



# In Vivo Assessment of The Anti-Inflammatory Effects of *Arnica montana* Using Albino Rats

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## ABSTRACT

This study investigates the anti-inflammatory effects of *Arnica montana* using rat models. Utilizing a controlled experimental design, we administered various doses of Arnica extract to male Wistar rats induced with inflammation via formalin or carrageenan injections. Parameters measured included paw edema, biochemical markers of inflammation, and histopathological changes. Results indicated a significant reduction in swelling and inflammatory markers in treatment groups compared to control. These findings support *Arnica montana's* potential as a natural anti-inflammatory agent and highlight its therapeutic value in managing inflammatory conditions. Further studies are warranted to elucidate its mechanisms of action.

**KEY WORDS:** Anti-inflammation, *Arnica montana*, preliminary phytochemical constituents, diclofenac, Glacial acetic acid.

## 1. INTRODUCTION

### 1.1 INFLAMMATION:

Inflammation is an important physiological reaction which occurs in response to a wide variety of injurious agents (example: bacterial infection, physical trauma, chemicals or any other phenomenon) ultimately aiming to perform the dual function of limiting damage and promoting tissue repair. Inflammatory processes are required for immune surveillance, optimal repair, and regeneration after injury. The inflammatory process protects our body from diseases by releasing cells and mediators that combat foreign substances and prevent infection. However, sustained, excessive or inappropriate inflammation is the cause of numerous diseases including rheumatoid arthritis, psoriasis and inflammatory bowel disease. Inflammation is a major component of the damage caused by autoimmune diseases, and is a fundamental contributor of various infectious and non-infectious diseases such as cancer. Diabetes cardiovascular disease, rheumatoid arthritis, Alzheimer's and arteriosclerosis. Depending on the intensity of this process, mediators generated in the inflammatory site can reach the circulation and cause fever<sup>1</sup>.

Inflammation is a complex pathophysiological process mediated by a variety of signaling molecules produced by leucocytes, macrophages and mast cells undergoing various cellular responses such as phagocytic uptake, and the production of inflammatory mediators such as nitric oxide (NO), prostaglandin E2 (PGE2) and tumor necrosis factor (TNF)- $\alpha$ , that bring about edema formation as a result of extravasation of fluid and proteins and accumulation of leucocytes at the inflammatory site. In addition, it is broadly accepted that cytokines. Produced by either immune or central nervous system cells, might directly sensitize the peripheral nociceptors<sup>2</sup>.

### Inflammatory mediators

The inflammatory response is a complex and highly regulated sequence of events that start with an initial production of pro-inflammatory mediators that recruit professional inflammatory cells to the site of injury to clear the offending trigger. Macrophages play major roles in the immune and inflammatory responses involved in host defence. Activated macrophages secrete a number of different inflammatory mediators, including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-18 (IL-18), interleukin-6 (IL6), reactive oxygen species (ROS), prostaglandin E2 (PGE2), nitric oxide (NO), etc.<sup>3</sup>

## 2. PLANT PROFILE

**Plant:** *Arnica montana*

**Common name** - Leopard's Bane

**Discovered By** - Hahnemann

**Family Name** - Asteraceae

**Mode of preparation**<sup>4,5</sup>:

We use the roots, flowers & leaves. Before using the flower's, we should clean them of the eggs, (*musca arnicae*) of a peculiar parasitic insect Which infests this plant, called Arnica, a species of staphylinus.

- We prepare the powder of the root by making three attenuations by trituration or dissolve "if in twenty parts of alcohol to make the tincture.
- Arnica is a perennial, plant; we gather the fresh plant. When it flower's in July and August to use it to make a tincture.
- For the fresh plant, we express the juke of the whole plant and then mix it with equal parts of alcohol to make the tincture.
- We must be careful not to expose the root of this plant too long in air because it loses a portion of its strength, but powdered root may be kept for long periods in well stopped bottles.
- Arnica is often used in gel (or) lotion form. This can apply topically to the affected area.

