SUMMARY OF DISSERTATION

Name of Doctoral candidate: **Ly Hai Trieu**

Dissertation title: **Study on the hypoglycemic effect and mechanism of *Ensete glaucum* (Roxb.) Cheesman seeds in experimental models**

Speciality: **Pharmacology - Clinical pharmacy** Code of speciality: **972.02.05**

Name of academic advisors:

1. **Dr. Le Van Minh**
2. **Assoc. Prof. Dr. Nguyen Thi Thu Huong**

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

Summary of the dissertation:

1. Objectives

- Evaluating the hypoglycemic effect and related effects of ethanol extract of *Ensete glaucum* seeds in the model of experimental hyperglycemia.
- Determining the mechanisms of hypoglycemic effect of ethanol extract and some isolated compounds of *Ensete glaucum* seeds in experimental models.

2. Methods

2.1. Oral glucose tolerance test

Mice were fasted overnight, oral glucose tolerance test (OGTT) was performed one hour after oral administration of test samples (the extract at doses of 12.5, 25, and 50 mg/kg; glibenclamide at dose of 5 mg/kg). Plasma glucose level was determined at 0, 30, 60, and 120 min after oral glucose at dose of 2 g/kg. The evaluated criteria include plasma glucose concentration at each time point, the percentage of hypoglycemia compared to the control group at the same time, and the area under the curve (AUC) of glucose.

2.2. Study design to evaluate the effect of the ethanol extract in streptozotocin (STZ)-induced hyperglycemic mice

Mice were fasted overnight and tail blood was collected to determine baseline fasting blood glucose (FBG). Normoglycemic mice were injected (*i.p.*) with a single dose of 170 mg/kg of STZ. On day 7, tail blood was collected to determine FBG levels. Mice with FBG higher than 200 mg/dL were selected for the test. Mice divided into physiological group

were injected (*i.p.*) with 0.1 M sodium citrate solution, pH = 4.5 (STZ solvent) at the same time as STZ injection.

Mice were given oral test samples (the extract at doses of 12.5, 25, and 50 mg/kg, glibenclamide at dose of 5 mg/kg) once a day in the morning for 7 consecutive days. After 1 hour of oral administration on day 7, the evaluated criteria include:

Evaluation of hypoglycemic effect: FBG; OGTT.

Evaluation of the effect of improving liver and kidney damage: Biochemical indices: AST, ALT, ALP, GGT, creatinine and BUN in serum; Histopathological examination of liver and kidney; Levels of oxidative stress markers in liver and kidney tissues: MDA and GSH; Levels of inflammatory markers in liver and kidney tissues: TNF-α and IL-6.

Evaluation of the effect of stimulating pancreatic β cells to secrete insulin: Fasting blood insulin concentration.

Evaluation of pancreatic protection effect: Size and number of pancreatic islets; Expression levels of some proteins in the cell apoptosis pathway (Bax, Bcl-2, Cytochrome c, cleaved caspase-3, Poly(ADP-ribose) polymerase (PARP), p-p38 MAPK, ERK1/2, JNK1 , p-AMPK, and NF-κB p65) in pancreatic tissues by Western blot; Levels of oxidative stress markers in pancreatic tissues: MDA and GSH; Levels of inflammatory markers in pancreatic tissues: TNF-α and IL-6.

Evaluation of the effect of increasing insulin sensitivity: Expression level of p-AMPK in liver tissues by Western blot.

2.3. The assays to evaluate the effect of reducing or slowing down glucose absorption

- *In vitro* α-amylase inhibitory assay;
- *In vitro* α-glucosidase inhibitory assay;
- *Ex vivo* inhibition of intestinal glucose absorption.

2.4. Study design to evaluate the effect of stimulating insulin-secreting pancreatic β cells and protecting islet cells in the *in vitro* pancreatic islet models

On isolated pancreatic islets stimulated by glucose: Isolated pancreatic islets were co-treated with glucose (2.8 and 16.8 mM) and extract/compounds at different concentrations for 60 min. Glimepiride was used as a positive control. The effect of the extract and compounds on stimulating pancreatic β cells to secrete insulin was assessed using the glucose-stimulated insulin secretion (GSIS) assay.

