EVALUATION OF ACUTE TOXICITY AND ANALGESIC ACTIVITIES OF MEKONG CISSUS CAPSULES ON EXPERIMENTAL ANIMALS

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Mekong Cissus capsules, a herbal preparation primarily containing Cissus quadrangularis L., were tested for both acute toxicity and pain-relieving effects in mice. The evaluation of acute oral toxicity in Swiss mice followed the procedures outlined by the WHO and the Litchfield-Wilcoxon method. Analgesic activity was assessed using three experimental models: hot plate, Von Frey, and acetic acid-induced writhing tests. No mortality or clinical sign of acute toxicity was observed, and the maximum tolerated dose was estimated at 15000 mg/kg. In the hot plate test, Mekong Cissus capsules at 288 and 864 mg/kg/day showed a tendency to prolong latency to thermal stimuli, although the changes were not statistically significant. In the Von Frey test, the higher dose (864 mg/kg/day) significantly increased both pain threshold and latency compared with baseline and control ($p < 0.05$), and produced a greater effect on applied force than the lower dose ($p < 0.05$). In the writhing test, both doses significantly reduced acetic acid-induced writhing counts from the 5th minute onward compared with the control group.

Keywords: Mekong Cissus capsules, Cissus quadrangularis, acute toxicity, analgesic, mice.

I. INTRODUCTION

Musculoskeletal (MSK) disorders - including low back pain, osteoarthritis, rheumatoid arthritis, and other soft-tissue conditions - represent a major and growing cause of global disability and health-care need. Global burden estimates consistently place MSK conditions among the top contributors to years lived with disability worldwide. In 2017, MSK disorders ranked fifth among all diseases in disability-adjusted life-years (DALYs) and ranked first in years lost due to disability globally.[1] Between 1990 and 2021, population aging was the largest contributor to the increase in incident cases, prevalent cases, and DALYs, significantly increasing the global burden of MSK disorders, which in turn is projected to escalate healthcare demands and economic costs.[2]

Pain is often the early and most prominent symptom in MSK pathologies. Disorders causing musculoskeletal discomfort remain a complex issue for patients and medical practitioners. Uncontrolled long-term musculoskeletal pain can greatly reduce a patient's quality of life, and is therefore a primary therapeutic target in both acute and chronic care.[3]

Current pharmacologic management of MSK pain relies principally on non-steroidal anti-inflammatory drugs (NSAIDs), paracetamol and, when indicated, opioid analgesics and

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Received: 06/10/2025
Accepted: 02/11/2025

adjuvant agents.[4] While many patients obtain symptomatic relief, each drug class carries important limitations. NSAIDs are effective for inflammatory and nociceptive pain but are associated with well-documented gastrointestinal and cardiovascular risks and can adversely affect renal function when used long-term or in vulnerable patients.[5] Opioids remain an option for severe acute or cancer pain but carry risks of dependence, overdose, and other adverse effects that limit their suitability for chronic non-cancer pain.[6] In addition to NSAIDs and opioids, glucocorticoids remain an important treatment option in musculoskeletal disorders, particularly in minimally invasive procedures to control MSK pain, providing short-term pain relief and functional improvement. Glucocorticoids fall within the most potent anti-inflammatory agents with diverse mechanisms of action. They can precipitate several side effects that include hyperglycemia, hypertension, insomnia, and fluid retention.[4] The pain in question is persistent, and due to the fact that current medications are known for their drawbacks, there is an urgent and continuous need for new analgesic medications to be effective and safer, as well as having improved long-term tolerability.

Against this background, traditional medicinal plants and herbal formulations have attracted renewed interest as potential sources of safer and more effective analgesic agents. With centuries of traditional medical practice and a wealth of biodiversity, Vietnam provides numerous native medicinal species that possess distinct therapeutic properties and a long heritage of use.[7]

Cissus quadrangularis L. is a traditional medicinal plant most commonly used in India and found extensively in the tropical regions of Asia and Africa for bone and joint health.[8] Previous global research highlights its proven potential for analgesic and anti-inflammatory effects, as well as bone healing properties, positioning it as a promising candidate for musculoskeletal disorders.[9,10] However, specifically in Vietnam, *Cissus quadrangularis L.* has not yet been subject to specific pharmacological or toxicity studies. Therefore, conducting an initial assessment of the acute toxicity and analgesic activities of Mekong Cissus capsules, containing *Cissus quadrangularis L.,* in experimental models is essential. This study aims to provide scientific data on the safety of the product in preclinical models. It also serves as a starting point for further research into its potential for pain relief and possible future use in clinical practice.

