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TO STUDY THE ANTI-INFLAMMATORY ACTIVITY OF ACONITUM *FEROX* (WOLF'S BANE)

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ABSTRACT

Ethanolic extract of **ACONITUM FEROX ROOT** was used for the present project work. The Successive extract was subjected to preliminary phytochemical testing for the detection of major chemical groups. **ANTI-INFLAMMATORY ACTIVITY** of plant was determined using ***CARRAGEENAN* INDUCED PAW EDEMA MODEL**. Four groups of mice were prepared, each group having five mices. First group was control group. 0.1 ml of 1% *carrageenan* was given to this group. Second group was given standard Indomethacin 5gm/kg. Third group was given drug 250 mg/Kg and fourth group was given 500 mg/Kg of Aconitum ferox. Four readings of paw were taken using Plethysmometer. There was significant reduction in paw volume in Indomethacine standard group ($p<0.05$) as compared to control group at 4 hour readings. There was dose dependent decrease in paw volume in ethanolic extract treated groups. The ethanol extract (250and 500 mg/kg) significantly ($p<0.05$) and dose-dependently inhibited carrageenan-induced rat paw edema (65.31 % and 77.85 %, respectively) when compared with control group after 1 hour of carrageenan injection.

KEYWORDS: Carrageenan, paw edema volume, anti-inflammatory activity, Aconitum ferox, Indomethacin.

1. INTRODUCTION

1.1 Disease[1-2]

Living tissues respond locally (inflammation is a reaction) to both endogenous and external stimuli. The term is derived from the Latin word "inflamer" which means "to burn". Inflammation is the body's attempt to neutralise or eliminate foreign invaders, get rid of irritants, and prepare the ground for tissue restoration. Living tissue's severe reaction to any type of insult is inflammation. It entails a carefully planned series of cellular and fluid changes in living tissue. It is defined as the local response of living mammalian tissues to injury due to any factors including pathogens, damaged cells and toxic compounds. The process is designed by immune cells invading the tissues just like an army in full battle mode.[24] "Immunity or immune reaction" and "inflammatory response" by the host are both protective mechanisms in the body—inflammation is the visible response to an immune reaction, and activation of immune response is almost necessary before inflammatory response shows.

Signs of Inflammation: There can be four primary indicators of inflammation as:

- Rubor (redness)
- Tumor (swelling)
- Calor (heat)
- Dolor (pain)
- Functio Laesa (loss of function)

Clinical Manifestation of Inflammation

- Fever
- Changes of WBC's
- Proliferation of mononuclear phagocyte system
- Alteration of parenchymal cells.
- Redness
- Swollen joint that is warm to touch
- Joint pain
- Joint stiffness
- Loss of joint function
- "Flu-like" symptoms
- Chills
- Fatigue
- Loss of energy
- Headaches
- Loss of appetite

1.2 Plant profile

1.2.a Aconitum ferox[3]

In past years, most of the disease used to be treated by administration of plants, plants products or multiple plant products combination. *Aconitum ferox* (syn. *A. virorum), commonly referred to as Aconitum virorum, is a kind of monkshood that belongs to the Ranunculaceae family.* The commonly name by which it is most often known is

Indian Aconite or Wolf's bane.

Fig. 1: *Aconitum ferox* Flower.

Aconite, Monkshood, wolf's bane, leopard's bane, Indian vish, bikh, mouse bane, women's bane, devil's

Aconitum ferox is also known by the common names helmet, queen of all poisons, and blue rocket. Although other Ayurvedic herbs like Ahiphena, Bhanga, Dhatura, Langali, Karvira, and Jayapal are poisonous as well, Vatsanabha is the most lethal of them all. Its heavy dose may cause fatal effects even it may lead to death also. The main ingredient in the Indian poison is aconitum ferox.

Fig. 2: *Aconitum ferox* dried roots.

