

Anti-inflammatory activity of *Ajmodadi Churna* extract against acute inflammation in rats

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ABSTRACT

Background: Ayurvedic polyherbal formulations are widely prescribed for a wide range of inflammatory conditions, yet, despite widespread use, there has been no systematic documentation of their safety and efficacy. **Objective:** The present study was undertaken to evaluate the anti-inflammatory activity of aqueous extracts of *Ajmodadi churna* (AJM) in rats. **Materials and Methods:** Carrageenan-induced hind paw edema and air pouch inflammation models were used for the study. **Results:** The extracts showed significant antiinflammatory activity, reducing paw edema volume by 0.417 ± 0.097 and 0.379 ± 0.049 , respectively. In the carrageenan-induced air pouch model, AJM reduced total leukocyte count by 73.09 ± 7.13 and 62.17 ± 10.53 , granulocyte count by 69.48 ± 5.44 and 63.33 ± 4.13 , and myeloperoxidase activity by 14.84 ± 0.91 and 18.44 ± 3.18 , respectively, compared to controls. **Discussion and Conclusion:** AJM significantly reduced paw edema, during the second phase of edema development. In the carrageenan-induced air pouch model, AJM inhibited cellular infiltration into the air pouch fluid. We conclude that AJM is an effective candidate for prevention or treatment of acute inflammation

Key words: *Ajmodadi churna*, granulocyte count, myeloperoxidase activity, paw edema, total leukocyte count

INTRODUCTION

Inflammation represents a highly coordinated sequence of events in which tissues respond to physical trauma, noxious chemicals, or pathogens.^[1] Inflammatory processes are involved in immune surveillance and optimal repair and regeneration, following injury.^[2] However, sustained excessive or irrelevant inflammation is the cause of many diseases including rheumatoid arthritis, psoriasis, and inflammatory bowel disease. Inflammation is a major component of the damage caused by autoimmune diseases and is a key contributor to pathologies such as cancer, diabetes, and cardiovascular disease.^[3] Use of herbal extracts for treatment of inflammatory diseases is

well documented in Ayurveda, the medicinal system of ancient India.^[4]

Ajmodadi churna (AJM) is a polyherbal Ayurvedic medicine traditionally used as a carminative, antispasmodic, wormifuge, or in sciatica.^[5,6] The antiinflammatory activity of its six ingredients, *Trachyspermum ammi*,^[7] *Cedrus deodara*,^[8] *Piper longum*,^[9] *Terminalia chebula*,^[10] *Argyreia nervosa*,^[11] and *Zingiber officinale*,^[12] has previously been studied on an individual basis. In addition to their antiinflammatory properties, all the ingredients have been shown to possess additional biological activity. *Trachyspermum ammi* has also been reported to possess analgesic, antibacterial, antifilarial, antifungal, antiviral, and gastroprotective activities.^[13] *Embelia ribes* exhibits a wide range of activity: Hepatoprotective, analgesic, amylase and trypsin inhibitory, antibacterial, antioxidant, antidiabetic, anticonvulsant, adaptogenic, antifertility, anticancer, antihyperlipidemic, and antifungal.^[14] In *Cedrus deodara*, antioxidant^[15] and anticancer^[16] activity is reported to be significant. *Plumbago zeylanica* reported to possess anticancer,^[17-19] antibacterial,^[20] antiinflammatory,^[21] antileishmanial,^[22] antifungal,^[23] hyperglycemic,^[24] and hypolipidemic^[25] activities. *Piper longum* exhibits antioxidant, antiallergic, antidiabetic, anticancer, immunomodulatory, antiviral, antiulcer, antidepressant, and hepatoprotective activities.^[26] *Anethum graveolens* has been reported to possess anticancer, antidiabetic, antioxidant, antisecretory, antispasmodic,

