Reviewer's report

Title: Effect of a Herbal-Leucine mix on the IL-1beta-induced cartilage degradation and inflammatory gene expression in human chondrocytes

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Reviewer: Mehdi Shakibaei

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Title: “Effect of a Herbal-Leucine mix on the IL-1beta-induced cartilage degradation and inflammatory gene expression in human chondrocytes”

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Summary:
This manuscript attempts to highlight the beneficial role of a herbal and amino acid mixture containing extract of the Uncaria tomentosa, Boswellia spp., Lepidium meyenii and L-Leucine in osteoarthritis. The authors used an in vitro model of primary human OA chondrocytes and OA cartilage explants. They have demonstrated that herbal-Leucine mixture prevent iNOS, MMP-9 and MMP-13 expression and NO production in IL-1beta-stimulated OA chondrocytes. Moreover, in the presence of herbal-Leucine mixture (HLM) up-regulation of ACAN and COL2A1 expression in IL-1beta-stimulated OA chondrocytes was also noted. The authors conclude that HLM could be chondroprotective and anti-inflammatory agent in arthritis.

Comments to the Authors,

Although the approach to use a herbal-Leucine mix extracts in osteoarthritis as a therapeutic for the treatment of joint disorders is by itself a valuable idea, many concerns are raised:

1. The introduction should contain literature data informing the reader what is unknown and what remains to be studied etc, and not only a summary of what the state of knowledge is on each topic.

2. The major concern is the characterization of such extracts. It is usual that herbal extracts of the same plant give different results due to differences in chemical composition (plant origin, extraction conditions, artifacts formed during plant conservation or extraction, etc.). Therefore, to give reproducible results extracts must be perfectly characterized with respect to chemical composition. How do we know the effect of every active compound in a composition of plant extracts?

Again, the results indicate that these extracts counteract IL-1beta effects. With such complex extracts, the possibility exists of non specific effects.

3. The authors have to perform experiments with control cells (with normal, healthy chondrocytes), not only with OA-chondrocytes. They have to
demonstrate that the induced mechanism is specific to “healthy and unhealthy” chondrocytes. It is important to show data from control experiments.

4. It has been previously shown that pro-inflammatory effects of IL-1# and TNF-# in OA are regulated by activated transcription factor “Nuclear transcription Factor #B”. herbal-Leucine mixture extracts most probably inhibit NF-kB signaling pathway though their ability to scavenge free radicals. This was not investigated in this work. Why did the authors not look for activation of NF-kB?

5. It would make sense to show a picture of the structure in the manuscript. The behaviour of the cells in the presence or absence of the extract should be shown.

The spectrum of the methods performed is very limited for specific demonstration and evaluation of the complex process of ECM production by chondrocytes. Some histological data needs to be supplemented with Alcian blue staining for proteoglycans and GAGs.

6. All extracellular matrix components are measured exclusively at the gene expression level. This, by itself, has very little value. Proteins are the key players in chondrocyte biology. Messenger RNA is simply the transcript and may not reflect important change at the protein level. Therefore, it would make more sense to evaluate these also with western blot analysis at the protein level.

7. Fig. 3A/B: Why did the authors treat the chondrocytes with IL-1β for 6 h for MMP-9 and 24 h for MMP-13? Why did they not use the same incubation time for IL-1β for both MMPs? This should be clarified.

8. Fig. 4A and B are the same pictures like Fig. 3A and B, but in the results and Fig. Legends of the MS state they should be for ACAN (A) and COL2A1 (B). This should be clarified.

**Level of interest:** An article of limited interest

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.