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Ameliorative effect of Koflet formulations against pyridine-induced pharyngitis in rats

G.L. Viswanatha [a], Mohamed Rafiq [a,*], A.H.M. Thippeswamy [a], H.C. Yuvaraj [a], K.J. Kavya [a], Mirza Rizwan Baig [a], D.A. Suryakanth [a], Mohammed Azeemuddin [a], P.S. Patki [b], H.B. Pushpalatha [c], Prafulla S. Chaudhari [c], Ramakrishnan Shyam [d]

[a] Department of Pharmacology, R&D Center, The Himalaya Drug Company, Bangalore 562 162, India
[b] Head-Medical Services & Clinical Trials, R&D Center, The Himalaya Drug Company, Bangalore 562 162, India
[c] Formulation & Development, R&D Center, The Himalaya Drug Company, Bangalore 562 162, India
[d] Chief Scientific Officer, R&D Center, The Himalaya Drug Company, Bangalore 562 162, India

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ABSTRACT

In present study two formulations of Koflet (syrup and lozenges) were evaluated against pyridine-induced pharyngitis in rats. Topical application of 10% pyridine showed extravasation of Evans blue stain as a characteristic feature of on-going inflammation. In addition, the levels of TNF-α ($p < 0.01$) and IL-6 ($p < 0.01$) were significantly increased compared to control. Further, histopathology of the pharyngeal tissue showed submucosal gland hypertrophy, severe mucosal inflammation characterized by presence of mononuclear cells and neutrophils along with haemorrhages and congestion; however, saline applied animals (normal control) showed normal cytoarchitecture of the pharynx. Interestingly, pretreatment with dexamethasone (1 mg/kg, p.o.), Koflet lozenges (KL) (500 and 1000 mg/kg, p.o.) and Koflet syrup (KS) (2 and 4 ml/kg, p.o.) for 7 days showed significant and dose dependent protection by decreasing the EB dye extravasation, and serum levels of TNF-α and IL-6. In addition, histopathological findings have further supported the protective effect of Koflet formulations. These findings suggest that, both Koflet syrup and Koflet lozenges are highly effective in treating non-infectious type of pharyngitis. Among the two formulations KS was found to be more potent than KL, and possible mechanism of action thought to be mediating through inhibition of TNF-α and/or phospholipids–arachidonic acid pathway.

1. Introduction

The inflammation of the mucus membrane of pharynx is termed as pharyngitis, commonly known as sore throat [1], it is the most common and frequent among the upper respiratory tract diseases, which is accompanied by fever and/or cough [2]. In United states, acute pharyngitis accounts for about 1–2% of overall visits to the outpatient departments (OPD) and emergency departments [3]. Pharyngitis is known to be commonly associated with symptoms such as hoarseness, sore throat, cough, pain, difficulty in swallowing, airway obstruction, due to pathologic features like mucosal inflammation and submucosal oedema [4]. The frequent causes of pharyngitis is mainly due to infections associated with virus, bacteria

* Corresponding author at: Department of Pharmacology, R&D Center, The Himalaya Drug Company, Makali, Bangalore 562 162, Karnataka, India. Tel.: +91 80 23714444, +91 9844108957; fax: +91 80 23714471.
E-mail addresses: glv_000@yahoo.com (G.L. Viswanatha), dr.rafiq@himalayahealthcare.com (M. Rafiq).

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and rarely due to candidal, fungal and parasites (infectious pharyngitis) [5], apart from infectious causes tracheal intubation during medical procedures, smoking, snoring, shouting, drugs such as ACE inhibitors, chemotherapy, corticosteroids, exposure to pesticides and environmental factors such as pollution, temperature, humidity/air conditioning are the non-infectious causative factors (non-infectious pharyngitis) [6,7]. Additionally, the diseases such as GERD (gastroesophasengeal reflux disease), thyroiditis are well known to cause non-infectious type of pharyngitis [8,9].

In spite of many available treatment strategies for pharyngitis, the side/adverse effects associated with them always made the scientists to think about the better, safe medicine. However, currently there is a lack of rigorous trials (both preclinical and clinical) for the treatment of non-infectious pharyngitis, one of the important factor hampering the efforts in identifying the effective new treatments is the lack of a suitable animal model for non-infectious pharyngitis [5]. In this context, we have developed a novel animal model for non-infectious pharyngitis in rats using pyridine as an inducer [10], it was found to be useful in screening the beneficial effect of synthetic, plant based medicines in treating non-infectious pharyngitis. In continuation, the present study was aimed to evaluate Koflet syrup, Koflet lozenges against pyridine-induced pharyngitis in rats.

