MINISTRY OF EDUCATION&TRAINING MINISTRY OF HEALTH

VIETNAM UNIVERSITY OF TRADITIONAL MEDICINE

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STUDY ON THE SAFETY AND THE EFFECTS OF HSN REMEDY ON DYSLIPIDEMIA ADJUSTMENT IN CLINICAL AND EXPERIMENTAL SETTINGS

SUMMARY OF DOCTORAL DISSERTATION

Hanoi, 2018

THIS DISSERTATION HAS BEEN CARRIED OUT AT VIETNAM UNIVERSITY OF TRADITIONAL MEDICINE

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Argumentant 1:

Argumentant 2:

The thesis will be defended at the Board of Examiners of Vietnam University of Traditional Medicine at ……, on ……., 2018.

The thesis will be available at the library of Vietnam University of Traditional Medicine.

RATIONALE

Dislipidemia is a term used to describe some of the chronic diseases characterized by changes in blood lipids indicators. Patients with dyslipidemia tend to suffer from many risk factors such as atherosclerosis, myocardial infarction, etc. Along with the development of society, the disease patterns also change. According to the World Health Organization (WHO), the proportion of people with lipid disorders in the world is increasing. In 2008, the prevalence of adult people with dyslipidemia was 39%, of which Europe estimated the highest rate of dyslipidemia with 54%, followed by America with 48%. Africa and Southeast Asia had a lower rate of dislipidemia with 22.6% and 29% respectively, and the incidence of dyslipidemia was directly proportional to the per capita income in the whole country.

Along with the development of modern medicine, traditional medicine has been asserting itself and making great contributions to the health care for the community at the same time. In terms of traditional medicine, the manifestations of lipid disorders, atherosclerosis, overweight, etc are described in a number of diseases caused by phlegm-dampness.

HSN remedy is made up of a combination of six medicinal materials with the rheumatism prevention, phlegm elimination. To better understand the effectiveness of HSN remedy, we conducted a research titled *"Study on the safety and the effects of HSN remedy on dyslipidemia adjustment in clinical and experimental settings"* with three main aims as follows:

(1) To determine the acute toxicity and semi-chronic toxicity of HSN remedy.

(2) To study the effectiveness of dyslipidemia adjustment of HSN remedy in experimental setting.

(3) To evaluate the HSN treatment results of dyslipidemia in clinical setting.

SIGNIFICANCE OF THE STUDY

As new contributions of this dissertation scientific significance. The study on using HSN to adjust dyslipidemia in clinical and experimental setting has shown some reliable result which is the basis for subsequent studies on larger models in order to discover more medicinal products originated from herbs for dyslipidemia treatment.

Practical significance

As we know that dyslipidemia is matter of great concern to researchers in the world as well as in our country. Dyslipidemia is a risk factor leading to cardiovascular events such as hypertension, atherosclerosis, myocardial infarction, etc. Therefore, HSN was taken into account to provide scientific evidence of the effects of dyslipidemia adjustment as well as adverse effects (if any) in the clinical and experimental trials.

Some new contributions

The effects on dyslipidemia adjustment in experimental setting:

In the endogenous model: HSN is effective to regulate dyslipidemia selectively.

In the exogenous model: There is a significant reduction of TG concentration when using HSN drug in both two doses low dose (clinically administered dose) and high dose (three times higher than the clinical dose) after 4 weeks and it tends to make the reduction of TC and LDL-C concentrations.

The effects on dyslipidemia adjustment in the clinical setting:

- After 30 days of treatment, with HSN, there has been the reduction in the following indicators: TC (16.6%), TG (24.6%), LDL-C (16.5%), TC / HDL- C (19.6%), LDL-C / HDL-C (18.0%); however, there has been an increase in HDL-C with 7.3%.

- After 30 days of treatment, with HSN, there haven't been clinical and subclinical side effects such as changes in the hematopoietic system, liver and kidney functions in patients with dyslipidemia.

STRUCTURE OF THE STUDY

The dissertation consists of 127 pages, of which: Rationale (2 pages), Overview (43 pages), Materials, objectives and methods (14 pages), Research results (34 pages), Discussions (28 pages), Conclusion (2 pages), Recommendations (1 page). There are 114 references used in the research, in which there are 75 documents in Vietnamese, 29 documents in English and 10 documents in Chinese. The thesis has been presented and illustrated through 38 tables, 11 fugures, 12 images and diagrams.

Chapter 1. OVERVIEW

1.1. The concepts of blood lipids and lipid metabolism

1.1.1. The composition of lipids

Definition

Blood lipids are lipid components found in plasma, including Cholesterol, triglycerides, phospholipids and free fatty acids.

Classification

Lipids are of many types and can be sorted in many ways, however they are usually classified into two broad categories: derived lipids and complex lipids.

1.1.2. The composition of blood lipoprotein

Definition

Owing to lipid molecules are insoluble in water, so they circulate in the blood in the form of a combination of specific proteins called apoproteins which form lipoproteins.

1.1.3. Lipoprotein metabolism

Lipids and lipoproteins have different metabolic pathways depending on their origins:

Exogenous metabolism: The exogenous cycle accounts for about 25%, primarily from food, through LDL and LDL-mediated apoprotein B receptors in the cell membrane.

Endogenous metabolism: The endogenous cycle makes up about 75%, through HMGCoA enzyme (hydroxyl methyl co-enzyme) reductase.

1.2. Dyslipidemia syndrome

1.2.1. Definition

Dyslipidemia syndrome is a change and/or an increase of serum lipid levels.

1.2.2. Classification

There are many ways to classify dyslipidemia, here we introduce the commonly used classification: the Fredrickson's method. In 1965, in terms of lopoprotein composition, Fredrickson classified the dyslipidemia into five types, by using electrophoretic and hyperintense techniques. In 1970, a group of authors divided type II into II_a và II_b, from which it has became an international classification.

When referring to the causes of dyslipidemia, it may be primary causes (single or multiple mutations) and secondary causes (sedative lifestyle combined with the diet of too much saturated fatty acids and cholesterol; or diseases such as diabetes, hypothyroidism...)

