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Investigation for subchronic oral toxicity and evaluation of analgesic activities of *Stephania tetrandra* S. Moore liquid extract in experimental animals

Thanh Mai Phuong[1], Trang Tran Thi Thu[2], Khanh Do Thi Hong[3], Ngoc Tran Thi Hong[3], Dat Tran Tien[3], Trang Nguyen Thi Mai[3], Minh Phan Hong[3*]

[1]Hanoi Medical University, 1 Ton That Tung Street, Trung Tu Ward, Dong Da District, Hanoi, Vietnam
[2]Hanoi University of Pharmacy, 13-15 Le Thanh Tong Street, Phan Chu Trinh Ward, Hoan Kiem District, Hanoi, Vietnam
[3]University of Medicine and Pharmacy, Vietnam National University - Hanoi, 144 Xuan Thuy Street, Dich Vong Hau Ward, Cau Giay District, Hanoi, Vietnam

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Abstract:

This research evaluates subchronic toxicities and analgesic effects of *Stephania tetrandra* S. Moore (*S. tetrandra*) liquid extract through oral administration in experimental animals. Over 4 weeks of subchronic toxicity research, *S. tetrandra* was administered to Wistar rats at a dose of 1.6 g/kg body weight (BW) (the dose is equivalent to the expected human dose) and 4.8 g/kg BW (three times the human expected dose), based on World Health Organization (WHO) and Organisation for Economic Co-operation and Development (OECD) recommendations. The analgesic effect was assessed using three models: hot plate, tail-flick, and acetic acid-induced writhing in *Swiss* mice. Regarding the subchronic toxicity test, after oral administration of *S. tetrandra* liquid extract, haematological parameters, hepato-renal functions, and microscopic images of the liver and kidney were unchanged compared with the control group. As a result, *S. tetrandra* liquid extract at both doses of 3.2 g/kg/day and 9.6 g/kg/day tended to reduce pain in the models. The low dose significantly showed peripheral analgesic effects, while the high dose demonstrated better analgesic activity at both central and peripheral sites. In conclusion, *S. tetrandra* liquid extract did not produce subchronic toxicities in *Wistar* rats, and has shown experimental analgesic effects.

Keywords: **analgesic activity, experimental animals, *Stephania tetrandra* S. Moore (*S. tetrandra*), subchronic toxicity.**

Classification number: **3.3**

1. Introduction

Nature has been a source of medicinal agents since ancient times, and medicinal plants form a wide variety of traditional medicines used in various countries worldwide [1]. The exclusive use of herbal drugs for managing various ailments continues due to easy access, better compatibility, and economic reasons. According to the World Health Organization (WHO), up to 80% of the population in developing countries uses traditional medicine for primary health care. However, the lack of evidence-based approaches and toxicological profiling of herbal preparations remains the biggest concern regarding the use of medicinal plants. Thus, evaluating their toxicity plays a vital role in recognising these effects, in support of their characterisation, evaluation for human risk, and thus proposing measures to mitigate the risk, particularly in early clinical trials [2].

Toxicity refers to unwanted effects on biological systems. The toxicity of a substance can be impacted by many factors, such as the route of exposure (skin absorption, ingestion, inhalation, or injection), the exposure time (acute or chronic administration), the physical form of the toxin (solid, liquid, or gas), the organ system involved (cardiovascular, nephro-, haemo-, nervous-, or haematopoietic-system), and genetic structure [3]. Subchronic systemic toxicity is defined as adverse effects occurring after repeated or continuous administration of a test sample for up to 12 weeks or not exceeding 10% of the animal's lifespan [4, 5]. In Vietnam, *S. tetrandra* has been recently exploited and used as medicine. Historically, *S. tetrandra* has been used since ancient times and in folklore to treat joint pain, muscle fatigue, etc., but no report available on the safety of the product and little scientific evidence is available on the topical analgesic activity. Therefore, our intention was to investigate subchronic toxicities and antinociceptive activities of *S. tetrandra* liquid extract in animals in this study.

