Research Article *doi: 10.47866/2615-9252/vjfc.4078*

Study on the anti-arthritic effects of *Tinospora sinensis* Merr.

Nguyen Duc Thanh[1*], Vy Quoc Tuan[1], Nguyen Dang Long Vu[1], Nguyen Quang Dung[1], Pham Van Toan[1], Nguyen Cong Cuong[1], Nguyen Thi Van Anh[2]

[1]Vietnam Military Medical University, Hanoi, Vietnam

[2]Vietnam University of Traditional Medicine, Hanoi, Vietnam

(Received: 10/05/2023; Accepted: 26/05/2023)

Abstract

The *Tinospora sinensis* Merr. was ultrasonically extracted using 80% ethanol at a temperature of 50°C. The resulting solution was distilled to remove the solvent under reduced pressure, yielding the extract. The extract was then dissolved in distilled water, and this solution was administered to the experimental mice. The results showed that even at the maximum allowable dose, no mice experienced mortality. The anti-inflammatory effects of *Tinospora sinensis* Merr. extract were tested in experiments. The results demonstrated that at a dose equivalent to 1.6 g of plant material per kilogram of body weight per day, the extract exhibited acute anti-inflammatory activity in a model of carrageenan-induced foot edema in mice, comparable to a dose of 80 mg/kg body weight per day of aspirin. Additionally, at a dose equivalent to 2.8 g of plant material per kilogram of body weight per day, the extract showed chronic anti-inflammatory effects in a model of amian-induced granuloma in white mice. These findings suggest a novel direction in using plant-derived medicines, specifically the *Tinospora sinensis* Merr.

Keywords: *Tinospora sinensis Merr. extract, anti-arthritic, in vivo.*

1. INTRODUCTION

Tinospora sinensis (Merr.), belonging to the Menispermaceae family, is a perennial plant [1]. In Vietnam, *Tinospora sinensis* Merr. grows wild everywhere, in both mountainous and plain areas, and is abundant in the northwest mountainous region [1]. According to traditional medicine, *Tinospora sinensis* Merr. is used to treat back pain, knee weakness due to kidney deficiency, fever, low immunity, sciatica, injuries from falls or swollen feet due to excessive walking, as well as general health improvement [2].

Until now, there have been research studies worldwide on the effects of *Tinospora sinensis* Merr. Narendra Naik D and colleagues [4] demonstrated the hepatoprotective effect of the alcoholic root extract of *Tinospora sinensis* Merr. on the mouse liver injury model induced by carbon tetrachloride (CCl4). Another study by A. Srinivasa Rao [5] investigated

* *Corresponding author: 0987396956* *Email: nguyenducthanh@vmmu.edu.vn*

the diuretic effect of the extract of *Tinospora sinensis* Merr., which was found to be equivalent to the standard dose of Furosemide (20 mg/kg). Xiong Hui and colleagues [6] concluded that *Tinospora sinensis* Merr. significantly reduces swelling, pain sensation, and severity of joint diseases while also reducing the proliferation of synovial fluid and infiltration of inflammatory cells in collagen-induced arthritis in mice.

In Vietnam, there have been some studies on the therapeutic effects of medicinal plants for arthritis treatment, such as the study on the role of Tho Phuc Linh in herbal remedies for rheumatoid arthritis by Do Trung Dam and Doan Thanh Hien (1996), or the study on the anti-inflammatory effects of flavonoids extracted from the roots of Cao Cang plant conducted by Nguyen Thi Vinh Hue and colleagues (2007), etc. However, until now, there has been no experimental study in Vietnam on the effects of *Tinospora sinensis* Merr. on low-grade arthritis.

In this study, we conducted the extraction of *Tinospora sinensis* Merr. using ethanol with the assistance of ultrasound. Subsequently, we tested the acute toxicity and the acute anti-inflammatory effect on carrageenin-induced arthritis in rats and the chronic anti-inflammatory effect on experimental granuloma formation in mice.

2. MATERIALS AND METHODS

2.1. Subjects

The stems of the *Tinospora sinensis* Merr. were harvested in Lang Son from February to March 2023. After removing the non-utilizable parts, they were dried at 110°C for 10 minutes to eliminate microorganisms. Subsequently, the samples were further dried at a temperature of 60°C until the moisture content reached below 13%, according to Annex 9.6 of the Vietnamese Pharmacopoeia. The botanical name of the sample was identified by the Department of Botany and a voucher specimen was deposited at the Department of Chemistry, Military Medical Academy.

