Original Article

Phyto-therapeutic Potential of *Aconitum ferox* Roots in CFA-induced Arthritis in Rat Model

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ABSTRACT

Background: To investigate the anti-arthritic potential of ethanolic extract of *Aconitum ferox* root (EAF) and isolation of a compound from the active fraction. **Materials and Methods:** The EAF obtained from successive solvent extraction was evaluated for antirheumatic action by complete Freund's adjuvant (CFA) induced arthritis in adult Lewis rats (150-200gm) at 10mg/kg dose for prophylactic and therapeutic effect after acute toxicity study. Paw volume, body weight changes, locomotor activity, hemoglobin (Hb) count, erythrocyte sedimentation rate, LPO, and SOD levels were assessed in arthritic animals. These were supported by histological and radiological studies of the affected joints. Statistical analysis was performed using one way ANOVA followed by Dunnett's test. $p<0.05$ was considered statistically significant. A compound isolated was characterized by spectroscopic and chromatographic techniques. **Results:** The EAF 10mg/kg showed a significant reduction in paw volume (58.32%), body weight, ESR (8.1-7.5 mm/h), and LPO level (37.5nmol/g). There was a significant increase in hemoglobin count (13.42g/dl), locomotors activity (92.95%), and SOD level (201U/mg). The isolated compound after TLC, UV, FTIR, and LCMS analysis was identified as delphinine. **Conclusion:** The data shows that EAF has anti-arthritic activity at 10mg/kg, p.o., and a compound delphinine isolated from it.

Keywords: Aconite, Anti-arthritic, Delphinine, Alkaloid, *Vatsanabh*, CFA.

INTRODUCTION

Arthritis with rheumatism is a chronic inflammatory progressive and autoimmune disease with clinical features of pain, limited movement of joints, and damaged cartilage with bone erosion.[1] Globally 1% of the adult population and about <1% of the Indian population are affected by Rheumatoid Arthritis (RA).[2] An inflammatory reaction, morphological changes, an abnormal increase in the number of synovial cells, inflammation, and damage in joints are the pathological distinctive features of RA.[3-4] The inflammation in synovial membrane and injury in the joints are the consequences of disturbance in the balance between the pro-inflammatory and anti-inflammatory state in RA conditions.[5] The conventional treatment approaches for RA conditions involves the strategic use of disease-modifying anti-rheumatic drugs (DMARDs) like sulphasalazine, leflunomide, methotrexate, hydroxychloroquine, non-steroidal anti-inflammatory drugs (NSAIDs) like etoricoxib, corticosteroids like prednisolone, methylprednisolone, immuno-suppressant, and biological agents. Those are associated with unavoidable adverse effects.[6]

Those adverse effects and the high cost of treatment in musculoskeletal disorders provoke patients for alternative methods to get symptomatic relief and this increases the use of complementary and alternative medicine.[7] Thus various clinical diseases are treated with various medicines of natural origin especially from plants or herbs and their exact mechanism of action is still needs more exploration.[8] Various treatment strategies for chronic diseases e.g. RA and

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other inflammatory complications from herbal and other complementary therapies are well documented.[9] Still NSAID's are involved in the first line of treatment for arthritic patients even they are not effective on the production of inflammatory cytokines whereas they are inhibiting cyclooxygenases (COXs) and reducing the generation of prostaglandins.[10] Therefore the herbal medicines, complementary and alternative strategies for therapeutic benefits in arthritis should be economical, effective on inflammatory conditions with minimal side effects and safety evidences.

Vatsanabha (*Aconitum ferox*), is a poisonous plant mainly found in the Himalayan region.[11] It has been used in the traditional system of medicine in China and India. As per Ayurveda, the herb is considered hot and toxic, increasing *pitta* dosha and is prepared in extremely small doses.[12] Traditionally the roots after the ayurvedic *shodhan* process; the "purified" root tubers are used to treat various clinical conditions e.g. digestive problems, colic, gastric disorders, respiratory problems (coughs, asthma, bronchitis), weak hearts, nasal catarrh, tonsillitis, sore throat, neuralgia, painful inflammations, leprosy, skin afflictions, paralysis, fever, cholera, debility, as a sedative, diaphoretic, gout, diabetes and in rheumatism (paste form).[12] Various ayurvedic formulations contain *Aconitum ferox* which are used in different conditions e.g. *Mahamrutynjaya* rasa, contain *Aconitum ferox, Piper longum and Piper nigrum.* As far as is known the antiarthritic potential of AF roots was not scientifically validated. Hence, the present study was carried out with the aim to experimentally validate the antiarthritic potential of the fractions obtained from AF in CFA induced arthritic rats and identification of the compound.

MATERIALS AND METHODS

Plant Material and Extract

The plant was selected on the basis of morphology and was collected from Vindhya herbals barkheda pathani Bhopal (M.P.) where it is widely used for commercial purposes. The plant was authenticated from the Department of Botany at Safia Science College, Bhopal (M.P.) by Dr. Zia Ul Hasan with a voucher Specimen no. 316/Bot/Safia/12. The roots were cleaned, dried in the shade; to protect their chemical constituents not to getting degraded at a high temp. The moderately coarse powder of the *Aconitum ferox* roots (80g) was subjected to defatting with Petroleum ether (40-60°C) till it gets completely defatted followed by successive soxhlet extraction with chloroform, ethyl acetate, ethanol, and water in increasing polarity of solvents. The solvent was removed by distillation and the last trace of solvent was removed under vacuum by rota-vaccum evaporator. The extracts obtained were weighed to a constant weight and percentage w/w basis was calculated and stored in an airtight container for further use (Table 1).[13-15]

Instruments and Chemicals

Diclofenac sodium, Freund's adjuvant was obtained from Sigma Aldrich Chemical Co., Bengaluru, EDTA (Merck Laboratories Pvt. Ltd.), DTNB (Tokyo Chemical Industry Co. Ltd.), Thio-Barbituric Acid, Trichloro acetic Acid (Sunchem India). All other chemicals used in the study were of analytical grade.

LC–MS (6130B Single Quadrupole LC/MS spectrometer, Agilent, US) with electrospray ionization detection was carried out using an Poroshell 120 C_{-18} reversed phase column (2.1 × 100 mm I.D., 2.7 µm; US), Bruker-Alpha II FTIR, Cooling centrifuge (Remi-electrotechnic limited), UV Spectrophotometer (Pharmaspecs shimadzu), Actophotometer (Teknik), Hot plate (Lyzer India), Rotatory Flash Evaporator (IKA RV10), trinocular microscope, and other instruments were used.