Table 1: Taxonomical hierarchy.[4]

Kingdom	Plantae
Subkingdom	Tracheobionta
Division	Spermatophyta
Subdivision	Magnoliophyta
Class	Magnoliopsida
Subclass	Magnoliidae
Order	Ranunculales
Family	Ranunculaceae (buttercup family)
Genus	*Aconitum*
Species	*Ferox* Wall

Table 2: Vernacular name.[5]

Hindi	Vish, Bachang
English	Wolf's bane, Indian Aconite
Sanskrit	Bachnag, Meetavish
Gujarati	Mahoor, Bachang
Malayalam	Vatsanabhi
Tamil	Vasnumbi
Bihari	Dakara
Telugu	Nabhi, Vasnabhi

Medicinal Uses[6]

It is used in Ayurvedic treatment of fever, spleen disorders, pain, digestion, anorexia, gout, cough, asthma, anorexia, spleen disease, gout, cough, asthma, eye infections, inflammation, otitis, relieve headache, sciatica, backacheAconitum ferox is pungent, bitter and astringent in taste. It serves as a catalyst for other medications, or yogavahi.

The dried root has sedative, alterative, stimulant, anaesthetic, arthritic, diaphoretic, and diuretic properties. Taking aconite root orally is dangerous. Aconite contains a strong, fast-acting poison that causes severe side effects such as nausea, vomiting, weakness, sweating, breathing problems, heart problems and death. It is best harvested in the autumn as soon as the plant dies down. This plant is extremely dangerous, thus it should only be used with great care and under a professional's guidance. It has been traditionally used in India and Nepal in the treatment of -

- Neuralgia
- Leprosy
- Cholera
- Fever due to cold

- Pneumonia
- Rheumatism

Chemical Constituents[7]

Toxic alkaloids including pseudo-aconitine, bikhaconitine, chasmaconitine, and indaconitine are found in roots. It is regarded as one of the most deadly plants in the world and the most poisonous plant found in the Himalayas because it contains significant amounts of the exceedingly toxic alkaloid pseudaconitine, also known as nepaline after Nepal.The tuber of Vatsanabha contains 0.4–0.8% diterpene alkaloids and the concentration of aconite in the fresh plant is between 0.3% and 2.0% in tubers and 0.2% and 1.2% in the leaves. Wintertime is when aconite is present in the maximum concentration. The major alkaloids are aconitine, Pseudaconitine, Bikhaconitine, Di-Acetyl Pseudaconitine, Aconine, Picro-Aconine, Veratry Pseudaconitine, Chamaconitine, Veratryl Gama Aconine, and Di-Ac–Y-Aconitine. Its main alkaloid, aconitine, has the chemical formula C34H47NO11 and is only very slightly soluble in water. It is more soluble in ether or chloroform. It is a neurotoxin that opens TTX-sensitive Na+ channels in the heart and other tissues, and is used for creating models of cardiac arrhythmia.

Roots contain toxic alkaloids, Pseudo-Aconitine, Bikhaconitine, Chasmaconitine, Indaconitine, etc. The poisonous substance obtained from this herb is called aconitine. Aconitine (Acetylbenzoylaconine, $C_{33}H_{47}NO_{11}$). Additionally, the roots of aconitum plants contain isoquinolines, quaternary ammonium compounds, and catecholamine alkaloids. It should contain not less than 0.6 per cent of alkaloids of aconite, of which not less than 30 per cent should be Aconitine. Pseudaconitine, an alkaloid, is present in significant concentrations.

2. MATERIAL AND METHODS

2.1 Collection of Aconitum ferox Plant- Fresh whole plant of ***Aconitum ferox*** is cultivated in Darjeeling hills of West Bengal, Laddakh. The dried roots of Aconite plant are collected from Chitrakoot region.

2.2 Authentication- Root of the selected plant's ***Aconitum ferox*** cultivated in Darjeeling hills of West Bengal, Laddakh. Identified and authentication by **Prof. P.JAYARAMAN M.sc,Ph.d, Director, Plant Anatomy Research Center (PARC)** west Tambaram, Chennai -45 Reference : Nair, N.C & Henry, A.N. Flora of Tamil nadu, India and date of authentication 01/02/2020 , I : pg-167 .1983, Ibid II : - .1987 , ibid III .1989.

