

ห้องสมุดงานวิจัย สำนักงานคณะกรรมการวิจัยแห่งชาติ



E47343

**EFFECT OF *VERNONIA CINEREA* LESS. EXTRACTS ON NICOTINE  
WITHDRAWAL SYMPTOMS IN MICE**

**PATTACHAI PINNAK**

**A Thesis Submitted to the Graduate School of Naresuan University  
in Partial Fulfillment of the Requirements  
for the Master of Science Degree in Pharmacology and Biomolecular Sciences  
(International Program)**

**May 2012**

**Copyright 2012 by Naresuan University**

600254867

ห้องสมุดงานวิจัย สำนักงานคณะกรรมการการวิจัยแห่งชาติ



E47343

**EFFECT OF VERNONIA CINEREA LESS. EXTRACTS ON NICOTINE  
WITHDRAWAL SYMPTOMS IN MICE**



**PATTACHAI PINNAK**

**A Thesis Submitted to the Graduate School of Naresuan University  
in Partial Fulfillment of the Requirements  
for the Master of Science Degree in Pharmacology and Biomolecular Sciences  
(International Program)**

**May 2012**

**Copyright 2012 by Naresuan University**

This thesis entitled “Effect of *Vernonia cinerea* Less. extracts on nicotine withdrawal symptoms in mice” submitted by Pattachai Pinnak in partial fulfillment of the requirements for the Master of Science Degree in Pharmacology and Biomolecular Sciences (International Program) is hereby approved.

*Sutisa Thanoi*.....Chair

(Associate Professor Sutisa Thanoi, Ph.D.)

*Thanasak Teaktong*.....Committee

(Thanasak Teaktong, Ph.D.)

*Sakonwan Praputbut*.....Committee

(Assistant Professor Sakonwan Praputbut, Ph.D.)

*Parirat Khonsung*.....Committee

(Parirat Khonsung, Ph.D.)

**Approved**

*K. Papatwibul*.....

(Assistant Professor Kanungnit Papatwibul, Ph.D.)

Dean of the Graduate School

4 May 2012

## ACKNOWLEDGEMENT

First of all, I would like to acknowledge the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education and Faculty of Pharmaceutical Sciences, Naresuan University for the financial support of this study.

I would like to express my appreciation to my advisor, Dr. Thanasak Teaktong for his valuable guidance during my graduate study. He has provided a great opportunity and continuous encouragement for my master degree study.

Grateful thanks is also gone to my co-advisor, Assistant Professor Dr. Sakonwan Praputbut for her suggestions and ideas that have allowed me to advance my research particularly, materials and directions in the western blot study.

I would like to thank Associate Professor Dr. Sutisa Thanoi for providing directions and suggestions in the anatomy of mouse brain sections.

I also would like to thank all the members of my thesis committee, Associate Professor Dr. Nantaka Khorana and Dr. Parirat Khonsung for their valuable comments.

I would like to extend my thanks to Miss Jutamas Kampeerapong, an educator of the Department of pharmaceutical sciences, for her academic guidance.

I would like to give my thanks to my parents and Miss Linda Kaewluang who have listened and encouraged me from the beginning to the end of this thesis.

Finally, I would like to thank the others, who inspired me but are not named in this acknowledgement.

Pattachai Pinnak

**Title** EFFECT OF VERNONIA CINEREA LESS. EXTRACTS ON NICOTINE WITHDRAWAL SYMPTOMS IN MICE

**Author** Pattachai Pinnak

**Advisor** Thanasak Teaktong, Ph.D.

**Co-Advisors** Assistant Professor Sakonwan Praputbut, Ph.D.

**Type of Degree** Thesis M.Sc. in Pharmacology and Biomolecular Sciences (International Program), Naresuan University, 2011

**Keywords** nicotine, withdrawal symptoms, *Vernonia cinerea*, nicotinic receptor, muscarinic receptor

### ABSTRACT

**E 47343**

Nicotine is considered to be the primary component of tobacco smoke and it has been found to be the main cause of tobacco addiction. Currently available smoking cessation agents (i.e., nicotine replacement therapy, bupropion and varenicline) have limited efficacy and relapse rates are reported to be high, revealing a continuing need for development of alternative and more efficacious smoking cessation pharmacotherapies. *Vernonia cinerea* Less. has been reported to have many medicinal properties including analgesia, antipyretic effect, anti-inflammation, and smoking cessation. Although several studies have been reported efficacy of *V. cinerea* in reducing tobacco smoking, mechanisms of action of *V. cinerea* have not been understood. The aims of this study were evaluate effect of *V. cinerea* on nicotine withdrawal mice and determine the possible mechanisms of action of *V. cinerea* extracts (VE) involved with nicotinic and muscarinic receptors, which have been reported possible role in nicotine addiction.

