Evaluation of Anti-inflammatory and Anti-arthritic Activity of Ajmodadi Churna- A Polyherbal Formulation

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ABSTRACT

Anti-inflammatory and anti- arthritic activity of aqueous extract of Ajmodadi Churna (AJM) were evaluated by three methods namely, Carrageenan paw edema, Carrageenan induced Air Pouch Model in Rats and Freunds' complete adjuvant Arthritis. The Carrageenan paw edema was carried out to test the effect of the extract on acute phase of inflammation. Carrageenan induced Air Pouch Model was used for local inflammation and Freunds' complete adjuvant Arthritis was used for evaluation of chronic inflammation. Results showed that AJM have significant anti-inflammatory activity and Anti-arthritic Activity in both the doses (200 mg/kg and 400 mg/kg) when compared to the Diclofenac but higher dose was found more effective.

Key Words: Anti-inflammatory, Anti-arthritic Activity, Freunds' complete adjuvant Arthritis, Ajmodadi churna.
DOI: 10.24896/eijppr.2016651 **eIJPPR 2016; 6(5): 72-76**

INTRODUCTION

Inflammation is the body's immediate response to damage its tissues and cells by pathogens, noxious substances such as chemicals, or physical injury [1]. Inflammatory processes are required for immune surveillance, optimal repair, and regeneration after injury [2]. However, sustained, excessive or inappropriate inflammation is the cause of numerous diseases including rheumatoid arthritis, psoriasis and inflammatory bowel disease. Inflammation is a major component of the damage caused by autoimmune diseases, and is a fundamental contributor to diseases such as cancer, diabetes and cardiovascular disease [3]. Locally, inflammation develops in the classical forms of swelling, redness, heat and often pain. The common instigators of inflammation are at one end of a large range of adverse conditions that induce inflammation, and they trigger the recruitment of leukocytes and induction or activation of inflammatory mediators such as kinins, cyclooxygenase products and cytokines. Many of these molecules are produced locally and have proven involvement in tissue inflammation, and are thus key targets for therapeutic intervention in a range of diseases [3-4]. Clinical treatment of inflammatory diseases is dependent on drugs, which belong either to the non-steroidal or to the steroidal chemical groups [5]. The continued search for potentially effective and safe anti-inflammatory agents cannot be over-emphasized. The use of herbal extracts and nutritional supplements either as an alternative or as a complimentary medicine to conventional chemotherapy for the treatment of inflammatory diseases is well documented in Ayurveda, which is an alternative medicinal system that has been practiced primarily in the Indian subcontinent for 5000 years [6]. As a result of adverse side effects, like gastric lesions, caused by NSAIDs and tolerance and dependence induced by opiates, the use of these drugs as anti-inflammatory and analgesic agents have not been successful in all the cases. Therefore, new anti-inflammatory and analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates.

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Received: 18 May 2016; **Revised:** 25 October 2016; **Accepted:** 27 October 2016

During this process, the investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant-based drugs [7].

Ajmodadi churna is a polyherbal Ayurvedic medicine used as carminative and anti-spasmodic, has strong wormifuge and helps in all painful conditions like sciatica, rheumatoid arthritis and stiffness in back and also restores normal digestive functions also used in diarrhea [8,9]. This formulation is prepared from parts of 11 different plants that are used in traditional medicine for a variety of purposes [10] such as (a) reduction of pain (*Cedrus deodara, Plumbago zeylanica*) (b) reduction of inflammation (*Zingiber officinale,, C. deodara, Argyreia nervosa* and *Terminalia chebula*), and (c) anti-pyretic activity (*Zingiber officinale, Piper longum*, *Piper nigrum* and *C. deodara*). Included are also plants able to (a) improve appetite and digestion (*Anethum graveolens*, *Trachyspermum ammi, C. deodara,* and *Z. officinale*), (b) act as immunostimulants (*Piper longum* and *Piper nigrum*), (c) act as diuretics (e.g. *Anethum graveolens* and *C. deodara*), (d) laxatives (e.g. *Terminalia chebula*) and (e) anthelmintic action (*Embelica ribes*).