**Symptoms of *Arnica montana***

- Sore, Lame, Bruised feeling alloves Body as if beaten.
- The pain of Arnica patient that is similar to the pain which produce after getting blood injuries.
- The patient is oversensitive to pain Arnica for injury.
- It is especially suited in all cases of injury both Acute and Chronic.
- Arnica reduces the pain of affected parts.
- It heals the injured blood vessels

**Acute injuries** - Which are caused by:

- Blunt instrument
- Blow fall
- Confusion
- Traumatic and mechanical injuries
- Accident

During acute injuries the patient is very much sensitive to touch.

After affected part's look's Bluish Blackish in color

**Chronic injuries** - which are received from years ago now still causing some symptoms, discomfort in a patient.

- Master Samuel Hahnemann has recommended of Arnica in injury even received years ago.
- Chronic injuries results in constant pain, Tremors, Rheumatism, paretic & paralytic condition Arnica work's wonderfully in these cases. It cures all the symptoms from diseases.

**Arnica and hair fall**

- Its work application Local of *Arnica* produces extraordinary Growth of hair and prevents hair falling.
- Arnica is used topically for wide range of conditions, including bruises, sprains, muscle aches, wound healing, joint pain, inflammation from insect bites, and swelling from broken bones more recent studies suggest it may also be helpful in the treatment of burns.
- In addition to its. Anti-inflammatory properties, Arnica montana has been shown to have pain relieving effects.
- It's often used to alleviate headaches, toothaches and other types of pains.
- While *Arnica montana* is generally, considered Safe, it's important to use it as directed and consult with a health care professional.



**Fig. No: 1** *Arnica montana* plant

***Arnica montana* description<sup>6-8,9-10</sup>:**

**Color:** Bright yellow petal ray flower's surrounding a central cluster of small, yellow tubular disk flowers.

**Odour:** Discreet of neutral with a hint of cut grass

**Taste:** The taste is acrid, bitterish, and durable.

**Height:** Grows to 1 to 2 feet tall.

**Flowers:** Large, bright yellow or orange, daisy-like flowers with numerous ray florets and disc florets.

**Stems:** Round and hairy, with 1 to 3 flower stalks.

**Leaves:** Bright green, with basal leaves having rounded tips and stem leaves being lance-shaped.

**Rhizome:** Has a woody, brownish, horizontal rhizome.

**Habitat:** Moist, grassy upland meadows in the hills and mountains of northern and central Europe and Siberia. Prefers slightly alkaline, moist, sandy loam in well-drained locations in full sun to partial shade.

**Medicinal Uses:** Dried flower heads have been used in herbal medicine for centuries.

**Aim-** Aim of the study is evaluation of anti-inflammatory activity of *Arnica montana*.

**Objective-** The collection of plant material and extraction. preliminary phytochemical studies, pharmacological studies:

**1. Plan of work**

- Procedure of powder *Arnica Montana*.
- Extraction of powder material with suitable solvents.

**2. Preliminary phytochemical studies**

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

**3. Pharmacological studies**

- *In-vivo* screening for the evaluation of Anti-inflammatory activity.

### 3. METHODOLOGY

#### 1. Collection of Plant Material

Collection of plant material & extraction of fresh plant *Arnica montana* was collected in Nalgonda & were authenticated by Dr. K. N Venkateshwara Rao sir department of Pharmacognosy. A voucher specimen was deposited in the college herbarium. The Whole plant was shade dried and coarsely powdered. The coarse powder was subjected to extraction with solvents of Ethanol, methanol, chloroform, n-hexane, benzene by Maceration process and extract was concentrated dryness in a vacuum.

#### 2. Preparation of Plant Material

Once collected, the entire plant was subjected to a thorough cleaning process to remove any contaminants or debris. After cleaning, the plant material was air-dried in shade to prevent degradation of sensitive phytochemicals. Once completely dry, the plant was finely ground into a coarse powder using a mortar and pestle or mechanical grinder.