Phytochemical Analysis

The dried extracts were subjected to phytochemical analysis for identification of carbohydrates, proteins, amino acids, fats and oils, steroids, triterpenoids, glycosides, flavonoids, alkaloids, phenolic compounds, volatile oil, tannins, and resins using the conventional protocol (Table 2).[16-17]

Animals

The experiment was carried out on healthy albino Lewis rats of either sex weighing between 200-250 g. were used for the evaluation of acute oral toxicity test and anti-arthritic activity. Animals were provided by the authorized animal house of Sapience Bioanalytical Research Lab, Bhopal, Madhya Pradesh, India. The animals were used after an acclimatization period of 10 days in the laboratory environment. The experiment was approved by the Institutional Animal Ethical Committee as per the committee for the Purpose of

Table 1: Percentage yield of different extracts of *Aconitum ferox.*

Sl. No.	Solvent	Color of Extract	% Yield w/w
1	Petroleum ether	Yellowish white	11.35
2	Chloroform	Yellowish brown	15.59
3	Ethanol	Reddish Brown	41.70
4	Water	Brownish Black	22.51

Table 2: Qualitative analysis of different extracts of *Aconitum ferox.*

Sl. No.	Chemical Tests	Pet. Ether	Chloroform	Ethanol	Aqueous
1.	**Carbohydrates**				
a.	Molisch test	-	-	-	+
b.	Fehlings test	-	-	-	+
c.	Benedicts test	-	-	-	-
2.	**Proteins**				
a.	Biuret's test	-	+	+	-
b.	Millon's test	-	+	-	-
3.	**Amino Acids**				
a.	Ninhydrin test	-	+	-	-
4.	**Fats and oils**				
a.	Filter paper test	+	-	-	-
5.	**Steroids and Triterpenoids**				
a.	Salkowski reaction	+	-	-	-
b.	Libermann-Burchard reaction	+	-	+	-
6.	**Glycosides**				
a.	Raymonds test	-	-	+	-
b.	Keller-Killani test	-	-	-	-
c.	Foam test	-	-	+	+
7.	**Flavonoids**				
a.	Shinoda test	-	-	+	+
b.	Lead acetate test	-	-	+	+
8.	**Alkaloids**				
a.	Dragendorff's test	-	+	+	+
b.	Mayer's test	-	+	+	-
c.	Wagner's test	-	+	+	+
9.	**Phenolic Compounds**				
a.	5% $FeCl_3$ solution	-	-	+	+
10.	**Volatile oil**				
a.	Sudan red	-	-	-	-
11.	**Tannins**				
a.	Vanilline HCL	-	+	-	+
b.	Gelatin	-	-	-	-
12.	**Resin**				
a.	Ferric chloride test	-	+	-	-

'-' means negative result, '+' means positive results.

control and supervision of experiments on animal guideline. (CPCSEA Reg. no.: 1413/PO/a/11). All animals were kept in standard cages and maintained at 25°C. Under 12 hr dark/ light cycle. The animals were fed with standard rat feed and water was given after specific intervals prior to the experiment animals were kept for 12 hr fasting.

Acute Oral Toxicity Study and Selection of Doses

An oral dose of EAF was determined through the acute oral toxicity test in rats at different doses. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300, and 2000 mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals, the use of an additional upper dose level of 5000 mg/kg body weight may be considered. At the dose of 50 mg/kg there was no mortality rate but above that it is, so 1/5th of this dose i.e. 10 mg/kg was selected for the study.[18]

Induction of arthritis

Arthritis was induced in rats by a single intradermal injection of 0.1 ml of Complete Freund's Adjuvant into the tail base of the rats.[19] EAF (10mg/kg body weight, orally) and the reference drug diclofenac sodium (10mg/kg body weight, orally) were administered for 28 consecutive days (prophylactic treatment) and from the 14th day of CFA injection (Therapeutic treatment).

Experimental Design

Group I served as a control group it received 0.5% CMC (5ml/kg), Group II serves as Arthritic Control Group received 1mg/0.1ml of CFA, Group III served as Standard received Diclofenac sodium 10mg/kg p.o.,[20-21] Group IV and V served as Test groups received 10mg/kg p.o of *Aconitum ferox* root (AFR) extract for 28 days and 10mg/kg p.o. of *Aconitum ferox* root extract for 14 days. The extract was evaluated for antirheumatic action by complete Freund's adjuvant (CFA) induced arthritis in adult Lewis rats (150-200gm) at 10mg/kg dose for prophylactic and therapeutic effect after acute toxicity study.

Arthritic Assessment

To follow the course of the disease, swelling of the hind paw was determined with vernier calipers and body weight was measured by electronic weighing balance. The paw volume was periodically measured on 0th, 7th, 14th, 21st, and 28th day (after injection of CFA) thereafter to confirm the reduction of RA.[22]

Hematological Assessment

Hemoglobin (Hb) content was determined in rat blood by the Sahli's Hemoglobinometer in pre and post arthritic conditions. The Hb content of standard drug and various extract-treated groups were compared with the control group.

The erythrocyte sedimentation rate (ESR) was also measured in the blood collected from rats through orbital puncture on the 7th, 14th, 21st and 28th day and was determined by the specific method.[23] The antioxidant parameters e.g. lipid peroxidation (LPO) and superoxide dismutase (SOD) was estimated in rat plasma by enzymatic testing methods.[24]

Histological Studies

At the end of the study, on the day of 29th the hind limbs were removed, immediately fixed in 10% formaldehyde. The tissue was embedded in paraffin blocks and cut into 4 µm sections of sagittal slices with a microtome. Sections were then stained with hematoxylin and eosin (H&E), and morpho-pathological assessment was performed to evaluate the effect of treatment.

Radiographic Analysis

At the end of the experiment, on the 29th day after adjuvant injection, hind limbs of the treated and untreated rats were fixed in 10% formaldehyde and limbs send to pathology for radiographic assessment of joint tissues.

Statistical Analysis

All the data expressed as a mean ± SEM (standard error mean) (six animals per group) were evaluated by one-way analysis of variance (ANOVA), followed by Dunnett's test and values of $p<0.05$ (or less) were considered as statistically significant.

