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, anticancer, immunomodulatory, antiviral, and gastroprotective activities.[13] Plumbago zeylanica is reported to possess antioxidant and anticancer activities. Piper longum exhibits antioxidant, antiallergic, antidiabetic, antifungal, immunomodulatory, and hypolipidemic activities. Piper longum has been reported to possess antioxidant, antidiabetic, antioxidant, and hepatoprotective activities.
insecticidal, and diuretic properties.\textsuperscript{[27]} *Piper nigrum* is said to possess antioxidant, antiplatelet, antihypertensive, antitumor, antithyroid, antiasthmatic, and hepatoprotective activities.\textsuperscript{[28]} *Terminalia chebula* exhibits antiviral, antibacterial, antioxidant, antibacterial, radioprotective, wound healing, and immunomodulatory activities.\textsuperscript{[29]} *Argyreia nervosa* is used in wound healing, rheumatic disorders, leukorrhoea, ulcer, cancer, and syphilis; also as a diuretic and to prevent contraception.\textsuperscript{[30]} *Zingiber officinale* exhibits immunomodulatory, antitumorigenic, antiinflammatory, antiapoptotic, antihyperglycemic, antilipidemic, and antiemetic actions.\textsuperscript{[30]} In addition, the combination, *Piper longum*, *Piper nigrum*, and *Zingiber officinale*, known in Ayurveda as Trikatu, is a significant bioenhancer.\textsuperscript{[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.\textsuperscript{[19]} All the ingredients were powdered separately, passed through a 80 nº 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 desiccator 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 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 1/10\textsuperscript{th} and 1/20\textsuperscript{th} of the maximum tolerated dose.

**Carrageenan-induced hind paw edema in rats**

The acute antiinflammatory effect was evaluated by carrageenan-induced hind paw edema.\textsuperscript{[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 administrated orally 1 hour before injection of carrageenan. Rats’ paw volumes were measured by digital Plethysmometer at hours 0, 3, and 5, after injection.\textsuperscript{[33]}

**Carrageenan-induced air pouch inflammation in rats**

On day 1, the rat’s dorsal sides were shaved (1 cm\textsuperscript{2}) under ether anesthesia and disinfected, then air cavities were produced by subcutaneous injection of 20 ml of sterile

| Table 1: Ingredients of *Ajmodadi churna* |
|------------------------------------------|
| **Ingredients** | **Parts** | **Quantity** (gm) | **Voucher specimen no.** |
| Ajmoda (*Trachyspermum ammi*) | Fruit | 12 | PP 5 |
| Vidanga (*Embelia ribes*) | Fruit | 12 | PP 576 |
| Saindhava lavana (*Rock Salt*) | Salt | 12 | - |
| Devdaru (*Cedrus deodara*) | Wood | 12 | PP 577 |
| Chitraka (*Plumbago zeylanica*) | Aerial parts | 12 | PP 578 |
| Pipalimula (*Piper longum*) | Stem | 12 | PP 575 |
| Satapuspa (*Anethum graveolens*) | Fruit | 12 | PP 579 |
| Pipali (*Piper longum*) | Fruit | 12 | PP 7 |
| Marica (*Piper nigrum*) | Fruit | 12 | PP 3 |
| Pathya (*Terminalia chebula*) | Fruit | 60 | PP 531A |
| Vrddhadaruka (*Argyreia nervosa*) | Root | 120 | PP 580 |
| Nagara (*Zingiber officinale*) | Rhizome | 120 | PP 2 |
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 μl sample/standard/50 mM phosphate buffer (pH 6) (blank) into the respective well. Then, 250 μl 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 μl 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±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±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 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, antiinflammatory agent, for treatment of inflammatory disorders and conditions. Its antiinflammatory activity on humans under clinical conditions should now be evaluated.