The powdered *Tinospora sinensis* Merr. was obtained by grinding the plant material into a coarse powder. A total of 100g of the powder was then subjected to ultrasonic extraction using 80% ethanol at a temperature of 50°C. After the extraction process, the solvent was distilled under reduced pressure, yielding 25.3 g of concentrated extract. This extract was subsequently dissolved in distilled water in a 1 : 1 ratio to prepare a concentrated liquid for further studies.

Wistar white rats (190 ± 20 g) and Swiss white mice (20 ± 2 g) of both sexes were used in this study. The animals were provided by the Laboratory Animal Breeding Department, Military Medical Academy, and were housed under stable laboratory conditions. They were fed a standard laboratory diet and had free access to water, which was boiled and allowed to cool before being provided to them.

Aspirin and methylprednisolone originated from the United States and meet the USP43 standard. Ethanol is sourced from Vietnam and meets the Vietnamese Pharmacopoeia

(DĐVN V) standard. Distilled water is produced at the Department of Chemistry - Vietnam Military Medical University and the Department of Pharmacy - Vietnam University of Traditional Medicine

2.2. Instruments, chemical and standard material

2.2.1. Oral toxicity test LD_{50}

In this study, a dose escalation of the extract was administered to mice to determine the LD_{50}, which is the dose that causes death in 50% of the experimental mice. LD_{50} was determined in white mice using the oral route according to the Litchfield-Wilcoxon method and the guidelines provided by the World Health Organization (WHO) [8]. The white mice used in the study were of the same breed, healthy, weighing between 18 - 22 g, and 5 - 6 weeks old. They were kept stable in a controlled environment and divided into 4 groups, with each group consisting of 6 - 7 mice:

Control group: drink distilled water

DC01 group: The mice were to drink the extract at the highest possible dose of 30 g/kg body weight.

DC02 group: The mice were to drink the extract at a dose of 20 g/kg body weight.

DC03 group: The mice were to drink the extract at a dose of 10 g/kg body weight.

Before administering the extract to the mice, they were subjected to a 16-hour fasting period with access to water as per their requirement. The extract was diluted with distilled water to achieve a volume of 0.5 mL/10g body weight for oral administration to the mice. The mice were kept under normal nourishment conditions and their behavioral activities, food and water intake, fur, and skin conditions were monitored. The mortality rate of the mice was recorded within 72 hours after administration. The highest dose that did not cause any mouse mortality (0%), the lowest dose that resulted in complete mouse mortality (100%), and intermediate doses were determined. Based on these results, a linear graph was constructed to determine the LD_{50} of the studied extract.

2.2.2. Evaluation of acute anti-inflammatory effects

Evaluation of acute anti-inflammatory effects according to Winter's method [7]. Sewer rats are randomly divided into 4 batches, each batch of 06 (n = 6). Sewer rats abstain from food 12 hours before taking the drug. Conduct acute inflammatory edema by injecting a mixture of 1% carrageenin solution (dispensing just before injection) dose 0.05 mL/animal into the subcutaneous tissue of the rat's feet. After 3 hours of carrageenin injection, give the rat the test product or distilled water with the same volume of 1.00 mL/100 g of body weight.

Group 1 (control group): drink distilled water.

Group 2 (comparison group): Oral aspirin 80 mg/kg body weight/day

Group 3 (dose 1 group): Oral *Tinospora sinensis* Merr. extract, dose 1, equivalent to 1.6 g dried herbs/kg body weight/day (equivalent dose to humans, extrapolation coefficient 6.47)

Group 4 (dose 2 group): Oral *Tinospora sinensis* Merr. extract, dose 2, equivalent to 3.2 g dried herbs /kg body weight/day (double dose for humans, extrapolation coefficient 6.47)

Measure the mouse foot (to the ankle joint) with the Ugo-Basile Plethysmometer inflammation measuring device at time points 1, 3, 5, and 7 hours after dosing. Compare the degree of inhibition of rat's paw edema between the groups at the same time and the same batch at the time of the study.

