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# Evaluation of Analgesic Effects of Herbal Formula Qt-2 on Experimental Mice Model

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ABSTRACT

Osteoarthritis is a prevalent condition worldwide, causing significant pain and disability. The use of a traditional Vietnamese remedy, QT-2 decoction, has been employed for its potential therapeutic effects on osteoarthritis. However, there is a lack of scientific evidence supporting its analgesic properties. Therefore, the aim of this research was to assess the analgesic effect of QT-2 decoction in mice models. The study evaluated both the central and peripheral analgesic effects of QT-2 decoction using the hot plate test and the writhing test with acetic acid, respectively. The results revealed analgesic effects of QT-2 decoction. According to statistically significant results, QT-2 administration at doses of 21.4 g/kg/day and 42.8 g/kg/day demonstrated an analgesic effect on hot plate and delayed the onset of colic in the acetic acid-induced pain model by exerting peripheral analgesic effects. These findings indicate the potential of QT-2 decoction to alleviate pain both centrally and peripherally suggesting that QT-2 decoction holds promise as an effective treatment option for pain management.

***Keywords***: Analgesic effect, QT-2, traditional medicine, herbal medicine

## Introduction

Osteoarthritis (OA) represents a significant global health burden, contributing to pain, disability, and socioeconomic costs.[1] The prevalence of OA has shown a substantial increase worldwide in recent decades, with cases rising by 113.25% from 1990 to 2019, reaching 527.81 million individuals. The geographic distribution and affected joints have also demonstrated variation in OA prevalence.[2] Addressing this growing burden necessitates a focus on prevention and early intervention. Traditional medicine, also known as Eastern medicine, holds a significant position in the healthcare systems of several Asian countries. In recent times, there has been growing evidence supporting the use of traditional remedies in the management of osteoarthritis.[3–5] Traditional herbal medicine has played a prominent role in Vietnamese healthcare, with a rich history of utilizing diverse combinations of herbs to treat various ailments.[6] Within this tradition, specific decoctions have been developed for managing osteoarthritis, such as QT2. This decoction consists of *Notopterygium incisum, Rhizoma curcumae longae, Angeliae sinensis, Astragalus membranaceus, Ledebouriellae sesloides, Paeonia liacliflora, Glycyrrhizae glaba, Zizyphus jujubae, Cinnamomum casia, Spina gleditschea*. In Oriental medicine, these herbs facilitate energy flow, adjust meridians, strengthen Qi, promote blood and balance overall harmony. According to the principles of Traditional Oriental Medicine, the QT-2 decoction is believed to possess various functions, including reducing leprosy, activating meridians and blood circulation, tonifying the kidneys, and promoting kidney health.

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The composition of QT-2 includes herbs containing essential oils, saponins, alkaloids, flavones, and glycyrrhizin, which exhibit pharmacological effects such as pain relief, anti-inflammatory properties, and immune enhancement. Some researchers have consistently shown similarities between the pharmacological effects: *Cinnamomum casia*, one of the components of the QT-2 remedy, has been found to possess anti-inflammatory and pain-relieving effects.[7–10] These effects are attributed to the inhibition of histamine and PGE synthesis and release, scavenging of free radicals, inhibition of IgE and inflammatory mediator release, and complement activity inhibition. Cinnamon essential oil has vasodilatory effects, improves blood circulation, inhibits acute inflammatory agents, reduces vascular permeability, and exhibits inhibitory effects on chronic inflammation in experimental granulomatous models. In animal studies, cinnamon extract, cinnamic aldehyde, and cinnamic acid have been shown to increase pain thresholds, which are associated with muscle development and relaxation.[9] *Angeliae sinensis* has demonstrated analgesic effects in the acetic acid-induced cramps and hot plate tests, as well as anti-inflammatory effects in the formaldehyde-induced inflammation model.[11] *Notopterygium incisum* has shown positive effects in treating rheumatism and joint pain, particularly in cases of rheumatoid arthritis where effective pain relievers and anti-inflammatory drugs are required. Notopterol found in *Notopterygium incisum*, has been identified as the analgesic component in acetic acid-induced pain tests in rats. Additionally, it has been shown to possess anti-inflammatory effects through tests on vascular permeability. *Astragalus membranaceus* has demonstrated anti-osteoporotic effects in a steroid-induced osteoporosis model by increasing bone formation rates and enhancing immunity while exhibiting anti-free radical and anti-aging properties. *Glycyrrhizae glaba* exhibits varying degrees of corticosteroid-like anti-inflammatory activity, and its main components responsible for the anti-inflammatory effects are glycyrrhetat and glycyrrhretinic acid salts, flavonoids, and licorzence.[12] *Rhizoma curcumae longae* stimulates liver cell secretion, while curcumin induces gallbladder contractions, lowers cholesterol levels, and possesses anti-inflammatory and analgesic effects.[13]

