Reviewer's report

Title: MAK-4 and -5 supplemented diet inhibits liver carcinogenesis in mice

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Reviewer: Maria Marino

Reviewer's report:

General

In the present study the Authors evaluated the cancer inhibiting effects of two herbal mixtures, MAK-4 and MAK-5, in vitro and in vivo. Furthermore, the ability of MAK-4 and MAK-5 to scavenge free radicals and reactive oxygen species (ROS) and regulate connexins, markers of tissue integrity and tumor formation, have been also investigated.

The cancer inhibiting effects of these herbal mixtures has been studied in quite few papers, but the main originality of this manuscript concerns the confront between in vivo and in vitro results.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. The Authors stated in methods (pg. 9) that the mice/group were fed a pellet diet containing: MAK-4 (4%, w/w) MAK-5 (1%, w/w). It is not clear if the final animal pellet were obtained with the herbal mixtures or with the aqueous extracts of MAK-4 or MAK-5. If present, this difference could explain the divergent effect of MAK-4 in cancer growth in vitro vs in vivo experiments.

2. The Author indicate in the introduction and in the discussion that multiple antioxidants are present in MAK-4 and MAK-5 (i.e. alpha-tocopherol, beta-carotene, ascorbate, bioflavonoid, catechin, polyphenols, riboflavin, and tannic acid), but the water solubility of some of these substances is very low. In the absence of a precise antioxidant content the Authors should be cautious about generalizing.

3. MAK-4 seemed to enhance cell transformation only in transformed cells, whereas it showed a decrease in % tumor positive mice and in nodule number/mouse (see fig.1). This discrepancy should be discussed in the discussion.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Table 1 change comma with dot. In the legend of this figure the Authors stated that the relative number of foci is the number of foci obtained from the treatment groups divided by those obtained from the no-treatment group, but 13.0/15.3 is 0.84 not 1.54.

The statistical analysis (Two Way ANOVA Test followed by Post Hoc Bonferroni analysis) should be inserted in all figure legends. Some data does not seem significant with respect to the control (figure 1 panel B, figure 3 panel A, QR), thus the use of letters instead of asterisks could better indicate the significant difference presented.

Discretionary Revisions (which the author can choose to ignore)

Tables 1, 2, and 3 could be pasted.

What next?: Accept after minor essential revisions

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

Quality of written English: Acceptable
**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

'I declare that I have no competing interests'