




Ethnopharmacological communication

Modulation of inflammatory markers by the ethanolic extract of *Leucas aspera* in adjuvant arthritis

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Abstract

Aim of the study

To investigate the anti-inflammatory and antioxidant potential of ethanolic extract of *Leucas aspera* (EELA) in adjuvant arthritis.

Materials and methods

Complete Freund's adjuvant served to induce arthritis. EELA was administered in two doses along with vehicle control (0.1% carboxymethyl cellulose) and positive control (Diclofenac). Levels of tumour necrosis factor (TNF-) α , C-reactive protein (CRP), Interleukin-2 (IL-2), Cathepsin D, activities of antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) were estimated in plasma/hemolysate and tissue. HPLC analysis of EELA was also performed.

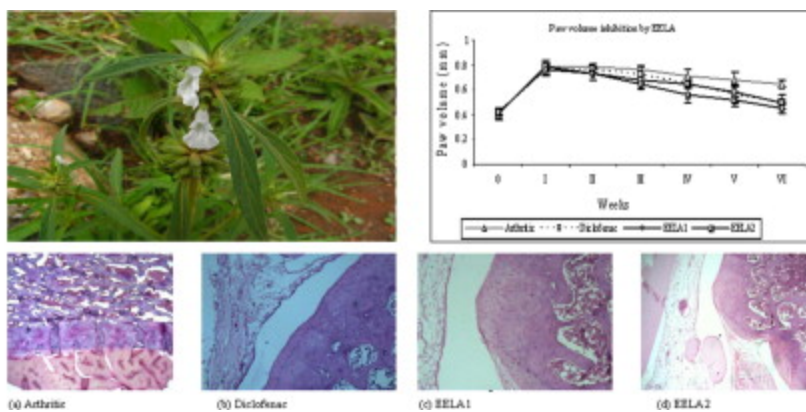
Results

EELA exhibited significant anti-inflammatory ($p < 0.001$) and antioxidant activity ($p < 0.001$). It did not show mortality up to 2000mg/kg body weight. Histopathological studies confirmed complete cartilage regeneration and near normal joint in EELA2 treated arthritic rats.

Conclusion

This study highlighted the antioxidant and anti-inflammatory potential of *Leucas aspera*. Three major families of compounds present in EELA may explain these activities: catechins (epicatechin, beta epicatechin), flavonoids (procyanidin), phytosterols (beta-sitosterol) apart from glycosides, phenolic compounds and tannins.

Graphical abstract



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Introduction

Free radicals and the associated oxidative stress have been implicated in the etiology of rheumatoid arthritis (RA). The pro-inflammatory cytokines released by arthritic joints activate neutrophils that play a key role in the pathogenesis of synovial inflammation. They generate ROS (reactive oxygen species) which cause the release of collagenases and elastases that contribute to cartilage destruction (Mythilipriya et al., 2007). Recently, there has been an increasing interest in free radical scavenging substances derived from herbs and the role of potential herbs in anti-arthritic therapy has been evaluated (Jiang and Qiang, 2003). *Leucas aspera* (thumbai) belonging to the family Labiateae, is an herbaceous annual

distributed throughout India. In traditional medicine, the leaves find their use in chronic rheumatism and juice for skin eruptions (Kirtikar and Basu, 1991). Literature reveals that extracts of *Leucas aspera* exhibit analgesic, anti-inflammatory and anti-pyretic efficacies (Saundane and Satyanarayanan, 2000). The anti-inflammatory activity of different fractions of *Leucas aspera* in cotton pellet and carrageenan induced paw edema has been proved (Goudgaon et al., 2003). Extracts prepared from root parts of the plant showed significant antinociceptive, antioxidant and cytotoxic activities (Rahman et al., 2007). Compounds isolated from *Leucas aspera* include a hydroxyl tetra triacontan-4-one, aliphatic ketones (Misra et al., 1993), nicotine (Mangathayaru et al., 2006b), alpha-farnesene, alpha-thujene, menthol from leaf volatiles and amyl propionate, isoamyl propionate from flower volatiles (Mangathayaru et al., 2006a). *In vitro* antioxidant activity guided fractionation of *Leucas aspera* yielded eight lignans and four flavonoids (Sadhu et al., 2003).

The present study focuses on the effect of *Leucas aspera* on adjuvant induced arthritic rats by analysing the markers of inflammation, antioxidant status and histological changes of knee joints. HPLC analysis was performed to detect the phenolic and bioflavonoid constituents of EELA.

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Section snippets

Drugs and chemicals

Complete Freund's adjuvant (CFA) was purchased from Sigma Chemical Company, USA. ELISA kits for TNF- α from Pierce Endogen, USA; CRP from Immunology Consultants Laboratory, USA and IL-2 from Bender Med Systems GmbH, Europe. Other chemicals used were of analytical grade.

Plant material

Aerial parts of the plant *Leucas aspera* were collected from Kanchipuram district, Tamilnadu, India and taxonomically identified by the Plant Anatomy Research Center, Chennai (PARC/2007/362).

In vitro antioxidant studies

In the antioxidant investigation, EELA showed maximum efficiency in scavenging DPPH, the stable free radical donor. The antioxidant activity expressed in terms of IC₅₀ value was found to be 929, 1114, 77.29 and 71.4 µg/ml for n-hexane, chloroform, ethyl acetate and ethanolic extracts. Since the antioxidant property of EELA was favourable, it was subjected to further studies.

HPLC analysis

HPLC analysis of EELA demonstrated the presence of EC (4.66 mg), β, 1-4 EC (1.98 mg), Pcy (2.78 mg) and beta-sitosterol (0.89

Conclusion

In the light of the above results, it might be concluded that EELA abates disease progression in RA. This anti-inflammatory and antioxidant potential might be attributed to the presence of EC, Pcy, phenolics and tannins in EELA apart from other bioflavonoids such as acacetin and apigenin present in the plant (Sadhu et al., 2003). These results justify the ethnomedicinal use of this plant in chronic rheumatism.

Acknowledgements

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2016, International Journal of Biological Macromolecules

Citation Excerpt :

...In rheumatoid arthritis (RA) patients, several oxidative stress markers are useful to evaluate the progression of disease and elucidate the pathogenesis of disease [25]. A large number of studies have well indicated that ROS plays an important part in pathogenesis and progression of OA and RA in experimental animal models [8,9,26,27]. H₂O₂ is regarded as a common form of ROS, and accumulation of H₂O₂ is involved in progression of OA....

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2014, Brain Research Bulletin

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...As far as we are aware, this is the first work to report that this plant is able to prevent these deleterious effects. Our promising results could be explained by GPx modulation, conducted by the occurrence of phenolic compounds in AE, as already reported (Kripa et al., 2011; Yuan et al., 2014). It is important to mention that GPx, removes toxic peroxides and is one of major enzymes of the GSH system to control the thiol status in the brain (Aksenov and Markesbery, 2001)....

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[Cocoa intake attenuates oxidative stress associated with rat adjuvant arthritis](#)


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...SOD and catalase activities in the C10 and Q groups were not significantly different from the AA rats (Figs. 3 and 4). The beneficial effect of a 5% cocoa diet on antioxidant systems in adjuvant arthritis is in

line with the effect of other flavonoids or compounds of a botanical origin on this experimental inflammatory model [24,30], although these studies found a clinical improvement on arthritis. The reduction of oxidative stress by a 5% cocoa diet (Fig. 2), as well as the decrease in TNF- α secretion produced by a similar diet as reported in a previous study [7] could be responsible for the reduction in splenic SOD....

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