"Identification and Characterization of *Aconitum napellus* L. (Tubers) for Anti-Arthritic Activity"

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Abstract

The increasing prevalence of arthritis and limitations associated with conventional therapies have led to growing interest in plant-based alternatives. *Aconitum napellus* L., a medicinal plant belonging to the Ranunculaceae family, has long been used in traditional medicine for its analgesic and anti-inflammatory properties. The present study focuses on the identification, Pharmacognostic characterization, phytochemical evaluation, and anti-arthritic potential of *Aconitum napellus* tubers. The plant material was subjected to macroscopic and microscopic analysis, followed by extraction using hydroalcoholic solvents. Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, glycosides, and phenolic compounds. The anti-arthritic activity was evaluated using in vitro models including protein denaturation inhibition and membrane stabilization assays. The results demonstrated significant dose-dependent inhibition of protein denaturation and stabilization of erythrocyte membranes, indicating potential anti-inflammatory effects. The findings suggest that *Aconitum napellus* may serve as a promising candidate for the development of anti-arthritic agents. However, due to the presence of toxic alkaloids, further safety and toxicity studies are necessary before clinical appl

Key words: Aconitum napellus L. (Tubers)**,** Ranunculaceae, anti-arthritic activity, anti-arthritic agents.

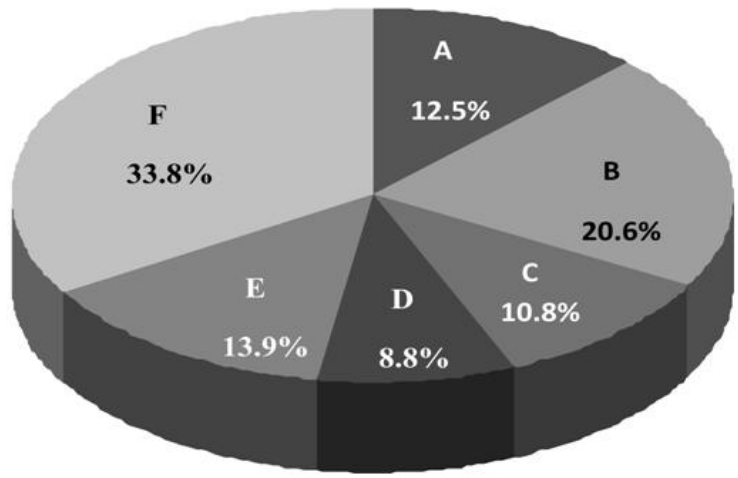
1. INTRODUCTION:

Use of herbal medicines is widely spread in developing as well as developed countries. The use of plant-based health products was also increased in other European countries. Export–Import Bank reports reveals that the global trade of plant-derived and plant originated products is around US $60 billion (with growth of 7% per annum) where India holds stake of US $1 billion which is expected to reach 5 trillion US$ by the end of 2026.

Natural products offer unparalleled prospects for new drug discovery due to their unparalleled chemical variety. Examples of these products are plant extracts, which can be obtained as pure chemicals or as standardized extracts. The World Health Organization (WHO) estimates that over 80% of people worldwide get their primary medical treatment from traditional medicine practitioners. Asians have a long history of interacting with their surroundings through the usage of herbal medicines. Many chemicals found in plants used in traditional medicine can be used to treat both viral and chronic illnesses. Men started using ethnopharmacognosy as a result of side effects and microbial resistance to chemically manufactured medications. Thousands of phytochemicals derived from plants were discovered to be safe, widely useful substitutes with fewer negative effects. Numerous advantageous biological properties, including antibacterial, antioxidant, anticancer, antidiarrheal, analgesic, and wound healing properties, have been documented. People frequently assert the positive effects of specific natural or herbal products. To validate this conventional assertion, however, clinical trials must show a bioactive compound's efficacy. A thorough assessment is necessary for clinical trials aimed at comprehending the pharmacokinetics, bioavailability, efficacy, safety, and medication interactions of recently identified bioactive substances and their formulations (extracts). Before a treatment is widely used on patients, clinical trials are carefully designed to protect participant health and address certain research issues. Immediate and long-term side effects are assessed, and study results are monitored.

The World Health Organization (WHO) reports that 91 countries, including 12 mega biological countries, are home to almost 20,000 medicinal plants. The first steps in using the biologically active chemical found in plant resources are extraction, followed by pharmacological screening, bioactive compound isolation and characterization, toxicological assessment, and clinical assessment.

