Annals of Pharmacology and Pharmaceutics

Research Article
Published: 06 Mar, 2020

Evaluation of Anti-Arthritic Potential of *Arisaema propinquum* Schott Rhizomes against Complete Freund's Adjuvant Induced Arthritic Rats

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Abstract

Ethnopharmacological relevance: *Arisaema propinquum* Schott (Araceae) commonly known as cobra lily. Traditionally the rhizomes were used as vermifuge, in rheumatism, as stomach-ache and in snake bites.

Aim: The present study was designed to evaluate the anti-arthritic potential of methanolic and aqueous extract of *Arisaema propinquum* Schott rhizomes in experimental animal models.

Material and Methods: *A. propinquum* Schott rhizomes were defatted with hexane and then extracted with methanol using hot extraction method in a soxhlet apparatus. Aqueous extract was prepared by decoction method. Both the extracts were evaluated at two doses 200 mg/kg and 400 mg/kg, against Freund's complete adjuvant induced chronic immunological arthritis in rats. Paw edema, paw diameter, body weight and nociceptive threshold were measured during the study. Biochemical parameters like hemoglobin content, red and white blood cells, erythrocyte sedimentation rate, platelet count were estimated. Serum parameters like COX-2, IL6 and TNF-α were also estimated for assessing the anti-arthritic potential of methanolic and aqueous extract of *A. propinquum* rhizomes. Histopathological studies were also done on day 21, after animals were sacrificed.

Result: Dose dependent and significant inhibition of paw edema was observed in animals treated methanolic and aqueous extract of *A. propinquum*. The anti-arthritic activity of extract was also supported by the results of body weight, biochemical parameters and nociceptive threshold. Treatment with the extract in experimental animals also decreased the histopathological alterations induced by Freund's complete adjuvant in experimental animals.

Conclusion: In the present study, both methanolic and aqueous extract protects synovial membrane by improving the health status through hematinic parameters and exhibits promising anti-arthritic activity. This finding thus supports the traditional claim of *A. propinquum* Schott rhizomes for use in rheumatism.

Keywords: ***Arisaema propinquum;*** **Freund's complete adjuvant; Rheumatism; Paw edema; Biological parameters; Nociceptive threshold**

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Received Date: *07 Feb 2020*
Accepted Date: *03 Mar 2020*
Published Date: *06 Mar 2020*

Citation:
Ahad Mir P, Mohi-ud-din R, Akbar Dar M, Bader NG. Evaluation of Anti-Arthritic Potential of Arisaema propinquum Schott Rhizomes against Complete Freund's Adjuvant Induced Arthritic Rats. Ann Pharmacol Pharm. 2020; 5(2): 1180.

Introduction

Rheumatoid Arthritis (RA) is a widespread autoimmune disease characterized by pain, morning stiffness, synovial and peripheral joint inflammation, articular tissues destruction and joint deformation [1,2]. RA is the most prevalent disorders affecting 0.5% to 1.0% of total population, with females being affected three times more than males [3,4]. RA cause severe disability restricts the quality of life and causes premature death [5]. RA affects not only the body but also affects the mental condition and also confers an increased risk of many other diseases including cardiovascular disease, pulmonary dysfunction and renal disorders [6-8].

Rheumatoid arthritis begins with the inflammation of the synovial membrane, takes proliferative nature (pannus formation) resulting in damage of cartilage and bones [9,10]. The aetiology of the disease are still not fully understood, however it is believed that RA is likely to be multi-factorial with intense interactions amongst genetic, environmental, biomechanical factors, neuro-immunological interactions and altered articular microvascular function [11]. Chemokines, as well as other inflammatory mediators play key roles in the pathogenesis of RA. Imbalance between

pro- and anti-inflammatory cytokine activities favors the induction of autoimmunity, chronic inflammation and thereby joint damage [12].

The various treatment methods used for the treatment of arthritis include physical therapy, medications and surgery. The various drugs include glucocorticoids (cortisone and prednisone etc.) NSAIDS(Ibuprofen and naproxen etc.), disease-modifying anti-inflammatory drugs (Methotrexate (MTX) and leflunomide etc.), and biological response modifiers like TNF-α blocking agents, anti-CD 20 therapy (rituximab) and abatacept which are often required to inhibit or halt the underlying immune processes. However, besides the expensive cost and numerous side effects, researchers are directed towards herbal system of medicine for the discovery of drugs with potent activity and no or minimum side effects [13,14].

Arisaema propinquum Schott also known as Wallich's cobra lily or simply cobra in English, Hapat makhi in Kashmiri belongs to family Araceae. Literature survey of the plant revealed its traditional use in the treatment of diabetic neuropathy and rheumatoid arthritis [15]. Poultice prepared from chopped tubers are used to treat chronic boils. Water extract of its bulbs is used for skin eruptions and paste prepared from roots is applied externally for Erysipelas and scabies [16,17].

Materials and Methods

Plant material

The rhizomes of *Arisaema propinquum* Schott were collected from Doodhpathri, Dist. Budgam, Jammu and Kashmir. The plant was authenticated by the centre of Plant Taxonomy, Department of Botany, University of Kashmir, Hazratbal and specimen was kept in KASH herbarium under a specific voucher number 1895-KASH.

