Trop J Nat Prod Res, November 2023; 7(11):5178-5182
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

The *In Vivo* Anti-Inflammatory Effects of Qt-2 (A Traditional Medicine Remedy) Water Extract

Tuan T. Vo[1], Tuan A. Phan[2,4], Thuy T. Lam[3], Nguyet T. Tran[5], Hoang M. Le[2]*

[1]*Faculty of Traditional Medicine, University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, Vietnam*
[2]*Department of Traditional Medicine, Can Tho University of Medicine and Pharmacy, Can Tho, Vietnam*
[3]*Kien Giang Traditional Medicine and Pharmacy Hospital, Kien Giang, Vietnam*
[4]*Ha Noi college of Pharmacy, Ha Noi, Vietnam*
[5]*Faculty of Public Health, Can Tho University of Medicine and Pharmacy, Can Tho, Vietnam*

ARTICLE INFO

Article history:
Received 13 July 2023
Revised 01 November 2023
Accepted 21 November 2023
Published online 01 December 2023

ABSTRACT

This study investigated the *in vivo* anti-inflammatory effect of QT-2, a traditional medicinal remedy renowned for its potential in treating arthritis. We assessed its acute and chronic anti-inflammatory properties at two different doses (11.8 g/kg/day and 23.6 g/kg/day) using three *in vivo* models. The results revealed significant anti-inflammatory effects in the first carrageenan-induced paw edema model. QT-2 extract demonstrated a discernible reduction in paw edema volume, statistically comparable to the diclofenac 15 mg/kg treated group. In the second peritonitis model, both doses of QT-2 extract exhibited pronounced anti-inflammatory potential, leading to significant reductions in transudate volume, protein concentration within the transudate, and lowered optical density, comparable to the diclofenac 15 mg/kg treated group. In the third anti-granuloma model, both QT-2 extract doses caused marked reductions in the weight of dried and fresh granulomas, equivalent to the Prednisolone 15 mg/kg treated group. These results demonstrate the anti-inflammatory capability of QT-2 for inflammatory-related conditions.

Keywords: QT-2 extract; anti-inflammatory effects; herbal remedy. .

Introduction

Inflammatory arthritis refers to a series of arthritic symptoms that include joint pain, swelling, warmth, tenderness, and morning stiffness lasting for at least an hour.[1] The inflammatory response within the affected joint involves an increased number of cells and the presence of inflammatory substances, which can lead to joint irritation, cartilage degradation, and synovial swelling. Among the various types of inflammatory arthritis, rheumatoid arthritis (RA) is the most prevalent.[2] According to a systematic review, the global prevalence of RA between 1980 and 2019 was estimated at 460 cases per 100,000 population.[3] The World Health Organization reported that in 2019, approximately 18 million people worldwide were living with rheumatoid arthritis.[4] While RA is a systemic autoimmune disease that affects multiple organ systems, it primarily affects the joints of the hands, wrists, feet, ankles, knees, shoulders, and elbows. Despite recent therapeutic advancements, the etiology of RA remains poorly understood, and a definitive cure has yet to be discovered. It is recognized that RA is a complex disease influenced by both genetic and environmental factors, contributing to its variable prevalence across different populations and within countries.[5]

According to Traditional Medicine theory, rheumatoid arthritis is classified as an impediment disease or arthralgia syndrome within the context of inflammation and pain. Due to the invasion of external pathogenic forces like wind, cold, and dampness, this condition is characterized by the obstruction of qi and blood in the meridians. The typical manifestations include soreness, pain, numbness, a heavy sensation, joint and limb swelling, and restricted movements. Pain arises from the compromised functioning of the body's systems, leading to stagnation of blood and fluid, thereby causing discomfort and pain.[6,7] Numerous studies have demonstrated the promising efficacy of traditional herbal decoctions in alleviating symptoms and slowing down the progression of rheumatoid arthritis.[8–10] However, long-term oral administration of these decoctions may be associated with certain side effects. Therefore, it is important to strike a balance between the potential benefits and risks of prolonged herbal usage.[11] Further research and development are needed to explore safer and more effective treatment approaches for rheumatoid arthritis, aiming to maximize therapeutic outcomes while minimizing adverse effects.