MATERIALS AND METHODS

1. Subject

The preparation of Mekong Cissus capsules

Mekong Cissus capsules were prepared from *Cissus quadrangularis* L. extract at a ratio of 1:6 (1ml extract equivalent to 6g raw material). All components were in dried form and complied with the Vietnamese Pharmacopoeia V.[11] Each capsule contained 400mg, and the recommended adult dosage was one capsule three times daily (equivalent to 24 mg/kg/day for an average adult weighing 50kg).

Experimental animals

Swiss albino mice of both sexes, weighing 20 ± 2g, were obtained from the National Institute of Hygiene and Epidemiology. The animals were housed in cages (10 per cage) under controlled environmental conditions, including a temperature of 25 ± 2°C, humidity of 50 ± 5%, and a 12-hour light/dark cycle. They were provided with standard chow (National Institute of Hygiene and Epidemiology) and water ad

libitum. Before the experiment, the mice were acclimatized to laboratory conditions for seven days. The Scientific Board Committee of Hanoi Medical University reviewed and approved the study protocol.

Drugs and chemicals

Enteric-coated aspirin tablets 500mg (Aspirin pH8) (Mekophar, Vietnam); Paratramol tablets (containing 325mg paracetamol and 37.5mg tramadol hydrochloride) (Pharmaceuticals Works Polpharma S.A., Poland); acetic acid ≥ 99.5% (Xilong Scientific Co., Ltd., China); Hot Plate model DS37 (Ugo-Basile, Italy); and Dynamic Plantar Aesthesiometer 37450 (Ugo-Basile, Italy) were used in this study.

2. Methods

Acute toxicity study

This study was conducted in accordance with World Health Organization (WHO) guidance.[12] Before oral administration of Mekong Cissus capsules, mice (10 per group) were fasted for 12 hours, with doses raised progressively according to their tolerance. Clinical signs of toxicity and mortality were monitored over a 72-hour period. All animals that died were necropsied for gross pathological examination. The median lethal dose (LD_{50}) was determined using the Litchfield–Wilcoxon method.[13] Surviving animals were further observed for seven days to detect any delayed toxic manifestations.

Evaluation of analgesic activity

The central analgesic activity was evaluated using two experimental models: the hot plate test and the Von Frey test, while the peripheral analgesic effect was assessed through the acetic acid-induced abdominal writhing model.

Hot Plate Test (Thermal Stimuli): The hot plate assay was performed following standard procedures, with the plate maintained at 56°C.[14] After mice were placed on the heated surface, the latency time (in seconds) to the first nociceptive response - such as licking of the right hind paw - was recorded. Animals with baseline responses shorter than 8 seconds or longer than 30 seconds were excluded. The treatments were given once per day over a period of three consecutive days, including distilled water, paratramol (a fixed-dose combination of paracetamol at 216 mg/kg/day and tramadol at 25 mg/kg/day), or Mekong Cissus capsules at two dose levels (288 and 864 mg/kg/day) given by oral gavage at 0.2 ml/10 g body weight. Pain thresholds were assessed both before dosing and one hour after the final administration.

