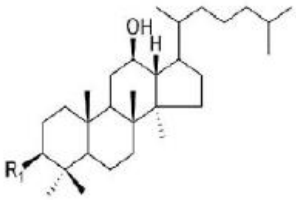
A. Protopanaxadiol-type



		R_1	R_2
1	ginsenoside Ra_1	–O–glc^{2}–^{1}glc	–O–glc^{6}–^{1}ara(P)^{4}–^{1}xyl
2	ginsenoside Ra_2	–O–glc^{2}–^{1}glc	–O–glc^{6}–^{1}ara(f)^{2}–^{1}xyl
3	ginsenoside Rb_1	–O–glc^{2}–^{1}glc	–O–glc^{6}–^{1}glc
4	ginsenoside Rb_2	–O–glc^{2}–^{1}glc	–O–glc^{6}–^{1}ara(P)
5	ginsenoside Rb_3	–O–glc^{2}–^{1}glc	–O–glc^{2}–^{1}xyl
6	ginsenoside Rc	–O–glc^{2}–^{1}glc	–O–glc^{2}–^{1}ara(f)
7	ginsenoside Rd	–O–glc^{2}–^{1}glc	–O–glc
8	(20*S*)-ginsenoside Rg_3	–O–glc^{2}–^{1}glc	OH
9	ginsenoside Rh_2	–O–glc	OH
10	ginsenoside Rs_1	–O–glc^{2}–^{1}glc^{3}–Ac	–O–glc^{6}–^{1}ara(P)
11	ginsenoside Rs_2	–O–glc^{2}–^{1}glc^{3}–Ac	–O–glc^{6}–^{1}ara(f)
12	quinquenoside R_1	–O–glc^{2}–^{1}glc^{6}–Ac	–O–glc^{6}–^{1}glc
13	compound K	OH	–O–glc
14	Chikusetsusaponin III	–O–glc^{2}(6\ ^{1}xyl)–^{1}glc	OH





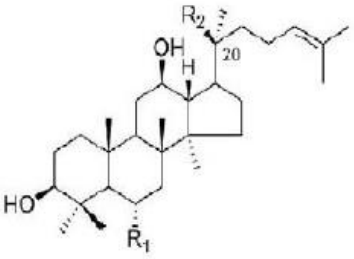


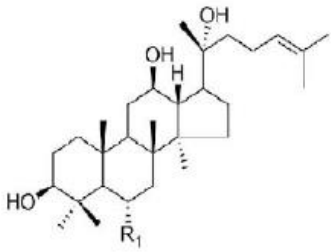
		R_1			R_1			R_1
15	ginsenoside Rg_5	–O–glc^{2}–^{1}glc	**16**	ginsenoside Rk_1	–O–glc^{2}–^{1}glc	**17**	ginsenoside Rp_1	–O–glc^{2}–^{1}glc

glc: β-D-glucopyranosyl ara(p): α-L-arabinopyranosyl ara(f): α-L-arabinofuranosyl

xyl: β-D-xylopyranosyl rha: α-L-rhamnopyranosyl glcUA: β-D-glucuronic acid

B. Protopanaxatriol-type

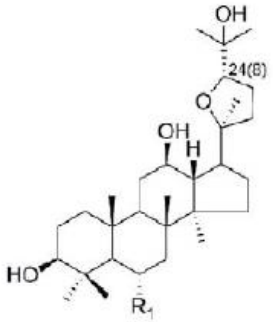




		R_1	R_2
18	ginsenoside Re	–O–glc^{2}–^{1}rha	–O–glc
19	ginsenoside Rf	–O–glc^{2}–^{1}glc	OH
20	ginsenoside Rg_1	–O–glc	–O–glc
21	(20*S*)-ginsenoside Rg_2	–O–glc^{2}–^{1}rha	OH
22	ginsenoside Rh_1	–O–glc	OH
23	20-glucoginsenoside Rf	–O–glc^{2}–^{1}glc	–O–glc
24	notoginsenoside R_1	–O–glc^{2}–^{1}xyl	–O–glc
25	notoginsenoside R_2	–O–glc^{2}–^{1}xyl	OH

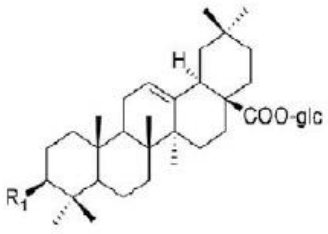
		R_1
21R	(20*R*)-ginsenoside Rg_2	–O–glc^{2}–^{1}rha

C. Ocotillol-type



		R_1
26	Majonoside R_2	–O–glc^{2}–^{1}xyl

D. Oleanic acid-type



		R_1
27	ginsenoside Ro	–O–glcUA^{2}–^{1}glc
28	Chikusetsusaponin IV	–O–glcUA^{4}–^{1}ara(f)
29	Chikusetsusaponin IVa	–O–glcUA

Figure 2.6 Structures of ginsenosides, based on chemical structure Protopanaxadiol (A), protopanaxatriol (B), ocotillol (C) and oleanic acid (D) (101).

Many studies reported an antidepressant-like effect of ginseng extract and ginsenosides. Acute single treatment of ginsenoside Rg1, Rb1, Rd and Re significantly decreased immobility time in the mice forced swimming and tail suspension tests (27, 99). Long-term treatment of ginseng total saponin (GTS), ginsenoside Rg3 and Rb1 significantly decreased immobility time in forced swimming test and tail suspension test in mice (23-26). The antidepressant-like effect of GTS and ginsenosides have been shown to involved the increases of BDNF and monoamine levels in the prefrontal cortex and hippocampus. It was shown that long-term treatment of GTS increased monoamine and BDNF levels in rat hippocampus (23, 24). Additionally, long-term treatment of ginsenoside Rg1 increased BDNF levels in mice hippocampus (27) while ginsenoside Rb3 increased monoamine and BDNF levels in mice prefrontal cortex, hippocampus and amygdala (28).

As an adaptogen ginseng extract and ginsenosides can increase corticosterone levels in normal rodents (29, 30) while decrease corticosterone levels in rodents with high corticosterone conditions (30, 31). Hiai *et al*. (29) reported that ginseng saponin mixture significantly increased plasma ACTH and corticosterone in normal rats. Moreover, GTS and ginsenoside Rc showed significantly increased plasma ACTH and corticosterone in normal mice (30). Hiai *et al*. (29) suggested that ginseng extract increased plasma corticosterone levels by direct activation of hippocampus, hypothalamus and pituitary gland but not the action on adrenal cortex. Previous studies showed that pretreatment of dexamethasone, a selective blocker of corticosterone-preferring receptors in the hypothalamus and pituitary gland, inhibited ginseng extract-induced corticosterone release (103). Moreover, ginseng saponin significantly increased plasma ACTH, corticosterone and adrenal cAMP in intact rats but not in hypophysectomized rats indicating that ginseng extracts act on hypothalamus and pituitary gland but not on adrenal cortex (104). The activation of hypothalamus and pituitary gland of ginseng extract involved an increase of HPA axis positive feedback as ginseng extract and ginsenoside Rg1 increased type 1 corticosterone-preferring receptor capacity in the hippocampus (105). On the other hand, in mice with high corticosterone conditions, ginseng extract can decrease corticosterone to normal levels (31). Kim *et al*. (31) showed that GTS reduced plasma corticosterone levels in morphine-induced thymic apoptosis mice. GTS and ginsenoside Rc decreased corticosterone levels in the immobilization-stressed mice and in the ACTH-induced increase of plasma corticosterone level (30).

In addition, most components of ginseng extract, saponin, presents a chemical structure similar to corticosterone (Figure 2.7). Therefore, saponins showed an affinity to corticoticosteroid receptor (106). Previous studies reported molecule of saponins in ginseng extract had affinity to type 1 corticosterone-preferring receptor in hippocampus and hypothalamus resulting in the increase of corticosterone-preferring receptor capacity to activate HPA axis and high corticosterone levels (105, 106). On the other hand, some studies found that molecules of ginseng saponins had affinity to type 2 glucocorticoid receptor leading to an increase of negative feedback and a decrease of corticosterone levels in high corticosterone level conditions (31, 106, 107).

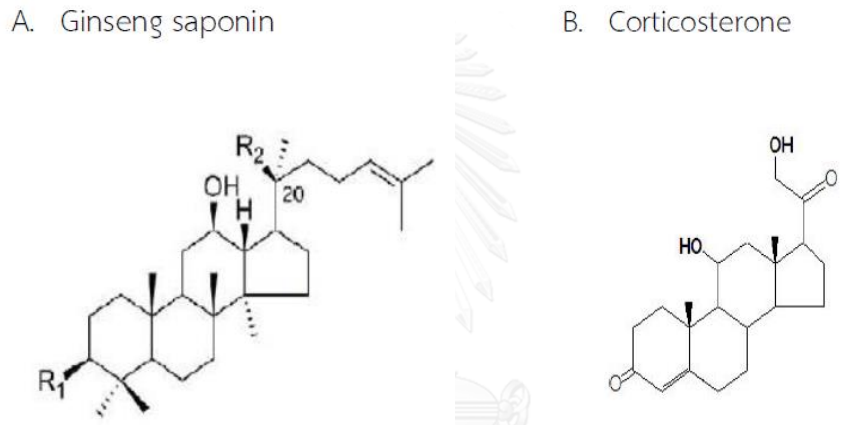


Figure 2.7 Structures of ginseng saponin (A) and corticosterone (B) (101, 108)

2.7 Model validation for ethanol-induced depressive behaviors in mice

There are several methods used to induce alcohol dependence in rodents including polydipsia technique (109, 110), liquid diet technique (110-112), ethanol inhalation (113, 114), and ethanol injection (37). Ethanol inhalation is mostly used to induce alcohol dependence in rodents (113, 114). In addition, previous studies demonstrated that ethanol inhalation can induce depressive behaviors in rodents (13, 77, 84, 115, 116). For example, Getachew *et al*. (13) reported that rats giving ethanol vapor 3 hours for 10 consecutive days presented depressive behaviors in forced swimming test and increased blood alcohol concentration to 150 mg% which was similar to blood alcohol concentration of heavy alcohol consumption in human. Ethanol intraperitoneally injection was also used to induce alcohol dependence and alcohol withdrawal symptoms. Shibasaki *et al*. (37) reported that mice alternately given either saline or ethanol (2 g/kg i.p.) for 8 days in conditioned place preference showed alcohol-addictive behaviors and discontinuation of ethanol injection caused alcohol withdrawal symptoms. The present study is the first study to induce depression in ethanol-treated mice using ethanol intraperitoneal injection. To imitate previous study by Shibasaki *et al*. (37), ethanol either 2 or 3 g/kg i.p. were given to mice for 4 consecutive days. However this procedure failed to induce depression in forced swimming test (Figure 2.8A). Therefore ethanol either 2 or 3 g/kg i.p. was given to separate groups of mice for 8 consecutive days. It was shown that ethanol (3 g/kg) significantly increased immobility time compared to controls ($p<0.01$, Tukey's *post-hoc* test) (Figure 2.8A) without changes in locomotor activity (Figure 2.8B). Therefore, ethanol (3 g/kg) given for 8 consecutive days was selected to induce depressive behaviors in ethanol-treated mice in this study.