Isolation and Characterization of Phytoconstituent from Active Fraction

The active alcoholic extract of *Aconitum ferox* (10g) was taken for isolation of phytoconstituents from it. The method followed for the isolate is mentioned in Figure 1. The conventional method was applied with slight modification for the isolation of compounds (e.g. alkaloids).[25] Percentage yield and melting point were determined. Thin layer chromatographic studies were also performed for single compound confirmation. The isolated compound was then undertaken for spectroscopic studies e.g. UV, FTIR, and LCMS analysis for characterization.

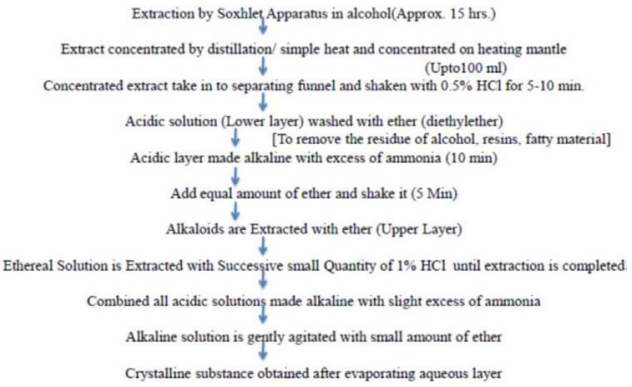


Figure 1: Isolation from Alcoholic Fraction of *Aconitum ferox*.

RESULTS

The successive solvent extraction of *Aconitum ferox roots* with increasing polarity of solvents results in following percentage yields (Table 1). The greater yield of alcoholic extract reflects the presence of more polar compounds in the drug.

Preliminary phytochemical screening showed that petroleum ether extract contains steroids and fats; chloroform extract contains protein, amino acids, alkaloids, tannins, resins. Ethanolic extract showed alkaloids, triterpenoids (steroids), proteins, flavanoids, and phenolic compounds. Aqueous extracts have shown the presence of carbohydrates, flavonoids, phenolic compounds, alkaloids, and tannins (Table 2).

The EAF 10mg/kg showed remarkable reduction in paw volume, body weight, ESR and LPO level. There was a significant increase in hemoglobin count (13.42g/dl), locomotor activity (110) and SOD level (20 IU/mg). Later on, the radiological and histological studies supported the anti-arthritic activity of EAF by showing lesser abnormalities in the groups when compared to the groups with arthritis.

Assessment of Paw Volume by using Vernier Caliper

The measurements of paw volume on day 0, 7th, 14th, 21st, and 28th day of the experiment was done by Vernier calipers (Figure 2 and 3).

Body Weight

In our study, it is evident from the observations that the magnitude of weight loss is associated with the extent of inflammation in joints. The rats with arthritis after adjuvant injection showed notable weight loss in the 1st week and normal weight gain in the following weeks. The group treated with the standard drug and the first treatment group showed less weight loss (Figure 4).

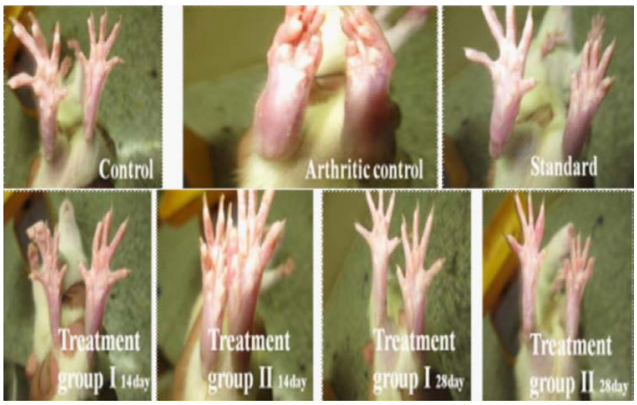


Figure 2: CFA induced inflammation in rats.

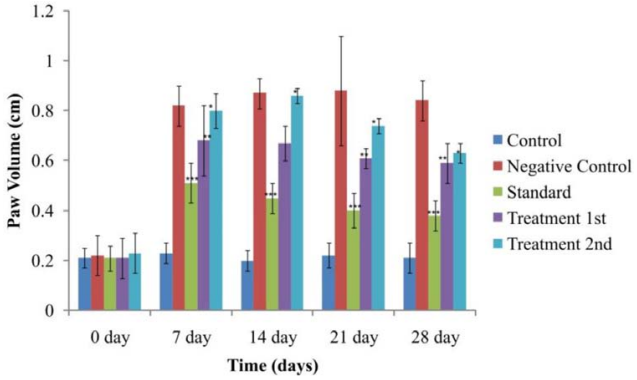


Figure 3: Mean change in paw volume using Vernier Caliper.

Values are Mean ± S.E.M, n = 6 animals in each group. Results are Mean ± S.E.M. ANOVA, Dunnet's test ***p< 0.001, **p<0.01, *p<0.05 (treated groups Vs arthritic control group II).

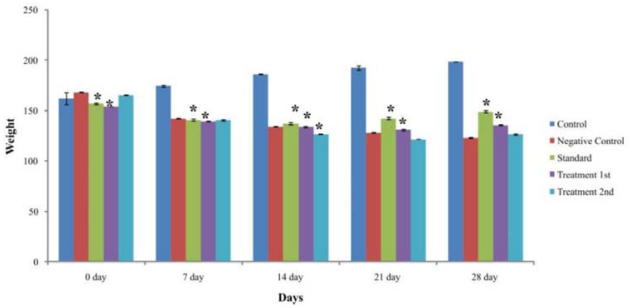


Figure 4: Effect of *Aconitum ferox* on Animal Body Weight Changes.

Values are Mean ± S.E.M, ANOVA, Dunnett test *p<0.05, n = 6 animals in each group.

Assessment of Locomotor Activity

The movement of animals in the Actophotometer was measured on the 0, 7th, 14th, 21st and 28th day of experiment for the assessment of locomotor activity (Figure 5).