Preparation of extracts

Extraction was carried out according to standard procedures using analytical grade solvents. The coarse powder of the rhizomes (500 mg) was loaded in soxhlet apparatus and was extracted with methanol using hot extraction method. Aqueous extract was prepared by decoction method. Both the extracts were concentrated on rotary evaporator (IKA RV 10) under reduced pressure. The dried crude methanolic and aqueous extract of *A. propinquum* was collected and preserved in an airtight glass container at 4°C to 8°C.

Chemicals: All the chemicals used were of analytical grade and were procured from registered dealers like Central Drug House (P) LTD. Bombay, Hi Media Laboratories Pvt. Ltd. Mumbai and Rankem Pvt. Ltd. Haryana, India. CFA was procured from *In vivo* Gen- San Diego, USA.

Preparation of standard

The reference anti-inflammatory drug Indomethacin was dissolved in normal saline for the study. The drug solution was freshly prepared and administered orally at dose 10 mg/kg.

Animals

Male Albino rats (wistar strain), weighing 150 g to 200 g were obtained from animal house of Indian Institute of Integrative Medicine-Jammu (IIIM-Jammu), Jammu and Kashmir, India, approved by CPCSEA, Government of India. For carrying out animal studies, the protocol was approved by Institutional Animal Ethical Committee under Registration No. 801/03/CA/CPCSEA. The animals were housed in polypropylene cages and maintained under standard environmental conditions: 25°C ± 2°C, 12:12 h light: dark cycle and 45% to 55% humidity, with free access to food and water *ad libitum* and received human care according to requirements of National Institute of Health (NIH) guidelines for care and use of laboratory animals.

Experimental procedure

Acute oral toxicity study: The acute toxicity study was performed as per Organization for Economic Cooperation and Development (OECD) Guidelines No. 425. Initially one animal was administered with aqueous extract of *P. alpinum* rhizomes at a dose of 2000 mg/kg b.w, p.o and observed for 0 h, 1 h, 2 h, 4 h and 6 h then at 24 h. The animal survived and therefore four more animals were dosed at 2000 mg/kg b.w, p.o and were observed similarly. All five animals survived. Same method was used for methanolic extract. All the animals survived indicating LD_{50} is greater than 2000 mg/kg for all the extracts. On the basis of acute toxicity study $1/10^{th}$ and $1/5^{th}$ of the dose i.e. 200 mg/kg and 400 mg/kg b.w were selected for further experimental evaluation.

Chronic immunological FCA-induced arthritis in rats: Adjuvant arthritis was induced in rats according to the method described by Newbould, with slight modification [18,19]. In this method, the initial hind paw volumes (both left and right) of the experimental animals were measured by mercury plethysmometer. The animals were anaesthetized with ether followed by 0.1 mL of CFA (heat killed Mycobacterium tuberculosis in sterile paraffin oil) injection into the sub plantar tissue of the right hind paw. Inflammatory edema of the hind paw peaking at 7^{th} to 8^{th} day was indicative of successful induction of adjuvant arthritis.

Experimental procedure: The animals (albino rats) were divided into seven groups of six animals each. Baseline recording of the paw volume was made by using mercury plethysmometer.

Group I: Vehicle control (1% CMC solution, 10 mL/kg, orally).

Group II: FCA control (0.1 mL CFA).

Group III: Standard (10 mg/kg orally).

Group IV and V: Methanolic extract (200 mg/kg and 400 mg/kg body weight/rat/day, orally).

Group VI and VII: Aqueous extract (200 mg/kg and 400 mg/kg body weight/rat/day, orally).

After thirty minutes of vehicle/drug administration, arthritis was induced by sub plantar injection of FCA. This was designated as day 1^{st}. After immunization with FCA, all groups were maintained on vehicle/drug treatment up to 21 days. Anti-arthritic activity of methanolic and aqueous extract was evaluated on paw volume, joint diameter, and pain withdrawal latency on day 1^{st}, 3^{rd}, 5^{th}, 7^{th}, 9^{th}, 11^{th}, 13^{th}, 15^{th}, 17^{th}, 19^{th} and 21^{st}. Moreover body weights of animals were monitored regularly during the course of the experiment. On day 21^{st} all the experimental animals were scarified by cervical dislocation, the tibiotarsal joint was dissected and collected into 10% formalin solution for histopathological study. The blood was collected through heart puncture and centrifuged. Serum was separated for the measurement of biological parameters like TNF-alpha, COX-2 and IL-6 using Elisa kits. Whole blood was used for the measurement of various hematological parameters like RBC, Hb, ESR and Total WBC.

Parameter assessment

Paw volume: Paw volume was measured using Plethysmometer

Table 1: Effect of methanolic and aqueous extracts of *Arisaema propinquum* on complete Freund's adjuvant induced paw edema.