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insecticidal, and diuretic properties.^[27] *Piper nigrum* is said to possess antioxidant, antiplatelet, antihypertensive, antitumor, antithyroid, antiasthmatic, and hepatoprotective activities.^[28] *Terminalia chebula* exhibits antiviral, antibacterial, antioxidant, antibacterial, radioprotective, wound healing, and immunomodulatory activities.^[10] *Argyrea nervosa* is used in wound healing, rheumatic disorders, leucorrhoea, ulcer, cancer, and syphilis; also as a diuretic and to prevent contraception.^[29] *Zingiber officinale* exhibits immunomodulatory, antitumorogenic, antiinflammatory, antiapoptotic, antihyperglycemic, antilipidemic, and antiemetic actions.^[30] In addition, the combination, *Piper longum*, *Piper nigrum*, and *Zingiber officinale*, known in Ayurveda as Trikatu, is a significant bioenhancer.^[31]

However, no scientific data on antiinflammatory activity of the overall formulation are available. The present study evaluated the potential use of aqueous extracts of AJM to treat acute inflammatory toxicity.

MATERIALS AND METHODS

Plant materials and chemicals

All the 12 ingredients of AJM were procured from Jogappa Shanbhag Ayurvedic Pharmacy, Udupi, Karnataka, India, and were authenticated by Dr. Gopal Krishna Bhat, Department of Botany, Poorna Prajna College, Udupi, Karnataka. Voucher specimens of the same were deposited in the museum of the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal. [Table 1]. All chemicals used in the experiment were of analytical grade.

Ajmodadi churna preparation

The churna was prepared according to the method given in Ayurvedic Formulary of India.^[5] All the ingredients were powdered separately, passed through a 80 # sieve, and then mixed together in specified proportions to yield a uniformly blended churna.

Aqueous extract preparation

Powdered AJM (100 g) was macerated with chloroform-water (1:99 v/v) for 7 days with intermittent shaking. The resulting aqueous extract was concentrated under reduced pressure at 40°C and lyophilized (-40°C) to obtain a solid brown residue (yield: 24.6 g), which was stored in a dessicator until use.

Experimental animals

Female Wistar rats weighing 130–150 g were used. All animals were housed in polypropylene cages in a temperature-controlled room at 24 ± 1°C. They were fed with pelleted rat feed manufactured by Hindustan Lever Ltd, Mumbai, and enjoyed free access to water throughout

Table 1: Ingredients of *Ajmodadi churna*

Ingredients	Parts	Quantity (gm)	Voucher specimen no.
<i>Ajmoda (Trachyspermum ammi)</i>	Fruit	12	PP 5
<i>Vidanga (Embelia ribes)</i>	Fruit	12	PP 576
<i>Saindhava lavana (Rock Salt)</i>	Salt	12	-
<i>Devdaru (Cedrus deodara)</i>	Wood	12	PP 577
<i>Chitraka (Plumbago zeylanica)</i>	Aerial parts	12	PP 578
<i>Pipalimula (Piper longum)</i>	Stem	12	PP 575
<i>Satapusp (Anethum graveolens)</i>	Fruit	12	PP 579
<i>Pipali (Piper longum)</i>	Fruit	12	PP 7
<i>Marica (Piper nigrum)</i>	Fruit	12	PP 3
<i>Pathya (Terminalia chebula)</i>	Fruit	60	PP 511A
<i>Vrddhadaruka (Argyrea nervosa)</i>	Root	120	PP 580
<i>Nagara (Zingiber officinale)</i>	Rhizome	120	PP 2

the experiment. Rats were acclimatized at least 1 week before starting the experiment. For Carrageenan induced hind paw edema and air pouch inflammation models, rats were divided into four groups with six rats in each group. Group I was control group and received 0.25% CMC. Diclofenac sodium served as the standard drug and was given to the group II. The *churna* extract (200 mg/kg and 400 mg/kg) were administered to the group III and group IV respectively.