MATERIALS AND METHODS

Materials

All chemicals were purchased from Sigma Chemical Co. Ltd., St. Louis, USA.

Plant materials [11]

All these ingredients were procured from the local market of Udupi, Karnataka, India, authenticated and churna was prepared according to formula prescribed in Auyrvedic Formulary of India 2003 demonstrated in Table 1.

Preparation of Ajmodadi churna - The churna was prepared as per the procedure given in Ayurvedic Formulary of India. All the ingredients were powdered separately, passed through 80 # sieve and then mixed together in specified proportions to get uniformly blended churna.

Preparation of the extracts - Aqueous extract (AJM) - The powdered churna 100g was macerated with chloroform: water (1:99) for 48 h. The filtered the extract by vacuum filtration method and evaporated the solvent at 40°C on water bath then concentrated extract was subjected to stored in a refrigerator.

Animals

Female Wistar rats weighing 130–150 g were procured from Animal house of Manipal University, Manipal. All animals were housed in polypropylene cages in a temperature-controlled room at 24±1°C. The animals were fed with pelleted rat feed manufactured by Hindustan Lever Ltd, Mumbai with free access to water throughout the experiment. The rats were acclimatized at least one week before starting the experiments. In all experimental models of inflammation the studies were carried out using six rats in each group. This study got clearance from the Institutional Animal Ethical Committee (No. IAEC/ KMC/76/2009-2010).

Drug administration

All the extracts were dissolved in 1% CMC in water. The vehicle alone served as control. Diclofenac (10mg/kg p.o), served as standard [12].

Carrageenan induced rat paw edema

The acute anti-inflammatory effect was evaluated by carrageenan induced rat paw edema according to the method of Winter *et al*. (1962) [13]. Edema was induced by injection of 1% suspension of carrageenan in 0.9% sterile saline solution into the right plantar region of the rat. The plant extract (200 and 400mg/kg), Diclofenac (10mg/kg body weight), or vehicle was administrated orally 1 h before injection of carrageenan. The paw volumes of rats were measured by plethysmometer in 0, 3rd and 5th hour after injection [14].

Carrageenan induced Air Pouch Model in Rats [12]

Preparation of air pouch- On day 1, the dorsal side of the animal was shaved (1cm^2) under ether anaesthesia and disinfected and an air cavity was produced by the subcutaneous injection of 20 ml of sterile air with a 28 gauge needle. An additional 20 ml of air was injected on day 3 and experimental animals were grouped. On day 5 of the initial air injection, 1ml of 1%w/v carrageenan dissolved in saline was injected directly into the pouch to produce an inflammatory response for all groups except negative control which received 1ml of saline only. After 6 hours of carrageenan injection, the animals were sacrificed under ether anesthesia and 5ml of ice-cold saline was injected into the pouch.

Table 1. Ingredients of Ajmodadi Churna

S. NO.	INGREDIENTS	QUANTITY (gm)
1	*Ajmoda (Trachyspermumammi)*	12
2	*Vidanga (Embeliaribes)*	12
3	*Saindhavalavana*	12
4	*Devdaru (Cedrus deodara)*	12
5	*Chitraka (Plumbagozeylanica)*	12
6	*Pipalimula ((Piper longum(stems))*	12
7	*Satapuspa (Anethumgraveolens)*	12
8	*Pipali (Piper longum)*	12
9	*Marica (Piper nigrum)*	12
10	*Pathya (Terminalia chebula)*	60
11	*Vrddhadaruka (Argyreia nervosa)*	120
12	*Nagara (Zingiber officinale)*	120

Table 2. Alterations in hematological parameters in CFA-induced arthritis in rats