1.2.3. Treatment

The methods used to interfere with dyslipidemia disorder include lifestyle changes (Acording to many specialists, dietary changes, exercise and weight control are the basis of treatment of dyslipidemia. These basic interventions can achieve efficacy at different levels in over 90% of patients); drug therapy (LDL-C indicator is considered the primary goal, according to the recommendation of ESC/EAS, 2016); and several prescribed drug groups such as reductase inhibitors, fibric acid (fibrate), bile acid sepeestrants (resin), nicotinic acid (niacin), inhibitors of cholesterol absorption inhibitors have been indicated (Ezetimib).

1.3. Dyslipidemia in traditional medicine

1.3.1. Transformation and transportation of the fluid and humor in the body

In terms of traditional medicine, fluid and humor are all normal fluids in the body, in which fluid is turbid while humor is transparent. Fluid and humor are

the basis of life, dirived from nutriments from food. Thanks to the Qi transformation of the triple Burner throughout the body, fluid and humor aim at nourishing organs, muscles, meridian vessels and skin.

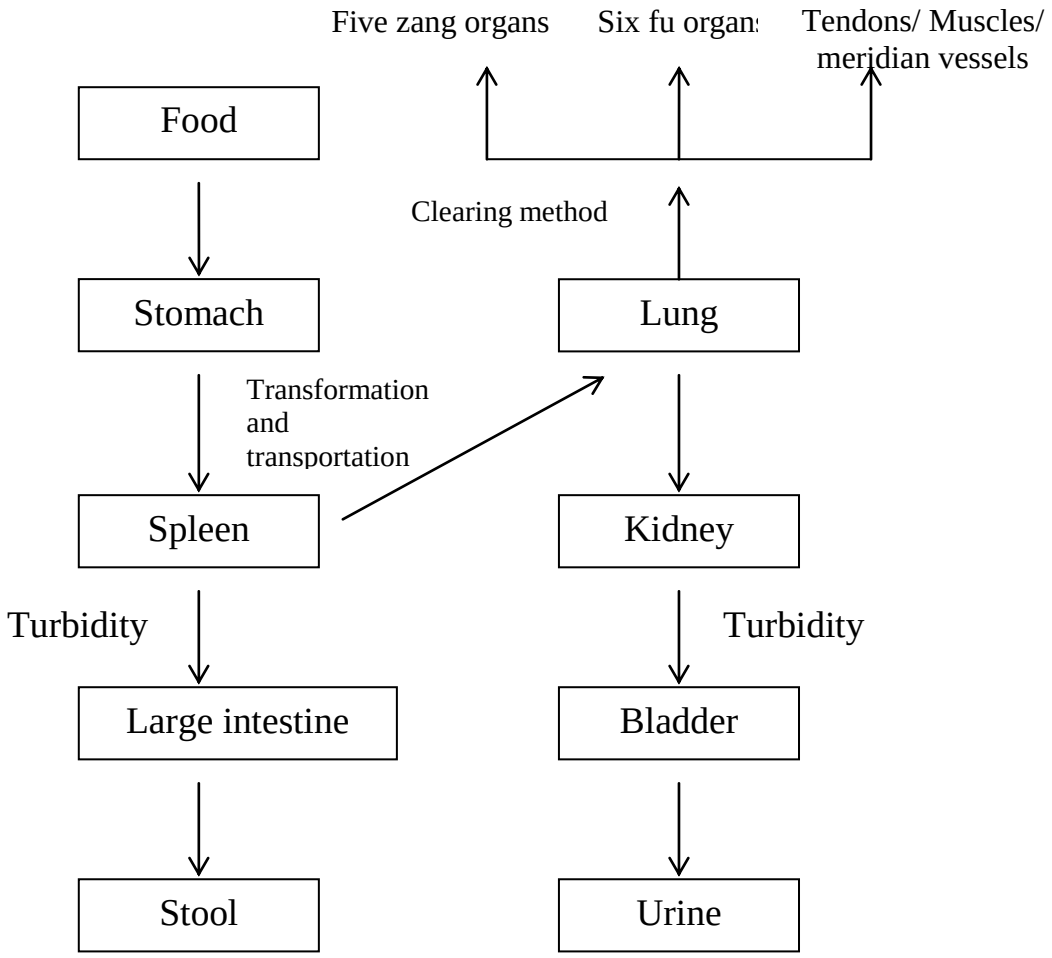

Diagram 1.1. Transformation and transportation of the fluid and humor

Mechanism of disease:

- Spleen is the root of phlegm: owing to deficiency of spleen-qi, water and food cannot transform, so the movement of lucid substance can't be upward, the turbidity can't be downward, the essence of water and foot can't be activated, creating phlegm turbidity which causes deseases. On the other hand, the deficiency of spleen causes the failure in controlling water, so creating phlegm.

- Kidney is the root of the phlegm: because of the deficiency of kidney-yang, the fire does not warm up spleen, water-dampness and fluid and humor can’t be transformed into qi, and creating phlegm. Owing to the deficiency of kidney-yin and the deficiency of fire at lower Burner, fluid and humor boils, so transforming into phlegm.

- The deficiency of lung qi: it cam’t regulate the water passage so water comes to a standstill and becomes phlegm or lung-yin deficiency yin xu and fire excess, xu fire boils fluid and humor to form phlegm.

1.3.2. Treatment principles

Diseases caused by phlegm-dampness are often derived from deficiency in origin and excess in superficiality, so it must be pay attention to both origin and superficiality when treating the deseases. It mean that when there is dampness-phlegm, we not only treat dampness-phlegm but also pay attention to the origin of the disease. Depending on how severse the deseases are, the treatment of phlegm in terms of traditional medicine is divided into three methods: phlegm elimiation, phlegm dissipation, phlegm regulation.

1.4. Some studies on the traditional medicine treatment of dyslipidemia

At present, there are many clinical and experimental studies on the drugs used to treat dyslipidemia in Vietnam and all over the world. Some popular medicinal products such as Malus Doumeri, Salvia Miltiorrhiza Bunge, Achyranthes Bidentate or some remedies such as Nhi Thang Thang, Ban Ha Bach Truat Thien Ma Thang must be mentioned. However, it is necessary to continue studying and developing more about medicinal herbs as well as remedies to be able to apply better in the future.

1.5. Overview of HSN remedy for the treatment of dyslipidemia

Many Vietnamese medicinal herbs have been used by the K'Ho ethnic group in Dat Ta district, Lam Dong province to treat some deseases such as

obesity, hypertension, liver disease, kidney disease. From 1991 to 2005, dozens of good remedies of ethnic minority people were inherited and applied to treatment by Pham Ngoc Thach Traditional Medicine Hospital in Lam Dong, including HSN remedy for lipidemia reduction from available medicinal herbs. This HSN remedy is inherited from the results of the research implemented by Nguyen The Thinh in 1996 with the title "An initial evaluation of the effect of HSN remedy on hyperlipidemia treatment" and the medical knowledge of the local K'Ho community.

