Researching the dyslipidemia treatment effect of Ha mo Nk total extract in experiment

Pham Thuy Phuong[1], Hoang Trong Quan[1], Nguyen Pham Ngoc Mai[2]
Nguyen Thanh Luan[1], Trinh Vu Lam[1], Le Minh Trung[1]
[1]Viet Nam University of Traditional Medicine
[2]University of Science and Technology of Hanoi

SUMMARY

Objective: *To study the acute toxicity and dyslipidemia treatment effects of Ha mo NK total extracts in experiment.*

Subjects and methods: *Study the acute toxicity on Swiss albino, both genders, weighing 18 - 22g. Study the dyslipidemia treatment effects on Swiss albino, both genders, weighing 25 ± 2g using empirical endogenous model.*

Results*: The LD_{50} of Ha mo NK total extract has not been determined, there were no signs of acute toxicity at a dose of 59.52 grams of Ha mo NK/kg (12.33 times higher than the expected clinical dose). The Ha mo NK total extract at a dose of 4.8g/kg/day (equivalent to the expected clinical dose) and 14.5g total extract/kg/day has the effect of reducing the concentration of total cholesterol and non-HDL-Cholesterol with total cholesterol reduction levels of 20.4% and 18.1% respectively compared to the model batch, the difference is significant statistics at p<0.01, tends to increase HDL-Cholesterol concentration, and reduce Triglyceride in white mice modeled with P-407-induced dyslipidemia.*

Conclusion: *The total extract Ha mo NK at a dose 12.33 times higher than the expected human dose but has no acute toxicity in mice, orally. The total extract of Ha mo NK at a dose of 4.8g/kg/day (equivalent to the expected clinical dose) and 14.5g/kg/day (3 times the expected clinical dose on humans) tended to increase HDL-Cholesterol index, and reduce Triglyceride; and had the effect of reducing total cholesterol (TC) and non-HDL-Cholesterol index in white mice modeled with dyslipidemia by P-407.*

Keywords*: Total extracts, dyslipidemia.*

INTRODUCTION

Dyslipidemia is one of the leading risk factors for the development and progression of atherosclerosis - the leading cause of morbidity and mortality in the United States and in most developed countries. In 2016, coronary and cerebrovascular atherosclerosis caused approximately 18 million deaths worldwide, accounting for > 30% of all deaths [1]. A report from the American Heart Association about Heart Disease and Stroke Statistics-2019 showed that, more than 800,000 peoples died of cardiovascular disease, representing nearly one-third of all deaths in The United States of America [2].

In Vietnam, dyslipidemia tend to increase rapidly with rapid social development. Therefore, dyslipidemia is one of the current public health concerns. Treatment with modern medicine has made great progress but

Corresponding author: Pham Thuy Phuong
Phone number: (+84) 983654033
Email address: thuyphuongydhctvn@gmail.com
DOI: https://doi.org/10.60117/vjmap.v60iĐặc biệt 02.377

Received: 19/8/2024
Reviewed: 24/10/2024
Accepted: 08/3/2025

still has some side effects such as: Digestive disorders, muscle pain, increased liver enzymes,...[3].

In addition to treating dyslipidemia with modern medicine, traditional medicine literature also presents a number of methods for treating these diseases. However, these treatment methods are often built on an ancient theoretical foundation and valuable treatment experiences left by our ancestors. Proving, studying the scientific basis, finding new effects of traditional medicine and creating conditions for the modernization of traditional medicine are necessary.

The remedy Ha mo NK is Vietnamese remedy of the former physician Nguyen Kieu which has the energy circulating effect, cooling and eliminate stagnation, phlegm-dampness according to traditional medicine. In clinical practice, certain results have been achieved in the treatment of patients with dyslipidemia. To clarify the effect of the total extract of Ha mo NK in the treatment of dyslipidemia, the research team conducted the project with the following objectives: To study the acute toxicity and dyslipidemia treatment effects of Ha mo NK total extracts in experiment.

