SUMMARY OF DISSERTATION

Name of Doctoral candidate: **Nguyen Thu Hien**
Dissertation title: **"Study on the anti-dementia and the antidepressant-like effects of *Ocimum sanctum* L. on mice experiments".**
Specialty: **Pharmacology - Clinical pharmacy**
Code of specialty: **9720205**
Name of academic advisors:

1. Assoc. Prof. Dr. Nguyen Minh Khoi

2. Dr. Le Thi Xoan

Name of academic institute: **National Institute of Medicinal Materials**

Summary of the dissertation:

1. Objectives

- Study on ameliorative effects of *Ocimum sanctum* L. on cognitive deficits in olfactory bulbectomized mice (OBX) and putative mechanisms underlying its actions.

- Study on the antidepressant-like effects of *Ocimum sanctum* L. in olfactory bulbectomized mice (OBX) and unpredictable chronic mild stress-induced depression in mice (UCMS).

2. Methods

2.1. Study on ameliorative effects of *Ocimum sanctum* L. on cognitive deficits in olfactory bulbectomized mice (OBX) and putative mechanisms underlying its actions

- The anti-dementia effects of the 70% ethanol extract of *Ocimum sanctum* (OS): Behavioral tests: spatial and nonspatial working memory performances were measured using a modified Y maze test (Y-maze) and a novel object recognition test (ORT), respectively.

- The underlying mechanisms of OS: Histological studies: Microscopic analysis of lateral ventricles, Immunohistochemistry - cholinergic neurons in the medial septum and newly generated neurons in the hippocampal dentate gyrus were analyzed by staining choline acetyltransferase- (ChAT-) immunopositive cells and doublecortin- (DCX-) immunopositive cells, respectively; Neurochemical studies: the expression levels of vascular endothelial growth factor (VEGF) and VEGF receptor 2 (VEGFR2)

genes in the hippocampus of mice were analyzed using quantitative real-time PCR (qRT-PCR), the expression levels of VEGF protein in the hippocampus of mice were analyzed using Western blot analysis, acetylcholinesterase (AchE) activity in the mouse brain was performed using *ex vivo* measurements of AChE activity.

- The effects of OS extract further fractionated with n-hexane (OS-H), ethyl acetate (OS-E), and n-butanol (OS-B) on the spatial cognitive deficits of OBX mice were elucidated by the modified Y-maze test.

- The anti-dementia effects of ursolic acid and oleanolic acid, two major constituents in the ethyl acetate fraction of OS (OS-E): behavioral tests: short-term spatial and long-term spacial memory performances were measured using a modified Y maze test (Y-maze) and the Morris water maze (MWM) test, respectively.

- The underlying mechanisms of ursolic acid: Neurochemical studies: the expression levels of VEGF and ChAT protein in the hippocampus of mice were analyzed using Western blot analysis, acetylcholinesterase (AchE) activity in the mouse brain was performed using *ex vivo* and *in vitro* measurements of AChE activity.

2.2. Study on the antidepressant-like effects of *Ocimum sanctum* L. in olfactory bulbectomized mice (OBX) and unpredictable chronic mild stress-induced depression in mice (UCMS)

- The antidepressant-like effects of OS extract further fractionated with n-hexane (OS-H), ethyl acetate (OS-E), and n-butanol (OS-B) in OBX were evaluated by using behavioral tests: the tail suspension test (TST) and forced swimming test (FST).

- The antidepressant-like effects of OS-B were evaluated by using behavioral tests: th UCMS-induced anhedonia in mice was analysed by the sucrose preference test (SPT), while behavioural despair was assessed using the tail suspension test (TST) and forced swimming test (FST). Locomotor activities and grooming behaviour of mice were elucidated using the open-field test (OFT).

- The possible mechanism of OS-B via monoaminergic systems were demonstrated by administrating of ρ-chlorophenylalanine (PCPA), a tryptophan hydroxylase inhibitor, and α-methyl-ρ-tyrosine (AMPT), a tyrosine hydroxylase inhibitor in the tail suspension test.

3. Results and conclusion

3.1. The ameliorative effects of *Ocimum sanctum* L. on cognitive deficits

- OS (400 mg/kg, p.o.) and OS-E (200 and 400 mg/kg, p.o.) treatment attenuated OBX-induced impairment of spatial (Y-maze) and non-spatial (ORT) working memories.

- The administration of OS can lessen the cognitive deficits and neurohistological damages of OBX and these actions are, at least in part, mediated by the enhancement of central cholinergic systems and VEGF expression. In detail, OBX induced degeneration of septal cholinergic neurons, enlargement of the lateral ventricles, and suppression of hippocampal neurogenesis. OS and donepezil (DNP) treatment depressed these histological damages. OS administration reduced *ex vivo* activity of acetylcholinesterase in the brain. OBX diminished the expression levels of genes coding vascular endothelial growth factor (VEGF) and VEGF receptor type 2 (VEGFR2). Treatment with OS and DNP reversed OBX-induced decrease in VEGF gene and protein expression levels without affecting the expression of the VEGFR2 gene.

- Ursolic acid (UA, 6 and 12 mg/kg) and oleanolic acid (OA, 24 mg/kg), two major constituents in OS-E, attenuated the OBX-induced cognitive deficits in the modified Y maze test (Y-maze) and the Morris water maze (MWM) test.

- The effects of UA are, at least in part, mediated by normalizing the function of central cholinergic systems and VEGF protein expression. Specifically, UA (6 mg/kg) and donepezil reversed the OBX-induced down-regulation of vascular endothelial growth factor (VEGF) and choline acetyltransferase (ChAT) expression levels in the hippocampus. UA inhibited the *ex vivo* activity of acetylcholinesterase with similar efficacy to donepezil. UA inhibited the *in vitro* activity of acetylcholinesterase (IC50 = 106.5 μM), while the effects of OS, OS-E, and other isolated compounds were negligible. These findings suggest that UA and OA are responsible for the anti-dementia action of OS extract, whereas UA possesses a more potent anti-dementia effect than its isomer OA.

3.2. The antidepressant-like effects of *Ocimum sanctum* L.

- Treatment of OS extract and n-butanol fraction (OS-B) (400 mg/kg/day, p.o.) reduced OBX-induced despair behaviors in the tail suspension test (TST) and increased the climbing time in the forced swimming test (FST).

- The UCMS procedure for 5 weeks induced anhedonia in the saccharose consumption test (SPT), and this symptom was significantly ameliorated by the administration of OS-B (100 mg/kg) as well as imipramine (IMP, 8.0 mg/kg, i.p.) during the UCMS period. Moreover, the OS-B and IMP treatment attenuated the UCMS-induced enhancement of behavioural despair in the TST and FST. In OFT, mice subjected to UCMS showed a decrease in grooming behaviour, and the effect of UCMS was reversed by OS-B and IMP administrations. No significant difference in locomotor activities between each animal group was observed.

- The amelioration effects of OS-B and IMP on UCMS-induced behavioural despair in the TST were abolished by administrating of ρ-chlorophenylalanine (PCPA, 80 mg/kg, i.p), a tryptophan hydroxylase inhibitor, and α-methyl-ρ-tyrosine (AMPT, 100 mg/kg), a tyrosine hydroxylase inhibitor. The present results suggest that OS-B attenuates UCMS-induced depression-like symptoms via monoaminergic systems including in the noradrenergic, dopaminergic, and serotonergic systems.

Hanoi, September, 2022

ACADEMIC ADVISORS **DOCTORAL CANDIDATE**

Assoc. Prof. Dr. Nguyen Minh Khoi **Dr. Le Thi Xoan** **Nguyen Thu Hien**