2.3 Chemical and Reagents: Carrageenan, ethyl alcohol, acetone, chloroform, Mayer's reagent, Wagner's reagent, Dragendorff's reagent, Molisch's Reagent, Hager's reagent, Borntrager's Reagent, Benedict's Reagent.

2.4 Apparatus: - Plethysmometer, Soxhlet Apparatus, Electronic Balance, Heating Mantle, Petri Dish/ Glass rod, Funnel/ Measuring Cylinder.

2.5 Extraction Process[8]

The hot continuous extraction technique is another name for extraction. The Soxhlet extractor setup consists of a round bottom flask, siphon tube, distillation path, condenser, cooling water inlet, cooling water outlet, heat source and thimble. In this method, sample dried, powdered, grinded into small particles and placed in a porous bag or "thimble" made from a strong filter paper, which is placed, is in thimble chamber of the Soxhlet apparatus. This method is not suitable for thermolabile compounds as extended heating may lead to degradation of compounds. This method maintains a relatively high extraction temperature with heat from the distillation flask. A fine coarse powder was obtained which was sieved through #40 to obtain uniformity. The powdered obtained was extracted in ethanol. To combat the crude medicine, petroleum ether or hexane were typically used to the powdered root. Defatting assists in removing the oily, fatty, and other low polar common components from the plants, making it easier to process them further with a yield of 8.87%. Soxhlet assembly was performed on 200 grammes of the powdered root in 1.5 litres of 60% ethanol. The extract was then concentrated under pressure until dry, yielding 16.4 g (8.2%) of a dark greenish solid. The extracts were then made to powder under reduced pressure.

3.1 Qualitative Analysis: Different biochemical parameters like reducing sugar, Flavonoid, Terpenoid, Tannin, Saponin, Anthraquinone, glycosides, alkaloidsetc. were tested.

3.1.a Test for Alkaloids[9,10]

Mayer's Test: Extract treated with Meyer's reagent gives cream coloured precipitate which indicates the presence of Alkaloids**.**

Dragendorff's Test: Addition of Dragendorff's reagent (solution of Potassium Bismuth Iodide) to the extract gives reddish brown coloured precipitate.

3.1.b Test for Carbohydrates

Fehling's Test: Extract on boiling with equal proportions of Fehling's A and Fehling's B solution gives yellow to brick red coloured precipitate.

Molisch's Test: To the extract added few drops of Molisch's reagent and 1-2 ml conc. Sulfuric acid slowly seeps through the test tube's sides. When a violet ring forms at the junction, there are carbs present.

3.1.c Test for Proteins

Biuret Test: Extract on treatment with 4% Sodium hydroxide and 1% Copper sulphate solutiongives violet or pink colour.

3.1.d Test for Glycosides

Borntrager's Test: Extract when shaken with Benzene gently for few minutes. The organic layer on treatment with Ammonia, give rose pink color in Ammoniacal layer.

3.1.e Test for Saponins

Froth test: 20ml of distilled water was used to dilute the extracts, and this solution was agitated in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foams indicates the presence ofsaponins.

3.2 Experimental Animals

Studies were carried out using Wistar rats of either sex weighing 180–200 g. They were obtained from central animal facility of **Shri Ram College of Pharmacy, Banmore, M.P., and India (SRCP/M. Pharma/IAEC/55/18-1)** and was maintained in polypropylene cages on rodent pellet condition of controlled temperature (22±2^{o}C) and acclimatized to 12/12 h light/dark cycle. Free access to food and water was allowed until 2h before the experiment. The care and maintenance of the animals was as per the approved guidelines of the "Committee for the purpose of control and supervision of experiments on animals (CPCSEA)". All experimentson animals were conducted according to the guidelines of establishment's ethical committee on animal experimentation.

The rats were randomly allocated into five groups of five rats each for the four Different experimental animal models. We have used Rat models for testing Anti-inflammatory.

3.3 Chemicals: Carrageenan, Standard drug (Indomethacin).

Table 3: Experimental Design.