OBJECTIVES AND RESEARCH METHODS

Research materials

The total extract of Ha mo NK is extracted from the Ha mo NK medicine of the former physician Nguyen Kieu, meeting basic standards. The combined formula of the extract rich in active ingredients in the remedy was determined. Tran bi, Nguu tat and Hoe hoa extract were calculated based on the content of hesperdine, saponin total and rutin in the water extract of the respective remedy. Extracts rich in active ingredients of medicinal herbs such as Re co tranh, Thao quyet minh and La sen extract are mixed in the formula based on extrapolation between the water extract of each medicinal herb and the water extract of the whole remedy by the mass of common dry medicinal herbs through polysaccharides, total anthranoids and total flavonoids corresponding to the amount of medicine in the remedy. Ty giai, Ban ha nam and Ha kho thao extract are mixed in a formula rich in active ingredients based on the ratio between the mass of the obtained dried extract and the original medicinal material.

Expected clinical dosage: 20.1 grams of total medicinal extract/day, equivalent to 1 decoction of 98 grams of medicinal herbs.

Ingredients of the remedy

No.	Medication name	Scientific name	Dose (g)
1	Tran bi	*Pericarpium Citri reticulatae perenne*	6
2	Ty giai nam	*Rhizoma Smilax ferox*	12
3	Nguu tat	*Radix Achyranthis bidentatae*	12
4	Than re co tranh	*Rhizoma Imperatae cylindricae*	12
5	Thao quyet minh	*Semen Cassiae torae*	12
6	Hoe hoa	*Flos Styphnolobii japonicI imaturi*	12
7	La sen	*Folium Nelumbinis nuciferae*	12
8	Ha kho thao	*Spica Prunellae Vulgaris*	12
9	Ban ha nam	*Rhizoma Typhonium trilobatum*	8

Research subjects

***Acute toxicity*:** Healthy Swiss white mice, both genders, weighing 18 - 22g, provided by the National Institute of Hygiene and Epidemiology. The mice were kept in the laboratory of the Department of Pharmacology for 5-10 days before the study and throughout the study period with standard food for mice (provided by the National Institute of Hygiene and Epidemiology), drinking water freely.

Effects of dyslipidemia treatment on experimental endogenous models: Healthy Swiss white mice, both genders weighing 20 ± 2g, provided by the Central Institute of Hygiene and Epidemiology. Animals were kept for 7-10 days before the study and throughout the study period with standard food and drinking water freely at the laboratory of the Department of Pharmacology - Hanoi Medical University.

Research methods

***Acute toxicity*:** Mice were divided into different groups, each group of 10, and were given the total extract of Ha mo NK with increasing doses in the same volume to determine the lowest dose that causes 100% death of mice and the highest dose that does not died mice (causes 0% death of mice). All dead mice were autopsied to assess gross lesions. From there, a linear graph was constructed to determine the LD_{50} of the test drug. Then continue to monitor the condition of the mice until the end of the 7th day after taking the reagent [4],[5].

***Research on the effects of treating dyslipidemia on a model of dyslipidemia caused by endogenous mechanisms*:** Prepare a 2% P-407 solution by mixing 0.4g of P-407 in 0.9% saline solution to 20 mL, and refrigerate overnight to increase the solubility of P-407 [6]. The needle and syringe used to inject mice were soaked in ice water before use. White mice were divided into 5 groups, each group of 10 mice. The groups were injected and given medicine as follows:

Group 1 (Biomarker Group): Inject peritoneally with 0.9% physiological saline with a volume of 0.1mL/10g of mouse body weight and drink distilled water.

Group 2 (Model): Inject intraperitoneally 2% P-407 solution at a dose of 200 mg/kg (0.1mL/10g) and drink distilled water.

