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

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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.

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ABSTRACT

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 IC50 = 1.145 mg/ml and IC50 = 2.487 mg/ml, respectively. For western blot study, VE had no significant effects on α7nAChRs 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 [^{3}H]nicotine binding in mouse brain, however, there were no significant different on [^{3}H]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.

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ABBREVIATIONS

1-D	=	one dimension
α7	=	alpha7
μg	=	microgram
μl	=	microliter
μM	=	micromolar
°C	=	degree Celsius
[^{3}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-1β	=	interleukin-1β
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-α	=	tumor necrosis factor-alpha

ABBREVIATIONS (CONT.)

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