INTRODUCTION

Sleep is an important indicator of health and well-being in life. Sleep deficiency can lead to physical and mental health problems, injuries, loss of productivity, and even a greater risk of death. Numerous epidemiological studies have discovered that about 20% - 30% of the adult population, this rate increases in the elderly.

Along with the development of modern medicine, traditional medicine has affirmed itself and at the same time has made significant contributions to the health care for the community. Insomnia is described in the scope of "shimian" of traditional medicine, the main manifestation is hard to fall asleep, hard to stay asleep.

Traditional medicine (traditional medicine) has effective drugs for insomnia.

The remedy Duong tam an than of Thanh Hoa Traditional Medicine Hospital is originated from TianWanBuXinDan remedy, which has been modified based on practical treatment experience at the hospital. The remedy needs to be scientifically evaluated and comprehensive according to the regulations of the Ministry of Health to be widely applied in clinical. Therefore, we carried out the thesis: "Research on toxicity, sedative effects on experimental and clinical treatment of non-organic insomnia “Duong tam an than” liquid" with the following objectives:

1. Determine the acute toxicity and sub-chronic toxicity of “Duong tam an than” liquid on experimental.
2. Evaluate the sedative effects of “Duong tam an than” liquid on experimental animal models.
3. Evaluate the effect of “Duong tam an than” liquid on non-organic insomnia patients.

NEW CONTRIBUTIONS OF THE THESIS

Scientific significance

The study has obtained specific, reliable results on sedative effects on experimental and clinical studies, as a basis for further studies on a larger model for more herbal developes which treatment of nonorganic insomnia.

Practical significance

Non-organic insomnia has been receiving a great deal of public attention, local and international researchers even, because of directly affects to health and quality of daily life, memory, concentration, alertness and mood, which also results in reduce learning ability, less working efficiency, fatigue, loss of appetite, etc. This means that non-organic insomnia can even lead to serious accidents which boosts increase the incidence of disease or dead even, etc. Therefore, the studied "Duong tam an than" liquid has provided scientific evidence on the effect of regulating sleep as well as undesirable effects in case it happens. Thereby contributing to providing a herbal medicine product to help treat a common clinical disease.

NEW CONTRIBUTIONS

**Acute toxicity, subchronic toxicity of "Duong tam an than" liquid on experimental animals*

- Acute toxicity of "Duong tam an than" liquid in albino mice orally: at the dose of 17g condensed liquid/kg/day (≈ 11 clinical dose) (=38,42g of dried herb/ kg/day). In result, mice did not die within 24 hours but became inactive, overslept and suffered from diarrhea did not die within 24 hours, were inactive, slept, and diarrhea; LD50 has not been determined.

- Subchronic toxicity of "Duong tam an than" liquid in albino rats via oral use: at dose of 9,24 g condensed liquid/kg/day (= 20.88 g of dried herb/ kg/day, conversion factor is six) and 27, 72 g condensed liquid/kg/day (= 62.64 g of dried herb/kg/day ≈ 3 clinical dose) using continuously for 8 weeks showed no effect on general condition, weight, and indicators of hematopoietic function evaluation, liver function, level of liver cell destruction and filtering function of the kidney, and anatomic pathology of the liver and the kidney.

** Sedative effect of DTAT liquid on experimental*

1 hour and 3 hours after admission, DTAT liquid in both clinical dose (41.76 g dried herb/kg/day) and 3 fold clinical dose (125.28 g dried herb / kg / day) shows the sedative effect:

- Increase the number of entries, the time spent on the open arm; reduce the rate of the avoidance on the open arm. Reduce the time spent on the close arm Reduce the number of times the mouse moves horizontally and vertically.
- Reduce the grip strength time and reduce the grip of white mouse on Rotarod horizontal rod and on grip strength apparatus. The effect between two doses is similar.

** Sedative effect of sedative liquid sedative on clinical:*

After 30 days of treatment, "Duong tam an than" liquid has shown good effects on treating insomnia patients:

Reduce time of falling asleep: The rate of patients falling asleep in <15 minutes and 15- <30 minutes respectively increased from 0% and 4.55% to 67.27% and 30.00% ($p < 0.05$). Increasing time of sleep per night: From 3.46 ± 0.95 hours / night to 6.46 ± 0.97 hours / night ($p < 0.05$). The rate of patients with sleep efficiency ≥ 85% and 75% - <85% increased from 0% and 1.82% before treatment to 65.46% and 28.18%, respectively. Significantly improved the average PSQI score: reduced from 14.16 points to 3.84 points ($p < 0.05$).

** No clinical and subclinical unwanted effects have been seen during the course of the medication.*

THESIS STRUCTURE

The thesis consists of 150 pages: Introduction 02 pages; Overview 39 pages; Material, objects and methods 21 pages; Results 43 pages; Discussion 42 pages; Conclusion 02 pages; Recommend 01 page. There are 135 references used; including 44 Vietnamese documents, 68 English documents and 23 Chinese documents. The thesis is presented and illustrated through 38 tables, 12 charts, 5 images and diagrams.

CHAPTER 1
OVERVIEW
PHYSIOLOGICAL OF SLEEP

Definition

Sleep is the normal physiological state of humans. Sleep - that is the long suppressed state of the body, caused by the reorganization of the complex of endogenous and exogenous elements that characterize day-night oscillations and ensure the function of the brain in the awake state. Sleep is regulated in a relatively fixed and repetitive way.

1.2. DEFINITION, AETIOLOGY AND PATHOPHYSIOLOGY, DIAGNOSIS AND TREATMENT BY MORDEN MEDICINE

1.2.1. Definition of non-organic insomnia (F51.0)

Non-organic is called chronic insomnia, primary insomnia is defined as: A condition of unsatisfactory quantity and/or quality of sleep, which persists for a considerable period of time, including difficulty falling asleep, difficulty staying asleep, or early final wakening, often appearing suddenly after psychological, social or stress factors.

1.2.2. Aetiology and pathophysiology

*** Aetiology**

Due to psychological, emotional: Insomnia often occurs later a psychological trauma or series of adverse events in life. Psychological trauma or stress as a trigger for insomnia. Often, the state of insomnia increases at the time of psychological trauma.

*** Pathophysiology**

Now, It has been shown that the role of serotonin in general sleep and sleeplessness in particular.

In insomnia, there is no real damage, serotonin levels in synap neuron and in cerebrospinal fluid decreased by 20-30%.

1.2.3. Diagnostic

*** Diagnostic criteria for non-organic insomnia (F51.0) according to ICD-10**

1. Disturbance of sleep onset or sleep maintenance, or poor sleep quality.
2. Sleep disturbances occur at least three times a week over a period of 1 month.
3. The insufficient sleep duration and quality is coupled with a high degree of suffering or impairs daily activities.
4. Absence of any known causative organic factor, such as a neurological or other medical condition, psychoactive substance use disorder or a medication.

1.2.4. Treatment of non-organic insomnia in modern medicine

1.2.4.1. Treatment principles: There are two major groups: psychotherapy and pharmacology. These two groups can be combined.

The drugs that treat insomnia are not real

* The Benzodiazepine Group (BZD) and the non-benzodiazepine group

* Antidepressants. The barbiturates.