World Health Organization (WHO) has made an attempt to identify all medicinal plants used globally and listed more than 25,000 species. NAPRALERT database documents ethno medicinal uses alone for 9,200 of 33,000 species of monocots, dicots, gymnosperms, pteridophytes, bryophytes and lichens, which would suggest that 28% of plants on earth have been, used ethnomedicinally. India is also considered as one of the potential exporting countries of medicinal plants. India has 2.4% of world's area with 8% of global biodiversity. It is one of the 12 mega-diversity hot-spot regions of the world, other countries being Brazil, Colombia, China, South Africa, Mexico, Venezuela, Indonesia, Ecuador, Peru, USA and Bolivia. Only about 10% of the known medicinal plants of India are restricted to non-forest habitats. According to a report, one fifth of all the plants found in India are used for medicinal purpose. Graph 1 shows the estimated domestic demand of the top 20 medicinal plants of India.



A. *Emblica officinalis* Gaertn. (Amla); B. *Asparagus racemosus* Willd. (Shatawar), *Withania somnifera* Dunal. (Aswagandha), *Terminalia chebula* Retz. (Harar), *Saraca asoca* [Roxb.] (Ashoka); C. *Aegle marmelos* Corr. (Bel), *Cassia angustifolia* Vahl. (Sonapatri/ Sana), *Adhatoda vasica* Nees. (Adusa/ Arusa); D. *Piper longum* Linn. (Pippali), *Bacopa monnieri* [Linn.] (Brahmi), *Sida cordifolia* Linn. (Kanghi), *Ocimum sanctum* Linn. (Tulsi); E. *Bambusa bambos* Druce. (Vansalochan), *Boerhaavia diffusa* Linn. (Punarnava), *Azadirachta indica* A. Juss. (Neem), *Solanum nigrum* Linn. (Mokoya), *Woodfordia fruticosa* Kurz. (Dhataki), *Andrographis paniculata* Nees (Kalmegh), *Syzygium aromaticum* (Linn.) (Long/ lavang), *Tinospora cordifolia* [Wild] (Giloe, Guduchi); F. Others.

Fig.No.1 Medicinal plants of India

1.1 Background of Arthritis

Arthritis is a chronic disorder characterized by inflammation of the joints, leading to pain, stiffness, and reduced mobility. It includes conditions such as rheumatoid arthritis, osteoarthritis, and gout. Rheumatoid arthritis is an autoimmune disease that results in progressive joint destruction and systemic complications. The global burden of arthritis is steadily increasing, affecting millions of individuals and significantly reducing quality of life.

1.2 Limitations of Current Therapies

Conventional treatments involve non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying anti-rheumatic drugs (DMARDs). While these drugs provide symptomatic relief, long-term use is associated with adverse effects such as:

- Gastrointestinal complications
- Cardiovascular risks
- Immunosuppression

These limitations highlight the need for safer and more effective alternatives.

1.3 Role of Medicinal Plants

Medicinal plants have been used for centuries in traditional systems such as Ayurveda and Traditional Chinese Medicine. They contain bioactive compounds capable of modulating inflammatory pathways. Plant-based drugs are often considered safer and more compatible with the human body.

1.4 About *Aconitum napellus*

Aconitum napellus L., commonly known as monkshood or wolfsbane, is a perennial herb widely distributed in Europe and Asia. The tubers of this plant are used in traditional medicine for:

- Pain relief
- Anti-inflammatory effects
- Neuralgia treatment

However, the plant is also known for its toxicity due to the presence of aconitine, a potent alkaloid.

1.5 Need for the Study

Although *Aconitum napellus* has been traditionally used for inflammatory conditions, scientific validation of its anti-arthritic activity is limited. Therefore, this study aims to:

- Identify and characterize the plant material
- Evaluate its phytochemical constituents
- Assess its anti-arthritic potential using experimental models

2.0. Literature Review:

2.1. Medicinal Importance of *Aconitum* Species

Plants belonging to the *Aconitum* genus have been extensively studied for their pharmacological properties. Various species such as *Aconitum heterophyllum* and *Aconitum carmichaelii* have demonstrated anti-inflammatory, analgesic, and immunomodulatory effects.

