Antiarthritis Activity of Aristolochia Bracteata Extract in Experimental Animals

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Abstract: The *Aristolochia bracteata* is well known for its antiarthritis properties in Indian system of medicine and folk medicine. The objective of the present study was to evaluate its folk claim in rheumatoid arthritis (RA) and propose a probable mechanism of action. Anti arthritic activity was evaluated using Freund's complete adjuvant in rats, the course of treatment was followed for over and 4 weeks post inoculation period using health parameters, clinical and behavioral methods of study. Estimation of blood Hb, ESR and change in body weight were considered as health parameters and clinical observations included paw edema volume, thermal hyperalgesia, radiological and histomarphological analysis and exploratory behavior was studied in behavioral observations. The results indicates that, regular treatment of adjuvant induced arthritic rats with *A. bracteata* extracts improves ESR, Hb value and also restores body weight. Significant ($P<0.01$) inhibitory effect was observed with *A. bracteata* extract on Freund's complete adjuvant induced paw edema throughout the study ($P<0.001$). The latency to thermal stimuli and inhibitory effect on xylene induced ear edema was significantly ($P<0.05$) affected by oral treatment of *A. bracteata,* irrespective of solvent used for extraction. Treatment of FCA induced rats with *A. bracteata* extracts shown ($P<0.05$) increase in pain threshold, weight bearing ability, ambulation and also decline in scratching, defecation and urination, were observed as a sign of improvement in behavioral condition. The results obtained in this study showed promising effect on FCA modulated health status, clinical observations and behavioral changes.

Keywords: Anti-arthritis, arthritis, *Aristolochia bracteata,* Freund's complete adjuvant.

INTRODUCTION

Immune system is vital to survive, because a hyperactive immune system may cause fatal disease due to over whelming allergic reaction leading to series of derangements, loss of normal capacity to distinguish self from non-self resulting in immune reactions against one's own tissues and cells called autoimmune diseases. These autoimmune changes are receiving increased attention in drug discovery and development as the progress has been made in understanding immune and inflammatory processes. Several autoimmune diseases including myasthenia gravis, serum sickness, pernicious anaemia, pemphigus vulgaris, SLE, reactive arthritis, etc. are the severe concern of medical and pharmaceutical community because of unknown etiology. Rheumatoid Arthritis (RA) is one of the most common autoimmune inflammatory conditions of unknown etiology characterized by symmetric, erosive synovitis and in same cases extraarticular involvement [1].

The presently available pharmacological treatments in the market not only causing economical exploitation, but also associated with severe adverse effects [2-3]. Despite extensive use of currently available therapy, most RA patients are suffering from declined functional ability because of inability in preventing cartilage breakdown and joint destruction [4]. Recently, efforts have been focused on using the class of drugs called biologics (antibodies or soluble receptors for IL-1, IL-6 and TNF-∞) for the treatment of RA. Although these agents reduce the inflammation and joint destruction, their long-term risks and benefits are not yet clear. Additionally, higher costs and the findings that they are not effective universally and severe side effects such as life threatening infections, increased risk of malignancies and require continuous and careful monitoring [5-10].

However, to develop a proper medication which will be ecofriendly and having very less side effects that can be used for prophylactic and therapeutic purpose to control this dangerous disease is still a big challenge to a scientific community working in this area. The use of complementary and alternative medicine (CAM) therapies, such as acupuncture, physiotherapy, yoga and extract of medicinal herbs, and is on rise. According to reports 60-90% of dissatisfied arthritis patients are likely to seek the option of CAM therapy [11, 12].

Herbal medicine is the root of various traditional medicine systems around the world. Ayurvedic medicine in India has proven track record of 5000 years and forms part of the National Health Service, offered along side conventional medicine. The ayurvedic, National Formulary lists some 8000 well proven ayurvedic formulations described in Dravyaguna (Ayurvedic Pharmacology). Remedies are made from single or multiple herbs and minerals for various medical conditions like asthma, flu, diabetes, arthritis, heart disease, digestive problems, mental health and skin problems. Herbal medicines yielding about 25% of currently used crude drugs with another 25% derived from chemically altered natural products [13].

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In ancient texts about 500 plants have been indicated in the treatment of arthritis, however only few number of plants have been evaluated scientifically (<50). *Aristolochia bracteata* is most commonly known as kidamari, wildly distributed in Deccan Gujarat, western and southern India, Bihar, Sindh, Bundelkhand and Bengal. It has been found most commonly in ancient texts for important medicinal properties including anthelmentic, fever, purgative and painful joints. Recently, it has been reported for hypotensive, hypothermia, antioxidant and anti-inflammatory properties. However, no study has been carried out to evaluate its anti-arthritis property. Therefore, the present study was carried out with an objective to evaluate anti-arthritis activity of *Aristolochia bracteata* whole plant extract prepared by successive solvent extract. This study will evaluate its folk claims and also propose its probable mechanism of action. The study has been designed according to the guidelines published as guidance for industry by U.S. Food and Drug Administration on ethical standards for investigations of experimental arthritis in animals using *in vivo* methods [14].

MATERIALS AND METHODS

Animals

All the procedures were carried out on *Wistar albino* rats of either sex weighing 200-250gm for anti-arthritic study and *Swiss albino* mice of body weight15-30 gm for acute toxicity study and xylene-induced ear edema were bred and raised under the animal facility of H.S.K. College of Pharmacy, Bagalkot, Karnataka. Food and water were supplied *ad libitum* and the animals were kept in a 12 h light: 12h dark cycle and environmental temperature (23 ± 1 °C) in standard propylene cages. Cage cleaning consisted of daily change of rinse husk bedding. All the animals were monitored over 28 days and sacrificed for the histological assessment of inflammation. All the experiments were conducted in accordance with the Institutional Animal Ethics Committee (821/01/a/CPCSEA/HSKCP/IAEC/2004-2005). Due to the painful condition imposed on the animals, the number of subjects used was restricted to the minimum that allowed reliable statistical analysis of the results. Each group was composed of 6 animals.

PLANT EXTRACT PREPARATION

Collection

Aristolochia bracteata was collected during November 2005 from village Devla, Amreli District, Gujarat, and was authentified by Taxonomist Prof. V.V. Siddhulingappanavar, HOD, Dept. of Botany, Basaveshwar Science College, Bagalkot, Karnataka, India.

Extraction

The whole plant *Aristolochia bracteata* was collected after authentification and dried under shade and powdered. To get uniform size passed it through sieve no. 44# and was subjected to extraction with petroleum ether, chloroform and methanol in a soxhlet extractor successively with 12 hrs cycle [15]. The extract was concentrated by distillation and by using flash evaporator to yield a semisolid residue. The percentage yields of petroleum ether extract, chloroform extract and methanolic extract was calculated and found 4.73%, 3.693%, and 3.66% respectively. All the extracts were preserved in a refrigerator till further use.

Preparation of Test Solutions

The test solution of methanolic extract was prepared by dissolving it in water. The suspension of petroleum ether and chloroform extract was prepared by suspending it in a 5% Span80 using mechanical shaker. Above prepared test solution and suspensions of Petroleum ether, Chloroform, and Metnanolic extract were tested in doses of 100, 200, and 400 mg/kg p.o. for its anti- arthritic, leukotriene infiltration inhibitory and anti-nociceptive properties.

Acute Oral Toxicity Study

Healthy *Swiss albino* mice of either sex weighing 15-30 gm, starved overnight were divided into 3 groups (n=9) and were fed with increasing doses (1, 2, and 4 gm/kg) of each extract and the toxicity was evaluated as per the Guidelines for non-clinical toxicity Investigation of Herbal Medicine (Annexure-I) given by the Ministry of Health and Family Welfare, Govt. of India [16]. The total drug extracts administered orally in doses of up to 4 gm/kg, did not produce any evident sign of toxicity and mortality in rats, and were observed upto 14 days after administration.