[*]Corresponding author: Email: phanhongminh.hmu@gmail.com

2. Materials and methods

2.1. Preparation of S. tetrandra liquid extract

S. tetrandra were harvested in Ba Vi, Hanoi, Vietnam. The samples were assessed by the Department of Medicinal Materials at the University of Medicine and Pharmacy, Vietnam National University - Hanoi. The tubers were washed and dried at 60°C. A total of 800 g of the dried tubers were ultrasonically extracted with 96% ethanol for 45 minutes and then evaporated under reduced pressure to obtain the extract (97 g) with an extraction yield of 12.1%. The extracts were stored in a refrigerator at 2-8°C. The extract was completely dissolved in distilled water before administration.

2.2. Preparation of machinery and chemicals

+ Codeine phosphate was provided by the Central Institute of Drug Testing.

+ Acetic acid solution was supplied by Guangdong Guanghua Sci-Tech Co., Ltd, China.

+ Aspirin tablets (100 mg) were obtained from Traphaco Joint Stock Company, Vietnam.

+ Distilled water.

+ Sodium chloride 0.9% solution was acquired from Braun, Vietnam.

+ 70% alcohol was sourced from HD Pharma, Vietnam.

- Machines and tools for research:

+ Precisa LX2200c electronic scale (Switzerland).

+ Hot plate machine model - DS37 (Ugo-Basile, IDEA).

+ Dynamic Plantar pain response meter Aesthesiometer 37450 (Ugo Basile, Italy).

+ Special needles for administering medicine to mice.

+ Various tools and materials used in the research (cotton, trays, gauze, scissors, pints, etc.).

2.3. Experimental animals

This study utilised *Wistar* rats weighing 150-200 g obtained from the Vietnam Military Medical University and *Swiss* mice weighing 20-22 g sourced from the National Institute of Hygiene and Epidemiology, Vietnam. The animals were divided into cages (each containing 10 mice or rats) and housed in a room maintained at a temperature of 22±2°C and 18-20% humidity. They were subjected to a 12-hour light/dark cycle and had free access to food and water ad libitum. The animals were acclimated to the housing conditions for at least one week prior to the commencement of the study.

2.4. Subchronic toxicity study

A subchronic toxicity study was conducted according to WHO and OECD guidelines [6, 7]. The study spanned a continuous four weeks. *Wistar* rats were divided into three groups of ten animals each:

- Group 1 (control group) received distilled water.

- Group 2 was orally administered *S. tetrandra* liquid extract at a dose of 1.6 g/kg/day (equivalent to the human recommended dose, conversion ratio 6);

- Group 3 was orally administered *S. tetrandra* liquid extract at a dose of 4.8 g/kg/day (3 times higher than that of Group 2).

Animals received oral administration of distilled water and *S. tetrandra* liquid extract at a volume of 10 ml/kg body weight daily for four consecutive weeks and were observed daily to detect clinical signs and determine time points for laboratory tests. The liquid extract was dissolved in distilled water (the solvent for *S. tetrandra* liquid extract) daily before administration to the rats.

The parameters monitored during the study included general conditions, mortality, and clinical signs such as:

- Body weight changes.

- Haematopoietic function: red blood cells (RBC), haemoglobin (HGB), haematocrit, total white blood cells (WBC), WBC differentials, and platelet count (PLT).

- Serum biochemistry tests: aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, albumin, total cholesterol, and creatinine levels.

These parameters were checked before treatment, two weeks, and four weeks after treatment. At the end of the experiment, all animals underwent a full gross necropsy. The livers and kidneys of 30% of the rats from each group were taken for histopathological examinations. The micro-histological examination was carried out at the Department of Pathology, 103 Military Hospital, Hanoi, Vietnam.

2.5. Analgesic activity

Hot plate test: The hot plate device used in this research was the Ugo Basile, Model DS 37, and was maintained at 54.5-56.5°C. Mice were randomly divided into four groups of ten each. The experiment involved placing mice on a hot plate within a beaker and observing their response to heat-induced pain. The baseline reaction time was considered the mean reaction time obtained before the oral administration of normal saline, codeine phosphate at a dose of 20 mg/kg/day, and *S. tetrandra* liquid extract at doses of 3.2 and 9.6 g/kg/day, defined as the normal reaction of the animal

to temperature. Thirty minutes after treatment, the reaction time (in seconds) was recorded when mice licked their fore or hind paws and then jumped, indicating their response to heat-induced pain. Animals showing a reaction time greater than 30 seconds were discarded. A cut-off time of typically 30 seconds was established to minimise the risk of tissue damage from prolonged exposure to hot surfaces [8, 9].

