Journal of Chemical and Pharmaceutical Sciences

Journal of Chemical and Pharmaceutical Sciences-JCHPS (2025) 18 (4): 23-30
Online ISSN: Online 2349-8552, Print ISSN: 0974-2115
Research Article | Pharmaceutical Sciences | MCI Approved | Peer-Reviewed | Referred | OA Journal |

Pharmacological Investigation of The Analgesic Effects of *Arnica montana* in Mice

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Received: 15 Aug 2025 / Accepted: 20 Sept 2025 / Published online: 01 Oct 2025
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ABSTRACT

The present study was undertaken to evaluate the analgesic activity of *Arnica Montana*; a medicinal plant traditionally used for its anti-inflammatory and pain-relieving properties. The aerial parts of the plant were collected, shade-dried, coarsely powdered, and subjected to methanolic extraction using the maceration method. Preliminary phytochemical screening of the extract revealed the presence of several bioactive constituents such as alkaloids, tannins, saponins, flavonoids, phenols, terpenoids, steroids, and sesquiterpenoids. The analgesic activity was assessed using Swiss albino mice (200–250 g) under standard laboratory conditions, divided into four groups: a control group receiving saline, a standard group treated with pentazocine (10 mg/kg), and two test groups receiving *Arnica Montana* extract at doses of 100 mg/kg and 200 mg/kg respectively. The analgesic activity was evaluated using the hot plate method, where the reaction time to pain stimulus was recorded. The results demonstrated a dose-dependent increase in analgesic activity of the extract, comparable to the standard drug. Acute toxicity studies conducted according to OECD guideline 423 revealed no signs of toxicity or mortality at a limit dose of 2000 mg/kg, indicating the extract is safe for oral administration. Delayed toxicity studies were also performed, where all animal groups were observed for 14 days post-treatment for any behavioral or physiological changes; no adverse effects were noted. The study concludes that *Arnica Montana* possesses significant analgesic activity and is safe at the tested doses, supporting its traditional use in pain management and encouraging further pharmacological investigations.

KEY WORDS: *Arnica montana*, Analgesic activity, Mice models, Pain assessment, *In vivo* study

1. INTRODUCTION:

Analgesics are the drugs which relieve or suppress the pain sensation by their action on the CNS without causing loss of consciousness. An analgesic drug, also called simply an analgesic, antalgic, pain reliever, or painkiller, is any member of the group of drugs used for pain management. Analgesics are conceptually distinct from anesthetics, which temporarily reduce, and in some instances eliminate, sensation, although analgesia and anesthesia are neuro physiologically overlapping and thus various drugs have both analgesic and anesthetic effects.

Pain cannot be measured; it is only felt by an individual. It can be explained as an unpleasant sensation resulted from some diseases or infection. Clinically, pain can be divided in three categories.

1.1.1 ACUTE PAIN:

The Acute Pain has the quick onset of action, often resulting from a specific injury or condition. It's generally short-lived usually lasting less than three months. It serves as a warning signal to the body, indicating that something is wrong.

1.1.2 CHRONIC PAIN:

Chronic pain is defined as pain that persists for more than three months, or beyond the expected healing time. Unlike acute pain, which serves as a warning signal, chronic pain can continue even after the initial injury or illness has healed.

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1.1.3 PSYCHOGENIC PAIN:

Essentially, psychogenic pain refers to physical pain that is significantly influenced by psychological, emotional, or behavioral factors. It's important to emphasize that this pain is real. Individuals experiencing psychogenic pain are not faking it. The term psychogenic pain is becoming less used, as modern medical understanding recognizes that all pain experiences have biological, psychological, and social components.

The Analgesic administered to an individual should be ideal and safe. The intensity of pain is not proportional to the large size of wound and severity of tissue damage. In the year 1946, during 2nd world war, recently wounded 215 injured men were questioned. There wounds, were severe, tissue damage was of large area. All were almost rational having equal injuries among injured person's only 24% patients reported "Bad pain". The other wounded patients expressed only moderate, mild pain or no pain.

1.2 SYMPTOMS:

- Appetite loss
- General ill feeling and Fatigue
- Headache
- Itching and Dry skin
- Nausea
- Weight loss
- Abnormally Dark or Light skin
- Bone pain
- Drowsiness
- Muscle twitching or cramps
- Breathe odor.