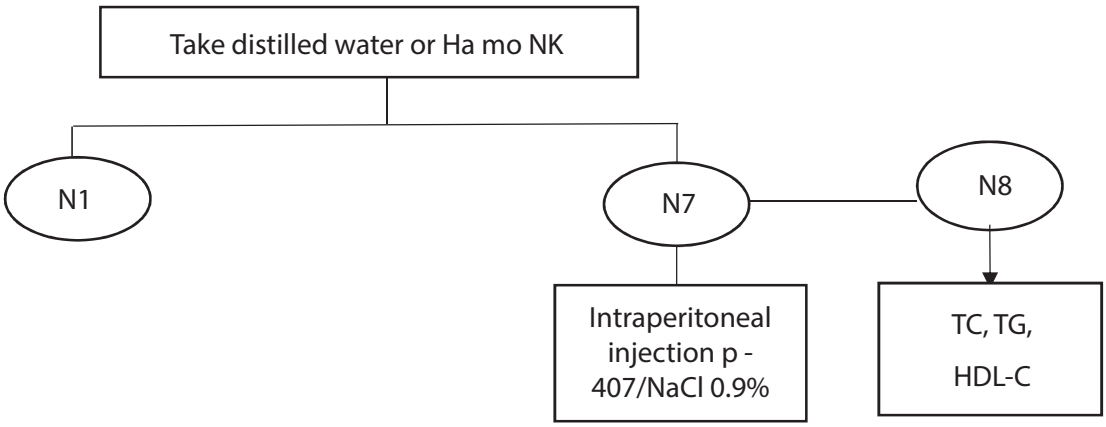
Group 3 (Take atorvastatin): Inject intraperitoneally with 2% P-407 solution at a dose of 200 mg/kg (0.1mL/10g) and take with Atorvastatin at a dose of 100 mg/kg.

Group 4 (Treatment group 1): Intraperitoneal injection of 2% P-407 solution at a dose of 200 mg/kg (0.1 mL/10g), take Ha mo NK at a dose of 4.8g of total extract/kg/day (equivalent to the expected clinical dose, calculated by a factor of 12).

Group 5 (Treatment group 2): Intraperitoneal injection of 2% P-407 solution at a dose of 200 mg/kg (0.1 mL/10g), take Ha mo NK at a dose of 14.5g of total extract/kg/day (3 times the expected clinical dose).

White mice were given distilled water and reagent for 7 consecutive days before being injected intraperitoneally with P-407 solution. After being injected with P-407, the mice were completely fasted but still allowed to drink water freely. After 24 hours of being injected with P-407, all mice had their carotid artery blood collected for quantitative testing of Total cholesterol (TC), Triglyceride (TG), and High Density Lipoprotein Cholesterol (HDL-C). Non-HDL-C was calculated according to the formula: Non-HDL-C = TC – HDL-C (mmol/L).

Research diagram:



Data processing methods

The data were statistically processed using the Student T-test statistical algorithm using Microsoft Excel software, biomedical statistical methods using the student t-test and pre-post test (Avant-après) (X± SD).

Convention (compared to control group): *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$. The difference is statistically significant when $p<0.05$.

Ethics in research

Ensuring and fully comply with ethical regulations in Biomedical research.

RESULTS

Acute toxicity results

White mice were given the total extract of Ha mo NK from the lowest dose to the highest dose. The group of mice took up to a dose of 0.25 ml/10g, 4 times in 24 hours of the most concentrated solution. Monitoring showed that the total of Ha mo NK had no signs, no abnormal symptoms appeared within 72 hours after taking the test drug.

Table 1. Results of acute toxicity research of total extract Ha mo NK

Group of mice	n	Dose (ml/kg)	Dose (g Ha mo NK/kg)	Mortality rate (%)	Other unusual signs
Group 1	10	30	17.85	0	No
Group 2	10	45	26.78	0	No
Group 3	10	60	35.71	0	No
Group 4	10	75	44.64	0	No
Group 5	10	100	59.52	0	No

The groups of mice that drank the whole extract of Ha mo NK at doses from 30ml/kg corresponding to 17.85 grams of total extract of Ha mo NK total extract/kg to the maximum dose of 100ml/kg corresponding to 59.52 grams of total extract of Ha mo NK total extract/kg did not show any signs of acute toxicity.