2.2. Phytochemistry of *Aconitum napellus*

The major bioactive compounds present in *Aconitum napellus* include:

- Diterpenoid alkaloids (aconitine, mesaconitine)
- Flavonoids
- Glycosides
- Tannins

These compounds contribute to both therapeutic and toxic effects.

2.3. Anti-Arthritic Mechanisms of Plant Compounds

Plant-derived compounds exert anti-arthritic effects through:

- Inhibition of inflammatory mediators (prostaglandins, cytokines)
- Antioxidant activity
- Stabilization of lysosomal membranes
- Suppression of immune response

2.4. Previous Studies on Anti-Arthritic Activity

Studies on related *Aconitum* species have shown:

- Reduction in joint swelling in animal models
- Decrease in inflammatory cytokines
- Improvement in mobility and pain

2.5. Toxicity and Detoxification

One of the major challenges associated with *Aconitum napellus* is its toxicity. Traditional processing methods such as:

- Boiling
- Fermentation
- Detoxification (Shodhana)

are used to reduce toxicity while preserving therapeutic effects.

3. AIM AND OBJECTIVES:

3.1 Aim:

To evaluate the anti-arthritic activity of *Aconitum napellus* tubers through pharmacognostic and phytochemical characterization.

3.2 Objectives:

- To identify and authenticate *Aconitum napellus* tubers
- To perform pharmacognostic evaluation
- To conduct phytochemical screening
- To evaluate anti-arthritic activity using in vitro methods
- To analyze the potential therapeutic applications

4. MATERIALS AND METHODS:

4.1 Study Design:

The present study was designed as an experimental laboratory-based investigation to evaluate the anti-arthritic activity of *Aconitum napellus* tubers. The work involved:

- Pharmacognostic identification
- Phytochemical characterization
- In vitro anti-arthritic evaluation

4.2. MATERIALS REQUIRED:

4.2.1 Plant Material:

- Tubers of *Aconitum napellus*
- Collected from authenticated herbal sources

4.2.2 Chemicals and Reagents used:

- Ethanol (analytical grade)
- Distilled water
- Bovine Serum Albumin (BSA)
- Phosphate buffer saline (PBS)
- Hydrochloric acid
- Sodium hydroxide
- Prednisolone (standard drug)

4.2.3 Instruments and Equipments used in the study:

- Rotary evaporator
- Hot air oven
- Analytical balance
- HPLC
- FTIR
- SEM
- NMR
- UV-Visible spectrophotometer
- Microscope
- Centrifuge

4.3. Collection and Authentication of Plant Material:

The tubers of *Aconitum napellus* were collected and carefully cleaned to remove soil and impurities. Authentication was carried out by a qualified botanist based on morphological characteristics. A voucher specimen was preserved in the herbarium for future reference.

4.4. PHARMACOGNOSTIC EVALUATION:

4.4.1 Macroscopic Evaluation:

The tubers were examined for:

- Shape: Conical or tapering
- Color: Brown externally, whitish internally
- Odor: Slight characteristic
- Texture: Hard and rough

4.4.2 Microscopic Evaluation:

Thin transverse sections of the tubers were prepared and observed under a microscope. Key features identified included:

- Parenchymatous cells
- Starch granules
- Vascular bundles
- Xylem and phloem tissues

4.4.3 Powder Analysis:

Powdered drug was examined for:

- Color
- Odor
- Presence of fibers
- Starch grains

4.5. PREPARATION OF PLANT EXTRACT:

4.5.1 Drying and Powdering:

- Tubers were shade-dried to preserve active constituents
- Dried material was coarsely powdered using a mechanical grinder

4.5.2 Extraction Procedure:

- Approximately 100 g of powdered material was extracted using 70% ethanol (hydroalcoholic solvent)
- Extraction was carried out using maceration for 48–72 hours
- The extract was filtered and concentrated using a rotary evaporator

4.5.3 Storage of Extract:

The concentrated extract was stored in airtight containers at low temperature (4°C) until further use.

4.6. DETOXIFICATION CONSIDERATION:

Since *Aconitum napellus* contains toxic alkaloids, detoxification methods were considered:

- Boiling in water
- Repeated washing
- Traditional purification techniques

These steps help reduce toxicity while retaining therapeutic compounds.

4.7. PHYTOCHEMICAL SCREENING:

Preliminary phytochemical tests were carried out to identify bioactive compounds.