Day	1st	5th	9th	13th	17th	21st
Normal	0.68 ± 0.47	0.96 ± 0.95	0.96 ± 0.95	0.96 ± 0.95	0.78 ± 1.22	0.78 ± 1.22
Toxic	1.8 ± 1.55	6.02 ± 1.41[a]*	11.96 ± 1.67[a]*	14.9 ± 0.77[a]*	15.84 ± 1.27[a]*	16.24 ± 1.55[a]*
Standard	1.65 ± 0.5	4.1 ± 0.45[b]*	10.42 ± 1.97	10.52 ± 2.11[b]*	7.26 ± 2.84[b]*	3.06 ± 2.09[b]*
Alc. Prop. 400	1.58 ± 0.04	4.22 ± 0.53[b]*	10.78 ± 0.64	11.82 ± 0.64[b]*	8.4 ± 1.27[b]*	3.58 ± 0.8[b]*
Alc. Prop. 200	1.64 ± 0.49	5.66 ± 0.64	11.06 ± 0.43	12.96 ± 0.43	10.92 ± 0.52[b]*	6.16 ± 1.59[b]*
Aq. Prop. 400	1.33 ± 0.55	4.78 ± 0.52[b]*	10.98 ± 0.97	12.34 ± 0.89[b]*	9.18 ± 1.51[b]*	5.86 ± 0.82[b]*
Aq. Prop. 200	1.55 ± 0.96	5.84 ± 0.39	11.58 ± 0.45	14.44 ± 0.43	11.05 ± 0.45[b]*	7.86 ± 0.87[b]*

Results are expressed as Mean ± SEM (n=6), the comparisons were made by ANOVA followed by post-hoc tests, [a]*P-value <0.05 compared to normal control and [b]*P<0.5 compared to toxic control

Table 2: Showing the effect of alcoholic and aqueous extracts of *Arisaema propinquum* on joint diameter.

Day	1st	5th	9th	13th	17th	21st
Normal	0.10 ± 0.0	0.10 ± 0.03	0.10 ± 0.03	0.10 ± 0.03	0.10 ± 0.03	0.10 ± 0.03
Toxic	0.15 ± 0.15	1.10 ± 0.07[a]*	2.04 ± 0.10[a]*	3.22 ± 0.09[a]*	3.66 ± 0.10[a]*	4.90 ± 0.12[a]*
Standard	0.18 ± 0.01	0.56 ± 0.05[b]*	0.96 ± 0.09[b]*	0.78 ± 0.05[b]*	0.50 ± 0.70[b]*	0.24 ± 0.05[b]*
Alc. Prop. 400	0.14 ± 0.05	0.84 ± 0.50	1.56 ± 0.05	1.28 ± 0.05[b]*	1.00 ± 0.03[b]*	0.64 ± 0.04[b]*
Alc. Prop. 200	0.21 ± 0.16	1.00 ± 0.05	1.88 ± 0.03	1.94 ± 0.05[b]*	1.54 ± 0.50[b]*	1.12 ± 0.03[b]*
Aq. Prop. 400	0.15 ± 0.15	0.64 ± 0.05[b]*	1.36 ± 0.05[b]*	1.66 ± 0.02[b]*	1.20 ± 0.03[b]*	0.84 ± 0.02[b]*
Aq. Prop. 200	0.17 ± 0.17	1.14 ± 0.05	2.42 ± 0.08	2.34 ± 0.05[b]*	1.78 ± 0.03[b]*	1.20 ± 0.07[b]*

Results are expressed as Mean ± SEM (n=6), the comparisons were made by ANOVA followed by post-hoc tests, [a]*P-value <0.05 compared to normal control and [b]*P<0.5 compared to toxic control

on day 0 (initial paw volume) before CFA injection and on alternative days i.e. 1st, 3rd, 5th, 7th, 9th, 11th, 13th, 15th, 17th, 19th and 21st. Difference between final and initial paw volume was calculated as change in paw volume [20].

Joint diameter: Joint diameter was measured using vernier caliper on day 0 before CFA injection and on alternative days i.e. 1st, 3rd, 5th, 7th, 9th, 11th, 13th, 15th, 17th, 19th and 21st. Difference between final and initial joint diameter was calculated [20].

Anti-nociceptive activity: The method described by Uma-Devi was used for this experiment. Animals were tightly held leaving tail hanging out freely and dipped into a water bath containing warm water maintained at a temperature of 50°C ± 1°C. The time taken for the animal to flick its tail or withdraw it from the warm water known as the Pain Reaction Time (PRT) was recorded for all the rats. The reaction time was determined before and after oral administration of extracts [21].

Measurement of organ weight: On 21st day after scarifying the animals with cervical dislocation, the spleen and thymus were removed and the weight of the organs was recorded and corrected for 100 g body weight.

Blood and serum analysis: On day 21st, blood was withdrawn from each animal through heart puncture into a test tube containing anticoagulant (EDTA). The blood collected was divided into two parts. To first part, hematological parameters like Red Blood Cell (RBC) count, White Blood Cell (WBC) count, Hemoglobin (Hb), and Erythrocyte Sedimentation Rate (ESR) were determined using standard procedures and to another part, serum were separated by centrifuging blood at 3000xg for 10 min for estimation of biological precursors like TNF-α, IL-6 and COX-2 using ELISA kits.