Effect on Hb Count in Adjuvant-induced Arthritis in Rats

The Hb content measured on 0, 7th, 14th, 21st and 28th day of the experiment showed remarkable elevation in

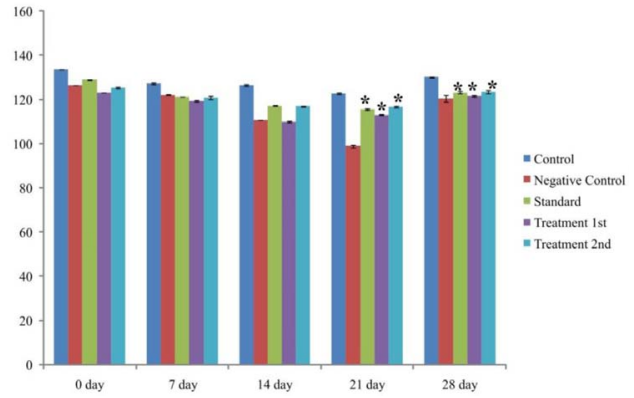


Figure 5: Evaluation of Locomotor Activity by Actophotometer.

Results are Mean± S.E.M. ANOVA, Dunnett's test *p<0.05, n = 6 animals in each group.

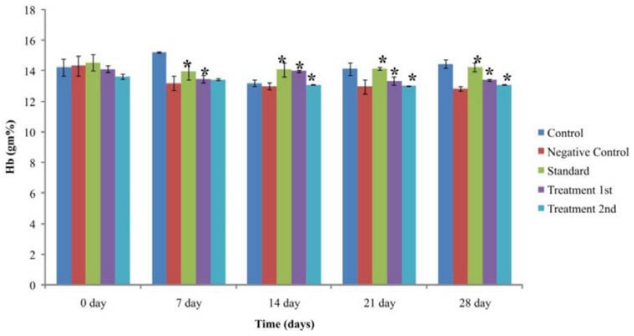


Figure 6: Effect of *Aconitum ferox* on Hemoglobin count.

Values are Mean ± S.E.M, ANOVA, Dunnett test *p<0.05, n = 6 animals in each group.

Hb content in arthritic rats in comparison with rats of other groups (Figure 6).

Effect of *Aconitum ferox* on ESR

A significant reduction in ESR was observed in AF treated groups when compared to the group treated with vehicle. The levels of ESR were decreased from 8.1 to 7.5 millimeter per hour (mm/h) in group V and 7.9 to 7.1 (mm/h) in group IV of groups treated with AF. The level of ESR was significantly increased in group II from 4.2 to 13.84 (Table 3).

Effects of *Aconitum ferox* on LPO and SOD activity

In the Treatment group SOD level elevated significantly. Lipid peroxidation was determined in the form of malondialdehyde (MDA) in liver and was reduced remarkably. The observed reduction was found statistically significant when compared to the arthritic control group (Table 4).

Radiology studies

In radiological studies, the normal control group showed no malformations in the ankle joint, whereas the arthritic joint of negative control showed swelling in

Table 3: Effect of *Aconitum ferox* on ESR.

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
Control	4.6±0.26	4.8±0.64	4.3±0.53	4.5±0.21	4.7±0.23
Negative Control	4.2±0.28	6.3±0.46	9.7±0.54	12.9±0.26	13.8±0.15
Standard	5.3±0.23	5.8±0.21*	6.8±0.46*	7.1±0.22*	6.9±0.19*
Treatment 1st	4.8±0.41	5.1±0.46*	7.9±0.11*	7.5±0.27*	7.1±0.02*
Treatment 2nd	4.9±0.27	5.3±0.15	8.1±0.29*	7.9±0.28*	7.5±0.31*

Values are Mean ± S.E.M, ANOVA, Dunnett test *p <0.05, n = 6 animals in each group.

Table 4: Effect of *Aconitum ferox* on LPO and SOD activity.

Groups	LPO (MDA nmol/gm proteins)	SOD (IU/ mg protein)
Control	28.7 ±0.11	272.60 ±0.04
Negative control	108.4±0.35	81±0.05
Standard	41.01±0.13*	211±0.03*
Treatment 1st	37.5±0.55*	201±0.05*
Treatment 2nd	77.4±0.69*	144±0.09*

Values are Mean ± S.E.M, ANOVA, Dunnett test *p <0.05, n = 6 animals in each group.

Where A. Normal Control, B. Negative Control, C. Standard group, D. Treatment -I, E. Treatment-II

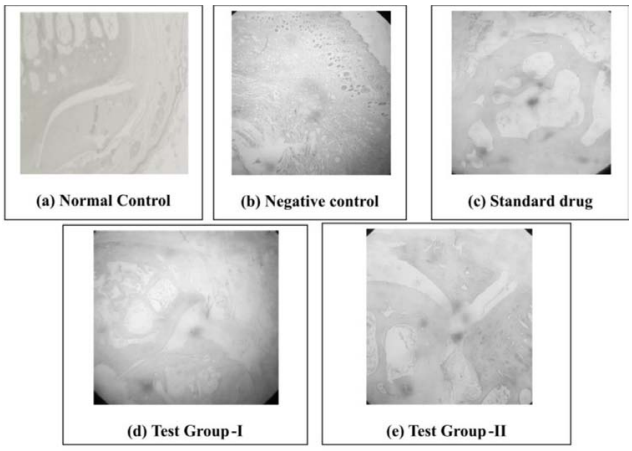
Figure 7: Radiology of Hind Paw.

Where A. Normal Control, B. Negative Control, C. Standard group, D. Treatment-I, E. Treatment-II.

soft tissue along with lessening of the joint spaces which entail the destruction in bones in arthritis (Figure 7A to E). The group of animals that received the standard drug showed minor swelling of joints and prevented the condition of bony destruction. Groups (Treatment 1st and 2nd) showed no bony destruction and no selling of joints. The group that received more doses of extract (Treatment I e.g. 10mg/kg for 28 days) has shown lesser swelling in soft tissues and lessening of joint spaces when it was compared with the negative control group and Treatment II (10mg/kg for 14 days). Therefore the drug has prophylactic and therapeutic potential.

Histopathology of Joints

The examination of histopathological modifications of the interphalangeal joints in various groups are demonstrated in Figure 8a to e, the control group Group I (a) exhibited the histopathological features



A= Control Group, B= Arthritic Control Group, C= Standard Group, D=Treated 1st and E= Treated 2nd Group

Figure 8: Histopathology of joints.