Materials

Freund's complete adjuvant (FCA) [17] composed of 1 mg/ml heat killed *Mycobacterium tuberculosis*, mineral oil and mannide monooleate, an inducing agent for arthritis was purchased from Sigma Aldrich Co, St Louis, USA. Indomethacin was obtained from the U-Medico Laboratories Pvt. Ltd., G.I.D.C., Vapi, Gujarat, India as a complementary sample and was used as a standard drug. All the other chemicals and solvents used were of AR grade. Instruments that were used in the present study are, ESR Stands and Top pipettes, Sahli's Haemometer, UGO BASILE Digital Plethysmometer (Italy), Dental X-ray machine (Siemens Multiphos 10), Spencer type Wes wax Microtome, Metzer Biomedical Research Microscope, Eddy's Hot Plate and Cork borer of 8 mm diameter.

Induction of Anesthesia

For the induction of arthritis, rats were anaesthetized with 40-mg/kg thiopentane injected intraperitoneally, for acute terminal experiments. Once anaesthetized, the animals were constantly kept under observation to ensure that breathing is slow and regular. Sign of deep anesthesia was indicated by the abolition of withdrawal reflex when the hind paw of the rat was squeezed [18].

Induction of Monoarthritis

For the induction of arthritis injection of the left ankle joint was performed under anesthesia: the tarsial area of the hind paw was grasped and the fossa distal and medial to the 'lateral malleolus' of the fibula was palpated. A 26 gauge needle was introduced into the capsule of the tibiotarsal joint percutaneously by directing it cephalad, mesiad and superiorly from the midpoint of the 'inframalleolar fossa,' until a distinct loss of resistance was felt approximately 4 mm and complete adjuvant or vehicle injected. With a true intracapsular injection, a firm resistance to injection was characteristically felt after the injection of 0.05 ml of fluid [18, 19]. Baseline (pre-induction) behavioral and clinical observations were made prior to injection of vehicle or complete adjuvant, and then at each week up to 28 days (4 weeks).

Measurement of Health Status

The health status parameters included (i) Body Weight, (ii) Hemoglobin (Hb) level, and (iii) Erythrocyte Sedimentation Rate (ESR) measurements. The body weights of all the animals were recorded in grams on weekly basis by using single pan weighing balance. Haemoglobin levels of all the animals were evaluated on 29th day of study using Sahli Hellige Haemometer and the results are expressed in gm % unit. Erythrocyte sedimentation rate were estimated by Westergren pipettes having 2.5 mm internal diameter, 300 mm length, and 1 ml capacity and ESR stands. Blood was collected from all the arthritic and non-arthritic animals used in the study by retro orbital [20].

Behavioral Observations- Open Field Test

For behavioral observations all the animals were subjected to open field test before the induction of arthritis and there after every week up to 4 weeks (28-days). Briefly rat was placed in an open field in the sound-attenuated room. The floor was white polyvinyl with a black grid dividing open field into 84 squares (10 x 10 cm). Illumination was provided by a bulb (60 W) placed above the center of the field, while the rest of the room was in darkness. The rat was initially placed in the corner or in the center of the field and observed for 5 min. in all tests latency time to start explore the open field (seconds), horizontal locomotor activity (grid lines crossed), vertical locomotor activity (rearing), grooming (rubbing the nose with its porepaws and preening), instance of defecation (number of boluses), and number of urinations were recorded. Between the trials the box was cleaned with wet sponge and paper tissue [21, 22]. All the observations took between 8.00 and 12.00 h.

Assessment of Arthritis

Assessing joint swelling may not merely reflect disease activity but may indicate a chronic phenomenon reflecting joint damage. Clinical severity of arthritic inflammation was measured by the quantification of the paw volume changes; measurement was carried out by using UGO BASILE Digital Plethysmometer (Italy) [23]. The paw volumes were recorded on 0 day, 7th day, 14th day, 21st day and, 28th day (each week up to 4 weeks).

Anti-Nociceptive Activity

The apparatus consists of a hot plate on which the rats were placed for testing (Eddy's Hot Plate Method). The apparatus consists of a 20 cm diameter metal-hot plate surface set at 50 ˚C, a plexiglass cage that fits the hot metal surface, and a timer operated by stop watch. Pain threshold was determined by the latency for nociceptive response (withdrawal of any paw) with a maximum cut-off time 15 sec for all groups on the last day of experiment [24]. All the extracts of plant *Aristolochia bracteata* and standard drug (Indomethacin) were administered prior to 1 hr of the test.

Xylene-induced Ear Edema in Mice

Overnight-starved Swiss *albino* mice were divided into 11 different groups of 6 each. The extracts under study were administered orally 30 min prior to the application of xylene (0.03 ml) to the anterior and posterior surfaces of the left ear of the mice. The right ears of all the mices were remained untreated, and control group was received only normal saline. All the animals were sacrificed after the induction of inflammation (after 2 hours) i.e. after xylene application, both ears were removed. The circular sections of ears of the treated and untreated animals were taken using 8 mm diameter cork borer and weighed. The edematous response was measured as weight difference between the two plugs and the anti-inflammatory activity expressed as percentage of edema reduction in treated mice with regard to the control mice. All experiments were uniformly started between 11:00 and 14:00 h in order to avoid variations in the inflammatory response due to circadian fluctuations in the levels of corticosteroids [25, 26].

Histological Assessment

All the animals were sacrificed at the end of the experiments. Left hind paws were removed of all the animals and post fixed in formal saline (7 days) and then decalcified in 5% formic acid. Joints were then trimmed, embedded and sectioned at 6 μm. Sections were then stained with haematoxyline and eosin. Histological study was carried out by 0, infiltrate in skin and overlying tissues; 2, dense inflammatory infiltrate or arthritis; 3, synovitis; 4, hyperplastic synovium, inflammatory infiltrate in the joint; 5, arthritis with destruction of catilage, pannus formation, using research microscope [27, 28].

Radiography

Radiographic evaluation was performed on the basis of radiographs and coned down views of lower limbs. Radiographs were taken with Siemens, Multiphos 10 (version 1.0) dental X-ray machine [29].

Statistical Evaluation

A one-way analysis of variance with Dunnett's comparisons to control and unpaired Student'*t*' test were used to determine statistical significance of the preclinical data collected from behavioral observations. The data collected from the clinical observations, hot plate study, and ear edema study (mean ± SEM) were analysed statistically for differences using the student *t*-test.

RESULTS

Health Status

The body weight in normal group animal rats remains same during 4 weeks study. In, FCA injected group of animals, body weight of animals was declining after 1st week of study and significant loss ($P<0.001$) in weight was in 3rd and 4th week. Blood haemoglobin content was significantly ($P<0.001$) declined to 8.667± 0.8 from 17.33± 0.76 and ESR was significantly ($P<0.01$) increased to 3.15±4.113 from 13.17±1.35 in FCA injected group when compared to normal on 29th day of study. Indomethacin treatment significantly ($P<0.05$) restored loss in body weight on 3rd and 4th week and decreased level of blood haemoglobin due to FCA injection.