QT-2 decoction has gained popularity as a treatment option for various forms of osteoarthritis and musculoskeletal disorders. At the Kien Giang Traditional Medicine and Pharmacy Hospital in Vietnam, it is

commonly prescribed by medical practitioner as a normal therapy due to its notable analgesic properties. Despite its widespread clinical use, the efficacy of QT2 for these conditions has not been scientifically studied. Thus, the objective of this research is to provide empirical evidence that supports the extensive utilization of QT-2 in clinical practice. This study aims to examine the analgesic effects of QT-2 and contribute to a better understanding of its mechanisms of action. By conducting experiments on mice, we intend to investigate the specific mechanisms through which QT-2 exerts its analgesic properties. The findings obtained from this study have the potential to not only validate the use of QT-2 in pain management but also offer valuable perceptions into the underlying mechanisms that drive the therapeutic effects of traditional herbal decoctions.

## Materials and Methods

*QT-2 preparation*

Table 1 displays the detailed composition of the remedy, including the specific ingredients used in its formulation. The herbs utilized in this study were sourced from Kien Giang Traditional Medicine and Pharmacy Hospital in Vietnam, in accordance with Vietnamese Pharmacy V (2017) guidelines. The dosages of these ingredients were measured in grams of dried herbs and were determined based on a total remedy weight of 91 grams, intended for daily consumption by an individual. Human dosage was determined using a reference body weight of 50 kg, resulting in 1.2 g/kg as the recommended dosage. To determine the appropriate dosage for mice, a conversion factor of approximately 11.76, based on the assumption of human-to-mice conversion, was applied. Consequently, the calculated adequate amount for mice was 21.4 g/kg. In addition, the effective dosage for mice was estimated to be approximately 7.764 g/kg. This calculation was based on the human dosage of 1.2 g/kg, multiplied by a conversion factor of 6.4715 specifically for mice. To administer the remedy to the mice, a specialized curved needle was utilized to facilitate the oral insertion of the remedy directly into the stomach.[14]

*Animal experiments*

The study protocols were approved by the Medical Science Council of Can Tho University of Medicine and Pharmacy, Can Tho, Vietnam (acceptance code 721/QD-DHYDCT). Adult Swiss mice, weighing between 18 and 22 grams, were obtained from the Laboratory Animal Breeding Board, Military Medical University, Vietnam. In preparation for the study, the mice were acclimatized in the laboratory for at least one week.[15,16] During this period, standard diets were provided, and clean drinking water was available ad libitum. Daily observations were conducted to ensure the well-being of the mice, and experimental data were regularly collected and recorded.

Once the mice had adapted to the laboratory conditions, they were randomly divided into four groups (n = 10) for the analgesic activity experiments, based on two evaluation models. The groups included the control group, the Codeine (Codeine phosphate group, 20 mg/kg), and two different dose groups for the QT-2 treatment (21.4 g/kg and 42.8 g/kg). In the second experiment, the CDP group was replaced with a group receiving a dose of Aspegic (Lysine acetylsalicylate, 180 mg/kg). All drugs were administered orally to the respective groups for five continuous days. Controls received the same volume of distilled water (10 mL/kg) as the drug group.

