Journal of Integrative Medicine 16 (2018) 72–76

Contents lists available at ScienceDirect

Journal of Integrative Medicine

journal homepage: www.jcimjournal.com/jim
www.journals.elsevier.com/journal-of-integrative-medicine

Commentary

Arnica montana experimental studies: confounders and biases?

Bellavite Paolo, Marzotto Marta *, Bonafini Clara

Department of Medicine, University of Verona, Strada Le Grazie 8, Verona 37134, Italy

ARTICLE INFO

Article history:
Received 5 July 2017
Accepted 27 November 2017
Available online 21 February 2018

Keywords:
Arnica montana
Gene expression
Ethanol
Statistics

ABSTRACT

Arnica montana is a popular traditional remedy widely used in complementary and alternative medicine, in part for its wound-healing properties. The authors recently showed that this plant extract and several of its homeopathic dilutions are able to modify the expression of a series of genes involved in inflammation and connective tissue regeneration. Their studies opened a debate, including criticisms to the "errors" in the methods used and the "confounders and biases". Here the authors show that the criticisms raised on methodology and statistics are not consistent and cannot be considered pertinent. The present comment also updates and reviews information concerning the action of *A. montana* dilutions in human macrophage cells while summarizing the major experimental advances reported on this interesting medicinal plant.

Please cite this article as: Bellavite P, Marzotto M, Bonafini C. *Arnica montana* experimental studies: confounders and biases? *J Integr Med.* 2018; 16(2): 72–76.

1. Introduction

Experimental homeopathy is a field that has attracted the interest, curiosity and doubts of the scientific world for years. Research at the laboratory level on homeopathy investigates various areas, from physical–chemical studies on highly diluted medicines to biological studies with *in vitro* and *in vivo* models, and the results support evidence of an effect that is not merely placebo [1–5]. All the experiments conducted to verify the activity of the homeopathic preparations require scrupulous settings that make possible precise measures of even small events with high reproducibility and discrimination against any systematic or random errors. Careful design of controlled experiments with adequate biological replicates is important, especially given the current lack of a unique physicochemical description of high dilutions or of the factors that may affect the transmission of information at the biological level.

Our research team has been engaged in homeopathic research for a long time. Together with other research groups worldwide, we have made theoretical contributions to homeopathic concepts such as the "simile" mechanism of action [6–9] and to experimental studies in animals [10,11] and in cell lines (neurons and macrophages) [12–15]. Today, the availability of modern, high-throughput laboratory technologies, based on the study of gene expression, has enabled us to demonstrate a biological basis, both cellular and molecular, for the medicinal action, completing certain pieces of a very complex mosaic. The ability of highly diluted compounds to modulate gene expression in human/animal cells and unicellular organisms has been reported previously by a number of authors [9,16–24]. Ribonucleic acid (RNA)-seq technique, in particular, is a reliable and well established procedure, both for preparing libraries of transcripts and for bioinformatic analysis and statistics. In addition, collaboration among researchers with advanced technical expertise and skill in setting up biological studies with high dilution medicines ensures reliable results. This appears to be a good direction for future homeopathic experimental research [25,26].

Starting from year 2011, our publications have been the subject of several critical annotations by Dr. Salvatore Chirumbolo [27–30], and a summary of our technical responses was reported immediately afterward [31]. More recently, we used real-time polymerase chain reaction to investigate the effects of *Arnica montana* on gene expression of the THP-1 myelomonocytic cell line, differentiated by phorbol-myristate acetate and interleukin-4 in the wound-healing phenotype [32]. These findings also drew critical commentary by Chirumbolo and Bjørklund [33], based on some recalculations and extrapolations from the values of standard errors of the mean. Our reply, accepted by the journal *Frontiers in Immunology*, showed that those recalculations were wrong [15].

A subsequent series of our studies on the effects of *A. montana*, based on RNA-seq methodology, were published by PlosOne in

* Corresponding author.
E-mail address: marta.marzotto@univr.it (M. Marzotto).

https://doi.org/10.1016/j.joim.2018.01.001
2095-4964/

November 2016 [14]. This article was the subject of a recent commentary by Chirumbolo and Bjørklund [34], published in the *Journal of Integrative Medicine*.

Such debate has enabled us to define in more detail the experimental methods applied, providing a useful tool for experimental homeopathic research.