Group	RBC (×10^6/mm^3)	WBC(×10^3/mm^3)	ESR (mm/h)	Hb (mg%)
CFA control	8.14±0.32	13.82±0.88	13.66±0.33	12.94±0.31
Diclofenac	10.28±0.24***	8.84±0.69***	10.33±0.33***	15.14±0.73**
AJM 100	9.18±0.20*	9.92±0.29***	12±0.57*	12.59±0.35
AJM 200	9.87±0.17***	9.10±0.52***	10.66±0.33***	13.90±0.38*

Exudate harvesting- The pouch was gently massaged for a minute and cut open carefully and exudate was collected. Total leukocyte count of lavage fluid was estimated using cell counter (Veterinary cell counter, ERMA).

Freunds' complete adjuvant Arthritis [15]

Induction of inflammation and treatment Measurement of paw edema- Inflammation induced by complete Freund's adjuvant (CFA) was elicited as described by Wilson *et al.* (2006). Rats were briefly anesthetized with ether (for 2–3 min) and 100 µl of the complete Freund's adjuvant (CFA, 1mg/ml of heat killed *Mycobacterium butyricum* was intradermally injected into the ventral surface of the right hind paw (intraplantar injection) and subject to treatment according to the following scheme (n=6). Group 1: 0.25% CMC (Control group), Group 2: Diclofenac Sodium (5mg/kg p.o), Group 3: AJM (100 mg/kg p.o), Group 4: AJM (200 mg/kg p.o). On day 0th after an initial measurement of paw volume, paw volumes were measured on day 7, 14 post injections. On day 15 rats with developed arthritis were selected and randomized into treatment and control groups. Treatments were given orally, from day 15 to day 28. Paw volumes were measured on day 21 and day 28. The percent changes in paw volume before and after treatment in the groups were calculated and compared among the groups.

Haematological parameters

The RBC, WBC, ESR and Hemoglobin (Hb) were evaluated using routine laboratory methods [16].

Radiological Analysis

On day 21, animals were anesthetized with anesthetic ether. Radiographs of the adjuvant-injected hind paws were taken with a X-ray instrument (Kodak, Japan) computerized radiographic systems (Japan). The film focus distance was 60 inches and the machine was operated at 43 kV peak, 6 mA for 0.6s. The X-ray image of the adjuvant-injected limb of each rat was evaluated for radiographic changes [16, 17].

Statistical analysis

Statistical significance (p) was calculated by one-way ANOVA between the control group and the AJM treated group followed by Dunnett's post hoc test of significance where, $p < 0.05$, $p < 0.01$ and $p < 0.0001$ considered being significant, very significant and highly significant respectively. All datas are expressed as mean ± S.E.M (n=6 mice per groups).

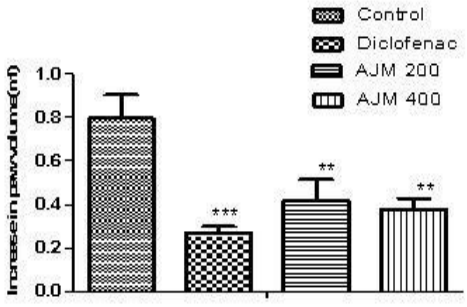

Figure 1. Effect of AJM on Carrageenan induced rat paw edema

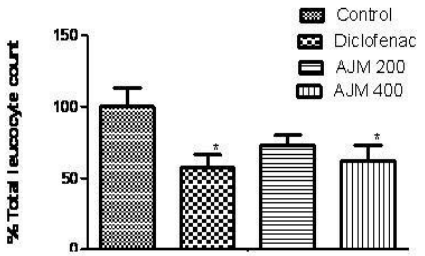

Figure 2. Effect of AJM on total leukocyte count in fluid collected from air pouch

