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Extraction, characterization and evaluation of *Kaempferia galanga* L. (Zingiberaceae) rhizome extracts against acute and chronic inflammation in rats

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A R T I C L E I N F O

Chemical compounds studied in this article:
Diclofenac (PubChem CID: 5018304)
Ethyl p-methoxycinnamate (PubChem CID: 5281783)

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A B S T R A C T

Ethnopharmacological relevance: The rhizomes of an acaulescent perennial herb, *Kaempferia galanga* Linn (Family: Zingiberaceae), used as traditional ayurvedic herb to get relief from indigestion, swelling, pain, high blood pressure and dyslipidemia.

Aim of the study: To prepare and characterize various extracts of *Kaempferia galanga (K. galanga)* for their comparative evaluation for the identification of the most efficacious extract and its possible pharmacological implication in acute and chronic inflammatory paradigm.

Materials and methods: Dried and powdered rhizome of *K. galanga* was subjected to alcoholic extraction as well as successive extractions with various solvents. After phytochemical characterization, all the extracts were standardized for the presence of ethyl-*p*-methoxycinnamate. The extracts, and the isolated compound, were tested against carrageenan-induced acute inflammation in rats. The most promising extract was tested against adjuvant-induced chronic inflammation in rats. Further, local myeloperoxidase (MPO) levels were investigated to establish the possible mechanism of action.

Results: Among the extracts, petroleum ether extract (SKG-1) and crude alcoholic extract (KG) had the maximum quantity of ethyl-*p*-methoxycinnamate. SKG-1 (300 mg/kg) was found effective against acute inflammation in rats. Further, SKG-1 (100 mg/kg) reversed the inflammation and elevated MPO levels found in the chronic model.

Conclusions: The results suggest that among all the extracts of *K. galanga*, SKG-1 effectively suppresses the progression of acute and chronic inflammation in rats by inhibition of neutrophil infiltration.

1. Introduction

Inflammatory response to the tissue stress and/or injury, involves the migration of immune cells to protect the tissue from further damage. Persistent injury results in unresolved inflammation, which progresses into chronic stage leading to inflammatory disorders. Inflammation has been studied universally in an attempt to combat its deleterious effects on the human body. Though, inflammation plays a central role in the pathogenesis of various disorders such as rheumatoid arthritis, cancer, diabetes, obesity, cardiovascular complications etc., its impact and role in the pathogenesis may vary from disease to disease (Kotas and Medzhitov, 2015).

Kaempferia galanga L. (*K. galanga*) family Zingiberaceae, is an acaulescent perennial plant that grows in southern China, Indo-China, Malaysia, India and Bangladesh (Kanjanapothi et al., 2004). The rhizome of this plant has been used traditionally for the treatment of a variety of disorders. The rhizome is rich in essential oils and is used traditionally for the treatment of indigestion, cold, pectoral and abdominal pain, headache, and as expectorant, diuretic and carminative (Achuthan and Padikkala, 1997). Also, the cytotoxic (Jagadish et al., 2010), antihypertensive (Othman et al., 2002), hypolipidemic (Achuthan and Padikkala, 1997) and larvicidal activity (Choochote et al., 1999) of the rhizome have been reported.

Apart from the above traditional uses, *K. galanga* extracts are used for the treatment of swelling (Mitra et al., 2007; Umar et al., 2011). A recent report demonstrated the acute anti-inflammatory effect of *K.*

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galanga rhizome extract plaster (Riasari et al., 2016). Anti-nociceptive potential of *K. galanga* leaves' aqueous extract in acute rodent model of inflammation and pain (Sulaiman et al., 2008) was documented. Similarly, acute anti-nociceptive effect was elicited by *K. galanga* whole plants' (including rhizomes and aerial parts) methanolic extract (Ridtitid et al., 2008). Particularly, the alcoholic extract obtained from *K. galanga* rhizome extract showed significant anti-inflammatory effect against acute and sub-acute inflammation in rats (Vittalrao et al., 2011).

Based upon the above literatures, it is justified to explore the effect of *K. galanga* rhizome extract on chronic inflammatory condition, which has not been investigated till date. Also, recent report compared the acute anti-inflammatory effect of various extracts of *K. galanga* (2 g/kg) obtained after serial extraction process (Umar et al., 2012). However, these finding were obtained at 2 g/kg oral dose against the acute inflammatory condition. Thus, the present work was aimed at comparing crude alcoholic and successive extracts of rhizome of *K. galanga* to find the most efficacious extract at lower than already tested doses. Further, the study was aimed at exploring the effect of *K. galanga* in a chronic inflammatory condition similar to rheumatoid arthritis.

2. Materials and methods

2.1. Plant material

The rhizome of *K. galanga* was obtained from Abirami Botanicals, Tuticorin, Tamilnadu, India and authenticated by G K Bhat, Professor of Botany (Rtd), Udupi, India. A voucher specimen (No.: PP614) has been retained for future reference.