Tail-flick test: The tail-flick method was applied to evaluate the analgesic activity in mice. Reaction latencies were the index used in this study, measured by placing the tip (last 3 cm) of the tail on the radiant heat source. Removing the tail from the radiant heat source was considered the endpoint. A time limit of 15 seconds was used to avoid heat damage to the tail. Mice were divided into four groups (n=10) and administered with normal saline, codeine phosphate at a dose of 20 mg/kg/day, and *S. tetrandra* liquid extract at doses of 3.2 and 9.6 g/kg/day. The latency of the tail-flick reaction was recorded 30 minutes after dosing [10].

Acetic acid induced writhing in mice: Swiss mice were randomly divided into four groups (n=10). Group I was treated with distilled water (20 ml/kg), Group II with codeine phosphate at a dose of 20 mg/kg/day; Groups III and IV received *S. tetrandra* liquid extract at doses of 3.2 and 9.6 g/kg/day, respectively, for five consecutive days. On the last day, one hour after codeine phosphate and *S. tetrandra* liquid extract administration, groups II, III, and IV received 1% acetic acid at a dose of 10 ml/kg b.w, intraperitoneally, and writhing reflex was observed for 30 minutes. The mice exhibited writhing movements (stretching of hind limbs and bending of the trunk).

2.6. Statistical analysis

All data were expressed as mean ± standard deviation. The statistical analysis of the experimental data was carried out to identify differences between each treated group and the control group using Student's t-test and paired t-test. A difference was considered statistically significant at $p \leq 0.05$.

3. Results

3.1. Acute and subchronic toxicity study

3.1.1. Acute toxicity

Swiss mice were administered *S. tetrandra* liquid extract in doses ranging from the highest dose that did not cause death (65.18 g/kg) to the lowest dose that resulted in 100% mortality (195.29 g/kg). The administrations were given three times within 24 hours, with each administration separated by 3 hours. As a result, *Swiss* mice exhibited mortality and some abnormal signs at a dose of 81.38 g/kg (Table 1).

Table 1. Results of acute toxicity studies according to a dose of *S. tetrandra* liquid extract.

Group	n	Dose (ml/kg)	Dose (g/kg)	Lethal dose	Abnormal sign
Group 1	10	15	48.83	0	No
Group 2	10	20	65.10	0	
Group 3	10	25	81.38	20	Convulsions, cyanosis
Group 4	10	30	97.66	40	
Group 5	10	35	113.95	40	
Group 6	10	40	130.22	60	
Group 7	10	45	146.50	70	
Group 8	10	50	162.79	80	
Group 9	10	55	179.18	90	
Group 10	10	60	195.29	100	

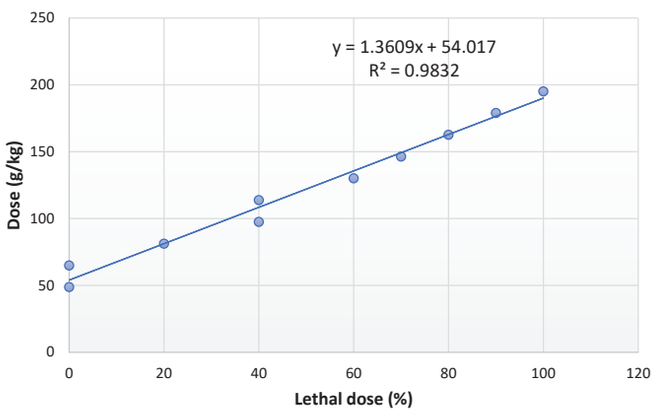


Fig. 1. Graph representing the dose vs. the lethal dose of *S. tetrandra* liquid extract.

From the graph above (Fig. 1), a 50% lethal dose of extract can be calculated as: LD50 = 1.3609x50+54.017=122.1 g/kg. This calculation includes adults weighing 50 kg. The extrapolation coefficient for mice was 12 and for rats was 6.