- Ingredients: Rhizoma Lasiae 20g, Folium Nelumbilis Nucifera 20g, Pericarpium Citri Reticulatae Perenne10g, Herba et Radix Scopariae 20g, Fructus Docyniae 10g, Fructus Schizandrae 20g.

- Effects: relieve dampness, fortify the spleen, resolve phlegm and supportively improve healthy qi for the entire body, improve the functions of lung, spleen and kidneys.

Chapter 2. STUDY MATERIALS, OBJECTIVES AND METHODS

2.1. STUDY ON TOXICITY

2.1.1. Study materials

Drugs used in research

- Acute toxicity: HSN 100ml was boiled away by the rotary machine and was vacuumed under pressure to get the extract 5:1 ratio, it means that 20ml/1 scale drug is equivalent to 100g medicinal materials.

- Sub-chronic toxicity: HSN 100ml for study was calculated on the basis of a human dose multiplied by 6, equivalent dose for rats.

2.1.2. Research Participants

- Study on acute toxicity: 100 male and female white mice of Swiss, weighting 18 - 22g were used to evaluate acute toxicity of the sample.

- Study on sub-chronic toxicity: 30 male and femaile white rats of Wistar, weighing 200 ± 20g were used to evaluate sub-chronic toxicity of the sample.

2.1.3. Research methods

- Study on acute toxicity: Acute toxicity is realized under the method of Litchfield-Wilcoxon.

- Study on sub-chronic toxicity: Sub-chronic toxicity is proceeded under the Regulation on the assessment of safety and effectiveness of traditional medicines in pursuance of Decision No. 371/BYT in 1996.

2.2. EXPERIMENTAL STUDY

2.2.1. Study materials

Drugs used in the experimental setting

- Human dose: HSN 100ml is taken with 1 traditional recipe/day/person, equivalent to 100g of medicinal materials

- Mice dose: 1ml/100g mice in all lots.

- Controlled drug: Atorvastatin 10mg (STADA-Vietnam).

2.2.2. Research participants

- Model of endogenous dyslipidemias: 50 male and female white mice of Swiss, weighing 25 ± 2 g were used to evaluate.

- Model of exogenous dyslipidemias: 50 white mice rats of Wistar, weighing 200 ± 20g were used to evaluate.

2.2.3. Research methods

- Model of endogenous lipid metabolism: it is realized under the method of Poloxamer-407 by Millar et al.

- Model of exogenous lipid metabolism: it is proceeded by the model of Nassiri et al with the adjustment of concentration of cholic acid and PTU.

2.3. CLINICAL STUDY

Research materials

- HSN is prepared in liquid form in the ratio of 1 to 1

- Controlled drug: fenosup lidose 160mg (fibrates)

Research participants:

The participants were 150 patients diagnosed with dyslipidemia syndrome. All the patients, who came for inpatient and outpatient treatment at Ha Dong General Hospital Traditional Medicine voluntarily participated in the study, agreed to be fully tested, and adhered to the treatment procedure. The patients who were selected for the study must meet the criteria of selection and exclusion of the study.

Research methods:

The open-clinical trial compared with controlled group has been . There has been a comparision before and after treatment. performed. The study sample consisted of 150 patients divided into three groups, 50 patients in each group will use remedy in 30 successive days as follows:

Group 1: The patients take 100ml HSN once a day.

Group 2: The patients take fenosup lidose 160 mg/(1 tablet) once a day

Group 3: The patients take the mixture of HSN 100ml and fenosup lidose 160 mg/(1 tablet) once a day.

All patients were instructed to apply the diet for those with dyslipidemia during the course of the study. Basing on the theory of traditional medicine, the patients in each group have been divided into three main syndrome categories: phlegm turbidity and stasis, deficiency of liver-kidney yin, deficiency of spleen-kidney yang.

The effects of drug have been evaluated after 30 day treatment through the indicators of weight, body mass index (BMI), blood pressure, complete blood count, lipidemia (TC, TG, HDL-C, LDL-C) glucose, urea, creatinine, uric acid, AST and ALT.

2.4. RESEARCH LOCATION

The study was conducted at Department of Traditional Medicine, Ha Dong General Hospital.

2.5. RESEARCH ETHICS

The study has been conducted after the research proposal was approved by the Scientific Council (on January 31, 2015) and the Ethics Council (on March 3, 2017) and consented by Tue Tinh Institute of Traditional Medicine and

Pharmacy, Department of Pharmacology of Hanoi Medical University, and Ha Dong General Hospital.

2.6. DATA ANALYSIS

The data have been processed by the biomedical statistical algorithm on Microsoft office Excel, and SPSS 20.0.

Chapter 3: THE RESULTS OF THE STUDY

3.1. The results of the study on acute toxicity and sub-chronic toxicity

3.1.1. The results of the study on acute toxicity

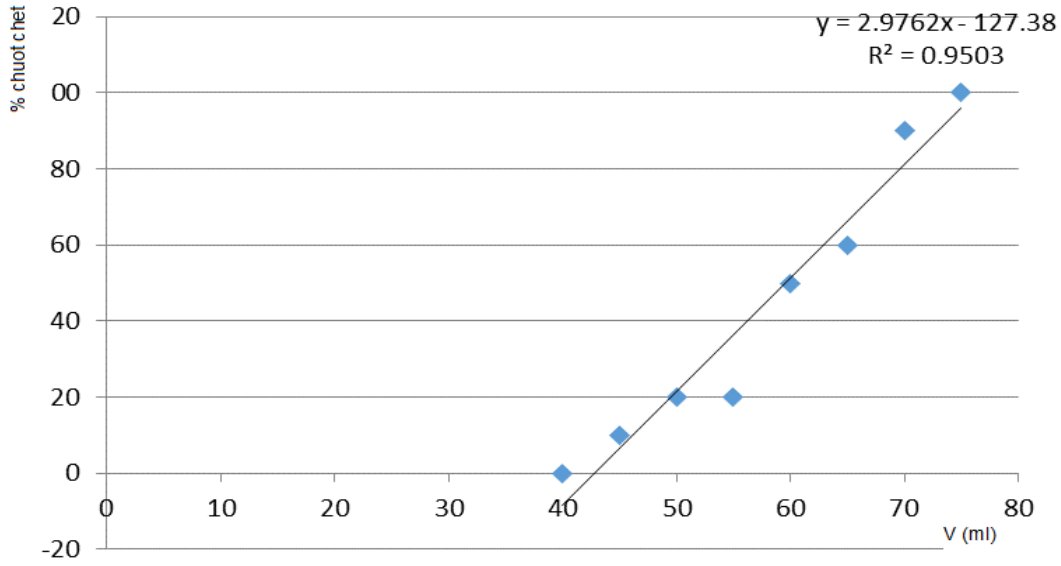

Diagram 3.1. The linear connection between the HSN remedy and the death rate of experimental mice