Histological analysis: After sacrifice on day 21st, ankle joints were separated and immersed in 10% buffered formalin followed by decalcification in 5% formic acid. The tissue was embedded in paraffin and sectioned at 5 µ thickness. The sections were stained with hematoxylin and eosin. An experienced pathologist (Dr. Mohammed Maqbool Darzi) evaluated the slides under light microscope for the presence of hyperplasia of synovium, fibrosis, destruction of joint space and inflammatory cells.

Statistical analysis: The results were expressed as mean ± SD. Statistical comparison was made between the drug-treated group and arthritic-control group, determined by two-way ANOVA followed by post-hoc test comparison test using Graph pad prism statistical computer software. P<0.01 or P<0.05 was considered as statistically significant.

Results

Effect of extracts on paw volume

Sub plantar immunization with CFA in experimental animals produced an increase in paw volume in all rats compared to vehicle control. Both methanolic and aqueous extract of *A. propinquum* Schott rhizomes showed significant dose dependent reduction in paw volume when compared with toxic group. Methanolic extract at the dose of 400 mg/kg and 200 mg/kg showed significant P<0.05 paw edema inhibition by 80.17% and 63.30%, while as its aqueous extract (400 mg/kg and 200 mg/kg) showed paw edema inhibition of 70.81% and 61.69% as compared to standard indomethacin 10 mg/kg showed potent anti-arthritic activity with 89.28% paw volume reduction under same experimental conditions (Table 1 and Figure 1).

Effect of extracts on joint diameter

The joint diameter of both hind limbs of all experimental animals were measured on day 0 before CFA injection and taken as baseline values (Table 2 and Figure 2). The joint diameter remains almost unchanged in normal control during the study. In CFA-induced arthritis group (toxic) joint diameter significantly increases (P-value

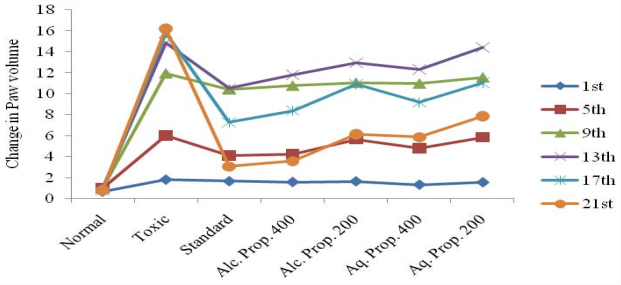

Figure 1: Results are expressed as Mean ± SEM (n=6), the comparisons were made by ANOVA followed by post-hoc tests, [a]*P-value <0.05 compared to normal control and [b]*P<0.5 compared to toxic control.

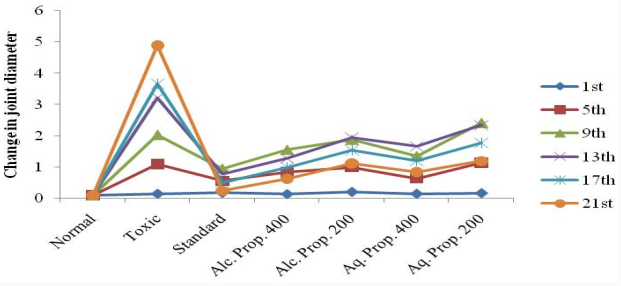

Figure 2: Results are expressed as Mean ± SEM (n=6), the comparisons were made by ANOVA followed by post-hoc tests, [a]*P-value <0.05 compared to normal control and [b]*P<0.5 compared to toxic control.

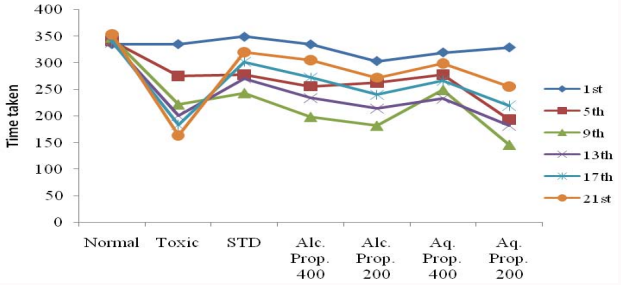

Figure 3: Results are expressed as Mean ± SEM (n=6), the comparisons were made by ANOVA followed by post-hoc tests, [a]*P-value <0.05 compared to normal control and [b]*P<0.5 compared to toxic control.

<0.05) as compared to normal control. Significant (P-value <0.05) decrease in joint diameter was observed in animals treated with the methanolic and aqueous (200 mg/kg and 400 mg/kg) extract of *A. propinquum* Schott rhizomes as well as standard drug indomethacin. On intergroup comparison joint diameter was significantly reduced in indomethacin 10 mg/kg treated group followed by methanolic extract 400 mg/kg treated group as compared to CFA control group.

Effect of extracts on body weight

Immunization with CFA in experimental animals showed less body weight gain as compared to vehicle control possibly due to immune response generation (Table 3). Significant weight gain was observed in animals treated with the standard indomethacin, methanolic and aqueous extract (200 and 400 mg/kg) as compared to toxic group.