1.3. DEFINITION, AETIOLOGY AND PATHOPHYSIOLOGY, DIAGNOSIS AND TREATMENT BY TRADITIONAL MEDICINE

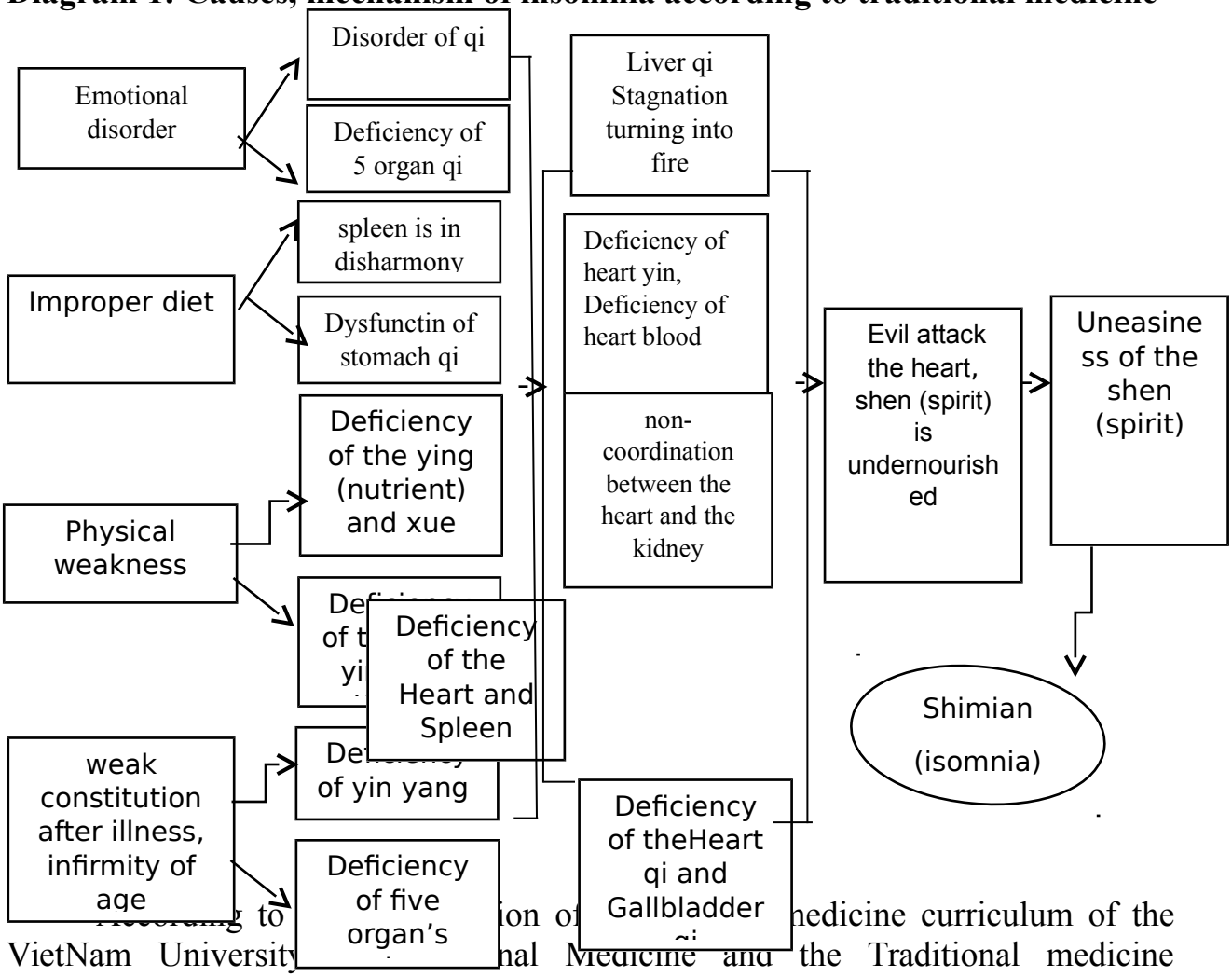
1.3.1. The definition of traditional medicine about insomnia:

Insomnia is depicted in the scope of "shimian" of traditional medicine, in the sense of “shi” is loss,”mian” is sleeping, “shimian” means insomnia. Insomnia is the inability to obtain sufficient sleep, difficulty falling asleep or being unable to asleep throughout the night. The degree of insomnia is ranged from mild, with difficult falling asleep; shallow sleep, waking up frequently during the night and difficulty returning to sleep, waking up too early in the morning, to heavy, with inable to sleep at all during the night

1.3.2. Aetiology and pathophysiology

Aetiology: Emotional disorder, improper diet, weak constitution after illness, infirmity of age, congenital insufficiency and timidness.

Diagram 1: Causes, mechanism of insomnia according to traditional medicine



According to ... ion of ... nedicine curriculum of the VietNam University ... nal Medicine and the Traditional medicine department at Hanoi Medical University.

- Deficiency of the Heart and Spleen

- Deficiency Hyperactivity of fire due to Yin deficiency (non-coordination between the heart and the kidney)
- Deficiency of heart yin, Deficiency of heart blood
- Deficiency of Heart qi and Gallbladder qi
- Liver qi Stagnation turning into fire

1.4. OVERVIEW OF "DUONG TAM AN THAN" REMEDY

The remedy "Duong tam an than" was originated from the " Tian wang bu xin dan" remedy in "Jiayidexiaofang" to treat xingui syndromes with with hyperactivities of fire due to yin deficiency, the remedy is modified by practical experience, specifically as follows:.

Remove 3 drugs: Radix Scrophulariae, Radix Rehmaniae, Radix Asparagi because the cold, dampness herbal is not suitable for deficiency of spleen yin. Add 4 drugs: Semen Cassiae, Radix Pseudoginseng,Fructus Amoni, Radix Astagali, in order to increase the sedative effect, enrich blood, invigorate the spleen, promote qi, spleen and stomach organ will not be overloaded, reduce the stagnation of the remedy, lead drugs into the heart and spleen organ.

After adjustment, the remedy has the composition and content of the ingredients: Radix Codonopsis 16g; Radix Polygalae 8g; Radix Pseudoginseng 4g; Radix Platycodonis 10g; Radix Salviae multiorrhizae 16g; Radix Angenicae sinensis 10g; Fructus Schisandrae 8g; Radix Ophiopogonis 10g; Semen Thujae orientalis 12g; Radix Astagali 30g; Semen Ziziphi jujubae 16g; Fructus Amoni 6g; Poria Cocos 16g; Semen Casiae torae 12g.

The drug is excellently formulated to form liquid polyethylene sterilized bag, content of 340mL liquid/day divided 2 bags (170ml/bag), (equivalent to 174g dried pharmaceuticals/day = 3.48g dried medicinal herbs/kg weight ≈ 6,8ml/kg/day). Dosage: 1 bags (170ml/bag) po bid.

CHAPTER 2
MATERIALS AND METHODS

2.1 EXPERIMENTAL RESEARCH

2.1.1. Materials

"Duong tam an than" liquid as the overview stated, was concentrated on a boiler system under normal pressure, obtained extract with a ratio of 4,411: 1 (340 ml /day ≈ 77.07 g condensate/day ≈ 1. condensate/kg/day (≈ 3,48g dry medicinal herbs/kg).

