

# Comments on the Retraction by PLoS ONE of a Laboratory Study on *Arnica montana*

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Homeopathy

## Abstract

In June 2019, the journal PLoS ONE retracted an original research article, published in 2016, which described the effects of homeopathic *Arnica montana* on interleukin-4 treated human macrophages. The results showed an increase in extracellular matrix gene expression, including the gene encoding fibronectin, which is one of the main proteins involved in connective tissue healing. Here, the authors of the article discuss the critical points raised by the journal in the retraction note, with a focus on the specific methodological aspects of research on high dilutions of natural compounds. The editorial arguments made to justify the retraction did not prove any methodological errors, nor scientific misconduct. As a general rule, when a study published by a group of researchers raises scientific doubts because the results appear at variation with the commonly accepted knowledge in a field, the study is repeated by other scholars and any contrasting results are published and/or discussed. Therefore, retraction of the *Arnica m.* study by PLoS ONE is a violation of the conventions of scientific publication and knowledge-sharing methods derived from honest experimental method.

## Keywords

- ▶ *Arnica montana*
- ▶ gene expression
- ▶ paper retraction
- ▶ homeopathic dilutions
- ▶ scientific literature

On June 20, 2019, PLoS ONE retracted our article '*Arnica montana* Stimulates Extracellular Matrix Gene Expression in a Macrophage Cell Line Differentiated to Wound-Healing Phenotype',<sup>1</sup> which provided new and original data, obtained with correct methods, on the effects of *Arnica montana* (*Arnica m.*) on human macrophages. As the corresponding author (PB) and first author (MM), we believe it is important to present to the scientific community our response to the Editor's published retraction note.<sup>2</sup>

The results presented in the retracted study showed for the first time that the regulating action of *Arnica m.*, even at high dilutions, led to an increase in extracellular matrix gene expression in a macrophage cell line after pre-treatment with interleukin-4 (IL-4), including the gene encoding fibronectin, which is one of the main proteins involved in connective tissue healing.<sup>1</sup> The in-vitro action also aligns with published clinical

studies indicating that this medicinal product can have positive effects on trauma healing and post-operative recovery.<sup>3-11</sup>

The manuscript was sent on February 5, 2016, accepted on August 26 after a long and thorough review process, and was published on November 10, 2016. After publication, the paper received dozens of citations by other peer-reviewed scientific journals. The retraction of the paper more than 30 months after the publication is not motivated by misconduct, by statistical errors, or by other methodological defects. Here, we summarize the main issues of the debate, discussing the points made by the PLoS ONE Editor for the general interest they raise in the context of the debate on the scientific bases of homeopathy.

The retraction note<sup>2</sup> states that '*concerns were raised about the concentration of Arnica m. used in the experiments, and that the reported gene expression changes are within the range of what would be expected for standard noise within an RNA-seq dataset*' and that '*In Figs 1 and 2, the article reports an absorption spectrum and nanoparticle spectrum analysis for the Arnica m. 1c starting material, but not for the 2c or other solutions used in the study, or for a control solution. This raises*

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concerns on whether there is sufficient evidence to demonstrate that biochemically active ingredients remain in the diluted solutions used in the experiments'. However, as far as the concentration of active principles in *Arnica 2c* is concerned, our article is extremely clear. The concentration of *Arnica m. 2c* used in our study was admittedly low, as we clearly noted, where we used solutions diluted over 10,000 times from starting crude extract, and the final concentration of active principles (sesquiterpene lactones) in the final cell assay was approximately 10 nanomolar. We have shown only the spectrum of 1c because this dilution was better characterized due to the sensitivity of instruments used. We also recorded the spectra of 2c dilution, but we have not reported them in the article because, as expected from a 100x diluted solution, the absorption peak was around the detection power of the spectrophotometer. The difference between a 1c and 2c dilution will be reported in a forthcoming article (manuscript in preparation).