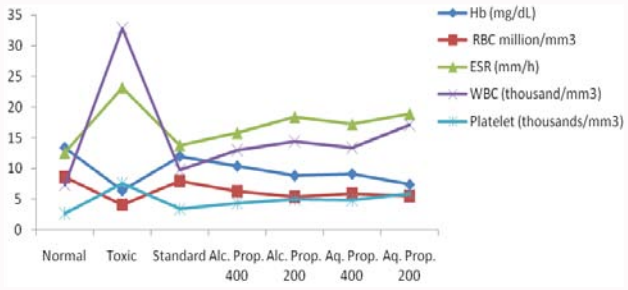

Figure 4: Results are expressed as Mean ± SEM (n=6), the comparisons are made by ANOVA followed by tukey test [a]***P<0.001 compared to normal control and [b]***P<0.001 compared to toxic control.

Table 4: Effect of methanolic and aqueous extract of *Arisaema propinquum* Schott on spleen and thymus.

	Dose (mg/kg)	Organs	
		Spleen (g)	Thymus (g)
Normal	--	0.59 ± 0.64	0.67 ± 0.29
Toxic	--	1.08 ± 0.59	0.38 ± 0.06
Indomethacin	10	0.64 ± 0.68*	0.61 ± 0.08
Alc. Propinquum	400	0.69 ± 0.56**	0.58 ± 0.12***
Alc. Propinquum	200	0.76 ± 0.71*	0.53 ± 0.09*
Aq. Propinquum	400	0.75 ± 0.54**	0.55 ± 0.10**
Aq. Propinquum	200	0.89 ± 0.65	0.41 ± 0.15

Effect of extracts on body organs

Spleen and thymus were dissected after sacrificing the animals on 21st day and the weight of the organs were recorded (Table 4). The results showed increase in spleen weight and decrease in thymus weight in CFA control as compared to normal control. The increase in spleen weight was significantly (P<0.05) inhibited in animals treated with the standard indomethacin, methanolic and aqueous extract (200 and 400 mg/kg) as compared to toxic control. Similarly the decrease in thymus weight was significantly increased (P<0.01) in animals treated with the methanolic and aqueous extract of *A. propinquum* Schott rhizomes as compared to toxic control.

Effect of extracts on nociceptive threshold

Consistent decrease in nociceptive threshold was observed in CFA treated compared to normal control animals (Table 5 and Figure 3). Pre-treatment with methanolic and aqueous extract (200 and 400 mg/k) of *A. propinquum*as well as indomethacin (10 mg/kg) showed significant and dose dependent antinociceptive activity (P-value

Table 3: Effect of methanolic and aqueous extract of *Arisaema propinquum* Schott on body weight.

Treatment days	Toxic	Standard	Alc. Prop. 400	Alc. Prop. 200	Aq. Prop. 400	Aq. Prop. 200
1st	197 ± 0.12	184 ± 0.33	183 ± 0.19	184 ± 0.04	179 ± 0.05	177 ± 0.19
7th	193 ± 0.31	184.4 ± 0.21	185 ± 0.15	186.4 ± 0.05	180.4 ±	178.4 ± 0.16
14th	197 ± 0.16	189 ± 0.99	186 ± 0.39	188 ± 0.12	181.8 ± 0.33	179.4 ± 0.46
21st	187 ± 0.46	195.4 ± 0.04**	191 ± 0.15	193 ± 0.97	184 ± 0.07**	182.8 ± 0.03***

Results are expressed as Mean ± SEM (n=6), the comparisons were made by ANOVA followed by tukey's tests, *P-value <0.01 and *P-value <0.001 compared to toxic control

Table 5: Antinociceptive activity alcoholic and aqueous extracts of *Arisaema propinquum* in arthritic rats.

Day	1st	5th	9th	13th	17th	21st
Normal	335.60 ± 5.92	341.60 ± 3.50	347.60 ± 5.51	339.60 ± 5.73	342.00 ± 10.05	354.00 ± 3.45
Toxic	335.60 ± 5.92	275.60 ± 3.61^{a*}	222.40 ± 3.26^{a*}	201.60 ± 5.24^{a*}	184.00 ± 5.90^{a*}	164.00 ± 2.02^{a*}
Standard	349.60 ± 16.45	278.00 ± 9.76	243.60 ± 12.19	271.60 ± 9.50^{b*}	302.60 ± 9.10^{b*}	320.60 ± 9.29^{b*}
Alc. Prop. 400	335.20 ± 2.17	256.00 ± 5.41	199.00 ± 2.91	234.20 ± 4.36^{b*}	273.00 ± 3.36^{b*}	305.20 ± 3.20^{b*}
Alc. Prop. 200	303.40 ± 0.60	263.00 ± 2.02	182.40 ± 3.23^{b*}	214.60 ± 2.56	240.80 ± 2.81^{b*}	272.60 ± 3.32^{b*}
Aq. Prop. 400	319.80 ± 2.87	277.80 ± 7.43	249.60 ± 8.17	233.40 ± 3.15^{b*}	267.00 ± 3.78^{b*}	299.20 ± 0.80^{b*}
Aq. Prop. 200	329.00 ± 5.58^{b*}	193.00 ± 1.37^{b*}	146.60 ± 3.70^{b*}	182.40 ± 1.82	220.20 ± 3.65^{b*}	255.80 ± 2.85^{b*}

Results are expressed as Mean ± SEM (n=6), the comparisons were made by ANOVA followed by post-hoc tests, ^{a*}P-value <0.05 compared to normal control and ^{b*}P<0.05 compared to toxic control

Table 6: Effect of methanolic and aqueous extract of *Arisaema propinquum* Schott rhizomes on hematological parameters.