VE significantly reduced total abstinence signs of mice in dose dependence manner compared to nicotine withdrawal group both at the first day and the last day after stopping nicotine administration ( $p < 0.05$ ). Moreover, VE significantly reduced total square enter compared to control group in locomotor activity test although VE was slightly effective on anxiety-like behaviors with significant reduced close-arm entries on the elevated plus maze (EPM) apparatus at concentration of 500 mg/kg of VE compared to control mice. VE showed affinity to nicotinic and muscarinic receptor

with  $IC_{50} = 1.145$  mg/ml and  $IC_{50} = 2.487$  mg/ml, respectively. For western blot study, VE had no significant effects on  $\alpha 7nAChRs$  and  $M5mAChRs$  protein expression but trends of reduction in both receptors were appeared in VE groups compared to nicotine withdrawal group. Autoradiographic study failed to detect [ $^3H$ ]nicotine binding in mouse brain, however, there were no significant different on [ $^3H$ ]scopolamine binding for muscarinic receptor in VTA, NAcc, and hippocampus in mice brain between VE treated groups and nicotine withdrawal group.

These results suggest that VE have possible properties to be used as therapeutic agent for smoking cessation due to its ability to reduce nicotine withdrawal symptoms. However, the reduction in abstinence signs by VE treatment may be not associated with nicotinic and/or muscarinic receptors. VE may be involved with reduction of nicotine withdrawal symptoms in other mechanisms such as dopamine and NMDA receptors. Therefore, further studies will be required to find out the other mechanisms of action of VE that involved with nicotine withdrawal symptoms.

## LIST OF CONTENTS

Chapter	Page
<b>I INTRODUCTION.....</b>	<b>1</b>
The rationale for the study.....	1
Hypothesis of the study.....	2
Objectives of the study.....	2
Expected outputs of the study.....	2
Expected outcomes of the study .....	2
<b>II REVIEWS OF RELATED LITERATURE AND RESEARCH.....</b>	<b>3</b>
Reward system.....	3
Drug addiction.....	4
Nicotine and addiction.....	5
Nicotinic and muscarinic receptors in drug addiction.....	6
The effect of nicotine on animal behaviors.....	7
Smoking cessation.....	8
<i>Vernonia cinerea</i> Less.....	10
<b>III RESEARCH METHODOLOGY.....</b>	<b>13</b>
Animals.....	13
Materials.....	13
Instruments.....	16
Experimental procedures.....	16
Statistical analysis.....	24
<b>IV RESULTS AND DISCUSSION.....</b>	<b>25</b>
Effect of VE on nicotine withdrawal symptoms in mice.....	25
The affinity of VE on nicotinic and muscarinic receptors.....	41

## LIST OF CONTENTS (CONT.)

Chapter	Page
Effect of VE on nicotinic and muscarinic receptors expression in mouse brain.....	43
<b>V CONCLUSIONS.....</b>	<b>47</b>
<b>REFERENCES.....</b>	<b>48</b>
<b>APPENDIXS.....</b>	<b>59</b>
<b>BIOGRAPHY.....</b>	<b>77</b>



## LIST OF TABLES (CONT.)

Table	Page
15 Close-arm entry of mice at the 7 <sup>th</sup> day after nicotine withdrawal expressed in every minute.....	70
16 Data of nicotinic receptor displacement assay .....	72
17 Data of muscarinic receptor displacement assay.....	73
18 Data of nicotinic receptor expression by western blot assay.....	74
19 Data of muscarinic receptor expression by western blot assay.....	75
20 Data of muscarinic receptor expression by autoradiographic study.....	76