3.1.2. Subchronic toxicity

General condition: Animals exhibited normal locomotor activities and good feeding habits. None of the animals in any of the treated groups showed any macroscopic or gross pathological changes compared with the control group.

Body weight changes: Fig. 2 shows that after two weeks and four weeks, the body weight in all groups increased slightly compared with the time point "Before treatment". There was no significant difference in body weight between the *S. tetrandra* liquid extract-treated groups and the control group ($p>0.05$).

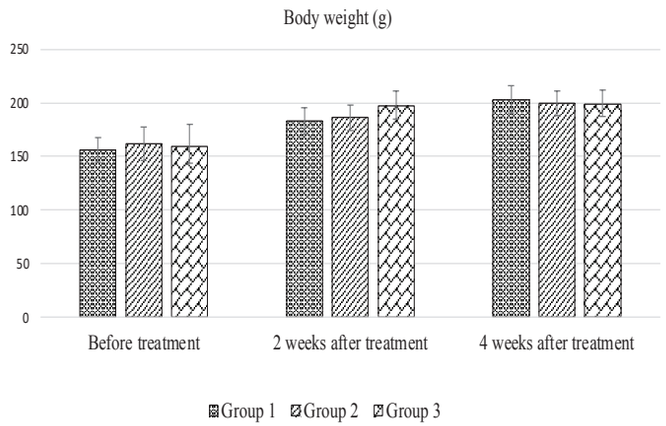


Fig. 2. The effect of *S. tetrandra* liquid extract on body weight.

Table 2. The effect of *S. tetrandra* liquid extract on hematopoietic function.

Parameters	Group	Before treatment	After treatment	
			Two weeks	*Four weeks*
Red blood cells count (T/L)	Group 1	8.41±0.69	8.24±1.01	8.07±1.09
	Group 2	8.96±1.89	8.99±0.64	8.12±0.86
	Group 3	8.30±0.42	8.12±0.86	8.53±0.65
	p	*>0.05*	*>0.05*	*>0.05*
Haemoglobin level (g/dL)	Group 1	11.08±1.11	11.21±1.06	11.57±0.94
	Group 2	11.65±1.73	11.68±1.03	11.82±0.93
	Group 3	1093±1.27	11.01±0.91	11.61±1.15
	p	*>0.05*	*>0.05*	*>0.05*
Haematocrit (%)	Group 1	44.60±3.07	43.02±4.16	44.12±2.91
	Group 2	43.81±4.60	42.04±3.85	42.66±2.63
	Group 3	43.87±4.80	42.12±6.09	43.84±2.15
	p	*>0.05*	*>0.05*	*>0.05*
Platelet count (G/L)	Group 1	558.50±109.76	572.70±111.23	601.50±112.65
	Group 2	567.50±111.34	581.20±110.29	582.30±99.07
	Group 3	539.40±114.21	551.40±95.99	548.60±106.10
	p	*>0.05*	*>0.05*	*>0.05*
Total WBC count (G/L)	Group 1	6.68±1.07	7.20±1.76	7.09±1.19
	Group 2	6.85±1.70	7.19±2.03	6.99±1.73
	Group 3	6.34±0.93	673±1.53	6.85±1.56
	p	*>0.05*	*>0.05*	*>0.05*
Lymphocytes (%)	Group 1	79.38±5.32	75.00±5.27	78.33±5.35
	Group 2	75.92±6.54	78.48±3.77	76.32±5.78
	Group 3	75.82±4.38	77.94±9.09	76.28±5.36
	p	*>0.05*	*>0.05*	*>0.05*
Neutrophils (%)	Group 1	8.44±2.84	7.74±1.26	8.58±2.25
	Group 2	7.93±2.20	7.75±1.35	7.23±2.06
	Group 3	8.96±2.61	7.45±2.05	9.08±2.40
	p	*>0.05*	*>0.05*	*>0.05*

p: comparison with the control group and "Before treatment".

The effect of S. tetrandra liquid extract on the haematological system: There were no significant differences in red blood cell count, haematocrit, haemoglobin level, platelet count, total WBC count, and WBC between the *S. tetrandra* liquid extract-treated groups and the control group (p>0.05) (Table 2).