A= Control Group, B= Arthritic Control Group, C= Standard Group, D=Treated 1st and E= Treated 2nd Group.

of the normal ankle joint. The joints of arthritic rats of negative control group II, exhibited distinguishing malformations from the normal joint e.g. formation of edema, degeneration associated with partial cartilage erosion, bone marrow destruction and huge accumulation of infiltrated effusions of inflammation in the articular surface (Figure 8b). The group III(c) was treated with standard drug exhibited lesser cellular infiltrates and normal bone marrow. The groups treated with extracts for 28 days e.g. group IV and V (d and e) exhibited lesser signs of inflammation like normal bone marrow, meager cellular infiltrate and absence of edema formation. There was significant prevention of the signs of inflammation on the joints of the rat ankle in 28 days. The groups treated with the drugs not exhibited any degeneracy of ankle joints when compared with the negative control.

Characterization of Compound

A crystalline buff white colored powder 167mg (0.167% w/w) of 197-199°C melting point was obtained which was characterized by various chromatographic and spectroscopic techniques. The isolated compound in UV spectroscopic analysis has shown λ_{max} 368nm indicating the presence of conjugated unsaturation in the molecule (Figure 10). The compound was buff white-colored and crystalline, shown single spot in thin layer chromatographic studies in solvent system Benzene: Chloroform: Methanol (20:20:60) with detecting agent (iodine vapors and dragondorrf's reagent) (Figure 9).[26-27] The FTIR spectra of isolated compound shown characteristic bands at 3654.98, 3537.92, 2500.3 (free OH and OH stretch), 3432.41, 3270.77 (NH stretch), 2256.50,2187.91, 2105.87 (-C=C-

Figure 9: TLC of isolate.

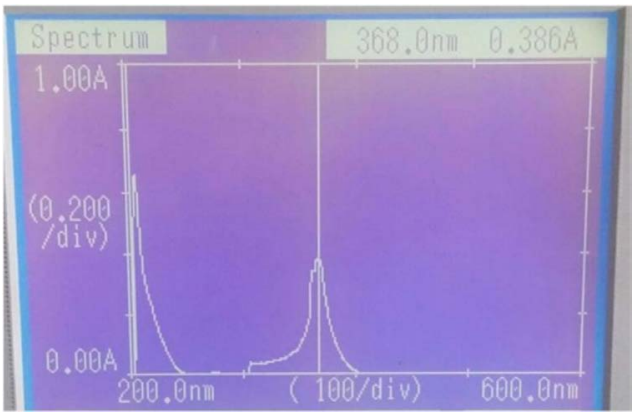


Figure 10: Ultraviolet Spectra of the Isolate from AF.

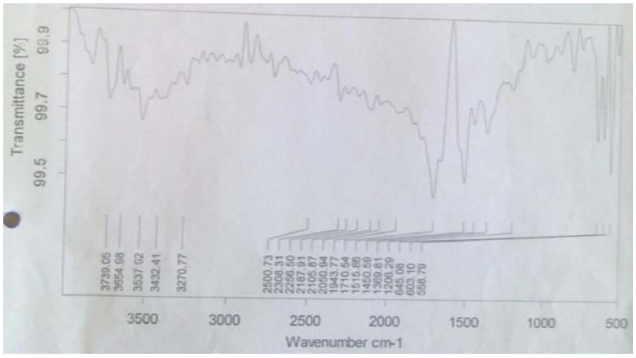


Figure 11: FTIR Spectra of the Isolate from AF.

stretch), 1710.54 (C=O stretch, unsaturated conjugated acid), 1369.81(C-H bending) and 1206.29 (C-O stretch) (Figure 11). LC-MS spectroscopy showed the molecular ion peaks at 600.0 that correspond to the molecular formula, $C_{33}H_{45}NO_9$. Ion peaks were also observed at m/z 571.8, 487.5, 393.8, 214.9 (Figure 12,13).

DISCUSSION

The extraction of phytoconstituents by the Soxhlet method is recommended over other methods of extraction as it is also a continuous hot percolation

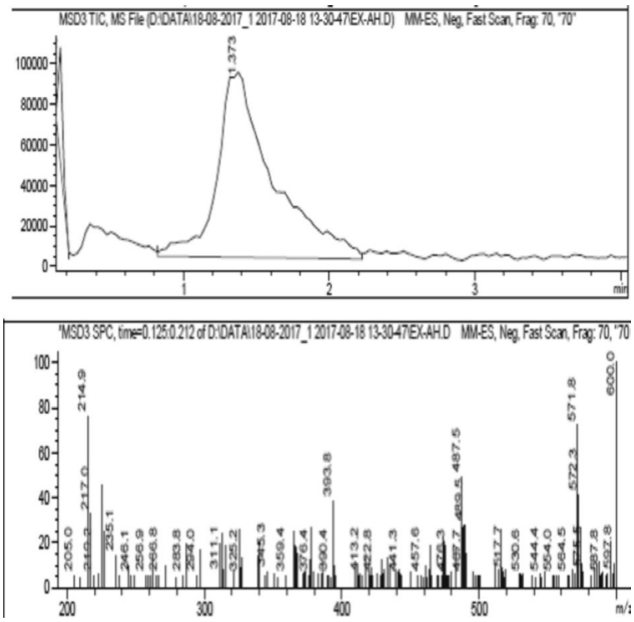


Figure 12: LCMS Data of isolate from AF.

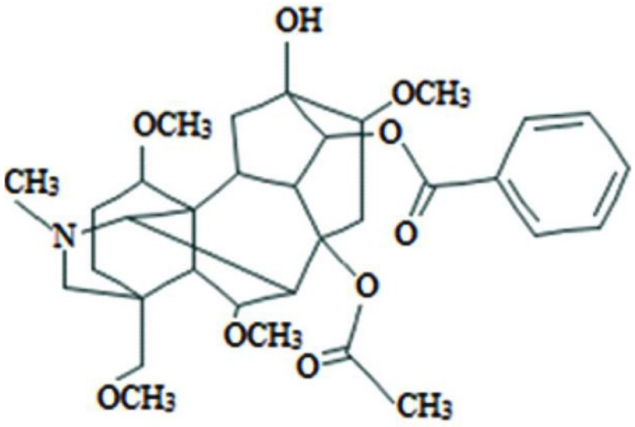


Figure 13: Delphinine.

method that gives better penetration of solvent in the plant tissues and separating the constituents from the matrix by making contact of the fresh solvent with the cellular matrix in a continuous manner. This suggested us to select this method for extraction. The successive solvent extraction method was employed to screen the compounds from the complex mixture present in the selected drug part as per the polarity of solvents and constituents. The constituents get separated as per their polarity in different solvents which was later on confirmed by the preliminary phytochemical screening tests. The test result also suggested the selection of alcoholic extract for evaluation of anti-arthritic potential showing the presence of some category of phyto-constituents may be responsible for the activity.