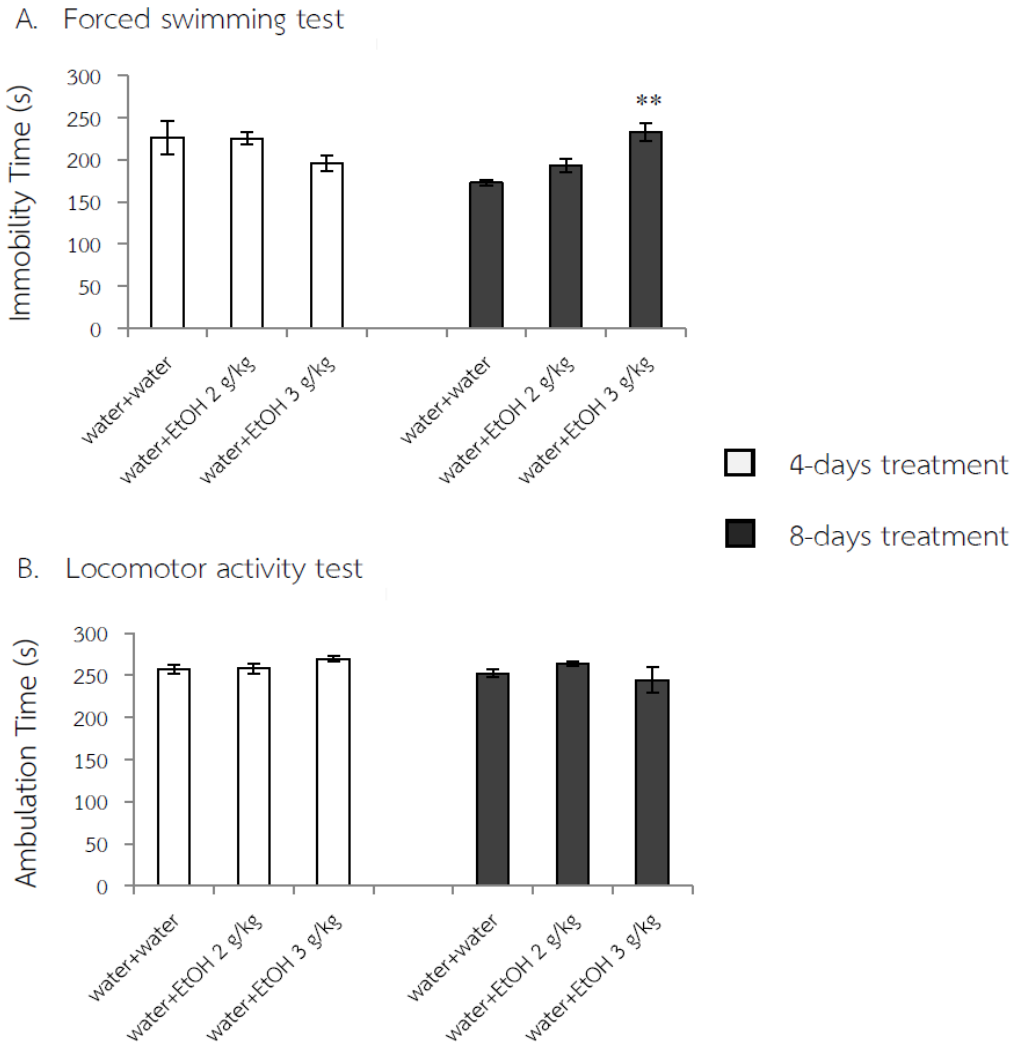


Figure 2.8 The immobility time (A) and ambulation time (B) of mice in preliminary experiment. Mice received either sterile water (10 ml/kg i.p.) or ethanol (2 or 3 g/kg i.p.) 1 hour after receiving sterile water (10 ml/kg p.o.) for 4 and 8 days. Forced swimming test and locomotor activity were performed 1-day after the last treatment. ***p<0.01* compared to water-treated controls (Tukey's *post-hoc* test).

2.8 Forced swimming test

Animal models of depression include forced swimming test, tail suspension test, chronic mild stress test, learned helplessness test, olfactory bulbectomy model and transgenic mice models. Each model possesses different advantages, limitations and different levels of validity (117). Forced swimming test (FST) was selected in this study. FST is widely used for screening compounds with antidepressive effect because of its reliability and good predictive validity (118). The principle of FST is to use stress-induced depression (117, 118). Each mouse or rat is put into a high cylinder containing water and forced to swim in the narrow area. When mouse or rat cannot escape from the cylinder, despair behaviors occur. Immobility time, defined as time that mouse's or rat's body silently floats or has only small movement to maintain its head above water level, is recorded to indicate despair behavior. Compounds with antidepressive property should decrease immobility time compared to controls. Swimming time and climbing time can also be used as parameters. Many previous studies used forced swimming test to screen antidepressant drugs (85, 119-121) and herbal medicines with antidepressive property including ginseng extract and ginsenosides (23-27, 99). Getachew *et al*. (13, 84) also used forced swimming test to determine antidepressive effect of desipramine and imipramine in alcohol-induced depression rats. Therefore, FST is the appropriate model to determine ethanol-induced depression and antidepressant-like effect of ginseng extract G115 in the present study.

2.9 Western blot analysis

Western blotting known as protein blotting or immunoblotting is a core technique in cell and molecular biology research used to detect the expression of specific protein in a complex mixture extracted from cells (122). To detect expression of BDNF protein, several methods including western blotting (27, 123) and ELISA (12, 58) can be performed. Although either ELISA or western blotting can be used to detect BDNF in the cells, western blotting is used in the present study because it is more specific than ELISA (124). In western blotting, protein mixtures i.e. from brain homogenates are separated by network of gel electrophoresis. Each

protein is separated depending on its molecular weight. Then, a target protein is transferred onto the membrane and incubated with the unconjugated primary antibody to bind specifically with the target protein. After the primary antibody incubation is complete, the blotting membrane is washed and incubated with the HRP conjugated secondary antibody. HRP conjugated secondary antibody bind specifically with the primary antibody leading to strong signal and increased specificity to detect the target protein. The substrate is used to interact with HRP enzyme. Then, the blot is detected the band of target protein by using luminescent image analyzer with chemiluminescent method (122).

2.10 Corticosterone ELISA

ELISA test is used to detect quantity of the interested substances including hormones, microbes, antibodies, proteins and peptides in samples using specific antibodies and color substrates. ELISA are divided into 4 types; direct ELISA, indirect ELISA, sandwich ELISA and competitive ELISA (125). In this study, competitive corticosterone ELISA (Abcam®, UK) was used to determine corticosterone concentrations in mice serum. The minimum concentration of corticosterone detected in this system is 0.3 ng/ml.

In competitive corticosterone ELISA, the corticosterone specific antibody was precoated in 96-well plates. Either standards or samples is added to each well and incubated with biotinylated corticosterone. Biotinylated corticosterone will bind with excess corticosterone specific antibody that has no corticosterone in the standard or samples binding. Then, other components of samples and excess biotinylated corticosterone are washed. The streptavidin-peroxidase conjugate is added to conjugate with biotinylated corticosterone then unconjugated streptavidin-peroxidase are washed out. Tetramethyl benzidine (TMB) or chromogen substrate is added. The chromogen substrate is catalyzed by streptavidin-peroxidase to produce a blue color product which later changed to yellow after adding stop solution (Figure 2.9). The density of yellow coloration is inversely proportional to the amount of corticosterone captured in plate. The absorbance of each well is read by microplate reader at a wavelength of 450 and 570 nm. The

absorbance at wavelength 450 nm is subtracted by the absorbance at wavelength 570 nm to correct of the optical density value (125).

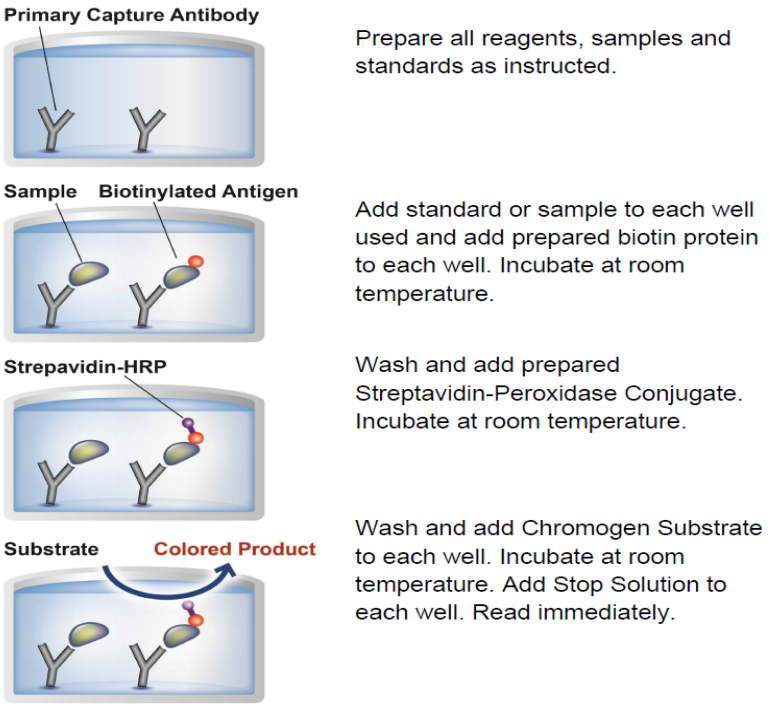


Figure 2.9 Competitive corticosterone ELISA

CHAPTER 3
MATERIALS AND METHODS

3.1 Animals

Male ICR mice, weighing 15-20 g, were purchased from National Laboratory Animal center (Salaya, Nakornpathom, Thailand). Mice were housed in groups of 6-8 per cage in a temperature and relative humidity-controlled environment (25±2 ºC, 50±10%) under a 12-h light/dark cycle (light on at 6.00 AM) with free access to food and water. Mice were allowed to acclimatize in the housing environment for 1 week before beginning of the experiment. All behavioral tests were performed between 8.00 AM to 4.00 PM. Mice were randomly assigned to different groups and moved to the experimental room in their home cages at least 1 hour before behavioral testing. All procedures with animals were conducted according to guidelines of the Animal Care and Use Committee, National Institute of Health and approved by the Research Ethical Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University (Approval number 14-33-003).