The claim that the reported gene expression changes could be due to a purported "standard noise" is not acceptable just because we employed specific statistics, designed to exclude random events and false-positive results. Since the expected differences in gene expression were small, the experimental design and statistics were defined consequently, choosing a high accuracy level in sequencing (obtained with high read depth), and using five independent experiments performed in triplicate, with appropriate batch-controlled model and Wald statistic test, corrected for false discovery rate (FDR) (0.05). The FDR is a statistical method to reduce the risk of false-positive conclusions when multiple comparisons are made, typically in -omic assays, where gene-by-gene assessments are required. RNA-seq data were analyzed with the package DESeq2 to test for differential expression. DESeq2 is a widely used and robust tool that applies an algorithm to control the expected FDR below a specified level given a list of independent  $p$ -values. The outcome of the application of the FDR correction is that many genes, even with  $p$  (not adjusted)  $< 0.05$ , actually are discarded because of the risk of being false positive. Such restriction on the number of statistically significant conclusions ensures reliable results and is always used in RNA-seq dataset analysis. Furthermore, gene-by-gene comparisons between *Arnica m. 2c* and control (the same solvent without *Arnica m.*) were analyzed using a statistical model that properly minimized the inter-experimental variations—basically a paired approach. The small changes in the differentially expressed genes were reproduced in all the experiments, as reported in the article. How much this small change can be independently reproduced must be discovered by other researchers, as is customary in science. In fact, an analysis performed by PLoS ONE on the data sent by us confirmed the significant increase in expression of fibronectin, as documented by previous correspondence with its Editor. Furthermore, our research team has recently confirmed the regulating action of *Arnica m.* on fibronectin gene expression with the real-time polymerase chain reaction method (manuscript in preparation). Of this latest experimental evidence, we notified the PLoS ONE Editor in a previous correspondence. Finally, the increase of gene expression was associated with a statistically

significant increase of fibronectin protein release by IL-4-treated macrophages in the culture medium.<sup>1</sup>

The retraction note then criticizes the results obtained with high dilutions: 'Follow-up experiments using pooled samples of cells treated with more dilute solutions (3c, 5c, 9c, and 15c) yielded results in approximately the same range of fold change, as reported in Fig 5, calling into question the specificity of the reported results'. However, this is actually one of the major results of the article, which the PLoS ONE Editor appears not to believe, despite the experimental and statistical evidence we provided. The interest and novelty of our research are actually the discovery that the same gene set modified by *Arnica 2c* in macrophages is modulated also by *Arnica 3c* (100x diluted as compared with 2c): the effect of a 3c dilution is only slightly smaller than that of a 2c, although the expected concentration of active ingredients is 100 times lower. The "non-specificity" of results with higher dilutions (5c, 9c, and 15c) can be excluded from concern since the changes are statistically significant as compared with a control ("placebo") solution. It should be noted that the RNA-seq analyses were made by independent researchers in a different university department and the researchers were not aware of the solutions used in the different samples. Clearly, this result suggests the existence of non-linear sensitivities and responses in the cells employed. It can be understood that a reviewer not familiar with high-dilution pharmacology may have doubts about the "specificity" of these effects, but in science the experimental results should have priority over pre-judgement and even accepted theories. The results obtained with IL-4-treated macrophages were replicated also with endotoxin-treated macrophages but not with resting macrophages, suggesting that only stressed cells become sensitive to high dilutions of *Arnica m.*<sup>12</sup>

An important methodological aspect, which PLoS ONE reviewers of the original manuscript had appreciated but was ignored by experts in this latter re-review, is that the tested samples were prepared by the special method of dilution followed by strong shaking (succussion), which is characteristic of homeopathic pharmacopoeia. Sequential dilution and succussion in the homeopathic production process change the physical-chemical properties of the solutions, indicating that succussion may have an important influence on treatment effectiveness.<sup>13–15</sup> According to current views in the literature, these changes are related to nano-heterogeneities of water solutions (e.g., nanostructures, clusters, or coherence domains)<sup>15–22</sup> and so highlight the need for further research. Science has always progressed when current theories were tested and even challenged by experimental evidence, not when experimental evidence was censored for not agreeing with dominant ideas.

Finally, the retraction note raises a criticism on possible competing interests: 'The Competing Interests statement was incorrect for this article and should have explicitly stated that Boiron Laboratories, a company that provided funding support for this study, markets homeopathic products including various dilutions of *Arnica m.*' We refute the argument that we omitted possible relevant information on this point. Actually, we declared that this work was supported by Boiron Laboratoires, Lyon, within a research agreement in partnership

with the University of Verona, and that the funder had no role in data collection and analysis, interpretation, decision to publish, or writing the manuscript. We declared that the tested medicine, at the 1c dilution, was provided by Boiron. *Arnica m.* is not a new product in development, nor is it patented by Boiron: it is a common medicine in the homeopathic repertoire, produced by all similar companies, and has been marketed for decades worldwide. It is evident that the study was not finalized to improve the market of the funder, but to obtain new knowledge in the field and to be shared with the scientific community.

We reject the retraction decision by PLoS ONE and are firmly convinced that this study is of interest for the scientific community because it identifies a new path that should not be disregarded by modern medicine.

#### Highlights

- Recently, the journal PLoS ONE has retracted an article showing the effects of *Arnica montana* on human macrophages.
- The arguments made to justify the retraction did not prove any methodological errors, nor misconduct.
- The reported effects on gene expression were admittedly low but statistically significant.
- Retracting the *Arnica m.* study appears as a violation of the conventions of the experimental method.

#### Conflict of Interest

None declared.

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