2.1.2. Subject study

Healthy Swiss albino mice (both sexes), weighing about 24–25 g and Wistar rats (both sexes), weighing about 150–200 g

2.1.3. Location, time of study

- Location: Department of Pharmacology, Hanoi Medical University.
- Time: From 5/2015 to 11/2015.

2.1.4. Method

2.1.4.1 Acute toxicity study test

Study of acute toxicity and the LD50's determination of the testing "Duong tam an than" liquid experimented in albino mice by oral route.

2.1.4.2. Subchoronic toxicity study test

The study of subchronic toxicity was performed in albino rats by oral route, following WHO's protocol

2.1.4.3 Experimental model to study the sedative effect of "Duong tam an than" liquid.

*** Elevated plus maze testing**

Activity cage testing is done by the method of G. Olayiwola et al.

*** Rotarod testing**

Rotarod testing was used to study the sedative effect of drug, The **rotarod test** was carried out **based** on a previous study (**Shiotsuki et al**)

*** Activity cage testing**

Activity cage testing is carried out by the method of Mill J et al (2002).

*** Grip strength testing**

Grip strength testing of the mouse was performed by the method of Robert M.J. Deacon.

2.2. CLINICAL RESEARCH

2.2.1. Material

2.2.1.1. Studied drug: "Duong tam an than" liquid: Ingredient, content and clinical dose as shown in the overview.

2.2.1.2. Controlled drug: bagged fluid "tianwangbuxindan", packed in polyethylene bags, sterilized, dosage: 1 bags (170ml/bag) po bid. Ingredients included : Radix Codonopsis 16g; Radix Polygalae 8g; Radix Platycodonis 10g; Radix Salviae multiorrhizae 16g; Radix Angenicae sinensis 10g; Fructus Schisandrae 8g; Radix Ophiopogonis 10g; Semen Thujae orientalis 12g; Semen Ziziphi jujubae 16g; Semen Casiae torae 12g; Radix Scrophulariae 12g, Radix Rehmaniae 16g, Radix Asparagi 12g

2.2.2. Subjects:

Patients who are diagnosed with non-organic insomnia, were treated inpatient at Thanh Hoa Mental Hospital and Thanh Hoa Traditional Medicine Hospital.

The first patient visit was on January 2016, and recruitment was completed on February 2017. 165 patients, aged 20-60 years, were selected based on the non-organic insomnia diagnosis criteria of modern medicine and type of disease of

traditional medicine. Using pairing method, these patients were divided to 2 groups with the ratio of 2: 1.

2.2.2.1. Selection criteria according to modern medicine

*** Diagnostic criteria for non-organic insomnia (F51.0) according to ICD-10**

2.2.2.2. Selection criteria patients according to traditional medicine

Examination	Deficiency of heart yin	Deficiency of heart blood
Observation	Pale face, the tongue is red and dry without fur or with thin yellow fur	Pale or yellow face, the tongue is pale with a white thin or non coating, tend to be frightened.
Listening	Small voices, clear, breath is not foul.	Small voices, clear, breath is not foul.
Questioning	Palpitations, chest oppression, insomnia, nightmares, night sweating and dry lips and throat, feverish sensation in palms and soles of the feet, fever occuring at the same time of day or low grade fever, dizziness, ringing in the ears, irregular menstrual cycleand light menstrual flow	Palpitation, insomnia, nightmares, poor memory, a lusterless complexion, dizziness, Bleeding into the skin can occur ,irregular menstrual cycle, light red,heavy menstrual flow,menorrhagia or light menstrual flow, missed period.
Palpation and touch	the pulse is thready and rapid.	the pulse is thready and weak.

2.2.3. Method

Prospective controlled clinical trials.

- 165 patients met the research criteria were divided into 2 groups using matching method:

+ Studied group: 110 patients treated with "Duong tam an than" liquid, treatment course of 30 days continuously.

+ Controlled group: 55 patients treated with “Tianwangbuxindan” bagging decoction of 30 day treatment course continuously.

- Clinical symptoms evaluation, clinical assessment and functional tests were conducted before treatment (D0), after 15 days of treatment, after 30 days of treatment (D30).

- Blood tests were done before and after 30 days of treatment

***Location of research**: Thanh Hoa Mental Hospital, Thanh Hoa Traditional Medicine hospital

* Time: from 1/2016 - 12/2017.

2.2.4. Statistical methods

T- Test student, SPSS program and EXCELL.2000.

2.3.5. Research ethics

This clinical research was conducted upon the approval of the Medical Ethics Committee of Thanh Hoa Mental Hospital and Thanh Hoa Traditional Medicine and Pharmacy Hospital (Appendix 5).

CHAPTER 3: RESEARCH RESULTS

3.1. RESEARCH RESULTS BASED ON EXPERIMENTAL EXPERIENCES OF DUONG TAM AN THAN LIQUID

3.1.1. Results of acute toxicity study

Table 3.1: Research results of acute toxicity according to the dose of Duong tam an than liquid reagent (YXAS)

Lot of rat	**n**	**Dose (g/kg)**	**Mortality rate (%)**	**Other unusual signs**
Lot 1	10	6,8	0	No death, no diarrhea, inactivity, getting much sleep.
Lot 2	10	10,2	0	No death, no diarrhea, inactivity, getting much sleep.
Lot 3	10	13,6	0	No death, no diarrhea, inactivity, getting much sleep.
Lot 4	10	17,0	0	No death, inactivity, getting much sleep, 40% of rats in this lot got diarrhea

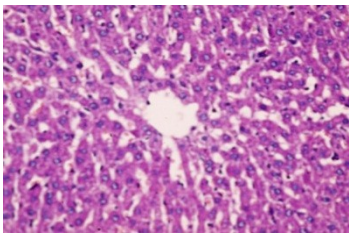
YXAS liquid reagent showed no acute toxicity at high condensed liquid of 17g / kg = 38.42 g dried herb, (≈ 11 fold clinical dose). When the white rats was drunk the YXAS liquid reagent, LD50 has not been determined**.**

3.1.2. Results of subchronic toxicity

YXAS liquid did not show subchronic toxicity on white rats by oral method: dose of 9,24 g condensed liquid/kg/day (= 20.88 g of dried herb/ kg/day) and dose of 27, 72 g condensed liquid/kg/day (= 62.64 g of dried herb/kg/day) using this liquid for 8 weeks continuously did not affect the general condition, weight, and indicators of hematopoietic function evaluation, liver function, level of liver cell destruction and filtering function of the kidney, and anatomic pathology of the liver and the kidney.

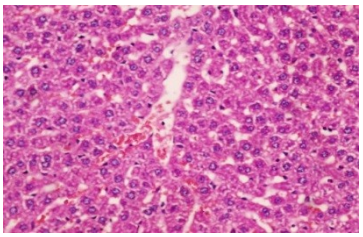
Changes of histopathology:

In general: in all experimental white rats including controlled lot and two treated lots, before and after using YXAS liquid dose of 9,24 g/kg/day and 27,72 g/kg/day in 8 weeks showed normal size , color and density, no change of disease of the organs such as: heart, lungs, liver, spleen, pancreas, kidneys and digestive system in general.