Results of treatment effects on dyslipidemia in endogenous models

Table 2. Modeling dyslipidemia using Poloxamer 407

Lipid index	Biomarker Group (n = 10) ($\overline{X} \pm SD$, mmol/L)	Model group (n = 10) ($\overline{X} \pm SD$, mmol/L)
TC	4.18 ± 1.12	9.93 ± 1.10 ***
TG	0.97 ± 0.16	8.19 ± 2.65 ***
HDL-C	0.80 ± 0.23	1.52 ± 0.40 ***
Non-HDL-C	3.38 ± 1.03	8.41 ± 1.29 ***

Intraperitoneal injection of 2% P-407 solution at a dose of 200 mg/kg (0.1mL/10g) has a clear effect of causing lipid disorders: In the model group, TG increased 8.4 times; TC increased 2.4 times; HDL-C increased 1.9 times and non-HDL-C increased 2.5 times.

Table 3. Effect of Ha mo NK on index total cholesterol in white mice

Research group (n= 10)	Total Cholesterol index (mmol/L)	Reduction compared to model group	p vs group 2	p vs group 3
Group 1: Biomarker	4.18 ± 1.12			
Group 2: Model	9.93 ± 1.10			
Group 3: Atorvastatin 100mg/kg	7.68 ± 2.13	22.7%	< 0.01	
Group 4: Ha mo NK 4.8g /kg/day	7.94 ± 1.76	20.4%	< 0.01	> 0.05
Group 5: Ha mo NK 14.5g /kg/day	8.13 ± 1.30	18.1%	< 0.01	> 0.05

Total cholesterol Index of white mice in the model group and drug groups at 24 hours after intraperitoneal injection of P-407 solution to cause dyslipidemia by endogenous mechanism.

The group taking Atorvastatin at a dose of 100mg/kg clearly reduced total cholesterol index compared to the model group (reduced by 22.7%), the difference was statistically significant ($p<0.01$).

Both groups taking Ha mo NK at a dose of 4.8g/kg/day and Ha mo NK at a dose of 14.5g/kg/day reduced total cholesterol levels compared to the model group (reduced by 20.4% and 18.1%), the difference was statistically significant ($p<0.01$). There was no difference when compared to the group using Atorvastatin ($p>0.05$).

Table 4. Effect of Ha mo NK on Triglyceride index in white mice

Research group (n=10)	Triglycerid index (mmol/L)	Reduction compared to model group	p vs group 2	p vs group 3
Group 1: Biomarker	0.97 ± 0.16			
Group 2: Model	8.19 ± 2.65			
Group 3: Atorvastatin 100mg/kg	9.80 ± 3.29	- 19.7 %	> 0.05	
Group 4: Ha mo NK 4,8g /kg/day	7.26 ± 2.16	11.4 %	> 0.05	> 0.05
Group 5: Ha mo NK 14,5g /kg/day	7.35 ± 2.23	10.3 %	> 0.05	> 0.05

Triglyceride index of white mice in model groups and drug groups at 24 hours after intraperitoneal injection of P-407 solution to cause dyslipidemia by endogenous mechanism.

The group taking Atorvastatin at a dose of 100mg/kg did not reduce Triglyceride index compared to the model group (p>0.05).

The groups taking Ha mo NK at a dose of 4.8g/kg/day and the group taking Ha mo NK at a dose of 14.5g/kg/day both had a tendency to reduce Triglyceride concentration compared to the model group (11.4% and 10.3%), however, the difference was not statistically significant (p>0.05).