Von Frey Test (Mechanical Stimuli): Mechanical nociception was evaluated using the Dynamic Plantar Aesthesiometer (Ugo Basile, Italy). Forty mice were randomly allocated to four groups, each comprising ten animals. The treatment regimen consisted of distilled water (control), paratramol as the positive control, and Mekong Cissus capsules administered at 288 or 864 mg/kg/day. Treatments were given orally for three consecutive days. On the third day, mechanical sensitivity was tested one hour after the final dose by applying a von Frey filament to the right hind paw through the wire grid floor, gradually increasing the force until the paw was withdrawn. The instrument automatically recorded the withdrawal. Baseline measurements were taken after one training session conducted before treatment initiation, and results were compared between pre- and post-treatment values.[15]

Acetic Acid-Induced Writhing Test (Chemical Stimuli): The abdominal writhing assay was conducted as previously described.[16] Mice were divided into four groups (n = 10 per group) and treated orally for three consecutive days with either distilled water (control), aspirin (150 mg/kg/day), or Mekong Cissus capsules at 288 and

864 mg/kg/day. On the third day, one hour after the last treatment, each mouse received an intraperitoneal injection of 0.2 ml of 1.0% acetic acid solution prepared in normal saline to induce nociception. The animals were next positioned in the observation chamber, and the number of abdominal constrictions - characterized by pelvic rotation, abdominal contractions, and extension of the hind limbs - was recorded over a 30-minute period.

Statistical analysis

The data were expressed as the mean ± standard deviation (SD), and statistical analysis was carried out employing Student's T-test. In the study of histological grading, nonparametric tests (Mann-Whitney U test) were used. Both analyses were performed using SPSS 25.0. The p-value < 0.05 was statistically significant.

III. RESULTS

1. Acute toxicity study

Table 1. Acute toxicity study of Mekong Cissus capsules

Group	n	Dose (ml/kg)	Dose (mg/kg)	The proportion of deaths (%)	Other abnormal signs
1	10	25	5,000	0	No
2	10	50	10,000	0	No
3	10	75	15,000	0	No

In the study on acute oral toxicity, Mekong Cissus capsules were given at doses from 25 ml/kg up to 75 ml/kg, and no death was observed either within the initial 72 hours or throughout the following seven-day monitoring period. Furthermore, no clinical sign of acute toxicity, such as piloerection, lacrimation, or alterations in locomotor activity and respiration, was observed. Based on the data presented in Table 1, the maximum tolerated dose of Mekong Cissus was determined to be 15000 mg/kg.