1.3 PATHOPHYSIOLOGY:

The Pathophysiology of analgesic drugs involves diverse mechanisms depending on the specific drug class. Generally, Analgesics aim to disrupt the pain pathway, which involves both peripheral and central nervous system components Non-Steroidal Anti-inflammatory drugs (NSAID's) like Ibuprofen primarily inhibit cyclooxygenase (COX) enzymes, reducing the production of prostaglandins, which are inflammatory mediators that sensitize pain receptors. In contrast, opioids analgesics such as morphine bind to opioid receptors in the CNS, modulating pain signal transmission and perception. This action triggers a cascade of events that ultimately decreases the release of neurotransmitters involved in pain signaling Acetaminophen, while its extract mechanism is not fully understood, is believed to act centrally by inhibiting COX enzymes, particularly in the brain. Understanding these varied pathophysiological pathways is crucial for selecting appropriate analgesic drugs and managing pain effectively.

1.5 TREATMENT:

Non-opioid and opioid analgesics are the main drugs used to treat pain. Anti-depressants, Anti- convulsant and other CNS active drugs may also be used for Chronic or neuropathic pain and are first line therapy for some conditions. Neuraxial infusion, Nerve stimulation, injection therapies, and neural blockade can help selected patients. Cognitive behavioral interventions may reduce pain and pain related disability and help patients cope.

1.7 PLANT PROFILE:

Common Name: Arnica, Mountain Arnica, leopard's bane, wolf's bane, mountain tobacco.

Family: Asteraceae

Botanical Name:

Arnica Montana, Arnica chamissonis, Arnica card folia.

Parts used:

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Aerial portions, especially the flowers.

Figure No: 1 ***Arnica Montana***

Description:

Arnica Montana is a perennial herbaceous plant known for its bright yellow-orange, daisy like flowers. It is native to Europe and Siberia & thrives in mountainous regions with poor, acidic soil. The plant is widely used in herbal medicines for it anti-inflammatory& Analgesic properties, but there is insufficiently high-quality clinical evidence for such effects, & its toxic when taken or applied to internally or applied to injured skin.

Chemical constituents: chemical Structure of helenalin.

The main constituents of *Arnica Montana* are essential oils, fatty acids, thymol; pseudo guaianolide sesquiterpene lactones and flavone glycosides. Pseudo guaianolide sesquiterpene constitute 0.2-0.87 of the flower head of *Arnica Montana.* The quality & chemical constitution of the plant substances -Arnica flowers be monitored by near-infrared spectroscopy. *Arnica Montana* is a flowering plant about 18-60 cm tall aromatic fragrant, herbaceous perennial. Its basal green ovate leaves with rounded tips are bright colored & level to the ground. In addition, they are somewhat downy on their upper surface, veined & aggregated in rosettes. By contrast the upper leaves an opposed, spear shaped & smaller which is an exception within the Asteraceae.

The flowering season is between May & August (central Europe)

The 2 in (5.1cm) flower beds are composed of orange yellow disc florets in the center which are externally bordered by 10-15 yellow ray florets. The achenes have a one-piece rough paper which opens in dry conditions. *Arnica Montana* is a hemicryptophyte, which helps the plant to survive the extreme overwintering conditions of its habitat.

CULTIVATION:

Arnica Montana is propagated from seed. Generally, 20% of seeds do not germinate. For large scale planting, it is recommended to raise plants first in nursery and then to transplant & them in the field. Seeds sprout in 14-20 days but germination rate depends highly on the seed quality. The Flowers are harvested when fully developed & dried without their bract nor receptacles. The roots can be harvested in autumn and dried as well after being carefully washed.

SYMPTOMS OF *Arnica Montana:*

Sore, lame, bruised feeling allows body as if beaten. The pain of Arnica patient that is similar to the pain which produce after getting blood injuries. It is especially suitable in all cases of injury- both in acute& chronic Arnica reduces the pain of affected parts it heals the injured Blood vessels.

FOR INJURIES & ACUTE PAIN:

Arnica is renowned for helping with bumps, bruises, pain & swelling caused by minor acute injuries or muscle overuse. It is commonly used as both a homeopathic medicine and topically as an herbal salve or cream Arnica strongly modulates inflammation and may work by dilating capillaries, which increases blood flow to and from the injury. This increased blood flow brings healing substances to the areas and moves stagnant blood, like you see in bruises. The result decreases swelling and faster healing time. I like to use *Arnica Montana* as soon as the bruises are injury occurs.

