Author's response to reviews

Title: A 90 day chronic toxicity study of Nigerian herbal preparation DAS-77 in rats

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RESPONSE TO REVIEWER'S COMMENTS

Reviewer #1

1. The identity of the constituent plants, the form of the product and the fact that it was constituted in distilled water was initially mentioned in the manuscript under methods (herbal product). The issue of yield is not relevant in this case but the ratio of the two plants has now been included.

2. The fact that animals were inbred Wistar rats and mice has been mentioned under methods (experimental animals).

3. It was possible for us to administer the test product at the dose of 2000 mg/kg. The volume administered was determined by the concentration of test product solution that we prepared. For example, considering the dose of 2000 mg/kg, animal weight of 150 g and test product concentration of 300 mg/ml, the volume for administration will be 1 ml which can even be given to rats in divided doses.

4. The essence of the reversibility study was to determine if the effects induced by the test product will be reverse upon cessation of treatment for some time. In other words, were the effects reversible or irreversible?

5. We have tried as much as possible to limit the number of Tables and Figures in the manuscript hence the approach we have adopted. In any case the results displayed in the Tables and Figures have been extensively described in the result section.

6. The issue of reproducibility is germane in the communication of research findings. In respect of the acute toxicity study as concerns the i.p. route, we mentioned under methods (acute toxicity study) that the test herbal product was administered at doses of 250, 500, 1000, 2000, and 3000 mg/kg. We also mentioned under result (acute toxicity) that mortality was 0% and 100% respectively at the extremities of doses, 250 and 3000 mg/kg. These are concrete data that guarantee reproducibility.
7. Values with signs of statistical significance as explained in legends are simply significant while those without such signs are simply not significant. We have reported precisely what we obtained in our statistical analysis.

8. The method of protein assay has now been mentioned to be the Biuret method under method (Measurement of in vivo antioxidants and malondialdehyde (MDA) levels) and references have been updated accordingly.

9. It is our opinion that the issue of antioxidants has been adequately explained under discussion section. In respect of Table 9, results of experiment and statistical analysis have been displayed as obtained. The activities of CAT, SOD and peroxidase were indeed lower relative to control at the 400 mg/kg dose in respect of rat brain but there was no statistically significant difference. The units are correct as displayed. The issue of main and reversibility study has been explained in section 4 above and that of Tables being too complex has been addressed in section 5 above.

10. We used animals that were available to us at the time of conduct of this experiment. What we did to reduce sex oriented variability is to ensure that we had equal number of male (14) and female (6) rats across all the groups. In respect of male rats, what we found and have reported is the reduction in sperm count and motility and increases in sperm abnormality at the highest dose of 2000 mg/kg. These effects were however reversed upon cessation of treatment for one month.

Reviewer #2

Minor Essential Revisions:

1. The title of the manuscript has been changed to “A 90 day chronic toxicity study of Nigerian herbal preparation DAS-77 in rats” as can be observed in the title page.

2. The proportion of the constituent plant materials of the herbal product has been mentioned under methods (herbal product).

Major Compulsory Revisions:

1. The assertion of CNS stimulatory activity as a result of behavioural manifestation in the acute toxicity study for the i.p. route has been deleted in the discussion section.

2. Data on body weight of rats, food and water intake were actually collected every week till the 90th day but no dramatic observations were made hence the presentation of the results as a point data taking into consideration the average of the respective data over the different weeks. The results for change in weight, food and fluid intake have been presented in one table so as to keep the overall number of tables as minimal as possible.

3. We have reported precisely our observation from the results obtained in the study. One cannot say categorically that it is impossible for the lower dose of 400 mg/kg to boost the immune response and for the higher dose of 2000 mg/kg to suppress the immune response. The product under investigation is herbal in nature and we are talking about in vivo experiment not in vitro.
4. The explanations given in 3 above suffice. Experimental and statistical analyses have been displayed as obtained. Again we are dealing with herbal product here not pure compound, and in vivo experiment not in vitro. Establishment of time-effect relationship was not part of the scope of our thinking in the design of the experiment and you will agree with me that references that are in agreement with what we have done abound.

5. Increase in the level of ALT and AST indeed may reflect damage to the liver. Our explanation has been made clearer in the discussion section that reduction in the level of these parameters may suggest a potential for hepatoprotective action which need an independent study for probing the herbal product in this direction. The result is certainly not from experimental error as being suggested.

RESPONSE TO EDITORIAL REQUESTS
The observations have been not and applicable corrections effected.

In general, corrections in the manuscript are reflected in green text.