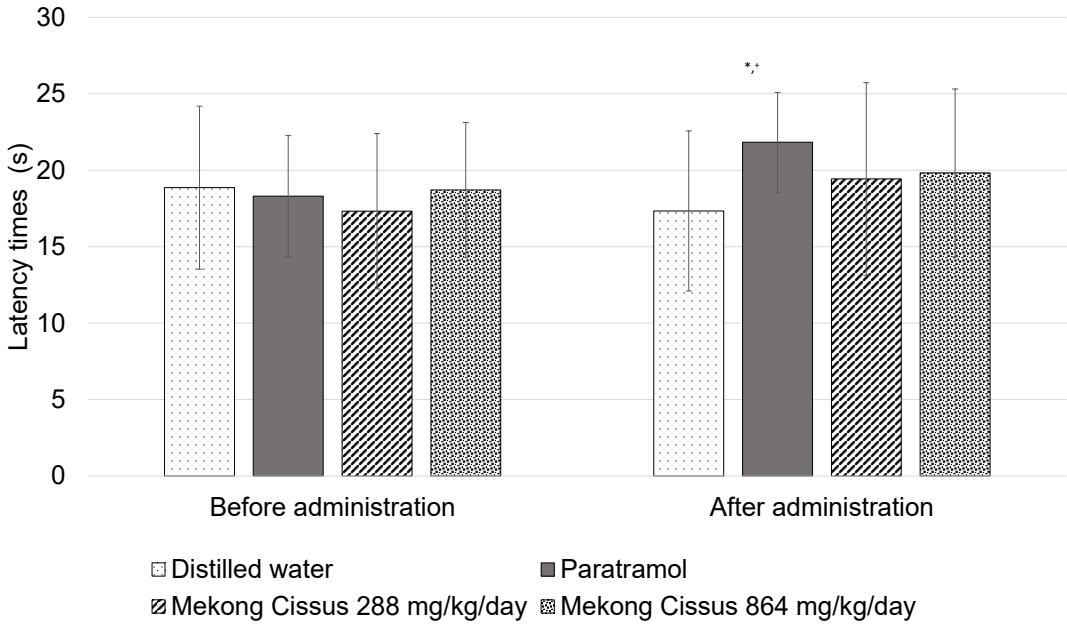
Analgesic activity

Hot Plate Test

In the hot plate test, paratramol (positive control) produced a significant increase in reaction latency after three days of treatment compared with both baseline values and the control group ($p < 0.05$). Administration of Mekong Cissus at both 288 and 864 mg/kg/day tended to extend the mice's response time to heat stimuli relative to baseline and controls, but the changes were not statistically significant ($p > 0.05$). No significant difference was observed between the Mekong Cissus groups and the positive control, nor between the two dose levels of Mekong Cissus ($p > 0.05$). Latency times are presented in Chart 1.

Von Frey Test

Paratramol administered for three consecutive days showed significant anti-nociceptive activity, with an increased paw withdrawal threshold reflected in both applied force ($p < 0.05$) and latency time ($p < 0.01$) compared with baseline, and showed a significant increase compared with the control group ($p < 0.05$). Mekong Cissus capsules at 288 mg/kg/day induced only a non-significant trend toward increased pain threshold and latency ($p > 0.05$), whereas the 864 mg/kg/day dose produced a significant increase relative to baseline and control ($p < 0.05$). The higher dose also elicited a more pronounced effect than the lower dose on applied force ($p < 0.05$), while for latency time, it showed only a clearer upward trend without reaching statistical significance.



**p < 0.05 compared to control; +p < 0.05 compared to before dosing.*

Chart 1. Effect of Mekong Cissus capsules on the pain threshold of mice in the hot plate test

Table 2. Effect of Mekong Cissus capsules on the paw withdrawal threshold of mice in the Von Frey test

Groups (n = 10)	Force (g)		Latency time (s)	
	Before dosing	After dosing	Before dosing	After dosing
Control	11.85 ± 3.70	11.28 ± 1.75	3,30 ± 1,01	3,60 ± 0,78
Paratramol	10.89 ± 3.25	14.45 ± 4.10^{*+}	3,16 ± 0,99	4,89 ± 1,59^{*++}
Mekong Cissus capsules 288 mg/kg/day	10.65 ± 3.97	11.64 ± 2.87	3,09 ± 1,18	3,69 ± 0,96
Mekong Cissus capsules 864 mg/kg/day	11.11 ± 3.85	14.55 ± 3.27$^{*+\$}$	3,17 ± 1,02	4,73 ± 1,45^{*+}

**p < 0.05 compared to control; +p < 0.05, ++p < 0.01 compared to before dosing; $p < 0.05 compared to Mekong Cissus capsules 288 mg/kg/day*

Acetic Acid-Induced Writhing Test

Table 3 presents the analgesic effect of Mekong Cissus capsules on acetic acid-induced writhing in mice. In the aspirin group, the average writhing responses were consistently lower than those of the control group during the first 20 minutes following acetic acid injection. Five minutes after intraperitoneal administration of acetic acid, Mekong Cissus capsules at both doses (288 and 864 mg/kg/day) significantly reduced writhing counts compared with the control group. At 288 mg/kg/day, significant reductions were observed at 5-10 mins ($p < 0.01$) and at subsequent intervals of 10-15, 15-20, 20-25 and 25-30 mins ($p < 0.05$). At 864 mg/kg/day, writhing counts were significantly

reduced at all intervals from 5 to 25 min ($p < 0.01$). The higher dose showed a more pronounced analgesic effect than the lower dose, although the difference did not reach statistical significance ($p > 0.05$).