Picture 3.1 (Rat no. 305)

Picture 3. 1: Microscopic morphology of liver of lot 1 rats*(Rat no. 305)* (HE x 400)

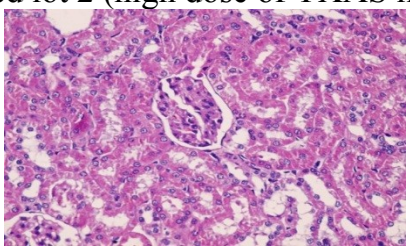


Picture 3.2 (Rat no. 189)

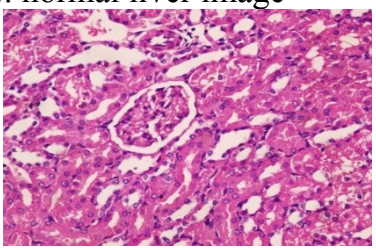
Picture 3.2: Microscopic morphology of liver of lot 2 rats*(Rat no. 189)* (HE x 400)

Microscopic morphology of liver*:* + Controlled lot: normal liver image

+ Treated lot 1 (low dose of YXAS liquid - *Picture* 3.1): normal liver image
+ Treated lot 2 (high dose of YXAS liquid - *Picture* 3.2): normal liver image



Picture 3.3 (Rat no. 302)



Picture 3.4 (Rat no. 189)

Picture 3.3: Microscopic morphology of kidney of lot 1 rats (Rat no. 302) (HE x 400)

Picture 3.4: Microscopic morphology of kidney of lot 2 rats *(Rat no. 189)*(HE x 400)

Microscopic morphology of kidney*:* + Controlled lot: normal kidney image
+ Treated lot 1 (low dose of YXAS liquid - *Picture* 3.3): normal kidney image
+ Treated lot 2 (high dose of YXAS liquid - *Picture* 3.4): normal kidney image

3.1.3. Research results of sedative effects of Duong tam an than liquid on experiment

3.1.3.1. Elevated plus maze testing

Table 3.2. Effects of Duong tam an than liquid(YXAS) to time, number of keeping opened branch, the time to close the closed branch, and the ratio of avoiding opened branch

Lot	**The time spent on the open arm (seconds)**	**The time spent on the close arm (seconds)**	**The number of entries**	**The rate of the avoidance on the open arm (%)**
Lot 1 (Sham)	53,10 ± 20,12	173,60 ± 38,75	2,30 ± 1,25	76,26 ± 8,47
Lot 2 (Diazepam dose of 2,4g/kg)	115,30 ± 31,67	128,80 ± 40,25	8,70 ± 3,62	53,06 ± 11,36
p (compared to lot 1)	p < 0,001	p < 0,05	p < 0,001	p < 0,001
Lot 3 (Duong tam an than liquid dose equivalent to clinical dose)	111,40 ± 42,3	106,90 ± 38,6	5,80 ± 2,49	50,97 ± 19,67
p (compared to lot 1)	p < 0,001	p < 0,01	p < 0,001	p < 0,01
p (compared to lot 2)	p > 0,05	p > 0,05	p > 0,05	p > 0,05
Lot 4 (Duong tam an than liquid dose 3 fold clinical dose)	123,40 ± 46,15	135,50 ± 36,03	4,10 ± 2,28	56,63 ± 12,17
p (compared to lot 1)	p < 0,001	p < 0,05	p < 0,05	p < 0,001
p (compared to lot 2)	p > 0,05	p > 0,05	p <0,01	p > 0,05
p (compared to lot 3)	p > 0,05	p > 0,05	p > 0,05	p > 0,05

Comment: Rats in 2 lots used diazepam and YXAS liquid both showed relieved anxiolytic compared to sham, with p<0,05. Rats of lots used YXAS liquid was similar and there was no difference compared to diazepam (p> 0.05).

*3.1.3.2. Research results **of** Rotarod rotary axis model*

Table 3.3: Effect of Duong tam an than liquid (YXAS) on the gripping time of the rats on rotarod rotating axis.

Lô	*Time that rats sticked with rotarod rotating axis*		
	Start time of the study	1 hour after taking medicine	3 hours after taking medicine
Lot 1 (Sham)	247,8 ± 70,3	251,3 ± 48,2	249,6 ± 70,8
Lot 2 (Diazepam dose of 2,4g/kg)	238,0 ± 67,3	170,9 ± 50,0	219,6 ± 82,4
p (compared to lot 1)	*p > 0,05*	*p < 0,05*	*p > 0,05*
Lot 3 (Duong tam an than liquid dose equivalent clinical dose)	229,0 ± 95,2	178,5 ± 63,8	239,1 ± 62,6
p (compared to lot 1)	*p > 0,05*	*p < 0,05*	*p > 0,05*
p (compared to lot 2)	*p > 0,05*	*p > 0,05*	*p > 0,05*
Lô 4 (Duong tam an than liquid dose 3 fold clinical dose)	232,9 ± 123,6	146,2 ± 94,0	244,3 ± 54,0
p (compared to lot 1)	*p > 0,05*	*p < 0,05*	*p > 0,05*
p (compared to lot 2)	*p > 0,05*	*p > 0,05*	*p > 0,05*
p (compared to lot 3)	*p > 0,05*	*p > 0,05*	*p > 0,05*

Comment: at 1 hour after rats taking diazepam and rats of lots used YXAS liquid showed sedative effect by reduction the gripping time of the rats on rotarod rotating axis compared to sham ($p<0.05$). The effect of two YXAS liquid doses was similar and equivalent to diazepam ($p>0.05$)

3.1.3.3. Research results of the activity log model

Table 3.4: Effects of Duong tam an than liquid (YXAS) to horizontal movement of the rats

Lot	The number of horizontal movement		
	Before research	1 hour after taking medicine	3 hour after taking medicine
1 (Sham)	241,80 ± 40,93	247,40 ± 37,30	247,20 ± 33,99
2 (Diazepam dose of 2,4g/kg)	245,20 ± 59,62	201,00 ± 47,40	196,53 ± 64,15
p (compared to lot 1)	p > 0,05	p < 0,05	p < 0,05
3 (Duong tam an than liquid dose equivalent clinical dose)	243,40 ± 44,72	209,10 ± 40,75	208,43 ± 40,18
p (compared to lot 1)	p > 0,05	p < 0,05	p < 0,05
p (compared to lot 2)	p > 0,05	p > 0,05	p > 0,05
4 (Duong tam an than liquid dose equivalent clinical dose)	246,40 ± 47,40	208,30 ± 41,16	202,21 ± 46,27
p (compared to lot 1)	p > 0,05	p < 0,05	p < 0,05
p (compared to lot 2)	p > 0,05	p > 0,05	p > 0,05
p (compared to lot 3)	p > 0,05	p > 0,05	p > 0,05

Comment: Rats of 2 lots used diazepam and YXAS liquid showed decrease activity compared to sham at the times after taking medicine: reduction of the number of

horizontal movement (p<0.05). Rats of lots used two YXAS liquid doses was no difference compared to diazepam (p> 0.05).