After preliminary phytochemical screening tests the ethanolic extract was taken for evaluation of *in vivo* anti-arthritic activity as it has shown the presence of major phyto-constituents, may be responsible for the anti-arthritic and anti-inflammatory activities as reported in previous literatures (e.g. alkaloids, triterpenoids (steroids), proteins, flavanoids and phenolic compounds).[28]

Around 1% of the adult population worldwide is suffering from RA in which the causative factor is not known.[29-30] The evaluation of the uniqueness of the drugs for treatment of RA in humans can better be studied in CFA induced arthritis in rats. This model is useful to assess the anti-inflammatory and anti-arthritic potential of drugs.[31] It has various resemblances with that of RA in humans making it a more suitable model for the study.[32]

Swelling in paw is an index of determining the magnitude of inflammation in CFA induced rats and reduction in paw swelling indicates the anti-arthritic activity of numerous drugs.[33-34] It was used to assess the activity of AF at the dose of 10 mg/kg per oral. AF treated groups exhibited significant decrement in paw volume when correlated with the arthritic control group and remarkable weight loss. (Figure 2-4).

It also suggests the rapport between the intensity of inflammation and gain or loss in body weight. (Figure 4).[35] The loss in appetite, enormous release of pro-inflammatory mediators and/or reduced absorption capacity and free radicals may result in loss of weight loss.[36] This was noticed in the negative control group of arthritic rats. Recovery of arthritis by evaluating the augmentation in body weight is an easy tool while studying in CFA rat models. In present investigations AF extracts showed protective effect against weight loss in arthritis.

The remarkable decrease in locomotors counts in CFA-induced arthritic rats can be a result of the active response of immunological reactions against the antigens which invade and the corresponding increase in AF treated groups showed its immune-modulatory effect.[37] The minor movement of animals in the actophotometer while studying activity especially in groups with arthritis maybe a result of rigid joints and joints pain (Figure 5).

Blood examination, like Hb, pack cell volume and white blood cell count reflects the function of bone marrow.[38] The decrease in hemoglobin content is also a representation of the anemic condition in arthritic rats, supported by records on akin arthritis-inducing models. It may be the result of the irregular storage of iron in the synovial tissue and reticuloendothelial system (Figure 6).[39]

The increasing ESR values are characteristics of chronic arthritic conditions. As the drug is decreasing the ESR it is remodeling the health conditions in comparison to the negative control group (Table 3).

Lipid peroxidation is an important etiological factor for cell membrane damage, increased levels of MDA and myeloperoxidase (MPO) results in oxidative damage of the gut mucosal membrane via lipid peroxidation.[40] The polyunsaturated fatty acid founds in the membrane lipids are the major target of ROS which leads them to lipid peroxidation causing damage. In our investigations, we found the increased levels of the end product of peroxidized lipid decompositions e.g. MDA levels in arthritic rats. An elevated level of MDA indicates the production of ROS and our finding was consistent with the previous reports. In our study, treatment of arthritic rats with *Aconitum ferox* resulted in remarkable cut-backs in plasma concentrations of MDA. The SOD activity was remarkably decreased in rats with arthritis in comparison to the animals of the normal control group. Excessive reactive oxygen species (ROS) generated could have reduced the SOD, making the synovial fluid and collagen of the joint more vulnerable to the oxygen radicals mediated damage in arthritic rats. The treatment of AF to arthritic rats emanated in the remarkable increase in SOD activity and reduced the level of LPO approximately to the normal control group exhibiting scavenging of ROS or antioxidant potential (Table 4).[41]

The joint of arthritic rat group II (negative control) showed prominent deformities from the joint of the normal control group e.g. formation of edema, bone marrow destruction, worsening of cartilage erosion and considerable intrusion of exudates of inflammation. The rats treated with the standard drug exhibited less cellular infiltrate and normal bone marrow. Treatment with AF for 28 days revealed fewer signs of inflammation like normal bone marrow, meager cellular infiltrates and dearth of edema formation, whereas the treatment for two weeks exhibited lesser cartilage destruction and cellular infiltrations on the articular surface.[42] The drug showed significant avoidance of the signs of inflammation on joints in four weeks in comparison to two-week treatments. (Figure 8)

Radiographic modifications are useful diagnostic tools in arthritic conditions indicating the grimness of the disease. Swelling in Soft tissues, erosions in bones and lessening of joint spaces are radiographic signs helping to assess the earlier or final stages of arthritic conditions (Figure 7).[42-43] Treatment with AF for four weeks has shown remarkable effect by preventing the destruction in bones with lesser swelling in soft tissues and lessening of joint spaces when compared with the *Aconitum ferox*

treated group for two weeks. The experimental results of the study showed that AF extract has an effective anti-arthritic activity against CFA-induced rheumatoid arthritis.

The active ethanolic extract was selected for the isolation of alkaloids from it as the preliminary observations showed the presence of some valuable phyto-constituents. The classical approach of alkaloid isolation as per the peach and Tracey was followed with slight modification by using less toxic solvents. The treatment of alcoholic extracts with acid converts alkaloidal bases into salt and washing with non-polar solvents e.g. ether, removes the residues of fatty materials or impurities, if any remains. Alkali treatment to washed portion helps to liberate the free alkaloidal base which later on can be extracted in the non-polar solvents e.g. ether. The reiteration of steps supports better purification and crystallization of the substance. The compound isolated from the active fraction of EAF was characterized by thin layer chromatography, UV, FTIR and LCMS studies (Figure 9-13). The chemical structure of the compound was interpreted as delphinine when also compared the spectra with spectral libraries.[44-45]

CONCLUSION

In conclusion, *Aconitum ferox* at the stated dosage level of 10 mg/kg, p.o. exhibited reduction in the volume of edematous rat paw and it could normalize the hemoglobin, locomotors and biochemical irregularities in adjuvant-induced arthritis in both the developing and developed phases of arthritis induced by CFA. Further, the radiological and histopathological studies supported the antiarthritic potential of *Aconitum ferox* CFA induced arthritis. A compound delphinine was characterized from an active fraction.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