Table 3. Effect of Mekong Cissus capsules on acetic acid-induced writhing in mice

Groups (n = 10)	Number of writhings within 30 minutes					
	0-5 mins	5-10 mins	10-15 mins	15-20 mins	20-25 mins	25-30 mins
Control	4.50 ± 1.58	16.60 ± 4.60	10.50 ± 3.92	6.80 ± 1.48	5.80 ± 1.40	3.80 ± 1.81
Aspirin 150 mg/kg/day	1.80 ± 1.14***	7.00 ± 2.87***	6.30 ± 2.16**	5.10 ± 1.91*	5.30 ± 2.63	3.10 ± 1.66
Mekong Cissus capsules 288 mg/kg/day	4.30 ± 1.77##	9.60 ± 3.72**	6.70 ± 2.75*	4.40 ± 2.59*	4.00 ± 1.63*	2.20 ± 0.79*
Mekong Cissus capsules 864 mg/kg/day	3.10 ± 1.45#	10.00 ± 3.74**	5.20 ± 1.69**	3.80 ± 1.99**	3.90 ± 1.52**	2.80 ± 0.79

**p < 0.05, **p < 0.01, ***p < 0.001 compared to control; #p < 0.05, ##p < 0.01 compared to aspirin*

IV. DISCUSSION

Acute toxicity of Mekong Cissus capsules

Evaluating toxicity is an essential step in drug research and development. To date, the acute toxicity of Mekong Cissus capsules has not been thoroughly investigated in Vietnam. Acute toxicity studies are designed to examine the potential harmful effects of a single or multiple doses administered within 24 hours via a specific route.[12] Since the intended clinical use of Mekong Cissus capsules is oral, the present study employed the oral route to evaluate acute toxicity. In this experiment, oral administration at the maximum tested dose of 15000 mg/kg produced no mortality or clinical signs of toxicity during the initial 72 hours or throughout the subsequent seven-day observation period. These findings provide preliminary evidence that the formulation does not induce acute toxic effects at the investigated doses.

Furthermore, Mekong Cissus was tolerated at up to 15000 mg/kg, corresponding to approximately 52.08 times the recommended human dose (based on an extrapolation factor of 12 for mice), which is substantially higher than the expected therapeutic dose in humans. This wide safety margin is particularly meaningful for a herbal product, as herbal medicines are generally anticipated to exhibit lower toxicity compared to synthetic pharmaceuticals. In line with WHO definitions, Mekong Cissus capsules can therefore be considered a safe herbal formulation.[17] The oral LD_{50} of Mekong Cissus capsules could not be determined in mice.

Analgesic activities of Mekong Cissus capsules

Pain is described by the International Association for the Study of Pain (IASP) as “An unpleasant sensory and emotional experience associated with, or resembling that associated

with, actual or potential tissue damage".[18] Anti-nociceptive activity of Mekong Cissus capsules was assessed via the acetic acid-induced writhing test as a chemical nociceptive model, the hot plate test as a thermal stimulus, and the Von Frey test as a mechanical stimulus.[19] Among these, the hot plate and Von Frey tests are regarded as models of central analgesia, whereas the acetic acid-induced writhing test is indicative of peripheral analgesic activity.

The hot plate test, a specific method for evaluating central analgesic activity, was used to assess the activity of Mekong Cissus capsules, as it captures not merely simple reflexes but also intricate, higher-order pain behaviors coordinated above the spinal cord.[20] This model used heat as the noxious stimulus, and paratramol was used as the positive control. Tramadol, an ingredient in paratramol, is a 4-phenyl-piperidine analogue of the opioid drug codeine. It is a centrally acting weak μ-opioid receptor analgesic.[21] Mekong Cissus capsules showed a trend toward prolonged pain response times at both tested doses, but the analgesic effect did not reach statistical significance. In the hot plate test, the painful stimulus comes from heat applied to the skin, and the nociceptors involved are mainly thermoceptors. Pain transmission involves pathways extending from the periphery, through the spinal cord, and up to supraspinal centers in the brain, resulting in complex behavioral responses such as paw licking. The absence of a notable effect from Mekong Cissus capsules indicates that the compound may not efficiently block the fast transmission of signals from the peripheral nerves to the brain. Consequently, the capsules do not appear to possess analgesic efficacy via a neural mechanism involving thermal stimulation of the skin.