FOR CHRONIC PAIN:

In addition to being used for acute injuries, Arnica has can also relieve chronic pain and inflammation like the pain and swelling of arthritic joints. One study compared the use of Arnica gel to Ibuprofen gel in 204 patients diagnosed

with osteo arthritics in their hands. The Study showed that the Arnica gel was just as effective as Ibuprofen at relieving pain intensity, reducing the severity of morning Stiffness & increasing hand function capacity.

SIDE EFFECTS:

In fact, Arnica is considered poisonous. When taken by mouth it can cause vomiting, heart damage, organ failure increased bleeding, coma & death.

PLANT PREPARATION:

Arnica flower can be used fresh or dried & infused into water, Oil, or alcohol. Some herbalists recommend using the leaves, especially if the plant isn't abundant in your areas. First prefer to use Arnica buds, harvesting them just before they open to a full bloom. Dried Arnica buds can retain more of their petals than drying fully opened Arnica flower. Arnica flower, like many plants in the Asteraceae family, can turned into Fluff ball when they are dried. Arnica can be made into a strong tea and used externally as a wash. Herbalists Beverly Gray recommends the tea as a foot bath to relieve tried feet. An infused oil can be made into a salve or cream. A tincture (alcohol extraction) can be used externally as a liniment. Dosage externally is as needed. Sometimes people develop a Sensitivity to Arnica & start to experience redness or irritation.

Issues with Internal uses: -

Problems associated with Arnica's internal use include lesions within the digestive tract & cardio toxicity.

SPECIAL CONSIDERATIONS:

Taking the Arnica internally can lead to serious complication. It is recommended for external use only. It is commonly recommended not to apply Arnica to broken Skin. Touching Arnica while harvesting it can cause contact dermatitis in some sensitive individuals. Some people are allergic to Arnica. The Aim of the study is to evaluation of Analgesic activity of *Arnica Montana* by using Mice models.

OBJECTIVE: Collection of plant material and extraction, Preliminary phytochemical studies.**,** Pharmacological studies.

PLAN OF WORK:

I. Collection of plant material and extraction

- Identification of Plant material.
- Extraction of powdered material with suitable solvents.

II. Preliminary phytochemical studies

- Preparation of various extracts.
- Qualitative phytochemical test for detection of various plant constituents.

III. Pharmacological studies

- *In vivo* screening for the evaluation of analgesic activity.

3. METHODOLOGY

3.1 Experimental design for Analgesic activity:

- The Mice were divided into 4 groups and each group consists of 6 animals.
- Drugs/vehicle for administered to animals prior 60 min to study.
- Group-1: Control administered Normal saline orally.
- Group-2: Receive *Arnica Montana* 100mg/kg body weight orally.
- Group-3: Receive *Arnica Montana* 200mg/kg body weight orally.
- Group-4: Receive standard drug Diclofenac sodium 10mg/kg for body weight orally.

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3.2 Extraction procedure (Maceration):

Powdered material was purchased in online via Amazon Platform.

i. Preparation of plant material:

- Weigh 50 grams of *Arnica Montana* powder.
- Ensure the powder is coarsely powdered, dry, and free from foreign matter.

ii. Selection and Preparation of solvent:

- Select an appropriate solvent methanol.
- Measure 5-10 times the volume of the plant material.

iii. Maceration Process:

- Transfer the *Arnica Montana* to a clean, dry glass container.
- Add the solvent to cover the powder completely.
- Stir the mixture thoroughly to wet all the plant material.
- Close the container with a tight-fitting lid.

iv. Duration and Storage:

- Allow the mixture to stand for 7 days at room temperature, with occasional shaking/stirring.
- Keep the container in a cool, dark place to prevent solvent evaporation and degradation of active compounds.

v. Filtration:

- After 7 days, filter the mixture through Whatman filter paper.

vi. Storage:

- Store the final extract in an amber-colored bottle at low temperature (4-8°C) to avoid degradation.