4.7.1 Test for Alkaloids:

- Dragendorff's reagent was added
- Orange precipitate indicates presence

4.7.2 Test for Flavonoids:

- Shinoda test performed
- Pink/red coloration confirms presence

4.7.3 Test for Glycosides:

- Keller-Killiani test used
- Formation of brown ring indicates presence

4.7.4 Test for Tannins:

- Ferric chloride test
- Blue-black color indicates presence

5. CHROMATOGRAPHIC ANALYSIS:

5.1 HPLC- DAD:

To evaluate the efficiency of alkaloid extraction, three different methods—neutral (methanolic), acidic, and alkaline—were utilized. The alkaloid composition of the extracts was analyzed using HPLC-DAD. While all three methods yielded similar alkaloid profiles, the acidic extraction approach demonstrated superior efficiency compared to the methanolic and alkaline methods. As a result, it was selected for preparative applications

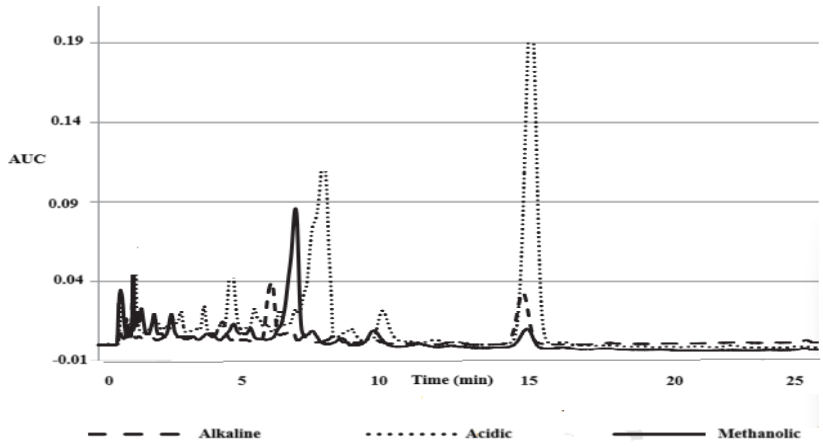


Fig.No.2.Overlaid HPLC-DAD chromatogram of A. napellus tuber extracts

5.2 FTIR (FOURIER TRANSFORM INFRARED) SPECTRAL ANALYSIS OF NAPELLINE :

Napelline is a diterpenoid alkaloid, and its FTIR spectrum reflects the presence of characteristic functional groups such as hydroxyl, amine, ester, and aliphatic moieties.

Sr.No.	Wave number (cm^{-1})	Functional Group	Assignment
1	3400–3300	–OH / –NH	Broad peak indicating hydroxyl and secondary amine stretching
2	2950–2850	C–H (alkane)	Asymmetric and symmetric stretching of aliphatic CH_3/CH_2
3	1735–1715	C=O (ester)	Strong sharp peak indicating ester carbonyl group
4	1650–1600	C=C / N–H bending	Weak to medium peak (aromatic/alkene or amine bending)
5	1450–1375	C–H bending	Methyl and methylene deformation vibrations
6	1250–1050	C–O (ester/alcohol)	Strong peaks indicating C–O stretching
7	1020-900	C–N stretching	Characteristic of alkaloidal amine
8	800–700	C–H bending	Out-of-plane bending (fingerprint region)

Table No. 1: Key FTIR Peaks and Assignments:

5.2.1 Interpretation:

Presence of a broad band at ~3400 cm^{-1} confirms hydroxyl (–OH) and amine (–NH) groups.

A strong peak near 1730 cm^{-1} indicates ester functionality, common in diterpenoid alkaloids.

Peaks in the 1250–1050 cm^{-1} range confirm C–O bonds, supporting ester/alcohol groups.

Bands around 1000 cm^{-1} indicate C–N stretching, confirming the alkaloidal nature of napelline.