Table 3.5: Effects of Duong tam an than liquid (YXAS) on vertical movement of rats

Lot	The number of vertical movement		
	Before research	1 hour after taking medicine	3 hour after taking medicine
1 (Sham)	19,30 ± 6,07	20,60 ± 5,46	20,20 ± 3,65
2 (Diazepam dose of 2,4g/kg)	18,87 ± 5,14	12,67 ± 3,81	13,53 ± 3,58
p (compared to lot 1)	p > 0,05	p < 0,05	p < 0,05
3 (Duong tam an than liquid dose equivalent clinical dose)	19,14 ± 4,69	16,00 ± 3,04	15,64 ± 3,61
p (compared to lot 1)	p > 0,05	p < 0,05	p < 0,05
p (compared to lot 2)	p > 0,05	p > 0,05	p > 0,05
4 (Duong tam an than liquid dose 3 fold clinical dose)	19,79 ± 6,99	14,93 ± 4,45	14,43 ± 4,20
p (compared to lot 1)	p > 0,05	p < 0,05	p < 0,05
p (compared to lot 2)	p > 0,05	p > 0,05	p > 0,05
p (compared to lot 3)	p > 0,05	p > 0,05	p > 0,05

Comment: Rats of 2 lots used diazepam and YXAS liquid showed decrease activity compared to sham at the times after taking medicine: reduction of the number of vertical movement (p<0.05). Rats of lots used two YXAS liquid doses was no difference compared to diazepam (p> 0.05).

3.1.3.4. Research results of the grip measurement model:

Table 3.6: Effects of Duong tam an than liquid (YXAS) on the grip force of the rats

Lot	*The grip force of the rats* (g)	
	1 hour after taking medicine	3 hours after taking medicine
Lot 1 (Sham)	353,20 ± 60,85	352,13 ± 63,13
Lot 2 (Diazepam dose of 2,4g/kg)	261,73 ± 62,64	286,20 ± 72,83
p (compared to lot 1)	*p < 0,001*	*p < 0,05*
Lot 3 (Duong tam an than liquid dose equivalent clinical dose)	226,93 ± 71,52	247,33 ± 63,06
p (compared to lot 1)	*p < 0,001*	*p < 0,001*
p (compared to lot 2)	*p > 0,05*	*p > 0,05*
Lot 4 (Duong tam an than liquid dose 3 fold clinical dose)	248,20 ± 46,89	250,40 ± 41,06
p (compared to lot 1)	*p < 0,001*	*p < 0,001*
p (compared to lot 2)	*p > 0,05*	*p > 0,05*
p (compared to lot 3)	*p > 0,05*	*p > 0,05*

Comment**:** at the times after taking diazepam and YXAS liquid, rats reduced the grip force compared to sham (p<0.001). There was no difference in sedation level of two YXAS liquid dose compared to diazepam (p>0.05).

3.2. CLINICAL RESEARCH RESULTS OF DUONG TAM AN THAN LIQUID

3.2.1. Characteristics of the research object.

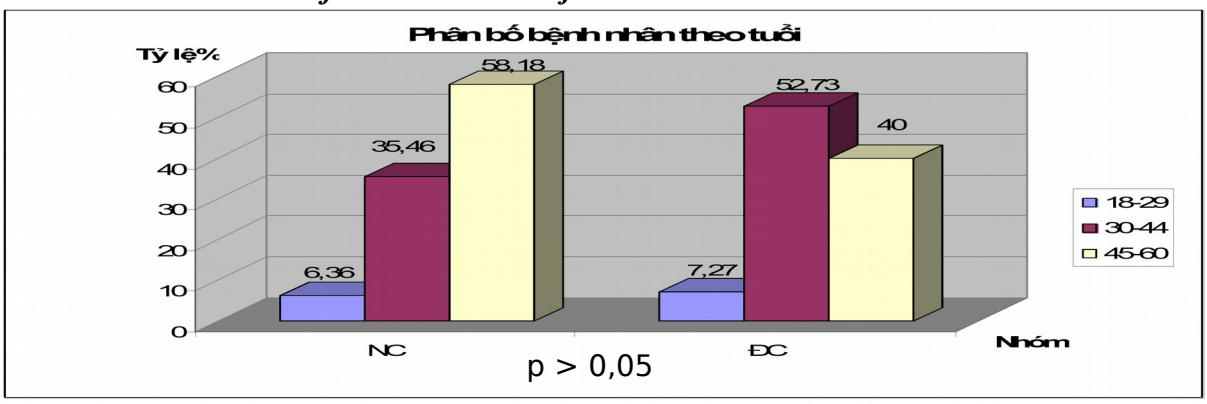


Chart 3.1: Distribution of patients by age.

Comment: The age group had the highest rate of insomnia was 45 - 60 years old and 30 - 44 years old and there is no difference in the rate of disease by age between 2 groups (p> 0.05).

Table* 3.7*: Distribution of patients by gender

The group / Gender	Studied group (n=110)		Controlled group (n=55)	
	Amount	Ratio %	Amount	Ratio %
Male	29	26,36	10	18,18
Female	81	73,64	45	81,82
Total	110	100	55	100
p	p(NC-ĐC) > 0,05			

Comment: The rate of female insomnia was higher than that of men (p <0.05). There was no difference between the two groups with p> 0.05.

3.2.2. The effects of Duong tam an than liquid.

Table 3.8: The change of time going to sleep over time

Time / Day		< 15 minutes		15-<30 minutes		30-<60 minutes		≥ 60 minutes		p
		n	%	n	%	n	%	n	%	
D0	Studied group (n=110)	0	0	5	4,55	20	18,18	85	77,27	p> 0,05
	Controlled group (n=55)	0	0	3	5,45	12	21,82	40	72,73	
D15	Studied group (n=110)	17	15,45	48	43,64	45	40,91	0	0	p< 0,05
	Controlled group (n=55)	0	0	17	30,91	33	60,00	5	9,09	
D30	Studied group (n=110)	74	67,27	33	30,00	3	2,73	0	0	p< 0,05
	Controlled group (n=55)	16	29,09	24	43,64	15	27,27	0	0	
p(D0-D15)				< 0,0001						
p(D0-D30)				< 0,0001						

Comment: After treatmented, at the times of the study, the change in the time of falling asleep of the studied group had a clearer improvement than before treatmented and the controlled group (with p <0.05).

Table 3.9: The change of the sleeping hours each night over time of 2 groups

Unit: hours

The group Day	Studied group (n=110) $\overline{X}$ ± SD	Controlled group (n=55) $\overline{X}$ ± SD	p(Studied group - Controlled group)
D0	3,46 ± 0,95	3,51 ± 0,69	P > 0,05
D15	5,39 ±1,26	4,16± 1,09	p < 0,05
D30	6,46 ± 0,97	5,03 ± 0,72	p < 0,05
p(D0-D15)	< 0,0001	< 0,0001	
p(D0-D30)	< 0,0001	< 0,0001	

Comment: At 15 days and 30 days after treatmented, both groups reduced the average hours of sleep in a night compared to before treatmented (p<0.05). However, the studied group‘s sleep hours had higher than the controlled group, with p<0.05.