## LIST OF TABLES

Table	Page
1 Application of <i>Vernonia cinerea</i> Less.....	12
2 Animals treatments.....	17
3 Muscarinic receptor level in mouse brains measured by [ <sup>3</sup> H]-scopolamine binding.....	46
4 Total abstinence sign score of mice at the first day after nicotine withdrawal.....	61
5 Total abstinence sign score of mice at the 7 <sup>th</sup> day after nicotine withdrawal.....	61
6 Total abstinence sign score of mice at the first day after nicotine withdrawal expressed in every 5 minutes.....	62
7 Total abstinence sign score of mice at the 7 <sup>th</sup> day after nicotine withdrawal expressed in every 5 minutes.....	63
8 Locomotor activity of mice at the first day after nicotine withdrawal expressed in every minute.....	64
9 Locomotor activity of mice at the 7 <sup>th</sup> day after nicotine withdrawal expressed in every minute.....	65
10 Open-arm entry of mice at the first day after nicotine withdrawal expressed in every minute.....	66
11 Open-arm entry of mice at the 7 <sup>th</sup> day after nicotine withdrawal expressed in every minute.....	67
12 Percent time spent in open-arm entry of mice at the first day after nicotine withdrawal.....	68
13 Percent time spent in open-arm entry of mice at the 7 <sup>th</sup> day after nicotine withdrawal.....	68
14 Close-arm entry of mice at the first day after nicotine withdrawal expressed in every minute.....	69

## LIST OF FIGURES

Figure	Page
1 A simple diagram of rodent brain reward circuit [22]. Abbreviations: PMT, pontomesencephalic tegmental; VTA, ventral tegmental area; NAcc, nucleus accumbens; PFC, prefrontal cortex; DA, dopamine; GABA, gamma aminobutyric acid.....	4
2 A chemical structure of nicotine.....	5
3 <i>Vernonia cinerea</i> Less.....	10
4 Some abstinence signs in mice including nose scratching (A), ear scratching (B), body lifting (C), body scratching (D), dog shaking (E), and rearing (F).....	19
5 Elevated plus maze apparatus.....	20
6 Open-field apparatus for testing locomotor activity.....	20
7 Rodent's skull diagram [74].....	23
8 Total abstinence sign score of mice at the first day after nicotine withdrawal (day 15 of the experiment). (a, significant difference compared to control group; b significant difference compared to nicotine withdrawal group; c significant difference compared to mecamylamine received group, $p \leq 0.05$ , $n = 6$ ).....	26
9 Mean of nicotine abstinence signs versus time, day 15 of the experiment. (A) Rearing (B) Nose scratching (C) Ear scratching (D) Dog shake (E) Body scratching (F) Chewing (G) Abnormal constriction (H) Body lifting. (a significant difference compared to control group; b significant difference compared to nicotine withdrawal group; c significant difference compared to mecamylamine received group, $p \leq 0.05$ , $n = 6$ ).....	27

## LIST OF FIGURES (CONT.)

Figure	Page
10 Total abstinence sign score of mice at the 7th day after nicotine withdrawal, day 21 of the experiment. (a significant difference compared to control group; b significant difference compared to nicotine withdrawal group; c significant difference compared to mecamylamine received group, $p \leq 0.05$ , $n = 6$ ).....	29
11 Mean of nicotine abstinence signs versus time, day 21 of the experiment. (A) Rearing (B) Nose scratching (C) Ear scratching (D) Dog shake (E) Body scratching (F) Chewing (G) Abnormal constriction (H) Body lifting. (a significant difference compared to control group; b significant difference compared to nicotine withdrawal group; c significant difference compared to mecamylamine received group, $p \leq 0.05$ , $n = 6$ ).....	30
12 Locomotor activity (LMA) of mice at 1 day after nicotine withdrawal (day 15). (A) Mean of total square enter. (B) Mean of square enter versus time. (a significant difference compared to control group; b significant difference compared to nicotine withdrawal group, $p \leq 0.05$ , $n = 6$ ).....	32
13 Locomotor activity (LMA) of mice at 7 days after nicotine withdrawal (day 15). (A) Mean of total square enter. (B) Mean of square enter versus time. (a significant difference compared to control group; b significant difference compared to nicotine withdrawal group, $p \leq 0.05$ , $n = 6$ ).....	34
14 Anxiety-like behaviors of mice at 1 day after nicotine withdrawal. (A) Total open-arm entry. (B) Open-arm entry versus time. (C) Percent time spent in open-arm.....	36
15 Anxiety-like behaviors of mice at 7 days after nicotine withdrawal. (A) Total open-arm entry. (B) Open-arm entry versus time. (C) Percent time spent in open-arm.....	37