Parameters	Hb (mg/dL)	RBC million/mm^3	ESR (mm/h)	WBC (thousand/mm^3)	Platelet (thousands/mm^3)
Normal	13.42 ± 0.10	8.62 ± 0.11	12.60 ± 0.40	7.30 ± 0.55	2.64 ± 0.17
Toxic	6.43 ± 0.45^{a*}	4.14 ± 0.17^{a***}	23.2 ± 0.66^{a***}	32.86 ± 3.38^{a***}	7.57 ± 0.23^{a***}
Standard	12.04 ± 0.08^{b***}	7.93 ± 0.02^{b***}	13.8 ± 0.37^{b***}	9.80 ± 0.29^{b***}	3.36 ± 0.17^{b***}
Alc. Prop. 400	10.42 ± 0.13^{b***}	6.35 ± 0.05^{b***}	15.8 ± 0.244^{b***}	12.96 ± 0.13^{b***}	4.30 ± 0.14^{b***}
Alc. Prop. 200	8.86 ± 0.28^{b***}	5.45 ± 0.23 ns	18.4 ± 0.244^{b***}	14.38 ± 0.16^{b***}	5.00 ± 0.11^{b***}
Aq. Prop. 400	9.14 ± 0.02^{b***}	5.98 ± 0.01^{b***}	17.2 ± 0.20^{b***}	13.35 ± 0.19^{b***}	4.88 ± 0.17^{b***}
Aq. Prop. 200	7.42 ± 0.17^{b***}	5.60 ± 0.05 ns	18.9 ± 0.20^{b***}	17.12 ± 0.44^{b***}	5.93 ± 0.01^{b***}

Results are expressed as Mean ± SEM (n=6), the comparisons are made by ANOVA followed by tukey test ^{a***}P<0.001 compared to normal control and ^{b***}P<0.001 compared to toxic control

Table 7: Effect of methanolic and aqueous extract of *Arisaema propinquum* Schott rhizomes on IL-6, TNF-α and COX-2 levels.

Parameters	IL-6 (pg/mL)	TNF-α (pg/mL)	COX-2 (ng/mL)
Normal	12.79 ± 2.21	10.73 ± 0.97	10.46 ± 0.84
Toxic	236.65 ± 18.70^{a***}	196.68 ± 10.51^{a***}	210.14 ± 10.03^{a***}
Standard	91.61 ± 1.75^{b***}	21.81 ± 1.85^{b***}	28.37 ± 0.36^{b***}
Alc. Prop. 400	143.44 ± 3.32^{b***}	45.25 ± 1.37^{b***}	48.83 ± 0.73^{b***}
Alc. Prop. 200	192.09 ± 5.07^{b***}	60.23 ± 3.75^{b***}	63.40 ± 2.01^{b***}
Aq. Prop. 400	184.74 ± 2.52^{b***}	51.82 ± 1.75^{b***}	62.72 ± 1.30^{b***}
Aq. Prop. 200	201.82 ± 1.30^{b*}	76.18 ± 4.60^{b***}	66.92 ± 2.54^{b***}

Results are expressed as Mean ± SEM (n=6), the comparisons are made by ANOVA followed by tukey test ^{a***}P<0.001 compared to normal control and ^{b***}P<0.001 compared to toxic control

<0.05) from day 10th as compared to toxic group.

Effect of extracts on hematological parameters

Significant increase (P<0.001) in WBC, ESR and platelet count and decrease in RBC and Hb level was observed in CFA control animals as compared to normal control (Table 6 and Figure 4). Immunization with the methanolic and aqueous extracts (200 mg/kg and 400 mg/kg) as well as standard indomethacin (10 mg/kg) Significantly (P<0.001) reduced the WBC, ESR and platelet count level and increase the RBC and Hb level as compared to CFA control rats.

Effect of extracts on biochemical estimation

Injection of adjuvant in the hind paw causes significant increase (P<0.001) in IL-6, TNF-alpha and COX-2 levels in all rats as compared to control rats. Both methanolic and aqueous extract (200 mg/kg and 400 mg/kg) significantly decrease all these biological precursors as compared to CFA control group. Standard drug indomethacin (10 mg/kg) significantly reduced the biological precursors to a greater extent than the methanolic extract followed by aqueous extract. The IL-6 level, TNF-α and COX-2 levels of the standard groups were found to be 91.61 ± 1.75 pg/mL, 21.81 ± 1.85 pg/mL and 28.37 ± 0.36 pg/mL respectively, which was least as compared to extract treated group (Table 7 and Figure 5).