There is a long history of traditional medicine in Vietnam, and traditional herbal medicine has been widely used alongside conventional treatment.[12–14] QT-2 decoction containing 10 herbs is a commonly prescribed remedy in Vietnamese traditional medicine. It is used as a remedy for a variety of musculoskeletal conditions. It is frequently prescribed in routine clinical practice as a standard therapy for patients with rheumatoid arthritis or osteoarthritis due to its notable anti-inflammatory and analgesic properties. However, despite its widespread use in clinical settings and the efficacy of QT-2 in alleviating these conditions, there has been a paucity of reports of its bioactivity.[15] The objective of this research was to investigate the anti-inflammatory effects of QT-2 using mouse and rat models. In this study, we aimed to provide scientific evidence supporting the potential benefits of QT-2 in reducing inflammation and pain. Furthermore, this study sought to contribute to the advancement of traditional medicine by establishing a theoretical foundation for the future development of herbal products based on the QT-2 formulation.

*Corresponding author. E mail: lmhoang@ctump.edu.vn
Tel: +84-973431666

Citation: Vo TT, Phan TA, Lam TT, Tran NT, Le HM. The *In Vivo* Anti-Inflammatory Effects of Qt-2 (A Traditional Medicine Remedy) Water Extract. Trop J Nat Prod Res. 2023; 7(11):5178-5182. http://www.doi.org/10.26538/tjnpr/v7i11.21

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Materials and Methods

Formulation of QT-2

QT-2 comprises dried herbs, including *Cinnamomum casia, Notopterygium incisum, Spina gleditschea, Angeliae sinensis, Astragalus membranaceus, Rhizoma curcumae longae, Ledebouriellae sesloides, Glycyrrhizae glaba, Paeonia liacliflora, Zizyphus jujubae* combined in a ratio of 9:8:12:12:8:8:4:12:10:8, respectively. The daily required dose of the mixture is 91 grams to be ingested. This remedy was first formulated by 'Phu Tin' in Vietnam, following the standards outlined in the Vietnamese Pharmacopoeia (fifth edition). For dosing in humans, a standardized body weight of 50 kg was employed, resulting in a recommended dose of 1.82 g per kilogram. To determine the suitable doses for rats, a conversion factor of approximately 6.47, based on the assumption of human-to-rat conversion, was applied. Consequently, the calculated adequate quantity for rats was 11.8 g/kg body weight. This dose was administered orally to the experimental animals using an oro-gastric needle.

Animal experiments

The study protocol was approved by Can Tho University of Medicine and Pharmacy's Medical Science Council, Vietnam (approval code 721/QD-DHYDCT). Rats (160-180 g) were procured from Military Medical University's Laboratory Animal Breeding Board, Vietnam. Rats were acclimatized for a week and were provided standard diets and water *ad libitum*.[16] Daily monitoring ensured their well-being with systematic data collection. They were randomly divided into four groups (n=10) for anti-inflammatory experiments using three models (n=40). Groups included controls, diclofenac sodium (15 mg/kg), and two QT-2 extract treatment groups (11.8 g/kg and 23.6 g/kg). In the second experiment, the diclofenac sodium rats received a dose of 10 mg/kg. In the third experiment, Prednisolone (15 mg/kg) was used. Drugs were administered orally for five days, and control rats received distilled water equivalent to the drug volume.

Carrageenan-Induced Edema model

The induction of hind paw edema using carrageenan is a widely employed animal model to assess the anti-inflammatory potential of pharmacological substances, as demonstrated in a previous study.[17] Forty rats were used for this experiment. The rats were administered either the extract, Diclofenac sodium (15 mg/kg), or distilled water for five consecutive days before the induction of inflammation. On the fifth day, one hour after the administration of the extract, Diclofenac sodium (15 mg/kg) or distilled water, inflammation was induced by injecting 0.1 mL of a 1% carrageenan solution (dissolved in physiological saline) into the pad of the right hind paw of each rat. The rats were allowed unrestricted access to water and fasted overnight. The volume of the paw, up to the ankle joint, was measured at specific time intervals using a Plethysmometer: before inducing inflammation (V0), 2 hours after inducing inflammation (V2), 4 hours after inducing inflammation (V4), 6 hours after inducing inflammation (V6), and 24 hours after inducing inflammation (V24).