2. *A. montana* drug composition

Chirumbolo and Bj∅rklund [34] criticize the molar estimation of sesquiterpene lactones in *A. montana* dilutions. We do not agree with this remark. In actual fact, in our study, *A. montana* mother tincture is chemically characterized in accordance with the European Pharmacopoeia Guidelines [35], which conventionally measure the quality of *A. montana* preparation in terms of the content of sesquiterpene lactones, considered to be the active ingredients of reference, even if a complex variety of chemical ester variants and other chemical compounds could be present as a function of the cultivar, the part of the plant and the timing and methods of harvesting. Sesquiterpene lactones are determined by liquid chromatography and expressed as dihydrohelenalin tiglate. It follows that estimating the average molar weight of sesquiterpene lactone esters as 340 g/mol [36] is adequate for converting chromatographic quantification into molar concentration. Note that we had already published a rebuttal reaffirming the validity of our calculation of standard sesquiterpene lactones in our samples [15] in response to a previous letter from Chirumbolo and Bjørklund [33].

A major argument of Chirumbolo and Bjørklund [34] is that they think we used 51.1 mmol/L ethanol in our assay system, a dose which they consider to be too high and toxic for cells. However, the two authors made an error in their calculations: as is clearly stated in the methods of our paper, all the dilutions tested in cells (2 c and higher) were prepared in 0.3% ethanol and then further diluted 1/10 in culture medium. Since ethanol in final cell culture was 0.03%, and the molar mass of ethanol is 46.07 g/mol, with a density of 0.7893 g/cm^3, this corresponds to 5.1 mmol/L and not 51.1 mmol/L.

Taking into consideration the presence of factors that may alter cell viability is a mandatory step, especially in *in vitro* experiments. It was precisely for this reason that we chose to dilute both the medicinal product and its vehicle (control), originally hydroalcoholic solutions at 30% v/v ethanol, as a large part of homeopathic medicinal products, a total of 1000 times the concentration present in cell cultures. This operation guarantees the non-toxicity of the ethanol, whether in the *verum* or placebo, as shown by suitable cell viability assays [13–15]. The residual ethanol (0.03%) could have an effect on the background activity of the cells, but it would be identical in the medicine and in the control. Consequently, all considerations about the purported confounding effects on gene expression and cell viability ascribed to ethanol in control and in *A. montana*-treated samples should be considered to be wrong and misleading, since they refer to ethanol doses ten times higher than the actual ones. Note that a refutation of Chirumbolo's erroneous opinions on the toxicity of ethanol in homeopathic medicines has already been published [12].

Throughout the text, Chirumbolo and Bjørklund [34] suggested a possible biasing effect on the control samples due to a supposed difference in the handling of the *A. montana* dilutions and of the control vehicle (the preparations and the treated samples). This is an important point in the design of experiments with highly diluted medicine, giving us the opportunity to emphasize how these controls were properly performed in our experiments. Actually, as described by Marzotto et al. [14], the control solutions (solvents) in all the reported experiments were prepared under the same procedure as the drug dilutions, just without the plant extract, as indicated. All the procedural handling was conducted in parallel, including filtering of the first 1c solution of the *A. montana* or of the ethanol vehicle (the same batch of the *verum*, provided by the pharmaceutical manufacturer). The time of conservation was exactly the same. Control and *A. montana* samples were subjected to matching experimental steps, from the cell cultures to RNA-seq and bioinformatic pipeline.

3. Clarification of protocol and statistics

Chirumbolo et al. [34] hint at purported "biases" due to the pooling of RNA samples. Actually, the pooling of equal amounts of carefully quantized RNAs from replicated experiments in the same cell line—as we did in our experiments—is a conventional procedure for evaluating the presence of general trends in gene expression, in cases where the number of test samples must be minimized. As confirmation of the reliability of the results from the pooled samples, in our RNA-seq study (not DNA-microarray), we analyzed the gene expression values of *A. montana* 2 c and of the control-treated samples, both as five separate samples and as a pool, and the values matched very well, as reported in Fig. 5 and Table S1 [14]. Moreover, in a recent paper [37], it was reported that *A. montana* 2c up-regulated the same gene set both in THP-1 cells activated with lipopolysaccharide 10 ng/mL and in cells in a resting condition. These data confirmed that such differentially expressed genes (DEGs) are the true target of *A. montana* 2 c and excluded the possibility of control biases. In any case, the significant genes differentially expressed upon *A. montana* 2 c treatment, as reported by Marzotto et al. [14], were derived from a statistical analysis of five independent experiments and not from pooled samples. We calculated the *P* values of DEGs with *A. montana* 2 c by DESeq2, one of the most modern and rigorous statistical methods, specifically designed and applied to RNA-seq dataset experiments [38]. The few genes that emerged from our analysis as targets of *A. montana* action on human macrophages have an elevated biological significance and internal coherence; moreover, increased fibronectin secretion was observed at the protein level as well. Therefore, the criticism of Chirumbolo and Bjørklund [34] regarding the statistical approach we used to infer the significance of DEGs, claiming that a greater number of replicated samples was necessary and a more rigorous test should have been used, is unwarranted.