Group	Name of Group	Treatment	Dose
I	Control	Normal Saline	0.9ml/kg
II	Standard Drug	Indomethacin	5mg/kg
III	Test Group(A)	*Aconitum ferox Root Extract*	250mg/kg.
IV	Test Group(B)	*Aconitum ferox Root Extract*	500mg/kg

4. RESULTS AND OBSERVATION

The plant material's phytochemicals were removed. The solvent, ethyl alcohol was used for the separation of chemical component.

4.1 Phytochemical Screening[11-16]

Using a conventional screening test, the extract was checked for the presence of several elements. An established methodology was utilised to check for the presence of substances such as steroids, alkaloids, tannins, flavonoids, glycosides, etc.

Table 4: Phytochemical Screening.

S.NO	Test	Result
1	**Carbohydrates**	
I	Barfoed Test	+
II	Benedict Test	+
III	Fehling's test	+
IV	Molish Test	+
2	**PROTEINS**	
I	Biuret Test	+
II	Millon's Test	+
III	Ninhydrin Test	+
IV	Xanthoproteic Test	+
3	**GLYCOSIDES**	
I	Borntrager's Test	+
II	Killer-Killani test	+
III	Raymond's Test	+
4	**SAPONINS**	
I	Faom Test	_
II	Froth Test	_

(+) Indicates positive result, (–) Indicates negative result

4.2 Evaluation of Anti-Inflammatory Activity[17,18]

4.2.a Carrageenan Induced Paw Edema in Rats

In the present study, anti-inflammatory activity was determined in wistar rats of either sex according to the method of Winter et al. All groups were injected with 1% carrageenan (in 1 % CMC) solution into the sub-plantar region of rat right hind paw. The first group is referred as Control Group as they received normal saline, whereas third and fourth groups received low dose (250mg/kg) and higher dose (500mg/kg) respectively of *Aconitum ferox* through oral gavage. Second group served as standard, received Indomethacin (5mg/kg) through oral gavage. Before 1 hour of injecting of carrageenan the rats were treated with different doses of *Aconitum ferox* Indomethacin and normal saline. The volume of paw oedema was measured by dislocation Displays the water column in an Orchid Scientific Plethysmometer after 1, 2, 3, and 4 hours after a carrageenan injection.

% of paw oedema inhibition is equal to Oc - Ot / Oc x 10. Oc represents the average increase in paw volume (average inflammation) of the rats in the control group at a given time.

O_t = was the average inflammation of the drug treated (i.e., plant extracts or test drug aspirin) rats at the same time. The difference in the initial 0h and volume at +1h indicate paw edema at 1h following carrageenan administration. Accordingly paw edema at +1, +2 +3, and +4 hours was calculated.

4.2.b Plethysmometer[19,20]

a plethysmometer created for quick, accurate detection of

paw swelling or inflammation in small rodents. The volume is determined by weight and specific gravity, based on the principles of Archmides. The exact volume of the animal paw will be displaced when it is dipped into the water in the cell up to the pre-marked point on the paw. Stable measurements may be taken thanks to a finger rest.

It is designed to provide a highly useful tool in the measurement of small volume changes. This test is often used to assess experimentally generated inflammation in rodents and to check for pharmacological agents' possible anti-inflammatory or anti-oedema effects.

Fig. 3: Plethysmometer.

4.3 Evaluation[21]

The average reaction time for each group that received therapy was calculated and contrasted with the results for each group before to treatment. The formula for percentage increase in reaction time (I%) is I% = (It - Io)/Io 100. t, Io, and It = reaction time at time = reaction time at time, t, and Io = reaction time at time zero (0 h). The animals were subjected to the same test procedure at +1, +2, +3, +4 and +5 hr. after the administration of test/standard/control drug. Standard ($p<0.05$) as compared to control group at 4-hour readings. There was dose dependent decrease in paw volume in ethanolic extract treated groups. The Ethanolicextract (250mg/kg and 500mg/kg) significantly ($p<0.05$) and dose-dependently inhibited carrageenan- induced rat paw oedema (65.31% and 77.85%, respectively) when compared with control group after 1 hour of carrageenan injection.