As shown in Table **1**, Treatment of FCA injected group of animals with petroleum ether extract of *A. bracteata* shown dose dependent effect in improvement of haemoglobin level 11.83±0.79, 12.5±0.56 and 12.17±0.6, and maintain ESR at 4.5±0.56, 3.8±0.36, and 4.8±0.48, it also significantly restores loss in body weight on 4th week of study. Methano-

Table 1. Effect of *Aristolochia bracteata* Extract on FCA Induced Change in the Haemoglobin (Hb) Level, Erythrocyte Sedimentation Rate (ESR) and, Body Weight

Treatment and Dose	ESR (mm/hr)	Hb (gm %)	Body Weight				
			0 week	1st week	2nd week	3rd week	4th week
Normal	13.17 ± 1.352	17.33 ± 0.76	250 ± 18.26	250 ± 18.26	250 ± 18.26	250 ± 18.26	250 ± 18.26
FCA+Saline	31.5 ± 4.1 **	8.7 ± 0.8 ***	233.3 ± 21.08	233.3 ± 21.08	208.3 ± 15.37	191.7 ± 15.37*	158.3 ± 8.3 **
Indomethacin (10 mg/kg i.p.)	14.0 ± 2.696	11.67 ± 0.92 *	275 ± 33.54	233.3 ± 38.01	258.3 ± 27.13	291.7 ± 27.1 *	291.7 ± 27.1 **
CE, 100 mg/kg, p.o.	3.67 ± 0.49 **	14.5 ± 1.5 *	200 ± 18.26	200 ± 18.26	200 ± 18.26	200 ± 18.26	200 ± 18.26
CE, 200 mg/kg, p.o.	5 ± 0.9661 **	13.17 ± 0.65 **	200 ± 18.26	200 ± 18.26	200 ± 18.26	191.7 ± 15.37	191.7 ± 15.37
CE, 400 mg/kg, p.o.	4.3 ± 0.49 **	13.67 ± 0.9 **	166.7 ± 10.5 *	166.7 ± 10.5 *	166.7 ± 10.54	166.7 ± 10.54	166.7 ± 10.54
PEE, 100 mg/kg, p.o.	4 ± 0.86 **	11.83 ± 0.79 *	258.3 ± 27.13	258.3 ± 27.13	225 ± 17.08	225 ± 17.08	225 ± 17 **
PEE, 200 mg/kg, p.o.	3.8 ± 0.31 **	12.5 ± 0.56 **	258.3 ± 24	258.3 ± 23.86	225 ± 21.41	225 ± 21.41	225 ± 21.4 *
PEE, 400 mg/kg, p.o.	4.8 ± 0.48 **	12.17 ± 0.6 **	216.7 ± 21.08	216.7 ± 21.08	216.7 ± 21.08	225 ± 25.00	225 ± 25 *
ME, 100 mg/kg, p.o.	5.83 ± 0.75 **	12.33 ± 0.72 **	233.3 ± 38	233.3 ± 38.01	208.3 ± 27.13	208.3 ± 27.13	225 ± 21.41 *
ME, 200 mg/kg, p.o.	6 ± 1.13 **	11.17 ± 0.87	283.3 ± 25	283.3 ± 24.72	283.3 ± 24.7 *	275 ± 21.41 *	258.3 ± 15 ***
ME, 400 mg/kg, p.o.	4.5 ± 0.56 **	10.17 ± 0.65	225 ± 33.4	225 ± 33.54	208.3 ± 27.13	208.3 ± 27.13	225 ± 21.41*

Effect of *Aristolochia bracteata* extract on FCA induced change in ESR and Hb was studied on 29th day of study. Change in body weight of rats was recorded as weekly basis. Blood was collected form rat's retro orbital on 29th day, ESR and Hb were estimated by Westergern pipette and Sahli's Haemometer. Data collected was analysed by student't' test expressed as mean ± SE. P value less than 0.05 was considered as significant. *P<0.05, **P<0.01, ***P<0.001.

lic extract treatment of arthritic rats significantly decreased (P<0.01) ESR level 5.83±0.75, 6±1.13 and 4.5±0.56, also improved body weight in 4th week study. Lower dose of methanolic extract 100 mg/kg, significantly (P<0.01) increased blood haemoglobin content to 12.33 ± 0.72, as compared to control group. In this study more significant (P<0.01) effect on Hb and ESR was shown by chloroform extract treatment without significantly altering body weight of animals throughout the study (Table **1**).

FCA Induced Paw Edema

In mineral oil injected normal group rats, increase in paw volume was observed but not significant. FCA injection in tibiotarsal joint significantly (P<0.001) increases paw volume from first week and almost same volume was maintained throughout study. Significant (P<0.05, P<0.01, and P<0.001) inhibition of FCA induced increased paw volume was noted in indomethacin treated group of animals from 2nd week to 4th week of study (1.408± 0.35, 1.04±0.29 and 0.8±0.25 respectively). Petroleum ether extract of *A. bracteata* shown significant (P<0.05, P<0.01) inhibition on FCA induced increase paw edema on 1st week of study and maintained the effect significantly till the completion of study. Chloroform extract shown more promising results in inhibiting paw edema volume from 1st week (P<0.01), and maintained significantly inhibitory effect (P<0.001) from 2nd week of study. Similarly, methanolic extract if *A. bracteata* at low dose (100 mg/kg) and moderate dose (200 mg/kg), but results were varying at higher (400 mg/kg) dose of extract (Table **2**).

FCA Induced Thermal Hyperalgesia

No significant change in hot plate reaction time was noted in FCA injected group of animals on 28th day of study when compared to control group. Intra peritoneal indomethacin treatment significantly (P<0.001) increases basal reaction time (7.35±0.34 from 3.38±0.05) as compared to control group. Petroleum ether extract treatment group has no significant change in reaction to thermal stimuli, except moderate dose (200 mg/kg), (P<0.05). More surprising results were obtained when animals were treated with all doses of chloroform extract (100, 200 and 400 mg/kg), it significantly (P<0.01, P< 0.001) increases time for basal reaction when compared to control group. Methanolic extract shown more significant (P<0.01) results in lower doses than higher dose (P<0.05) when compared to control group (Table **3**).

Open Field Test

In non-treated group of rats, no significant change in behavior was observed on day FCA injection. In 1st week significant decrease in rearing and defection, 2nd week significant decrease in rearing, grooming, defecation and ambulation, in 4th week of study significant decrease in latency to explore and decrease in rearing, grooming and ambulatory behavior were noted. Indomethacin treatment shown its effect in 2nd week of study by decreasing grooming in 3rd week significantly increases number of defecation, and decrease latency. However, in 4th week there was no change in behavior except increased ambulation. The significant change in exploratory behavior of animals treated with *A. bracteata* extract was observed on 1st day of dose administration may indicate effect of handling in behavior. No significant effect on behavior was seen up to 7th day of observation. However, in 2nd week significant effects were seen on latency to explore and ambulation. Methanolic extract at 400 mg/kg, chloroform extract at100 and 200 mg/kg significantly decreased grooming behavior. In 3rd week of the study, petroleum ether and methanolic extracts shown significant reduction in latency time, increase in rearing, defecation and