Peritonitis model

The anti-inflammatory effects were assessed using the peritonitis model, as described in a previous study.[18] The rats were administered either the extract, Diclofenac sodium (10 mg/kg), or distilled water for five consecutive days before inducing peritonitis. On the fifth day, one hour after administering the extract, Diclofenac sodium (10 mg/kg), acute peritonitis was induced by injecting a solution consisting of carrageenan (0.05 g) and formaldehyde (1.4 mL) diluted in 100 mL of physiological saline. The injection volume was 1 mL per 100 g of rat body weight, delivered into the peritoneal cavity. Following 24 hours of peritonitis induction, the rats were euthanized, and the peritoneal fluid was collected. The volume of the peritoneal fluid was measured, and the number of white blood cells (leukocytes) per milliliter, as well as the protein content in the peritoneal fluid, were quantified.

Anti-granuloma model

The procedure was according to the method described in a previous study.[19] Thirty minutes after pretreatment with distilled water, prednisolone (15 mg/kg), or QT-2 extract (11.8 g/kg and 23.6 g/kg), the rats were lightly anesthetized with Calypsol and sterile cotton pellet (30 ± 0.1 mg) were implanted subcutaneously on both sides of their backs.[19] The rats continued to receive the respective treatments for an additional 6 days. On the 7th day, the rats were euthanized, and the granulomas surrounding the cotton pellets were dissected and separated. The granulomas were accurately weighed using an analytical balance to the nearest 10^{-4} g. The sterile cotton pellets were subsequently dried to a constant weight and then reweighed. The actual weight of the granulomas was determined by subtracting the weight of the cotton pellet (in mg/100 g body weight of the rat). The average weights of both fresh and dried granulomas among the rat groups were used to assess the inhibitory effect on chronic inflammation. Additionally, three granuloma rats were randomly selected from each group for microscopic pathology examination. After collecting and fixing the granuloma tissues, they were dehydrated and embedded in melted paraffin wax. The granuloma slices were then prepared, and hematoxylin-eosin staining was applied. Finally, histopathological examination was conducted under a x400 microscope.

Statistical analysis

The data are presented as mean values plus standard deviations (mean ± SD). Statistical tests were performed using SPSS 22.0 using the ANOVA test, and the significance level was set at $p < 0.05$.

Results and Discussion

Anti-inflammatory activity in carrageenan-induced paw edema rats model

In the carrageenan-induced paw edema rat model, carrageenan, a polysaccharide structurally similar to bacterial components, elicits a non-specific immune response mainly involving macrophages and neutrophils. This immune response is characterized by vasodilation, leukocyte infiltration, and increased production of autacoids such as nitric oxide, histamine, serotonin, kinins, and prostaglandins, which act as neurotransmitters or neuromodulators.[20] The intraplantar injection of 0.1 mL of 1% carrageenan into the rat hind paw increased paw thickness, particularly exhibiting significant paw edema at 4 hours post-inflammation, which gradually decreased by 6 hours (Table 1). The QT-2 extract groups and diclofenac group exhibited a significant reduction in percentage increase of paw volume compared to the control group ($p < 0.01$) at 2, 4, and 6 hours post-injection. At 24 hours, there was no significant difference in paw edema between the test and control groups, indicating the waning of the inflammatory process induced by carrageenan. Although the QT-2 extract groups showed a dose-dependent reduction in paw edema, the differences were not statistically significant ($p > 0.05$) at all time intervals. Moreover, the increase in rat paw volume in the QT-2 groups did not significantly differ from the diclofenac at 15 mg/kg ($p > 0.05$). The results demonstrate that the extract at a dose of 23.6 g/kg/day exhibits a good anti-inflammatory effect equivalent to Diclofenac at a dose of 15 mg/kg/day.

Anti-inflammatory effect on peritonitis model.

The peritonitis model is another model used to evaluate the acute anti-inflammatory effects, focusing on assessing the inhibition of increased vascular permeability (resulting in increased exudate production) and leukocyte migration to the inflammatory site. Also, in acute antigen-induced inflammation, polysaccharides participate in the immune response mediated by B lymphocytes. Antigens that are not thymus-dependent, such as polysaccharides, are recognized by B lymphocytes, which produce specific antibodies without the assistance of T lymphocytes. Table 2 shows that both doses of QT-2 extract (11.8 g/kg/day and 23.6 g/kg/day) significantly reduced the volume of inflammatory exudate in the peritoneal cavity, with statistical significance ($p<0.01$). The effect of QT-2 extract at a dose of 23.6 g/kg/day was equivalent to that of Diclofenac sodium at a dose of 15 mg/kg ($p>0.05$). Compared to the control group, both the QT-2 extract group at a dose of 23.6 g/kg/day and the Diclofenac 15 mg/kg group showed a noticeable reduction in the number of leukocytes and protein content in the inflammatory exudate. The group receiving QT-2 at a dose of 11.8 g/kg/day also exhibited a reduction in leukocyte count