The paper cited [34] also criticized the statistical test used to compare expression trends of the set of 20 DEGs after treatment with higher *A. montana* dilutions (2 c, 3 c, 5 c, 9 c, 15 c) and the control. The authors wrongly declared that the criticized paper used only the Friedman test (defined as not effective), while Marzotto et al. [14] applied the Friedman test (as a non-parametric analysis of variance), followed by the Wilcoxon-signed rank test (as a paired comparison post-hoc test). Chirumbolo and Bjørklund [34] purport to recalculate *P* values with the Wilcoxon–Mann–Whitney and Kolmogorov–Smirnov tests, concluding with the contention that most of our results are false and not meaningful. This re-analysis is erroneous for a series of reasons:

(1) The Wilcoxon–Mann–Whitney and Kolmogorov–Smirnov tests are not appropriate for this analysis, because those tests, unlike the Wilcoxon-signed rank test, must be applied to independent samples, but the geneset expression profiles of treated and control samples found in Tables S1 and S2 are absolutely matched or dependent samples. This means that, in their own statistical tests, Chirumbolo and Bjørklund [34] compared expression profiles without any matching of genes between the treated and the control, i.e., the

expression value of gene A in the treated sample is not matched with the value of the same gene A in the control, and this obviously results in a misleading procedure.

(2) Moreover, Marzotto et al. [14] compared profiles of a biologically coherent geneset, i.e., down-regulated genes separately from up-regulated ones, and not the entire list, which includes genes with opposite expression trends.

(3) Marzotto et al. [14] clearly stated that the list reported in Table S2 included other candidate DEGs that were analyzed to understand the role of *A. montana* in the extracellular matrix network. Even if genes were apparently modulated by *A. montana* 2 c, most of those were not modulated by higher *A. montana* dilutions and their role deserved further investigation. So the re-analysis of Chirumbolo and Bjørklund [34], as reported in their Table 2, was not carried out correctly, nor does it add anything to the results already presented. Notably, the genes on Tables S1 and S2 are different populations, and thus cannot be merged to perform a new statistical analysis, as reported in the text on "summing all data reported in (a) and (b)".

The criticism regarding a presumably irregular homoscedasticity of RNA-seq dataset is surprising. The Bartlett's test suggested by Chirumbolo and Bjørklund [34] simply assesses for non-normality of the distributions, but this was already known, and clearly reported by Marzotto et al. [14] Actually, the distribution of the expression values of the genome, as in the case of a restricted gene list, is not normal, and other models are used to prepare the data for a statistical analysis [38]. The RNA-seq dataset obtained by Marzotto et al. [14] was submitted to quality control analysis, as is customarily required for publication in public databases (as requested by PlosOne). Cluster analysis of samples, using Euclidean distance, confirmed that all datasets from replicated experiments had high similarity scores (>0.85) and were not identified as outliers. Moreover, different genes have intrinsically different variability, depending on their level of expression and on different regulation mechanisms, but this cannot be due to bias in the handling of control samples. Notably, a criticism based on the analysis of standard error of mean distribution and the presence of outliers was already fully refuted as being totally worthless by Olioso et al. [15].

In chapter 7, the authors introduce the themes of hydro-alcoholic solution inhomogeneity and the possible presence of nanobubbles, issues that have recently drawn the interest of scientists working with theoretical and experimental chemo-physics in the field of high dilutions. As a general suggestion for further investigation of the characteristics of nanostructures, an area of research still in the preliminary phase, this is acceptable. In fact, we reported the presence of nanostructures in the *A. montana* 2 c dilution, without further defining the nature of these structures. To a certain extent, the presence of nanostructures in *A. montana* 1 c is to be expected, as the method of preparation involves the plant material being macerated, triturated and vortically mixed in an ethanol solution in glass containers [39]. The control solution itself might contain nanostructures originating from glass contaminants, but in the case of the control preparation used in this study, they were not detected.