#### 3. Extraction Process

The coarse powder of *Arnica montana* was subjected to a series of solvent extractions to isolate its bioactive compounds. The extraction was performed using different solvents, including ethanol, methanol, chloroform, and n-hexane, to assess the efficacy of each in extracting valuable compounds.

**Solvent Selection:** Each solvent was selected based on its polarity, with polar solvents like ethanol and methanol expected to extract polar compounds, while non-polar solvents like chloroform and n-hexane were aimed at extracting non-polar compounds.

**Extraction Procedure:** The powdered material was soaked in each solvent for [specific time, e.g., 24 hours) in a closed container. The mixture was then filtered using filter paper to separate the liquid extract from the solid residue.

#### 4. Concentration of Extracts

The filtered extracts were concentrated using a rotary evaporator at a controlled temperature (generally below 40°C) to avoid thermal degradation of sensitive components. The final extracts were stored in airtight containers under refrigeration until further analysis

#### 5. Assessment of Extracts

The extracts obtained were then subjected to qualitative and quantitative analysis to determine the presence of phytochemicals such as alkaloids, flavonoids, tannins, and essential oils. Various analytical techniques (e.g., chromatography, spectrophotometry) may be employed to identify and quantify these compounds.

#### 6. Safety and Ethical Considerations

All methods used in this research adhered to safety guidelines and ethical considerations regarding the collection and use of plant materials.

### 4. MATERIALS AND METHODS

#### MATERIALS:

1. **Raw material:** Dried flowers of *Arnica montana* ensure they are sourced from a reliable supplier to guarantee quality.
2. **Solvent:** suitable solvent for extraction, commonly used solvents are Ethanol (ethyl alcohol), olive oil (for oil infusion), Glycerine (for glycerite preparation)
3. **Equipment:** Glass jar for extraction, Filter paper Measuring cylinder, Glass rods, Dark glass bottles for storage, Beaker, PH meter
4. **Dosing equipment:** syringe and feeding tube.
  - Monitoring equipment-recording behavior of rats.
  - Safety equipment - rat's holder, handling equipment, lab coat, gloves.

## 5. Maceration extraction method.

- Plant material
- Solvent
- Container
- Measuring tools/cylinder
- Glass rod
- Filter paper
- Storage bottles
- Labeling materials

## 6. Administration requirements:

- **Extracted drug (arnica montana)**
- **Rat models** - Healthy rats like age, weight of status.
- **Dosing method** - Oral (or) subcutaneous injection (or) topical application
- **Dosing equipment** - Syringe and feeding tube
- **Control group**
- **Ethical approval**- treatment of animal Subjects.
- **Monitoring equipment** - scales for measuring weight and recording behavior of rats
- **Data recording materials** - digital devices, Note books for observation's
- **Safety Equipment** - personal protective equipment for researchers handling the rats and the extract.

## Material requirements

- **Animals**
- **Rat's** - Sufficient number of rat's
- **Housing** - Appropriate cages with bedding, food and water for the rat's
- **Surgical instruments** - (e.g.: cotton pellet-induced granuloma). (glove's)
- **Arnica montana**
- **Standardized extract** - (arnica montana)
- **Tincture** - Tincture of Arnica montana with a known concentration
- **Vehicle** – (e.g. water, oil, saline)



Fig. No: 2 Arnica montana powder (plant extract)

## Inflammation induction materials

- Acetic acid induced paw edema method
- Formalin induced paw edema.
- Cotton pellet's

### 1. Measurement Tools

- **Plethysmometer** - To measure paw swelling.
- **Microscope** - for histological analysis to examine tissue samples.
- Paw Edema meter
- Thermometer.

### 2. Additional materials

- **Syringes and Needles** - For injecting
- **Gavage Needles** - For oral administration.
- **Weighing Scale** - To weigh the rats and record their body weight
- **Lab coat**- For safety and hygiene.
- **Gloves** - for handling the rat's and chemicals
- **Sterile containers** - for storing and preparing Solution's (vials & tubes).
- Data sheets & recording equipment.

