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Original Research Article

Anti-arthritic activity of leaves and oil of *Aquilaria agallocha*

Habibur Rahman[1], M. Chinna Eswaraiah[2], A. M. Dutta[3]

[1] Associate. professor, Department of Pharmacology, Anurag Pharmacy College, Kodad, Andhra Pradesh-508206, India.
[2] Principal, Anurag Pharmacy College, Kodad, Andhra Pradesh-508206, India.
[3]Professor, Department of Chemistry, Assam Down Town University, Guwahati, Assam

***Corresponding Author:**
Habibur Rahman
Email: habiburruh@gmail.com

Abstract: The present study conducted for *Aquilaria agallocha* of family Thymelaeaceae for anti-arthritic activity. The ethanolic extract of *Aquilaria agallocha* (EEAA) leaves and the *Aquilaria agallocha* oil (AAO) from Heart Wood were studied using *in-vitro* BSA denaturation method and *in-vivo* Freund's adjuvant induced arthritic rat model. The inhibition of protein denaturation *in-vitro* model and paw volume, hematological parameters and radiology of hind legs were studied. EEAA (100, 250 and 500 µg/ml) showed 34.09%, 36.95% and 43.13% inhibition respectively and AAO (100, 250 and 500 µg/ml) showed 23.68%, 48.21% and 56.71% inhibition of protein denaturation respectively and are comparable with Diclofenac (100, 250 and 500 µg/ml) showed 39.58%, 75.83% and 77.51% . In FA Arthritic model EEAA (200 mg/kg and 400 mg/kg) inhibited the increase in paw volume and maximum inhibition were 21.20% and 25.34% respectably on 21th day. The percentage of inhibition was found gradually increasing with the day of treatment. Treated group with AAO (125 mg/kg and 250 mg/kg) inhibited the increase in paw volume and maximum inhibition were 19.78% and 27.88% respectably on 13th and 21th day respectably. Further the hematological and radiological also studies revealed the antiarthritic activity of EEAA and AAO.

Keywords: *Aquilaria agallocha*, anti-arthritic activity, Freund's adjuvant, BSA denaturation.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by persistent inflammation of multiple peripheral joints. It is one of the most common inflammatory diseases due to chronic inflammatory proliferation of the synovial linings of diarthrodial joints, which leads to aggressive cartilage destruction and progressive bony erosions. If untreated, rheumatoid arthritis often leads to progressive joint destruction, disability, and premature death[1].

Till it is believed that cause of rheumatoid arthritis is unknown, but it is supposed to be triggered by the combination of genetic susceptibility and exposure to environmental factors[2]. A critical role for T cells in the pathogenesis of RA is suggested by the strong association between RA and certain human leukocyte antigen (HLA) haplotypes. Recent data suggest that the destruction of rheumatoid joints is initiated by complex cell-cell interaction between antigen presenting cells and CD4+T cells. However, it is thought that these cell-cell interaction result in the activation of macrophages and induction of the inflammatory process, culminating in degradation and resorption of cartilage and bone. Pro inflammatory cytokines particularly TNF and interleukin 1(IL-1) are critical components of this process[3-4].

Rheumatoid arthritis is consistent worldwide affecting about 0.5-1.0% of the population[5]. It usually occurs in people between 25 and 55 years of age. Women are more prone than men at a ratio of 3 to 1. Nearly 46 million U.S. adults were reported to be arthritic, among this prevalence rate a quarter million are children and 60% are women. While in India, the prevalence of rheumatoid is reported to be about 0.75% and this prevalence rate is higher than that reported from China, Indonesia, Philippines and rural Africa[6].

The drugs commonly in use for the treatment of arthritis include NSAIDS (eg- Ibuprofen and naproxen) and glucocorticoids (eg-cortisone and prednisone) to suppress the symptoms , while disease-modifying antirheumatic drugs (DMARDs) such as anti-tumour necrosis factor (TNF)-α therapy (eg-etanercept, infliximab and adalimumab), anti-CD20 therapy (eg-rituximab) and abatacept are often required to inhibit or halt the underlying immune process[2,3].

However, all of these agents are associated with numerous side effects. In recent days, researchers are directed towards traditional system of medicine for the discovery of drugs that are long acting effect with minimum side effects[7]. In India, many Ayurvedic practitioners are using various indigenous plants for the treatment of different types of arthritic conditions.