4. Conclusions

We hope that these clarifications are welcome, in the interests of providing correct and truthful information to readers on the action mechanisms of *A. montana,* which we discovered and published [14,15,37].

Our results are complemented by a series of laboratory and clinical studies that, in recent years, have confirmed the potential of *A. montana* as a plant capable of regulating mechanisms of inflammation and the healing of wounds (Table 1).

Certainly, the vastness of the subject, and above all the variety of medicinal formulations and dilutions employed by the different authors, make it difficult to draw conclusions on the efficacy and therapeutic capabilities of the plant, and further studies are desirable.

Basic research in homeopathy is a new field. It is fascinating but challenging, because it deals with difficult-to-solve technical issues

Table 1
Summary of laboratory and clinical studies on *Arnica montana*.

Study type	Tested formulation	Setting	Evidence	References
In vitro	*A. montana* 30 c	*Escherichia coli* exposed to ultraviolet irradiation	Up-regulation of repair genes and amelioration of the oxidative stress	[40]
	A. montana 2–15 c (nonlinearity)	Cultivated macrophages differentiated by interleukin (IL)-4	Increase in gene expression of some CXC chemokines and bone morphogenetic protein, decrease in MMP-1	[32]
	A. montana 2–15 c (nonlinearity)	Cultivated macrophages differentiated by IL-4	Increase in gene expression and production of fibronectin	[14]
	Pure helenalin, dihydrohelenalin and chamissonolid	T-cells, B-cells and epithelial cells	Inhibition of activation of transcription factor NF-κB	[41]
	Pure helenalin	$CD4^+$ T-cells	Immunosuppressive effects	[42]
	Whole extract	Murine macrophages challenged with lipopolysaccharide	Inhibition of nitric oxide production without cytotoxicity	[43]
Laboratory animals	*A. montana* 6 c	Rat paw edema induced by carrageenan	Treated rats presented less intense edema. Animals that presented precocious edema were less responsive to *A. montana*	[44]
	A. montana 6 c	Rat paw edema induced by nystatin	Reduction in edema (post-induction effect)	[45]
	Traumeel S, a homeopathic formulation containing *A. montana* and other plant extracts	Rat paw edema induced by blood	Reduction in edema (post-induction effect)	[46]
	A. montana 4 x	Rat paw edema induced by carrageenan	No effect	[47]
	A. montana 30 c	Rat	Protects against hepatic mitochondrial peroxidation	[48]
	Whole *A. montana* extract (75 mg/kg)	Rat paw arthritis induced by collagen	Improvement of arthritis index, decrease in pro-inflammatory cytokines	[49]

Table 1 (*continued*)

Study type	Tested formulation	Setting	Evidence	References
	Whole *A. montana* extract (50 mg/kg)	Citric-acid-induced cough reflex in guinea pig	Reduction in the cough efforts by and bronchodilator activity of *A. montana* complex	[50]
	A. montana 3D gel combined with microcurrent	Rat (linear incision wound model)	Improved wound healing	[51]
Clinical studies	*A. montana* gel plus hydroxyethylsalicylate	Ankle sprains	Alleviates pain	[52]
	A. montana gel	Hand osteoarthritis	*A. montana* gel probably improves symptoms as effectively as a gel containing non-steroidal anti-inflammatory drug	[53]
	A. montana 30 x	Knee surgery	Reduced pain and swelling after cruciate ligament surgery (not after arthroscopy)	[54]
	Various (Review)	Rhinoplasty	Eyelid edema and ecchymosis during the first 7 days postoperatively showed a statistically significant decrease in the *A. montana* administration groups versus the control group	[55]
	A. montana 1 M and *A. montana* 12 c	Rhinoplasty	Reduced edema during the early postoperative period	[56]
	A. montana 1 M and 12 c	Rhinoplasty	*A. montana* seemed to accelerate postoperative healing	[57]
	A. montana 1 M and 12 c	Rhytidectomy	Reduction in ecchymosis	[58]
	Topical *A. montana* cream	Rhinoplasty	Regression of postoperative edema and ecchymosis in treated patients	[59]
	A. montana 1000 Korsakovian dilution	Mastectomy	The per-protocol analysis revealed a lower mean volume of blood and serum collected in drainages with *A. montana*	[60]
	Topical hydrogel pads (OcuMend, Cearna Inc., Chicago, IL) containing *A. montana* 50 M 50% and Ledum 50 M	Oculofacial procedures, including blepharoplasty, browpexy, and rhinoplasty	Subjective physician-patient rating scores showed accelerated healing in *A. montana*-treated patients compared with expected healing	[61]
	Homeopathic complex (*A. montana* and other components, each at 6c dilution)	Osteoarthritis	Treatment group significantly outperformed the control group with regard to pain, daily functioning, and motion	[62]
	A. montana 4 x	Hallux valgus surgery	Equivalent of diclofenac in wound healing, lower efficacy in pain scores	[63]
	A. montana 200 c	Wisdom teeth removal	No effects on pain and swelling	[64]
	A. montana 30 c, 6 c	Carpal tunnel syndrome	No effects on pain and swelling	[65]
	A. montana 30 c	Tonsillectomy	Reduction in pain, not of analgesic consumption	[66]
	A. montana 30 c	Hysterectomy	No effects on pain and recovery rate	[67]