*Hotplate-induced Central pain model*

The assessment of analgesic activity was performed using the hot plate latency test, a well-established method developed by Woolfe and Macdonald.[17] This test relies on thermal stimuli principles to evaluate the analgesic effects of the reagent. In the experiment, mice were subjected to pain by placing their hind paws on a hot plate maintained at a constant temperature of 56°C.[18] The reaction time, defined as the duration taken for the mice to reflexively lick their hind paw, was measured. Mice with reaction times below 8 seconds or above 30 seconds were excluded from the study. The response times to thermal stimulation were compared before and after the administration of the reagent, both within groups and between different groups of mice. The experiment was conducted at two distinct time points: prior to administering the reagent and after five consecutive days of administration. On the 5th day, one hour after the mice received the drug, they were placed on the hot plate with a stable temperature of 56°C. The pain tolerance time, measured from the moment the mice placed their paw on the hot plate until they licked their hind paw, was recorded. The analgesic effect was evaluated by calculating the percentage prolongation of pain tolerance time in the mice. Mice that exhibited response times below 8 seconds or above 30 seconds during the initial pre-treatment assessment conducted by the researchers were excluded from further analysis.

*Acetic acid-induced peripheral pain model*

The mice in the study were received either the drugs or distilled water on a daily basis for five continuous days. On the fifth day of the experiment, 60 minutes after the oral administration, the mice received an intra-peritoneal injection of a 0.6% acetic acid solution at a dose of 0.1 mL per 10 g of body weight. After the injection of acetic acid into the abdominal cavity, the mice displayed observable signs of cramping pain, including abdominal retraction, pressure on the floor, and elongation of the trunk and hind legs. The onset of pain was meticulously documented for each individual mouse in every batch, representing the time elapsed from the injection of acetic acid to the occurrence of the first cramping episode. Additionally, Following acetic acid injection, 5-minute intervals of abdominal writhing were recorded until the end of 25 minutes.[19] By comparing the remedy with the control group, the analgesic effects of the remedy were assessed. Using the following formula, the percentage inhibition of abdominal cramps was calculated to determine the treatment's analgesic effect:

$$C\% = \frac{Mc - Mt}{Mc} \times 100$$

In the formula, C (%) represents the rate of inhibition of abdominal writhing observed in the drug trial batch. Mc represents the mean number of cramps observed in the animals in the control group, while Mt represents the mean number of cramps observed in the animals in the treated group.

*Data analysis*

The mean ± standard error of the mean (SEM) was used to represent the data obtained from the experiments, providing an indication of the central tendency and variability of the results. The variance of the data was analyzed using SPSS 22.0 software, applying the Student's t-test to determine statistical significance. A significance level of $p < 0.05$ was chosen to assess the differences between the control and treated groups, indicating the presence of statistically significant findings.

**Table 1:** Constituent elements of the QT-2 Herbal Preparation

| Scientific name | Quantity (gram) |
|---|---|
| *Notopterygium incisum* | 09 |
| *Rhizoma curcumae longae* | 08 |
| *Angeliae sinensis* | 12 |
| *Astragalus membranaceus* | 12 |
| *Ledebouriellae sesloides* | 08 |
| *Paeonia liacliflora* | 08 |
| *Glycyrrhizae glaba* | 04 |
| *Zizyphus jujubae* | 12 |
| *Cinnamomum casia* | 10 |
| *Spina gleditschea* | 08 |

