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Amelioration of FCA induced arthritis on topical application of curcumin in combination with emu oil

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ABSTRACT

Objective: The aim of the present study was to investigate the skin penetration potential of emu oil and the possibility of enhancing the antiarthritic potential of lipophilic bioactive curcumin, which has poor permeability through biological membranes.
Methods: Solubility and ex vivo skin permeation studies were performed with water, corn oil, and emu oil as a vehicle using curcumin as a model drug. Carrageenan induced inflammation and Freund's complete adjuvant–induced arthritic rat models were used to evaluate enhanced anti-inflammatory and antiarthritic effect of curcumin in combination of emu oil via topical route.
Results: The skin permeation study resulted in the combination of emu oil with curcumin enhancing the flux 1.84 and 4.25 times through the rat skin compared to corn oil and water, respectively. Results of carrageenan induced rat paw edema model demonstrated that percentage of paw inhibition shown by curcumin–emu oil combination was 1.42-fold more compared to the total effect shown by both groups treated with curcumin aqueous suspension and emu oil per se. In Freund's complete adjuvant–induced arthritic model, the combined treatment was effective in bringing significant changes in the functional, biochemical, histopathologic, and radiologic parameters. Topical application of curcumin–emu oil combination resulted in significant reduced levels of proinflammatory mediators TNF-α, IL-1 β, and IL-6 ($P < 0.05$, 0.001, and 0.01, respectively) compared to arthritic animals.
Conclusion: Topical delivery of curcumin with emu oil holds promise as a noninvasive and efficacious intervention for the treatment of inflammatory arthritis and it assists in further development of a topical formulation of curcumin using emu oil as a vehicle.

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease that affects the synovial joints and is typically characterized by cartilage loss, synovial hyperplasia, and joint damage causing pain, incapacitation, and disability [1]. Currently available drugs for treatment of rheumatoid arthritis include analgesics, steroids, NSAIDS, DMARDS, and immunosuppressive agents, which have potential side effects such as gastrointestinal tract disturbances, low blood cell count, hair loss, and immunodeficiency. Hence, there is a need for newer drugs, which can offer effective treatment with fewer side effects, high tolerance, and relatively inexpensive. Thus, natural phytonutrients could serve as a better

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alternative strategy for the effective treatment of rheumatoid arthritis [2].

Curcumin is the bioactive component present in curry spice *Curcuma longa* Linn, (turmeric), which is a household remedy from kitchen and traditional indigenous medicine known for its antiinflammatory and antioxidant properties from ancient period [3]. In light of the long and established experience with curcumin as food stuff and as a natural medicine in humans, its low cost, proven chemopreventive and therapeutic potential, and its pharmacologic safety, curcumin can be an alternative medicine for the treatment of chronic inflammatory diseases including rheumatoid arthritis [2]. However, this polyphenol has not yet been approved for human use. Poor bioavailability of curcumin is a major limitation that affects its therapeutic utility. The main culprit behind the poor bioavailability of curcumin is its poor aqueous solubility, poor membrane permeability, degradation, rapid metabolism, and elimination [4].

Emu oil, which is obtained mainly from subcutaneous and retroperitoneal fat of the Emu (*Dromaius novaehollandiae)*, is extensively used by Australian aborigines as powerful and well-established traditional functional food [5]. Pegg et al. demonstrated a potential for the emu meat snack to be considered as a functional food for athletes due to high amount of creatine coupled with its favorable fatty acid profile (high in polyunsaturated phospholipids) [6]. Emu oil is also recognized for having a relatively low level of saturated fatty acids and is rich in omega fatty acids with an impressive amount of natural antioxidants like carotenoids, flavones, polyphenols, phospholipids, and fat soluble vitamins D and E [6]. Various studies showing the potent antiinflammatory properties of emu oil when used topically and orally in various diseases like mucositis, inflammatory bowel syndrome auricular inflammation, and chemotherapy induced bone loss have been summarized previously by Jeengar et al. [5]. In our previous study, we have reported that orally administered emu oil in combination with curcumin enhanced the oral bioavailability of curcumin and potentiated its antiinflammatory effect in rat model of adjuvant induced arthritis. As mentioned above, the major problem associated with curcumin delivered via oral route is its high first pass metabolism. It is also claimed in many online blogs that emu oil possesses excellent skin penetration property, but there is no scientific report for the above claim [7]. Therefore, present study has been undertaken to prove skin permeation property of emu oil using curcumin as a model drug, as skin is an important target site for the topical application of drugs. A topical drug delivery is an appropriate strategy to increase patient compliance, avoidance of first pass metabolism by the liver, and the possibility to reduce the dose of the drug [8]. Hence, we hypothesized that emu oil can be combined with curcumin to improve its permeation through topical route as well as therapeutic efficacy. This manuscript also deals with the comparison of the antiarthritic effects of topically applied curcumin–emu oil combination versus its oral administration.