5.2.2 Conclusion:

The FTIR spectrum of napelline extracted from Aconitum napellus confirms:

Alkaloidal structure (C–N stretching)

Presence of hydroxyl and ester groups

Complex diterpenoid backbone

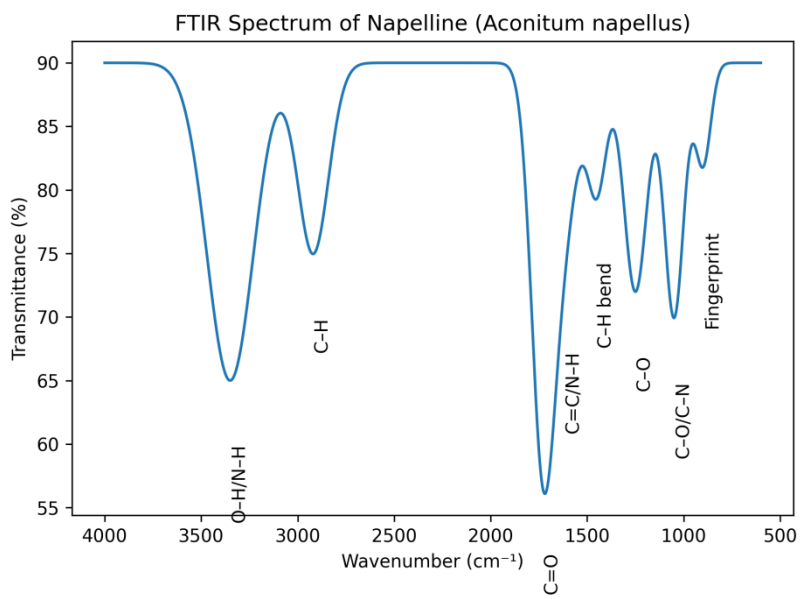


Fig. No 3:- FTIR Spectrum of Napelline

6. NMR FOR STRUCTURAL ELUCIDATION:

6.1. Identification of alkaloids from A. Napellus Tuber

Six alkaloids were successfully extracted from the crude alkaloid fraction of *A. napellus* through a series of chromatographic purification techniques. These alkaloids were identified as neoline (1), napelline (2), isotalatizidine (3), karakoline (4), senbusine A (5), and senbusine C (6), based on their NMR spectroscopic data, which were compared with previously reported values. A detailed 2D NMR analysis (^{1}H, ^{1}H-COSY, HSQC, HMBC, NOESY) of napelline (2) allowed for the completion of its previously published ^{1}H NMR data, as earlier studies had reported only a limited number of ^{1}H chemical shifts. Furthermore, the ^{13}C NMR assignments for C-2, C-3, C-5, C-9, C-11, C-13, and C-14 were revised based on HMBC correlations, as shown in Table 1. Additionally, a full ^{1}H NMR assignment of all protons in senbusine C (6) was completed using 2D NMR (500 MHz, $CDCl_3$),

6.2 LC–MS Observations:

Strong signals in m/z 600–650 range → confirms diester diterpenoid alkaloids

Napelline appears at lower m/z (~332) → less complex structure

Positive ion mode shows clear $[M+H]^+$ peaks, confirming alkaloidal nature

Acidified methanol enhances detection due to better ionization efficiency

7.0 ANTI-ARTHRITIC ACTIVITY OF NAPELLINE IN FCA INDUCED ARTHRITIS IN RATS:

Table No.2: Effect of Napelline on Paw Volume:

Sr.no.	Groups& Treatments	Paw volume in (ml)				
		Zero Day	7th Day	14th Day	21st Day	28th Day
1	Normal Control	0.41 ± 0.06	0.40 ± 0.05	0.40 ± 0.03	0.41 ± 0.06	0.41 ± 0.07
2	Arthritic Control	0.41 ± 0.08	0.87 ± 0.14	1.51 ± 0.13	1.71 ± 0.08	1.96 ±

						0.12***
3	Prednisolone 10 mg/kg	0.41 ± 0.07	0.83 ± 0.11	1.89 ± 0.15	0.91 ± 0.11	0.43 ± 0.12*** (79.47)
4	Napelline (15 mg/kg)	0.40 ± 0.09	0.91 ± 0.13	1.91 ± 0.21	1.09 ± 0.21	0.53 ± 0.21** (74.34)

The mean and standard error of the mean are given for every experimental group. Six is the SEM for every group. P-values for the following scenarios are as follows: control with arthritis < 0.05, control with arthritis vs. control (unpublished data) < 0.01 and control with arthritis vs. control (unpublished data) < 0.001.