## Results and Discussion

*Central analgesic effect on hotplate-induced pain mode*

In Table 2, it is observed that there was no significant difference in the time taken for pain response among the groups before drug administration. However, after administration, the QT-2 groups at both doses showed significantly longer pain response times compared to the control group ($p < 0.05$ and $p < 0.01$). Administration orally of QT-2 at doses of 21.4 g/kg/day and 42.8 g/kg/day exhibited a substantial analgesic effect, comparable to the standard group receiving Codeine at 20 mg/kg. The pain response time of mice in the QT-2 group at 42.8 g/kg was longer than in the QT-2 group at 21.4 g/kg, indicating a tendency of the hot plate analgesic effect to increase with higher doses, despite not being statistically significant ($p > 0.05$). Moreover, when comparing the mice within the same group before and after drug administration, the pain response time in the QT-2 groups at both doses was significantly longer than the pre-treatment time point ($p < 0.05$ and $p < 0.01$, respectively). The obtained results provide evidence supporting the analgesic effect of QT-2 as demonstrated by the Hotplate method. In this experimental model, pain is induced by applying heat to the skin, activating various types of heat-sensitive receptors that transmit the pain signal. This signal is then transmitted from the periphery to the spinal cord and eventually to the brain, eliciting a reflex response of hind leg licking in mice. The observed prolongation of pain response time in the QT-2 groups suggests that QT-2 may act by inhibiting the pain center or increasing the pain threshold at analgesic receptors, similar to the mechanism of action of morphine. Notably, two specific ingredients present in the QT-2 formulation, *Cinnamomum cassia* and *Spina gleditsiae*, are known to possess notable analgesic and anti-inflammatory properties, these ingredients may contribute to the overall analgesic effect of QT-2.[20–23]

*Analgesic effect on acetic acid-induced pain model*

In Table 3, it can be observed that both the QT-2 group and the Aspegic group showed a statistically significant delay in the onset of pain compared to the control group ($p < 0.05$). The duration of pain in the QT-2 groups at both doses and the Aspegic group was similar and did not show a statistically significant difference ($p > 0.05$).

Table 4 reveals that compared to the control group, both the QT-2 groups and the Aspegic group had a significantly lower total number of writhing episodes ($p < 0.01$). Additionally, during the 25-minute observation period, the percentage reduction in the number of writhing episodes was 32.77% in the Aspegic group at 180 mg/kg/day, 28.99% in the group that received QT-2 at a dose of 21.4 g/kg, and 34.24% in the group that received QT-2 at a dose of 42.8 g/kg. Thus, the group that received QT-2 at a dose of 42.8 g/kg exhibited the highest percentage reduction in writhing episodes. Furthermore, there was no statistically significant difference in the number of writhing episodes between the two QT-2 dose groups and the Aspegic group ($p > 0.05$) during the 25 minutes following acetic acid injection. Thus, from the above model, it is shown that QT-2 increases the dose by 21.4 g/kg and 42.8 g/kg with peripheral analgesic effect.

**Table 2:** Effect of QT-2 on the pain response time in the hot plate test (n = 10)

| Group | Before | After | Pain inhibition (%) | p value (before-after) |
|---|---|---|---|---|
| Control | 14.83 ± 3.30 | 14.39 ± 2.48 | - | > 0.05 |
| Codeine 20 mg/kg | 14.66 ± 2.04 | 19.95 ± 4.40$^{**}$ | 38.64 % | < 0.01 |
| QT-2 21.4 g/kg | 14.82 ± 2.75 | 17.92 ± 2.36$^{*}$ | 24.53 % | < 0.05 |
| QT-2 42.8 g/kg | 14.86 ± 2.31 | 19.42 ± 4.29$^{**}$ | 34.95% | < 0.01 |

$^{**}$ Compared with the control group, $^{*}p < 0.05$, $^{**}p < 0.01$;
$^{\#}$ Compared with the Codeine group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$.