Table 5. Effect of Ha mo NK on HDL-Cholesterol index in white mice

Research group (n=10)	HDL-C index (mmol/L)	p vs group 2	p vs group 3
Group 1: Biomarker	0.80 ± 0.23		
Group 2: Model	1.52 ± 0.40		
Group 3: Atorvastatin 100mg/kg	2.02 ± 0.69 (↑ 32.9 %)	> 0.05	
Group 4: Ha mo NK 4.8g /kg/day	1.64 ± 0.19 (↑ 7.9 %)	> 0.05	> 0.05
Group 5: Ha mo NK 14.5g /kg/day	1.55 ± 0.48 (↑ 1.97%)	> 0.05	> 0.05

HDL-Cholesterol index in the blood of white mice in model groups and drug groups at 24 hours after intraperitoneal injection of P-407 solution to cause dyslipidemia according to endogenous mechanism.

In both the group taking Atorvastatin 100mg/kg/day and the groups taking Ha mo NK at a dose of 4.8 g/kg/day and a dose of 14.5 g/kg/day, there was a tendency to increase the blood HDL-Cholesterol index compared to the model group, however, the difference was not statistically significant (p>0.05).

Table 6. Effect of Ha mo NK on non-HDL-Cholesterol index in white mice blood

Research group (n=10)	Non- HDL-Cholesterol index (mmol/L)	Reduction compared to model group	p vs group 2	p vs group 3
Group 1: Biomarker	3.38 ± 1.03			
Group 2: Model	8.41 ± 1.29			
Group 3: Atorvastatin 100mg/kg	5.66 ± 1.87	32.7 %	< 0.01	
Group 4: Ha mo NK 4.8g /kg/day	6.30 ± 1.62	25.1%	< 0.01	>0.05
Group 5: Ha mo NK 14.5g /kg/day	6.58 ± 1.11	21.8 %	< 0.01	> 0.05

Blood Non-HDL-Cholesterol index of white mice in model groups and drug groups at 24 hours after intraperitoneal injection of P-407 solution to cause dyslipidemia endogenous.

The group taking Atorvastatin at a dose of 100mg/kg and the 2 groups taking Ha Mo NK at a dose of 4.8g/kg/day and a dose of 14.5g/kg/day both significantly reduced Non-HDL-Cholesterol index compared to the model group, the difference was statistically significant (p<0.01). There was no difference when compared with Atorvastatin at a dose of 100mg/kg (p>0.05).

DISCUSSION

Medicines derived from plants and animals have been widely used in the world to support the treatment of diseases and maintain health. In developing countries, the use of traditional medicine is considered a commonly applied treatment method. Modern medicine when used can have many unwanted effects, so the trend of seeking traditional medicine is increasing. If in the past, the use of Vietnamese remedy according to folk experience was considered safe, without adverse reactions, now people are beginning to point out the adverse effects of many medicinal herbs when used alone or in combination. Therefore, toxicity research is a very important step before clinical use.

Table 1 shows: The group of mice took a dose of 0.25 ml/10g, 4 times in 24 hours of the most concentrated solution, observed that the high doses of total Ha mo NK did not show any signs, no abnormal symptoms appeared. The observation results showed that all mice in the groups did not show any special symptoms, ate and moved normally, had dry stools, and no mice died within 72 hours of taking the medicine. 07 days after taking Ha mo NK all mice were alive and no abnormalities were seen in all groups. Because there were no dead mice in any batch, the LD_{50} of Ha Mo NK orally could not be determined by the Litchfield - Wilcoxon method [5].

To research and evaluate a drug's effectiveness in treating dyslipidemia, it is first necessary to create an experimental model of dyslipidemia. In which, it may be a model of chronic dyslipidemia by exogenous mechanism by introducing cholesterol, sucarose for a long time through food or can cause acute hypercholesterolemia by endogenous mechanism by non-ionized surfactants that increase cholesterol synthesis or can combine both of these mechanisms. The research team chose the hyperlipidemia model based on the endogenous mechanism when evaluating the effects of Ha mo NK in experiments.