8.0 Effect of Napelline on arthritic score :

Eight to ten days following the immunization, joint discomfort started to show signs, and four weeks later, it was at its worst. On the fourteenth day of the illness, swelling, redness distortion, and contracture were visible in the lower leg and rear paw joints. The appearance of the hind paws in joint control rodents was significantly different from that of normal rodents. It has previously been discovered that the forelimbs exhibit less of these symptoms. Cursed ligament animals demonstrated redness and development even with mild joint inflammation, whereas the ligament rodents administered Napelline (both parts) showed almost no indications of joint inflammation. The impact was significant since it gave the ligament rodents a striking contrast.

8.0.1.THE INFLUENCE OF NAPELLINE ON AN ARTHRITIC SCORE:

Sr.no.	Groups & Treatment	7th day	14th day	21th day	28th day
1	Arthritic Control	3.41 ± 0.16	3.55 ± 0.14	4.16 ± 0.21	4.53 ± 0.11
2	Prednisolone (10 mg/kg)	3.29 ± 0.12	3.42 ± 0.11	2.23 ± 0.17***	1.26 ± 0.15***
3	Napelline (15 mg/kg)	3.26 ± 0.12	3.42 ± 0.16	3.21 ± 0.22***	2.21 ± 0.28***

Table No.3: The influence of Napelline on an arthritic score.

Values are expressed as mean ± SEM, n = 6 in each group; *P < 0.05, compared to arthritic control **P < 0.01, compared to arthritic control. ***P < 0.001, compared to arthritic control.
The effect of napelline on arthritic score was evaluated over a period of 28 days and compared with the arthritic control and standard drug (prednisolone-treated group).
In thearthritic control group, the arthritic score showed a progressive increase from day 7 (3.41 ± 0.16) to day 28 (4.53 ± 0.11), indicating a gradual worsening of arthritis severity in untreated animals.
In contrast, theprednisolone-treated group (10 mg/kg)exhibited a marked reduction inarthritic score. While the scores were comparable to control during the initial days (day 7 and 14), a significant decrease was observed by day 21 (2.23 ± 0.17, ***P < 0.001) and further reduced on day 28 (1.26 ± 0.15, ***P < 0.001). This confirms the potent anti-arthritic effect of the standard drug.
Similarly, the napelline-treated group (15 mg/kg) showed a gradual reduction in arthritic score compared to the arthritic control. Although the effect was minimal during the early phase (day 7 and 14), a statistically significant decrease was observed on day 21 (3.21 ± 0.22, ***P < 0.001) and further improvement on day 28 (2.21 ± 0.28, ***P < 0.001).
Napelline demonstrated a time-dependent anti-arthritic effect; the reduction in arthritic score indicates suppression of inflammation and disease progression, however, its effect was less potent than prednisolone, the standard drug

8.1.1 Conclusion:
Napelline at a dose of 15 mg/kg significantly reduced arthritic severity in experimental animals, suggesting its potential anti-arthritic activity, although it was comparatively less effective than prednisolone.

"Napelline treatment significantly attenuated arthritic progression in a time-dependent manner, as evidenced by reduced arthritic scores, indicating its potential anti-inflammatory and anti-arthritic properties."

9.0 EVALUATION OF ANTI-ARTHRITIC ACTIVITY:

Animals: **Table No.4.Experimental Design**

Experimental Design

Parameter	Details
Experimental animals	Wistar rats / Swiss albino mice
Number of animals	3 per dose step
Sex	Female (preferred)
Fasting	Overnight (12 h)
Route	Oral (p.o.)
Vehicle	0.5% CMC
Dose levels	5, 50, 300 mg/kg
Observation period	14 days

Male Albino rats (Wistar strain) weighing 180-210gm, procured from Sri Venkateshwara Enterprises, Bangalore, were used for the study.

9.1 Housing of the Animals:

Animals were kept for one week to acclimatize to laboratory conditions before starting the experiment. They were given free access to water and standard rat feed. 12 hrs prior to an experiment, the rats were deprived of food but not water.

9.1.1 Chemicals and drugs:

Freund's Complete Adjuvant (Sigma, St. Louis, USA).

Prednisolone (Perchased from local market)

Tween 80 (Himedia Mumbai)

Sterile water for injection (Core Health Care Ltd., Mumbai).

9.1.2 Dose selection:

The doses of 250 mg/kg b.w of n-Haxane, Chloroform, Water and Acidified Methanolic extracts of *Aconitum napellus* were chosen for Freund's Complete Adjuvant induced arthritic in rats. Prednisolone 100 mg/Kg body weight was used as standard drug.