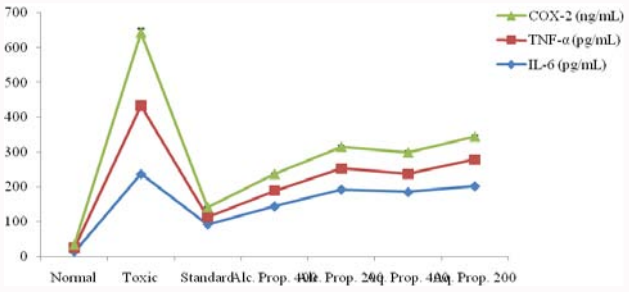

Figure 5: Results are expressed as Mean ± SEM (n=6), the comparisons are made by ANOVA followed by tukey test ^{a***}P<0.001 compared to normal control and ^{b***}P<0.001 compared to toxic control.

Histopathology of ankle joint

Histopathological studies stained with hematoxylin and eosin of normal tibiotarsal joint showed normal synovial membrane, with normal chondrocytes (Figure 6A). Immunization with CFA causes massive chondrocyte destruction, lymphocyte infiltration, cartilage destruction and pannus formation shown by toxic control (Figure

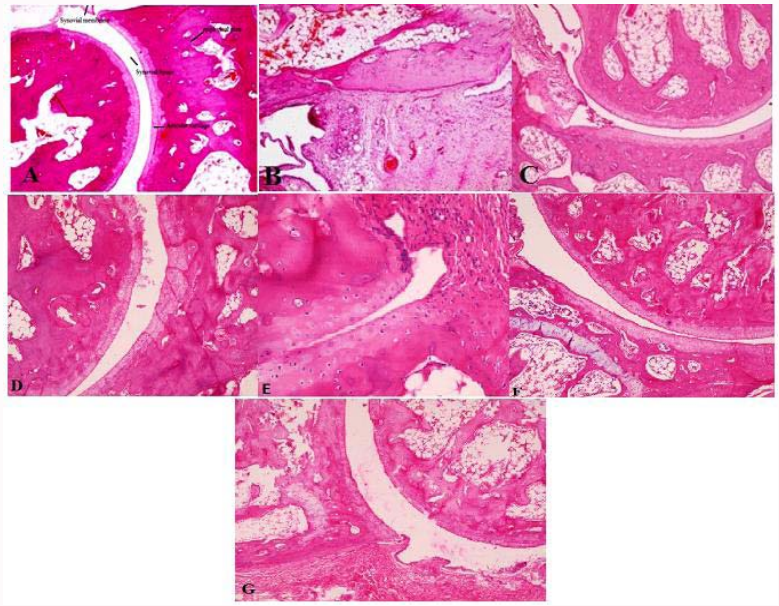

Figure 6: Histopathological analysis of tibiotarsal joint, 21 days after immunization with CFA compared with unimmunized albino rats, stained with H&E (A) Normal control, (B) Arthritic control, (C) Standard drug treated 10 mg/kg, (D) Alcoholic extract of *A. propinquum* 400 mg/kg, (E) Alcoholic extract of *A. propinquum* 200 mg/kg, (F) Aqueous extract of *A. propinquum* 400 mg/kg, (G) Aqueous extract of *A. propinquum* 200 mg/kg.

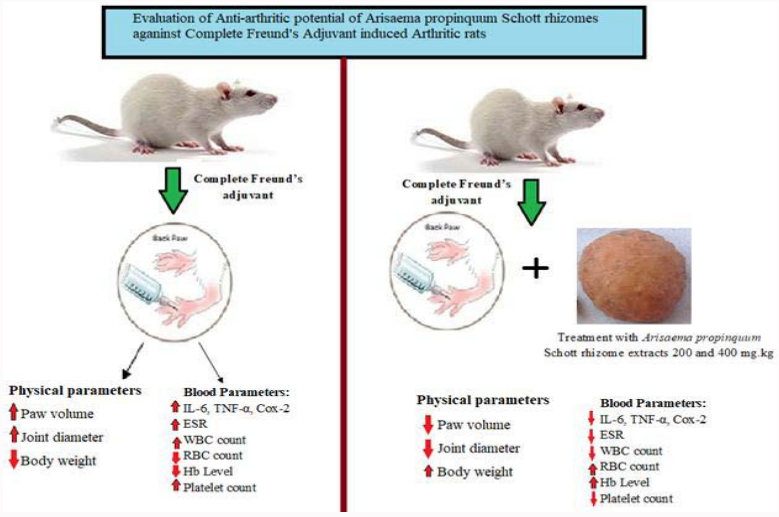

Figure 7: Evaluation of anti-arthritic potential of *Arisaema propinquum* Schott rhizomes against Complete Freund's Adjuvant Induced Arthritic rats.