3.2 Chemicals and Reagents

40% Acrylamide bis-solution (Bio-Rad Laboratories, China)

Amitriptyline (Sigma-Aldrich, St. Louis Mo, USA)

Ammonium persulfate (Amresco®, USA)

Albumin, bovine serum (Amresco®, USA)

BDNF rabbit polyclonal IgG primary antibodies (Santa Cruz BiotechnologyInc, USA)

Beta-glycerophosphate disodium salt hydrate (Sigma-Aldrich, St. Louis Mo, USA)

Bromphenol blue sultone (Sigma-Aldrich, St. Louis Mo, USA)

Butanol (VWR International Ltd, England)

DL-Dithiothreitol (Sigma-Aldrich, St. Louis Mo, USA)

ECL western blotting substrate (Thermo scientific, USA)

Ethanol (Macron®, USA)

Ethylene glycol tetraacetic acid (Sigma-Aldrich, St. Louis Mo, USA)

GAPDH primary antibodies (Abcam®, UK)

Ginseng extract G115 (Ginsana® S.A., Switzerland)

Glycerol (Analytical® Carlo Erba, Canada)

Glycine (Sigma-Aldrich, St. Louis Mo, USA)

37% Hydrochloric acid (Lab-Scan LTD, Thailand)

Mercaptoethanol (GE Healthcare Bio-Sciences, Sweden)

Methanol (RCL Labscan Limited, Thailand)

N, N, N', N'-tetramethyl-ethylenediamine (Sigma-Aldrich, St. Louis Mo, USA)

Pentobarbital sodium (Nembutal®, CEVA Sante' animale, Belgium)

Polyclonal goat anti-mouse immunoglobulins HRP (Dako, Canada)

Polyclonal goat anti-rabbit immunoglobulins HRP (Dako, Canada)

Ponceau S solution (Sigma-Aldrich, St. Louis Mo, USA)

Prestained protein ladder (Thermo Scientific, Lithuania)

Protease inhibitors (Sigma-Aldrich, St. Louis Mo, USA)

Recombinant human BDNF (Millipore®, California)

Skim milk powder (Fluka Analytical, Switzerland)

Sodium chloride (Ajax Finechem Pty Ltd, New Zealand)

Sodium dodecyl sulphate (Laboratory Reagents and Fine chemical, India)

Sodium fluoride (Laboratory Reagents and Fine chemical, India)

Sterile water for injection (A.N.B Laboratories, Bangkok)

Sucrose (Ajax Finechem Pty Ltd, New Zealand)

Tris base (Sigma-Aldrich, St. Louis Mo, USA)

Triton X (Fluka chemical, Switzerland)

Tween (Amresco LLC, USA)

3.3 Equipments

BL3205 Balance (Shimadzu Corp., Japan) for weighing mice

Centrifuge machine (Beckman Coulter, USA)

Corticosterone ELISA kit (Abcam®, UK)

Digital dry bath incubator (Becthai Equipment and Chemical Co., Ltd, Thailand)

Gel apparatus (Bio-Rad Laboratories, USA)

Himac CR20B3 Refrigerated centrifuge machine (Hitashi Corp., Japan)

Luminescent image analyzer (GE Healthcare Bio-Sciences, Sweden)

Manual homogenizer (Glas-col®, USA)

Micro centrifuge tubes 1.5 ml (Thermo®, India)

Mini rocker shaker (Biosan, Latvia)

Nanodrop 2000 spectrophotometer (Thermo scientific, USA)

Nitrocellulose membranes 0.2 µm (Bio-Rad Laboratories, Germany)

pH meter (Ohaus Corporation, USA)

Running apparatus (Bio-Rad Laboratories, USA)

TE124S Balance (Sartorius A.G., Germany) for weighing chemicals

Transfer apparatus (Bio-Rad Laboratories, USA)

VideoMOT2 program (TSE system International Group, Germany)

Vortex mixer (Scientific Industries, USA)

Western blotting filter paper 8 cm x 10.5 cm sheet (Thermo Scientific, USA)

3.4 Chemical Preparation

3.4.1 Ginseng extract G115

Ginseng extract G115 was dissolved in sterile water in the concentrations of 10, 20, 40 and 80 mg/ml. Mice were given ginseng extract G115 orally in a constant volume of 10 ml/kg body weight.

3.4.2 Amitriptyline

Amitriptyline was dissolved in sterile water in the concentration of 1 mg/ml. Mice were given amitriptyline by oral gavage size 18G in a constant volume of 10 ml/kg body weight.

3.4.3 Ethanol

Ethanol was diluted in sterile water to the concentration of 38% v/v. Mice in ethanol-treated group were injected by 38% ethanol intraperitoneally in a constant volume of 10 ml/kg body weight (equal to ethanol 3 g/kg body weight). Mice in water-treated group were injected sterile water (10 ml/kg).

3.5 Experimental design

Mice were firstly divided into 2 groups; water-treated and ethanol-treated groups (n = 48/group). To determine the antidepressive effect of ginseng extract G115, mice in water-treated group were divided again into 6 sub-groups (n = 8/group) and given either sterile water (10 ml/kg), amitriptyline (10 mg/kg) or ginseng extract G115 (100, 200, 400 or 800 mg/kg) orally 1 hour before injecting sterile water (10 ml/kg) intraperitoneally. To determine whether ginseng extract G115 can attenuate alcohol-induced depression, mice in ethanol-treated group were divided again into 6 sub-groups (n = 8/group) and given either sterile water (10 ml/kg), amitriptyline (10 mg/kg) or ginseng extract G115 (100, 200, 400 or 800 mg/kg) orally 1 hour before injecting ethanol (3 g/kg i.p.) (Figure 3.1). All mice were treated once daily for 8 consecutive days (Figure 3.1).

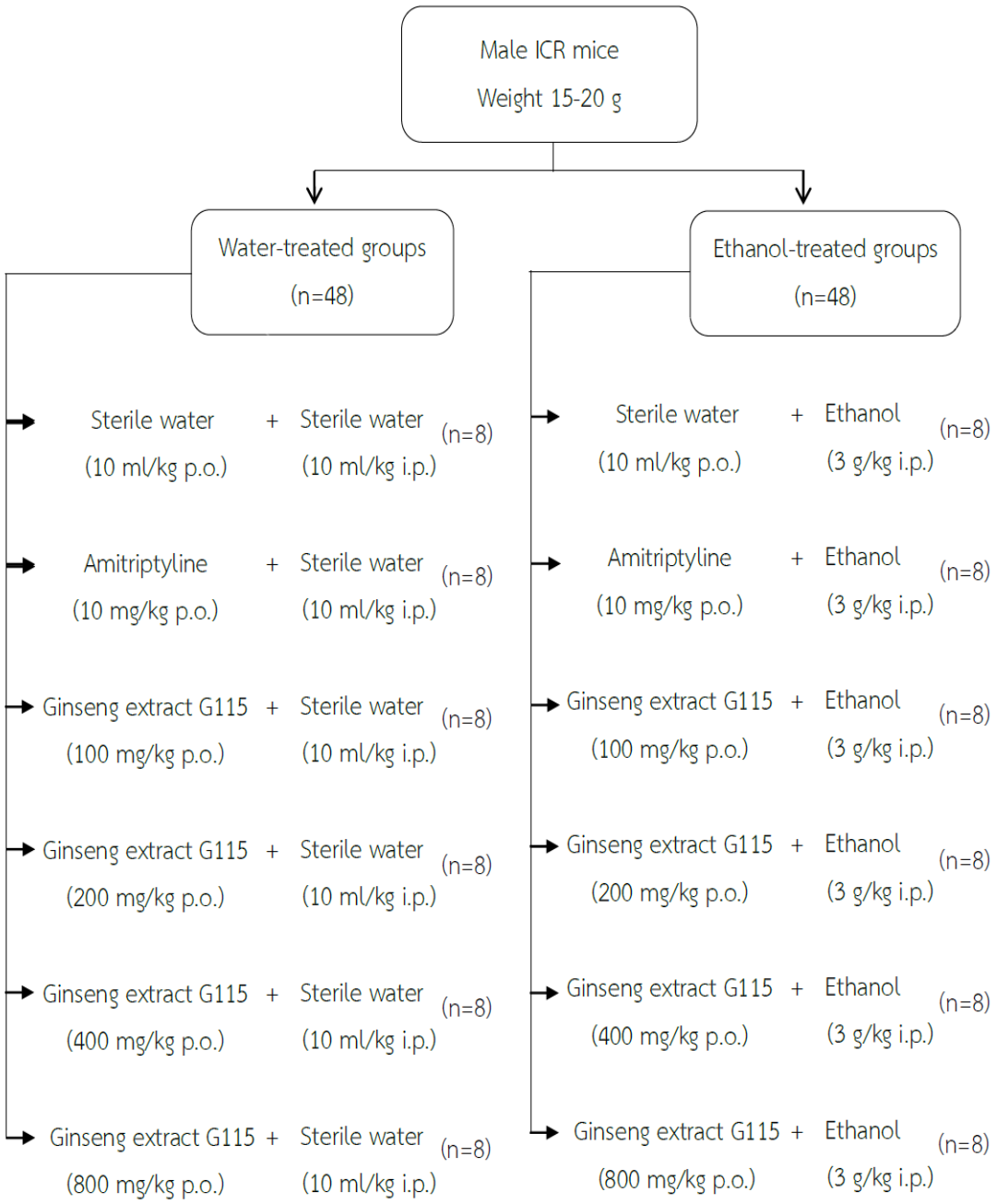


Figure 3.1 Schematic diagram of experimental groups

3.6 Behavioral tests

Behavioral testing including locomotor activity and forced swimming test were performed one day after the last treatment (day 9) (Figure 3.2). All behavioral tests were performed between 8.00 AM to 4.00 PM at Preclinical Efficacy and Safety Assessment Unit (PESA), Chulalongkorn University Drug and Health Products Innovation Promotion Center (CU.D.HIP), Faculty of Pharmaceutical Sciences, Chulalongkorn University.

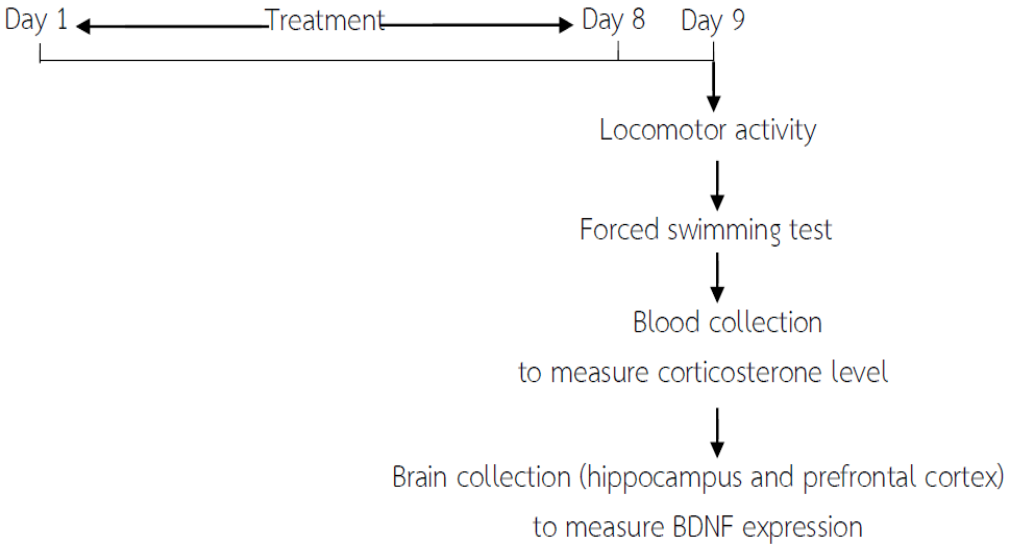


Figure 3.2 Schematic diagram of experimental methods

3.6.1 Forced swimming test

Forced swimming test was used to evaluate an antidepressive effect of substances. In this test, mouse was placed in a cylinder (height: 24 cm, diameter: 10 cm) containing 15 cm of water at 25±1 ºC for 6 minutes. Mouse swimming behaviors were recorded using video camera for further analysis of immobility time (defined as time that mouse floats with small movement to maintain its head above water level), swimming time (defined as time that mouse

moves on the water surface with a horizontal position) and climbing time (defined as mouse moves in upward-directed vigorous thrashing with the forepaws, usually along the side of the swim cylinder) (27, 99, 126).

3.6.2 Locomotor activity

Locomotor activity was examined to check whether ethanol, amitriptyline and ginseng extract G115 affect mouse's motor activity. Mouse was placed in a black square box (50x50x41 cm) for 5 minutes. Ambulation time was recorded and analyzed using VideoMOT2 system (13, 84).

3.7 Biochemical analysis

To determine the antidepressive mechanisms of ginseng extract G115, mice were euthanized using pentobarbital sodium (100 mg/kg i.p.) after behavioral tests. Blood was collected for further measuring of corticosterone level using corticosterone ELISA kit. Brain was removed quickly. Prefrontal cortex and hippocampus were dissected on ice and kept in freezer at -80 ºC for further measuring of BDNF levels using western blot analysis.