The endogenous hyperlipidemia model is performed by using non-ionic surfactants such as Tween 80, Triton WR-1339 or Poloxamer 407

(P-407) systemically without the need for exogenous lipid sources supplemented by food. After intraperitoneal injection of P-407, blood lipid concentrations began to increase and peaked after 24 hours, then gradually decreased to normal. Therefore, the research team chose the time 24 hours after P-407 injection to quantitatively evaluate the blood lipid index of experimental mice [6].

The research team used P-407 to create a model to evaluate the effect of Ha mo NK, the control drug used was Atorvastatin - a statin derivative. Atorvastatin has a structure similar to HMG-CoA, so it competitively inhibits HMG-CoA reductase, reduces cholesterol synthesis, and increases LDL receptors in the cell membrane, so it is the preferred choice in cases of hypercholesterolemia. Therefore, atorvastatin was chosen as the control drug in the study [7],[8].

The results on the endogenous model showed that: The group taking Atorvastatin at a dose of 100mg/kg clearly reduced the concentration of total cholesterol compared to the model group (reduced by 22.7%), the difference was statistically significant ($p<0.01$). In both groups taking Ha mo NK at a dose of 4.8g of total extract/kg/day and Ha mo NK at a dose of 14.5g of total extract/kg/day, the concentration of total cholesterol was reduced compared to the model group (reduced by 20.4% and 18.1%), the difference was statistically significant ($p<0.01$). There was no difference when compared to the group taking Atorvastatin ($p>0.05$).

The group using Atorvastatin at a dose of 100mg/kg and the 2 groups using Ha mo NK at a dose of 4.8g/kg/day and a dose of 14.5g/kg/day both significantly reduced Non-HDL-Cholesterol concentration compared to the model group, the difference was statistically significant ($p<0.01$). There was no difference when compared with Atorvastatin at a dose of 100mg/kg ($p>0.05$).

Evaluation of the effect of treating dyslipidemia on endogenous models through the Triglyceride index showed that the group using Atorvastatin at a dose of 100mg/kg did not reduce the Triglyceride index compared to the model group ($p>0.05$). The groups taking Ha mo NK at a dose of 4.8g/kg/day and the group using Ha mo NK at a dose of 14.5g/kg/day both tended to reduce the Triglyceride index compared to the model group (11.4% and 10.3%), however, the difference was not statistically significant ($p>0.05$).

In both the group using Atorvastatin 100mg/kg/day and the groups using Ha mo NK at a dose of 4.8 g total extract/kg/day and a dose of 14.5 g total extract/kg/day, there was a tendency to increase the blood HDL-Cholesterol index compared to the model group, however, the difference was not statistically significant ($p>0.05$).

Some medicinal herbs in the composition of Ha mo NK have also been proven to have lipid-lowering effects through the mechanism of changing the activity of the above enzymes. Research in China shows that Thao quyet minh is used to prevent and treat dyslipidemia through the mechanism of inhibiting cholesterol synthesis, ethanol extraction and water solvent extraction of Thao Quyet Minh reduce significantly TC, TG and Low Density Lipoprotein Cholesterol (LDL-C) index in serum, while increasing HDL-C index [9]. In addition, the presence of hesperidin in Tran Bi extract has been shown by Raushan Kumar et al. (2021) to have the effect of reducing TC, TG in white rats with dyslipidemia using P-407 [10]. Rauushan pointed out that hesperidin has a protective effect against oxidative stress and redox imbalance caused by P-407 in mice. Besides, flavonoid compounds such as quercetin in Ha Kho Thao, Thao Quyet Minh and La Sen help inhibit cholesterol biosynthesis by inhibiting HMG Co-A reductase, an enzyme that plays an important role in controlling the index of cholesterol. lipids in plasma and other tissues, at the same time increasing the expression of the C7αH enzyme, increasing the conversion of cholesterol into bile acids, thereby reducing the TC index in the blood,

contributing to the treatment of dyslipidemia caused by P- 407 causing [10].

