SUMMARY OF DISSERTATION

Name of Doctoral candidate: **Nguyen Thi Thanh Loan**

Dissertation title: **Evaluating the pharmacological effects of persimmon leaves (*Diospyros kaki* L.f.) in the prevention and treatment of cerebral ischemia in experimental models**

Specialty: **Pharmacology - Clinical pharmacy** Code of specialty: **9720205**

Name of academic advisors:

1. Dr. **Le Thi Xoan**
2. Assoc. Prof. Dr**. Pham Thi Van Anh**

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

Summary of the dissertation:

1. Objectives

- To evaluate the neuroprotective effects of the standardized extract from *Diospyros kaki* L.f. leaves (DK) and the potent flavonoids isolated from DK in experiments

- To evaluate the effects of the standardized extract from *Diospyros kaki* L.f. leaves on some risk factors associated with cerebral infarction in experiments.

2. Methods

2.1. Evaluating the neuroprotective effects of the standardized extract from Diospyros kaki L.f. leaves (DK) and the potent flavonoids isolated from DK on experiments

- *Middle cerebral artery occlusion (MCAO)-and-reperfusion model in mice:* The MCAO/reperfusion model was induced using a monofilament suture with a silicon-coated tip. The suture was introduced into the external carotid artery and then inserted into the internal carotid artery to block the middle cerebral artery origin in the circle of Willis before being fixed in position. The temporary suture was removed from the common carotid artery. After 60 minutes of occlusion, the monofilament was completely removed to allow reperfusion.

- *Evaluating the neuroprotective effects of DK*: The present study investigated the neuroprotective effects of DK against MCAO-induced cerebral ischemic injury, using pre-

treatment and post-treatment protocols, which started the administration 7 days before and immediately after the MCAO operation.

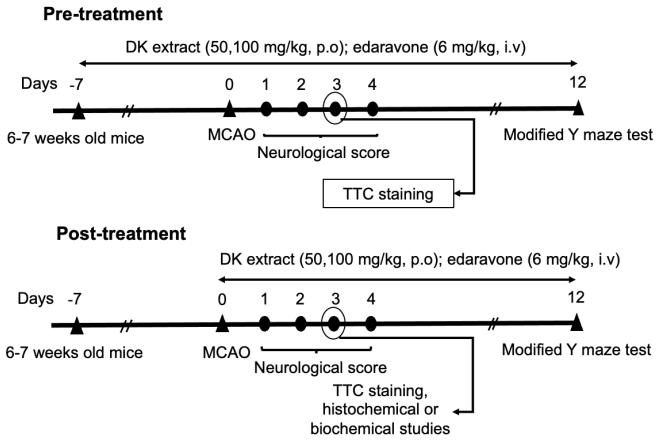

Figure 1. Experimental protocols

- *Evaluating the possible mechanisms underlying the effects of DK*: The number of different cells were determined using the appropriate stain techniques (euronal cells in the cerebral cortex, striatum, and hippocampus - Nissl staining; the number of poptotic cells in the cortex and striatum - TUNEL staining; the wet brain and the blood-brain barrier integrity - Evans blue dye extravasation); the antioxidant effects were assessed by measuring malondialdehyd (MDA) and glutathion (GSH) content in the brain cortex of ischemic mice; the expression VEGF, Akt, p-Akt and cleaved caspase-3 in the cerebral cortex of MCAO mice were assessed using Western blot analysis.

- *Clarifying isolated flavonoid components responsible for the effects of the DK extract, using an in vitro ischemia model caused by oxygen- and glucose-deprivation (OGD) in organotypic hippocampal slice cultures (OHSCs)*: DK or reference drugs were added to the culture medium 24 h before OGD and these treatments were continued for 24 h after the 60-min period of OGD. Neuronal cell damage of OHSCs was quantified by PI staining 24 h after exposure to OGD.