3.7.1 BDNF levels in the hippocampus and prefrontal cortex

Mouse hippocampus and prefrontal cortex from both brain hemispheres were homogenized in ice-cold lysis buffer and a cocktail of protease inhibitors. The homogenate was centrifuged at 13,000 rpm at 4 ºC for 10 minutes and supernatant was collected. The protein concentrations were measured by Nanodrop 2000 spectrophotometer. Equal amounts of protein samples (80 µg) were separated by electrophoresis using 14% SDS-PAGE gels. Then, the separated proteins were transferred onto the nitrocellulose membrane. The membrane was blocked in blocking buffer (5% skim milk or bovine serum albumin (BSA)) for 1 hour at room temperature. Subsequently, the nitrocellulose membrane was

incubated with the appropriate primary antibodies (anti-BDNF (1:150) and anti-GAPDH (1:20,000)) overnight at 4 ºC. The blot was incubated with secondary conjugated antibodies (anti-rabbit (1:2,000) and anti-mouse (1:2,000) IgG, HRP-linked antibody) at room temperature for 1 hour. The blot was developed using the enhanced chemiluminescence method by luminescent image analyzer. The signal was visualized with ECL western blotting substrate. Protein band densities were quantified with Image-J software and expressed as density of BDNF. Each sample of hippocampus and prefrontal cortex was measured duplicated and triplicated respectively. Density of BDNF was normalized with GAPDH (127).

3.7.2 Serum corticosterone levels

Whole blood was collected into eppendorf tubes and centrifuged at 3,000xg for 10 minutes. Supernatant (serum) was collected and then diluted 1:200 with dilute buffer. Corticosterone ELISA kit composed of 96-well plate precoated with corticosterone specific antibody. Twenty five-µl of either samples or corticosterone standards (concentrations of 100.000, 25.000, 6.250, 1.563, 0.391 and 0.000 ng/ml) was pipetted to each well. 25-µl of biotin protein was then added to each well. The 96-well plates were covered with a sealing tape and incubated for 2 hours at room temperature. Subsequently, the 96-well plates were rinsed 5 times with diluted wash solution. Fifty-µl of streptavidine-peroxidase conjugate was added to each well and incubated for 30 minutes at room temperature before rinsed 5 times with diluted wash solution. Fifty-µl of chromogen substrate was added to each well and incubated for 12 minutes. To stop the enzymatic reaction, 50-µl of stop solution was added to each well. Absorbance values were read immediately after adding the stop solution using microplate reader. Absorbance values at 450 nm were subtracted from values at 570 nm to correct absorbance values. Standard curves of each 96-well plate were

made and corticosterone concentration in each sample was interpreted directly from corresponding standard curve. Each sample was measured duplicated.

3.8 Statistical Analysis

All statistical analysis was performed using SPSS version 21. The results were expressed as mean ± S.E.M. of experimental data obtained from 6-8 mice. Data were analyzed statistically using one-way analysis of variance (ANOVA) followed by Tukey's or Dunnett T3's *post-hoc* test for multiple group comparison. Moreover, results from ethanol-treated controls (sterile water p.o. plus ethanol 3 g/kg i.p) were compared with results from water-treated controls (sterile water p.o. plus sterile water i.p) to examine depressive effect of ethanol using Independent t-test. Differences with $p<0.05$ were considered statistically significant.

CHAPTER 4
RESULTS

4.1 Forced swimming test

Forced swimming test was performed 1-day after the last treatment. Mice swimming activity were recorded and analyzed for immobility time, swimming time and climbing time.

4.1.1 Immobility time

Mice received water p.o. and ethanol (3 g/kg) for 8 days significantly increased immobility time compared to water-treated controls (*p<0.01*, independent t-test) (Figure 4.1A) indicating that ethanol can induce depression in mice.

The immobility time of mice in water-treated group receiving either sterile water, amitriptyline (10 mg/kg) or ginseng extract G115 (100, 200, 400 or 800 mg/kg) were 151.75 ± 7.28, 84.25 ± 11.02, 98.75 ± 8.21, 108.13 ± 11.55, 139.25 ± 12.11 and 85.63 ± 8.10 s respectively. One-way ANOVA revealed the effect of treatment on immobility time in water-treated group ($F_{0.05(5,42)}$ = 8.122, *p<0.001*). The tricyclic antidepressant, amitriptyline, significantly decreased immobility time compared to water-treated controls (*p<0.001*, Tukey's *post-hoc* test). In the same way, long-term treatment of ginseng extract G115 (100, 200 and 800 mg/kg) significantly reduced immobility time compared to water-treated controls (*p<0.01*, *p<0.05* and *p<0.001* respectively, Tukey's *post-hoc* test) (Figure 4.1B).

The immobility time of mice in ethanol-treated group, receiving either sterile water (10 ml/kg), amitriptyline (10 mg/kg) or ginseng extract G115 (100, 200, 400 or 800 mg/kg) orally 1 hour before injecting ethanol (3 g/kg) intraperitoneally were 200.63 ± 11.46, 101.88 ± 14.27, 109.63 ± 11.62, 134.88 ± 12.09, 155.38 ± 10.27 and 75.63 ± 6.36 s respectively. One-way ANOVA showed the effect of treatment on immobility time in ethanol-treated group ($F_{0.05(5,42)}$ = 15.465, *p<0.001*). Amitriptyline and ginseng extract G115 (100, 200 and 800 mg/kg) significantly reduced immobility time compared to ethanol-treated controls

(*p<0.001*, *p<0.001*, *p<0.01* and *p<0.001* respectively, Tukey's *post-hoc* test) (Figure 4.1C). These results indicated the antidepressant-like effect of ginseng extract G115 (100, 200 and 800 mg/kg) in normal and ethanol-treated mice.

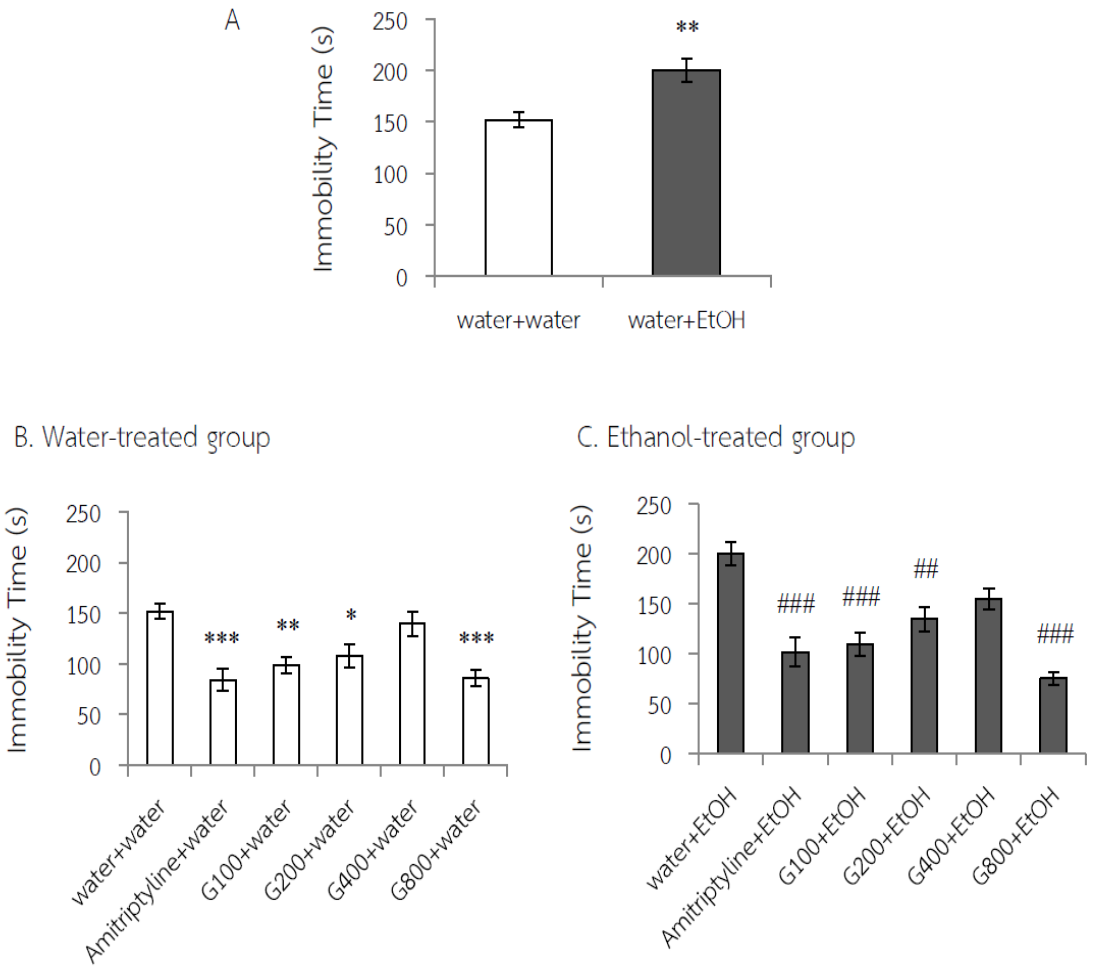


Figure 4.1 The immobility time of mice in forced swimming test. Mice received treatment for 8 days and forced swimming test was performed 1-day after the last treatment. The results are expressed as mean ± S.E.M. (A) Immobility time of mice in water-treated controls (water+water, n=8) and mice in ethanol-treated controls (water+EtOH, n=8) was compared. $^{**}p<0.01$ (independent t-test). (B) Immobility time of mice in water-treated group receiving either sterile water (n=8), amitriptyline (10 mg/kg, n=8) or ginseng extract G115 (100, 200, 400 or 800 mg/kg, n=8/group) orally 1 hour before injecting sterile water. $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ compared to water-treated controls (Tukey's *post-hoc* test). (C) Immobility time of mice in ethanol-treated group receiving either sterile water (n=8), amitriptyline (10 mg/kg, n=8) or ginseng extract G115 (100, 200, 400 or 800 mg/kg, n=8/group) orally 1 hour before injecting ethanol (3 g/kg). $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ compared to ethanol-treated controls (Tukey's *post-hoc* test).

4.1.2 Swimming time

Mice treated with ethanol (3 g/kg i.p. for 8 days) had significantly lower swimming time in forced swimming test than mice in water-treated controls (*p<0.05*, independent t-test) (Figure 4.2A).

The swimming time of mice in water-treated group receiving either sterile water, amitriptyline (10 mg/kg) or ginseng extract G115 (100, 200, 400 or 800 mg/kg) were 37.75 ± 8.00, 77.63 ± 9.70, 43.25 ± 14.66, 30.25 ± 5.20, 36.88 ± 7.11 and 80.38 ± 8.09 s respectively. One-way ANOVA revealed the effect of treatment on swimming time in water-treated group ($F_{0.05(5,42)}$ = 5.666, *p<0.001*). Long-term treatment of either amitriptyline (10 mg/kg) or ginseng extract G115 (800 mg/kg) increased swimming time compared to water-treated controls (*p<0.05*, Tukey's *post-hoc* test) (Figure 4.2B).