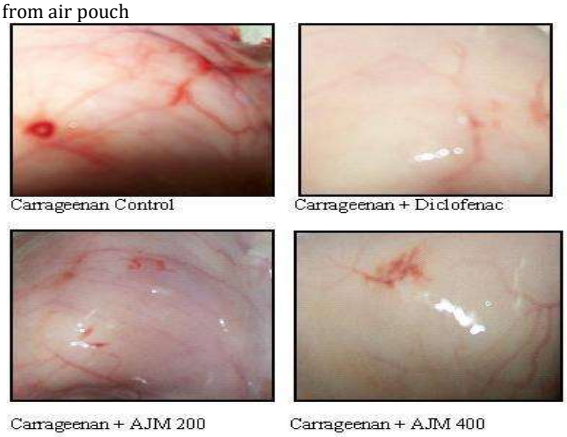

Figure 3. Photographs of exposed air pouch tissue

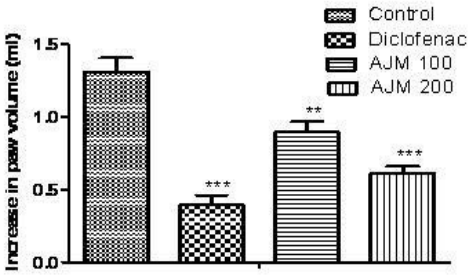

Figure 4. Effect of AJM on paw volume in adjuvant arthritis model

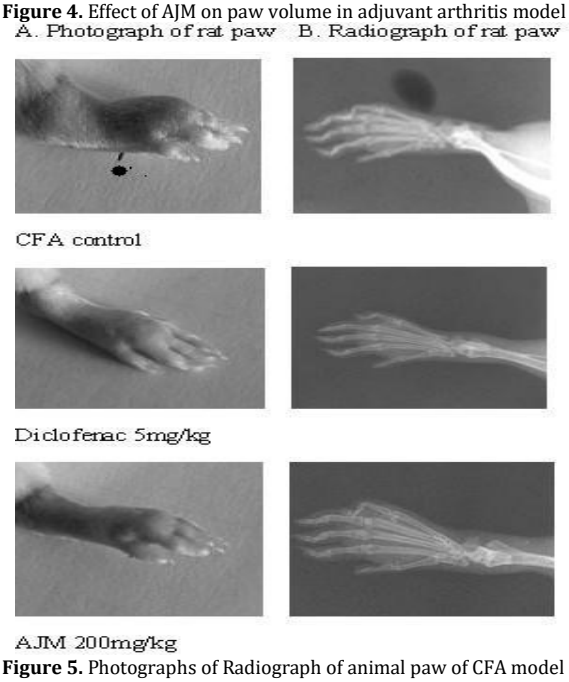

Figure 5. Photographs of Radiograph of animal paw of CFA model

RESULTS

Ajmodadi churna was prepared according to formula demonstrated in Table 1. Then the formulated churna was standardized for physico-chemical parameters and extracted by maceration and aqueous extract of churna (AJM) was used for inflammatory models.

The aqueous extracts of Ajmodadi churna at the doses of 200 and 400 mg/kg body weight, for acute models when administered orally significantly decrease in paw volume occurs in 3rd hour of study demonstrated in Figure 1 in Carrageenan induced rat paw edema model.

In Carrageenan induced Air Pouch Model in Rats the exposed tissue shows decrease in inflammatory response (Figure 3) and in total leukocyte count demonstrated in Figure 2.

The aqueous extracts of Ajmodadi churna at the doses of 100 and 200 mg/kg body weight, for chronic models when administered orally shown improvement in hematological and radiological changes of inflammation (Table 2 and Figure 5). Also significantly decrease in paw volume in 28th day of study (Figure 4).

DISCUSSION

Carrageenan induced rat hind paw edema has been widely used for the discovery and evaluation of anti-inflammatory drugs. The acute inflammation has two phases: the first phase (begins immediately after injection and last one hour) is characterized by the release of histamine and serotonin; and the second phase (begins after one hour and last three hours) is characterized by the bradykinin release via prostaglandins mediator pathways [18]. Both histamine and serotonin are characterized by the increase of vascular permeability. Prostaglandins mediate maximum vascular responses during the second phase of inflammation [19]. This may be due to the inhibition of cyclooxygenase enzymes that are involved in the formation of prostaglandins.