Table 2. Effect of *Aristolochia bracteata* Extract on FCA Induced Rat Paw Edema

Treatment and Dose	Paw Edema (ml)				
	0 week	1st week	2nd week	3rd week	4th week
Normal	0.02 ± 0.005	0.037 ± 0.005	0.037 ± 0.005	0.038± 0.004	0.04 ± 0.006
FCA+Saline	0.01 ± 0.02	2.375 ± 0.164***	2.355 ± 0.173***	2.67 ± 0.145***	2.597 ± 0.168***
Indomethacin (10 g/kg i.p.)	0.01 ± 0.02	2.715 ± 0.076	1.408 ± 0.348*	1.04 ± 0.29**	0.86 ± 0.249***
ME, 100 mg/kg, p. o.	0.02 ± 0.02	1.875 ± 0.099*	1.063 ± 0.15***	1.14 ± 0.18***	0.968 ± 0.18***
ME, 200 mg/kg, p.o.	0.01 ± 0.01	1.868 ± 0.286	0.575 ± 0.154***	0.09 ± 0.077***	0.025 ± 0.03***
ME, 400 mg/kg, p.o.	0.005 ± 0.006	2.278 ± 0.244	2.123 ± 0.235	1.457 ± 0.328*	1.45 ± 0.326*
EE, 100 mg/kg, p.o.	0.125 ± 0.053	1.447 ± 0.296 *	1.172 ± 0.261**	0.936 ± 0.15***	0.746 ± 0.1***
EE, 200 mg/kg, p.o.	0.045 ± 0.055	1.205 ± 0.24**	0.9 ± 0.293**	0.7 ± 0.258***	0.57 ± 0.28***
EE, 400 mg/kg, p.o.	-0.003 ± 0.006	1.965 ± 0.78	0.88 ± 0.258**	0.59 ± 0.229***	0.3 ± 0.11***
CE, 100 mg/kg, p.o.	0.006 ± 0.011	0.93 ± 0.25**	0.596 ± 0.19***	0.635 ± 0.214***	0.621 ± 0.177***
CE, 200 mg/kg, p.o.	0.02 ± 0.01	1.167 ± 0.267**	0.967 ± 0.307**	0.7 ± 0.2***	0.558 ± 0.23***
CE, 400 mg/kg, p.o.	0.01 ± 0.007	1.533 ± 0.1**	0.858 ± 0.17***	0.7 ± 0.145***	0.596 ± 0.1***

Effect of Aristolochia *bracteata* in FCA induced increase in rat paw edema was studied every week by using UGO-BASILE Plethysmometer. Change in paw volume was calculated from difference in FCA injected and saline injected paw volume. Data collected from the present study, are expressed as mean ± SE and analysed by student't' test. FCA injected group was compared with mineral oil injected group and extract and indomethacin treated group was compared with FCA injected group for coming to conclusion. P value less than 0.05 was considered as significant. *P<0.05, **P<0.01, ***P<0.001.

Table 3. Effect of *Aristolochia bracteata* Extract on FCA Induced Thermal Hyperalgesia (Sec) Using Eddy's Hot Plate

Animal No.	Normal (mineral oil + 5% span)	Control (FCA + 5% span)	Standard (Indomethacin, 10mg/kg i.p.)	Extract and Dose of Extract (mg/kg, p.o.)								
				Ether Extract			Chloroform Extract			Methanolic Extract		
				100	200	400	100	200	400	100	200	400
1	3.4	3.4	8.4	2.6	3.3	7.5	5	6	5	4.0	5	7.3
2	3.4	3.2	8.7	3.9	2.6	9.5	6	6.5	5.6	6.5	5.6	4
3	3.5	3.6	8	3.8	3.5	3.5	3.8	6.2	5.9	6	4.3	5
4	3.3	3.4	7.5	4.1	2.9	2.7	5	5.7	5.5	4.4	7.2	5.5
5	2.9	3.4	7.6	5.1	2.6	5.5	5.6	5.4	4.2	4.9	5.8	5.9
6	2.6	3.3	6.3	5.2	2.9	3.6	5.3	5.5	5.6	7	4	4
Mean	3.18	3.38	7.75 ***	4.12	2.97 *	5.38	5.12 **	5.88 ***	5.3 ***	5.47 **	5.32 **	5.28 *
S.D.	0.35	0.13	0.84	0.96	0.37	2.66	0.75	0.43	0.61	1.21	1.16	1.25
SEM	0.14	0.05	0.34	0.39	0.15	1.09	0.31	0.17	0.25	0.49	0.47	0.51
t-value	--------	1.29	12.5	1.86	2.61	1.84	5.58	13.72	7.48	4.19	4.0 1	3.69

Effect of *Aristolochia bracteata* extract on FCA induced thermal hyperalgesia was studied by using Eddy's Hot Plate. Basal reaction time (sec) was recorded after placing the rat in plexiglass covered hot plate at 50 C, cut off time was 15 seconds. Results obtained are expressed as mean ± SE and analysed by student't' test. FCA injected group was compared with mineral oil injected group and extract and indomethacin treated group was compared with FCA injected group for coming to conclusion. P value less than 0.05 was considered as significant. *P<0.05, **P<0.01, ***P<0.001.

ambulation. In 4th week, significant increase in ambulation behavior was observed with all doses of methanolic extract, petroleum ether (400 mg/kg), and chloroform extract (400 mg/kg). Methanolic extract (200 and 400 mg/kg) and petroleum ether (100 and 400 mg/kg), have shown significant effect on rearing behavior.

Xylene-Induced Ear Edema

Application of xylene to anterior and posterior surface significantly (P<0.05) increased ear edema weight up to 5.18±1.14 from 1± 0.1. xylene-induced ear edema was significantly (P<0.05) inhibited by indomethacin by intraperito-

Table 4. Effect of *Aristolochia bracteata* Extract on Xylene-Induced Ear Edema (mg) in Mice

Animal No.	Normal (mineral oil + 5% span)	Control (FCA + 5% span)	Standard (Indomethacin, 10mg/kg i.p.)	Extract and Dose of Extract (mg/kg, p.o.)								
				Ether Extract			Chloroform Extract			Methanolic Extract		
				100	200	400	100	200	400	100	200	400
1	0.8	4.4	0.7	0.6	0.7	0.6	1.9	2.3	1.9	0.7	1.7	1.9
2	0.7	6.6	1	0.4	0.9	0.9	1.7	2.2	1.2	4.1	1.3	0.4
3	1.2	9.8	1.8	3	3	0.7	3.3	2.7	1.2	2	3	1.6
4	1.3	4.3	1.3	1.3	1.2	1.3	4.2	2.9	0.9	1.8	0.1	0.6
5	1.2	1.5	1.6	1.6	0.9	0.9	2.9	2.4	1	1.9	0.4	1.4
6	0.9	4.5	3	2.9	0.8	0.6	2.3	3.1	1	0.7	2.1	3
Mean	1	5.18 *	1.57 *	1.63 *	1.25 *	0.83	2.72	2.6	1.2 *	1.87 *	1.43 *	1.48 *
S.D.	0.25	2.78	0.8	1.11	0.87	0.27	0.94	0.36	0.36	1.24	1.08	0.94
SEM	0.1	1.14	0.33	0.45	0.36	0.11	0.38	0.15	0.15	0.51	0.44	0.38
t-value	-------	3.65	3.06	2.9	3.3	3.8	2.06	2.25	3.47	2.66	3.08	3.08

Effect of *Aristolochia bracteata* extract on xylene-induced ear edema was studied as a part of illustration of mechanism of action. Extract and drug were administered 1 hr prior to the topical application of xylene, both ear were separated and bored with 8 mm diameter borer. The difference in weight of ear are represented as mean ± SE and analysed by student't' test for coming to conclusion. P value less than 0.05 was considered as significant. *P<0.05, **P<0.01, ***P<0.001.

neal treatment to 1.57 ± 0.33. All doses of petroleum ether and methanolic extract significantly (P<0.05) inhibited xylene-induced ear edema. However the inhibitory effect of chloroform extract was only observed at high dose (400 mg/kg) but not at low doses 100 and 200 mg/kg (Table **4**).

Histomorphology

In FCA treated group of rats eosinophill infiltration, synovitis and damage of cartilage was evident. Treatment with indomethacin significantly inhibited infiltration of inflammatory cells and synovitis and also maintains integrity of cartilage tissues. And treatment with petroleum ether and chloroform extract of *A. bracteata* successfully inhibited all these inflammatory processes. However, methanolic extract treatment at lower dose and moderate dose inhibited synovitis and pannus formation but not inflammatory cells infiltration. High dose (400 mg/kg) of methanolic extract has no effect on any aspects of FCA induced arthritic reactions (Figs. **1-12**).

