Reviewer’s report

**Title:** Immunomodulatory effects of preparations from Anthroposophical Medicine for parenteral use

**Version:** 1  **Date:** 6 September 2014

**Reviewer:** Ursula Wolf

**Reviewer’s report:**

Review manuscript: Immunomodulatory effects of preparations from Anthroposophical Medicine for parenteral use. Carsten Gründemann*, Christoph Diegel, Barbara Sauer, Manuel Garcia-Käufer, Roman Huber

General comments

The style of the manuscript is straightforward, the methodology and descriptive analysis sound, the results clearly stated and the conclusions are based on the results. Some additional information is desirable as indicated below.

Since it is beyond my expertise I cannot comment on the handling of the cells in the laboratory experiments.

The study should be particularly acknowledged not only because it is a high quality study but also because it contributes well to basic research in CM. Basic research facilitates to understand e.g. mode of actions and thus to advance knowledge which in turn enables further developments contributing to better treatments and patient care.

Careful, some characters can are not visible in the manuscript (see below)

Before the manuscript is ready for publication the following major and minor comments should be observed.

**Major comments**

**Abstract**

Mention that is an in vitro study

**Introduction**

Please give a reason why among the variety of anti-inflammatory drugs these were chosen

Please verify the correctness of this statement with respect to conventional medicine “Colchicum is a standard for gout treatment as well in conventional medicine as in AM”

“In vitro and in patients it has shown to modulate lymphocyte and monocyte function [4–6]…” Please be more specific, modulation with respect to what?

“Mandragora is used…..” Please adapt sentence, indications for Mandragora are mainly painful, degenerative and inflammatory disorders of joints and ligaments
but not mainly rheumatoid arthritis.

“Rosemary 5%.....” Please modify and mitigate this sentence. It is true that rosemary is used externally and internally in diabetic condition in AM; however claiming it as “promising treatment” in a scientific publication might be an overestimation. Also clarify whether the conclusion is based on Rosemary 5% or Rosemary bath.

While it is true that Bryophyllum 5% may be used in diabetic patients for a certain not necessarily diabetes related condition it is not the prevalent indication. Please provide more appropriate information.

“Other extracts of Kalanchoe....” , other to what?

Please explain why lymphocytes and DC among a large variety of immune-competent cells were chosen.

Material and Methods

Ethics statement

The information on having observed the Declaration of Helsinki is missing

Medication

“Rosmarinus 5% is derived from dried rosemary leaves which are put in boiling water (1:10). One ampoule (1ml) contains 0,5g of this rosemary tea and physiological saline.” Please describe the pharmaceutical process more clearly; in particular how the 5% are obtained. Also clarify what you mean by “tea” as this term is confusing and most probably not correct. Terms are extraction or extract.

Selection of human peripheral lymphocytes and purified CD4+ T cells

Please indicate whether the antibiotics used interfered with the immune-competency of the cells and/or the experiment and give a ratio for your answer/opinion

Results

Move first sentence “Biological....” in the M5M section as well as “Camptothecin....” as these are no results

“However, cells treated with different concentrations of Colchicum demonstrated that lymphocytes´ proliferation is strongly affected, whereas the presence of Rosmarinus just slightly diminished this parameter.” Please rephrase this sentence as it is not well to understand

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First sentence; was the result statistically significant

Injectable Rosmarinus preparation modulates cytokine secretion of dendritic cells

“But because we demonstrated an influence of Rosmarinus on dendritic cell maturation

we decided to further investigate its influence on dendritic cell function (Fig. 4).”

This procedure should have been mentioned in M&M. Otherwise it appears as an post-hoc analysis or provide a ratio in the M&M section why e.g. a step-wise approach was used
Page 12
“slightly inhibited…” and “affected”; be more precise in both

Discussion
“…had to fulfil all of the 3 following criteria:” Please explain why these were chosen?
“toxic solvents”; why should there be such solvents in registered medication. If
the authors had in mind that alcoholic extracts could damage the cells, then they
should modify the sentence accordingly
Also it is not well to understand why this “because they directly reach the
circulation and are bioavailable” should matter in an in vitro experiment
“In a first step toxicity of the used preparations up to undiluted concentrations
could be excluded.”; Please include (if possible) at some point in the discussion how
the concentrations used in the in vitro experiments relate to the ones used in
clinical application
“Colchicum did, however not exhibit immunomodulatory properties in human DC
in the tested concentrations.”; do these concentrations match the ones in the
ampoules?

Minor comments

Introduction
“They are clinically applied for the treatment of various diseases and we had in
our outpatient center which is visited by approximately 2000 patients with chronic
diseases per year, seen good single cases in patients with autoimmune diseases
during treatment with AM parenteralia.” Consider dividing this sentence into two
and correct the term “parenteralia”
Parentally applied instead of parental applied
Insert a sentence why lymphocyte viability as an outcome parameter was
selected
“Errant DC are, therefore, involved..”; consider deleting “therefore”

Material and Methods
Medication
“method 21…”, check whether term is correct
Delete “…of the parenteral preparations…” as ampoule is enough
“The mother extract is three times diluted 1:10 (D3) in physiological saline
according to the homeopathic pharmacopoeia. Mandragora D3 is an ethanolic
decoction of the Mandragora root. The mother extract is defined as D1 and two
times diluted 1:10 (D3) in physiological saline.” Provide an equal description as in
the way it presented it might confuse the reader
“......anthroposophical medications or controls as indicated”, >>> anthroposophical test medications or controls as indicated

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Data analysis instead of Analysis of data

“For statistical analysis, data were processed with Microsoft Excel and IBM SPSS software

20.0.”; include in “(“) the lieu

Results

In the pdf version some characters (e.g. alpha) are missing and/or replaced by others it appears as TNF-, LPS and IL-1, CD14

Discussion

“So it can be explained, that these preparations are clinically used for the treatment of inflammatory disorders but not yet have been tested regarding effects on cells of the human immune system.” >>> Thus it can be explained, that these preparations are clinically used for the treatment of inflammatory disorders but have not yet been tested regarding effects on cells of the human immune system.

randomly instead of arbitrarily

“key player”; consider term

References

Please give in brackets an English translation of the German references.

Also include English references if possible, as for e.g. the Weleda compendium

Ref 3, add number of edition and page number. Also delete colon

Ref 12 Carnisol not bold

References referring to websites, please include when they were last accessed

Captions

Some characters are not visible (see above)

Figures

1+2 the units on the y-axis are not visible as well as the information below the x-axis except the words of the test substances in the very last line

3 and 4 some information on top of the bars are not visible

Level of interest: An article of importance in its field

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.