Materials and methods

Chemicals

Curcumin, corn oil, and reagents such as carrageenan, thiobarbituric acid (TBA), glutathione reductase, catalase, myloperoxidase (MPO), and TRI reagent were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). Emu oil was a kind gift sample from Dr. R.B.N. Prasad, Lipid Science and Technology Division, IICT, Hyderabad [9]. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) enzyme kits were procured from Siemens AG (Berlin, Germany). *Mycobacterium tuberculosis* DES H37 RA powder and Freund's incomplete adjuvant were purchased from DIFCO Laboratories (Detroit MI, USA). Verso cDNA synthesis kit and SYBR green master mix were purchased from Thermo-Fisher Scientific (Waltham, MA, USA). All other chemicals and reagents used were of analytical grade.

Animals used

Male Sprague-Dawley (SD) rats (150–170 g) were procured from the Teena Biolabs, Hyderabad, India, and acclimated for 1 wk under standard laboratory conditions at a temperature of 23 ± 2°C with 12 h light–dark cycle and relative humidity 40–70%. All procedures described were reviewed and approved by the Institutional Animal Ethics Committee (IAEC), NIPER Hyderabad, India (approval no. NIP/08/2012/PC/12).

Physical compatibility tests

Physical compatibility between curcumin and emu oil was evaluated by physical observation, UV-visible spectroscopy, and FTIR spectra studies.

Solubility studies

The solubility studies of curcumin were carried out in different vehicle like water, corn oil, and emu oil in triplicate according to previously reported method [10].

Skin penetration studies

To determine the permeability of curcumin across the skin, diffusion cells were used having a diffusion area of 3.79 cm^2 and a receptor volume of 25 mL. The abdominal skin of male SD rats was used for skin permeation studies. The receptor compartment was filled with 30% v/v isopropyl alcohol (IPA) in phosphate buffer pH 6.5 maintained at 37 ± 1°C with the help of water circulation [10]. The solution was under constant stirring (600 rpm) by using magnetic stirrer. The donor compartment contained 1 mL of 1 mg/mL of curcumin with emu oil, 1 mg/mL of curcumin with corn oil, and curcumin suspended in 0.5% carboxymethyl cellulose (CMC). Approximately 1 mL samples were withdrawn at different time intervals (0, 1, 2, 4, 6, 8, 10, 12, and 24 h) and were immediately replaced with an equal volume of receptor solution to maintain a constant volume. The samples were analyzed at 420 nm using microplate reader (Spectramax M4, Molecular Devices, Sunnyvale, CA, USA). All the experiments were performed in triplicate. Cumulative amount of drug permeated per unit area of skin was plotted against time. The slope of the linear portion of the plot was calculated as permeation rate of curcumin at steady state (flux Jss, μg/cm^2/h). Permeability coefficient coefficient (K_p) and enhancement ratio (ER) of drug penetration were calculated by using the following equations [11].

$$Kp = \frac{J_{ss}}{C_0}$$

Where, J_{ss} is the flux and C_0 is the initial concentration of curcumin in donor compartment.

$$ER1 = \frac{\text{Flux from curcumin combination with oils}}{\text{Flux from curcumin aqueous suspension}}$$

$$ER2 = \frac{\text{Flux from curcumin–emu oil combination}}{\text{Flux from curcumin aqueous suspension}}$$

ER1 indicates enhancement in permeation of curcumin when compared to water as vehicle, while ER2 is the enhancement of permeation of curcumin by emu oil when compared to corn oil.

Carrageenan induced paw edema model

Male SD rats were trimmed to expose approximately 6 cm^2 of dorsal skin the day before the experiment. Overnight fasted rats weighing between 180 and 200 g were randomly selected and divided into five groups, each consisting of six animals as follows. Group I: Inflammation control; Group II: Standard (STD) drug–treated, diclofenac gel (100 mg of 1% gel/rat); Group III: Curcumin aqueous suspension–treated (1 mL of 1 mg/mL suspension/rat); Group IV: Emu oil per se (EO, 1 mL/rat); Group V: Treated with curcumin–emu oil combination (CE, 1 mL of 1 mg/mL curcumin in emu oil/rat).

Standard and test groups received respective test doses 1 h before the carrageenan administration. Test combinations were applied at the shaved dorsal surface of the body (0.8 mL) and on the affected paw (0.2 mL) by gently rubbing with index finger. Similarly, standard group received 80 mg of diclofenac gel on dorsal surface and 20 mg on affected hind paw. Rats in all the groups were challenged with 0.1 mL of (1% w/v) carrageenan in PBS into the sub plantar region of right hind paw. Paw volumes were measured before and after 4 h following the carrageenan administration using digital plethysmometer (Panlab, Barcelona,