2.2. Extraction process

2.2.1. Successive extraction

As reported earlier (Jagadish et al., 2010), the crushed pieces of shade-dried rhizomes were coarsely powdered (2 kg) and extracted using a soxhlet extractor for 18–20 h successively with petroleum ether (60–80 °C), ethyl acetate and ethanol. Using a rotary vacuum evaporator under reduced pressure and controlled temperature (40–50 °C), the extracts were concentrated and dried. The scheme of extraction of various extracts has been provided as the Supplementary file-1.

2.2.2. Crude extraction

As per the previous method reported (Jagadish et al., 2010), the powdered rhizomes (1 kg) were also subjected to extraction with ethanol in a soxhlet extractor for 18–20 h. Using a rotary vacuum evaporator under reduced pressure and controlled temperature (40–50 °C), the extracts were concentrated and dried (Supplementary file-1).

In our study, successive petroleum ether, ethyl acetate and alcohol extracts were designated as SKG-1, SKG-2 and SKG-3, respectively. Similarly, the crude alcoholic extract designated as KG and isolated compound was named ISO-2. These four extracts of *K. galanga* were subjected to phytochemical analysis (Table 1) as per standard protocols (Harborne et al., 1975; Kokate et al., 1995).

2.2.3. Isolation method of ISO-2 from petroleum extract

For isolation of ISO-2, the petroleum ether extract was kept for concentration in desiccator at 22–25 °C. White crystalline compound deposited at the bottom of the vessel was observed. The crystals were separated, washed and re-crystallized using petroleum ether. The purity of the recrystallized compound was confirmed by thin layer chromatography (TLC) [Rf: 0.8 [$CHCl_3$: Pet. ether (95:5)].

Table 1
Phytochemical tests of *Kaempferia galanga* L rhizome extracts.

Phytochemicals	SKG-1	SKG-2	SKG-3	KG
Carbohydrates	–	–	–	–
Alkaloids	–	–	–	–
Glycosides	–	–	–	–
Flavonoids	–	+	+	+
Phenolics	–	+	+	+
Fixed oils	+	+	+	+
Saponins	–	–	–	+
Steroids and sterols	+	–	–	+
Tannins	–	+	–	+
Triterpenoids	+	+	–	+

+ and – signs indicate the presence or absence of respective phytochemical in the extracts.

2.2.4. Spectral characterization of ISO-2

ISO-2 was subjected to UV, IR, ^1H NMR, ^{13}C NMR and mass spectral analysis (using GC–MS and TOF ES MS (Time of flight electrospray mass spectrometer). ISO-2 was analysed using GC-MS-QP5050 spectrophotometer (Shimadzu GC-17A) using DB-5 capillary column (30×250 µm×0.25 µm) and helium as carrier gas. The GC oven temperature was programmed from 50–250 °C at rate of 5 °C min^{-1}.

2.3. Standardization of extract of K. galanga *rhizome*

Standardization of rhizome of *K. galanga* was carried out with ethyl-*p*-methoxycinnamate (99.2%, Wuhan Hezhong Biochemical Manufacture co. Ltd, China) by reverse phase high performance liquid chromatography (RP-HPLC). This method employed optimized chromatographic conditions using a Waters' HPLC 2695 separation module with 2487 dual absorbance UV detector. millennium version 4.0 data station was applied for data collection. A Supelco C_{18} column (25 cm×4.6 mm i.d., 5 µ) was used and the mobile phase of a mixture of acetonitrile: 20 mM ammonium acetate buffer pH 4.5 (30:70% v/v) was delivered at a flow rate of 1.0 ml/min with detection at 285 nm. The mobile phase was filtered through a 0.2 µm membrane and degassed before pumping. The injection volume was 50 µl. Analysis was performed at ambient temperature. The developed method was validated as per ICH Q2 (R1) guidelines.

2.4. In vivo screening methods

2.4.1. Animals

Male Sprague-Dawley rats (175–225 g), were inbred in Central Animal Research Facility, Manipal University, Manipal, Karnataka, India were used. These animals were kept in plastic cages at a temperature of 25 ± 0.5 °C with 12 h light: dark cycle and humidity of 50 ± 5% RH. Animals were fed standard food pellet and water ad libitum. The experimental protocols had the approval of the Institutional Animal Ethics Committee, Kasturba Medical College (No. IAEC/KMC/35/2009-10).

2.4.2. Treatment regimen and dose selection

λ-Carrageenan and complete Freund's adjuvant (Sigma-Aldrich Co. LLC, St. Louis, MO, USA) were used to induce acute and chronic inflammation in rats, respectively. The standard drug diclofenac (10/5 mg/kg, p.o.), extracts (SKG-1, SKG-2, SKG-3, KG; 300 mg/kg, p.o.), and the isolated compound (ISO-2; 100 mg/kg, p.o.) were suspended in 0.25% w/v carboxymethylcellulose (CMC) and administered at a dose volume of 5 ml/kg, p.o. to rats. Control group was administered with saline or respective vehicle. The rhizome extract of *K. galanga* has been reported to be safe up to 5000 mg/kg orally (Kanjanapothi et al., 2004; Ridtitid et al., 2008; Umar et al., 2012). In the present study, doses of extracts tested were based upon the previously published reports (Umar et al., 2012; Vittalrao et al., 2011).

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