Ethical clearance

Ethical clearance was obtained from the institutional Animal Ethical Committee (No. IAEC/KMC/76/2009-2010).

Acute toxicity study

Graded doses (1200–4000 mg/kg) of AJM were tested for acute toxicity, according to OECD guideline no. 420. After drug administration, the animals were evaluated every 10 min for 4 hours and then at 24, 48, and 72 hours for changes in visual placing, stereotypy, passivity, grooming, restlessness, irritability, and other behavioral activities. Doses for the reported studies were chosen to be 1/10th and 1/20th of the maximum tolerated dose.

Carrageenan-induced hind paw edema in rats

The acute antiinflammatory effect was evaluated by carrageenan-induced hind paw edema.^[32] The edema was induced by injection of 1% suspension of carrageenan in 0.9% sterile saline solution into the rat's right plantar region. The churna extract (200 and 400 mg/kg), diclofenac sodium (10 mg/kg body weight), or vehicle was administered orally 1 hour before injection of carrageenan. Rats' paw volumes were measured by digital Plethysmometer at hours 0, 3, and 5, after injection.^[33]

Carrageenan-induced air pouch inflammation in rats

On day 1, the rat's dorsal sides were shaved (1 cm²) under ether anesthesia and disinfected, then air cavities were produced by 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 after the initial air injection, 1 ml of 1% w/v carrageenan dissolved in saline was injected directly into the pouch to produce an inflammatory response for all groups except the negative controls which received 1 ml of saline. Six hours after carrageenan injection, the animals were sacrificed under ether anesthesia, and 5 ml of ice-cold saline was injected into the pouch. The pouch was gently massaged for a minute, cut open carefully, and any exudate collected. Total leukocyte count of lavage fluid was measured using an Erma Veterinary cell counter, from Japan). The percentage myeloperoxidase (MPO) activity was determined by adding 50 μ l sample/standard/50 mM phosphate buffer (pH 6) (blank) into the respective well. Then, 250 μ l of O-dianisidine hydrochloride 0.167 mg/ml in 50 mM phosphate buffer (pH 6) containing 0.0005% hydrogen peroxide) was added. The plate was read after 5 and 15 mins at 490 nm. After 15 mins incubation, 50 μ l 4 M H₂SO₄ was added to stop the reaction and readings were taken. Percentage MPO activity compared to control was calculated and presented as mean % MPO activity \pm S.E.M.^[34,35]

Statistical analysis

Statistical significance (*P*) was calculated by one-way ANOVA between AJM-treated and control groups, followed by Dunnett's post hoc test of significance. All data were expressed as mean \pm S.E.M.

RESULTS

AJM did not produce any death till 72 hour at 4000 mg/kg, p.o. and no apparent adverse symptoms were observed. Figure 1 shows the inhibitory effect of aqueous extract of AJM on carrageenan-induced paw edema in rats. Maximum phlogistic response of carrageenan was observed at 1–3 hours after the injection in the control animals. Aqueous extract of AJM at doses of 200 and 400 mg/kg significantly decreases paw volume (0.417 ± 0.097 and 0.379 ± 0.049) at 1–3 hours after induction of paw edema. In carrageenan-induced air pouch inflammation, extract reduced total leukocyte count (73.09 ± 7.13 and 62.17 ± 10.53), granulocyte count (69.48 ± 5.44 and 63.33 ± 4.13), and MPO activity (14.84 ± 0.91 and 18.44 ± 3.18) in comparison to the control (100), respectively [Figures 2–3].