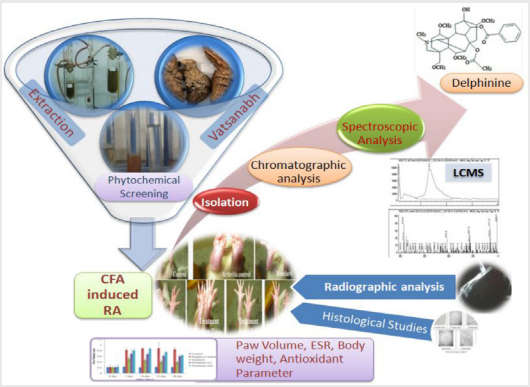
EAF: Ethanolic extract of *Aconitum ferox* root; **CFA:** Complete Freund's adjuvant; **Hb:** Hemoglobin; **AF:** *Aconitum ferox*; **LPO:** Lipid peroxidase; **SOD:** Superoxide dismutase; **ANOVA:** Analysis of Variance; **TLC:** Thin layer chromatography; **UV:** Ultraviolet Spectroscopy; **FTIR:** Fourier Transform Infrared Spectroscopy; **LCMS:** Liquid Chromatography Mass Spectroscopy.

REFERENCES

1. Rajkapoor B,. VR,. MG,. JA,. NH,. MS, *et al*. Effect of *Bauhinia variegata* on complete Freund's adjuvant induced arthritis in rats. J Pharmacol Toxicol. 2007;2(5):465-72. doi: 10.3923/jpt.2007.465.472.
2. Cui X, Wang R, Bian P, Wu Q, Seshadri VDD, Liu L. Evaluation of antiarthritic activity of nimbolide against Freund's adjuvant induced arthritis in rats. Artif Cells Nanomed Biotechnol. 2019;47(1):3391-8. doi: 10.1080/21691401.2019.1649269, PMID 31394949.
3. Chang X, He H, Zhu L, Gao J, Wei T, Ma Z, *et al*. Protective effect of apigenin on Freund's complete adjuvant-induced arthritis in rats via inhibiting P2X7/NF-κB pathway. Chem Biol Interact. 2015;236:41-6. doi: 10.1016/j.cbi.2015.04.021, PMID 25935278.
4. Lipsky PE. Rheumatoid arthritis. In: Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL, editors. Harrison's principles of internal medicine. 16th ed. New York: McGraw-Hill; 2005. p. 1968-77.
5. Chimenti MS, Triggianese P, Conigliaro P, Candi E, Melino G, Perricone R. The interplay between inflammation and metabolism in rheumatoid arthritis. Cell Death Dis. 2015;6:e1887. doi: 10.1038/cddis.2015.246, PMID 26379192.
6. Pincus T, Cutolo M. Clinical trials documenting the efficacy of low-dose glucocorticoids in rheumatoid arthritis. Neuroimmunomodulation. 2015;22(1-2):46-50. doi: 10.1159/000362734, PMID 25227901.
7. Cronan TA, Kaplan RM, Posner L, Blumberg E, Kozin F. Prevalence of the use of unconventional remedies for arthritis in a metropolitan community. Arthritis Rheum. 1989;32(12):1604-7. doi: 10.1002/anr.1780321217, PMID 2597213.
8. Ratheesh M, Helen A. Anti-inflammatory activity of *Ruta graveolens* Linn on carrageenan-induced paw oedema in rats. Afr J Biotechnol. 2007;6:1209-11.
9. Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Van Rompay M, *et al*. Trends in alternative medicine use in the United States, 1990-1997: Results of a follow-up national survey. JAMA. 1998;280(18):1569-75. doi: 10.1001/jama.280.18.1569, PMID 9820257.
10. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. Nat Rev Rheumatol. 2011;7(1):33-42. doi: 10.1038/nrrheum.2010.196, PMID 21119608.
11. Sajan LS, Ash BN, Bijaya LM, Mangala DM. Estimation of alkaloids and antibacterial activity of Aconitum spicatum Bruhl Stapf from Manaslu conservation area. Nepal J Sci Technol. 2012;13:67-71.
12. Sheokand A, Sharma A, Gothecha VK. Vatsanabha (*Aconitum ferox*): from Visha to amrita. Int. J. Ayurvedic Herb. Med. 2000;2:423-6.
13. Kokate CK. Practical pharmacognosy. 2nd ed. New Delhi: Vallabh Prakashan; 2012. p. 78-82.
14. Trease GE, Evans IC. Textbook of pharmacognosy. 12th ed. London: Bailliere Tindall; 2008. p. 21-2.
15. Ahmad W, HusainI A. N, AmirM, Sarafroz Md, AnsariMA, ZafarA, *et al*. Box–Behnken supported development and validation of robust HPTLC method: An application in estimation of punarnavine in leaf, stem, and their callus of *Boerhavia diffusa* Linn. 3 Biotech. 2020;10(4):165. doi: 10.1007/s13205-020-2154-1, PMID 32206499.
16. Khandelwal KR. Practical pharmacognosy. 20th ed Sethi VK, editor Nirali prakashan (Pune); 2010. Page no. 25.1 to 25.9.