Although the application of these medicaments has a sound tradition and a rational background according to the Indian system of medicine, perhaps it is essential to investigate the rationality of their use in modern scientific terms[7].

Agar wood (*Aquilaria agallocha* of family Thymelaeaceae) is extremely rare and precious plant available in North Eastern India, Bhutan and parts of South East Asia. The Oil of this plant is known as wood oil used in perfume industry and is well known in Middle East and Arab counties The plant has reported to possess anti-nociceptive and anti-inflammatory[8-9], anti-microbial[10], lower hypersensitivity reactions[11], laxative[12], anti oxidant activity[13], CNS activity[14], sedative effect[15] and anti-hyperglycaemic activity[16].

Hence, the aim of this study is to prove the therapeutic potential of *Aquilaria agallocha* as an anti-arthritic agent using *in-vitro* BSA denaturation method and *in-vivo* Freund's adjuvant induced arthritic rat model.

MATERIAL AND METHODS

Plant material

The leaves and Hard Woods of *Aquilaria agallocha* were collected from Local Vendors from Hojai, Nagaon Dist of Assam. The plant materials were authenticated by Prof (Dr.) K. Madhava Chetty, Taxonomist, SV University, Tirupati. A voucher specimen was kept in department for reference.

Extraction of Plant materials

The leaves were dried in shade at room temperature then subjected to size reduction to a fine powder with the help of electric grinder. The grinded plant material was subjected to Soxhlet extraction (45^0-55^0C) employing 95% Ethanol as solvent. Appearance of colourless solvent in the siphon tube was taken as the termination of extraction. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated.The percentage yield of the extract was 19.56%.

The oil was obtained by hydro distillation process from the hard wood as industrial scale and was directly obtained from supplier from Assam.

Chemicals and drugs

The chemicals used were Freund's Complete Adjuvant Injection (Sigma Chemicals), Ethanol (Rankem, New Delhi), and Diclofenac sodium (Akums Drugs and Pharmaceuticals, India). Ibuprofen (Nice Chemical Limited, Mumbai). All the chemicals and solvents used in this study were of pharmaceutical grade.

Experimental Animals

Rats of either sex weighing 150-200 g weight were used in experiment. Animals were obtained from Anurag Pharmacy College, Kodad. Animals were kept under standard conditions at 25 $\pm 2^0$C 12 hr light/dark cycle and given standard pellet diet and water. The animals were accustomed to the laboratory conditions for a week prior to the experimentation. Before using in experiment animals got clearance from CPCSEA. Registration No. 177/99/CPCSEA

Preliminary phytochemical tests

The ethanolic extract of *Aquilaria agallocha* leaves (EEAA) were tested for different phytoconsituents like alkaloids, glycosides, saponinins , tannins, protein, carbohydrates using standard procedures[17].

Physiochemical studies of oil

The oil obtained from Aquilaria agallocha were tested for qualitative tests for oraganoleptic characters, solubility, specific gravity, refractive index, saponification value, iodine value and chemical tests for oils.

Acute oral toxicity studies

Ethanolic extract of *Aquilaria agallocha leaves* (EEAA) was screened for toxicity by oral toxicity studies according to OECD guidelines 423 taking three female Wister rats with starting dose of 2000mg/kg body.

Acute oral toxicity study was carried out for *Aquilaria agallocha* oil (AAO*)* using Acute Toxic Class Method as described in OECD (Organization of Economic Co-operation and Development) Guidelines No. 423 with starting dose level of 2,000 mg/kg body weight

In-vitro Anti-arthritic activity using Bovine serum albumin (BSA) denaturation Method

The *in-vitro* antiarthritic activity was performed by using bovine serum albumin denaturation(BSA) method[18,19]. The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of *EEAA and AAO* (100, 250, 500 mcg/ml of final volume). pH was adjusted at 6.3 using a small amount of 1 N HCl. The samples were incubated at 37°C for 20 min and then heated at 57°C for 30 min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm for control test 0.05 ml distilled water was used instead of extracts while product control test lacked bovine serum albumin. The results were compared with diclofenac (100, 250 and 500 mcg/ml). The percentage inhibition of protein denaturation was calculated.