The acute toxicity and the estimated treatment indicators have been defined:

- LD50 = 59.58 (63.11 – 55.76)ml/kilogram = 297.9g HSN/kilogram

- TI= [297.9/2] x 50] : 12 = 12.41

3.1.2. The results of the study on sub-chronic toxicity

The sample of HSN has not induced sub-chronic toxicity in mice in both two doses of 12g/kg/day (this dosage is equivalent to human dose) and the dose of 36 g/kg/day (3 times higher than the first dose) in successive 4 weeks.

Histopathological changes

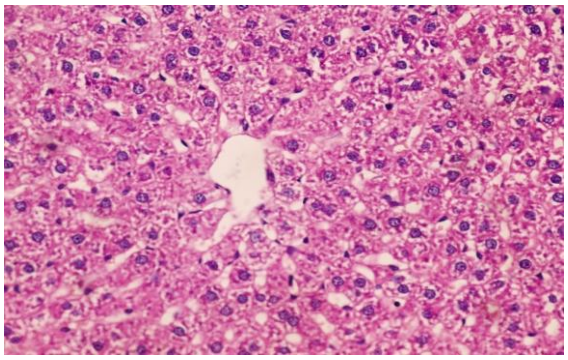

Fig. 3. 1. Microscopic morphology of liver in controlled lot (HE. 400). Liver cells are normal

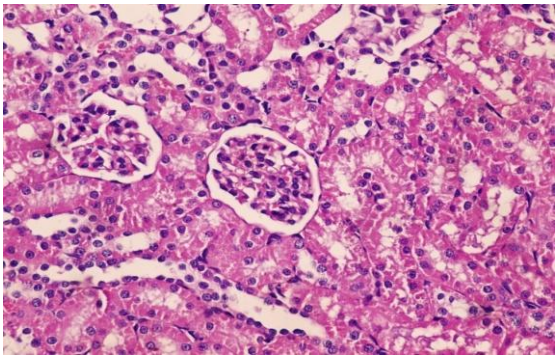

Fig. 3. 5. Microscopic morphology of kidneys in controlled lot (HE x 400). Kidney cells are normal

3.2. The effects of HSN on dyslipidemia adjustment in the experimental setting.

3.2.1. The effects on lipidemia adjustment in endogenous model

Table 3. 7. The effects of HSN on lipidemia concentration in endogenous model

Study lots	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	Non-HDL-C (mmol/L)
Lot 2: model (n=10)	7,80 ± 1,06	8,60 ± 1,38	2,07 ± 0,17	5,73 ± 1,13
Lot 3: Atorvastatin 100mg/kg (n=10)	5,13 ± 1,03 (↓ 34,23%)	7,56 ± 2,57 (↓ 12,09%)	1,90 ± 0,28	3,23 ± 1,11 (↓ 43,63%)
p compared to lot 2	p < 0,001	p > 0,05	p > 0,05	p < 0,001
Lot 4: HSN with low dosage (n=10)	6,40 ± 1,67 (↓17,95 %)	7,80 ± 2,08 (↓ 9,3%)	2,06 ± 0,39	4,34 ± 1,36 (↓ 24,26%)
p compared to lot 2	p < 0,05	p > 0,05	p > 0,05	p < 0,05
p compared to lot 3	p > 0,05	p > 0,05	p > 0,05	p > 0,05
Lot 5: HSN with high dosage (n=10)	6,36 ± 1,38 (↓ 18,46%)	7,53 ± 2,63 (↓ 12,44%)	2,02 ± 0,21	4,34 ± 1,23 (↓ 24,26%)
p compared to lot 2	p < 0,05	p > 0,05	p > 0,05	p < 0,01
p compared to lot 3	p < 0,05	p > 0,05	p > 0,05	p < 0,05
p compared to lot 4	p > 0,05	p > 0,05	p > 0,05	p > 0,05

3.2.2. The effects on lipidemia adjustment in exogenous model

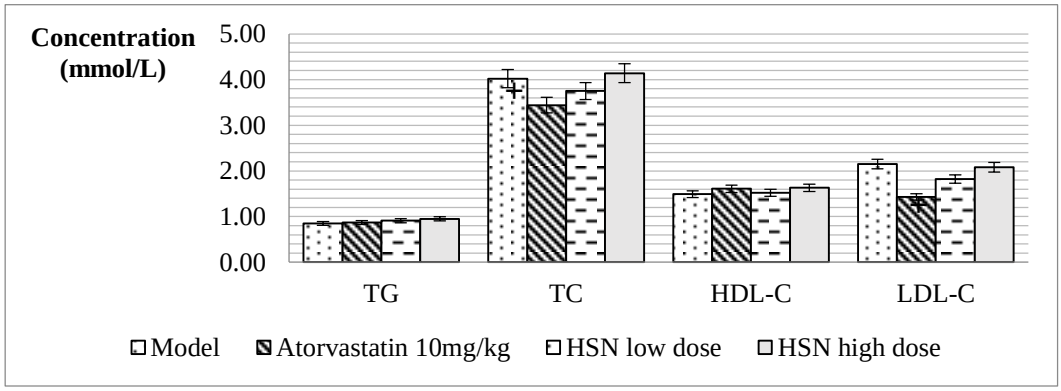

Figure 3. 3. The effects of HSN on lipidemia concentration in exogenous model after 2 weeks intake