## Acute toxicity study

1. **Objective** - Evaluate the acute toxicity of *Arnica montana* extract in rats.
2. **Study design**- Randomized, controlled study.
3. **Animals**- Healthy adult Albino rat's (200-250 grams)
4. **Dosing** - *Arnica montana* extract (100, 200, 400, 300 mg administered orally (or) intraperitoneally (or) Subcutaneous.
5. **Observations**-monitor for signs of toxicity
6. **Duration** - 14 days.
7. **End points**- Lose clinical signs body weight mortality.
  - The anti-inflammatory effect of *Arnica Montana* 6CH was evaluated using acute & chronic inflammation models
  - In the acute, model, carrageenan – Induced rat paws edema, the group treated with *Arnica montana* 6CH showed 30% inhibition composed to control (p<0.05).
  - Treatment with *Arnica* 6CH, 30 min prior to carrageenan, did not produce any inhibition, of the inflammatory process.
  - In the chronic model, Nystatin- Induced edema, the group treated 3 day's previously with *Arnica montana* 6CH had reduced inflammation 6h after the inflammatory agent was applied.
  - When treatment was given 6h after Nystatin treatment, there was no significant inhibitory effect.
  - In a model on histamine- Induced increase of vascular permeability, pretreatment with *Arnica montana* 6CH blocked the action of histamine in increasing vascular permeability.

## METHODS:

**Table No: 01 Animal grouping**

Groups	Group Name	Objective	Methodology
Group-1	Control group	Establish a base line for inflammation.	<ul style="list-style-type: none"> <li>• Select a group of rats and do not administer any treatment.</li> <li>• Induce inflammation using a method like Acetic acid injection in to the paw.</li> <li>• Measure the paw volume before and after the inflammation, induction at set intervals (e.g.: 30,60,120 min post induction)</li> </ul>

Group-2	Standard group	Compare the effectiveness of the test substance against a known Anti-inflammatory.	<ul style="list-style-type: none"> <li>Select a group of rats and administer a standard Anti-Inflammatory drug like Diclofenac at a therapeutic dose.</li> <li>Induce inflammation as in group A.</li> <li>Measure the paw volume at the same intervals as the Control group to assess the drug's effectiveness in reducing inflammation.</li> </ul>
Group-3	Test group-1	Evaluate the anti-inflammatory of a lower dose of the test substance.	<ul style="list-style-type: none"> <li>Select a group of rats and administer a lower dose of the test substance after inducing inflammation.</li> <li>Measure the paw edema volume at the same intervals as the other groups</li> <li>Record any observable behavioral changes, such as signs of pain or discomfort.</li> </ul>
Group-4	Test group-2	Assess the effect of a higher dose of the test substance.	<ul style="list-style-type: none"> <li>Select a group of rats and administer a higher dose of the test substance after inflammation.</li> <li>Measure the paw volume and any behavioral changes at the same intervals as the other groups.</li> <li>Compare the results to both the control and standard drug groups to evaluate the efficacy of the higher dose.</li> </ul>

## PROCEDURE

### ANIMAL GROUPING:

Select 24 male Albino Rat's and divide them in to 4 groups, which are as follows;

- i. Group 1 (control) - 6 Rats – Receiving normal saline.
- ii. Group 2 (standard) - 6 Rats-Receiving Diclofenac 100mg/kg body weight.
- iii. Group 3 (plant extract 1)-6 Rats-Receiving 100mg/kg body weight.
- iv. Group 4 (plant extract 2) -6 Rats - Receiving 200mg/kg body weight.



**Fig no: 3 Animal grouping**

## PREPARATION'S:

### A. Preparation of *Arnica montana* Extract

- weigh the required amount of *Arnica montana* Powder (e.g.: 100mg/kg body weight)
- Mix it in distilled water to form a uniform solution.
- Stirr well to avoid sedimentation.
- Cover the beaker with aluminum foil.

### B. Maceration

- Macerate the powder with 70% ethanol of 72 hr's.
- Filter and evaporate Solvent to get the dry extract.
- Transfer the extract in to Petri dish and cover with aluminum foil.



