Author's response to reviews

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

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Author's response to reviews: see over
To the Editor of
BMC Complementary and Alternative Medicine

Dear Editor,

Please find enclosed manuscript of:

**MAK-4 and -5 supplemented diet inhibits liver carcinogenesis in mice.**

By
Marialetizia Penza, Claudia Montani, Marija Jeremic, Giovanna Mazzoleni, W.L. Wendy Hsiao, Maurizio Marra, Hari Sharma and Diego Di Lorenzo

The work was reviewed according to the reviewers’ comments and questions, to which we have thoroughly answered.

**Answer to Rewiever 1**

**Question 1:** MAK-4 and MAK-5 were added to the pellet mixture as they are commercially provided. No extraction. Now this is indicated in Materials and Methods section, on p.9

**Question 2:** A sentence stating that the water solubility of the compounds present in MAK-4 and MAK-5 is unknown, was added at page 20 and in the answer to Question 3, below.

**Question 3:** The discrepancy between the *in vitro* and *in vivo* data, with regard to their anticarcinogenic properties, may be due to the fact that the *in vitro* experiments were performed with a water soluble fraction of MAK-4 and –5. Extraction may have out selected fractions of several chemicals that are important for the activity of MAKs for which the relative solubility in not known. This was added to the discussion, p.20.

**Minor essential revisions:**

**Table 1:** Done

**Statistical analysis:** We have double checked The statistical analysis and it is correct. We have added figure legends with the requested definition. We prefer to leave the asterisks as indicator of significant differences. The significativities that were between * p < 0.05 and ** p < 0.001 are now reported as * p<0.05.
Question 1: Carcinogenesis is a multistep process and the molecular changes that underpin tumor formation have been shown to involve activation of ras in different tissues of humans and animals (Feitelson MA, 2004; Benvenuti S, 2007). This knowledge lead to the establishment of models suitable for application to drug discovery cancer prevention studies (Dragani, 1991; Tsuda H, 2001). The following sentence and new references were added at the bottom of page 4: “Molecular changes that underpin tumour formation have been shown to involve activation of ras in different tissues of humans and animals (17, 18, 19, 20”).

Question 2: In our experiments we used MAK-4 and MAK-5 alone as well as in combination. The reason for using them in combination is based on suggestions from traditional medicine (Two reference added: 3 and 4), that indicate their complementary effect. No data about the biological reasons of their combinatorial effect are available.

Question 3: What makes the difference in using compounds of traditional medicines versus drugs, is that these natural plants preparations are complex mixtures of chemicals and phytochemicals, whose single effects are mainly unknown. Although several compounds contained in the plants used in MAK-4 and -5 have been characterized by modern chemistry and used by modern medicine, most of them are of unknown effect. Thus, it is very hard – if not impossible – to tell which ones of the chemicals present in these preparations are the most active. These are crude preparations containing thousands of chemicals. Fractionation can eventually be object of future work within our activity – which is ongoing in regard to the matter – at the Laboratory of Biotechnology in Brescia.

Minor essential revisions:

Reference no 54 (now 59): Done.

We look forward to receiving your reply.

Best regards,

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