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**References**

1. Weiss U. Inflammation. Nature 2008;454:427.
2. Vodovotz Y, Ceete M, Bartels J, Chang S, An G. Translational systems biology of inflammation. PLoS Comput Biol 2008;4: e1000014.
3. Lucas SM, Rothwell NJ, Gibson RM. The role of inflammation in CNS injury and disease. Br J Pharmacol 2006;147:S323-40.
4. Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of medicinal plants and natural products. Indian J Pharmacol 2000;32:581-118.
5. Anonymous. The Ayurvedic Formulary of India. Part 1, 2nd revised ed. Delhi: Controller of publications, Under Ministry of Health and Family Welfare, Govt of India; 2004.
6. Kulkarni PH. Bronchial asthma care in Ayurveda and holistic systems, 119 Indian medical science series. Delhi: Sri Satguru Publications; 2001.
7. Thangam C, Dhananjayan R. Anti-inflammatory potential of the seeds of Carum copticum Linn. Indian J Pharmacol 2003;35:388-91.
8. Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Saraf MN. Studies on the anti-inflammatory and analgesic activity of Cedrus deodara (Roxb.) Loud. Wood oil. J Ethnopharmacol 1999;65:21-7.
9. Mujumdar AM, Dhuley JN, Deshmukh VK, Naik SR. Antiinflammatory activity of piperine. Jpn J Med Sci Biol 1990;43:95-100.
10. Chatterpadhyay RR, Bhattacharyya SK. Terminalia chebula: A potential anti-infective and anti-inflammatory agent. Proc Soc Exp Biol Med 1962;111:544-7.
11. Modi AJ, Khadabadi SS, Farooqui A, Bhutada VS. Anti-Inflammatory activity of leaves of Argyreia nervosa in carrageenan-induced paw edema in rats. Phcog J 2010;2:229-32.
12. Thomson M, Al-Qattan KK, Al-Sawan SM, Alnagheeb MA, Khan I, Ali M, The use of ginger (Zingiber officinale Rosc.) as a potential anti-inflammatory and antithrombotic agent. Prostaglandins Leukot Essent Fatty Acid 2002;67:475-8.
13. Qureshi AA, Kumar KE. Phytochemical constituents and pharmacological activities of Trachyspermum ammi. Plant Archives 2010;10:955-9.
14. Ambati S, Jyothis V, Jyothis AV. Pharmacological, pharmacoagenic and phytochemical review of Embelia ribes. International Journal of Pharmacy and Technology 2010;2:525-39.
15. Tiwari AK, Srinivas PV. Free radical scavenging active components from Cedrus deodara. J Agric Food Chem 2001;49:4642-5.
16. Shashi B, Singh J, Rao JM, Saxena AK, Qazi GN. A novel lignan composition from Cedrus deodara induces apoptosis and early nitric oxide generation in human leukemia Molt-4 and HL-60 cells. Nitric Oxide 2006;14:72-88.
17. Nguyen AT, Malonne H, Duez P, Fastre R, Vanhaelen M, Fontaine J. Cytotoxic constituents from Plumbago zeylanica. Fitoterapia 2004;75:500-4.
18. Powolny AA, Singh SV. Plumbagin-induced apoptosis in human prostate cancer cells is associated with modulation of cellular redox status and generation of reactive oxygen species. Pharm Res 2008;25:2171-80.
19. Wang CC, Chiang YM, Sung SC, Hsu YL, Chang JK, Kuo PL. Plumbagin induces cell cycle arrest and apoptosis through reactive oxygen species/cJun N-terminal kinase pathways in human melanoma A375. S2 cells. Cancer Lett 2008;259:82-9.
20. Didry N, Dubreuil L, Trotin F, Pinkas M. Antimicrobial activity of aerial parts of Drosera peltata Smith on oral bacteria. J Ethnopharmacol 1998;60:91-6.
21. Ding YK, Chen ZJ, Liu SG, Che DN, Vetter M, Chang CH. Inhibition of Nox-4 activity by plumbagin, a plant-derived bioactive naphthoquinone. J Pharm Pharmacol 2005;57:111-6.
22. Hazra B, Sarkar R, Bhattacharyya S, Ghosh PK, Chel G, Dinda B. Synthesis of plumbagin derivatives and their inhibitory activities against Ehrlich ascites carcinoma and Leishmania donovani promastigotes in vitro. Phytother Res 2002;16:133-7.
23. Krishnaswamy M, Purushothaman KK. Plumbagin: A study of its anticancer, antifungal activities. Indian J Exp Biol 1980;18:876-7.
24. Olagunju JA, Jobi AA, Oyedappo O. An investigation into biochemical basis of the observed hyperglycaemia in rats treated with ethanol extract of P. zeylanica. Phytother Res 1999;13:346-8.
25. Sharma I, Gussain D, Dixit VP. Hypolipidaemic and antiatherosclerotic effect of plumbagin in rabbits. Indian J Physiol Pharmacol 1991;35:10-4.
26. Zaveri M, Khandhar A, Patel S, Patel A. Chemistry and pharmacology of Piper longum L. International Journal of Pharmaceutical Sciences Review and Research 2010;5:67-76.
27. Kaur GJ, Arora DS. Bioactive potential of Anethum graveolens, Foeniculum vulgare and Trachyspermum ammi belonging to the family Umbelliferae - Current status. J Med Plant Res 2010;4:87-94.
28. Singh A, Duggal S. Piperine-Review of Advances in Pharmacology. Int J Pharm Sci Nanotechnol 2008;2:615-20.
29. Krishnaveni A, Rani TS. Phyto-pharmacological review of Argynie nervosa. Int Res J Pharm 2011;2:28-31.
30. Ali BH, Blunden G, Tanira MO, Nemmar A. Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Rosc): A review of recent research. Food Chem Toxicol 2008;46:409-20.
31. Atal N, Bedi KL. Bioenhancers: Revolutionary concept to market. J Ayurveda Integr Med 2010;1:96-9.
32. Winter CA, Risley EA, Nauss GW. Carrageenan induced edema in hind paw of rats as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med 1962;111:544-7.
33. Ramprasad VR, Shanthi P, Sachandanadam P. Anti-inflammatory effect of Semecarpus anacardium Linn. Nut extract in acute and chronic inflammatory conditions. Biol Pharm Bull 2004;27:2028-31.
34. Ellis L, Gilston V, Soo CC, Morris CJ, Kidd BL, Winyard PG. Activation of the transcription factor NF-xB in the rat air pouch model of inflammation. Ann Rheum Dis 2000;4:303-7.
35. Whiteley PE, Dalrymple SA. Models of inflammation: Carrageenan air pouch in the rat. Curr Protoc Pharmacol 1998;561-566.
36. Garcia-Pastor P, Randazzo A, Gomez-Paloma L, Alcaraz MJ, Paya M. Effects of petrosaspongolide M, a novel phospholipase A2 inhibitor, on acute and chronic inflammation. J Pharmacol Exp Ther 1999;289:166-72.

37. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenan oedema in rats. J Pharmacol Exp Ther 1969;166:96-103.

38. Koelsch M, Mallak R, Graham GG, Kajer T, Milligan MK, Nguyen LQ, et al. Acetaminophen (paracetamol) inhibits myeloperoxidase-catalyzed oxidant production and biological damage at therapeutically achievable concentrations. Biochem Pharmacol 2010;79:1156-64.

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