The rat air pouch model is a convenient *in vivo* method to evaluate localized inflammation without systemic effects. Subcutaneous injection of air into the proximal area of the dorsum causes, over several days, a morphological change in the cellular lining of the pouch [20]. Localized inflammation, characterized by an infiltration of cells, an increase in exudate, and a marked production of biochemical mediators can be induced by intra-pouch administration of irritants such as carrageenan. This model has been used successfully in preclinical studies of non-steroidal anti-inflammatory drugs (NSAIDs) for use as therapeutic agents against the signs and symptoms of rheumatoid arthritis and other chronic inflammatory diseases [21]. Injection of a carrageenan solution into the pouch produces an inflammatory reaction that is characterized by an infiltration of cells, an increase in exudate, tissue damage and a marked production of biochemical mediators such as prostaglandins, leukotrienes, and cytokines. This unit describes a method to elicit a cellular influx in the six-day-old air pouch in the rat. Potential of anti-inflammatory drugs can be tested by measuring the reduction in carrageenan-induced inflammation. This may be due to the inhibition of biochemical mediators and significant antioxidant activity exhibited by the AJM.

CFA-induced arthritis is the most widely used chronic test model in which the clinical and pathological changes are comparable with those seen in human rheumatoid arthritis. Chronic inflammation in the CFA model is manifested as a progressive increase in the volume of the injected paw. It is noteworthy that the inhibitory effect of AJM (200 mg/kg/day) on the volume of the injected paw was comparable with that of Diclofenac (5 mg/ kg/day) on 28th day of study.

Other miscellaneous information related to the pathology of arthritis that has been obtained during this study includes radiographic examination of the paws, haematological parameters. The radiographic observations of the rats show that the treatment with AJM inhibited the arthritis-associated joint changes.

AJM 200 (0.417±0.097) and AJM 400 (0.379±0.049) showed significant reduction in paw volume in Carrageenan induced paw edema in compare to control (0.794±0.108). In Carrageenan induced Air Pouch Model showed AJM showed reduction in Total leukocyte count (62.17±10.53) and MPO (18.44±3.18) in compare to the control (100) respectively. In Freunds' complete adjuvant Arthritis there is decrease in paw volume (0.615±0.044), Total leukocyte count (9.10±0.52) and ESR (10.66±0.33) and an increase in Hb (13.90±0.38) and RBC count (9.87±0.17) when compared with control.

It has been reported that a moderate rise in the WBC count occurs in arthritic conditions. The present study reveals that AJM treatments tend to decreases the WBC count. In addition to this, other characteristic hematological alterations such as the decreased Hb and RBC count and increased erythrocyte sedimentation rate were also restored by the AJM treatments. It is proposed that the reduction in the Hb count during arthritis results from reduced erythropoietin levels, a decreased response of the bone marrow erythropoietin and premature destruction of red blood cells. Similarly, an increase in the ESR is attributed to the accelerated formation of endogenous proteins such as fibrinogen and such a rise in the ESR indicates an active but obscure disease process [22,23]. Thus, the reduction in the ESR and increase in the Hb count brought about by AJM treatment further support its anti-arthritic effect.

ACKNOWLEDGEMENT

The authors sincerely thank Manipal University, Manipal, India for providing all facilities to carry out this study.

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HOW TO CITE THIS ARTICLE: Srivastava, N., Dashora, N., Menaria, J. and Kumar, N., 2016. Evaluation of Anti-inflammatory and Anti-arthritic Activity of Ajmodadi Churna- A Polyherbal Formulation. *International Journal of Pharmaceutical and Phytopharmacological Research, 6*(5), pp.72-76, **DOI: 10.24896/eijppr.2016651**