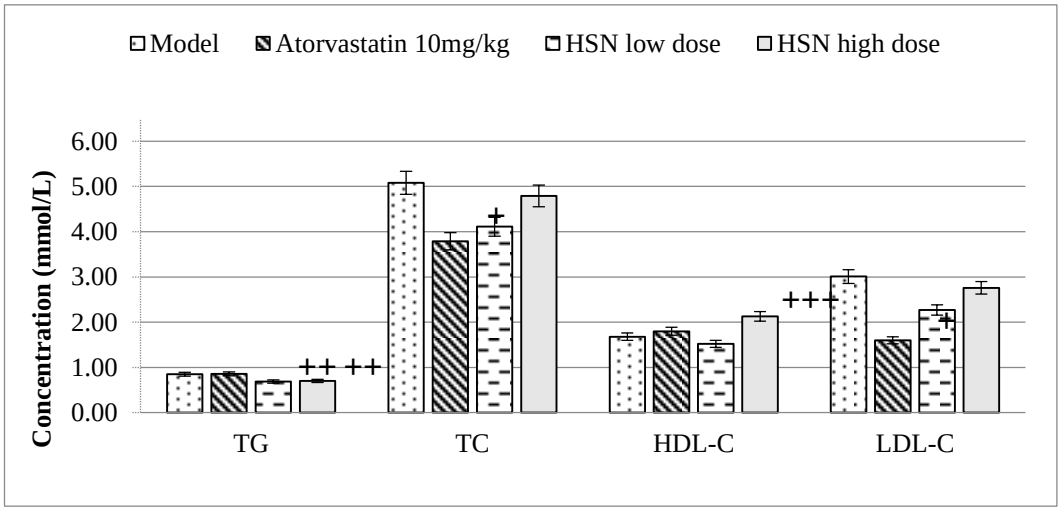

Figure 3. 4. The effects of HSN on lipidemia concentration in exogenous model after 4 weeks intake

3.3. The effects of HSN on dyslipidemia treatment in the clinical setting

3.3.1. General characteristics of study participants

Table 3. 10. Age distribution of study participants

Group / Age range	HSN (1) (n=50)		Fibrate (2) (n=50)		Mixture (3) (n=50)		Total (n=150)	
	Number of patients	Percentage (%)	Number of patients	Percentage (%)	Number of patients	Percentage (%)	Number of patients	Percentage (%)
< 50	3	6%	3	6%	2	4%	8	5.3%
50 – 59	19	38%	12	24%	10	20%	41	27.3%
60 – 69	21	42%	23	46%	25	50%	69	46%
≥ 70	7	14%	12	24%	13	26%	32	21.4%
$\overline{X}$ **±SD**	61.54 ± 9.9		63.88 ± 10.18		63.52 ± 9.84		62.98 ± 9.98	
p	$p_{1-2} > 0.05$		$p_{2-3} > 0.05$		$p_{1-3} > 0.05$			

3.3. Characteristics of dyslipidemia

Table 3. 14. Characteristics of disease patterns in terms of traditional medicine

Groups / Patterns	HSN (1) (n=50)		Fibrate (2) (n=50)		Complex (3) (n=50)		Total (n=150)	
	P	% (%)	P	%	P	%	P	%
Phlegm-turbidity stasis	34	68%	31	62%	30	60%	95	63.3%
Deficiency of Spleen-Kidney Yang	9	18%	8	16%	9	18%	26	17.3%
Deficiency of Liver - Kidney Yin	7	14%	11	22%	11	22%	29	19.4%
p	$p_{1-2} > 0.05$		$p_{2-3} > 0.05$		$p_{1-3} > 0.05$			

3.3.3. Changes in blood lipid indicators before and after treatment

Table 3.20. Changes of total cholesterol, triglycerides in patients after treatment

Groups / Points of time		HSN (1) (n=50)		Fibrate (2) (n=50)		Mixture (3) (n=50)		p
		($\overline{X}$ ±SD) (mmol/l)	Decrease percentage (%)	($\overline{X}$ ±SD) (mmol/l)	Decrease percentage (%)	($\overline{X}$ ±SD) (mmol/l)	Decrease percentage (%)	
TC	D_0	6.08±0.82		5.88±0.76		6.15±0.92		
	D_{30}	5.07±0.84	↓16.6%	5.02±0.96	↓14.6%	5.13±0.95	↓16.5%	$p_{1-2} > 0.05$ $p_{2-3} > 0.05$ $p_{1-3} > 0.05$
	p	$p_{0-30} < 0.001$		$p_{0-30} < 0.001$		$p_{0-30} < 0.001$		
TG	D_0	2.97±1.25		3.31±1.30		3.28±1.43		
	D_{30}	2.24±0.96	↓24.6%	2.38±1.05	↓28.1%	2.41±1.02	↓26.5%	$p_{1-2} > 0.05$ $p_{2-3} > 0.05$ $p_{1-3} > 0.05$
	p	$p_{0-30} < 0.01$		$p_{0-30} < 0.001$		$p_{0-30} < 0.001$		

Table 3.21. Changes of HDL-C, LDL-C indicators after treatment

Group / Points of time		HSN (1) (n=50)		Fibrate (2) (n=50)		Mixture (3) (n=50)		p
		($\overline{X}$ ±SD) (mmol/l)	Change (%)	($\overline{X}$ ±SD) (mmol/l)	Change (%)	($\overline{X}$ ±SD) (mmol/l)	Change (%)	
HDL-C	D_0	1.23±0.35		1.14±0.25		1.27±0.27		
	D_{30}	1.32±0.44	↑7.3 %	1.19±0.31	4.3%	1.37±0.65	↑7.9%	$p_{1-2} > 0.05$ $p_{2-3} > 0.05$ $p_{1-3} > 0.05$
	p	$p_{0-30} > 0.05$		$p_{0-30} > 0.05$		$p_{0-30} > 0.05$		
LDL-C	D_0	3.64±0.98		3.33±0.99		3.50±1.14		
	D_{30}	3.04±0.85	↓16.5%	2.82±0.84	↓15.3%	2.89±0.87	↓17.4%	$p_{1-2} > 0.05$ $p_{2-3} > 0.05$ $p_{1-3} > 0.05$
	p	$p_{0-30} < 0.001$		$p_{0-30} < 0.01$		$p_{0-30} < 0.01$		