In-vivo Freund's adjuvant induced arthritis Model in rats

Arthritis was induced in rats by the intraplantar injection of 0.1 ml of Complete Freund's Adjuvant (CFA) in the left hind paw[20,21]. Albino rats were divided into 7 groups of 6 rats in each. Group I served as normal (10 ml/kg of 2% Tween 80, p.o) which were given with vehicle only. Group II served as control (10 ml/kg of 2% Tween 80, p.o)injected with 0.1ml of Freund's adjuvant into sub plantar region of hind paw and group III (EEAA 200 mg/kg, p.o), Group IV(EEAA 400 mg/kg, p.o), Group V(AAO 125 mg/kg, p.o), Group VI(AAO 250 mg/kg, p.o) were test compounds with low and high dose of EEAA and AAO and group VII(Ibuprofen, 50 mg/kg p.o) served as standard, given with ibuprofen. All the groups except normal (group-I) will be injected with single dose of 0.1 ml of Freund's adjuvant and are to be treated with standard/extract for 12 consecutive days. Paw volumes of both paws are measured with Plethysmometer on day 0, 3,5,9,13,21 to note the primary lesionto note the primary lesion and to study the influence of standard and extracts on this phase. On day 21, Blood is collected through retro orbital puncture for serum analysis, later sacrificed by overdose of ether.

Estimation of Hematological parameters

The effect of the plant extracts (EEAA and AAO) for the estimation of hematological parameters was carried out. Blood of approximately 1 ml was collected from each group of the animal by retro-orbital puncture in to the EDTA coated tubes of accurately 1ml and shakes the tubes immediately, to mix up with the EDTA. Then the blood containing tubes are subjected to hemocytometer for the determination of White Blood Cells (W.B.C), Red Blood Cells (R.B.C), Hemoglobin (Hb) Platelets

Radiographic analysis

On day 21, animals were anesthetized with ketamine (45mg/kg). Radiographs of the FCA injected joints were taken with a Dental X-ray machine. Rats were placed on a radiographic box at a distance of 90 cm from the X-ray source. The X-ray image of the FCA injected joints of each rat was evaluated for radiographic changes.

Statistical Analysis

The statistical analysis was carried by one way ANOVA followed by Dunnet's multiple "t"test. P values < 0.05 (95% confidence limit) was considered statistically significant, using software Graph Pad Prism5.

RESULTS

Result of Preliminary Phytochemical Screening

Ethanolic extract of *Aquilaria agallocha* (EEAA) leaves were subjected for phytochemical screening and found EEAA of leaves to contain carbohydrates, flavonoids, glycosides, saponins, tannins and triterpines and Phenolic compounds and AAO from heartwood found to contain mainly Glycosides, Phenolic compounds, Tannins and Terpinoids.

Result of Preliminary physiochemical tests

The AAO was screened for various Physicochemical test as per the reported methods and found the oil Colour appeared as dark brown to bark yellow, Odour occurs as aromatic sweet, spicy fresh odour., specific gravity contains 0.952 at 25oC. The oil is soluble in organic solvents such as ethanol but insoluble in water, Saponification value 195, iodine value 186, and refractive index 1.52. Chemical tests also performed and confirmed as an essential oil.

Results of Acute Oral Toxicity Study

Acute oral toxicity studies of Ethanolic extract of *Aquilaria agallocha* (EEAA) *and Aquilaria agallocha* oil (AAO) were carried out according to OECD-423 guidelines in wister female rats. EEAA and AAO at a dose of 2000 mg/kg, p.o. exhibited normal behaviour, without any signs of passivity, stereotypy and vocalization. Their motor activity and secretary signs were also normal and no sign of depression. EEAA even showed no toxicity though repeated dose but AAO showed toxicity when repeated. LD ^{50} cut off value was obtained from the Flow Chart of OECD-423 and dose for Administration were taken as 1/20th and 1/10th of LD ^{50} as high and low dose of EEAA and AAO.

Results of *in-vitro* anti-arthritic activity on bovine serum protein denaturation method

The *in-vitro* anti- arthritic activity of EEAA and AAO has been done by on bovine serum protein denaturation method the results are given at Table-1. EEAA and AAO (100, 250 and 500 µg/ml) showed increase the absorbance. The percentage inhibition of protein denaturation was calculated and found for EEAA at 100, 250 and 500 µg/ml showed 34.09%, 36.95% and 43.13% inhibition respectively. AAO at 100, 250 and 500 µg/ml showed 23.68%, 48.21% and 56.71% inhibition of protein denaturation respectively. Diclofenac (100, 250 and 500 µg/ml) showed 39.58%, 75.83% and 77.51% inhibition of protein denaturation respectively.