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

and protein content in the inflammatory exudate. Therefore, on the model of peritonitis, the QT-2 group demonstrated a significant inhibitory effect on leukocyte number in the inflammatory exudate. This effect may be attributed to the ability to suppress the inflammatory process by reducing microvascular permeability, thereby decreasing the volume of inflammatory exudate and diminishing leukocyte transmigration.

Anti-granuloma activity effect

Table 3 presents the effect on the reduction of fresh and dried granuloma weight. Both prednisolone and QT-2, at two different dose levels, significantly reduced the volume of granulomas compared to the control group (p<0.01). The average mass of granulomas in the QT-2 group with a 23.6 g/kg dose exhibited a greater reduction compared to the 11.8 g/kg group, though this was not statistically significant. Furthermore, there was no significant difference in the reduction of granuloma volume between the QT-2 groups and the prednisolone group (p > 0.05). These findings demonstrate that QT-2 exhibits anti-granuloma activity at both dose levels, similar to the effects observed with prednisolone.

Figure 1 shows the histopathological changes in granulomatosis. The control group exhibited a high presence of degenerating polymorphonuclear leukocytes, with few lymphocytes and fibroblasts. In contrast, the prednisolone group and both QT-2 groups showed a decrease in polymorphonuclear leukocytes and degenerated cells, along with a reduction in lymphocytes and fibroblasts.

Prednisolone, a corticosteroid anti-inflammatory drug, exerts its therapeutic effects primarily by suppressing immune responses mediated by T lymphocytes.[21] In the experimental model of chronic inflammation, prednisolone is widely used for its potent anti-inflammatory properties. Both prednisolone and QT-2 at two different dose levels demonstrated significant reductions in granuloma weight compared to the control group (p<0.01). These results showed the possible anti-inflammatory effect of QT-2 decoction. QT-2's anti-inflammatory properties are attributed to the synergistic action of its herbal ingredients, among which *Cinnamomi ramulus, Spina gleditschea,* and *Glycyrrhizae glaba* are considered particularly important.[22–25] *Cinnamomi ramulus* essential oil demonstrated notable anti-inflammatory properties, as evidenced by its ability to impede the expression of key pro-inflammatory mediators such as TNF-α, IL-1β, NO, PGE2, COX-2, and inducible nitric oxide synthase (iNOS) in rats with carrageenan-induced paw edema. The essential oil's active compound, cinnamaldehyde, further exhibited anti-inflammatory effects by down-regulating pro-inflammatory cytokines through mitigating the release of ROS (reactive oxygen species) and inhibiting the activation of JNK1/2 and ERE1/2 signaling pathways in LPS-stimulated J774A.1 macrophages.[23,26] Extracts from *Spina gleditschea* inhibited cell proliferation, MMP-9 expression, and the production of prostaglandin E2 (PGE2) in cultured vascular smooth muscle cells and decreased cycloxygenase-2 (COX-2) expression.[24,27] One of the ingredients in QT-2 is *Glycyrrhizae glaba*, known for its anti-inflammatory effects in natural medicine. This anti-inflammatory effect has been attributed to its active components, including glycyrrhetinate salts, glycyrrhetinic acid, flavonoids, and liquiritin. *Glycyrrhizae glaba*'s ability to modulate inflammation further contributes to the overall therapeutic potential of QT-2. *Glycyrrhizae glaba* exhibits corticoid-like anti-inflammatory effects at various levels, possibly due to the presence of some anti-inflammatory principles (glycyrrhetinate salts, glycyrrhetinic acid, flavonoids, and liquiritin). Isoliquiritigenin (ILG) and liquiritigenin (LG) are the main bio-constituents of *Glycyrrhizae glaba*. By preventing NO, iNOS, and NF-kB/IkBa activation in RAW 264.7 macrophages, the two substances had anti-inflammatory effects.[28] In mice exposed to tert-butyl hydrogen peroxide (t-BHP), the hepatoprotective effects of LG dramatically decreased the raised serum levels of ALT, GGT, and AST and lowered the expression of TNF-a, IL-1b, and IL-6 mRNA.