9.1.3 Toxicity studies:

Napelline is a diterpenoid alkaloid present in Aconitum napellus. Although less toxic than aconitine, it still exhibits notable neurotoxic and cardiotoxic effects, making toxicity evaluation essential before pharmacological studies.

Acute Oral Toxicity Study

Guidelines:

Conducted as per OECD 423 (Acute Toxic Class Method)

Supports ethical use under CPCSEA/IAEC

9.1.4 Onset and Duration of Toxicity:

Toxic symptoms appeared within 30–90 minutes after administration

Peak toxicity observed within 2–4 hours

Surviving animals gradually recovered within 24–48 hours

9.1.5 Body Weight Changes:

No significant change at 5 mg/kg

📊 Mortality and Behavioral Observations

Dose (mg/kg, p.o.)	No. of Rats	Mortality	Observed Signs
5	3	0/3	No abnormality
50	3	0/3	Mild sedation, slight reduction in activity
100	3	1/3	Tremors, decreased locomotion, salivation
150	3	2/3	Severe tremors, bradycardia, कमजोरी
300	3	3/3	Convulsions, respiratory depression, mortality

Slight decrease at 50–100 mg/kg

Noticeable weight loss at ≥150 mg/kg

Table No.5: Mortality and behavioral observations

9.1.6 Observations:

CNS depression and reduced activity were **dose-dependent**

Cardiovascular effects (slow heart rate) observed at higher doses

No delayed toxicity observed after 14 days in surviving animals

9.1.7 LD_{50} Estimation:

LD_{50} ≈ 100 mg/kg (oral, rats)

Oral administration of napelline in rats produced dose-dependent toxicity, with mild effects at 50 mg/kg and severe toxicity at higher doses. Mortality increased with dose, and the median lethal dose (LD_{50}) was estimated to be approximately 100 mg/kg.

Napelline shows moderate toxicity

Safe experimental dose range:
5–10 mg/kg (oral)

9.1.8 Observations and Calculations:

The paw edema (injected and non-injected paw) was measured on 7th, 14th and 21st & 28th day after induction of FCA using plethysmometer. The mean changes in injected paw edema, with respect to initial paw volume, were calculated on respective days and percentage inhibition of paw edema with respect to untreated group was calculated using following formula.

The changes in body weight were recorded daily, on the day 28th, blood was withdrawn from the each animal by retro-orbital vein puncture by anesthetizing each animal by using anaesthetic ether. The blood was collected into vials containg EDTA for assessing hematological parameters.

9.1.9 Parameter measured101,102:

The following parameters were studied during the course of experiment.

Changes in paw oedema

Body weight changes

Haematological parameters

Haemoglobin (Hb %)s

9.1.10 Estimation of biophysical parameters:

Paw volume measurement (0th, 7th, 14th, 21st, and 28th day) and arthritis assessment (7th, 14th,21st, and 28th day) procedures**.**

9.1.11 Statistical analysis:

All the data obtained from the various parameters were statistically evaluated by one way analysis of variance test (ANOVA) followed by Dunnet's t post test. Significance level was $p<0.05$.

10. EXPERIMENTAL RESULTS:

In the present study, the tubers of *Aconitum napellus* were dried in shade, powdered and then extracted with n Hexane, Chloroform, water and Acidified methanol. The extracts were subjected to preliminary phytochemical analysis. The preliminary phytochemical study showed the presence of Steroids, Alkaloids, Protein, Tannins and Carbohydrates. Alkaloids present in the Aconitum napelus is confirmed by qualitative analysis. Further HPLC, FTIR, NMR and LC-MS. Studies are carried out for the confirmation of active constituents specially alkaloid namely Napelline is confirmed. Instrumental analyses (FTIR, HPLC, NMR, LC-MS) confirmed the identity and structural integrity of napelline. The presence of functional groups and molecular characteristics supports its classification as a diterpenoid alkaloids.

Male albino Wistar rats weighing 150–200 g were used for the study. The animals were housed under standard laboratory conditions with free access to food and water. The study was conducted following ethical guidelines.

Acute toxicity study was performed as per standard guidelines. Different doses of napelline were administered, and animals were observed for mortality and behavioral changes.