Result of anti-arthritic activity on Freund's adjuvant induced arthritis in rats

The results of anti-arthritic activity of EEAA and AAO on increase in paw volume and percentage reduction in paw volume in Freund's adjuvant induced paw oedema in rats given in Table-2 and shown in Fig-1. Freund's adjuvant treated group noted a significant increase in paw volume from 1st day to 21th day of the experimental study. On 0 day, Normal paw volumes were recorded as 0.823 ml and after injection with Freund's adjuvant the volume is gradually increased from 1st day to 21th day with a maximum 1.634ml respectively. The percent increase in paw volume is noted as 33.30%, 43.13%, 49.63%, 49.60 and 49.50%

on 3rd, 5th , 9th, 13th and 21th days of the study respectively. Treated group with EEAA (200 mg/kg and 400 mg/kg) inhibited the increase in paw volume and maximum inhibition were 21.20% and 25.34% respectably on 21th day. The percentage of inhibition was found gradually increasing with the day of treatment. Treated group with AAO (125 mg/kg and 250 mg/kg) inhibited the increase in paw volume and maximum inhibition were 19.78% and 27.88% respectably on 13th and 21th day respectably. The percentage of inhibition was found gradually increasing with the day of treatment. Where, Standard drug Ibuprofen at 50 mg/kg, showed maximum reduction 43.50 at 13th day. But, percentage of inhibition was irrespective of days of treatment and with minimum 23.34 at 3rd day.

Effect of EEAA and AAO in Hematological parameters on Freund's adjuvant induced arthritis in rats

The results of the Effect of EEAA and AAO in haematological parameters on Freund's adjuvant induced arthritis in rats in given in Table-3. Normal readings of blood parameters Hg (14.38±0.78) in g%, Total RBC (5.22 ±0.38 Millions/mm^3), Total WBC(7234±51.08 /mm^3) and Platelets (2.82±0.05, 2.82±0.05). Freund's adjuvant treated animals significantly decreased Hemoglobin level (10.52±1.20, $p<0.01$),Total RBC count (4.25±0.30 millions/mm^3, $p<0.05$) and Platelets (1.54±0.09, $p<0.001$) and increased total WBC count(1098±54.13, $p<0.001$). Groups treated with EEAA (200mg/kg and 400 mg/kg) and AAO (125 mg/kg and 250 mg/kg) dose dependent way significantly improved blood parameters and showed better result than Ibuprofen (50 mg/kg).

Radiographic Analysis

The effect of EEAA and AAO on Radiographic examination is shown in Fig-2(a) to Fig-2 (g). The complete Freund's adjuvant induced hind paw of arthritic rats control showed swelling, widening of joint spaces and cartilage destruction when compared with normal rats which not revealed narrowing of the joint spaces, and subsequent bone and cartilage destruction in the knee joint. EEAA and AAO at both dose level decrease in swelling and narrowing the spaces compared with control groups but not more than ibuprofen at the dose of 50mg/kg rats established almost normal appearance and regained normal architecture of ankle joint with mild soft tissue swelling.

Table-1: Effect of EEAA and AAO in *in-vitro* anti-antiarthritic activity on bovine serum protein denaturation method

Drug	Concentration (µg/ml)	% inhibition
Control	-	-
EEAA	100	34.09±1.22
EEAA	250	36.95±1.08
EEAA	500	43.13±1.32
AAO	100	23.68±2.25
AAO	250	48.21±1.52
AAO	500	56.71±2.42
Diclofenac	100	39.58±3.51
Diclofenac	250	75.83±2.92
Diclofenac	500	77.51±3.63

Values (Mean ± SD) for n=3

Table-2 : Result of Anti-arthritic activity of EEAA and AAO on Freund's adjuvant induced arthritis in rats