4.4 RESULTS

In carrageenan induced rat paw oedema there was significant reduction in paw volume in Indomethacin.

Table 5: Effect of Ethanolic extract of *Aconitum ferox* carrageenan induced rat paw oedema (Mean paw volume).

Treatment Group	MPV 1st hour	MPV 2nd hour	MPV 3rd hour	MPV 4th hour
Control	0.70±0.08	0.83±0.19	0.92±0.26	0.97±0.97
Standard drug	0.26±0.05*	0.22±0.09*	0.21±0.15*	0.16±0.81*
Aconite ferox (250mg/kg)	0.54±0.08	0.41±0.11	0.32±0.14*	0.28±0.52*
Aconite ferox (500mg/kg)	0.48±0.01	0.37±0.06*	0.25±0.06*	0.19±0.42*

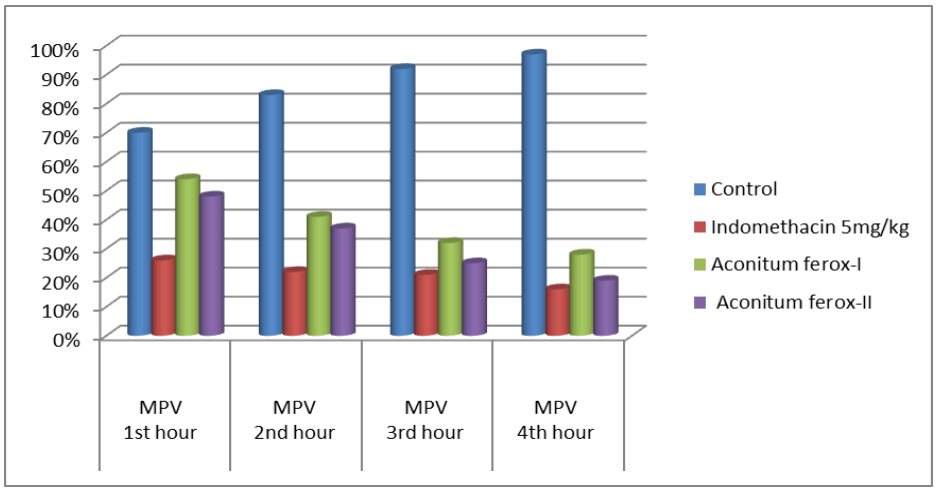

Fig. 4: Mean Paw volume (MPV).

Table 6: Effect of Ethanolic extract of *Aconitum ferox carrageenan* induced rat paw edema (Percentage of inhibition).

Treatment Group	(%) inhibition 1st hour	(%) inhibition 2nd hour	(%) inhibition 3rd hour	(%) inhibition 4th hour
Control	-	-	-	-
Standard drug	65.38%	73.98 %	82.58 %	84.87 %
Aconite ferox (250mg/kg)	29.05 %	50.10 %	57.98 %	65.31 %
Aconite ferox (500mg/kg)	36.08 %	53.11 %	71.84 %	77.85 %