The units of homeopathic dilutions cited are: "c", diluted at a ratio 1:100; "x" diluted at a ratio 1:10; "M", diluted at a ratio 1:100 for 1000 times. MMP-1: matrix metalloproteinase-1; NF-κB: nuclear factor-kappa B.

of the physicochemical nature of high dilutions, their action mechanisms, and the paradoxical reversal of the effects of drugs.

Competing interests

The authors declare no conflict of interest.

References

[1] Bellavite P, Marzotto M, Olioso D, Moratti E, Conforti A. High-dilution effects revisited. 1. Physicochemical aspects. Homeopathy 2014;103(1):4–21.

[2] Bellavite P, Marzotto M, Olioso D, Moratti E, Conforti A. High-dilution effects revisited. 2. Pharmacodynamic mechanisms. Homeopathy 2014;103(1):22–43.

[3] Mathie RT, Ramparsad N, Legg LA, Clausen J, Moss S, Davidson JR, et al. Randomised, double-blind, placebo-controlled trials of non-individualised homeopathic treatment: systematic review and meta-analysis. Syst Rev 2017;6(1):63.

[4] Frass M, Friehs H, Thallinger C, Sohal NK, Marosi C, Muchitsch I, et al. Influence of adjunctive classical homeopathy on global health status and subjective wellbeing in cancer patients—a pragmatic randomized controlled trial. Complement Ther Med 2015;23(3):309–17.

[5] Elia V, Germano R, Napoli E. Permanent dissipative structures in water: the matrix of life? Experimental evidences and their quantum origin. Curr Top Med Chem 2015;15(6):559–71.

[6] Bellavite P, Lussignoli S, Semizzi ML, Ortolani R, Signorini A. The similia principle: from cellular models to regulation of homeostasis. Br Homeopath J 1997;86(2):73–85.

[7] Bellavite P. Complexity science and homeopathy: a synthetic overview. Homeopathy 2003;92(4):203–12.

[8] Bellavite P, Olioso D, Marzotto M, Moratti E, Conforti A. A dynamic network model of the similia principle. Complement Ther Med 2013;21(6):750–61.

[9] Bellavite P, Signorini A, Marzotto M, Moratti E, Bonafini C, Olioso D. Cell sensitivity, non-linearity and inverse effects. Homeopathy 2015;104(2):139–60.

[10] Magnani P, Conforti A, Zanolin E, Marzotto M, Bellavite P. Dose-effect study of *Gelsemium sempervirens* in high dilutions on anxiety-related responses in mice. Psychopharmacology 2010;210(4):533–45.

[11] Bellavite P, Marzotto M, Olioso D, Cristofoletti M, Brizzi M, Tononi P, et al. Cellular and transcriptional responses of SH-SY5Y human neurocytes following *in vitro* exposure to *Gelsemium sempervirens*. Int J High Dilution Res 2012;11(40):144–6.

[12] Marzotto M, Olioso D, Bellavite P. Gene expression and highly diluted molecules. Front Pharmacol 2014;5:237.