Table 1: Effect of QT-2 extract on the rat paw volume

Group	Increase in paw edema (percentage) (Mean ± SD)			
	After 2h	After 4h	After 6h	After 24h
Control	72.13 ± 19.2	73.06 ± 19	63.96 ± 17.9	8.21 ± 4.5
Diclofenac 15 mg/kg	41.73 ± 7.90**	42.97 ± 7.67**	35.45 ± 9.7**	4.96 ± 3.03
QT-2 dose 11.8 g/kg	41.90 ± 9.1**	43.27 ± 9.2**	37.44 ± 14.9**	4.83 ± 3.43
QT-2 dose 23.6 g/kg	40.70 ± 8.8**	41.23 ± 8.9**	35.33 ± 9.7**	5.08 ± 2.77

*Compare with control group at the same time, *p<0.05, **p<0.01, n = 10 per group

Table 2: QT-2 extract effects on inflammatory exudate in peritonitis model

Group	Indicators in inflammatory exudate (Mean ± SD)		
	Volume of inflammatory exudate (ml)	Protein content (mg/dl)	WBC count (G/l)
Control	2.96 ± 0.8	3.79 ± 0.37	10.73 ± 3.1
Diclofenac 15 mg/kg	2.07 ± 0.5**	3.28 ± 0.3**	9.22 ± 2.3
QT-2 dose 11.8 g/kg	2.11 ± 0.5**#	3.28 ± 0.4**#	9.86 ± 2.8
QT-2 dose 23.6 g/kg	1.99 ± 0.6**#	3.18 ± 0.4**#	9.59 ± 2.2

**Compared with the control group, *p < 0.05, **p < 0.01;
Compared with the model group, #p < 0.05, ##p < 0.01. n = 10

Table 3: Effect of QT-2 extract on the weight of granulomas

Group	Average weight (Mean ± SD)	
	Average weight of fresh granulomas (mg/100g)	Average weight of dried granulomas (mg/100g)
Control	91.43 ± 4.49	28.58 ± 4.52
Prednisolone 15 mg/kg	65.50 ± 4.22**	21.10 ± 3.76**
QT-2 dose 11.8 g/kg	67.94 ± 5.19**	22.02 ± 2.66**
QT-2 dose 23.6 g/kg	65.56 ± 3.52**	21.07 ± 3.15**