3.3.4. Evaluation of the effects on treatment in terms of modern medicine

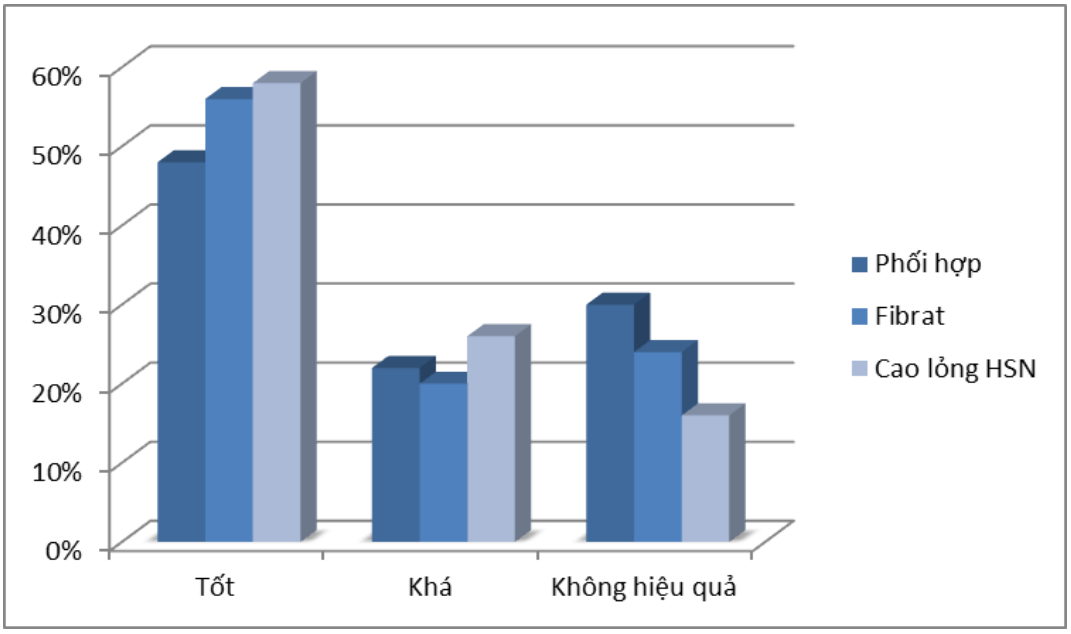

Figure 3.10. The results of dyslipidemia treatment in terms of modern medicine

3.3.5. Evaluation of the effects on treatment in terms of traditional medicine

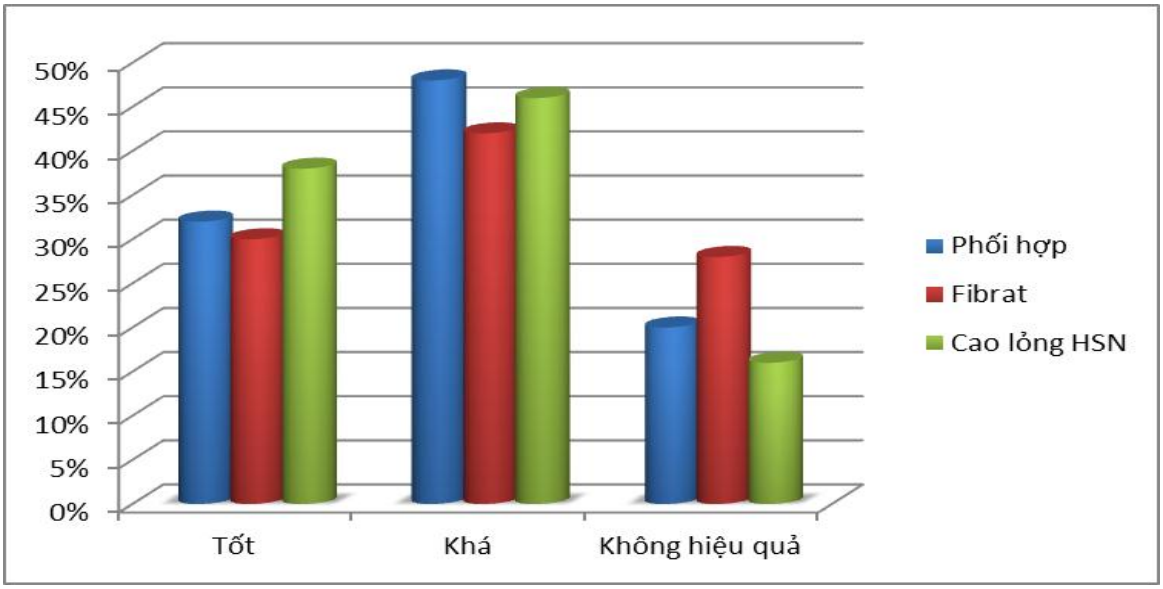

Figure 3.11. The results of dyslipidemia treatment in terms of traditional medicine

3.3.6. Evaluation of adverse drug effects

Table 3.26. Changes of some hematological and biochemical indicators after treatment

Group / Indicator	HSN (n=50) ($\overline{X}$ ±SD)		Fibrate (n=50) ($\overline{X}$ ±SD)		Mixture (n=50) ($\overline{X}$ ±SD)	
	D_0	D_{30}	D_0	D_{30}	D_0	D_{30}
Glucose (mmol/l)	5.86±0.75	5.95±1.12	6.26±1.52	6.16±1.35	6.08±1.22	6.21±1.26
	$p_{0-30} > 0.05$		$p_{0-30} > 0.05$		$p_{0-30} > 0.05$	
Urea (mmol/l)	5.22±1.34	5.45±1.07	5.56±1.11	5.52±1.40	5.49±1.4	5.66±1.26
	$p_{0-30} > 0.05$		$p_{0-30} > 0.05$		$p_{0-30} > 0.05$	
Creatinin (µmol/l)	74.0±13.65	76.68±11.96	78.88±14.12	78.5±14.83	75.86±13.82	75.74±13.57
	$p_{0-30} > 0.05$		$p_{0-30} > 0.05$		$p_{0-30} > 0.05$	
Uric acid (mmol/l)	372.2±87.0	358.3±89.5	379.5±86.9	354.6±101.1	346.24±97.6	337.68±99.3
	$p_{0-30} > 0.05$		$p_{0-30} > 0.05$		$p_{0-30} > 0.05$	
AST (U/L)	28.28±8.65	26.78±5.99	32.3±12.36	31.18±10.84	28.84±9.22	29.8±11.21
	$p_{0-30} > 0.05$		$p_{0-30} > 0.05$		$p_{0-30} > 0.05$	
ALT (U/L)	28.53±17.11	25.9±7.93	33.0±19.84	30.94±16.14	24.6±9.39	28.2±14.98
	$p_{0-30} > 0.05$		$p_{0-30} > 0.05$		$p_{0-30} > 0.05$	
Red Blood Cell Count (T/l)	4.12±0.32	4.21±0.28	4.33±0.41	4.52±0.22	4.45±0.62	4.42±0.46
	$p_{0-30} > 0.05$		$p_{0-30} > 0.05$		$p_{0-30} > 0.05$	
Hemoglobin (g/l)	125.43 ±14.21	124.12 ±12.26	123.25 ±14.11	126.43 ±11.65	121.41 ±15.15	123.43 ±14.29
	$p_{0-30} > 0.05$		$p_{0-30} > 0.05$		$p_{0-30} > 0.05$	
White Blood Cell Count (G/l)	6.23±1.23	6,34±1.31	6.73±1.23	6.84±1.62	6,49±1.72	6,53±1.13
	$p_{0-30} > 0.05$		$p_{0-30} > 0.05$		$p_{0-30} > 0.05$	
Platelet Count (G/l)	246.12 ±43.21	223.11 ±45.28	251.32 ±32.61	248.32 ±40.21	240.19 ±42.65	246.52 ±45.76
	$p_{0-30} > 0.05$		$p_{0-30} > 0.05$		$p_{0-30} > 0.05$	