2.2.3. Evaluation of chronic anti-inflammatory effects

To evaluate the chronic anti-inflammatory effect, the research is conducted according to Ducrot's method [3]. White rats are randomly divided into 4 batches, each batch of 08 rats (n = 8). All batches are induced with chronic inflammation by inoculating sterile 6 mg asbestos beads (dried at 120°C, 1 hour) impregnated with 1% carrageenin in the dorsal skin of each mouse. After implantation of granulomas, the rats were given distilled water and test preparations continuously for 07 days with the same volume of 0.20 mL/10 g of body weight.

- Group 1 (control group): drinking distilled water;
- Group 2 (comparison group): Oral methylprednisolone 10 mg/kg body weight/day;
- Group 3 (dose 1 group): Oral *Tinospora sinensis* Merr. extract, dose 1, equivalent to 2.8 g of dried herbs/kg body weight/day (equivalent dose to humans, extrapolation coefficient 11.76);
- Group 4 (dose 2 group): Oral *Tinospora sinensis* Merr. extract, dose 2, equivalent to 5.6 g of dried herbs/kg body weight/day (double dose for humans, extrapolation coefficient 11.76).

On the 8th day, rats were sacrificed, tumors were removed and fresh weight is weighed. 3 tumors were selected randomly from each batch for microscopic histopathology and judged by the following criteria:

- Average weight of inflammatory foci;
- Percentage of inhibition of granuloma formation compared with the control group;
- Pathological images were examined under the ZEISS Primostar optical microscope.

3. RESULT AND DISCUSSIONS

3.1. Result of acute toxicity test (LD_{50}) by oral route

According to the experiment, when the mice were administered doses of 10 g/kg, 20 g/kg, and 30 g/kg of the extract, none of the mice died. Therefore, no mortality was observed in the mice even at the maximum allowable dose of 30 g/kg. As a result, the LD_{50} of the extract could not be determined through oral administration. This also showed that oral decompression of bone pain cords was not toxic to rats.

3.2. Evaluation results of acute anti-inflammatory effects

Experimental layout process as in section 2.2.3, the results obtained are as presented in Table 1.

Table 1. *Average increase rate of mouse paws (%) at time points after inflammation*

Group	Average increase rate of mouse paws (%) at time points after inflammation				p
	After 01 hour (a)	***After 03 hours (b)***	***After 05 hours (c)***	***After 07 hours (d)***	
Group 1 (control group)	40.62 ± 12.99	91.22 ± 14.90	77.25 ± 12.16	62.35 ± 12.64	$P_{b.c.d-a} < 0.05$ $P_{c-b} > 0.05$ $P_{d-b} < 0.05$ $P_{d-c} < 0.05$
Group 2 (comparison group)	32.03 ± 5.35	56.62 ± 10.3	53.73 ± 5.78	39.87 ± 9.94	$P_{b.c.d-a} < 0.05$ $P_{c-b} > 0.05$ $P_{d-b} < 0.05$ $P_{d-c} < 0.05$
Group 3 (dose 1 group)	40.87 ± 13.90	72.87 ± 14.15	69.50 ± 13.78	58.48 ± 3.24	$P_{b.c.d-a} < 0.05$ $P_{c-b} > 0.05$ $P_{d-b} < 0.05$ $P_{d-c} < 0.05$
Group 4 (dose 2 group)	31.27 ± 14.10	59.98 ± 6.95	54.96 ± 12.44	43.97 ± 8.63	$P_{b.c.d-a} < 0.05$ $P_{c-b} > 0.05$ $P_{d-b} < 0.05$ $P_{d-c} < 0.05$
p	$P_{2.3.4-1} > 0.05$ $P_{4-1} < 0.05$ $P_{4.3-2} > 0.05$ $P_{4-3} > 0.05$	$P_{2.3.4-1} < 0.05$ $P_{3-2} < 0.05$ $P_{4-2} > 0.05$ $P_{4-3} > 0.05$	$P_{2.4-1} < 0.05$ $P_{3-1} > 0.05$ $P_{3-2} < 0.05$ $P_{4-2} > 0.05$ $P_{4-3} > 0.05$	$P_{2.4-1} < 0.05$ $P_{3-1} > 0.05$ $P_{3-2} < 0.05$ $P_{4-2} > 0.05$ $P_{4-3} < 0.05$	