*Compared with the control group, *p < 0.05, **p < 0.01; n = 10 per group

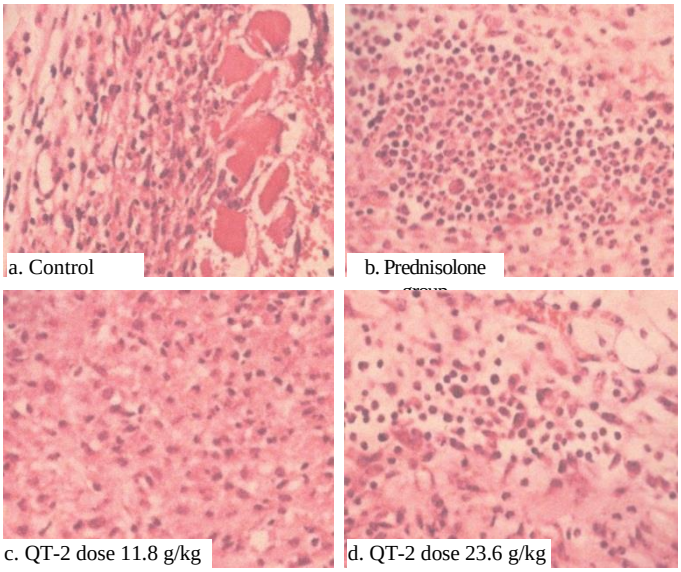

Figure 1: Chronic inflammation and histopathology of granulomatosis in rats

The anti-inflammatory and reduced expression of the brain-derived neurotrophic factor/tropomyosin receptor kinase B pathway were linked to the antidepressant and antianxiety properties of LG.[29]
The result of this study presents certain limitations. Firstly, the doses administered were determined based on the initial dry weight of the herbal material, quantified in grams. Specifically, 91 g of herbal material, typically intended for an individual weighing around 50 kg, was used, resulting in a calculated dose of 1.82 g/kg/day. To extrapolate this dose for rats (employing a conversion factor of 11.76), the original dose was multiplied, yielding an approximate dose of 21.4 g/kg/day. During the process of water extraction, 445 g of herbal material yielded a liquid extract at a 3:1 ratio, culminating in a final volume of 75 ml. Consequently, the 91 g of herbal material equates to an approximate volume of 30.3 ml. It is noteworthy that the conversion factor employed to estimate the effective dose for rodents constitutes an approximation and may not precisely reflect the true conversion factor. Inflammation, as the immune system's response to various harmful stimuli, holds a complex mechanism and is triggered by diverse factors, encompassing pathogens, cellular damage, and toxins. Such factors may induce acute and/or chronic inflammatory responses in multiple organs, potentially leading to tissue damage or diseases.[30] The assessment of inflammatory activity utilized induced granuloma, a widely accepted method for evaluating the anti-inflammatory effects of substances. While this approach provides valuable insights, it is imperative to recognize the intricate nature of the inflammatory process. Consequently, differences between murine and human inflammatory responses might impede a direct translation of results obtained from animal models to humans.

Conclusion

QT-2 extracts at the doses administered demonstrated significant anti-inflammatory effects in the three models used. There was a reduction in paw edema volume, which was statistically significant in the carrageenan-induced paw edema model. Both doses of QT-2 extract also exhibited potent acute anti-inflammatory activity in the peritonitis model in rats, accompanied by significant inhibition of transudate volume, protein concentration in the transudate, and reduced optical density. The QT-2 extracts also significantly inhibit chronic inflammation in the experimental animals, evidenced by a reduction in the weight of dried and fresh granulomas in the anti-granuloma model. This study validates the use of QT-2 extract in the management of inflammatory conditions in Vietnamese traditional medicine.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors would like to thank Can Tho University of Medicine and Pharmacy, which supported this study in part

References

1. Poudel P, Goyal A, Lappin SL. Inflammatory Arthritis. Treasure Island (FL): StatPearls; 2023.
2. Pisetsky DS, Ward MM: Advances in the treatment of inflammatory arthritis. Best Pract Res Clin Rheumatol 2012; 26:251–61.
3. Almutairi K, Nossent J, Preen D, Keen H, Inderjeeth C: The global prevalence of rheumatoid arthritis: a meta-analysis based on a systematic review. Rheumatol Int 2021; 41:863–77.
4. World Health Organization. Rheumatoid arthritis. [online] 2023. [cited 2023 Sep 1]. Available from: https://www.who.int/news-room/fact-sheets/detail/rheumatoid-arthritis.
5. Bullock J, Rizvi SAA, Saleh AM, Ahmed SS, Do DP, Ansari RA, Ahmed J: Rheumatoid Arthritis: A Brief Overview of the Treatment. Med Princ Pract 2019; 27:501–7.
6. Zhang E: Bi Syndrome (Arthralgia Syndrome). J Tradit Chin Med 2010; 30:145–52
7. Liu Z. Essentials of Chinese Medicine. London: Springer; 2010.
8. Defang L, Jiao Y, Mingdong Y, Ming Y, Yong L, Jun Z, Mingyang G, Mei Y, Weili Y, Wei Z, Hua L, Yonghe H: Effect of Sanhuangyilong decoction plus methotrexate on tumor necrosis factor-alpha and interferon-gamma in serum and synovial fluid in rheumatoid arthritis patients with symptom pattern of damp heat obstruction. J Tradit Chin Med 2016; 36:625–33.
9. Yang M, Guo M-Y, Luo Y, Yun M-D, Yan J, Liu T, Xiao C-H: Effect of *Artemisia annua* extract on treating active rheumatoid arthritis: A randomized controlled trial. Chin J Integr Med 2017; 23:496–503.
10. Ouyang G, Feng X, Xiao L, Huang Z, Xia Q, Zhu F: Effects of Chinese herbal medicine Qianggu Capsule on patients with rheumatoid arthritis-induced osteoporosis: a report of 82 cases. Zhong Xi Yi Jie He Xue Bao 2012; 10:1394–9.
11. Cameron M, Gagnier JJ, Chrubasik S: Herbal therapy for treating rheumatoid arthritis. Cochrane Database Syst Rev 2011:CD002948 doi:10.1002/14651858.CD002948.pub2.
12. Nguyen PH, Tran VD, Pham DT, Dao TNP, Dewey RS: Use of and attitudes towards herbal medicine during the COVID-19 pandemic: A cross-sectional study in Vietnam. Eur J Integr Med. 2021; 44:101328.
13. kDôTâťLi, Nguyên Xuân Duñg: Native drugs of Vietnam: which traditional and scientific approaches? J Ethnopharmacol 1991; 32:51–6.
14. Dung TN, Bodeker G: Tue Tinh: founder of Vietnamese traditional medicine. J Altern Complement Med 2001; 7:401–3.
15. Vo TT, Lam TT, Phan TA, Le HM: Evaluation of Analgesic Effects of Herbal Formula Qt-2 on Experimental Mice Model. Trop J Nat Prod Res. 2023; 7:3671–5.
16. Le HM, Huynh NTK, Vo TT, Nguyen TTB, Nguyen QM: Evaluation of Pain-Relieving Effect of The Herbal Remedy