Group No.	Treatment	Paw oedema volume in ml (Mean ± SEM)						% reduction in oedema volume				
		0 day	3 day	5 day	9 day	13 day	21 day	3 day	5 day	9 day	13 day	21 day
I	Normal (2% Tween 80)	0.823± 0.012	0.823± 0.012	0.823± 0.012	0.823± 0.012	0.823± 0.012	0.823± 0.012	-	-	-		-
II	Contro (2% Tween 80)	0.820± 0.022^{ns}	1.234± 0.016^{a}	1.447± 0.020^{a}	1.613± 0.022^{a}	1.634± 0.022^{a}	1.620± 0.018^{a}	33.30± 0.003#	43.13 ±0.003#	49.63 ±0.003#	49.60 ±0.003#	49.50 ±0.002#
III	EEAA (200 mg/kg p.o)	0.828± 0.020^{ns}	1.204± 0.018^{ns}	1.347± 0.020^{ns}	1.313± 0.019**	1.304± 0.020**	1.320± 0.018*	2.71± 0.02	6.93± 0.06	18.75± 0.13	21.20± 0.23	19.32± 0.14
IV	EEAA (400 mg/kg p.o)	0.825± 0.017^{ns}	1.200± 0.020^{ns}	1.317± 0.019^{ns}	1.328± 0.018**	1.301± 0.020**	1.210± 0.018**	3.45± 0.02	8.78± 0.002	17.24± 0.14	22.54± 0.24	25.34± 0.24
V	AAO 125 mg/kg p.o)	0.826± 0.018^{ns}	1.220± 0.012^{ns}	1.334± 0.018^{ns}	1.312± 0.018**	1.318± 0.020**	1.214± 0.016**	2.12± 0.002	7.50± 0.002	19.78± 0.25	19.60± 0.23	18.12± 0.20
VI	AAO (250 mg/kg p.o)	0.824± 0.018^{ns}	1.220± 0.016^{ns}	1.327± 0.018^{ns}	1.332± 0.018**	1.278± 0.022***	1.180± 0.016***	2.12± 0.05	14.65± 0.05	17.76± 0.32	22.50± 0.23	27.88± 0.34
VII	Ibuprofen, 50 mg/kg p.o)	0.824± 0.018^{ns}	0.940± 0.016**	1.020± 0.013***	1.002± 0.011***	0.918± 0.020***	0.930± 0.026***	23.34± 0.32	29.13± 0.05	37.80± 0.15	43.50± 0.17	42.12± 0.24

All values are expressed as mean ± SEM, n= 6, One way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test. The minimum value of $p<0.05$ was considered as significant. ^{a} as compared to normal group; ns-non significant, *$p<0.05$, **$p<0.01$, ***$P<0.001$ as compared with control group. Where, # means; % increase in oedema was compared with Normal groups.

Table-3: Effect of EEAA and AAO in haematological parameters on Freund's adjuvant induced arthritis in rats

Group No.	Treatment	Blood Parameter			
		Hg (g %)	**Total RBC Millions/mm^3**	**Total WBC/ mm^3**	**Platelets Lacks/ mm^3**
I	Normal (2% Tween 80)	14.38± 0.78	5.22±0.38	7234±51.08	2.82±0.05
II	Control (2% Tween 80)	10.52± 1.20$^{a\,**}$	4.25±0.30^{a*}	10980±54.13$^{a\,***}$	1.54±0.09$^{a\,***}$
III	EEAA (200 mg/kg p.o)	11.38± 2.00^{ns}	4.77±0.32^{ns}	10740±74.45^{ns}	2.53±0.02**
IV	EEAA (400 mg/kg p.o)	11.72± 2.10^{ns}	4.84±0.37*	8970±34.93*	2.34±0.04**
V	AAO 125 mg/kg p.o)	12.45± 2.16**	4.76±0.39^{ns}	8901±50.24**	2.56±0.02**
VI	AAO (250 mg/kg p.o)	12.47± 2.11**	4.90±0.29*	7600±44.20***	2.67±0.04**
VII	Ibuprofen, 50 mg/kg p.o)	10.80± 1.22^{ns}	4.52±0.42^{ns}	8404±34.80**	2.10±0.05*

All values are expressed as mean ± SEM, n= 6, One way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test. The minimum value of $p<0.05$ was considered as significant. ^{a} as compared to normal group; ns-non significant, *$p<0.05$, **$p<0.01$, ***$P<0.001$ as compared with control group.