After carrageenin injection, all mice showed clear paw edema. Compared to the control group at the same time points, the percentage increase in mouse paw volume for both the high-dose *Tinospora sinensis* Merr. group and the aspirin group tended to decrease, with the most significant reduction observed in all drug-treated groups at the 3-hour time point (statistically significant with $p < 0.05$). At the 5-hour and 7-hour time points after carrageenin injection, the percentage increase in paw volume tended to decrease in all four groups, with the most significant reduction observed in the Treatment 2 group and the control

group, showing statistically significant differences compared to the control group ($p < 0.05$). Compared to the reference group treated with aspirin at a dose of 80 mg/kg body weight/day at the same time point, the percentage increase in mouse paw volume in the Treatment 2 group was equivalent and did not show statistically significant differences ($p > 0.05$). Meanwhile, in the Treatment 1 group, the anti-edema effect was most pronounced and equivalent to the reference group at the 1-hour time point after carrageenin injection (1% increase). At the same time point, both doses of *Tinospora sinensis* Merr. used in the study, 1.6 g of herbal/kg body weight/day and 3.2 g of herbal/kg body weight/day, showed significant reduction in paw edema, and the differences were not statistically significant with $p > 0.05$ at the 1-hour, 3-hour, and 5-hour time points after carrageenin injection.

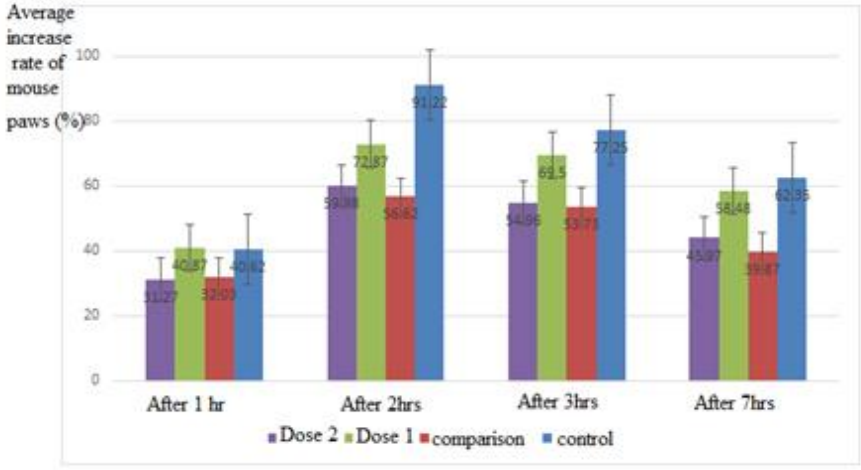


Figure 1. *Average percentage increase in mouse paw volume at the following time points caused inflammation*

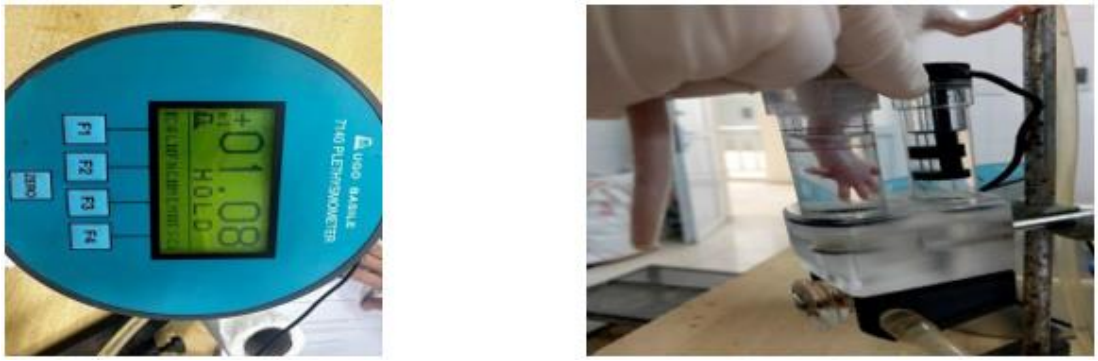


Figure 2. *White rat foot inflammation measurement*

Therefore, the high dose of *Tinospora sinensis* Merr. at the equivalent dose of 1.6 g herbs/kg body weight/day has an inhibitory effect on acute inflammation in the mouse paw edema model induced by 1% carrageenan at the 3-hour time point, which is comparable to the effect of aspirin at a dose of 80 mg/kg body weight/day.