Table 3.10: The change of sleep duration following Pittsburgh scale

Unit: Points

The group Day	Studied group (n=110) ($\overline{X}$ ± SD)	Controlled group (n=55) ($\overline{X}$ ± SD)	P(Studied group - Controlled group)
D0	2,90 ± 0,30	2,98 ± 0,135	p > 0,05
D15	1,74 ± 0,74	2,31 ± 0,66	p < 0,05
D30	1,03 ± 0,77	1,31 ± 0,98	p < 0,05
p(D0-D15)	< 0,0001	< 0,0001	
p(D0-D30)	< 0,0001	< 0,0001	

Table 3.11: Sleep eficiency over time

Efficiency Day		≥ 85%		75%-85%		65%-<75%		< 65%		p
		n	%	n	%	n	%	n	%	
D0	Studied group (n=110)	0	0	2	1,82	15	13,64	93	84,54	p> 0,05
	Controlled group (n=55)	0	0	1	1,82	7	12,73	47	85,45	
D15	Studied group (n=110)	38	34,54	52	47,27	16	14,55	4	3,64	p< 0,05
	Controlled group (n=55)	10	18,18	13	23,63	24	43,64	8	14,54	
D30	Studied group (n=110)	72	65,46	31	28,18	6	5,45	1	0,91	p< 0,05
	Controlled group (n=55)	21	38,18	19	34,55	13	23,64	2	3,64	
p(D0-D15)				< 0,001						
p(D0-D30)				< 0,001						

Comment: The improvement sleep eficiency over time in studied groups after treatmented was better compared to before treatmented and compared to controlled with p <0.05.

Table 3.12: Changes in sleep quality of patients over times.

Level / Moment		Very good		Good		Average		Poor		p
		n	%	n	%	n	%	n	%	
D0	Studied group (n=110)	0	0	0	0	71	64,55	39	35,45	p >0,05
	Controlled group (n=55)	0	0	0	0	35	63,64	20	36,36	
D15	Studied group (n=110)	37	33,64	51	46,36	17	15,45	5	4,55	p <0,05
	Controlled group (n=55)	9	16,36	15	27,27	21	38,18	10	18,18	
D30	Studied group (n=110)	63	57,27	39	35,46	7	6,36	1	0,91	p <0,05
	Controlled group (n=55)	13	23,64	28	50,91	11	20,00	3	5,45	
p(D0-D15)						< 0,001				
p(D0-D30)						< 0,001				

Comment: The improvement sleep quality over time in studied groups after treatmented was better compared to before treatmented and compared to controlled with p <0.05.

Table 3.13: The improvement of symptoms of early awakening over time.

Times/week / Day		0 time		1 time		2-3 times		> 3 times		p
		n	%	n	%	n	%	n	%	
D0	Studied group (n=110)	3	2,73	5	4,54	10	9,09	92	83,64	p >0,05
	Controlled group (n=55)	1	1,82	3	5,45	7	12,73	44	80,00	
D15	Studied group (n=110)	22	20,00	83	75,45	5	4,55	0	0	p < 0,05
	Controlled group (n=55)	6	10,91	36	65,45	13	23,64	0	0	
D30	Studied group (n=110)	69	62,73	37	33,64	4	3,64	0	0	p < 0,05
	Controlled group (n=55)	28	50,91	16	29,09	11	20,00	0	0	
p(D0-D15)		< 0,001								
p(D0-D30)		< 0,001								

Comment: The improvement of symptoms of early awakening over time in studied groups after treatmented was better compared to before treatmented and compared to controlled with p <0.05.

Table 3.14: The improvement of insomnia-related clinical symptoms of the two groups after treatment at different times.

Symptom / Day			Tired	Reduce concen-tration	Worry	Forge-tful	Tight	Lost weight	Dizziness t	P
D0	Studied group (n=110)	Amount	97	63	17	47	36	21	83	>0,05
		Ratio %	88,2	57,3	15,5	42,7	32,7	19,1	75,5	
	Controlled group (n=55)	Amount	48	32	8	22	19	10	41	
		Ratio %	87,27	58,18	14,75	40,00	34,55	18,18	74,55	
D15	Studied group (n=110)	Amount	13	10	7	17	5	5	15	<0,05
		Ratio %	11,82	9,09	6,36	15,45	4,55	4,55	13,64	
	Controlled group (n=55)	Amount	15	12	6	15	11	6	15	
		Ratio %	27,27	21,82	10,91	27,27	20,00	10,91	27,27	
D30	Studied group (n=110)	Amount	3	3	1	15	1	1	7	<0,05
		Ratio %	2,73	2,73	0,91	13,64	0,91	0,91	6,36	
	Controlled group (n=55)	Amount	6	8	3	15	5	6	13	
		Ratio %	10,91	14,45	5,45	27,27	9,09	10,91	23,64	
p0-p(15,30)			< 0,05	<0,05	<0,05	< 0,05	< 0,05	<0,05	< 0,05	

Comment: At 15 days and 30 days after treatmented, the improvement of clinical symptoms in studied groups after treatmented was better compared to before treatmented and compared to controlled, there was difference with p <0.05.

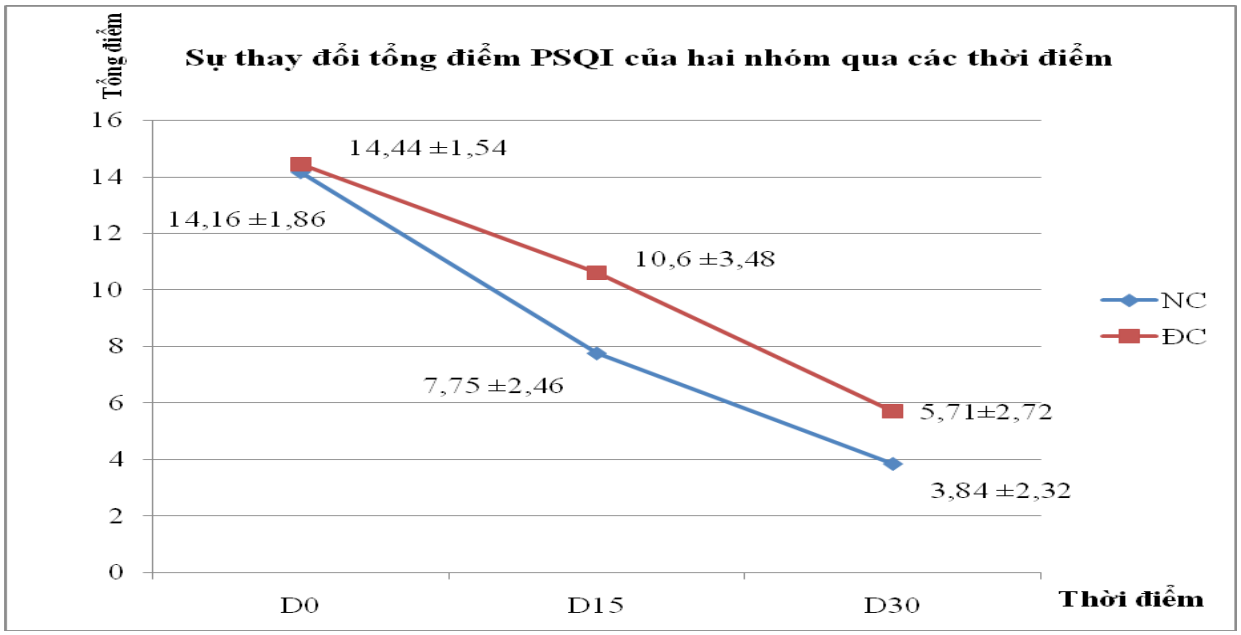


Chart 3.2: Changing of PSQI total score of the two groups over time (Unit: Score)

Comment: The improvement of PSQI total score in studied groups after treatmented was better compared to before treatmented and compared to controlled with p <0.05.

