

Assessment of anti-inflammatory activity of homeopathic *Arnica montana*

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Background: A homeopathic dilution of *Arnica montana* has been used for several years in traditional medicine to treat hematomas, contusions, sprains, rheumatic diseases, and superficial inflammations of the skin^{1,2}. However, its biological activity has not been sufficiently well-proven.

Therefore to provide scientific evidence we performed two studies to investigate the anti-inflammatory effect of *Arnica Montana* dilutions. We have used 2 models: in-vitro model Human umbilical vein endothelial cells (HUVECs) and in-vivo model skeletal muscle regeneration.

Aims: The aim of the in-vitro model was to measure the effect of *Arnica montana* 5 cH on the expression of intracellular cell adhesion molecule (ICAM-1) in HUVECs. Intercellular adhesion molecule-1 (ICAM-1) plays an important role in the inflammatory process and immune response^{3,4}. Whereas the aim of in-vivo model was to investigate the potential effect of different *Arnica montana* dilutions on regenerating skeletal muscles and inflammatory parameters.

Methodology: For the first study, in-vitro model consisted of incubate HUVECs confluent monolayers for 1 hour with *Arnica montana* 5 cH in cell culture dishes and co-incubated with TNFalpha (5 ng/mL) for 16 hours at 37°C to activated human umbilical vein endothelium cells (HUVECs). After treatment, the cells were fixed with paraformaldehyde, and labelled by adding anti-ICAM-1 monoclonal antibodies. Cells were examined under a fluorescence laser scanning confocal microscope. The expression of intracellular cell adhesion molecule (ICAM-1) was quantified by Biovays ImagePro (BIP) software.

The second model consisted of crush the Extensor digitorum longus (EDL)⁵ muscles of rats in blind on groups of 7 animals which received a daily treatment by IP injections of homeopathic dilutions of *Arnica montana* 3 xH, 9 cH or placebo. At day 8 post injury, EDL muscles were analyzed according to different parameters including assessments of structure of injured skeletal muscles, immunolabeled pro-(M1) and anti-(M2) inflammatory macrophages and vascular CD31+ cells using a computerized image analysis.

Results: On one hand, *Arnica montana* 5 cH attenuated significantly ICAM-1 expression in endothelial cells compared to placebo. This suggested that *Arnica* 5 cH has anti-inflammatory properties in the vasculature which contributes to modulate in reperfusion injury inflammation and vascular disease.

On the other hand, the in-vivo study didn't show significant amelioration of the amount of CD31+ cells and of histological aspect of regenerating muscles with *Arnica montana* 9 cH. However, this treatment significantly increased M2/M1 macrophages ratio compared to placebo.

Conclusion: Theses result provide evidence that *Arnica montana* dilution may have a positive effect on vascular cells and skeletal muscle repair through a modulation of inflammation.

keywords: *Arnica montana* , anti-inflammatory effect, HUVEC, ICAM-1, Extensor digitorum longus (EDL).

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Support: Boiron. I (We) had full access to all the data in this study and I (We) take complete responsibility for the integrity of the data and the accuracy of the data analysis”

Conflict of interest: authors declare there is no conflict of interest

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