3.3. Results of evaluation of chronic anti-inflammatory effects

3.3.1. Results of inhibiting granuloma formation

The three groups using the reference drugs and the two doses of *Tinospora sinensis* Merr. all showed a significant reduction in tumor weight compared to the pathological control group, with a statistically significant difference of $p < 0.05$. *Tinospora sinensis* Merr. extract at the doses of 2.8 g herbs/kg body weight/day and 5.6 g of herbs/kg body weight/day in white mice exhibited anti-inflammatory effects with inhibitory rates of tumor formation compared to the pathological control group of 28.10% ($p < 0.05$) and 30.05% ($p < 0.05$), respectively, and both had statistical significance. When comparing the two doses, there was no statistically significant difference ($p > 0.05$). It can be observed that the inhibitory effect on the inflammatory lesion is not dependent on the dosage of *Tinospora sinensis* Merr. extract in white mice at the two test doses. The reference group using methylprednisolone 10 mg/kg body weight/day showed a tendency to have a more pronounced inhibitory effect on tumor formation, but the difference compared to the two groups using *Tinospora sinensis* Merr. extract was not statistically significant with $p > 0.05$.

Table 2. *Results of inhibiting granuloma formation*

Group	***Mean weight of inflammation (mg)***	***% reduction compared to the group of diseases***
Group 1 (control group)	96.80 ± 4.93	
Group 2 (comparison group)	63.00 ± 5.82	34.92
Group 3 (dose 1 group)	69.60 ± 6.60	28.10
Group 4 (dose 2 group)	67.71 ± 5.76	30.05
P	$P_{2,3,4-1} < 0.05$ $P_{4,3-2} > 0.05$ $P_{4-3} > 0.05$	

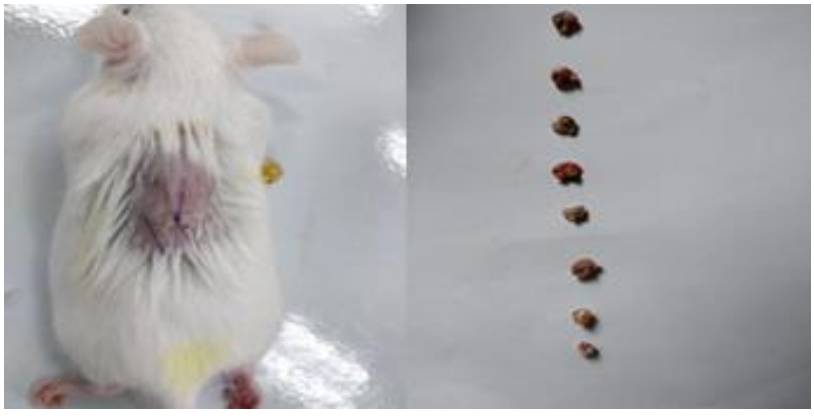
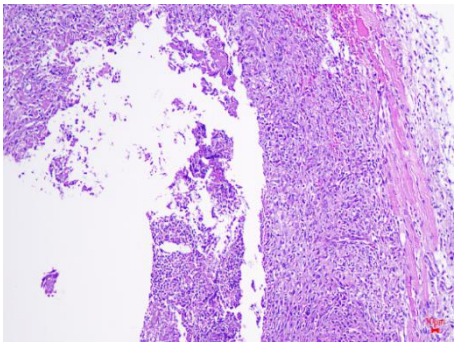
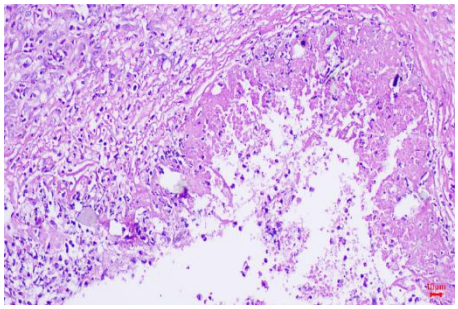
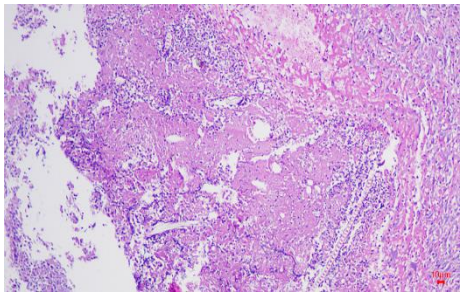