In ethanol-treated group, mice receiving either sterile water, amitriptyline (10 mg/kg) or ginseng extract G115 (100, 200, 400 or 800 mg/kg) orally 1 hour before injecting ethanol (3 g/kg) intraperitoneally showed swimming time of 15.88 ± 2.50, 51.75 ± 7.42, 69.50 ± 7.56, 20.25 ± 4.24, 38.13 ± 4.66 and 82.75 ± 11.28 s respectively. One-way ANOVA showed the effect of treatment on swimming time in ethanol-treated group ($F_{0.05(5,42)}$ = 15.003, *p<0.001*). Repeated treatment of either amitriptyline (10 mg/kg) or ginseng extract G115 (100 or 800 mg/kg) significantly increased swimming time compared to ethanol-treated controls (*p<0.01*, *p<0.001* and *p<0.001* respectively, Tukey's *post-hoc* test) indicating antidepressive effect of amitriptyline and ginseng extract G115 (100 and 800 mg/kg) in ethanol-treated mice (Figure 4.2C).

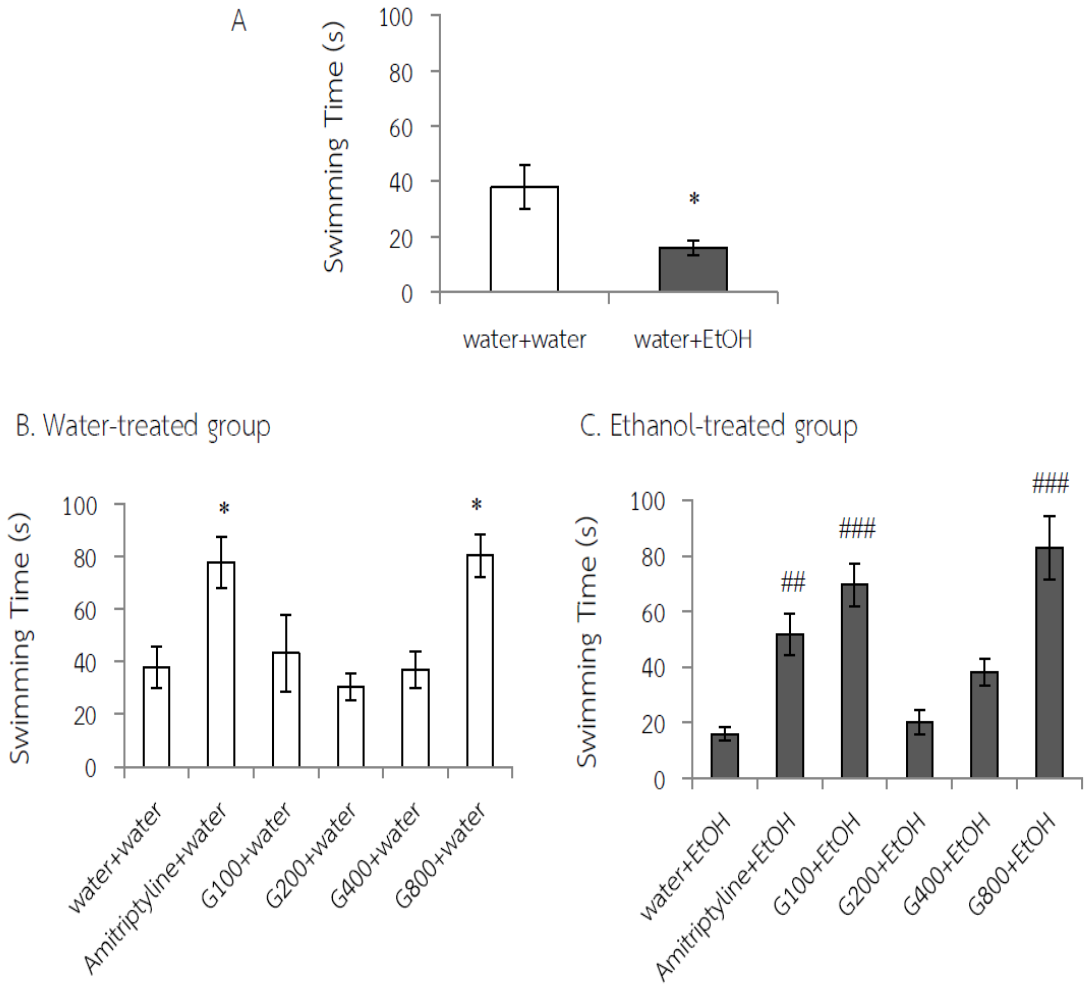


Figure 4.2 The swimming time of mice in forced swimming test. Mice received treatment for 8 days and forced swimming test was performed 1-day after the last treatment. The results are expressed as mean ± S.E.M. (A) Swimming time of mice in water-treated controls (water+water, n=8) and mice in ethanol-treated controls (water+EtOH, n=8) was compared. *$p<0.05$ (independent t-test). (B) Swimming time of mice in water-treated group receiving either sterile water (n=8), amitriptyline (10 mg/kg, n=8) or ginseng extract G115 (100, 200, 400 or 800 mg/kg, n=8/group) orally 1 hour before injecting sterile water. *$p<0.05$ compared to water-treated controls (Tukey's *post-hoc* test). (C) Swimming time of mice in ethanol-treated group receiving either sterile water (n=8), amitriptyline (10 mg/kg, n=8) or ginseng extract G115 (100, 200, 400 or 800 mg/kg, n=8/group) orally 1 hour before injecting ethanol (3 g/kg). $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ compared to ethanol-treated controls (Tukey's *post-hoc* test).

4.1.3 Climbing time

Mice treated with ethanol (3 g/kg i.p. for 8 days) had significantly lower climbing time in forced swimming test compared to water-treated controls (*p<0.05*, independent t-test) (Figure 4.3A).

The climbing time of mice in water-treated group receiving either sterile water, amitriptyline (10 mg/kg) or ginseng extract G115 (100, 200, 400 or 800 mg/kg) were 37.00 ± 3.74, 59.88 ± 3.77, 59.75 ± 2.99, 50.00 ± 4.64, 38.50 ± 8.03 and 33.25 ± 6.39 s respectively. One-way ANOVA revealed the effect of treatment on climbing time in water-treated group ($F_{0.05(5,42)}$ = 5.094, *p<0.01*). Repeated treatment of either amitriptyline (10 mg/kg) or ginseng extract G115 (100 mg/kg) increased climbing time compared to water-treated controls (*p<0.05*, Tukey's *post-hoc* test) (Figure 4.3B).

The climbing time of mice in ethanol-treated group receiving either sterile water, amitriptyline (10 mg/kg) or ginseng extract G115 (100, 200, 400 or 800 mg/kg) orally 1 hour before injecting ethanol (3 g/kg) intraperitoneally were 24.63 ± 3.21, 51.88 ± 6.20, 42.75 ± 6.84, 46.00 ± 6.39, 30.38 ± 3.24 and 35.50 ± 2.96 s respectively. One-way ANOVA showed the effect of treatment on climbing time in ethanol-treated group ($F_{0.05(5,42)}$ = 4.019, *p<0.01*). Amitriptyline (10 mg/kg) increased climbing time compared to ethanol-treated controls (*p<0.01*, Tukey's *post-hoc* test) while ginseng extract G115 treatment had no effect on climbing time in ethanol-treated group (Figure 4.3C).

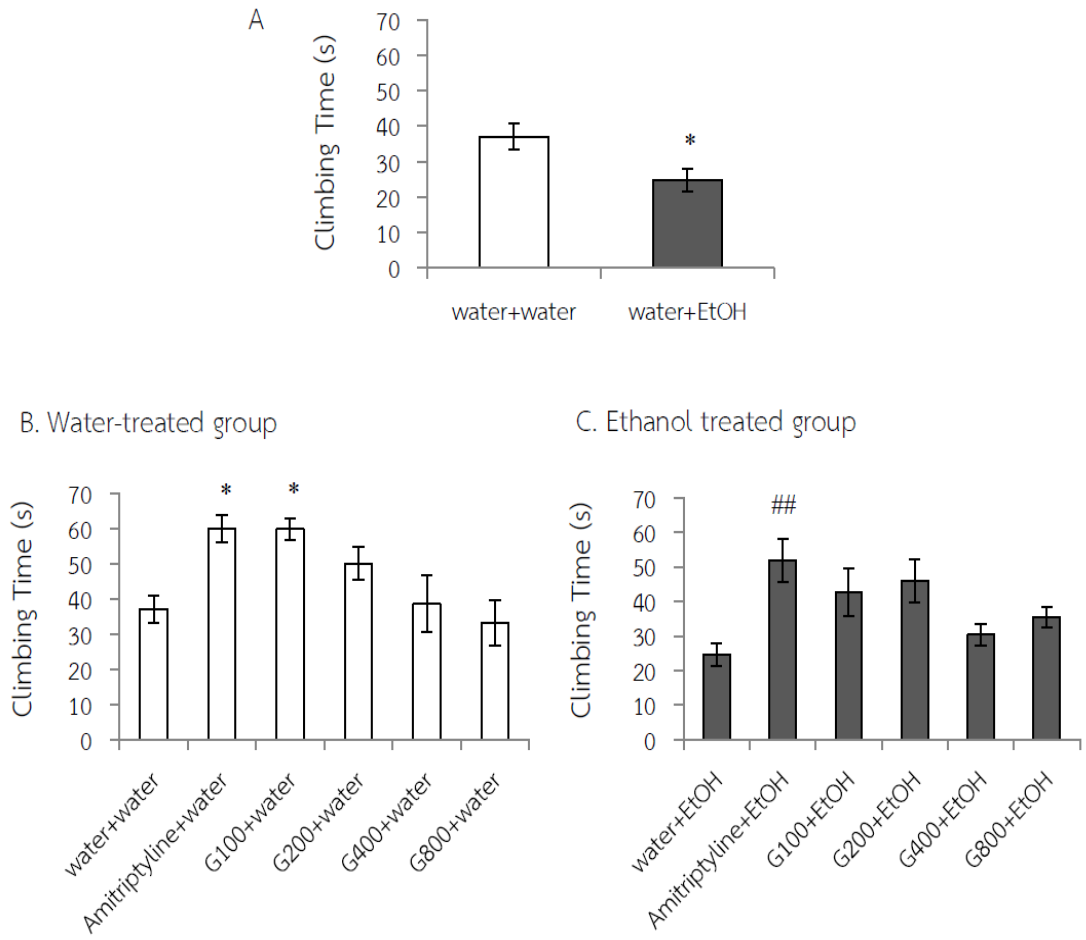


Figure 4.3 The climbing time of mice in forced swimming test. Mice received treatment for 8 days and forced swimming test was performed 1-day after the last treatment. The results are expressed as mean ± S.E.M. (A) Climbing time of mice in water-treated controls (water+water, n=8) and mice in ethanol-treated controls (water+EtOH, n=8) was compared. *$p<0.05$ (independent t-test). (B) Climbing time of mice in water-treated group receiving either sterile water (n=8), amitriptyline (10 mg/kg, n=8) or ginseng extract G115 (100, 200, 400 or 800 mg/kg, n=8/group) orally 1 hour before injecting sterile water. *$p<0.05$ compared to water-treated controls (Tukey's *post-hoc* test). (C) Climbing time of mice in ethanol-treated group receiving either sterile water (n=8), amitriptyline (10 mg/kg, n=8) or ginseng extract G115 (100, 200, 400 or 800 mg/kg, n=8/group) orally 1 hour before injecting ethanol (3 g/kg). ##$p<0.01$ compared to ethanol-treated controls (Tukey's *post-hoc* test).

4.2 Locomotor activity

Locomotor activity was performed 1-day after the last treatment to determine effects of ethanol, amitriptyline and ginseng extract G115 on mice motor activity. The ambulation time of mice in water-treated controls and ethanol-treated controls were 288.00 ± 2.33 and 287.00 ± 4.44 s respectively. Independent t-test showed no significant difference between these groups of mice (Figure 4.4A).