[13] Olioso D, Marzotto M, Moratti E, Brizzi M, Bellavite P. Effects of *Gelsemium sempervirens* L. on pathway-focused gene expression profiling in neuronal cells. J Ethnopharmacol 2014;153(2):535–9.

[14] Marzotto M, Bonafini C, Olioso D, Baruzzi A, Bettinetti L, Di Leva F, et al. *Arnica montana* stimulates extracellular matrix gene expression in a macrophage cell line differentiated to wound-healing phenotype. PLoS One 2016;11(11):e0166340.

[15] Olioso D, Marzotto M, Bonafini C, Brizzi M, Bellavite P. Response to commentary: *Arnica montana* effects on gene expression in a human macrophage cell line: evaluation by quantitative real-time PCR. Front Immunol 2016;7:320.

[16] Bigagli E, Luceri C, Bernardini S, Dei A, Dolara P. Extremely low copper concentrations affect gene expression profiles of human prostate epithelial cell lines. Chem Biol Interact 2010;188(1):214–9.

[17] Bigagli E, Luceri C, Bernardini S, Dei A, Filippini A, Dolara P. Exploring the effects of homeopathic *Apis mellifica* preparations on human gene expression profiles. Homeopathy 2014;103(2):127–32.

[18] Bigagli E, Luceri C, Dei A, Bernardini S, Dolara P. Effects of extreme dilutions of *Apis mellifica* preparations on gene expression profiles of human cells. Dose-Response 2016;14(1):1–7.

[19] Saha SK, Roy S, Khuda-bukhsh AR. Evidence in support of gene regulatory hypothesis: gene expression profiling manifests homeopathy effect as more than placebo. Int J High Dilution Res 2013;12(45):162–7.

[20] Olsen S. Effects of ultra-high dilutions of sodium butyrate on viability and gene expression in HEK 293 cells. Homeopathy 2017;106(1):32–6.

[21] Marotti I, Betti L, Bregola V, Bosi S, Trebbi G, Borghini G, et al. Transcriptome profiling of wheat seedlings following treatment with ultrahigh diluted arsenic trioxide. Evid Based Complement Altern Med 2014;2014:851263.

[22] Khuda-Bukhsh AR. An overview of research at University of Kalyani in exploring some basic issues of homoeopathy. Indian J Res Homoeopath 2017;11:147–57.

[23] de Oliveira CC, de Oliveira SM, Goes VM, Probst CM, Krieger MA, Buchi Dde F. Gene expression profiling of macrophages following mice treatment with an immunomodulator medication. J Cell Biochem 2008;104(4):1364–77.