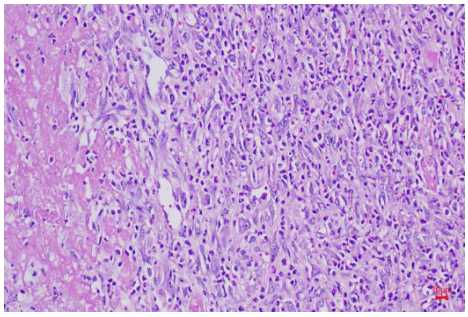
Figure 3. *Images of mice with granulomas and granulomas after dissection*

3.3.2. Results of the histopathological assessment of fibrous tissue

The results of the dissection and histopathological assessment of fibrous tissue in the studied rats are as Table 4 follows:

Table 4. *Results of the histopathological assessment of fibrous tissue*

Group	Image	Comment
Group 1 (control group)		In the subcutaneous tissue, there is a central inflammatory lesion characterized by necrotic tissue containing numerous neutrophils and scattered unidentified foreign bodies. Surrounding the necrotic tissue, there are inflammatory cells such as lymphocytes, macrophages, neutrophils, and proliferating fibroblasts. The infiltrated inflammatory cells within the muscle fibers cause degeneration and atrophy of the muscle cells. The connective tissue in the vicinity also exhibits degeneration and atrophy.
Group 2 (compari-son group)		In muscle tissue, there is a central focus of inflammation, which is a necrotic organization with the presence of N white blood cells and amorphous foreign bodies. Surrounding the necrotic tissue are inflammatory lymphocytes, macrophages, N-leukocytes, and mildly proliferative fibroblasts. Inflammatory cells invade the muscle fibers causing the muscle cells to degenerate and shrink. Skeletal muscle tissue shrinks and degenerates.
Group 3 (dose 1 group)		In muscle tissue, there is a central inflammatory focus, which is the presence of neutrophils and amorphous foreign bodies. Histiocytosis is composed of lymphoid inflammatory cells, macrophages, N white blood cells, and mild fibroblast proliferation. Inflammatory cells invade the muscle fibers causing the cells to degenerate and shrink. Skeletal muscle tissue shrinks and degenerates.

Group	Image	Comment
Group 4 (dose 2 group)		In muscle tissue, there is a central focus of inflammation, which is a necrotic organization with the presence of N white blood cells and amorphous foreign bodies. Surrounding the necrotic tissue are inflammatory lymphocytes, macrophages, N-leukocytes, and mildly proliferative fibroblasts. Inflammatory cells invade the muscle fibers causing the muscle cells to degenerate and shrink. Skeletal muscle tissue shrinks and degenerates.

The experimental results showed that Methylprednisolone 10 mg/kg body weight/day and the two samples of *Tinospora sinensis* Merr. extracts at equivalent doses of 2.8 g herbs/kg body weight/day and 5.6 g herbs/kg body weight/day all significantly reduced the weight of the experimental tumors compared to the pathological control group, with statistical significance ($p < 0.05$). There was no statistically significant difference when comparing the effects between the two study doses ($p > 0.05$). The inhibitory effect on tumor formation of Methylprednisolone at a dose of 10mg/kg body weight/day tended to be more pronounced, but compared to the two groups of mice treated with *Tinospora sinensis* Merr. extract, there was no statistically significant difference.

Histopathological images of the tumor specimens showed a central inflammatory focus with necrotic tissue containing neutrophils and amorphous foreign bodies. Surrounding the necrotic tissue were lymphocytic infiltrates, macrophages, neutrophils, and mild fibroblastic proliferation. The infiltrating inflammatory cells within the muscle fibers caused degeneration and atrophy of the muscle cells and fibrous connective tissue at various degrees.

Therefore, *Tinospora sinensis* Merr. extract at a dose equivalent to 2.8 g herbs/kg body weight/day had an inhibitory effect on chronic inflammation in the experimental model of amian-induced tumors in Swiss mice.