2. Materials and methods

2.1. Drugs and chemicals

Pyridine (SD Fine chemicals, Bangalore), Dexamethasone (Zydus Cadila Healthcare Ltd., Mumbai), Koflet syrup (The Himalaya Drug Company, Bangalore), Koflet lozenges (The Himalaya Drug Company, Bangalore), TNF-α and IL-6 ELISA Kits (Krishgen Biosystems, Mumbai) were used for the study, other solvents and chemicals used were highly pure and of analytical grade purchased from HiMedia Laboratories Pvt. Limited, India.

2.2. Experimental animals

Inbred Wistar rats (250–300 g) were used for the study. The animals were maintained in polypropylene cages at a temperature of 25 °C ± 1 °C and relative humidity of 45–55% in a clean environment under 12:12 h light–dark cycle. The animals had free access to food pellets (Pranav Agro Industry, Bangalore, India) and purified water.

All the experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) of The Himalaya Drug Company and were conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA), India.

2.3. Experimental protocol

2.3.1. Grouping and treatment schedule

Wistar rats (250–300 g) were divided into seven groups (G-I to G-VII, $n = 10$), G-I and G-II served as normal control and positive control; G-III served as standard and received dexamethasone (1 mg/kg, p.o.), G-IV and V have received 2 and 4 ml/kg, p.o., doses of Koflet syrup, while G-VI and VII have received Koflet lozenges at 500 and 1000 mg/kg, p.o. doses respectively for 7 days.

2.3.2. Induction of pharyngitis

On seventh day after administration of last dose of assigned treatments, EB dye (30 mg/kg, i.v.) was administered to all the animals via lateral tile vein. Ten minutes after the administration of EB dye, 10% pyridine was applied to the pharyngeal mucosa. In short, The tongue was slightly pulled out and pharynx area was opened deep into the oral cavity with the help of blunt forceps and the pyridine was applied with the help of cotton swab, gently for 5 s at each time point, for three times (approximately 50 μl). For G-I saline solution was applied similarly, since the pyridine solution was prepared in saline [10].

2.3.3. Induction of pharyngitis

After 60 min of pyridine/saline application, all the animals were sacrificed by exsanguination and the head portion was perfused with heparinised saline (40 IU/ml) to expel the intravascular EB dye. Then, the bilateral musculus masseter of the rat was incised and the lower jaw was removed to enable the extirpation of the pharynx. The portion of pharynx ranging from the caudal end of the soft palate to the epiglottis was isolated and weighed (approximately 40–50 mg).

The EB dye in the tissue was extracted in formamide at 55 °C for 24 h and determined spectrophotometrically at 620 nm, the tissue dye content was expressed as microgram of dye per gram of wet weight of the tissue (μg/g). Parallel to these experiments another set of experiments were run without administration of EB dye and the tissue samples collected were subjected to histopathological evaluation.

The blood samples were collected and serum was separated for the estimation of proinflammatory cytokines such as tumour necrosis factor alpha (TNF-α) and interleukin-6 (IL-6). These estimations were performed as per the user manual provided along with the respective ELISA Kits (Krishgen Biosystems, Mumbai, India).

3. Results

The polyherbal formulations, Koflet syrup and Koflet lozenges manufactured by Ms. The Himalaya Drug Company, Bangalore are well known for their beneficial effect in the treatment of pharyngitis and other upper respiratory tract diseases. The test formulations used in the present study (Koflet syrup and Koflet lozenges) are well known for the treatment of both infectious and non-infectious types of pharyngitis. However, there is a lack of scientific evidence related to their beneficial effect with respect to non-infectious type of pharyngitis. Incidentally, there is a paucity of scientific literature and reports related to screening models for non-infectious type of pharyngitis, hence in our previous study we have standardized a novel experimental animal model for non-infectious type of pharyngitis in rat using pyridine as a inducer [10]. In the present study, we have evaluated KS (2 and 4 ml/kg, p.o.)