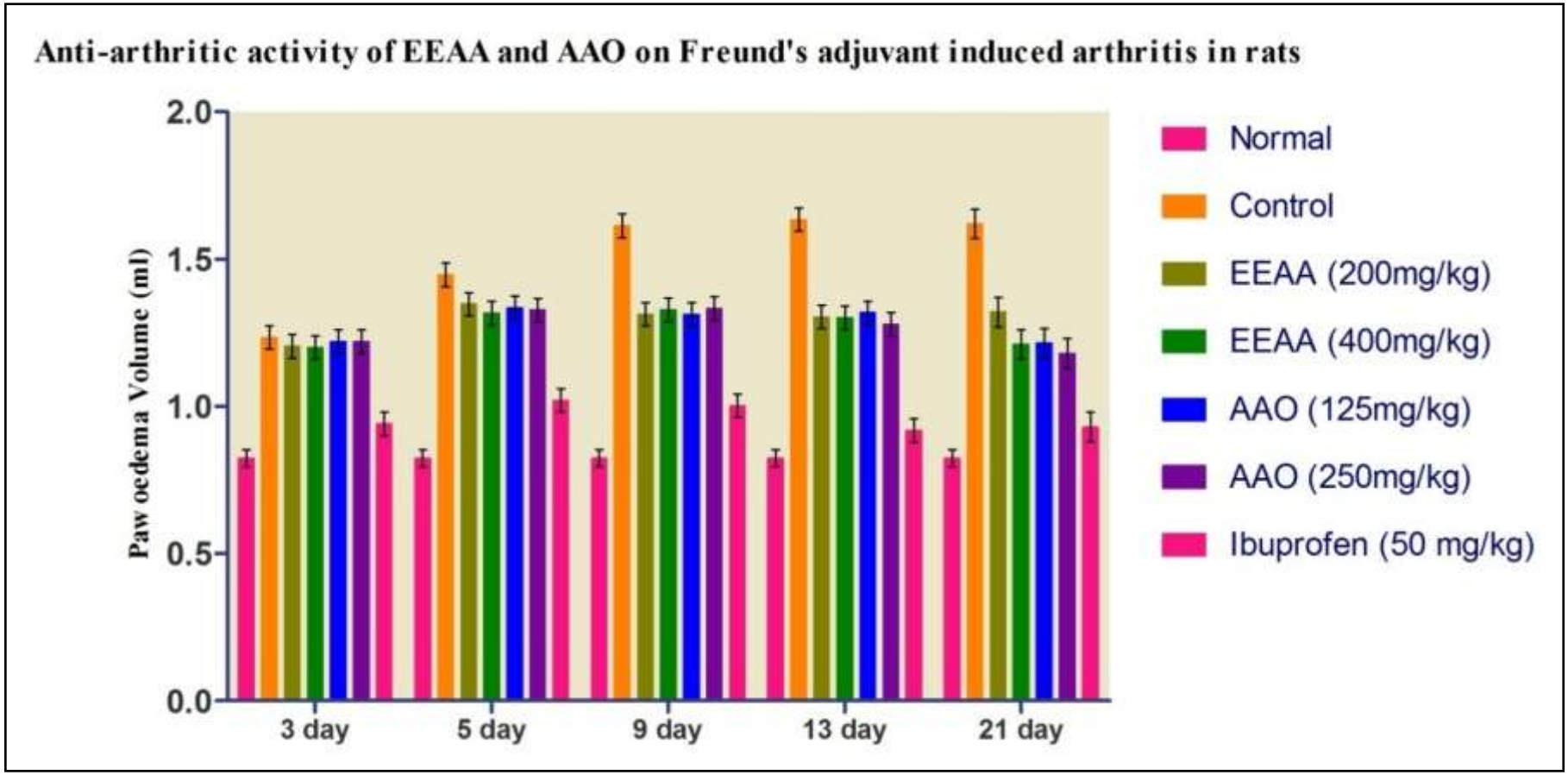

Fig-1: Anti-arthritic activity of EEAA and AAO on increase in paw volume on Freund's adjuvant induced arthritis in rats

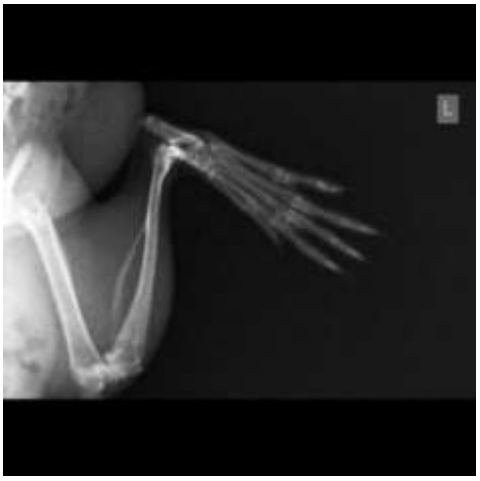

Fig-2 (a): Radiographic pic. (Normal)