4. CONCLUSION

This study extracted the herbal extract from *Tinospora sinensis* Merr. extract harvested in Lang Son, but the acute toxicity LD_{50} of the *Tinospora sinensis* Merr. extract extract in ethanol 800 was not determined. Even at the maximum allowable dose, no mice died. Regarding the anti-inflammatory effect of the *Tinospora sinensis* Merr. extract in the experimental model, the results showed that the high dose of *Tinospora sinensis* Merr. extract at the equivalent dose of 1.6 g herbs/kg body weight/day had an inhibitory effect on

acute inflammation in the mouse paw edema model induced by 1% carrageenan at the 3-hour time point, which was comparable to the effect of aspirin at a dose of 80 mg/kg body weight/day. Additionally, the dose equivalent to 2.8 g herbs/kg body weight/day showed anti-inflammatory effects in the chronic inflammation model induced by amian in Swiss mice.

REFERENCES

[1]. D. T. Loi, "Vietnamese Medicinal Plants And Medicinal Herbs," *Medical Publishing House*, pp 492-493, 2004 (in Vietnamese).

[2]. V. V. Chi, "Common botanical dictionary," *Science and Technics Publishing House*, 2003 (in Vietnamese).

[3]. R. Ducrot, L. Julon, et al., "Tumor screening methods in pharmacology," *Academic Press*, pp. 114-115, 1965.

[4]. D. N. Naik, S. S. Fathima, K. Durga, K. Ashwani, A. Elumalai, and R. Malothu, "Evaluation of hepato protective activity of ethanolic root extract of tinospora sinensis," *International Journal of Biological & Pharmaceutical Research*, vol. 4, no. 12, pp. 1065-1069, 2013.

[5]. Srinivasa Rao, "Evaluation of diuretic activity of aqueous and methanol extracts of tinospora sinensis in rats," *ActaBiomedica Scientia*, vol. 1, no. 2, pp. 58-60, 2014.

[6]. H. Xiong, X. Ding, H. Wang, H. Jiang, X. Wu, C. Tu, C. Wu, Y. Pi, G. Yang, Z. Zhao, and Z. Mei, "Tibetan medicine Kuan-Jin-Teng exerts anti-arthritic effects on collagen-induced arthritis rats via inhibition the production of pro-inflammatory cytokines and down-regulation of MAPK signaling pathway," *Phytomedicine*, vol. 57, pp 271-281, 2019.

[7]. C. A. Winter, E. A. Risley, and G. W. Nuss, "Carrageenin induced edema in hind paw of the rat as an assay for anti inflammatory drug," Proceeding of the Society for the Experimental Biology and Medicine (New York, N.Y.), vol. 111, pp 544-574, 1962.

[8]. World Health Organization, "Working group on the safety and efficacy of herbal medicine," *Report of Regional Office for the Western Pacific of the World Health Organization*, pp 33 -51, 1993.

Nghiên cứu tác dụng chống viêm khớp của dây đau xương (*Tinospora Sinensis* Merr.)

Nguyễn Đức Thanh[1], Vy Quốc Tuấn[1], Nguyễn Đăng Long Vũ[1], Nguyễn Quang Dũng[1], Phạm Văn Toản[1], Nguyễn Công Cương[1], Nguyễn Thị Vân Anh[2]

[1]Học viện Quân Y, Hà Nội, Việt Nam

[2]Học viện Y Dược học cổ truyền Việt Nam, Hà Nội, Việt Nam

Tóm tắt

Dây đau xương được chiết siêu âm bằng ethanol 80% ở nhiệt độ 50°C, dịch thu được cất loại dung môi dưới áp suất giảm thu được cắn. Cắn được hòa tan bằng nước cất thu được dịch chiết rồi tiến hành cho các lô chuột uống dịch chiết. Kết quả cho thấy khi dùng liều tối đa cho phép vẫn không xuất hiện chuột bị chết. Thử tác dụng chống viêm của cao dây đau xương trên thực nghiệm, kết quả cho thấy cao dây đau xương ở mức liều tương đương 1,6g dược liệu/kg thể trọng/ngày có tác dụng ức chế viêm cấp tính trên mô hình gây phù bàn chân chuột cống bằng carragenin 1% ở thời điểm 3 giờ tương đương với aspirin mức liều 80 mg/kg thể trọng/ngày và ở mức liều tương đương 2,8 g dược liệu/kg thể trọng/ngày có tác dụng chống viêm mạn tính trên mô hình gây u hạt thực nghiệm bằng amian ở chuột nhắt trắng. Đây là một hướng mới trong việc sử dụng thuốc có nguồn gốc thực vật nói chung và dây đau xương nói riêng.

Từ khóa: *Dây đau xương, viêm khớp, in vivo.*