6B). In contrast to these the rats treated with Indomethacin (10 mg/kg) showed significant protection against inflammatory response, with lowest irregular synovial space (Figure 6C). Animals treated with methanolic extract of *A. propinquum* (200 mg/kg and 400 mg/kg) showed epiphyseal plate and chondrocyte destruction, synovial membrane rupture and lymphocyte infiltration (Figure 6D and 6E). However the methanolic extract 400 mg/kg showed more protection than 200 mg/kg as compared to toxic control. Animals treated with aqueous extracts of *A. propinquum* (200 mg/kg and 400 mg/kg) showed significant protection against cell necrosis, punus formation with mild epiphyseal plate destruction (Figure 6F and 6G).

Discussion

CFA model is the most widely used chronic experimental model for polyarthritis that mimics the clinical features of human rheumatoid disease [22]. The first phase of the reaction i.e. soft-tissue thickening at the injected site is due to irritant effect of the adjuvant while as the late phase of the arthritis are presumed to be immunologic events [23]. Swelling of non-injected paw that is secondary lesions is due to manifestation of cell mediated immunity and the suppression of such secondary lesions by a drug shows its immunosuppressive activity.

Rheumatoid arthritis is an autoimmune disorder with unknown aetiology characterized by release of various inflammatory mediators. In recent researches role of various pro-inflammatory cytokines like tumor necrosis factor-α (TNF-α), interlukin-1b (1L-1b), 1L-6, 1L-8, MCSF, interferon's and Platelet Derived Growth Factor (PDGF) has been discovered for the limb and joint swelling along with pain, joint destruction, deformity and disability [24]. Measurement of paw volume is an important factor for evaluating degree of inflammation and therapeutic effect of drugs. The present investigation revealed that, immunization with CFA cause significant increase in paw volume in disease control group. Administration of methanolic and

aqueous extract of *A. propinquum* delayed the onset and reduced the severity of disease, shown by decreasing the paw volume *via* inhibiting the release of inflammatory mediators, hence representing its anti-inflammatory potential.

Alcoholic extract of *A. propinquum* (200 and 400 mg/kg) showed significant P<0.05 paw edema inhibition by 63.30% and 80.17% and reduced the joint diameter by 77.95% and 91.83% respectively. (Table 2 and 3) compared to toxic control while as its aqueous extract (200 mg/kg and 400 mg/kg) showed paw edema inhibition of 61.69% and 70.81% and reduced joint diameter by 72.24% and 78.36% respectively. Indomethacin (10 mg/kg) showed potent anti-arthritic activity exhibited by reduction in paw volume by 89.28% and reduction in joint diameter by 95.10%.

Rheumatoid arthritis is commonly accompanied with weight loss and loss of body mass, known as rheumatoid cachexia. The disease also leads to decreased physical activity and muscle strength [25]. Inflammatory condition reduces the intestinal absorption capacity of ^{14}C-glucose and ^{14}C-leucine and can be improved by using anti-inflammatory drugs [26]. Immunization with CFA in experimental animals showed less body weight gain as compared to vehicle control possibly due to immune response generation (Table 4). Significant weight gain was observed in animals treated with the standard indomethacin, methanolic and aqueous extract (200 mg/kg and 400 mg/kg) of *A. propinquum* Schott rhizomes as compared to toxic group.

The antinociceptive activity of methanolic and aqueous extract *A. propinquum* in CFA induced models was evaluated by tail flick method. Both the extracts significantly prolonged tail flicking reaction as compared to toxic group (Table 5).

Rheumatoid arthritis is commonly associated with anemia for which exact mechanism is not known. In this study, toxic group showed significant reduction in RBC count, Hb level and an increased Erythrocyte Sedimentation Rate (ESR) and WBC count. The groups treated with alcoholic and aqueous extracts of *Arisaema propinquum* (200 and 400 mg/kg) showed significant increase in RBC count, Hb level and decrease in erythrocyte sedimentation rate and WBC count indicating significant recovery from anemia. The decrease in hematological parameters like ESR, WBC and platelet count and increase in the Hb and RBC count by the extracts supports the anti-arthritic activity of these extracts.

Serum analysis showed significant increase in TNF-α, IL-6 and Cox-2 levels in arthritic control animals after CFA injection, which were significantly decreased in animals treated with standard drug indomethacin (10 mg/kg). Both alcoholic and aqueous extract (200 and 400 mg/kg) of *A. propinquum* showed significant decrease P<0.001 in serum IL-6, TNF-α and COX-2 levels compared to toxic control.

Histopathological studies revealed massive destruction in articular cartilage and chondrocytes after CFA injection as compared to normal group. Both methanolic and aqueous extracts of *A. propinquum* showed significant protection from tissue damage and punus formation in animals previously treated with CFA (Figure 6 and 7).

Conclusion

On the basis of present findings, it was concluded that both methanolic and aqueous extracts of *Arisaema propinquum* Schott rhizomes possess potent anti-arthritic activity. The activity of the extracts may be mediated by inhibiting inflammatory mediators like cytokines and leukotriene infiltration as evidenced in paw edema volume. Also, improvement in health parameters like HB, ESR and body weight demonstrates valuable effects while recovering from arthritis. Hence, the present results support traditional claim & thus reveals the promising anti arthritic potential of *Arisaema propinquum* Schott rhizomes used in the treatment of painful arthritis and other inflammatory conditions.

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