Table 3.27. Some adverse effects

Symptoms	HSN (n=50)		Fibrate (n=50)		Mixture (n=50)		Total (n=150)	
	Số BN	Tỷ lệ (%)	Số BN	Tỷ lệ (%)	Số BN	Tỷ lệ (%)	Số BN	Tỷ lệ (%)
Tiredness	1	2%	2	4%	1	2%	4	2.67%
Muscle aches	0	0%	1	2%	1	2%	2	1.33%
Rash	0	0%	0	0%	0	0%	0	0%
Dyspepsia	2	4%	1	2%	1	2%	4	2.67%
Diarrhea	1	2%	0	0%	1	2%	2	1.33%
Constipation	0	0%	0	0%	0	0%	0	0%

Chapter 4: DISCUSSIONS

4.1. STUDY ON ACUTE TOXICITY

4.1.1. Study on acute toxicity

Acute toxicity LD_{50} of HSN is 297.9 g/kg and the estimated therapeutic TI index is 12.41 g/kg. Thus, the safety range of HSN is acceptable with the dose of <1/10 LD_{50}. While in the clinical setting, we use the dose of HSN 2ml/kg/day for each patient, equivalent to 2g medicinal materials/kg/day, which is safe when used by the mouth.

4.1.2. Study on sub-chronic toxicity

There are not any sub-chronic toxicity with the sample of HSN in rats when they are taken at the dose of 12g/kg/day (equivalent to normal dose for humans) and at the dose of 36g medicinal materials/kg/day (3 times higher than the first dose) in four successive weeks.

All indicators of general condition such as weight, hematopoietic function, liver function, hepatic cell damage, kidney function and histopathology of liver and kidney are within normal levels. There is no significant difference from the controlled group and compared to this before with HSN treatment.

4.2. THE EFFECS OF HSN ON LIPIDEMIA ADJUSTMENTS IN EXPERIMENTAL SETTING

4.2.1. The effects of HSN on dyslipidemia adjustment in the endogenous model

In the model of endogenous dyslipidemia, we selected the HSN dose of 24g/kg/day (equivalent to normal dose for humans with coefficient of 12); and the dose of 72g/ kg/day (3 times more effective than the dose for person, with coefficient of 12).

Despite high TG concentrations, the low and high HSN doses tend to make the reduction of TG. These two HSN doses also make the decrease of TC and non-HDL-C indicators equivelent to each other. And there is statistical significant reduction compared with the model group ($p < 0.05$). Though, the effect of decreasing TC, TG and non-HDL-C levels in both groups using HSN is lower than that on the group using Atorvastatin 100 mg/kg there is no statistically significant difference among these groups ($p > 0, 05$).

4.2.2. The effects of HSN on dyslipidemia adjustment in the exogenous model

To evaluate the effect of HSN on dyslipidemia adjustment in the exogenous model, we have used two doses: the low dose of 12g/kg/day (the dosage is equivalent to the human dose, with coefficient of 6) and the high dose of 36g/kg/day in 4 successive weeks.

The results shows that after 2-week study of TG levels in Atorvastatin and HSN intake groups, there is no statistically significant change compared to the model group ($p > 0.05$). However, after 4 weeks of treatment, the low and high HSN intake groups cause the significant reduction of TG concentration compared to the controlled group and the difference is statistically significant ($p < 0.01$). The Atorvastatin causes the significant reduction of TC and LDC-C concentration compared to the controlled group ($p<0.05$). The low and high HSN intake groups tend to reduce TC and LDL-C concentration compared to the controlled group, and the effect of the reduction of TC and LDL-C in the low HSN intake group is better than the rest group. However, there is no statistically significant difference between them ($p > 0.05$).

4.3. THE EFFECT OF HSN ON DYSLIPIDEMIA TREATMENT IN CLINICAL SETTING

4.3.1. The effects of HSN on dyslipidemia treatment

4.3.1.1. Improving clinical symptoms

After 30 days of treatment, the manifestations of phlegm-stasis pattern such as severe headache, loss of appetite, indigestion, fatigue and especially the patients with string-like pulse symptom have decreased significantly in all three groups of patients.

4.3.1.2. The effects of HSN in the sub-clinical indicators

Cholesterol concentration

After 30 days of treatment, TC concentration has been significantly reduced in all three study groups compared with that test ($p<0.001$). In which, HSN makes the decrease of TC at 16.6% and 16.5%, respectively in HSN and Fibrate intake groups and 14.6% in the mixture intake group. The differences are not statistically significant in all three groups with $p> 0.05$.

Triglyceride concentration

After 30 days of treatment, TC concentration has been significantly reduced in all three study groups compared with that before test, and this difference is statistically significant ($p <0.01$). In which, Fibrate intake group makes the decrease of 28.1% compared to 26.5% in the mixture intake group and 24.6% in the HSN intake group. However, the differences are not statistically significant in all three groups with $p> 0.05$.

HDL-C concentration

After 30 days of treatment, HDL-C concentration in both two HSN and mixture intake groups have tended to increase of 7.3% and 7.9% respectively. But in the Fibrate intake group, HDL-C concentration has changed slightly. However, the differences among groups are not statistically significant with $p> 0.05$.

