Reviewer's report

Title: The safety of Homnawkod herbal formulary containing Aristolochia tagala cham. in Wistar rats

Version: 2 Date: 22 May 2012

Reviewer: Scott Jordan

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Major Compulsory Revisions:

1) The study described in this paper should have included a positive control group of animals administered pure AAI/II. This would have ensured that conclusions could have been made with respect to the appropriateness of the model used. This is important since no toxic effects were observed in this study, at least in the parameters investigated. It would have been important to observe if pure AAI/II induced toxicity or not, in this model.

2) In the Materials and Methods section, it is noted that the plant material was authenticated by two practitioners. There needs to be a description of how this authentication was done: were all 56 ingredients of HNK authenticated? How was each ingredient authenticated (macroscopic/microscopic/organoleptic, etc.)?

Minor Essential Revisions:

1) The English Usage in this paper is fairly good; however, some editing is required. The term "formulary" used in the paper should actually be "formula" (singular), or "formulae" (plural). The authors should be careful to define all acronyms upon the first use, and to use the acronym consistently after that point.

2) In the Discussion section, the authors need to discuss the differences in the UV spectra between the ATC, the HNK+ATC and the HNK groups that are noted on page 10. The results are noted, but no explanation is provided as to why these differences should have been produced.

3) A discussion should have been included as to how the results of this study compare to the other literature in animals given pure AAI/II or extracts of plants of the genus Aristolochia. Without the above mentioned positive control, such a comparison is very difficult; however, there needs to be some discussion as to whether the 21-day test period was appropriate to observe potential renal toxicity, and whether the biomarkers used would have detected renal toxicity after 21 days of exposure. For example, would histopathology have been a better method to detect early renal toxicity associated with low levels of AA?

4) Abstract: Here, one of the groups mentioned is "HNK containing 10 and 30 mg/kg ATC;" however, earlier in the paragraph, it is noted that HNK contains ATC. Did the authors mean to say that the actual dose group was ATC-removed
HNK with 10 and 30 mg/kg ATC added? Also in this section, HNK is defined as both "Homnawakod herbal formulary" and "Homnakawod Ayurved Siriraj herbal formulary." Later in the paper "Homnakawod" is used. A single name should be used. As previously noted, the term "formula" should be used here, not "formulary."

5) In the figures, there needs to be a footnote to describe what the star symbol represents.

6) On page 11, section 3.5, “Figure 5” should be changed to “Figure 7.”

7) The authors ultimately conclude that the quality of the original and modified HNK was similar, and that both produced no adverse effects in the model used. Given that AA has been identified as a genotoxic carcinogen in animals and humans, do the authors think that the modified HNK would be preferred over the original formula which contains ATC, especially since they believe that both formulae are equal in quality? There should be some discussion of this.

Discretionary Revisions:

It is suggested that in the introduction, the long listing of ingredients in the formula be removed and put in a table. This would be much clearer to the reader and make this section much more readable.

**Level of interest:** An article whose findings are important to those with closely related research interests

**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.

**Declaration of competing interests:**

I declare that I have no competing interests.