17. Ahmad W, Mujeeb M, Arif Zaidi SM, Ahmad S. Current strategy for research on quality identification of *Rheum emodi* Wall. Rhizome. Int J Drug Dev Res. 2013;5(1):1-10.
18. [cited 4/4/2022]Available from: https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl420.pdf.
19. Cuzzocrea S, Mazzon E, Bevilaqua C, Costantino G, Britti D, Mazzullo G, *et al*. Cloricromene, a coumarine derivative, protects against collagen-induced arthritis in Lewis rats. Br J Pharmacol. 2000;131(7):1399-407. doi: 10.1038/sj.bjp.0703695, PMID 11090113.
20. Kshirsagar AD, Panchal PV, Harle UN, Nanda RK, Shaikh HM. Anti-inflammatory and antiarthritic activity of anthraquinone derivatives in rodents. Int J Inflam. 2014;2014:Article ID690596. doi: 10.1155/2014/690596, PMID 25610704.
21. Vetal S, Bodhankar SL, Mohan V, Thakurdesai PA. Anti-inflammatory and anti-arthritic activity of type-A procyanidine polyphenols from bark of *Cinnamomum zeylanicum* in rats. Food Sci Hum Wellness. 2013;2(2):59-67. doi: 10.1016/j.fshw.2013.03.003.
22. Foyet HS, Tsala DE, Zogo Essono Bodo JC, Carine AN, Heroyne LT, Oben EK. Anti-inflammatory and anti-arthritic activity of a methanol extract from *Vitellaria paradoxa* stem bark. Pharmacogn Res. 2014;7(4):367-77. doi: 10.4103/0974-8490.159569, PMID 26692752.
23. Huxtable RJ. Activation and pulmonary toxicity of pyrrolizidine alkaloids. Pharmacol Ther. 1990;47(3):371-89. doi: 10.1016/0163-7258(90)90063-8, PMID 2290854.
24. Mahajan A, Tandon VR. Antioxidants and rheumatoid arthritis. J Indian Rheumatol Assoc. 2004;12:139-42.
25. Peach K, Tracey MV. Modern methods of plant drug analysis. New Delhi: Narosa Publisher House; 2010. p. 317-20.
26. Desai HK, Joshi BS, Panu AM, Pelletier SW. Separation of diterpenoid alkaloid mixtures using the Chromatotron. J Chromatogr. 1985;322:223-7. doi: 10.1016/S0021-9673(01)97676-9.
27. Harborne JB. Phytochemical methods. 2nd ed. Vol. 6. London: Chapman and Hall Publication; 1984. p. 24.
28. Singhai A, Singour PK, Pawar RS, Patil UK. Pharmacological Activities of Chandrakhya leaves. Int J Pharm Sci Drug Res. 2009;1(20):107-9.
29. Karmakar S, Kay J, Gravallese EM. Bone damage in rheumatoid arthritis: Mechanistic insights and approaches to prevention. Rheum Dis Clin North Am. 2010;36(2):385-404. doi: 10.1016/j.rdc.2010.03.003, PMID 20510240.
30. Bax M, Van Heemst J, Huizinga TW, Toes RE. Genetics of rheumatoid arthritis: What have we learned? Immunogenetics. 2011;63(8):459-66. doi: 10.1007/s00251-011-0528-6, PMID 21556860.
31. Lin B, Zhao Y, Han P, Yue W, Ma XQ, Rahman K, *et al*. Anti-arthritic activity of *Xanthium strumarium* L. extract on complete Freund's adjuvant induced arthritis in rats. J Ethnopharmacol. 2014;155(1):248-55. doi: 10.1016/j.jep.2014.05.023, PMID 24862493.
32. Tag HM, Kelany OE, Tantawy HM, Fahmy AA. Potential anti-inflammatory effect of lemon and hot pepper extracts on adjuvant-induced arthritis in mice. J Basic Appl Zool. 2014;67(5):149-57. doi: 10.1016/j.jobaz.2014.01.003.
33. Zhang X, Dong Y, Dong H, Zhang W, Li F. Investigation of the effect of phlomisoside F on complete Freund's adjuvant-induced arthritis. Exp Ther Med. 2017;13(2):710-16. doi: 10.3892/etm.2016.3995, PMID 28352356.
34. Rajendran R, Krishnakumar E. Anti-arthritic activity of *Premna serratifolia* Linn., wood against adjuvant induced arthritis. Avicenna J Med Biotechnol. 2010;2(2):101-6. PMID 23407688.
35. Mondal P, Das S, Mahato K. Evaluation of antiarthritic potential of the hydro-alcoholic extract of the stem bark of *Plumeria rubra* in Freund's complete adjuvant-induced arthritis in rats. IJPSR.2016;7:3675-88.
36. Singhai A, Patil UK. Amelioration of oxidative and inflammatory changes by *Peganum harmala* seeds in experimental arthritis. Clin Phytosci. 2021;7(1):1-11. doi: 10.1186/s40816-020-00243-3.
37. Aggarwal BB, Prasad S, Reuter S, Kannappan R, Yadev VR, Park B, *et al*. Identification of novel anti-inflammatory agents from Ayurvedic medicine for prevention of chronic diseases: Reverse pharmacology and bedside to bench approach. Curr Drug Targets. 2011 Oct;12(11):1595-653. doi: 10.2174/138945011798109464, PMID 21561421, PMCID PMC3170500.
38. Kaur M, Dangi CBS, Singhai A, Singh M, Kosta S, Singh H, *et al*. Toxicity Profile of ethanolic extract of *Lawsonia inermis* leaves in albino Wistar rats. World J Pharm Pharm Sci. 2014;3:835-48.
39. Borah DJ, Iqbal F. Anemia in recent onset Rheumatoid Arthritis. JK Sci. 2007;9(3):120-22.
40. Srivastava AK, Patil UK, Singhai A, Kumar M. Antioxidant activity of *Nelumbo nucifera Gaertn* stalks in rats. Int J Pharm Pharm Sci. 2015;7:368-73.
41. El-barbary AM, Khalek MAA, Elsalawy AM, Hazaa SM. Assessment of lipid peroxidation and antioxidant status in rheumatoid arthritis and osteoarthritis patients. Egypt Rheumatol. 2011;33(4):179-85. doi: 10.1016/j.ejr.2011.07.002.
42. Lawrence JS, Heberden O. Rheumatoid arthritis—nature or nurture? Ann Rheum Dis. 1969;29:357-79.
43. Rooney M, Condell D, Quinlan W, Daly L, Whelan A, Feighery C, *et al*. Analysis of the histologic variation of synovitis in rheumatoid arthritis. Arthritis Rheum. 1988;31(8):956-63. doi: 10.1002/art.1780310803, PMID 2457377.
44. [cited 4/4/2022]Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Delphinine#section=Computed-Properties.
45. Schneider W. Die Struktur des Diterpenalkaloids Delphinin 12. Mittlg: Aconitum-Alkaloide. Arch Pharm. 1960;293(6):577-85. doi: 10.1002/ardp.19602930603.

PICTORIAL ABSTRACT



SUMMARY

- The ethanolic extract of *Aconitum ferox* roots obtained after successive solvent extraction has shown significant antiarthritic potential in the CFA induced arthritic rats. The dose of *Aconitum ferox* is critical due to its toxicity.
- The histological and radiological studies of the joint supported the claim.
- The AF root extract also significantly affected the cellular oxidative stress.
- A molecule delphinine was isolated from the ethanolic extract with solvent-solvent extraction and separation with precipitation technique utilizing less toxic and commonly available solvents to prevent any residual solvent toxicity. TLC, UV, FTIR and LCMS studies helped in the characterization of the isolated phytoconstituent.

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