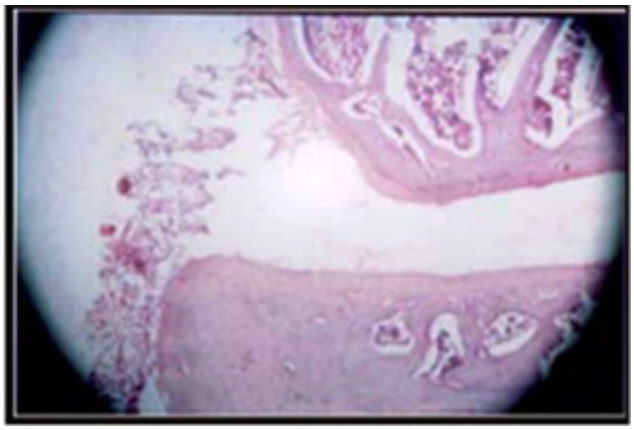

Fig. (1). Effect of Span 80 (5%) on mineral oil induced rats knee joint histopathology (H & E, X 100). Normal joint arachitecure.

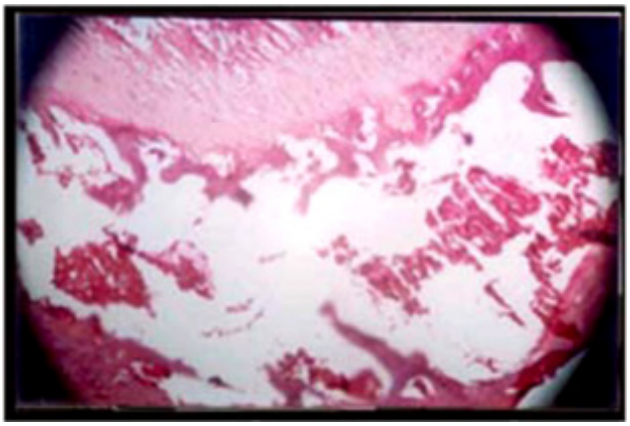

Fig. (2). Effect of Span 80 (5%) on FCA induced monoarthritic rats knee joint histopathology (H & E, X 100). Joint cartilage destruction and high inflammatory cellular infiltration.

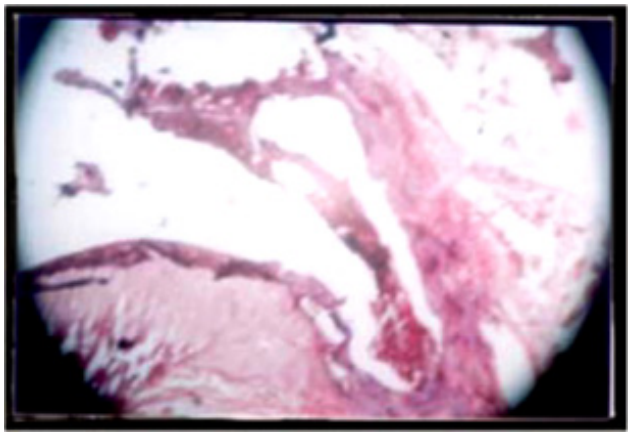

Fig. (3). Effect of Indomethacin (10 mg/kg i.p.) on FCA induced monoarthritic rats knee joint histopathology (H & E, X 100). Joint cartilage protection and low inflammatory cellular infiltration.

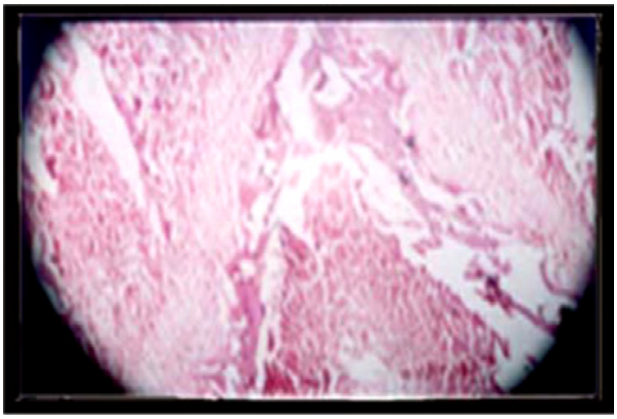

Fig. (4). Effect of Petroleum ether extract (100 mg/kg p.o.) on FCA induced monoarthritic rats knee joint histopathology (H & E, X 100). Low cartilage destruction and high inflammatory cellular infiltration.

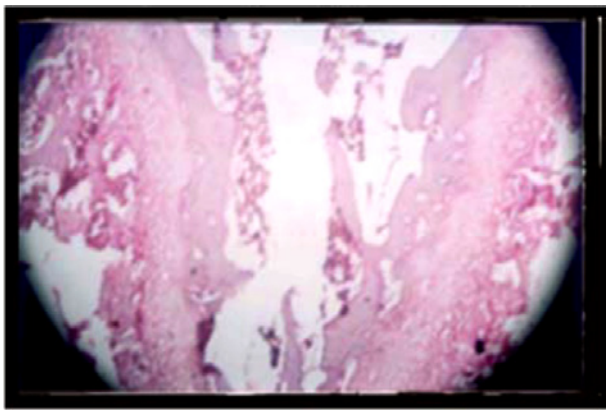

Fig. (5). Effect of Petroleum ether extract (200 mg/kg p.o.) on FCA induced monoarthritic rats knee joint histopathology (H & E, X 100). Low cartilage destruction and low inflammatory cellular infiltration.

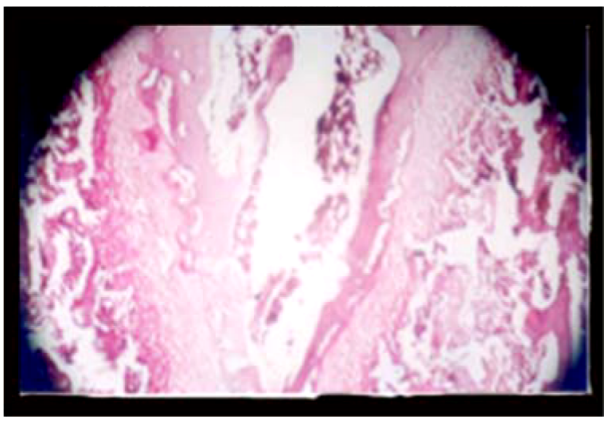

Fig. (6). Effect of Petroleum ether extract (400 mg/kg p.o.) on FCA induced monoarthritic rats knee joint histopathology (H & E, X 100). Very low cartilage destruction and low inflammatory cellular infiltration.

Radiography

FCA injected group of animals shown deformation and abnormality in toes. Treatment with petroleum ether and chloroform extract of *A. bracteata* restored normal architecture, whereas methanolic extract fails to maintain integrity of joints.

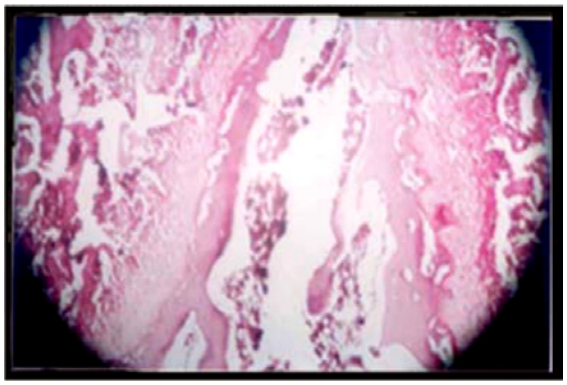

Fig. (7). Effect of Chloroform extract (100 mg/kg p.o.) on FCA induced monoarthritic rats knee joint histopathology (H & E, X 100). Low cartilage destruction and high inflammatory cellular infiltration.

