Reviewer's report

Title: Ameliorating effect of microdoses of a potentized homeopathic drug, Arsenicum Album, on arsenic-induced toxicity in mice

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Reviewer: Fred Wiegant

Level of interest: A paper whose findings are important to those with closely related research interests

Advice on publication: Accept after discretionary revisions

Discretionary revisions

In this paper, Mallick et al studied whether intoxication with arsenic trioxide can be ameliorated when oral administration of arsenicum album D30 or D200 are administered in mice intoxicated with arsenicum. In a well-controlled study the authors report some interesting data.

1. Is the question posed by the authors new and well defined?

The question that the authors of this group asked in this paper is not new. The group leaded by Khuda Bukhsh asked the same and similar questions in previous publications, but then the effect of homeopathic potencies on different parameters were reported. Also other (mostly French) research groups asked the same question (effect of homeopathic potencies of arsenic on arsenicum-intoxicated mice) in the seventies and eighties of the previous century.

2. Are the methods appropriate and well described, and are sufficient details provided to replicate the work?

The authors are not clear in describing the exact treatment protocol nor on how the potencies were produced. This part should definitely be clarified. Currently, it remains unclear how intoxication takes place. Is this occurring only once at the beginning of the experiment? Are mice chronically intoxicated and treated with homeopathic potencies at the same time? Or are mice first treated with potencies and subsequently intoxicated? Nor is it clear when homeopathic potencies are applied. Before intoxication, after intoxication, during chronic arsenite intoxication?

It also remains unclear whether the potencies are injected, put in drinking water, or pored on top of the food. The authors do not state this. Furthermore, how are the potencies prepared? By vigorous shaking with the hand, machine-made, is it by vortexing? and then how long?

The authors do not give a clear rationale for some Materials and Methods. For instance:
- Why did the authors select 6 time points at which fixation took place?, what was the rationale for
having different periods before fixation? Were these time points selected based on previous studies? (probably yes). The term 'fixation intervals' is not appropriate. I do not suppose that they fix everything at time point 0 and then refix the samples again at different moments in time for analysis. 'Interval before fixation' seems more appropriate.

-why did the authors use D30 and D200 (whereas in previous publications of this group C30 and C200 potencies were used).

Without any justification for these choices, the reader might get the impression that the choices were made as 'shots in the dark', and find out that the authors luckily hit some target.

3. Are the data sound and well controlled

Performed experiments seem to be sound and contain many controls.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?

The main problem is that the authors have a large amount of data to show and are a bit sloppy in describing the data. Thus, the description of the data is not done with clarity.

Three examples:
- ALT activity in blood
  In the second sentence the authors state: 'In As+D-30 group of mice, the enzyme activity was found to decrease appreciably (p-value ... etc) at all the fixation intervals'. However they do not say in comparison with what: the As-treated group or in comparison with the Dw control? Moreover, it is not true: the As+D-30 group is not decreased at 24 hours. At that time it is even increased with respect to Dw as well as with respect to the As-treated group.

GSH level in liver
  In the second sentence the authors state that the GSH level was increased at all fixation intervals. I do not understand why they talk about an increase whereas in essence it is a prevention of the As-induced decrease. In terms of regulation this might be a completely different mechanism and it is thus important to be precise in the description of the data.
  Some sentences down they state that 'the maximum level of decrease was effected in A-200 fed series'. However, according to the graphs they show (figure 5) it is in the A-30 fed series in which the maximum level of decrease is obtained.

GSH level in blood
  The authors describe a 'spectacular rise in GSH-level at all fixation intervals'. However there is no rise, there is a (spectacular) prevention of As-induced decrease in GSH-levels.
  In the last sentence of this text block, the authors describe 'an appreciable amount of decrease in GSH level was noted in As + A-30 or As + A-200 group at most fixation intervals, as compared to that of only A-30 or A-200 series'. However, what really happens is that A-30 and A-200 do not prevent the As-induced decrease in GSH levels. But more importantly from their data one can observe an initial stimulation or an enhancement of the As-induced decrease leading to a significant lower level at 7 days in blood. (a similar tendency is observed in liver). Unfortunately, this is not described nor discussed by the authors.
  This result might indicate the danger of using alcohol as vehicle for arsenic potencies. Apparently the vehicle, as a potency, might even enhance the toxicity of arsenic. The authors should describe and discuss this observation, also in relation to the fact that they recommend the potencies for human use. Might the use of alcohol as a vehicle not be an important drawback?

The authors are strongly urged to check the description, since at this moment a negative impression is obtained of the way their data are described.
In its current state the authors should realize that it takes time before the reader understands what has been done and what are the relevant lines or histograms to look at. Even the authors were apparently confused and/or overwhelmed by all the graphs, since figure 3 was also submitted as figure 4. Thus, figures 3 and 4 are now identical (AST activity in liver). The authors should submit the data of AST activity in blood as figure 4.

5. Are the discussion and conclusions well balanced and adequately supported by the data?

The current discussion is too much focussed on the effect of potencies on gene expression. This is strange since the authors did not study nor show effects on changes in gene expression. Only changes in the activity (not levels) of enzymes are described as well as changes in the levels of GSH. In principle, these activities and the GSH-level may well change without any alteration in the expression of the genes involved. The activity can be regulated for instance by phosphorylation or by the presence or absence of SH-groups, etc. Processes that may be affected by intoxication or possibly by homeopathic potencies. Therefore the lengthy space that is now dedicated to the discussion how gene expression may be affected by the potencies might be completely irrelevant to explain their data. The authors could better discuss or speculate how changes in the activity of enzymes may be affected by homeopathic potencies.

The main point of criticism is that the discussion is not focussed on the discussion of the reported results.

In the discussion the authors fail to compare and discuss their results with the results obtained in all previous studies using the same protocol (including the French studies) and thus do not take the opportunity to advance the (homeopathy-related) knowledge in this field.

Further homeopathy related issues could be discussed based on their results. For instance:  
- The authors chose to study the effect of two potencies of arsenicum album (D30 and D200). Both potencies are diluted beyond Avogadro's number. However they do not discuss the difference in effect between these potencies when applied to intoxicated mice. When they select two compounds, they should also discuss the similarities and differences between these two compounds. Why did they select these two potencies in the first place? According to homeopathy, the D200 should be more effective, since higher potencies are predicted to be increased in 'strength'. This is not observed in this study. The authors should at least state that based on their results they could not confirm any major difference in effect between D30 and D200. In case they do not want to discuss the similarities and differences, then the paper would improve in clarity and size if the data of either D30 or D200 are skipped altogether.

- Why did the authors not speculate on why A30/A200 is more toxic in the combination with Arsenic on GSH levels? Why do they emphasize the positive effect of the D-potencies, but not the negative effect of the A-potencies?

- What in their experience is preferred, the C-potencies which were used in their previous studies or the D-potencies which were used in this study?

These are the type of questions that might be relevant to include in the discussion. At least researchers in the field of homeopathy might benefit from the opinion in this respect of the group leaded by Khuda Bukhsh who has over the years accumulated a large expertise in this field. Unfortunately, the authors do not show any initiative in this direction.

6. Do the title and abstract accurately convey what has been found?
Sufficient

7. Is the writing acceptable
Yes

**Competing interests:**

None declared.