The ambulation time of mice in water-treated group receiving either sterile water, amitriptyline (10 mg/kg) or ginseng extract G115 (100, 200, 400 or 800 mg/kg) were 288.00 ± 2.33, 281.13 ± 5.25, 288.25 ± 2.23, 282.88 ± 4.14, 286.25 ± 2.56 and 286.13 ± 3.93 s respectively. One-way ANOVA showed no effect of treatment on ambulation time in water-treated group ($F_{0.05(5,42)} = 0.634$, $p>0.05$) (Figure 4.4B).

The ambulation time of mice in ethanol-treated group receiving either sterile water, amitriptyline (10 mg/kg) or ginseng extract G115 (100, 200, 400 or 800 mg/kg) orally 1 hour before injecting ethanol (3 g/kg) intraperitoneally were 287.00 ± 4.44, 283.25 ± 3.55, 286.75 ± 3.52, 286.88 ± 2.59, 281.13 ± 3.26 and 286.88 ± 3.91 s respectively. One-way ANOVA showed no effect of treatment on ambulation time in ethanol-treated group ($F_{0.05(5,42)} = 0.49$, $p>0.05$) (Figure 4.4C).

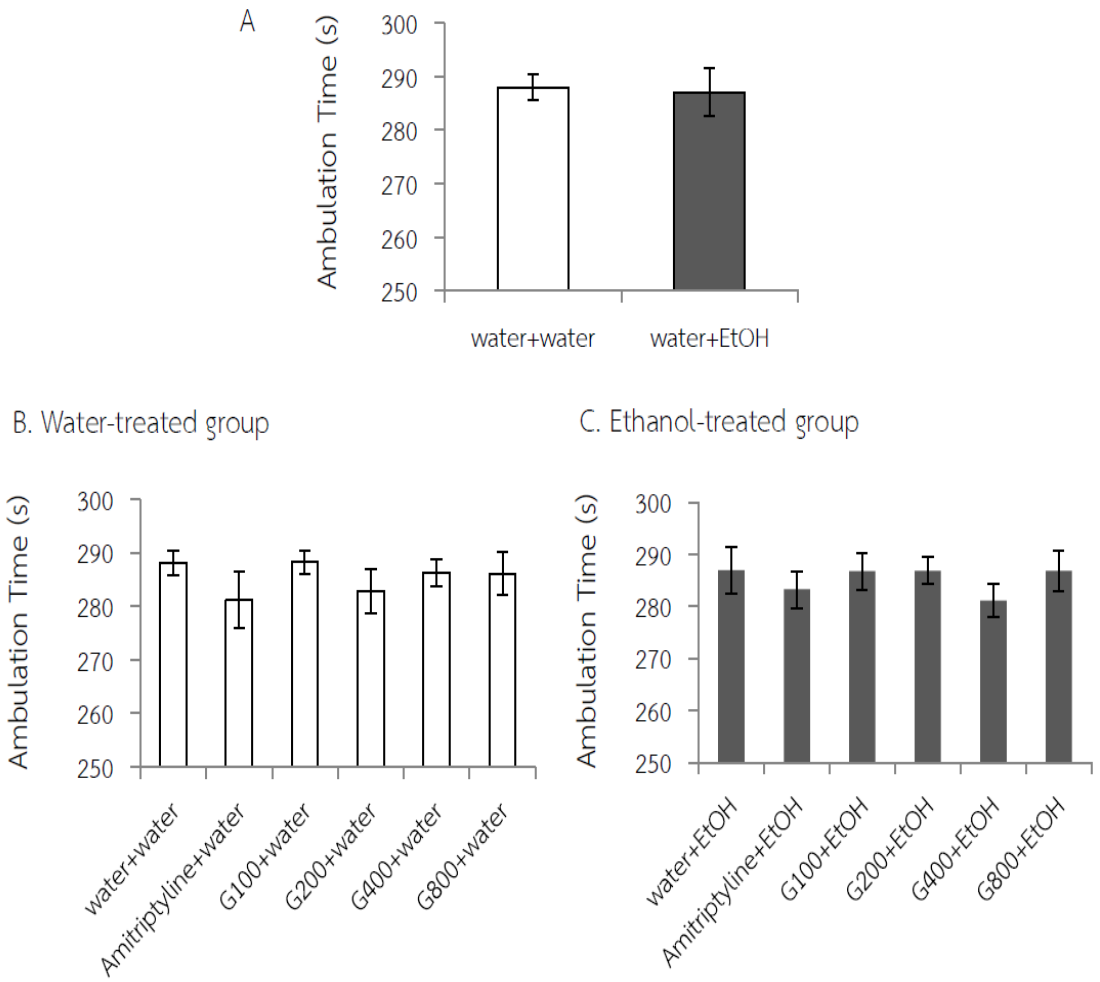


Figure 4.4 The ambulation time of mice in locomotor activity test. Mice received treatment for 8 days and locomotor activity test was performed 1-day after the last treatment. The results are expressed as mean ± S.E.M. (A) Ambulation time of mice in water-treated controls (water+water, n=8) and mice in ethanol-treated controls (water+EtOH, n=8) was compared. (B) Ambulation time of mice in water-treated group receiving either sterile water (n=8), amitriptyline (10 mg/kg, n=8) or ginseng extract G115 (100, 200, 400 or 800 mg/kg, n=8/group) orally 1 hour before injecting sterile water. (C) Ambulation time of mice in ethanol-treated group receiving either sterile water (n=8), amitriptyline (10 mg/kg, n=8) or ginseng extract G115 (100, 200, 400 or 800 mg/kg, n=8/group) orally 1 hour before injecting ethanol (3 g/kg).

4.3 BDNF levels in the hippocampus

Repeated ethanol treatment significantly decreased BDNF levels in the hippocampus compared to water-treated controls (*p<0.05*, independent t-test) (Figure 4.5A).

One-way ANOVA revealed the effect of treatment on hippocampal BDNF levels of mice in water-treated group ($F_{0.05(5,37)}$ = 10.426, *p<0.001*). Repeated treatment of ginseng extract G115 (100 and 200 mg/kg for 8 days) increased hippocampal BDNF in normal mice (*p<0.001* and *p<0.05* respectively compared to water-treated controls, Tukey's *post-hoc* test) (Figure 4.5B).

One-way ANOVA also showed the effect of treatment on hippocampal BDNF levels of mice in ethanol-treated group ($F_{0.05(5,36)}$ = 7.906, *p<0.001*). Repeated treatment of either amitriptyline (10 mg/kg) or ginseng extract G115 (100, 200, 400 or 800 mg/kg) in ethanol-treated mice significantly increased BDNF levels in the hippocampus compared to ethanol-treated alone (*p<0.05*, *p<0.01*, *p<0.05*, *p<0.01* and *p<0.05* respectively, Dunnett's T3 *post-hoc* test) (Figure 4.5C).

4.4 BDNF levels in the prefrontal cortex

Repeated treatment of ethanol significantly decreased BDNF levels in the prefrontal cortex compared to water-treated controls (p*<0.05*, independent t-test) (Figure 4.7A). One-way ANOVA revealed no effect of treatment on BDNF levels in the prefrontal cortex of mice in water-treated group ($F_{0.05(5,31)}$ = 1.816, *p>0.05*) (Figure 4.7B). In contrast, one-way ANOVA showed the effect of treatment on BDNF levels in the prefrontal cortex of mice in ethanol-treated group ($F_{0.05(5,31)}$ = 4.857, *p<0.01*). Repeated treatment of ginseng extract G115 (200 and 800 mg/kg) significantly increased BDNF levels in the prefrontal cortex compared to ethanol-treated controls (*p<0.01*, Tukey's *post-hoc* test) (Figure 4.7C)

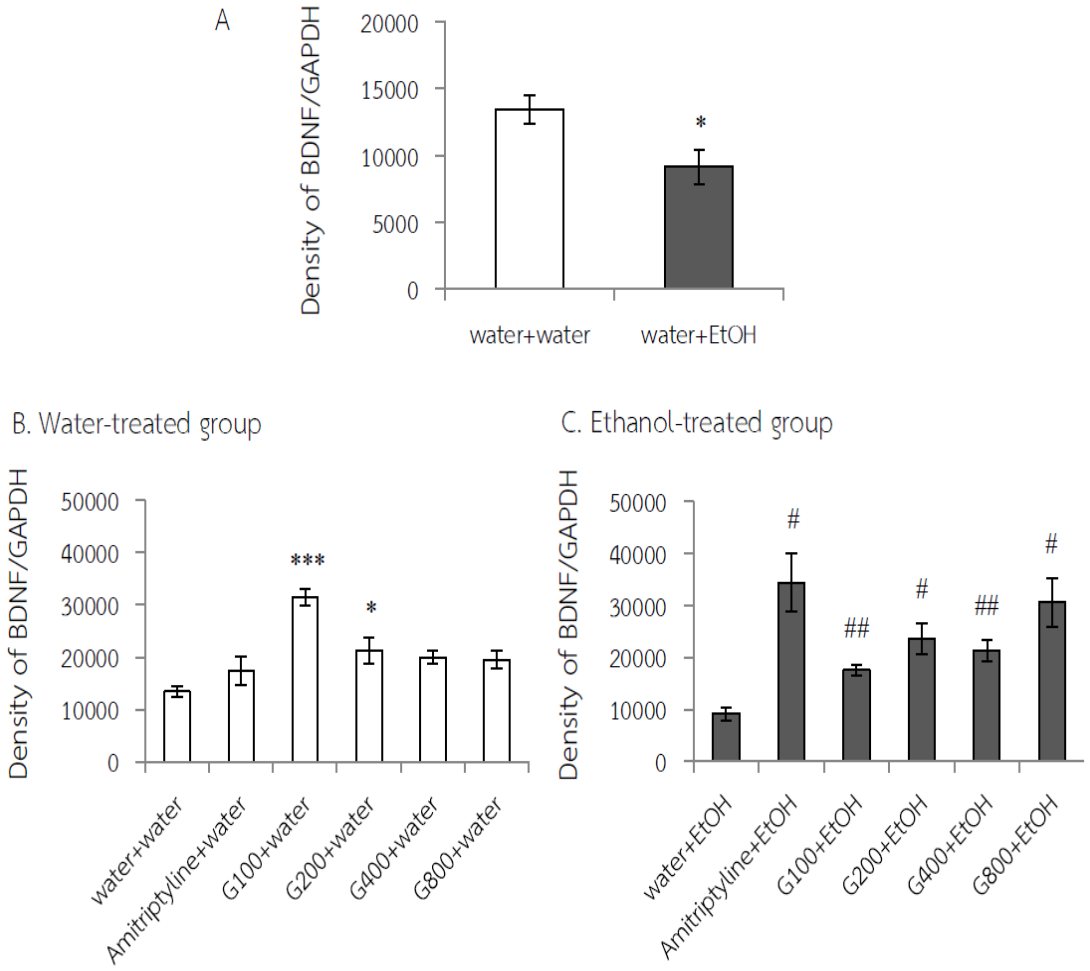


Figure 4.5 BDNF levels in mice hippocampus. The results are expressed as mean ± S.E.M. (A) BDNF levels in the hippocampus of mice in water-treated controls (water+water, n=8) and mice in ethanol-treated controls (water+EtOH, n=7) was compared. *$p<0.05$ (independent t-test). (B) BDNF levels in mice hippocampus in water-treated group receiving either sterile water (n=8), amitriptyline (10 mg/kg, n=6) or ginseng extract G115 (100, 200, 400 or 800 mg/kg, n=7-8/group) orally 1 hour before injecting sterile water. *$p<0.05$ and ***$p<0.001$ compared to water-treated controls (Tukey's *post-hoc* test). (C) BDNF levels in mice hippocampus in ethanol-treated group receiving either sterile water (n=7), amitriptyline (10 mg/kg, n=7) or ginseng extract G115 (100, 200, 400 or 800 mg/kg, n=6-8/group) orally 1 hour before injecting ethanol (3 g/kg). $^{\#}p<0.05$ and $^{\#\#}p<0.01$ compared to ethanol-treated controls (Dunnett's T3 *post-hoc* test).