[24] Kay P, Khuda-Bukhsh A. The contribution of homeogenomic and homeogenetic studies in the support of the practice of homoeopathy. Indian J Res Homoeopath 2016;10(2):101–7.
[25] Betti L, Trebbi G, Olioso D, Marzotto M, Bellavite P. Basic research in homeopathy and ultra-high dilutions: what progress is being made? Homeopathy 2013;102(2):151–4.
[26] Bellavite P, Betti L. Homeopathy and the science of high dilutions: when to believe the unbelievable. Int J High Dil Res 2012;11(40):107–9.
[27] Chirumbolo S. On *Gelsemium* and complementary and alternative medicine (CAM) in anxiety and experimental neurology. Neurol Ther 2015;4(1):1–10.
[28] Chirumbolo S. Gelsemine and *Gelsemium sempervirens* extracts in animal behavioral test: comments and related biases. Front Neurol 2011;2:31.
[29] Chirumbolo S. High diluted molecules and gene expression. Front Pharmacol 2014;5:183.
[30] Chirumbolo S. Dose response of high dilutions in gene expression and the concept of hormesis. Hum Exp Toxicol 2017;36(8):876–7.
[31] Bellavite P, Bonafini C, Marzotto M. Experimental neuropharmacology of *Gelsemium sempervirens*: recent advances and debated issues. J Ayurveda Integr Med 2017. in press.
[32] Olioso D, Marzotto M, Bonafini C, Brizzi M, Bellavite P. *Arnica montana* effects on gene expression in a human macrophage cell line. Evaluation by quantitative real-time PCR. Homeopathy 2016;105(2):131–47.
[33] Chirumbolo S, Bjørklund G. Commentary: *Arnica montana* effects on gene expression in a human macrophage cell line: evaluation by quantitative real-time PCR. Front Immunol 2016;7:280.
[34] Chirumbolo S, Bjørklund G. Homeopathic potencies of *Arnica montana* L. change gene expression in a Tamm-Horsfall protein-1 cell line *in vitro* model: the role of ethanol as a possible confounder and statistical bias. J Integr Med 2017;15(4):255–64.
[35] European Directorate for the Quality of Medicines & HealthCare. Guide for the elaboration of monographs on herbal drugs and herbal drug preparations (2007) [2017-05-01]. https://www.edqm.eu/medias/fichiers/Technical_Guide_for_the_Elaboration_of_Monographs_on_herbal_drugs_and_herbal_drug_preparations_2007.pdf.
[36] Staneva J, Denkova P, Todorova M, Evstatieva L. Quantitative analysis of sesquiterpene lactones in extract of *Arnica montana* L. by ^{1}H NMR spectroscopy. J Pharm Biomed Anal 2011;54(1):94–9.
[37] Marzotto M, Bonafini C, Olioso D, Bellavite P. Study of the action mechanisms of *Arnica montana* effects on macrophages. Int J High Dil Res 2016;15(4):32–5.
[38] Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014;15(12):550.
[39] Bell IR, Muralidharan S, Schwartz G. Nanoparticle characterization of traditional homeopathically-manufactured *Gelsemium sempervirens* medicines and placebo controls. J Nanomed Biotherapeut Discov 2015;5:136.
[40] Das S, Saha SK, De A, Das D, Khuda-Bukhsh AR. Potential of the homeopathic remedy, *Arnica montana* 30C, to reduce DNA damage in *Escherichia coli* exposed to ultraviolet irradiation through up-regulation of nucleotide excision repair genes. Zhong Xi Yi Jie He Xue Bao 2012;10(3):337–46.
[41] Lyss G, Schmidt TJ, Merfort I, Pahl HL. Helenalin, an anti-inflammatory sesquiterpene lactone from *Arnica*, selectively inhibits transcription factor NF-κB. Biol Chem 1997;378(9):951–61.
[42] Berges C, Fuchs D, Opelz G, Daniel V, Naujokat C. Helenalin suppresses essential immune functions of activated CD4^{+} T cells by multiple mechanisms. Mol Immunol 2009;46(15):2892–901.
[43] Verma N, Tripathi SK, Sahu D, Das HR, Das RH. Evaluation of inhibitory activities of plant extracts on production of LPS-stimulated pro-inflammatory mediators in J774 murine macrophages. Mol Cell Biochem 2010;336(1–2):127–35.
[44] Kawakami AP, Sato C, Cardoso TN, Bonamin LV. Inflammatory process modulation by homeopathic *Arnica montana* 6CH: the role of individual variation. Evid Based Complement Altern Med 2011;2011:917541.
[45] Macêdo SB, Ferreira LR, Perazzo FF, Carvalho JC. Anti-inflammatory activity of *Arnica montana* 6cH: preclinical study in animals. Homeopathy 2004;93(2):84–7.
[46] Lussignoli S, Bertani S, Metelmann H, Bellavite P, Conforti A. Effect of Traumeel S, a homeopathic formulation, on blood-induced inflammation in rats. Complement Ther Med 1999;7(4):225–30.
[47] Conforti A, Bellavite P, Bertani S, Chiarotti F, Menniti-Ippolito F, Raschetti R. Rat models of acute inflammation: a randomized controlled study on the effects of homeopathic remedies. BMC Complement Altern Med 2007;7:1.