"Hoang Ky Que Chi Ngu Vat Thang" in Animal Model. Trop J Nat Prod Res. 2023; 7:3153–7.
17. Ca W, Ea R, Gw N: Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med 1962; 111.
18. Clementi G, Caruso A, Cutuli VM, Prato A, Mangano NG, Amico-Roxas M: Anti-inflammatory activity of adrenomedullin in the acetic acid peritonitis in rats. Life Sci 1999; 65:PL203-208
19. Meshram GG, Kumar A, Rizvi W, Tripathi CD, Khan RA: Evaluation of the anti-inflammatory activity of the aqueous and ethanolic extracts of the leaves of *Albizzia lebbeck* in rats. J Tradit Complement Med 2016; 6:172–5.
20. Di Rosa M, Sorrentino L: The mechanism of the inflammatory effect of carrageenan. Eur J Pharmacol 1968; 4:340–2.
21. Fu X-Q, Cai J-Y, Huang Q-Y, Li D-J, Li N, Li M-J: Prednisone may induce immunologic tolerance by activating the functions of decidual immune cells in early pregnancy. Oncotarget 2017; 8:102191–8.
22. Bisht D, Rashid M, Arya RKK, Kumar D, Chaudhary SK, Rana VS, Sethiya NK: Revisiting liquorice (*Glycyrrhiza glabra* L.) as anti-inflammatory, antivirals and immunomodulators: Potential pharmacological applications with mechanistic insight. Phytomed Plus 2022; 2:100206.
23. Jia W, He X, Jin W, Gu J, Yu S, He J, Yi Z, Cai B, Gao H, Yang L: *Ramulus Cinnamomi* essential oil exerts an anti-inflammatory effect on RAW264.7 cells through N-acylethanolamine acid amidase inhibition. J Ethnopharmacol 2023; 317:116747.
24. Gao J, Yang X, Yin W: From Traditional Usage to Pharmacological Evidence: A Systematic Mini-Review of *Spina Gleditsiae*. Evid Based Complement Alternat Med 2016; 2016:3898957.
25. Li J, Huang H-Y, Lin Y-C-D, Zuo H, Tang Y, Huang H-D: *Cinnamomi ramulus* inhibits cancer cells growth by inducing G2/M arrest. Front Pharmacol 2023; 14.
26. Liu J, Zhang Q, Li R-L, Wei S-J, Huang C-Y, Gao Y-X, Pu X-F: The traditional uses, phytochemistry, pharmacology and toxicology of *Cinnamomi ramulus*: a review. J Pharm Pharmacol 2020; 72:319–42.
27. Lee S-J, Park S-S, Kim W-J, Moon S-K: *Gleditsia sinensis* thorn extract inhibits proliferation and TNF-α-induced MMP-9 expression in vascular smooth muscle cells. Am J Chin Med 2012; 40:373–86.
28. Leite C dos S, Bonafé GA, Carvalho Santos J, Martinez CAR, Ortega MM, Ribeiro ML: The Anti-inflammatory Properties of Licorice (*Glycyrrhiza glabra*)-Derived Compounds in Intestinal Disorders. Int J Mol Sci 2022; 23:4121.
29. Yin G, Cao L, Xu P, Jeney G, Nakao M, Lu C: Hepatoprotective and antioxidant effects of *Glycyrrhiza glabra* extract against carbon tetrachloride (CCl(4))-induced hepatocyte damage in common carp (*Cyprinus carpio*). Fish Physiol Biochem 2011; 37:209–16.
30. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L: Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 2017; 9:7204–18.