Table 3. The effect of *S. tetrandra* liquid extract on liver function.

Parameters	Group	Before treatment	After treatment	
			Two weeks	*Four weeks*
AST level (UI/L)	Group 1	84.98±8.15	79.82±13.76	79.98±20.88
	Group 2	85.12±9.06	80.62±14.77	86.14±22.52
	Group 3	87.34±9.17	87.85±14.06	89.00±21.27
	p	*>0.05*	*>0.05*	*>0.05*
ALT level (UI/L)	Group 1	36.08±4.11	38.00±6.06	33.57±3.94
	Group 2	32.25±6.73	33.68±8.03	34.82±4.93
	Group 3	35.13±5.27	34.01±5.81	34.69±7.85
	p	*>0.05*	*>0.05*	*>0.05*
Total bilirubin (mmol/l)	Group 1	13.35±0.46	13.55±0.75	13.39±0.42
	Group 2	13.52±0.38	13.53±0.29	13.57±0.35
	Group 3	13.31±0.53	13.40±0.30	13.52±0.28
	p	*>0.05*	*>0.05*	*>0.05*
Albumin concentration (g/dL)	Group 1	3.55±0.22	3.71±0.21	3.67±0.32
	Group 2	3.49±0.21	3.51±0.34	3.50±0.26
	Group 3	3.45±0.31	3.47±0.31	3.50±0.32
	p	*>0.05*	*>0.05*	*>0.05*
Total cholesterol concentration (mmol/l)	Group 1	1.66±0.29	1.62±0.30	1.65±0.27
	Group 2	1.65±0.29	1.63±0.24	1.59±0.23
	Group 3	1.59±0.31	1.55±0.23	1.72±0.29
	p	*>0.05*	*>0.05*	*>0.05*

p: compared with the control group and the time point "Before treatment".

The effect of *S. tetrandra* liquid extract on liver function: There were no significant differences in aspartate aminotransferase (AST), alanine aminotransferase (ALT) levels, total bilirubin, and albumin concentration between the *S. tetrandra* liquid extract-treated groups and the control group (p>0.05). The results are shown in Table 3.

The effect of S. tetrandra liquid extract on kidney function: Table 4 demonstrated that after two weeks and four weeks of treatment, *S. tetrandra* liquid extract caused no significant difference in serum creatinine levels between the control group and the two treated groups (p>0.05).

Table 4. The effect of *S. tetrandra* liquid extract on serum creatinine.

Group	Creatinine "Before treatment" (mg/dL)	Creatinine "After treatment" (mg/dL)	
		Two weeks	*Four weeks*
Group 1	0.78±0.05	0.81±0.06	0.79±0.03
Group 2	0.80±0.02	0.82±0.01	0.75±0.05
Group 3	0.79±0.04	0.83±0.07	0.78±0.07
p	*>0.05*	*>0.05*	*>0.05*

p: compared with the control group and the time point "Before treatment".

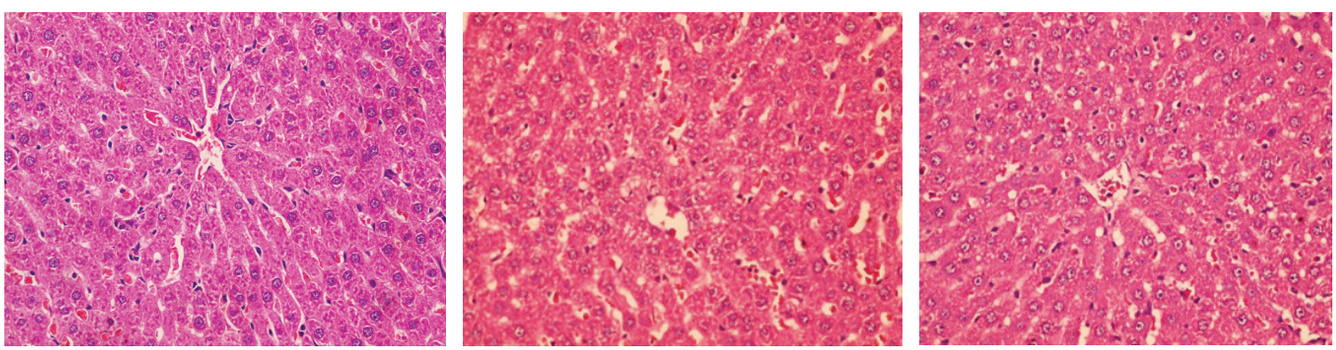
Histopathological examination: No gross lesions or changes in size were observed when all experimental rats underwent a full gross necropsy, which examined the hearts, livers, lungs, kidneys, and abdominal cavities.