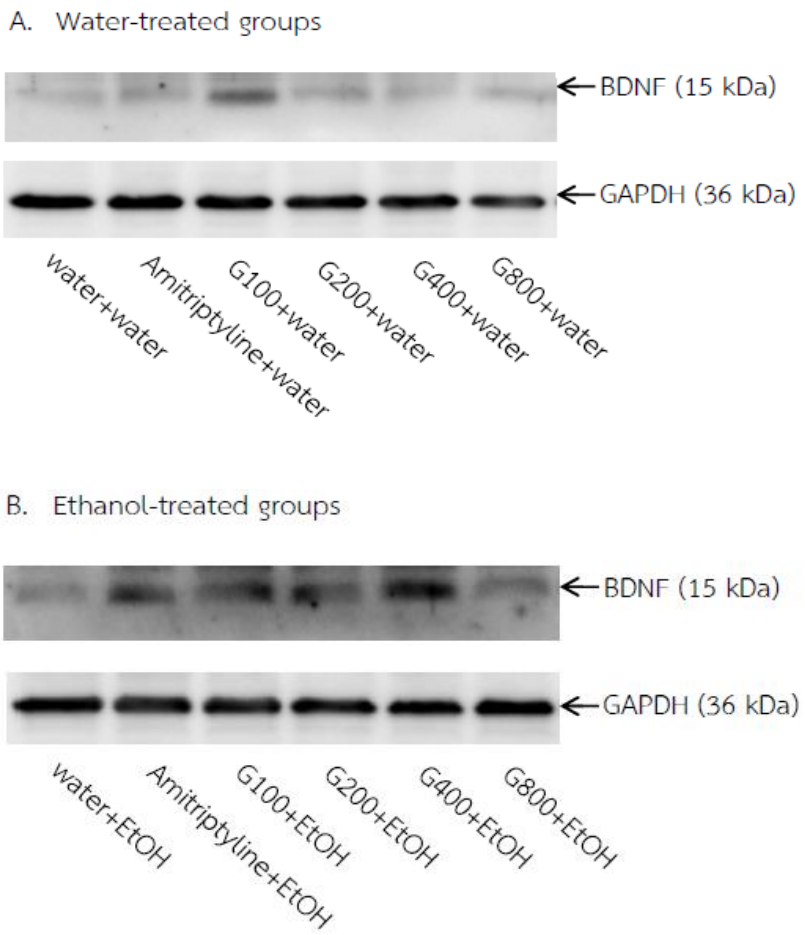


Figure 4.6 Expression of BDNF protein in mice hippocampus using western blot. (A) Expression of BDNF protein in mice hippocampus in water-treated group receiving either sterile water, amitriptyline (10 mg/kg) or ginseng extract G115 (100, 200, 400 or 800 mg/kg) orally 1 hour before injecting sterile water. (B) Expression of BDNF protein in mice hippocampus in ethanol-treated group receiving either sterile water, amitriptyline (10 mg/kg) or ginseng extract G115 (100, 200, 400 or 800 mg/kg) orally 1 hour before injecting ethanol (3 g/kg).

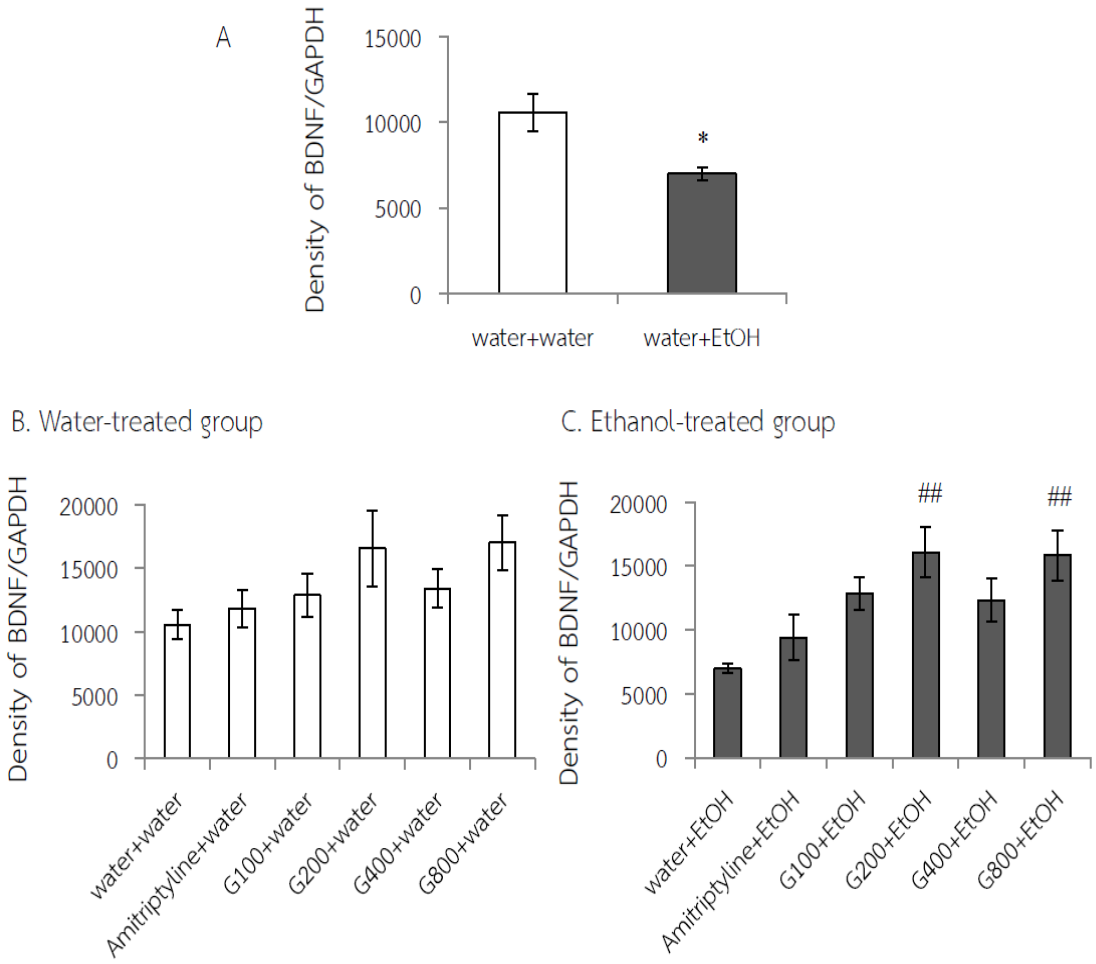


Figure 4.7 BDNF levels in the prefrontal cortex of mice. The results are expressed as mean ± S.E.M. (A) BDNF levels in the prefrontal cortex of mice in water-treated controls (water+water, n=6) and mice in ethanol-treated controls (water+EtOH, n=6) was compared. *$p<0.05$ (independent t-test). (B) BDNF levels in the prefrontal cortex of mice in water-treated group receiving either sterile water (n=6), amitriptyline (10 mg/kg, n=7) or ginseng extract G115 (100, 200, 400 or 800 mg/kg, n=6/group) orally 1 hour before injecting sterile water. (C) BDNF levels in the prefrontal cortex of mice in ethanol-treated group receiving either sterile water (n=6), amitriptyline (10 mg/kg, n=6) or ginseng extract G115 (100, 200, 400 or 800 mg/kg, n=6-7/group) orally 1 hour before injecting ethanol (3 g/kg). $^{\#\#}p<0.01$ compared to ethanol-treated controls (Tukey's *post-hoc* test).

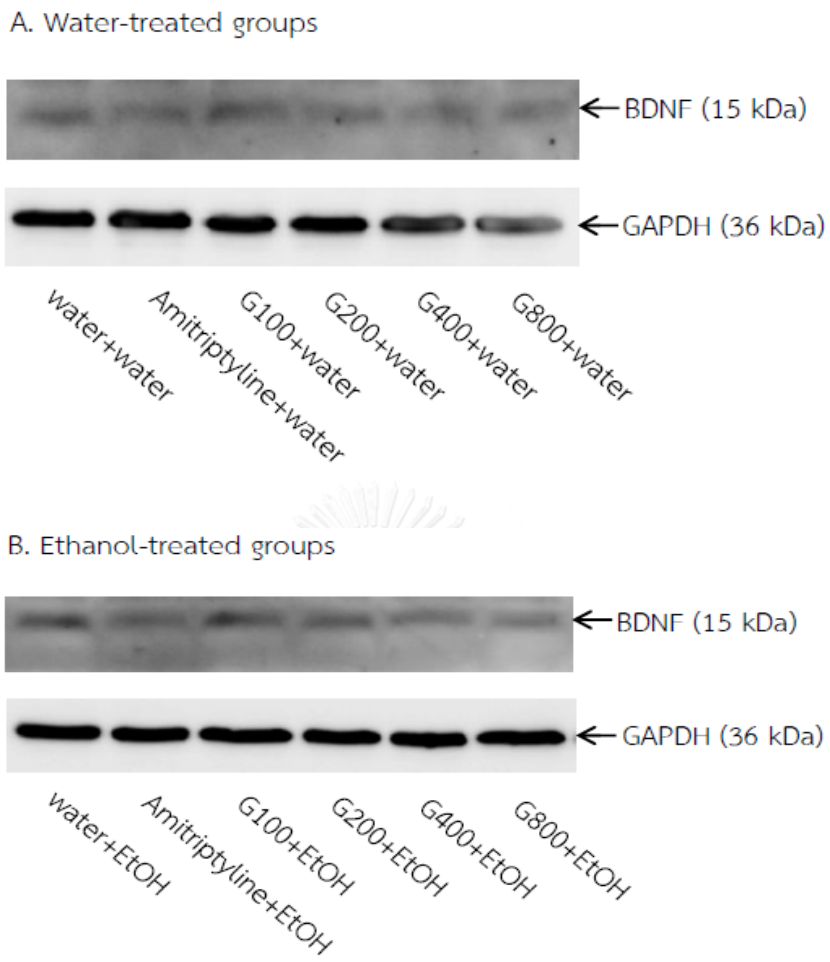


Figure 4.8 Expression of BDNF protein in the prefrontal cortex of mice using western blot. (A) Expression of BDNF protein in the prefrontal cortex of mice in water-treated group receiving either sterile water, amitriptyline (10 mg/kg) or ginseng extract G115 (100, 200, 400 or 800 mg/kg) orally 1 hour before injecting sterile water. (B) Expression of BDNF protein in the prefrontal cortex of mice in ethanol-treated group receiving either sterile water, amitriptyline (10 mg/kg) or ginseng extract G115 (100, 200, 400 or 800 mg/kg) orally 1 hour before injecting ethanol (3 g/kg).

4.5 Serum corticosterone levels

Serum corticosterone levels of mice in water-treated controls and ethanol-treated controls were 0.23 ± 0.08 and 0.29 ± 0.04 ng/ml respectively. Independent t-test showed no significant difference between these groups of mice (Figure 4.9A).