- *Investigating the neuroprotective effects of compound (**9**) (Kaempferol-3-O-(2"-O-galloyl-β-D-glucopyranoside)) on transient focal cerebral ischemic injury and underlying mechanisms using a mouse model of MCAO/reperfusion:*

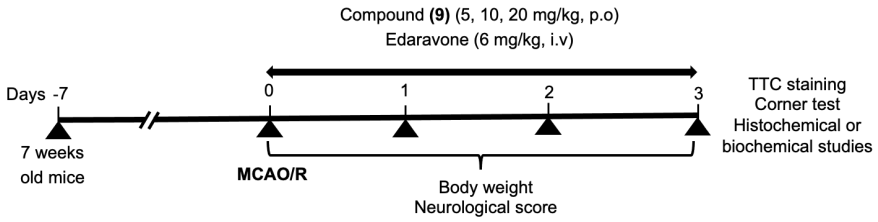

Figure 2. Experimental protocol

2.2. Evaluating the effects of DK on some risk factors related to cerebral infarction in experiments

- *Evaluating the anti-hypertensive effects of DK:* The cortisone acetate and NaCl 1% -induced hypertensive rats were treated daily with DK extract (50 and 100 mg/kg of body weight; p.o.). The blood pressure and heart rate of awake rats were measured using a non-invasive tail-cuff method. The heart weight and left ventricular wall thickness were measured. *In vitro* angiotensin-converting enzyme (ACE) inhibition activity of the DK extract and captopril were analysed.

- *Evaluating the effects of DK on dyslipidemia:*

Effects of DK in tyloxapol-induced dyslipidemic mice: Mice were treated daily with DK of fenofibrate for one week before tyloxapol injection (250 mg/kg, i.v). 20 h after the tyloxapol was administered, blood was collected to measure the serum lipid profiles.

Effects of DK in high-cholesterol diet fed rats: Rats were treated daily with DK extract or atorvastatin after 2h of an oral administration of oil-cholesterol mixture including cholesterol, cholic acid, propylthiouracil and peanut oil. Body weight, serum lipid profiles, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were evaluated.

3. Results and conclusion

3.1. Neuroprotective effects of DK and the potent isolated flavonoids from DK in experiments

- DK at doses of 50 and 100 mg/kg/day could exert protective and therapeutic effects on cerebral ischemia-induced brain damage by attenuating all the indices representing transient cerebral ischemia-induced damages, such as body weight loss, brain infarction, neurological impairments, and working memory deficits. This action was exerted via

exhibiting anti-oxidative, anti-apoptotic, and prosurvival properties while preserving the blood–brain barrier integrity by acting through the VEGF/Akt signalling pathway.

- Compound (**9**) (kaempferol-3-O-(2″-O-galloyl-β-D-glucopyranoside)) was the most promising potential neuroprotectant among nine flavonoids isolated from persimmon leaves. Compound (**9**) at concentration of 1 - 25 μM exhibited the neuroprotective activity against cerebral ischemic injury on an OGD-induced hippocampal cell damage model.

- Compound (9) at doses of 10 and 20 mg/kg displayed neuroprotective effects through ameliorating the indices representing transient cerebral ischemia-induced damages, such as body weight loss, brain infarction, neurological symptom scores, and behavior deficits. This action may be attributable to the preservation of blood-brain barrier integrity and suppression of oxidative stress caused by ischemic insult.

3.2. Effects of the standardized extract from *Diospyros kaki* L.f. leaves on some risk factors related to cerebral infarction in experiments

- DK at dose of 100 mg/kg significantly reduced systolic and diastolic blood pressure in the hypertensive rats. There were no significant changes observed in heart rate. In addition, the treatment of DK attenuated the increase in the heart weight and left ventricular wall thickness in hypertensive rats. DK extract also inhibited ACE activity in vitro with an IC_{50} of 4.71±0.53 μg/ml.

- DK at dose of 100 mg/kg could modulate dyslipidemia in tyloxapol-induced dyslipidemic mice via decreasing the levels of triglycerides, low density lipoprotein cholesterol, non-low density lipoprotein cholesterol, and increasing the levels of high density lipoprotein cholesterol, compared to the model group.

- DK at dose of 50 và 100 mg/kg possessed anti-dyslipidemic effects in high-cholesterol diet-induced dyslipidemia in rats. Treatment of DK extract decreased the serum total cholesterol, triglycerides and non-high-density lipoprotein cholesterol levels. Additionally, DK substantially diminished enzyme activity of serum AST and ALT, which were increased in the hypercholesterolaemia rats.

Hanoi, 30 November 2023

ACADEMIC ADVISORS **DOCTORAL CANDIDATE**

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