There was no significant difference in the histopathological examinations of the livers and kidneys between the *S. tetrandra* liquid extract-treated mice and the control group after four weeks of treatment (Figs. 3 and 4).

3.2. Analgesic activity

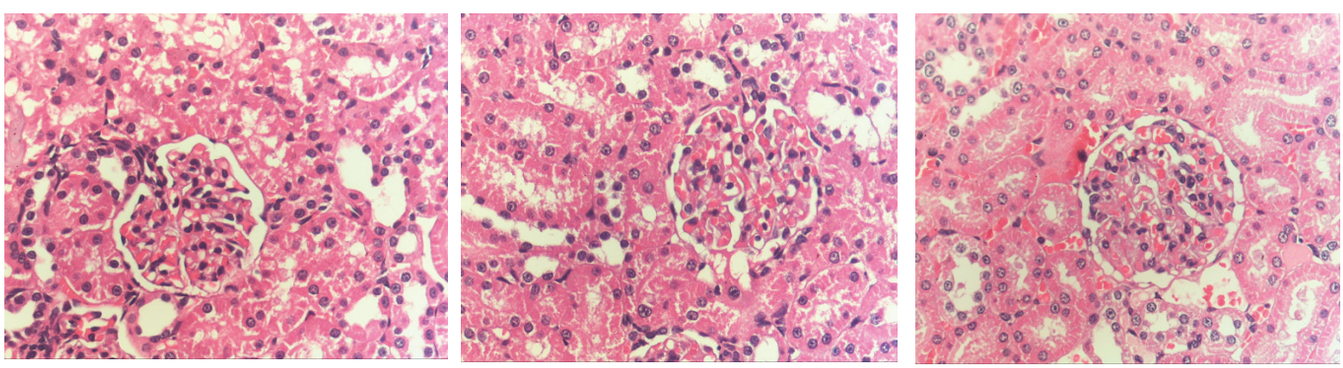
Hot plate test and tail flick test

Time (seconds): Observing Fig. 5, the treatment with codeine phosphate showed a significant prolongation of reaction time compared with the control group in both the tail-flick assay and hot plate test. The results obtained with the administration of *S. tetrandra liquid extract* increased the latency to pain on the hot plate (55.5±10°C) and in the tail-flick assay. The animals treated with *S. tetrandra* liquid extract at doses of 3.2 and 9.6 g/kg/day both showed increased resistance to the hot painful stimulation inflicted throughout this test. At the dose of 9.6 g/kg/day, the extract demonstrated significant results in antinociceptive activity, increasing the latency of tail movement in rats.



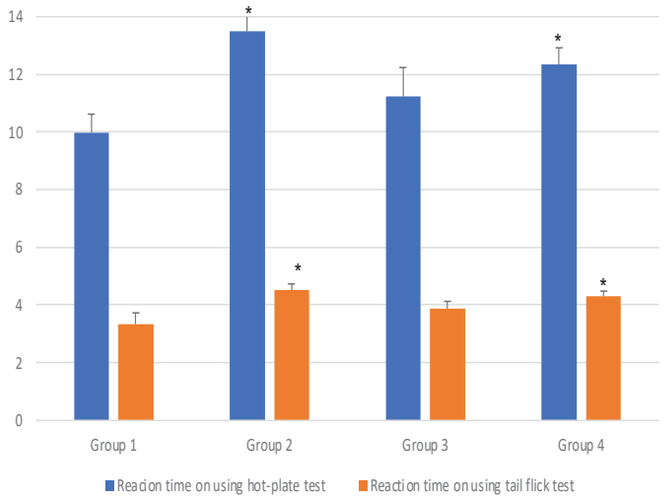
Group 1 Group 2 Group 3

Fig. 3. Histopathological morphology of liver (HE × 400).