Serum corticosterone levels of mice in water-treated group receiving either sterile water, amitriptyline (10 mg/kg) or ginseng extract G115 (100, 200, 400 or 800 mg/kg) were 0.23 ± 0.08, 0.44 ± 0.08, 0.44 ± 0.12, 0.54 ± 0.22, 0.75 ± 0.17 and 1.58 ± 0.24 ng/ml respectively. One-way ANOVA showed the effect of treatment on serum corticosterone levels in water-treated group ($F_{0.05(5,32)}$ = 8.682, *p<0.001*). Repeated treatment of ginseng extract G115 (800 mg/kg) significantly increased serum corticosterone levels compared to water-treated controls (*p<0.05*, Dunnett's T3 *post-hoc* test) (Figure 4.9B).

Serum corticosterone levels of mice in ethanol-treated group receiving either sterile water, amitriptyline (10 mg/kg) or ginseng extract G115 (100, 200, 400 or 800 mg/kg) orally 1 hour before injecting ethanol (3 g/kg) intraperitoneally were 0.29 ± 0.04, 0.35 ± 0.08, 0.70 ± 0.17, 0.34 ± 0.10, 0.66 ± 0.21 and 0.67 ± 0.11 ng/ml respectively. One-way ANOVA showed no effect of treatment on serum corticosterone levels in ethanol-treated group ($F_{0.05(5,30)}$ = 2.141, *p>0.05*) (Figure 4.9C).

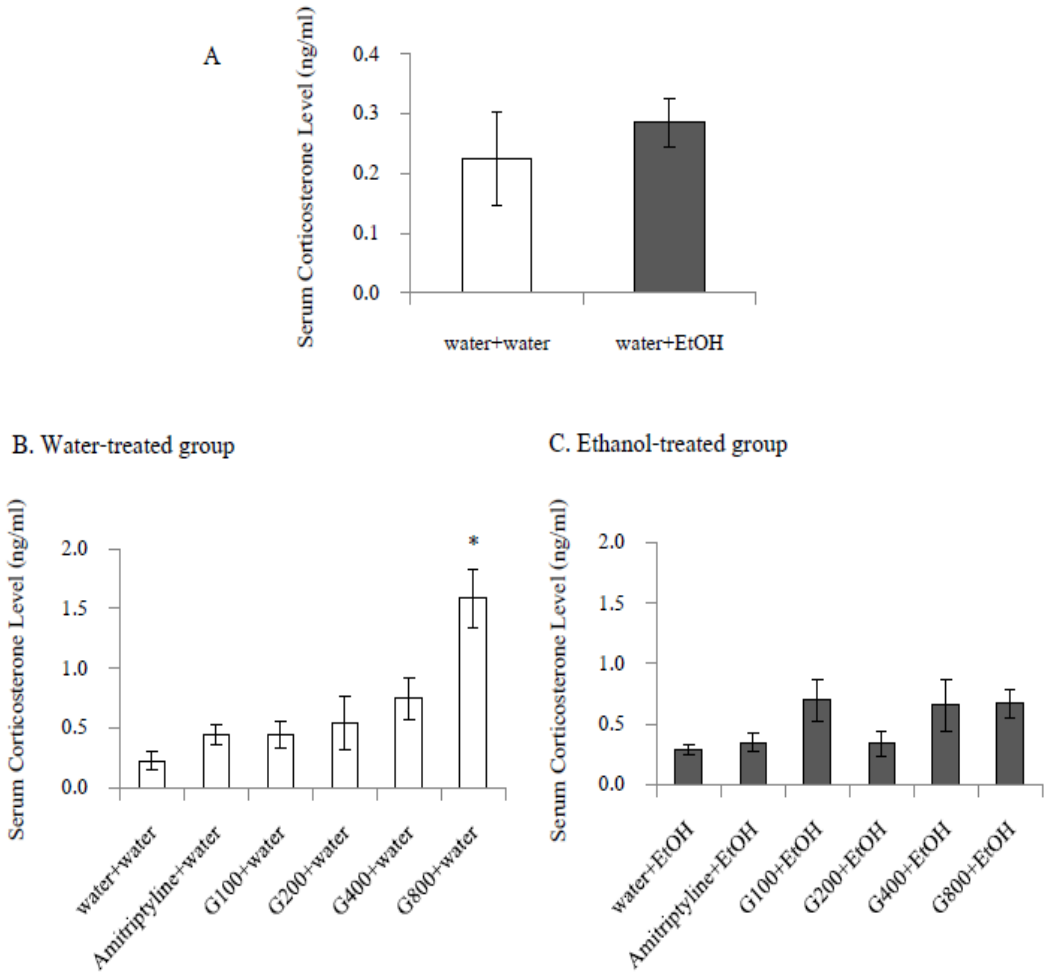


Figure 4.9 Serum corticosterone levels of mice. The results are expressed as mean ± S.E.M. (A) Serum corticosterone levels of mice in water-treated controls (water+water, n=6) and mice in ethanol-treated controls (water+EtOH, n=6) was compared. (B) Serum corticosterone levels of mice in water-treated group receiving either sterile water (n=6), amitriptyline (10 mg/kg, n=6) or ginseng extract G115 (100, 200, 400 or 800 mg/kg, n=6-7/group) orally 1 hour before injecting sterile water. *$p<0.05$ compared to water-treated controls (Dunnett's T3 *post-hoc* test). (C) Serum corticosterone levels of mice in ethanol-treated group receiving either sterile water (n=6), amitriptyline (10 mg/kg, n=6) or ginseng extract G115 (100, 200, 400 or 800 mg/kg, n=6/group) orally 1 hour before injecting ethanol (3 g/kg).

CHAPTER 5
DISCUSSION AND CONCLUSION

The present study aimed to determine the antidepressive effect of ginseng extract G115 in ethanol-treated mice using forced swimming test. Antidepressive mechanisms of ginseng extract G115 including alterations of serum corticosterone levels and BDNF levels in the hippocampus and prefrontal cortex were also examined.

Depression-like behaviors in mice was successfully induced by intraperitoneal injection of ethanol (3 g/kg for 8 days) in this study. Additionally, repeated ethanol administration caused decreases of BDNF levels in the hippocampus (Figure 4.5A) and prefrontal cortex (Figure 4.7A). These results are consistent with previous study showing that chronic alcohol exposure (95% ethanol inhalation 3 hours/day for 10 days) induced depressive-like behaviors and decreased BDNF levels in rat hippocampus (12). BDNF has a major role in psychiatric and mood disorders (63). In addition, the reduction of BDNF levels was reported in depression (58) and alcohol-dependent patients (60). Results from the present study suggested that ethanol induced depression-like behaviors in mice is likely due to decreases of brain BDNF levels.

Overstimulation of HPA axis and consequently increased serum cortisol levels has been reported in alcoholics and depression patients (14, 72, 74) and in acute ethanol-treated rats (75, 76). However, the present study showed no effect of repeated ethanol administration on serum corticosterone levels in mice (Figure 4.9A). Therefore ethanol-induced depression-like behaviors presented in this study did not involve the alteration of HPA axis. Factors that affect serum corticosterone levels in ethanol-treated mice including treatment duration, blood ethanol concentration, time for blood collection and mouse strain (128, 129). Kakihana and Moore (128) showed that morning plasma corticosterone levels of DBA/2J mice were higher than evening while Tabakoff, Jaffe and Ritzmann (129) found that morning plasma corticosterone levels of C57B1/6 mice were lower than evening.

Amitriptyline, used in this study as a positive control, showed an antidepressive effect in normal and ethanol-treated mice using forced swimming test. In addition, repeated amitriptyline administration significantly increased BDNF levels in the hippocampus of ethanol-treated mice. Previous studies also demonstrated that antidepressants such as nomifensine, imipramine, desipramine and fluoxetine

alleviated depression-like behaviors via increases of BDNF levels in the hippocampus, frontal cortex and amygdala (12, 130-132). In contrast, this study showed no effect of amitriptyline on serum corticosterone levels while Katz and Hersh (133) found that amitriptyline decreased plasma corticosterone levels in chronic stress-induced depression rats. This might be due to the differences in models to induce depression and species. To conclude, the present study suggested that the antidepressive mechanisms of amitriptyline in ethanol-treated mice is related to an increase of brain BDNF levels.

In the present study, an antidepressive effect of ginseng extract G115 was determined in normal mice (water-treated group). The results showed that ginseng extract G115 (100, 200 and 800 mg/kg) given orally for 8 days decreased immobility time in forced swimming test (Figure 4.1B). Morover, ginseng extract G115 (100 and 800 mg/kg) increased climbing time (Figure 4.3B) and swimming time (Figure 4.2B) respectively indicating the antidepressant-like effect of ginseng extract G115. These results are in agreement with previous studies reporting the antidepressant-like effect of ginseng extract and ginsenosides (23-27, 99).

An increase of BDNF levels in the hippocampus and prefrontal cortex is one of the proposed mechanisms of antidepressant drugs which explain the delay-onset of antidepressants (63). Previous studies demonstrated that antidepressive mechanisms of ginseng extracts and ginsenosides involved the increases of BDNF levels in the hippocampus and prefrontal cortex (23, 24, 27, 28). The present study showed that long-term ginseng extract G115 administration (100 and 200 mg/kg p.o. for 8 days) increased BDNF levels in mice hippocampus (Figure 4.5B) but not in the prefrontal cortex (Figure 4.7B) suggesting that antidepressive effect of ginseng extract G115 presented in normal mice might be partly due to an alteration of brain BDNF levels.

As an adaptogen, alterations of HPA axis activation and glucocorticoid levels are suggested to be one of mechanism of action of ginseng and ginsenosides (29, 30). The present study showed that high dose ginseng extract G115 treatment (800 mg/kg) increased serum corticosterone level in mice (Figure 4.9B) which might explain the antidepressive mechanism of high dose ginseng extract G115.

This study reported for the first time that long-term ginseng extract G115 administration (100, 200 and 800 mg/kg p.o. for 8 days) attenuated ethanol-induced depression in mice. The antidepressive mechanisms of ginseng extract G115 in ethanol-treated mice involved alterations of brain BDNF levels as repeated administration of ginseng extract G115 (100, 200, 400 and 800 mg/kg) in ethanol-

treated mice significantly increased BDNF levels in the hippocampus (Figure 4.5C). Additionally, ethanol-treated mice receiving ginseng extract G115 (200 and 800 mg/kg) had significantly higher BDNF levels in the prefrontal cortex than ethanol-treated alone (Figure 4.7C). Antidepressant drugs including nomifensine and imipramine were previously reported their antidepressive effect in ethanol-treated rats via an increase of BDNF levels in the hippocampus and frontal cortex (12). BDNF has a major role in neurogenesis, neural growth and survival of neurons in the prefrontal cortex and limbic system (61). The present study suggested that ginseng extract G115 attenuated ethanol-induced depression due to an increase of BDNF levels in the hippocampus and possibly prefrontal cortex leading to neuronal protection from toxicity of alcohol.

The attenuation of stress-induced high corticosterone levels has been suggested as one of mechanisms of action of ginseng as an adaptogen (30, 31). However the present study showed that ginseng extract G115 did not alter serum corticosterone in ethanol-treated mice (Figure 4.9C) indicating that antidepressive effect of ginseng extract G115 did not involve the alteration of serum corticosterone.

To summarize, the present study demonstrated the antidepressive effect of ginseng extract G115 (100, 200 and 800 mg/kg) in normal and ethanol-treated mice. The antidepressive mechanism of ginseng extract G115 in ethanol-treated mice was an increase of BDNF levels in the hippocampus and prefrontal cortex. Overall this study provided scientific information of the potential use and mechanism of ginseng extract G115 as an antidepressant to treat ethanol-induced depression.

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