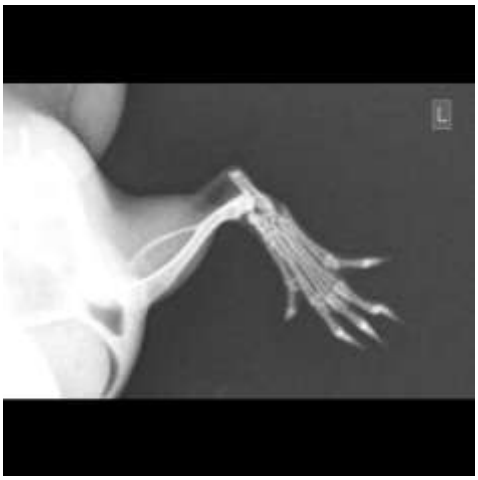

Fig-2 (b): Radiographic pic. (Control)

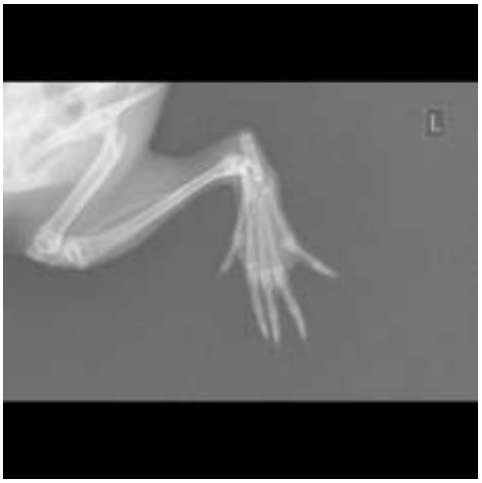

Fig-2 (c): Radiographic pic. EEAA (200 mg/kg, Low dose)

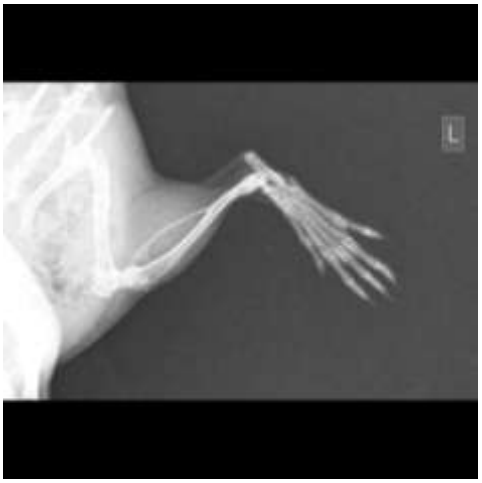

Fig-2(d): Radiographic pic. EEAA (400 mg/kg, high dose)

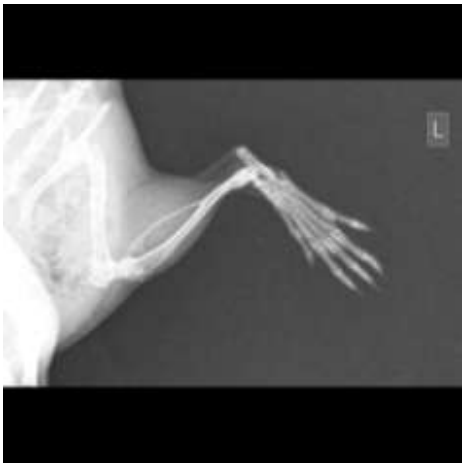

Fig-2 (e): Radiographic pic. AAO (125 mg/kg)

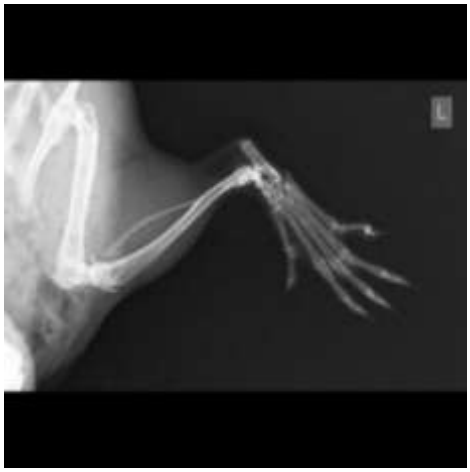

Fig-2 (f): Radiographic pic. AAO (250 mg/kg)

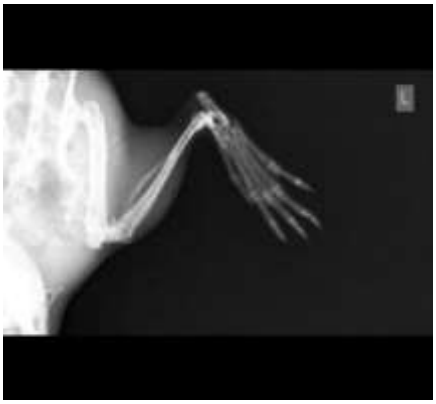

Fig-2 (g): Radiographic pic. Ibuprofen (50 mg/kg), Std.