LDL-C concentration

After 30 days of treatment, LDL-C concentration in all 3 groups have tended to decrease. The changes are statistically significant ($p < 0.01$) with the decrease of 16.5% in HSN intake group and 15.3% and 17.4% in fibrate and mixture intake groups respectively. The differences among these three groups are not statistically significant after 30 days of treatment with $p > 0.05$.

4.3.2 Evaluation of the effects of HSN on dyslipidemia treatment in terms of mordern medicine and traditional medicine.

In terms of modern medicine

After 30 days of treatment with HSN, based on the criteria of assessment of treatment effectiveness of modern medicine, we have found that 29 patients have achieved excellent results making up with 58%; and 13 patients have achieved good results making up with 26%. Thus, after 30 days, there have totally been 84% of patients achieving excellent and good result. These results are higher than that in both the Fibrat (76%) and mixture (70%) intake groups. However, the differences among these groups are not statistically significant.

In terms of traditional medicine

After 30 days of treatment with HSN, based on the criteria of assessment of treatment effectiveness of traditional medicine, we have found that 19 patients have achieved excellent results, accounting for 38%; and 23 patients have achieved good results with 46% and 8 patients have not been effective with the treatment (16%) and none of the patients has received worse effects. These results are better than the results of the Fibrat intake group with 30% of excellent, 42% of good; and 8% of worse results.

4.3.3. The adverse effects of HSN

In terms of clinical trial

During treatment, 3 patients in HSN intake group have showed digestive disorders (6%) and only one patient has been fatigued (2%). The symptoms are

mild and self-healing after a few days without treatment. There are 4 patients in each group of Fibrates and mixture suffering from some side effects such as fatigue, muscle pain, indigestion, diarrhea. Apart from these, none of other clinical adverse effects has been recorded.

In term of sub-clinical trial

After 30 days of HSN intake, we have found that there are not any significant changes in indicators such as red blood cell, white blood cell, platelet count and hemoglobin, as well as tests on glusose, urea, creatinine, AST, ALT ... The differences are not statistically significant for all indicators with $p > 0.05$.

However, these are subjective symptoms of the patients and these symptoms may be affected by patients' diet as well as exercise regimen, physical and environmental conditions; therefore, large-scale sample studies are needed to fully and comprehensively investigate undesirable effects.

Chapter 5: CONCLUSION

Basing on the research results, we have come to the following conclusions:

5.1. In terms of toxicity

Acute toxicity

The acute toxicity and expected therapeutic indicators have been defined :

- LD50 = 59.58 (63.11 - 55.76) ml/kg = 297.9 g of medicinal materials/kg

- TI = (297.9 / 2): 12 = 12.41

Of which, the dose of 2ml HSN/kg/day, equivalent to 2g medicinal materials/ kg/day has been used, which is safe when taken by the mouth.

Sub-chronic toxicity

The sample of HSN hasn't made any sub-chronics toxicity in mice at the dose of 12g/kg/day (this dosage is equivalent to human dose) and at the dose of 36g/kg/day (3 times higher than the first dose) in four successive weeks.

5.2. The effects on dyslipidemia adjustment in experiment setting

In the endogenous model

The dyslipidemia was selectively adjusted by HSN treatment with the following indicators: In the low dose of HSN intake group, there have been reductions in CT (17.79%), TG (12.09%), LDL-C (24.26), and an increase in HDL-C (92.52%). Whereas, in the high dose of HSN intake group, there have been decreases in CT (18.64%) TG (12.44%), LDL-C (24.26%), and an increase in HDL-C (88.78%).

In the exogenous model

- The HSN with dose equivalent to that in the clinical status has caused the noticeable reduction in triglycerid concentration compared to the controlled

group. The HSN with the dose three times as high as the dose of the clinical status has caused the significant reduction of triglycerid concentration and the significant increase of HDL-C concentration compared to the controlled group ($p<0,05$). The HSN hasn't made the decrease of TC and LDL-C concentration.

- The low and high HSN doses don't make the increase of liver enzyme activities (AST and ALT) in the blood of the mice after using medicine for four weeks successively.

5.3. The effects of HSN on dyslipidemia treatment in clinical setting

After 30 days of treatment, the dyslipidemia have been adjusted by the HSN at the dose of 100 ml/day. The changes of indicators have showed that: reduction in TC (16%), reduction in TG (24.6%), reduction in LDL-C (16.5%), reduction in HDL-C (7.3%), reduction in TC/HDL-C (19.6%) and reduction in LDL-C/HDL-C (18.0%).

- In terms of modern medicine, the effectiveness of dyslipidemia treatment with HSN has showed that: excellent results (58%), good (26%), ineffective (10%) and bad effects (6%).

- In terms of traditional medicine, the effectiveness of the HSN has showed significantly in patients with phlegm-stasis syndrome (88.2%), better than in the patients with spleen-kidney yang deficiency and liver-kidney yin deficiency patterns.

- After 30 days of treatment with, the HSN, there haven't been any clinical and subclinical side effects such as changes in blood function, liver function, and kidney function in patients with dyslipidemia .

RECOMMENDATIONS

- Basing on the results of acute toxicity which have showed that LD50 = 297.9 g/kg and TI = (297.9/2): 12 = 12.41, we recommend a further study to discover which medicinal materials cause toxicity.

- The time for the study on sub-chronic toxicity should be extended longer (2-3 m) because it usually takes 1-3 successive months for treatment of patients with dyslipidemia.

- There should be a study on the effectiveness of HSN on atherosclerosis reduction in experimental and clinical settings.

- The thesis has revealed some limitations on the number of participated patients and the research sites, so it is necessary to continue studying in clinical setting with a larger number of patients at many hospitals nationwide in order to make more persuasive results.

- Furthermore, it is advisable to study how to transfer HSN from liquid into capsules for patients easy to use, and extend expiration date.

RESEARCHER'S PUBLICATIONS RELATED TO THE DISSERTATION

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2. Trần Thị Hồng Ngãi, Nguyễn Duy Thuần, Nguyễn Thế Thịnh (2017). Nghiên cứu tác dụng điều chỉnh rối loạn chuyển hóa lipid máu trên mô hình nội sinh. Tạp chí Y dược cổ truyền Việt Nam số 10/2017, tr.30-34.