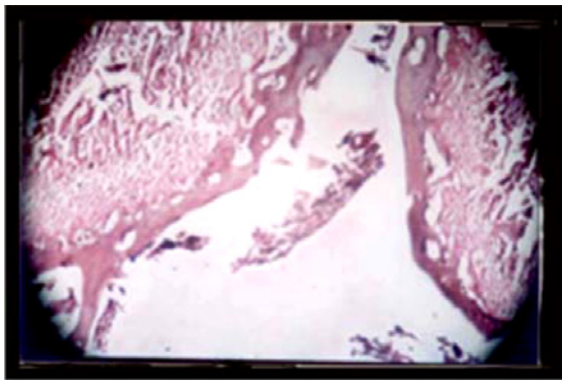

Fig. (8). Effect of Chloroform extract (200 mg/kg p.o.) on FCA induced monoarthritic rats knee joint histopathology (H & E, X 100). No cartilage destruction and low inflammatory cellular infiltration.

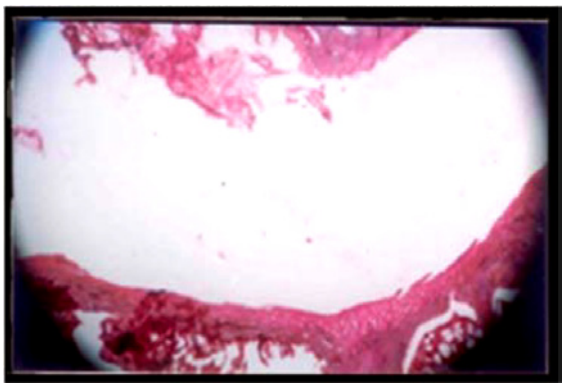

Fig. (9). Effect of Chloroform extract (400 mg/kg p.o.) on FCA induced monoarthritic rats knee joint histopathology (H & E, X 100). No cartilage destruction and no inflammatory cellular infiltration.

DISCUSSION

The Freund's complete adjuvant (FCA) induced arthritis model in rats is the most common model used by several

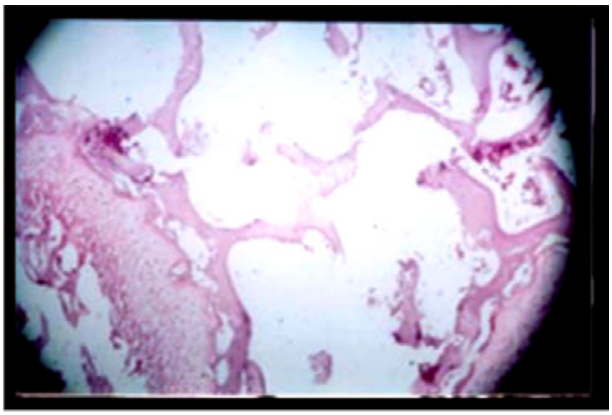

Fig. (10). Effect of Methanolic extract (100 mg/kg p.o.) on FCA induced monoarthritic rats knee joint histopathology (H & E, X 100). High cartilage destruction and absence of inflammatory cellular infiltration.

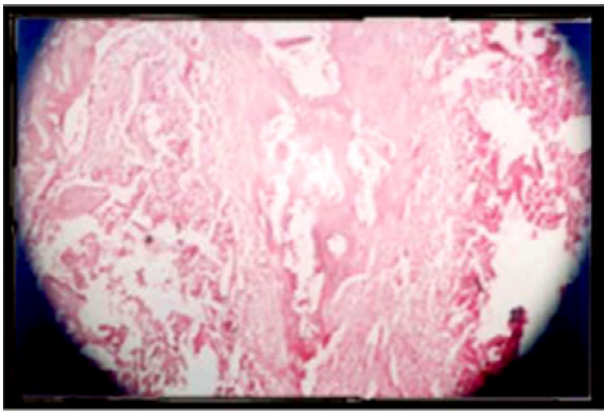

Fig. (11). Effect of Methanolic extract (200 mg/kg p.o.) on FCA induced monoarthritic rats knee joint histopathology (H & E, X 100). Low cartilage destruction and low of inflammatory cellular infiltration.

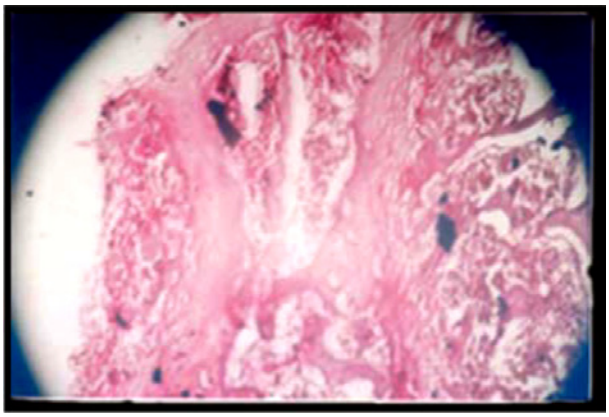

Fig. (12). Effect of Methanolic extract (400 mg/kg p.o.) on FCA induced monoarthritic rats knee joint histopathology (H & E, X 100). No change in cartilage architecture and low of inflammatory cellular infiltration.

scientists to evaluate potential anti-arthritic agents. This preclinical model predicted the activities of a number of compounds that are currently used in the treatment of rheumatoid arthritis are being tested in clinical trials. Recently, Baumgartner *et al*. distinguished 4 phases of arthritis on the basis of biochemical markers of arthritis [30] and different from clinical phases described by Houssay *et al*. (i) Day 1-4, , with acute local inflammation and systemic effects (liver); (2) Days 7-12, with remission of acute inflammation and periarthritis ; (3) Days 12-28, with chronic inflammation, periarthritis and osteogenic activity; (4) Day 35 onwards (indefinitely), with permanent articular deformity and minimal (burn-out) inflammation [31]. A general increase in 5-HT synthesis within the whole central nervous system during the acute phase of the disease (2-3 weeks postinoculation) with a specific, further enhancement restricted to the spinal cord during the post acute phase (4-6 weeks postinoculation) [22,32].

The results obtained in our study and indomethacin is similar to the results as predicted by earlier studies [33-35]. In various animal models to evaluate the effect of compounds upon the development of adjuvant induced arthritis. Body weight was considered as an indirect index of health status and recovery from disease [36-38]. In our study we followed US FDA guidelines on preclinical evaluation and considered ESR, Hb and body weight as an indirect index in restoration of health [14]. A dramatic cessation of growth and decline in body weight was indicated in control group of animals from first week of study similar to earlier study. Significant restoration and gain in body weight was evident, when treated with indomethacin as shown by previous study. It also improved blood Hb level without of significantly affecting ESR. All doses of petroleum ether, chloroform and methanolic extracts significantly restored ESR, blood Hb content and body weight change in 4^{th} week of study. However, more promising results were obtained with chloroform extract indicating more efficacies in recovering from FCA induced arthritis. The results support the involvement of antioxidant and anti-anaemic properties in maintenance [39].

