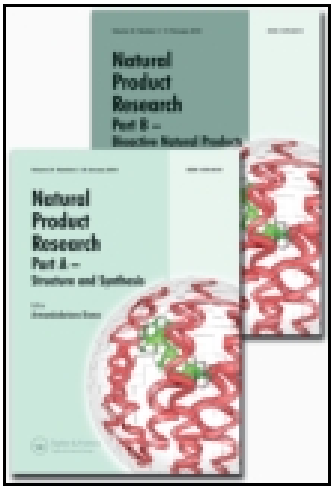

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Effect of Heterotheca inuloides essential oil on rat cytoskeleton articular chondrocytes

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SHORT COMMUNICATION

Effect of *Heterotheca inuloides* essential oil on rat cytoskeleton articular chondrocytes

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Osteoarthritis is characterised by progressive loss of articular cartilage through the increase of catabolic metalloproteinases, and chondrocyte cytoskeleton disruption has also been reported. In this regard, we studied the effect of *Heterotheca inuloides* essential oil (HIEO) on the distribution and immunolocalisation of actin, vimentin and tubulin of chondrocytes from cultured rat articular cartilage explants in the presence of the cytoskeleton disassembly agent acrylamide. After 48 h, chondrocytes treated with acrylamide showed changes in actin immunolocalisation and shrinkage, loss of tubulin compartmentalisation and vimentin collapse and redistribution. However, the immunostaining pattern of these three proteins in acrylamide- and HIEO-treated chondrocytes simultaneously retained their typical characteristics. These results suggest that HIEO promotes protein cytoskeleton reorganisation without providing a preventive effect of acrylamide-associated disassembly. However, it is also possible that HIEO prevents vimentin disorganisation by chemical interaction with acrylamide.

Keywords: *Heterotheca inuloides*; cytoskeleton; chondrocytes

1. Introduction

Osteoarthritis (OA) is a chronic degenerative joint disorder primarily characterised by articular cartilage destruction (Hunter et al. 2009). Besides the well-known imbalance between anabolism and catabolism in OA, the alterations of chondrocytes cytoskeleton have also been described. In human osteoarthritic chondrocytes, elongated protein network and disassembly of vimentin have been reported (Holloway et al. 2004; Blain et al. 2006). On the other hand, the alterations in distribution and content of vimentin, actin and tubulin were demonstrated in a rat OA model (Capín-Gutiérrez et al. 2004). Also, the reduction in the stiffness of vimentin in rat chondrocyte culture treated with acrylamide was described (Haudenschild et al. 2011). Thus, in the present study, we evaluated the effect of *Heterotheca inuloides* essential oil (HIEO), a Mexican traditional rheumatic remedy (http://www.medicinatradicionalmexicana.unam.mx/), on the

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acrylamide-disrupted cytoskeleton of rat chondrocytes analysed by immunofluorescence assays of actin, vimentin and tubulin.

2. Results and discussion

2.1. *Results*

The major components identified in the HIEO comprised monoterpenes (50.6%), sesquiterpenes (18.6%) and alcohols (11.2%). Among the major compounds identified, 27.7% of camphor was present, followed by 4-terpineol (11.5%), 1-hexanol (8.3%) and germacrene D (8.1%) (Table S1).

Non-cytotoxic concentration of HIEO (30 $\mu g\,mL^{-1}$) was used for immunolocalisation analysis (Figure S1). Actin in normal chondrocytes exhibited a wide, compartmentalised and homogeneous distribution throughout the cytoplasm (Figure S2(a)). Acrylamide-exposed cells (40 mM, 48 h) showed a concentric 'discoidal' distribution pattern (Figure S2(b)). Comparatively, in chondrocytes treated with acrylamide and HIEO (30 $\mu g\,mL^{-1}$), an actin redistribution similar to that of normal chondrocytes was observed (Figure S2(c)). Immunostaining of tubulin in normal chondrocytes was characterised by a coarse and fine granular pattern that was irregularly distributed throughout the cytoplasm (Figure S2(e)). Unlike this pattern, treatment with acrylamide induced tubulin condensation in some cells of the superficial and middle cartilage zone (Figure S2(f)). Interestingly, these changes were not detected in the presence of acrylamide and HIEO (Figure S2(g)). Finally, vimentin was arranged in the paranuclear regions of normal chondrocytes with a fine and coarse dot pattern (Figure S2 (i)). Treatment with acrylamide induced clear vimentin condensation and redistribution (Figure S2(j)); however, in cells with simultaneous treatments of the disassembly agent and HIEO, alterations were not observed (Figure S2(k)). The HIEO-treated cells conserved the normal pattern for the three analysed proteins, respectively (Figures S2(d), (h) and (l)). In additional experiments for the vimentin protein, in the cartilage treated with acrylamide at 76 h of exposition (Figure S2(m)) and in the cartilage treated with acrylamide 48 h before addition of HIEO (Figure S2(n)), the protein immunolocalisation was similar to normal chondrocytes. In contrast, the cartilage treated first with HIEO and with acrylamide 48 h later exhibited irregular vimentin immunodistribution and a speckled, compartmentalised appearance (Figure S2(o)). The same result was observed for celecoxib-treated cells (30 $\mu g\,mL^{-1}$; Figure S2(p)) as well as simultaneously for celecoxib with acrylamide (40 mM, 48 h; Figure S2(q)).

2.2. *Discussion*

Collapse and changes of vimentin filament distribution in chondrocytes exposed to acrylamide have been shown (Sager 1989; Trickey et al. 2004; Ofek et al. 2009); however, no effect has been observed on actin and tubulin. Interestingly, our results revealed changes in the immunolocalisation of all three proteins in chondrocytes from acrylamide-treated culture cartilage explants. On the other hand, the reorganisation of actin, tubulin and vimentin in chondrocytes treated with acrylamide and HIEO simultaneously was shown. Additionally for vimentin, the restoration pattern similar to normal was observed when the chondrocytes were initially exposed to acrylamide and then to HIEO, whereas chondrocytes exposed first to HIEO and subsequently to acrylamide demonstrated vimentin filament disassembly and collapse.

Therefore, it is likely that HIEO promotes protein reassembly and does not possess a protective effect, probably by a chemical interaction with acrylamide. At present, it would be important to assess the effect of the essential oil on osteoarthritic chondrocytes, which have cytoskeletal alterations and which do not require induced protein disassembly.

In support of the possible effect of HIEO, it is noteworthy that IL-1β comprises one of the major cytokines involved in the pathophysiology of OA (Kapoor et al. 2011). Potential inhibitors of this cytokine from *Mentha piperita*, *Origanum virens*, *Lavandula luiseri* and *Juniperus oxycedrus* essential oils (Neves et al. 2010), as well from *Uncaria guianensis* and *Lepidium meyenii* (Miller et al. 2006) have been described. In addition, IL-1β has been involved in the regulation of cytoskeleton-related genes and associated with the increase of cytoskeletal protein-related cellular chaperones (Joos et al. 2008; Calamia et al. 2011). Thus, it is possible that the components of *H. inuloides* exert an effect on cytoskeletal proteins via the IL-1β signalling pathway.

3. Conclusion

The data suggest that HIEO can avoid the protein cytoskeleton disassembly (actin, vimentin and tubulin) induced by acrylamide in chondrocytes from rat articular cartilage explants. Further experimental studies are required to elucidate the mechanisms responsible for this effect.

Supplementary material

Experimental details relating to this article are available online, alongside Table S1 and Figures S1 and S2.

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Conflict of interest

The authors have declared that there is no conflict of interest.

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