**Table 3:** Pain onset in colic pain with QT-2 in acetic-induced pain (n = 10)

| Group | Time of onset of colic pain (seconds, mean ± SD) |
|---|---|
| Control | 260.50 ± 66.88 |
| Aspegic 180 mg/kg | 353.00 ± 102.13$^{*}$ |
| QT-2 21.4 g/kg | 345.60 ± 100.61$^{*}$ |
| QT-2 42.8 g/kg | 350.90 ± 93.04$^{*}$ |

$^{**}$ Compared with the control group, $^{*}p < 0.05$, $^{**}p < 0.01$;
$^{\#}$ Compared with the Asperic group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$

According to Traditional Medicine theory, the pain associated with osteoarthritis is considered a manifestation of "impediment disease" within the realm of inflammation and pain. This concept refers to a blockage or obstruction that hinders the smooth flow of vital energies and fluids within the body. In this context, pain arises due to the inability of the system to function optimally, resulting in stagnation and accumulation of substances such as blood and fluid, which leads to pain and discomfort. The concept of "impediment" is used to describe both the symptoms of the disease, such as pain, numbness, fatigue, swelling, and aching, as well as the underlying pathological processes involving stagnation of meridians, qi, and blood. In the treatment of such conditions, the focus is on promoting the circulation of Qi and blood in the muscles, bones, and joints, and eliminating the pathological factors (such as wind, cold, dampness, or heat) that contribute to the disease. Additionally, nourishing the blood and strengthening the kidney function are important aspects of the treatment approach. The treatment strategy may vary depending on whether the condition is newly diagnosed or recurrent. In the case of a new condition, the emphasis is on expelling the pathogenic factors, while in chronic cases, the approach involves strengthening both the kidney, Qi, blood and expelling the pathogenic factors.[24,25] QT-2 is a formulation that draws from the ancient remedy called Juan-bi-tang, which has been traditionally used to treat musculoskeletal disorders. By incorporating additional ingredients such as *Cinnamomum cassia* and *Spina gleditsiae*, QT-2 aims to enhance the therapeutic effects of the original remedy. Clinical studies support the efficacy of Juan-bi-tang in treating these disorders, which have a long history in Traditional Medicine.[26]

This study still has some limitations. Firstly, it is important to note that the conversion factor used to estimate the effective dosage for mice is an approximation and may not precisely reflect the true conversion factor. Moreover, the study was conducted with a relatively small sample size, which may restrict the generalizability of the findings to a larger population. It is important to acknowledge that the use of mice as an animal model, although commonly employed in experimental research, does not perfectly mimic human physiology. Therefore, caution should be exercised when extrapolating the study results directly to humans. The evaluation of analgesic activity utilized the hot plate latency test, a widely employed research method. However, it is worth noting that it is worth noting that while thermal stimuli are commonly used in pain research, they may not fully capture the multidimensional nature of the pain experience in humans. Pain perception in humans is a complex phenomenon influenced by various factors, including psychological, emotional, and cognitive aspects. Lastly, the writhing test was employed to assess peripheral reduction by administering acetic acid to induce cramping pain in mice. While this test is commonly used to evaluate the pain-relief effects of drugs, it is

important to recognize that mice may not experience pain in the same way as humans do, thereby affecting the interpretation of the results.

## Conclusion

In conclusion, the experimental results indicate that the oral administration of QT-2 at doses of 21.4 g/kg/day and 42.8 g/kg/day demonstrated significant central analgesic effects as assessed by the Hotplate method. Additionally, the peripheral analgesic effects were observed through the writhing tests. These findings highlight the potential of QT-2 as a promising treatment option for pain management. Further investigations are required to elucidate its mechanism of action and explore its potential clinical applications.

## 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.

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**Table 4:** The effect of QT-2 on the number of abdominal writhes in a group of animals following acetic acid injection for 25 minutes (n = 10)

| Group | Number of abdominal writhes in 25 minutes after acetic acid injection (seconds, mean ± SD) | The percentage (%) reduction in the number abdominal writhes compared with the control group |
|---|---|---|
| Control | 47.60 ± 10.22 | - |
| Aspegic 180 mg/kg | 32.00 ± 8.56** | 32.77 % |
| Dose 21.4 g/kg | 33.80 ± 8.68** | 28.99 % |
| Dose 42.8 g/kg | 31.30 ± 7.99** | 34.24 % |

** Compared with the control group, *p < 0.05, **p < 0.01;
# Compared with the Asperic group, #p < 0.05, ##p < 0.01

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