**Fig. No: 4 Weight of *Arnica montana* powder**



**Fig. No: 5 Maceration**



**Fig. No: 6 Filtration**



**Fig. No: 7 Extract evaporation**

### C. Preparation of 1% Glacial acetic acid

- Take 1 ml of glacial acetic acid solution and dissolve it in to 100 ml of distilled water.

### D. Preparation of Diclofenac

- Take 75 mg/ml of diclofenac ampoule and dissolve it in with 10 ml of distilled water to formulate 10 mg/ml.

## ACETIC ACID INDUCED – PAW OEDEMA METHOD:

### Requirements:

**Rats-** (150-250gm Weight)

**Drugs** – 0.1ml of 1% v/v of acetic acid, 0.2ml of 75mg/ml Diclofenac

**Equipment** – plethysmometer



**Fig. No: 8 Plethysmometer**

- It is an equipment used to measure the paw volume
- A rat to perform the Anti-inflammatory Activity.

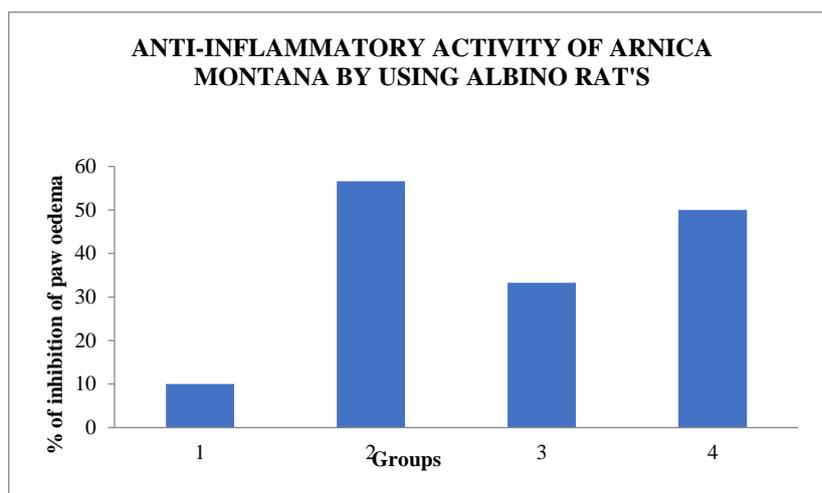
## RESULTS

**Table No: 01 Phytochemical tests**

Phytoconstituents	Phytoconstituents extract of Arnica Montana
Flavanoids	+
Tannis	+
Saponins	+
Glycosides	-
Terpenoids	+
Steroids	+
Phenols	+
Alkaloids	+

**Table No: 03 Anti-inflammatory activity by phelthysmometer.**

Treatment	Dose	30 Minutes		60 Minutes		120 Minutes	
		ml	%	ml	%	ml	%
Control	Vehicle	0.6 ml	100 %	0.6 ml	100 %	0.6 ml	100 %
Standard (Diclofenac)	10 mg/kg	0.4 ml	66.6 %	0.38 ml	65.5 %	0.34 ml	56.6 %
Test – 1	100 mg/kg	0.26 ml	43.3 %	0.25 ml	43.1 %	0.2 ml	33.3 %
Test – 2	200 mg/kg	0.36 ml	60 %	0.35 ml	58.3 %	0.3 ml	50 %



**Fig No:8 Anti-inflammatory activity of *Arnica montana* by using Albino Rat's**

## 7. CONCLUSION

The result obtained in Present study have shown the Anti-inflammatory activity of *Arnica montana*, the present study contains flavonoids, flavonoids are mostly responsible for reducing inflammation, the effect might be due to inhibition either inhibiting lysosomal enzymes, stabilizing the membrane, or by altering the endogenous, factor that are involved in migration of these substances to the inflammation, the present study the selected plant significantly shows in decreasing paw Edema in comparison with standard drug. These fundings are further support between the inflammatory markers and inflammatory activity. In selected plant (200mg/ kg) dose from the plant extract shows significantly decreasing paw Edema, so the plant exhibits better anti-inflammatory activity with is concentration.

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