Author’s response to reviews

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

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To
Ms Emma Veitch 12.07.03
Assistant Editor,
Biomedcentral, Middlesex House,
34-42Cleveland,
London W1T 4LB
Sub: Submission of revised ms for publication in Journal of BMC Compl. & Alt. Medicine

Dear Ms Emma Veitch,
I am submitting herewith electronic version of our revised ms entitled "Ameliorating effect of microdoses of a potentized homeopathic drug. Arsenicum Album, on arsenic-induced toxicity in mice" by P. Mallick, et al for favor of publication in your esteemed journal, BMC Compl. & Alternative Medicine. We have revised our paper in the light of suggestions and advice made by the three hon'ble reviewers.
We enclose a point by point answer to the queries made by the hon'ble reviewers and put our response and action in the following for your kind perusal.

With kind regards,
Yours sincerely,
A.R. Khuda-Bukhsh

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Reviewer No. 1. Dr. Philippe Belon.

We are extremely grateful and thankful to Dr. Belon for his kind and encouraging remarks on our works. Yes, we appreciate receiving several citations; a few relevant articles have now been cited in the text as additional references.
e.g. Cazin et al., 1987; Rey, L. 2003.

Reviewer No. 2. Dr. Fred Wiegant.

We are very much thankful to Dr. Fred Wiegant for his careful scrutiny of the ms and his constructive criticism. We have modified the ms by taking almost all of his suggestions which are as follows:
1. "Other French group asked the same question of effect of homeopathic potencies of arsenic on arsenicum-intoxicated mice": Yes, we have now included the reference and the work of the French group (Cazin et al., 1987). Others have worked on Acetyl Salicylic Acid on arterial thrombosis.

2. "The authors are not clear in describing exact treatment protocol nor how the potencies were produced.......pored on top of the food": We have clarified more on this point and made the description take care of all the points. Further, how the potencies were prepared have been elaborated.

3. Why the six time points selected? This has been answered in the Materials and Methods in part and in the Discussion in part.

4. Why did the authors use D-30 and D-200? We have clarified and have now used Ars Alb-30 and Ars Alb-200 in stead of using D-30 and D-200 which made confused reading.

5. The description of data is sloppy and not done with clarity in some cases: three examples: ALT activity in blood, GSH in liver and GSH in blood. Yes, indeed we appreciate the suggestion of the hon'ble referee and quite agree that the description needed a better deal with regard to some mistakes that inadvertently crept in the description, like Figure no., or a statement of fact. This has now been checked and rectified and the expressions like "suppression of increase:", or "intervals before fixation" or simply "fixation times" have been used instead , as suggested by the hon'ble referee.

6. "The current discussion is too much focussed on the effects of potencies on gene expression": We appreciate the suggestion of the hon'ble referee on this count and have now extended the discussion by comparing our results with that of other workers who worked on removal of arsenic intoxication. But to discuss how the enzyme activities may be affected by homeopathic potencies so much diluted, the discussion seemed to us to be necessary and relevant. We quite agree with the hon'ble referee that events like phosphorylation/dephosphorylation or the presence or absence of SH-groups etc. may affect enzyme activity. But then, the enzymes like tyrosine kinase or phosphatase would be necessary for any phosphorylation/dephosphorylation event to occur, and whenever the conditions will arise when phosphorylation would be necessary, there must be the synthesis (for supply) of the enzyme (kinase or phosphatase) which is under the control of specific genes. We are not in a position to suggest that any specific particle from the homeopathic drug itself could directly control the phosphorylation event by tagging and carrying the PO4 group or SH-group itself. Further, we have recently measured the expressions of two specific genes in mice (under induced carcinogenesis) by immuno-blot (Western) experiments, where in our initial studies, we have found that the gene products of the genes p53 (tumor-suppressor gene) and Bcl2 (apoptotic genes) have indeed been positively influenced by the potentized homeopathic drugs diluted much above Avagadro's limit! Several repeat experiments are underway at the moment. Therefore, we like this view or hypothesis to be under attention of other peers in this area simply as another line of thinking apart from the others that currently exist.

7. The two potencies, 30th and 200th, are the ones used in India very commonly. Yes, our studies did not demonstrate that the 200th potency was always superior in strength, but then we have only mentioned what we observed in our study. Now we have clearly mentioned this (in discussion).

8. Positive effects of D-potencies vis-a-vis negative effects of A-potencies have now been mentione more clearly.

Reviewer No. 3. Dr. W.B Jonas

We are thankful to Dr. Jonas for his first hand queries and comments and our responses to these are as follows:

1. How can one make a "tincture" out of a metal? Yes, it is quite possible as Arsenic trioxide is a powder soluble in water as well as in alcohol. Some metal compounds are indeed soluble in water or alcohol while the metal itself may not be soluble in them.

2. What force are the "jerks"..... Information available to us on potentization has been extended to cover some aspects. We have given the full address of the source (manufacturer of the drug) from where an interested reader can readily get the full information.

3. What is exact composition of rectified spirit? The rectified spirit contains approx. 90% alcohol and
the rest is water without any other known impurities or contaminants.
4. If the mice were orally fed...got? It has been mentioned that the mice were orally administered the exact amount of the drug or alcohol or water, as the case may be, by force feeding (putting the drop over its tongue deep inside the buccal cavity) with a suitable pipette.
5. What is blind checking method? It has been clarified now.
6. The frequency of dosing: Has been made easier to follow.
7. The statistical analysis: We have clarified on this point that we have only done the "t" test between two groups of experimental mice at a time, say between the drug-fed and the dw fed mice, between the drug fed and the alcohol fed mice, etc.
8. I think AST and GSH levels were measured in the blood, yet they are reported for both liver and blood. The activities of AST, ALT and GSH were measured in both blood (peripheral) and liver, separately, and clearly described like that.
9. In the present investigation, examination of MT proteins was not within the purview, partly for lack of infra-structural facilities and partly for paucity of funds.
10. Using genes to..... We have restricted ourselves to explaining the molecular mechanisms of action of potentized homeopathic drugs mainly within the animal subjects in this paper, leaving the other physical aspects. Several physicists proposed several mechanisms to explain how the medicinal properties can possibly be transferred to and retained by the "vehicle". However, this being an important issue and a part of the holistic mechanism of action, has been dealt with more elaborately by the corresponding author in a forthcoming review article to be published in the next issue of the mainstream journal, Molecular and Cellular Biochemistry, Kluwer Academic Publishers.
11. How do the authors know what there is in the brownies? We did not use the "brownies". We used the liquid form of the potentized drugs.
12. The figures are very confusing.... Combinations of bar and line graphs have been preferred to give the readers an opportunity to readily compare one data group with another in the manner one may desire to do it, as the data are quite extensive.