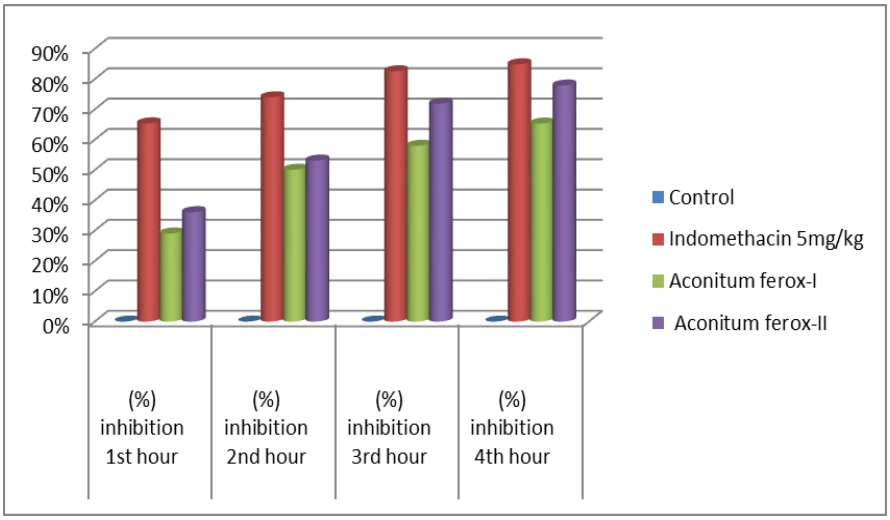

Fig. 5: Effect of Ethanolic extract of *Aconitum ferox* carrageenan induced rat paw edema (Percentage of inhibition).

5. DISCUSSION

This method was chosen for the present study as edema induced by carrageenan is the most popular and prominent experimental model in the discovery of new anti- inflammatory drugs. The carrageenan induced rat paw edema is considered as the most suitable in vivo model to study anti-inflammatory drugs both steroidal and non-steroidal since it involves several mediators. In this study, anti-inflammatory effects of ethanolic extract of *Aconite ferox* is investigated after sub-plantar injection of carrageenan in mouse paw. This study showed that the crude extracts (ethanol) of *Aconite ferox* possess anti-inflammatory activities in rat model. The observation that the ethanolic extract of *Aconite ferox* exhibited greater anti-inflammatory activity, more of the active principle (s) responsible for the anti-inflammatory activity might be present in higher concentration in the ethanolic extract. One of the primary indicators of inflammation is the occurrence of edoema. In addition, it is a method that been frequently used to assess the anti edematous effect of natural products. Furthermore, carrageenan is devoid of apparent systemic effects and exhibits a high degree of reproducibility. Carrageenan injections locally cause two distinct phases of inflammatory responses. Though it is difficult to speculate the time of onset for anti- inflammatory activity, ethanol extract of the dried roots of *Aconite ferox* for anti- inflammatory activity showed rapid onset, i.e. starting from eight minutes. The duration, however, seems to be fairly long. To get full pictures of their pharmacokinetic profiles, further investigation should be undertaken. Both extracts and fractions of *Aconite ferox* showed persistent anti- inflammatory activity during the four hours observation time. This study may give hint about the onset and duration of action of the extracts.

6. CONCLUSION

The results of this study show that dried root extracts of *Aconite ferox* roots exhibited anti-inflammatory activity with ethanol extract having higher activity. Phytochemical test of *Aconite ferox* extracts the alcoholic extracts revealed thepresence of Carbohydrate, Alkaloid and Glycosides, saponins.Further investigation, however, should be pursued after isolation and characterization of the active principle in order to come up with the active compound responsible for the anti-inflammatory properties of the *Aconite ferox*. However, otherstudies need to be performed to confirm the exact mechanism and anti-inflammatory activity of the plant in chronic inflammatory models. Proper regulatory mechanism is recommended to ensure safety & efficacy of herbal products. The wide range of chemical structures provided by natural sources is under investigation for their chemical as well as

pharmacological screening. It is possible to evaluate Indian traditional medicine by properly utilising a rich biodiversity and significant ancient texts in the context of current instruments & techniques.

7. FUTURE PROSPECTIVE

Aconite Plant is an extremely powerful and potentially toxic herb native to Asia. It has been used since thousands of years by practitioners of traditional medicine. Active constituent found Pseudaconitine is highly effective on microbial growth. It isa valuble plant which has bright medical future for humankind. Aconite not to be taken for pregnant and should never be used on broken skin. This plant has great approach for drug delivery system of micro particles. Significant basic and clinical research has been carried out on the medicinal plants and their formulations. Indian medicinal plants also provide a rich source for antioxidants that are known to prevent/delay different diseased states. Medicinal herbs as potential source of therapeutics aids have attained a significant role in health system all over the world. Information on the scientific basis of these plants will hopefully contribute to a greater understanding of Ayurveda and Indian herbal remedies worldwide. Rich dividends will result from this in the upcoming years. Those plants are used in herbal treatment of various diseases. As, the economic importance of medicinal plants is much more in our indigenous systems of medicine.

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