## LIST OF FIGURES (CONT.)

Figure	Page
16 Anxiety-like behaviors of mice at 1 day after nicotine withdrawal. (A) Total close-arm entry. (B) Close-arm entry versus time.....	39
17 Anxiety-like behaviors of mice at 7 days after nicotine withdrawal. (A) Total close-arm entry. (B) Close-arm entry versus time. (a significant difference compared to control group, $p \leq 0.05$ , $n = 6$ ).....	40
18 Receptor displacement curve for VE in mouse brains, (■) [ $^3\text{H}$ ]-nicotine and (●) [ $^3\text{H}$ ]-scopolamine binding.....	42
19 Alpha7 nicotinic receptor expressions probed with nAChR $\alpha$ 7 antibody were visualized using the enhanced chemiluminescence (ECL) detection kit and exposed to x-ray film.....	43
20 M5 muscarinic receptor expressions probed with mAChRm5 antibody were visualized using the enhanced chemiluminescence (ECL) detection kit and exposed to x-ray film.....	44
21 Autoradiographs of [ $^3\text{H}$ ]-scopolamine binding in the nucleus accumbens (left), hippocampus (center), ventral tegmental area (right).....	45

## ABBREVIATIONS

1-D	=	one dimension
$\alpha 7$	=	alpha7
$\mu\text{g}$	=	microgram
$\mu\text{l}$	=	microliter
$\mu\text{M}$	=	micromolar
$^{\circ}\text{C}$	=	degree Celsius
[ $^3\text{H}$ ]	=	tritium (radioactive)
ANOVA	=	analysis of variance
CAR	=	continuous abstinence rate
Ci/mmol	=	Curie per millimol
cm	=	centimeter
CNS	=	central nervous system
COX-2	=	cyclooxygenase-2
DA	=	dopamine
DAergic	=	dopaminergic
DOC	=	deoxycholate
ECL	=	enhanced chemiluminescence
EDTA	=	Ethylenediaminetetraacetic acid
EPM	=	elevated plus maze
FDA	=	Food and Drug Administration
Fig.	=	figure
fMol	=	femtomol
g	=	gravity
GABA	=	gamma aminobutyric acid
GTS	=	ginseng total saponin
IC50	=	half inhibitory concentration
ICR mouse	=	imprinting control region mouse
IL-1 $\beta$	=	interleukin-1 $\beta$
IL-6	=	interleukin-6
iNOS	=	inducible nitric oxide synthase

## ABBREVIATIONS (CONT.)

i.p.	=	intraperitoneal
kDa	=	kilo Dalton
LDT	=	laterodorsal tegmentum
LMA	=	locomotors activity
LPS	=	lipopolysaccharide
M	=	Molar
mAChR	=	muscarinic acetylcholine receptor
mAh	=	milliampere-hour
MEC	=	mecamylamine
MFB	=	medial forebrain bundle
mg	=	milligram
mg/kg	=	milligram per kilogram
ml	=	milliliter
mM	=	millimolar
mm	=	millimeter
NA	=	noradrenaline
nAChR	=	nicotinic acetylcholine receptor
NAcc	=	nucleus accumbens
nM	=	nanomolar
NO	=	nitric oxide
NRT	=	nicotine replacement therapy
PAR	=	prevalence abstinence rate
PFC	=	prefrontal cortex
pH	=	power of hydrogen ion concentration
PMT	=	pontomesencephalic tegmental
PVDF	=	polyvinylidene fluoride membrane
SDS	=	sodium dodecyl sulfate
SEM	=	standard error of mean
s.c.	=	subcutaneous
TNF- $\alpha$	=	tumor necrosis factor-alpha

## ABBREVIATIONS (CONT.)

VE	=	<i>Vernonia cinerea</i> Less. extracts
VP	=	ventral pallidum
VTA	=	ventral tegmental area
<i>V. cinerea</i>	=	<i>Vernonia cinerea</i> Less.
w/w	=	weight by weight