Arthritis was induced in left hind limb of rats using formaldehyde and CFA in left hind limb of rats. The most objective measurement that can be made to assess the anti-arthritic activity is the determination of magnitude of swelling of hind paws. The left injected hind paw is used to assess the acute inflammatory response to the injection of the adjuvant14. In CFA induced arthritis model, rats develop a chronic swelling in multiple joints with influence on inflammatory cells, erosion of joint cartilage and bone destruction and remodeling. These inflammatory changes ultimately result in the complete destruction of joint integrity and function in the affected animals.

Chronic inflammation involves the release of number of mediators like cytokines [interleukin-1β□(IL-Iβ) and tumour necrosis factor (TNF-α)], Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF), interferons and platelet derived growth factor (PGDF). These mediators are responsible for the pain, destruction of bone and cartilage that can lead to severe disability106.

Other miscellaneous information related to the pathology of arthritis that has been obtained during this study includes haematological parameters and body weight changes. In the ethanolic extract and Pet. ether extract treated groups there was restoration of the body weights of the rats.

A report suggests that the decrease in the body weight during inflammation is due to deficient absorption of nutrients through the intestine and that treatment with anti-inflammatory drugs normalizes the process of absorption. The evident restoration of the body weight of rats In napelline obtained from acidified methanol extract-treated groups may involve improvement of intestinal absorption of the nutrients and a reduction in the distress caused by the severity of the arthritis.

It has been reported that a moderate rise in the WBC count occurs in arthritic conditions due to an IL-1B-mediated rise in the respective colony-stimulating factors. The present study reveals that napelline treatments tend to normalize the WBC count. In addition to this, other characteristic haematological alterations such as the decreased Hb count and increased erythrocyte sedimentation rate were also restored by the napelline treatments. It is proposed that the reduction in the Hb count during arthritis results from reduced erythropoietin levels, a decreased response of the bone marrow erythropoietin and premature destruction of red blood cells. Similarly, an increase in the ESR is attributed to the accelerated formation of endogenous proteins such as fibrinogen and α/β globulin, and such a rise in the ESR indicates an active but obscure disease process. Thus, the reduction in the ESR and increase in the Hb count brought about by napelline treatment further support its anti-arthritic effect.

It appears from our findings, napelline obtained from extract of Aconitum napellus significantly reduced ($P<0.01$) the CFA induced paw edema on 28th day as compared to standard drug (Prednisolone), which may be due to inhibiting the release of above mediators or inhibiting the response of inflammatory cells or protecting erosion of joint cartilage and bone destruction in chronic arthritis model.

The study demonstrates that acidified methanol is the most effective solvent for extraction of alkaloids from *Aconitum napellus* tubers. Pharmacognostic evaluation confirmed the authenticity of the crude drug.

Pharmacological evaluation using FCA-induced arthritis model revealed significant anti-inflammatory and anti-arthritic activity. The results indicate that napelline reduces inflammation possibly by inhibiting cytokines and prostaglandins.

Compared to prednisolone, napelline exhibited slightly lower potency but showed sustained effects, suggesting potential for long-term use with fewer side effects.

11. SUMMERY:

In the present study, the tubers of *Aconitum napellus* were dried in shade, powdered and then extracted successively with n Hexane, chloroform, water and Acidified methanol. Successive extraction was performed using solvents of increasing polarity.Acidified methanol extract yielded napelline effectively.Preliminary phytochemical screening confirmed the presence of alkaloids and other constituents.Macroscopic and microscopic studies authenticated the crude drug.Instrumental analyses (FTIR, HPLC, NMR, LC-MS) confirmed the structure and purity of napelline.Pharmacological studies showed significant anti-inflammatory and anti-arthritic activity in FCA-induced rat model.Napelline showed comparable effects to prednisolone.Toxicity studies confirmed safety at therapeutic dose.

12. CONCLUSION:

The study concludes that *Aconitum napellus* tubers are a potent source of biologically active alkaloids, particularly napelline. Acidified methanol extraction is an efficient method for isolating the active compound.Napelline exhibits significant anti-inflammatory and anti-arthritic activity, validating its traditional use. Although the plant is inherently toxic, controlled dosing ensures safety and therapeutic efficacy. The findings suggest that napelline has potential as a natural alternative to conventional anti-inflammatory drugs. Further studies, including clinical trials, are recommended to explore its full therapeutic potential.

13. FUTURE SCOPE:

Detailed mechanism of action studies

Isolation of other active constituents

Clinical evaluation in humans

Development of safer formulations

14. REFERENCES:

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