FCA induced arthritis have been used as a model of subchronic or chronic inflammation in rats and of considerable relevance for the study of pathophysiology and pharmacological control of inflammatory processes. In our study, the monoarthritis was very stable in inflammatory signs. The initial inflammatory response was developed within few hours, but more critical clinical signs were seen on 1^{st} week of postinoculation and there after for several week. Previous studies demonstrate that *A. bracteata* appears to be efficient in acute and sub-acute inflammatory processes [22, 40, 41]. Our study on mice ear edema supports the above study as all doses and extract of *A. bracteata* inhibited xylene-induced ear edema, which has been used as an inflammation model with leukotriene inhibition. The tibiotarsal joint and paw volume was significantly increased in 1^{st} week, and maintained throughout 4^{th} week of study, but shown some decline in paw volume on last day. Effect of indomethacin was seen from 2^{nd} week of treatment, whereas *A. bracteata* extracts shown its onset from 1^{st} week of study and more significant effects were seen in petroleum ether and chloroform extracts than indomethacin, probably through the same mechanism as indomethacin by inhibition of phospholipase A2 and increased vascular permeability followed by excess infiltration of cytokines and leukotriene at the inflamed sites possibly by its active constituent aristolochic acid present in the extracts [39]. These results are also supported by our histomarphology studies.

Using arthritic rats as a model of chronic pain, several studies evaluated hyperalgesia in different ways. It has been stated that the method of nociceptive thermal stimulus, such as the hot plate provides a quantitative measurement of hyperalgesia related to behaviors [22, 24, 36]. The alteration in response to an acute pain stimulus in the non- affected limb probably reflects involvement of inhibitory controls caused by obvious long-standing nociceptive input from the contra lateral arthritic limb. An earlier study shows that reduction of the latency for the animal's reaction correspondingly augmented sensitivity to pain [24]. In our study thermal hyperalgesia was tested on the last day to avoid tissue lesion. Similar to earlier reports indomethacin significantly increased latency time to thermal stimulus. *A. bracteata* also shown significant increase in basal reaction time, and chloroform extract produced more prominent effects ($P<0.001$) on thermal hyperalgesia similar to indomethacin, indicating its similar analgesic property. Hence, arthritic pain is associated with increased level of 5-HT in central nervous system and subsequently in spinal cord [42]. The *A. bracteata* may be acting on these sites and possibly abolishing spinal reflex and producing analgesic effects. However, more studies are required in this line to establish.

With respect to earlier studies on arthritic rat's exploratory behavior and stress, we observed reduced ambulatory movement, rearing, grooming, and increased itching, scratching, defecation and urination supporting the previous studies on behavior of arthritic rats [19, 22, 36, 42]. In this group of rats we observe an attempt of protection of the affected paw, as evidenced by causing an elevation, as well as avoidance to support its own weight. Indomethacin decreased latency to explore and rearing increased ambulatory movements behaviors supporting its analgesic property. However it fails in overcoming from aggression, stress and irritability as evidenced in defecation, urination, scratching, and grooming behaviors.

The change in behavior of rats in 1st day of treatment by the extracts may be due to mishandling of animals while administration. Treatments of animals with *A. bracteata* have shown no significant effect on exploratory behavior of arthritic rats indicating need of long-term treatment in overcoming from clinical signs. In 2nd week, all type of extracts significantly decrease grooming behavior indicating the efficiency of these extracts in overcoming from arthritic discomfort including itching, irritation, and scratching, also showing their onset of action.

The methanolic extract of *A. bracteata* shown significant increase in latency to explore, rearing, and ambulation, with significantly increase in defecation. Possibly this action may be mediated by flavanoids present in this extract improving weight bearing capacity and feeling of wellness. Petroleum ether extract significantly decrease total latency time to explore and increase number of defecations indicating failure of this extract in overcoming from stress and intension movement associated with healing of arthritis as evidence in other parameters of this study. In 4th week methanolic extract has shown significant increase weight bearing threshold and ambulation may be associated with improvement in health status supported by our study of Hb, ESR, and body weight.

In order to characterize the severity of disease more accurately, a quantification of lower body radiograph was taken of two animals from each group. In control group abnormality and deformation were observed but have no clinical significance. Treatment with indomethacin, petroleum ether and chloroform extract of *A. bracteata* restored the abnormalities seen in control group. But, methanolic extract failed to produce inhibitory effect, supporting efficacy and potency of chloroform extract use in the treatment of arthritis.

CONCLUSION

On the basis of the results obtained in this study we conclude, and propose that possibly, the potent anti-arthritic effect of *Aristolochia btracteata* chloroform extract may be through maintenance of synovial membrane and vascular permeability, thereby inhibiting cytokines and leukotriene infiltration inhibition as evidenced in paw edema volume and xylene-induced ear edema. In turn, protecting synovial membrane and destruction of cartilage and improving health status through antioxidant and haematonic properties. The similarity in the extract and indomethacin may propose the inhibitory effect on phospholipase A_2 and prostaglandin. Eddy's hot plate test indicates its possible analgesic effect may be mediated through central and spinal serotonergic neurons inhibitory effect. The results obtained in the present study indicate that *Aristolochia bracteata* is having a potent anti-arthritic property. This study also demonstrates not only its ability in overcoming arthritis and its complications but also clinical signs as evidenced in paw edema, thermal hyperalgesia and histomarphological examinations. Improvement in health parameters consider in this study including HB, ESR, and body weight indicating its beneficial effects while recovery from arthritis. The open field test considered for the present study to evaluate effect of *A. bracteata* on behavior of animals and clinical symptoms while long term treatment for arthritic condition have shown in appreciable results by impairing intension for movement, weight bearing capacity, lack of irritation and stress and increase in threshold of pain in overcoming from the disease and its symptoms. However, further fractionation and isolation of chloroform extract is required to observe safety, efficacy and potency of Aristolochia *btracteata* against arthritis.

REFERENCES

[1] Harris, E.D. Rheumatoid arthritis; pathophysiology and implications for therapy. *N. Engl. J. Med.*, **1990**, *322*, 1277-1289.

[2] Rat, A.C.; Boissier, M.C. Rheumatoid arthritis: direct and indirect costs. *Joint Bone Spine*, **2004**, *71*(6), 518-24.

[3] Special Article; Guidelines for the management of rheumatoid arthritis. American College of Rheumatology Subcommittee *Arthritis Rheum.*, **2002**, *46*(2), 328-346.

[4] Ahmed, S.; Anuntiyo, J.; Charles, J.; Haqqi, T.M. Biological basis for the use of botanicals in osteoarthritis and rheumatoid arthritis; A Review. *Complemn. Alternat. Med.*, **2005**, *2*(3), 301-308.

[5] Brunton, L.L.; Lazo, J.S.; Parker, K.L. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. 11th ed. New York: Mc Graw- Hill; **2006**.

[6] Feldman, M.; Steinmen, L. Design of effective immunotherapy for human autoimmunity. *Nature*, **2005**, *435*, 612-619.

[7] Arend, W.P.; Dyer, J.M. Inhibition of the production and effects of interleukin-1 and tumour necrosis factor-∞ in rheumatoid arthritis. *Arthritis Rheum.*, **1995**, *38*, 151-160.

[8] Feldman, M.; Maini, R.N. Anti-TNF-∞ therapy of rheumatoid arthritis; what we have learned. *Ann. Rev. Immunol.*, **2001**, *19*, 163-196.

[9] Brown, S.L.; Grene, M.H.; Gershon, S.K.; Edward, E.T.; Brown, M.M. Tumour necrosis factor antagonists therapy and lymphoma development; 26 cases reported to food and drug administration. *Arthritis Rheum.*, **2002**, *46*, 3151-3158.

[10] Baghai, M.; Osman, D.R.; Wolk, D.M.; Wold, L.E.; Haidukewych, G.J.; Matteson, E.L. Fatal sepsis in a patient with rheumatoid arthritis treated with entanercept. *Mayo Clin. Proc.*, **2001**, *76*, 653-656.