Figure No: 02 Maceration procedure

3.3 Procedure (Eddy's Hot Plate Method):

a) Select healthy Swiss albino mice (20–25 g) and divide them into 4 groups in each 6 animals.
b) Fast the animals for 12 hours before the experiment with water.
c) Administer vehicle to the control group.
d) Administer *Arnica Montana* extract orally at low dose 100mg/kg BW to one test group.
e) Administer *Arnica Montana* extract orally at high dose 200mg/kg BW to another test group.
f) Administer a standard analgesic drug i.e. Diclofenac sodium to the standard group.
g) Record the baseline reaction time (paw licking or jumping) for all animals before treatment.

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h) Place each mouse on the hot plate maintained at 55 ± 0.5°C inside a glass cylinder.

i) Record the reaction time in seconds after 30, 60, 90, and 120 minutes of drug administration.

j) Maintain a cut-off time of 15 seconds to prevent injury.

k) Compare the increase in reaction time of test and standard groups with control to evaluate analgesic activity.

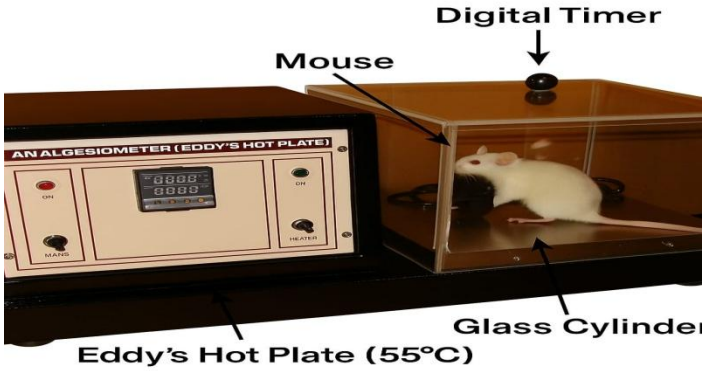

Figure No: 03 Eddy's Hot plate method

3.3. Preliminary phytochemical tests:

A Preliminary phytochemical investigation was carried out for all extracts by following the test methods.

Phytoconstituents	Phytoconstituents extract of *Arnica Montana*
Alkaloids	+
Tannins	+
Saponins	+
Terpenoids	+
Steroids	+
Phenols	+
Sesquiterpenoids	+
Flavonoids	+
Glycosides	-

3.4 DELAYED TOXICITY STUDIES:

In the present study, delayed toxicity evaluation was conducted to assess the long-term safety of *Arnica Montana* methanolic extract following its administration in mice. After completing the analgesic activity experiments, all animals including the control and treatment groups were carefully monitored over an extended period for any signs of delayed toxicity. This included daily observation of physical appearance, behavioural changes.

3.5 EXPERIMENTAL ANIMALS:

For the present study on the evaluation of analgesic activity of *Arnica Montana*, healthy male albino mice weighing between 200–250 grams were used as experimental animals. The animals were procured from a certified supplier and housed under standard laboratory conditions. They were maintained at a temperature of 23°C to 25°C with a relative humidity of 35% to 60% and exposed to a 12-hour light/dark cycle. All animals were fed with standard laboratory pellet diet and provided with water ad libitum throughout the study period. Prior to the commencement of the experiment, the mice were acclimatized to the laboratory environment for a period of 7 days to minimize stress. All experimental procedures were carried out in accordance with the guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India, and the study protocol was duly approved by the Institutional Animal Ethics Committee (IAEC) under the registration number NCOP/VK/318/PO/Re/S/2001/CCSEA/01/2025.

3.6 ACUTE TOXICITY STUDIES:

The acute toxicity study of *Arnica Montana* methanolic extract was conducted to determine the safety and tolerability of the extract following a single oral administration in mice. The study was performed as per the OECD guideline 423 (Acute Oral Toxicity – Acute Toxic Class Method). Healthy male albino mice weighing between 200–250 grams