Group 1 Group 2 Group 3

Fig. 4. Histopathological morphology of kidney (HE × 400).



* p<0.05; compared with control (Student's t-test).

Fig. 5. The effects of *S. tetrandra* liquid extract on reaction time on using hot plate and tail flick test.

research provides knowledge on the effects of repeated oral exposure and offers evidence for longer-term studies [6, 12]. Subchronic studies assess the undesirable effects of continuous or repeated exposure to plant extracts or compounds over a portion of an animal's average lifespan, such as in rodents, specifically providing information on target organ toxicity [13].

The most basic indicator to reflect organ and system toxicity is the body weight change index [13]. For all experimental animals, general signs should be observed daily, and body weight should be measured periodically [12]. Based on the obtained results, it can be asserted that *S. tetrandra* did not interfere with the animals' normal metabolism because there were no significant differences compared to the control group.

Table 5. Analgesic activity of *S. tetrandra* liquid extract by acetic acid-induced writhing method.

Group (n=10)	Number of writhing					
	0-5 min	*>5-10 min*	*>10-15 min*	*>15-20 min*	*>20-25 min*	*>25-30 min*
Group 1	10.70±2.09	17.20±3.80	15.60±4.61	13.80±3.80	10.90±3.21	6.90±1.84
Group 2	4.80±1.17***	14.20±1.05*	10.10±2.27*	9.20±2.06**	7.60±1.79*	4.50±1.13*
Group 3	7.60±2.22^{+++}	16.70±3.17	12.50±2.90*	9.70±2.27*	7.10±1.98*	5.50±1.18
Group 4	8.40±1.16^{+++}	15.90±3.16	11.80±2.07*	10.50±2.08*	6.80±2.22**	6.30±2.01^{+}

*p<0.05; **p<0.01; ***p<0.001 compared with control (Student's t-test); $^{+}$p<0.05; $^{++}$p<0.01; $^{+++}$p<0.001 compared with group 2 (Student's t-test).

Statistical analysis indicated that codeine phosphate significantly reduced the number of writhes when compared to the control (Table 5) (*p<0.05; p<0.01). Both dose levels of the test extract statistically significantly reduced the number of abdominal constrictions at three time points: >10-15 minutes, >15-20 minutes, and >20-25 minutes.

4. Discussion

4.1. Subchronic toxicity

Toxicity is the degree to which a substance can harm humans or animals. It can affect anything from a single cell to an organ or even the entire organism [11]. To evaluate the safety of drugs or products for human use, toxicity testing is first performed on various experimental animal models to detect toxicity and, based on that, to provide guidance and safety treatment for humans. Subchronic toxicity

The main function of the blood circulatory system is to deliver oxygen, nutrients, hormones, and drugs to cells, muscles, tissues, and organs. The haematopoietic system is one of the most sensitive targets of toxic compounds and is an essential parameter for the physiological and pathological status of humans and animals [6, 12]. Furthermore, such analysis is relevant to risk evaluation as changes in the haematological system have a higher predictive value for human toxicity when the data are translated from animal studies. After two weeks and four weeks of treatment, there was no significant difference in total red blood cells, haematocrit, haemoglobin level, platelet count, total WBC count, and WBC differentials between the *S. tetrandra* liquid extract-treated groups and the control group, so it can be concluded that the *S. tetrandra* liquid extract does not affect the haematological system.

Analysis of the kidney and liver is an important indicator in the assessment of the toxicity of drugs and plant extracts, as they are vital organs essential for the survival of living organisms. Biochemical indices were performed to assess plant product-induced changes in liver and renal function. Changes in serum transaminases are sensitive indicators to reflect the degree of hepatocellular injury. Transaminase would be released from hepatocellular injury when chronic liver cell damage occurs, increasing serum concentrations of these enzymes [11]. Creatinine levels can be used to describe the function of the kidneys [12]. There are no significant ALT and AST changes in both male and female rats at all doses, which indicates that *S. tetrandra* liquid extract had no deleterious effect on liver function. The blood biochemistry levels of the control and *S. tetrandra* liquid extract-treated rats at various doses presented no significant differences between the *S. tetrandra* liquid extract-treated groups and the control group ($p>0.05$). This evidence shows that *S. tetrandra* liquid extract did not affect liver and kidney functions.