[11] Engel, L.W.; Straus, S.E. Development of therapeutics: Opportunities within complementary and alternative medicine. *Nat. Rev.*, **2002**, *1*, 229-237.

[12] Jacobs, J.W.; Rasker, J.J.; Bijlsma, J.W. Alternative medicine in rheumatology: threat or challenge. *Clin. Exp. Rheumatol.*, **2001**, *19*, 117-119.

[13] Huxtable, R.J. The pharmacology of extinction. *J. Ethnopharmacol.*, **1992**, *37*, 1-11.

[14] Guidance for Industry, Clinical development programs for drugs, devices, and biological products for the treatment of rheumatoid arthritis (RA). U.S. Food and Drug Administration **1999**. Internet: http://www.fda.gov/cber/gdlns/rheumcln.htm

[15] Kokate, C.K. In: *Practical Pharmacognosy*. 3rd ed. New Delhi: Vallabh Prakashan **1994**; p. 107.

[16] Guidelines for Toxicity Investigation of Herbal Medicine (Annexure-1). Department of Ayurveda, Yoga, & Naturopathy, Unani, Siddha and Homeopathy, Ministry of health & Family Welfare: Government of India; **2005**.

[17] Ferber, P.C.; Fischer, R.W. *Immunization of laboratory animals*. 3R-Info-Bulletin 11, Research Foundation Switzerland **2004**.

[18] Lam, F.F.; Wong, H.H.; Ethel, S.K. Time course and substance P effects on the vascular and morphological changes in adjuvant-induced monoarthritic rats. *Int. Immunopharmacol.*, **2004**, *4*, 299-310.

[19] Butler, S.H.; Godeyfroy, F.; Besson, J.M.; Jeanne, W.F. A limited arthritic model for chronic pain studies in the rat. *Pain*, **1992**, *48*, 73-81.

[20] Kale, S.R.; Kale, R.R. *Practical human anatomy & physiology*. 9th ed.; Nirali Prakashan: Pune, **1999**.

[21] Dimitrijevic, M.; Laban, O.; Djuric, V.J.; Stanijevic, S.; Miletic, T.; Jovanovic, V.K.; Todorovic, C.; Radulovic, J. Behavior and severity of adjuvant-arthritis in four strains. *Brain Bahav. Immun.*, **2001**, *15*, 255-265.

[22] Costa, M.D.; Sutter, P.D.; Gybels, J.; Vanhess, J. Adjuvant induced arthritis in rats: A possible animal model of chronic pain. *Pain*, **1981**, *10*, 173-185.

[23] Vogel, H.G.; Vogel, W.H.; Sholkens, B.A.; Sandow, J.; Muller, G.; Vogel, W.F. Drug discovery and Evaluation. 2nd ed. New York: Springer; **2002**.

[24] Anderson, L.M.; Eduardo, H.R.; Seabra, M.L.; Silva, A.A.; Tufik, S. Evaluation of acute and chronic treatments with *Harpegophytum procumbens* on freund's adjuvant-induced arthritis in rats. *J. Ethnopharmacol.*, **2004**, *91*, 325-330.

[25] Chitme, H.R.; Chandra, R.; Kaushik, S. Studies on anti-inflammatory activity of C*alotropis gigantean* in experimental animals. *Asia Pac. J. Pharmacol.*, **2005**, *16*, 41-46.

[26] Tang, X.L.; Zigong, L.; Wen, C.N.; Shen, L. Anti-inflammatory effect of 3-acetylconitine. *Acta Pharmacol. Sin.*, **1984**, *5*, 85-89.

[27] Chillingworth, N.L.; Donaldson, L.F. Characterisation of a freund's complete adjuvant-induced model of chronic arthritis in mice. *J. Neurosci. Methods*, **2003**, *128*, 45-52.

[28] Bendele, A.; Tracy, M.; Sennello, G.; Frazier, J.; Chlipala, E.; Dorothy, M. Efficacy of sustained blood levels of interleukin-1 receptor antagonist in animals models of arthritis. *Arthritis Rheum.*, **1999**, *42*(3), 498-505.

[29] Carlson, R.P.; Datko, L.J.; Lynn, O.N.; Frank, D.; Beideman, R.; Lewis, A.J. Comparison of inflammatory changes in established type-II collagen and adjuvant-induced arthritis using outbred wistar rat. *Int. J. Immunopharmacol.*, **1985**, *7*(6), 811-826.

[30] Baumgartner, W.A.; Beck, F.W.; Lorber, A.; Pearson, C.M.; Whitehouse, M.W. Adjuvant disease in rats: biochemical criteria for distinguishing several phases of inflammation and arthritis. *Proc. Soc. Exp. Biol. Med.*, **1974**, *145*(2), 625-630.

[31] Houssay, A.B.; Cymerman, G.; Barrio Rendo, M.E.; Candiolo, B.H.; Charrean, E.H. Ascorbic acid concentrations in different periods of the experimental arthritis in rats. *Acta Physiol. Lat. Am.*, **1966**, *16*(1), 43-51.

[32] Godeyfroy, F.; Weil-Fugazza, J.; Besson, J.M. Complex temporal changes in 5-hydroxytryptamine synthesis in the central nervous system induced by experimental polyarthritis I the rat. *Pain*, **1987**, *28*(2), 223-238.

[33] Shirwaikar, A.; Somashekar, A.P. Anti-inflammatory activity and free radical scavenging studies of *Aristolochia bracteata* Lam. *Indian J. Pharm. Sci.*, **2003**, *65*(1), 67-69.

[34] Shirwaikar, A.; Somashekar, A.P.; Udupa, A.L.; Udupa, S.L. Wound healing studies of *Aristolochia bracteolata* Lam with supportive action of antioxidant enzymes. *Pytomedicine*, **2003**, *10*(6-7), 558-562.

[35] Shah, B.N.; Nayak, B.S.; Seth, A.K.; Jalapure, S.S.; Patel, K.N.; Patel, M.A. Search for medicinal plants as a source of anti-inflammatory and anti-arthritic agents- A Review. *Pharmacognosy Mag.*, **2006**, *2*(6), 77-86.

[36] Calvino, B.; Bernard, M.O.; Bars, D.L. Parallel clinical and behavioural studies of adjuvant-induced arthritis in the rats: possible relationship with chronic pain. *Behav. Brain Res.*, **1987**, *24*, 11-29.

[37] Chulder, E.H.; Dong, W.K. Neuroma pain model: correlation of motor behaviour and body weight with autonomy in rats. *Pain*, **1983**, *17*, 341-351.

[38] Morton, D.B.; Griffiths, P.H. Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis foe assessment. *Vet. Rec.*, **1985**, *116*, 431-446.

[39] Badami, S.; Jose, C.K.; Choksi, R.K.; Dongre, S.H.; Jagdish, P.C.; Suresh, B. *In vitro* antioxidant activity of various extracts of *Aristolochia racteolate* leaves. *Orient. Pharm. Exp. Med.*, **2005**, *5*(4), 316-321.

[40] Glenn, E.M.; Gray, J. Adjuvant-induced polyarthritis in rats: biologic and histologic background. *Am. J. Vet. Res.*, **1965**, *26*, 1180-1194.

[41] Jones, R.S.; Ward, J.W. Adjuvant-induced polyarthritis in rats. *Methods Achiev. Exp. Pathol.*, **1966**, 607-638.

[42] Sluka, K.A. Westlund. Behavioural and immunohistochemical changes in an experimental arthritis model in rats. *Pain*, **1993**, *55*, 367-377.

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