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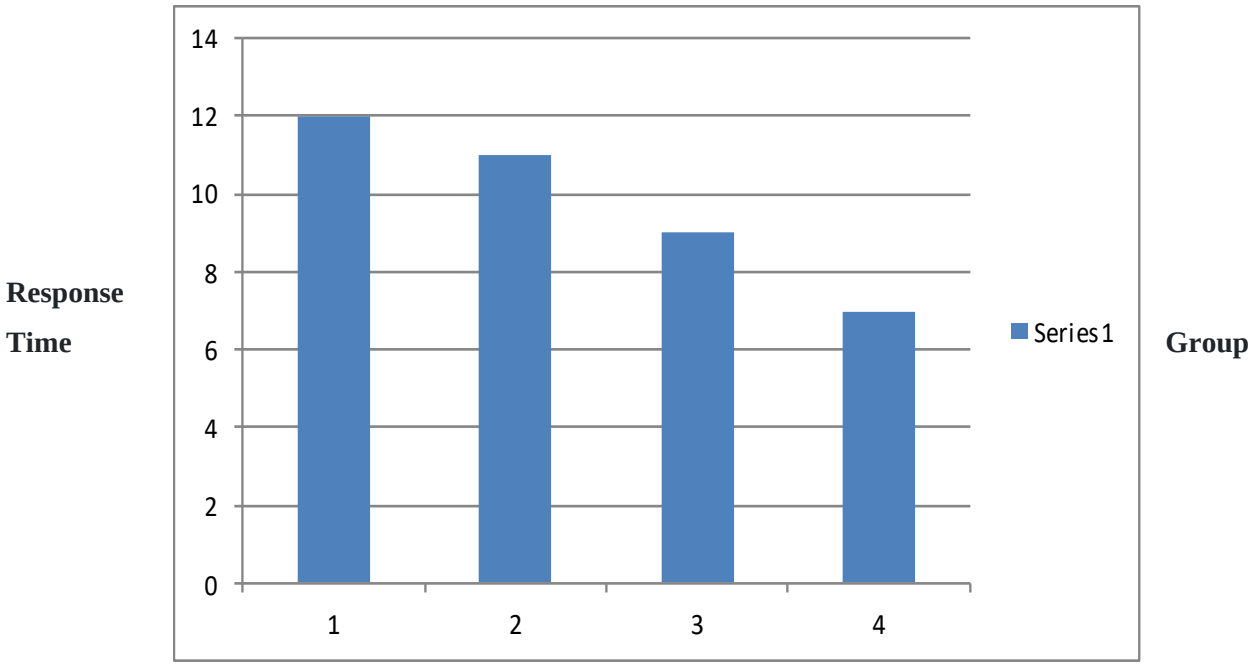
were selected and fasted overnight prior to dosing. A single high dose of 2000 mg/kg body weight of the methanolic extract was administered orally. The animals were then closely observed for the first 4 hours for any immediate signs of toxicity such as changes in skin, fur, eyes, mucous membranes, respiratory and autonomic activity, and behavioural patterns. Further observation was continued for 14 days to detect any delayed effects or mortality. Throughout the study, no toxic signs or behavioural abnormalities were observed, and no mortality occurred at the tested dose. This indicated that the extract of *Arnica Montana* is safe up to 2000 mg/kg and can be considered non-toxic in acute exposure, justifying the selection of lower doses (100 mg/kg and 200 mg/kg) for the analgesic activity evaluation.

4.RESULTS:

Table 1: Measurement of Parameters by using Hot plate method.

Group	Basal Reaction Time (Seconds)		Reaction after drug administration (Seconds)	
	Paw licking(sec)	Jumping Response(sec)	Paw licking(sec)	Jumping response(sec)
Group-1 (Control)	8±1	-	12±1	-
Group-2 (Plant extract 100mg/ml)	-	9±1	11±1	-
Group-3 (Plant extract 200mg/ml)	8±1	-	9±1	-
Group-4 (Standard)	-	8±1	7±1	-



Graph 01: Measurement of parameters observed in Eddy's hot plate method

5. CONCLUSION

The present study on the analgesic activity of *Arnica Montana* using mice models (such as Eddy's hot plate method) demonstrated that *Arnica Montana* exhibits significant Analgesic (pain relieving) effects in a dose dependent manner.

Mice treated with *Arnica Montana* extract showed increased latency time in response to thermal stimuli, indicating a reduction in pain sensitivity compared to the control group. The analgesic effect was more pronounced at higher

dosage and was comparable to the standard drug in some instances, suggested that *Arnica Montana* contains bio active compounds with potentials central and peripheral analgesic properties.

Thus, *Arnica Montana* may serve as a promising natural alternative or complementary agent for pain management, supporting its traditional use in Herbal medicine.

The present study shows that *Arnica Montana*shows better Analgesic activity at 200mg/kg dose of selected plant extract. Significant, Analgesic activity by 200mg/kg higher dose and the presence of Flavonoids, Saponins and Alkaloids are confirmed in the extract and also responsible for Analgesic activity.

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