Table 3.15: Variation of Alpha wave parameters on EEG of the two groups before and after treatment

Day / The group		D0 ($\overline{X}$ ± SD)	D30 ($\overline{X}$ ± SD)	p(D0-D30)
Studied group (n=110)	Frequency (period/sec)	10,18±1,52	9,98±1,40	p >0,05
	Index (%)	43,59±10,82	55,05±7,30	p <0,05
	Amplitude (µV)	38,36±12,82	51,77±12,20	p <0,05
Controlled group (n=55)	Frequency (period/sec)	9,91±1,11	9,73±0,99	p >0,05
	Index (%)	45,18±10,89	53,27±5,79	p <0,05
	Amplitude (µV)	43,91±10,92	48,55±6,78	p <0,05

Comment: The improvement of variation of Alpha wave parameters on EEG of the two groups after treatmented was better compared to before treatmented with p <0.05.

Table 3.16: Variation of Beta wave parameters on EEG of the two groups before and after treatment

Day The group		D0 $\overline{X}$ ± SD	D30 $\overline{X}$ ± SD	p(D0-D30)
Studied group (n=110)	Frequency (period/sec)	16,15±1,33	15,83±1,76	p>0,05
	Index (%)	52,32±11,41	45,14±8,03	p <0,05
	Amplitude (μV)	10,43±1,45	11,64±6,88	p>0,05
Controlled group (n=55)	Frequency (period/sec)	16,16±2,04	15,71±1,3	p>0,05
	Index (%)	50,09±11,57	46,55±7,69	p>0,05
	Amplitude (μV)	10,27±1,15	11,73±9,49	p>0,05

Comment: The improvement of variation of Alpha wave parameters on EEG of the two groups after treatmented was better compared to before treatmented with p <0.05. However, the change in the variation of the studied group compared to before treatmented was different with p<0.05.

Table 3.17: Changing of total PSQI score of 2 ti bing of traditional medicine in two groups after 30 days of treatment (D30)

Unit: Points

Ti bing of traditional medicine The group		Deficiency of heart yin (1) ($\overline{X}$ ± SD)	Deficiency of heart blood (2) ($\overline{X}$ ± SD)	p(1-2)
Studied group (n=110)	D0	13,78 ± 1,51	14,36 ± 1,99	p > 0,05
	D30	4,24 ± 2,82	3,63 ± 2,00	p > 0,05
Controlled group (n=55)	D0	14,36 ± 1,56	14,49 ± 1,54	p > 0,05
	D30	5,73 ± 2,83	5,70 ± 2,69	p > 0,05
p D0 (Studied group - Controlled group)		p >0,05	p >0,05	
p D30 (Studied group - Controlled group)		p <0,05	p <0,05	
p Studied group - Controlled group (D0-D30)		p <0,05	p <0,05	

Comment: after treatmented, PSQI average total score of deficiency of heart yin and deficiency of heart blood significantly improved in the studied group compared to the controlled group and compared with before treatmented was a difference with p <0.05.

3.2.3. Adverse effects of Duong tam an than liquid

Clinical

During treated 30 days of using Duong tam an than liquid, weight, pulse , blood pressure had no difference compared to baseline (p <0.05), no patients showed adverse effects such as nausea, digestive disorders, diarrhea, or pruritus….

Blood test

BUN, creatinine, SGOT, SGPT of the two groups before and after treatment are within the normal limit, the change has no statistical significance with p> 0.05.

CHƯƠNG IV: DISCUSSION

4.1. THE SAFETY OF "DUONG TAM AN THAN" LIQUID

4.1.1. acute toxicity of " Duong tam an than" liquid

Results of acute toxicity studies is showed in Table 3.1. Because there was no death rat, the LD50 of YXAS via oral use was not determined yet.

The acute toxicity was showed at highest dose of 17 g / kg (= 75 ml / kg ≈ 38.42 g dried herb ≈ 11 clinical dose this is the conversion ratio of mice: 10-13 times), rats drunk YXAS liquid with a maximum volume of 0.25ml / 10g mice, 3 times in 24 hours, rats did not die, were inactive, slept a lot, but up to 40% rats of the lot had diarrhea. Therefore the safe range of YXAS liquid is acceptable with effective dose <1/10 highest dose. This showed that the modification of the Tian wan bu xin to reduce the stickiness, stagnation and cold properties is necessary. Thus, it can be affirmed that a dose of 13.6g / kg (60ml / kg) of YXAS liquid did not caused acute toxicity on white rats.

4.1.2. Subchronic toxicity of " Duong tam an than" liquid

The research results showed that rats in 2 lots used YXAS liquid dose of 9.14 g condensed liquid/ kg body weight / day (clinical dose equivalent) for 8 weeks continuously, and 27.72 g condensed liquid / kg body weight / day (equivalent to 3 fold clinical dose) for 8 weeks constinuously.

All monitoring indicators for general condition, weight, and indicators of hematopoietic function evaluation, liver function, level of liver cell destruction and filtering function of the kidney, and anatomic pathology of the liver and the kidney were within the normal limits, there was no significant difference compared to the controlled group and compared to the baseline.

4.2. SEDATIVE EFFECTS OF DUONG TAM AN THAN ON EXPERIMENTAL

Lot of rats using Duong tam an than liquid and lot of rats using diazepam both showed inhibitor effects on the central nervous system, therefore increase the time spent on the open arm; increase the number of entries, reduce rate of the avoidance in the open arm due to reduce fear, reduce the time spent on the close arm on elevated plus maze testing. Activity cage testing also showed reduction of normal activity of rats, reduction of the number of horizontal movement and vertical movement. The rotarod rotating axis and the grip gauge showed reduction the time that rats sticked with rotarod rotating axis and the grip force of the rats. This is the evidence for sedative effects of lot of rats using YXAS liquid compared to sham and to lot of rats using diazepam, statistically significant at both 1 hour and 3 hours after taking liquid (with $p < 0.05$). The sedative effects of 2 YXAS doses was similar and there was no difference compared to diazepam with $p > 0.05$.

4.3. THE CLINICAL EFFECTS OF DUONG TAM AN THAN LIQUID

4.3.1. Discuss the therapeutic effect of Duong tam an than liquid

The evaluation results on the therapeutic effect of Duong tam an than liquid showed: The patients recognized a significant change in the time of falling asleep, the quality of sleep, the average hours of sleep in a night, sleep efficiency, Pittsburgh score (PSQI), reduce the number of early awakening and symptoms of clinical g of the patients was significantly improved on sleep quality in particular, and the quality

of life in general of the patient, which is liberation feeling worried too much of the patient when go to bed but sleep loss, or early awakening. The improvement in both groups after treatment was statistically significant compared to before treatment ($p < 0.05$). However, in the studied group, the results improved better than the controlled group, the difference was statistically significant with $p < 0.05$.