The liver and kidneys are important organs in the metabolism and elimination of drugs from the body. The histopathological examination revealed alterations in cell structure under the light microscope. Further histological study could furnish more information regarding the hepatotoxicity and nephrotoxicity of the *S. tetrandra* liquid extract. Our study showed no significant differences in histopathological examinations of the livers and kidneys between the *S. tetrandra* liquid extract-treated groups and the control group.

Overall, this study's findings indicated that no significant differences were observed in blood parameters, biochemistry parameters, and histopathological observations of liver and kidney tissues between the *S. tetrandra* liquid extract-treated groups and the control group.

4.2. Analgesic activity

In this study, the antinociceptive effect of *S. tetrandra* liquid extract was evaluated using classical *in vivo* models of nociception induced by thermal stimuli, as in the hot plate and tail-flick tests, as well as chemical stimuli, such as acetic acid-induced writhing in mice.

The hot plate test, similar to the tail-flick test, evaluates the effectiveness of pain relievers by indicators of response to heat-induced pain. The tail flick test is primarily a response at the spinal level, while the hot plate test response occurs at the supraspinal level. These methods are mainly used to evaluate centrally-acting analgesics [14]. Experimental evidence obtained from the hot plate and tail flick tests in mice showed a tendency to prolong the reaction time to thermal stimuli with *S. tetrandra* liquid extract at both tested doses, with a significant difference observed at the higher dose level of 9.6 g/kg/day.

Acetic acid-induced abdominal writhing is a sensitive procedure for establishing the efficacy of peripherally-acting analgesics. The reaction is thought to involve local peritoneal cells and be mediated by prostaglandin pathways [14]. The data in Table 5 show that *S. tetrandra* liquid extract tended to reduce the number of writhing motions. Both dose levels significantly reduced the number of abdominal cramps at three time points: >10-15 minutes, >15-20 minutes, and >20-25 minutes.

Pain and inflammation often occur together, possibly due to tissue damage, chemical irritation, or autoimmune processes. Stimuli that induce the release of chemical mediators of pain and inflammation [14]. These processes can lead to central sensitization and hypersensitivity. Its sub-plantar injection of acetic acid is followed by the sequential liberation of several mediators of inflammation, such as histamine, 5-hydroxytryptamine, bradykinin, and finally prostaglandins [14]. These mediators stimulate sensory nerve endings and cause pain. Therefore, the above models can be used to evaluate the analgesic activity of *S. tetrandra*.

From the above results, *S. tetrandra* liquid extract expressed both central and peripheral analgesic activities. The peripheral analgesic activity was clearly demonstrated at the two tested doses, while a significant central analgesic effect was observed only at the higher dose. Thus, the dose of 9.6 g/kg/day showed stronger analgesic activity than the dose of 3.2 g/kg/day. This finding suggests that the lower dose should be chosen in cases of mild pain, while the higher dosage appears to be more appropriate for more severe pain requiring central analgesia.

5. Conclusions

For a continuous four weeks, *S. tetrandra* liquid extract at oral doses of 1.6 and 4.8 g/kg/day did not create any toxic signs or symptoms of subchronic toxicity in Wistar rats. *S. tetrandra* liquid extract at the dose of 9.6 g/kg exhibited

pain-inhibitory activities that were characterised by a significantly prolonged reaction time in hot-plate and tail-flick tests and induced a significant decrease in the number of writhes in acetic acid-induced writhing in mice.

CRediT author statement

Thanh Mai Phuong: Conceptualisation, Methodology, Supervision; Trang Tran Thi Thu: Data curation, Writing - Original draft preparation; Khanh Do Thi Hong: Investigation, Writing - Reviewing and Editing; Ngoc Tran Thi Hong: Investigation; Dat Tran Tien: Software, Validation; Trang Nguyen Thi Mai: Project administration; Minh Phan Hong: Formal analysis, Writing - Reviewing and Editing, Methodology, Visualisation.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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