The Von Frey test, which uses a mechanical stimulus as the noxious agent, also assessed the central analgesic effect of Mekong Cissus capsules by evaluating the mice's pain reaction latency and the intensity of the force required to induce pain. The test itself is not inherently painful, but it can be used to gauge the effectiveness of analgesic agents. An elevated withdrawal threshold indicates a lower level of pain behavior.[22] The results showed that Mekong Cissus capsules at the 864 mg/kg/day dose significantly increased both the pain reflex threshold force and the pain response latency in the mice's algometer (Von Frey apparatus). Notably, the higher dose also elicited a more pronounced effect than the lower dose on applied force ($p < 0.05$). Thus, in contrast to the findings from the Hot Plate model, the Mekong Cissus capsules at 864 mg/kg/day demonstrated a very clear analgesic effect in the Von Frey model. The noxious agent in this model is mechanical stimulation, and the assessment threshold is the mice's pain tolerance threshold to the Von Frey filament. Mekong Cissus capsules seem to have a central analgesic effect on mechanical pain when administered at a high dose. Furthermore, as an agent derived from traditional medicine, the herbal component may require sufficient time to exert its full efficacy. The short administration period of only three days might be inadequate to demonstrate the analgesic effect against a mechanical cause at the low dose and against a thermal cause at both doses.

The acetic acid-induced writhing test was used as a model for evaluating peripheral analgesic activity, wherein acetic acid triggers local inflammation and pain. Acetic acid-induced writhing serves as a highly sensitive and practical model for analgesic drug research.[9] The mechanism of pain induction involves the chemical stimulation of resident

macrophages and mast cells in the peritoneum to release algogenic substances.[19] Aspirin, a non-steroidal anti-inflammatory drug (NSAID), inhibits the activity of the cyclooxygenase (COX) enzyme, leading to the suppression of prostaglandin (PG) formation, which causes pain and inflammation.[23] Mekong Cissus capsules, at both 288 and 864 mg/kg/day doses, produced a statistically significant reduction in the number of writhes compared to the control group, commencing from 5 minutes after intraperitoneal injection of acetic acid. There was a difference in the analgesic efficacy between the two doses of Mekong Cissus capsules. The 288 mg/kg/day dose maintained its analgesic effect until 30 minutes after nociception induction, whereas the 864 mg/kg/day dose only sustained the effect up to 25 minutes. However, given that the pain threshold varies among individual mice, it is difficult to rely on this specific temporal difference to draw a definitive conclusion regarding the difference in efficacy between the two doses. Nevertheless, it can be definitively stated that Mekong Cissus capsules possess a peripheral analgesic effect starting from the dose projected for clinical use.

This finding is strongly supported by existing evidence, as several previous studies worldwide on *Cissus quadrangularis* L., the constituent of this preparation, have already demonstrated its potent analgesic activity. According to Panthong A et al., *Cissus quadrangularis* L. has been shown to possess both central and peripheral analgesic effects. Notably, administration resulted in a significant decline in the count of writhing episodes during the acetic acid-induced pain response in mice.[24] Although not exactly analogous to the pain model used in this study, a report by Feigni et al. showed that in the Von Frey test evaluating the effect of *Cissus quadrangularis* L. extract on vincristine-induced neuropathic pain in mice, the ethanolic and aqueous extracts of *Cissus quadrangularis* L. (180 and 360 mg/kg) significantly increased the latency for all behavioral parameters studied ($p < 0.01$; $p < 0.001$), relative to the negative control group.[25]

These findings indicate that Mekong Cissus capsules exert analgesic effects via both central and peripheral pathways, with the central analgesic mechanism proving effective against mechanical stimuli. These results lay an essential groundwork for more detailed studies aimed at clarifying the specific analgesic mechanisms of the compounds derived from *Cissus quadrangularis* L.

V. CONCLUSIONS

No signof acute toxicity or mortality was observed in mice treated with Mekong Cissus capsules at 15000 mg/kg (approximately 52.08 times the recommended human dose). The oral LD_{50} was not determined in *Swiss* mice.

Mekong Cissus capsules demonstrated significant analgesic effects, involving both central and peripheral mechanisms. These effects were particularly notable in the Von Frey test at the 864 mg/kg/day dose and in the acetic acid-induced writhing model at both the 288 and 864 mg/kg/day doses.

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