CONCLUSION

On the acute toxicity of total extract of Ha mo NK in experiments

- LD_{50} in white mice of the total extract of Ha mo NK by oral route has not been determined.

- Total extract of Ha mo NK does not show acute toxicity at a dose of 59.52 grams of Ha mo NK/kg.

- Total extract Ha mo NK at a dose 12.33 times higher than the expected human dose but has no acute toxicity in mice, orally (Calculated for adults weighing 50 kg, extrapolation coefficient above mice: 12, maximum dose 20.1 grams of total extract Ha mo NK/day/person).

On the effect of treating lipid disorders of the total extract of Ha mo NK on experimental endogenous model

- The total extract of Ha mo NK at a dose of 4.8g/kg/day (equivalent to the expected clinical dose) and 14.5g/kg/day (3 times the expected clinical dose on humans) tended to increase HDL-Cholesterol index, and reduce Triglyceride.

- The total extract of Ha mo NK at a dose of 4.8g/kg/day (equivalent to the expected clinical dose) and 14.5g/kg/day (3 times the expected clinical dose on humans) had the effect of reducing total cholesterol (TC) and non-HDL-Cholesterol index in white mice modeled with dyslipidemia by P-407.

- Ha mo NK total extract at a dose of 4.8g/kg/day (equivalent to the expected clinical dose) tends to be more effective than a dose of 14.5g/kg/day (3 times the expected clinical dose in humans).

REFERENCES

1. **Global Health Estimates.** *Deaths by Cause, Age, Sex, by Country and by Region, 2000-2016*, Geneva, World Health Organization, 2018.
2. **Benjamin EJ, Muntner P, Alonso A, et al.** Heart Disease and Stroke Statistics- 2019. *A report From the American Heart Association,* 2019, Circulation 139: e56–e528.
3. **Ministry of Health Viet Nam.** *Roi loan chuyen hoa Lipid mau, Huong dan chan doan va dieu tri benh Noi tiet - Chuyen hoa*, Medical Publishing House, 2015, pp.255-262
4. **WHO.** Working group on the safety and efficacy of herbal medicine. *Report of regional office for the western pacific of the World Health Organization,* 2013.
5. **Litchfield JT, Wilcoxon F.** A simplified method of evaluating dose-effect experiments. *J Pharmacol Exp Ther,* 1949,96(2), pp.99-113.
6. **Millar JS, Cromley DA, McCoy MG, et al.** Determining hepatic triglyceride production in mice: comparison of poloxamer 407 with Triton WR-1339. *J Lipid Res.*, 2005, 46(9):2023-2028. doi:10.1194/jlr.D500019-JLR200.
7. **Kones R, Rumana U.** Current Treatment of Dyslipidemia: A New Paradigm for Statin Drug Use and the Need for Additional Therapies. *Drugs,* 2015, 75(11): 1187-1199. doi:10.1007/s40265-015-0428-4.
8. **Mohamed AS, Ibrahim WM, Zaki NI, et al**. Effectiveness of Coelatura aegyptiaca Extract Combination with Atorvastatin on Experimentally Induced Hyperlipidemia in Rats. *Evidence-Based Complementary and Alternative Medicine,* 2019: e9726137. doi:10.1155/2019/9726137.
9. **Dong X, Fu J, Yin X, et al. Cassiae semen.** A review of its phytochemistry and pharmacology. *Mol Med Rep*., 2017; 16(3): 2331-2346. doi: 10.3892/mmr. 2017.6880.
10. **Pham Thuy Phuong**. Effects of Ha mo NK hard capsule on experimental atherosclerosis model. *Journal of medical research,* 2021, No 5, pp.95-103.