By using EEG to evaluate the effect of Duong tam an than, the function of the brain and the effectiveness of the treatment has been objectively reflected. The index and amplitude of alpha wave and beta wave after treatment shows better improvement compared to baseline and controlled group, with $p < 0.05$. Meanwhile, the indices are still in normal range.

4.3.2 Mechanism of action of Duong tam an than liquid

Duong tam an than liquid has all of monarch, minister, assistant and guide herbs according to the structure of an ancient medicine. Codonopsis pilosula franch, astragalus membranaceus bge, ophiopogon japonicus are monarch herbs, in order to invigorates qi, nourish xin qi, zi yin, nourish yin fluids, reduce the fire deficiency, in order to keep the heart and the spirit from being disturbed. Panax pseudo- ginseng wall, Salvia miltiorrhiza bunge, Anggelia sinensis dielz enriches the blood, promote the blood, nourish yin; Thujae orientalis semen, Zyzyphus jujuba lamk, Polygala tenuifolia willd, Senna obtusifolia, Poria cocos are minister herbs, in order to calm the spirit. Schisandra chinensis, Zyzyphus jujuba lamk are assistant herbs, in order to keep xin qi, Amomum xanthioides wall, Platycodon grandifolium are guide herbs, in order to promote qi and lead the herbs up , sedative effect. Remedy in order to nourish the heart, calm the spirit method, zi yin, nourish qi blood, promote blood. Thus, the decisive role of heart yin and heart blood deficiency to the sleep is apparent.

On the other side, Duong tam an than liquid does not cause any damage to spleen qi, remove the stagnation properties of drugs, nourish yin, nourish blood, at the same time, promote qi to warm up the zhong jiao.

The experimental model also shows that in both liquid doses, the anti-anxiety effect of YXAS were equivalent to Diazepam with $p>0,05$ as mentioned above.

Sedative effect of the Duong tam an than liquid can be explained by the composition of the liquid wich many herbs such as Zyzyphus jujuba lamk, Thujae orientalis semen, Polygala tenuifolia willd, Poria cocos, Senna obtusifolia, Ophiopogon japonicus, Salvia miltiorrhiza bunge, Schisandra chinensis, which contain the active ingredients with sedative tranquility that has been scientifically proven.

4.3.3. Adverse effect of Duong tam an than liquid

During the treatment course of 30 days, patient's heart rate and blood pressure were stable, there was no nausea, diarhea, gastric pain, pruritus, headache and extremities edema. Besides, most of the patients commented that the YXAS has pleasant flavor and sweet tasting, thus easy to use, and this is also an advantage of YXAS liquid in clinical practice.

Impact on hematological and biochemical indicators: there was no significant difference between before and after treatment, with $p> 0.05$. All of the indicators were at normal physiological level.

CONCLUDE

From the results of the experimental and clinical study, we draw these following conclusion:

1. Acute toxicity, subchronic toxicity of YXAS liquid on experimental animals

1.1. Acute toxicity of YXAS liquid in white rats orally:

When using YXAS liquid at the dose of 17g condensed liquid/kg/day (=38,42 g of dried herb/ kg/day), rats did not die within 24 hours, were inactive, slept, and diarrhea; LD50 has not been determined.

1.2. Subchronic toxicity of YXAS liquid in white rats via oral use:

Dose of 9,24 g condensed liquid/kg/day (= 20.88 g of dried herb/ kg/day) and 27, 72 g condensed liquid/kg/day (= 62.64 g of dried herb/kg/day) using continuously for 8 weeks showed no effect on general condition, weight, and indicators of hematopoietic function evaluation, liver function, level of liver cell destruction and filtering function of the kidney, and anatomic pathology of the liver and the kidney.

2. Evaluation of sedative effects of "Duong tam an than" liquid on experimental animal models.

1 hour and 3 hours after taking YSAS liquid at clinical dose (41,76 g of dried herb/ kg/day) and 3 fold clinical dose (125,28 g of dried herb/ kg/day), the results showed the sedative effect:

Increase the number of entries, the time spent on the open arm; reduce the rate of the avoidance on the open arm. Reduce the time spent on the close arm, reduce the number of horizontal movement and vertically movement, reduce the time that rats sticked with rotarod rotating axis and the grip force of the rats. The effect is similar between these 2 doses.

3. The sedative effects of "Duong tam an than" liquid on clinical:

After conducting the research comparing 110 non-organic insomnia using Duong tam an than liquid to 55 patients using bagged fluid Tian Wan Bu Xin Dan for 30 days, the results showed:

* “Duong tam an than” liquid has a good effect in treating non-organic insomnia patients:

- Reduce the time of falling asleep: The rate fall sleep from <15 minutes and 15- <30 minutes, respectively increased from 0% and 4.55% to 67.27% and 30.00% ($p < 0.05$).

- Increasing sleep time per night: From 3.46 ± 0.95 hours / night to 6.46 ± 0.97 hours / night ($p < 0.05$).

- Sleep efficiency of ≥ 85% and 75% - <85% increased from 0% and 1.82% before treatment to 65.46% and 28.18%.

- Improve the symptoms of early waking, disturbances of the day

- Clearly improve the average PSQI score: Reduce from 14.16 points to 3.84 points ($p < 0.05$).

- The index and amplitude of alpha wave and beta wave after treatment shows better improvement compared to baseline and controlled group, with $p < 0.05$.

- The treatment effect of Duong tam an than liquid on heart yin deficiency tends to be better than heart blood deficiency ($p > 0.05$).

* *No clinical and subclinical adverse effects have been seen during the course of treatment.*

REQUEST

1. "Duong tam an than" liquid has a good effect in treating non-organic insomnia patients, so it is necessary to continue studying to evaluate the effect on a large number of patients and further research on pharmacological effects.

2. Due to the fact that there are only 2 products in liquid form: bagging and bottling, it is necessary to modernize the form of liquid products to satisfy the clinical needs for using this liquid. It should be further research on preparation in the form of tablets, capsules or freeze-dried to produce drugs in the form of soluble powder for easier storage, easier transportation and easier to use.

THE STUDY WORKS HAS BEEN DISCLOSURE RELATED TO THE THESIS CONTENT

1. Nguyen Van Tam, Nguyen Tran Thi Giang Huong, Pham Thi Van Anh, Do Thi Phuong (2016)

" Study on acute toxicity and effects of Yang xin an shen liquid on mental health on experimental hematological indexes". Vietnam traditional medicine research journal, No. 48 - 2016, page 26-35

2. Nguyen Van Tam, Nguyen Tran Giang Huong, Pham Thi Van Anh, Do Thi Phuong (2016)

" Study the effect of Yang xin an shen liquid on liver functions and kidney functions on experimental animals". Vietnam traditional medicine research journal, No. 48 - 2016, p. 70-77.

3. Nguyen Van Tam, Nguyen Tran Giang Huong, Pham Thi Van Anh,Do Thi Phuong, Nguyen Thi Loan (2017)

"Evaluate sedative and anxiolytic effects of the " Duong tam an than " extract in animals". Vietnam traditional medicine research journal,, October No. 02-2017, pp 215-219.

4. Nguyen Van Tam, Nguyen Tran Thi Giang Huong, Do Thi Phuong, (2019)

First evaluation of the effects of "Yang xin an shen liquid on non-organic insomnia patients" Vietnam traditional medicine research journal, No. 60 - 2019, page 13-21.