DISCUSSION

Aquilaria agallocha (EEAA) leaves and Aquilaria agallocha oil (AAO) of heartwood indicate that Aquilaria agallocha having both anti-inflammatory and anti-arthritic activity. These activity may be due to presence of phytoconstituents like glycosides, tannins, terpinoids, oleic acids, terpens and phenolic compounds in the extracts of EEAA and EEA. As previous literature suggests that phenolic compounds Terpeinoids, sesquiterpenes etc possesses anti-inflammatory and anti-arthritic activity[23-24].

Most of the investigators have reported that denaturation of protein is one of the cause of rheumatoid arthritis[25]. Production of auto-antigens in certain rheumatic diseases may be due to *in-vivo* denaturation of proteins. Mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding. Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation. In our present study, EEAA and AAO inhibited heat induced protein denaturation and is may one of the reasons of possessing anti-arthritic activity.

Fraud's Adjuvant-induced arthritis is the most frequently used for induction of rheumatoid arthritis, a chronic model in experimental animals. It seems that bacterial peptidoglycan and muramyl dipeptide are responsible for induction of arthritis. Since the composition of bacterial adjuvant is complex and the immune response is a multiple stage process of intercellular cooperation, the mechanism is unclear. RA, the common human autoimmune disease is characterized by chronic inflammation in joints followed by pannus formation with infiltered lymphocytes and fibrinoid joints of synovial membrane with concommittant destruction of cartilage and bone. Exact aetiological mechanism is not known but cytokines play a role in pathogenesis of RA. T-cells cause direct impact on TNF-α, IL1 and IL-6 and TNF-α plays a critical role in pathogenesis of RA and β- cells also play an important role through cell interaction with T-cells, dendritic cells, synovial nerves like cells (SNLC) and fibroblasts, leukotrienes were also seen[26-27]. In our study it is found that EEAA and AAO showed significant reduction in paw volume in FA induced arthritis rats. Probably the ant-arthritic activity of EEAA and AAO may due to inhibition of the chemical mediators involved in chronic development of RA.

It has been reported that a moderate rise in the WBC count occurs in arthritic conditions due to an IL-1B-mediated rise in the respective colony-stimulating factors. In addition to this, other characteristic haematological alterations such as the decreased Hemoglobin during arthritis results from reduced erythropoietin levels, a decreased response of the bone marrow erythropoietin and premature destruction of red blood cells[28]. The present study reveals that EEAA and AAO treatments tend to normalize the WBC count and hemoglobin level and hence it reveals protective effect against rheumatoid arthritis.

Radiographic changes in RA conditions are useful diagnostic measures which indicate the severity of the disease. Soft tissue swelling is the earlier radiographic sign, whereas prominent radiographic changes like bony erosions and narrowing of joint spaces can be observed only in the developed stages (final stages) of arthritis. The radiographic features of the rat joints in adjuvant induced arthritic model are shown in Fig-2(a) to Fig-2(g).

CONCLUSION

Ethanolic extract of *Aquilaria agallocha* (EEAA) leaves and *Aquilaria agallocha* oil (AAO) of heartwood exhibited significant anti-arthritic activity in both *in-vivo* and *in-vitro* methods.

Aquilaria agallocha oil (AAO) of heartwood exhibited relatively better activity than Ethanolic extract of *Aquilaria agallocha* (EEAA) leaves. The difference in the evaluated activities could be due to the presence of sesquiterpenes and phenolic in oil. The mechanism of anti-arthritic activity may be due to inhibition of chemical mediators involved in progression of arthritis and inhibition of protein denaturation, hematological and radiological analysis reveals their anti-arthritic activity.

From our study, we have made an attempt to prove its efficacy in experimental animals. Further study can be done in human subjects to make a good herbal formulation of anti-arthritic with *Aquilaria agallocha.*.

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