On the model of pancreatic islet damage with STZ: Isolated pancreatic islets were co-treated with STZ (5 mM) and extract/compounds at different concentrations for 24 hours. Glimepiride was used as a positive control. The effects of the extract and compounds was evaluated through:

- Evaluating the effect of stimulating pancreatic β cells to secrete insulin of the extract and compounds using the GSIS assay.
- Evaluating the protective effect of the extract and compounds on islet cell survival by MTT assay and of the extract by examining the protein expression of Bax, Bcl-2, cleaved caspase-3, and PARP by Western blot.

2.5. *In vitro* assay to evaluate the insulin sensitivity-increasing effect

- *In vitro* PTP1B inhibitory assay.

3. Results and conclusion

3.1. Hypoglycemic effect and related effects of ethanol extract of *Ensete glaucum* (Roxb.) Cheesman seeds in the model of experimental hyperglycemia

Hypoglycemic effect of the extract: The extract at doses of 12.5 and 25 mg/kg have hypoglycemic effect in oral glucose tolerance test on normal mice. The extract at doses of 12.5, 25, and 50 mg/kg have hypoglycemic effect in 170 mg/kg of STZ-induced hyperglycemic mouse model. The extract at doses of 25 and 50 mg/kg have hypoglycemic effect in oral glucose tolerance test on 170 mg/kg of STZ-induced hyperglycemic mouse model.

Effects of ameliorating liver and kidney damage of the extract: The extract at the dose of 50 mg/kg has the effect of enhancing liver and kidney function by reducing serum AST, ALT, creatinine and BUN levels, reducing MDA content, recovering histological structure of hepatic and renal tissues. The extract at the dose of 25 mg/kg has the effect of increasing hepatic GSH content.

3.2. Mechanism of hypoglycemic effect of ethanol extract and some isolated compounds of *Ensete glaucum* (Roxb.) Cheesman seeds in experimental models

The mechanisms of hypoglycemic effect of the extract include: **(1)** Reducing/slowing down glucose absorption by inhibiting α-amylase with IC_{50} of 222.80 µg/mL, α-glucosidase with IC_{50} of 1.58 µg/mL, and intestinal glucose absorption at concentrations of 2.5 and 5 mg/mL; **(2)** Stimulating pancreatic β cells to secrete insulin at concentrations of 50 and 100

μg/mL in the isolated pancreatic islet model and at doses of 25 and 50 mg/kg in 170 mg/kg of STZ-induced hyperglycemic mouse model; **(3)** Protecting the pancreatic islet cells at a concentration of 100 μg/mL may be through the anti-apoptotic mechanism in STZ-damaged isolated pancreatic islet model; protecting the pancreatic cells at the dose of 50 mg/kg via the mechanisms of anti-membrane lipid peroxidation, anti-inflammation and anti-apoptosis in STZ-damaged mouse model; **(4)** Increasing insulin sensitivity through the mechanism of hepatic AMPK activation at doses of 25 and 50 mg/kg in STZ-damaged mouse model and possibly the inhibition of PTP1B.

The mechanisms of hypoglycemic effect of some isolated compounds of *Ensete glaucum* seeds: Afzelechin and coniferaldehyde have effects that contribute to the hypoglycemic mechanism of ethanol extract of *Ensete glaucum* seeds as follows: **(1)** Reducing/slowing down glucose absorption by inhibiting α-glucosidase with IC_{50} of 184.63 and 52.84 μM, respectively; **(2)** Stimulating pancreatic β cells to secrete insulin at a concentration of 100 μM in the isolated pancreatic islet model with the predicted mechanism of binding and closing the ATP-sensitive potassium channel (K_{ATP}); **(3)** Protecting pancreatic β cells at a concentration of 100 μM in STZ-damaged isolated pancreatic islet model; **(4)** Increasing insulin sensitivity by inhibiting PTP1B with IC_{50} of 7.58 and 8.39 μM, respectively.

Ho Chi Minh City, 19 August 2024

Academic advisors **Doctoral Candidate**

Dr. Le Van Minh **Assoc. Prof. Nguyen Thi Thu Huong** **Ly Hai Trieu**