[48] de Camargo RA, da Costa ED, Catisti R. Effect of the oral administration homeopathic *Arnica montana* on mitochondrial oxidative stress. Homeopathy 2013;102(1):49–53.
[49] Sharma S, Arif M, Nirala RK, Gupta R, Thakur SC. Cumulative therapeutic effects of phytochemicals in *Arnica montana* flower extract alleviated collagen-induced arthritis: inhibition of both pro-inflammatory mediators and oxidative stress. J Sci Food Agric 2016;96(5):1500–10.
[50] Šutovská M, Capek P, Kočmalová M, Pawlaczyk I, Zaczyńska E, Czarny A, et al. Characterization and pharmacodynamic properties of *Arnica montana* complex. Int J Biol Macromol 2014;69:214–21.
[51] Castro FC, Magre A, Cherpinski R, Zelante PM, Neves LM, Esquisatto MA, et al. Effects of microcurrent application alone or in combination with topical *Hypericum perforatum* L. and *Arnica montana* L. on surgically induced wound healing in Wistar rats. Homeopathy 2012;101(3):147–53.
[52] Kučera M, Kolar P, Barna M, Kučera A, Hladiková M. Arnica/Hydroxyethyl salicylate combination spray for ankle distortion: a four-arm randomised double-blind study. Pain Res Treat 2011;2011:365625.
[53] Widrig R, Suter A, Saller R, Melzer J. Choosing between NSAID and arnica for topical treatment of hand osteoarthritis in a randomised, double-blind study. Rheumatol Int 2007;27(6):585–91.
[54] Brinkhaus B, Wilkens JM, Lüdtke R, Hunger J, Witt CM, Willich SN. Homeopathic arnica therapy in patients receiving knee surgery: results of three randomised double-blind trials. Complement Ther Med 2006;14(4):237–46.
[55] Lee HS, Yoon HY, Kim IH, Hwang SH. The effectiveness of postoperative intervention in patients after rhinoplasty: a meta-analysis. Eur Arch Otorhinolaryngol 2017;274(7):2685–94.
[56] Totonchi A, Guyuron B. A randomized, controlled comparison between arnica and steroids in the management of postrhinoplasty ecchymosis and edema. Plast Reconstr Surg 2007;120(1):271–4.
[57] Chaiet SR, Marcus BC. Perioperative *Arnica montana* for reduction of ecchymosis in rhinoplasty surgery. Ann Plast Surg 2016;76(5):477–82.
[58] Seeley BM, Denton AB, Ahn MS, Maas CS. Effect of homeopathic *Arnica montana* on bruising in face-lifts: results of a randomized, double-blind, placebo-controlled clinical trial. Arch Facial Plast Surg 2006;8(1):54–9.
[59] Simsek G, Sari E, Kilic R, Bayar Muluk N. Topical application of arnica and mucopolysaccharide polysulfate attenuates periorbital edema and ecchymosis in open rhinoplasty: a randomized controlled clinical study. Plast Reconstr Surg 2016;137(3):530e–5e.
[60] Sorrentino L, Piraneo S, Riggio E, Basilicò S, Sartani A, Bossi D, et al. Is there a role for homeopathy in breast cancer surgery? A first randomized clinical trial on treatment with *Arnica montana* to reduce post-operative seroma and bleeding in patients undergoing total mastectomy. J Intercult Ethnopharmacol 2017;6(1):1–8.
[61] Kang JY, Tran KD, Seiff SR, Mack WP, Lee WW. Assessing the effectiveness of *Arnica montana* and *Rhododendron tomentosum* (Ledum palustre) in the reduction of ecchymosis and edema after oculofacial surgery. Ophthal Plast Reconstr Surg 2017;33(1):47–52.
[62] Morris M, Pellow J, Solomon EM, Tsele-Tebakang T. Physiotherapy and a homeopathic complex for chronic low-back pain due to osteoarthritis: a randomized, controlled pilot study. Altern Ther Health Med 2016;22(1):48–56.
[63] Karow JH, Abt HP, Fröhling M, Ackermann H. Efficacy of *Arnica montana* D4 for healing of wounds after Hallux valgus surgery compared to diclofenac. J Altern Complement Med 2008;14(1):17–25.
[64] Kaziro GS. Metronidazole (Flagyl) and *Arnica montana* in the prevention of post-surgical complications, a comparative placebo controlled clinical trial. Br J Oral Maxillofac Surg 1984;22(1):42–9.
[65] Stevinson C, Devaraj VS, Fountain-Barber A, Hawkins S, Ernst E. Homeopathic arnica for prevention of pain and bruising: randomized placebo-controlled trial in hand surgery. J R Soc Med 2003;96(2):60–5.
[66] Robertson A, Suryanarayanan R, Banerjee A. Homeopathic *Arnica montana* for post-tonsillectomy analgesia: a randomised placebo control trial. Homeopathy 2007;96(1):17–21.
[67] Hart O, Mullee MA, Lewith G, Miller J. Double-blind, placebo-controlled, randomized clinical trial of homoeopathic arnica C30 for pain and infection after total abdominal hysterectomy. J R Soc Med 1997;90(2):73–8.