DISCUSSION

Aqueous extracts of AJM were investigated for their antiinflammatory activity in two study models. AJM significantly reduced paw edema, during the second phase

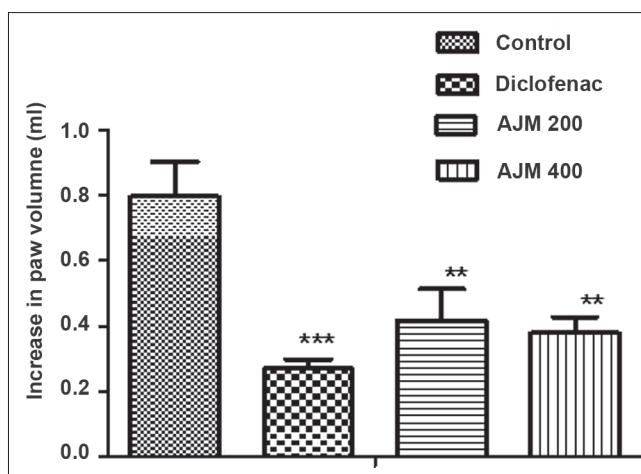


Figure 1: Inhibition of carrageenan-induced paw edema of rats by AJM. All results are expressed as mean \pm SEM, *n*=6. Results were analyzed by one-way ANOVA followed by post hoc Dunnett's test. ***P*<0.01 and ****P*<0.0001 as compared to carrageenan control

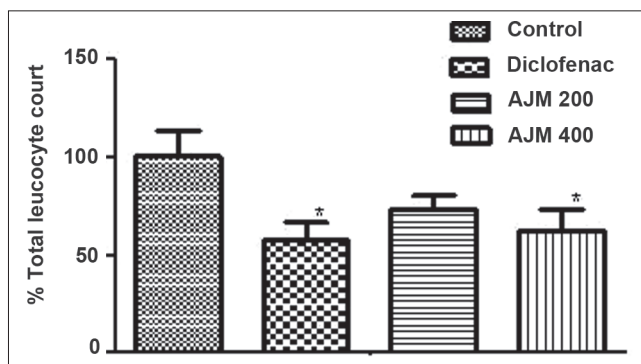


Figure 2: Effect of AJM on the infiltration of leukocytes in the carrageenan-induced air pouch inflammation in rats. Percentage leukocyte count with respect to normal control was calculated. Results were analyzed by one-way ANOVA followed by post hoc Dunnett's test. **P*<0.05 as compared to carrageenan control

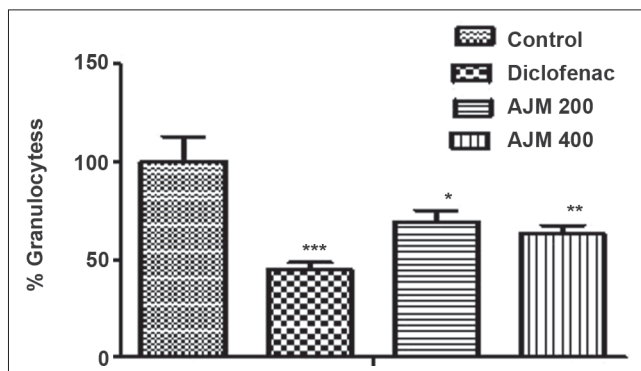


Figure 3: Effect of AJM on the infiltration of granulocytes in the carrageenan-induced air pouch inflammation in rats. Percentage leukocyte count with respect to normal control was calculated. Results were analyzed by one-way ANOVA followed by post hoc Dunnett's test. **P*<0.05, ***P*<0.01, and ****P*<0.0001, compared to controls

of edema development, between 1 and 4 hours, suggesting an inhibitory response in prostaglandin-mediated

inflammatory pathways.^[36,37] In the carrageenan-induced air pouch model, AJM inhibited cellular infiltration (neutrophils and granulocytes) into the air pouch fluid. Also, MPO from the neutrophils' azurophilic granules, is responsible for invoking tissue damage.^[38] Our results indicate that AJM has considerable therapeutic potential as an inhibitor of MPO-mediated tissue injury. This study suggests that AJM is a promising, plant-based, anti-inflammatory agent, for treatment of inflammatory disorders and conditions. Its anti-inflammatory activity on humans under clinical conditions should now be evaluated.

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