Reviewer's report

Title: Anti-tumor effect of Liqi, a Traditional Chinese Medicine prescription, in tumor bearing mice

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Reviewer: József Molnár

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Many herbal extracts used in the Chinese clinical practice are able to inhibit the growth of tumor cells. In the present work, extracts of mixtures of 4 selected herbs were prepared with boiling water and administered to mice bearing xenografts of different tumor cell lines. The antitumor effect was demonstrated by various methods including cytotoxicity, immunological and physical methods e.g. electric impedance. The idea and structure of protocol is properly worked out based on a special kind of formal logic. As an initiative authors collected publications on the effect of 'liqi' in vitro to define the main aims of the in vivo study.

If liqi prescription is from TCD, authors should mention the source by reference. By quoting the already known in vitro effects, the system was ready for screening anticancer effects of Liqi, a Traditional Chinese Medicine preparation in in vivo studies. In this aspect Deng-Bo Ji’s paper has a good perspective.

The Ms entitled „Antitumor effect of Liqi, a Traditional Chinese Medicine prescription, in tumor bearing mice” was revised in details. Some questions could be clarified.

Authors’ idea was good for the study but the presented methodology needs more details. Details of chromatography and references are not included, chromatography data are not presented to give evidence that the hypothesized flavonoids and terpenoids or saponins can be found in the hot water extracts or decoctums, since these compounds have poor water solubility. Consequently, authors measured the effect of water soluble compounds if the decoctum was dissolved in distilled water? The description of the applied main biological methods, from preparation of liqi, tumor cell culturing and cell cycle analysis, platelet aggregation assay needs some further details. Authors have to revise the concentrations e.g. RNaseA 200 mg/ml?? or propidium iodine final concentration 100 mg/ml???.These concentrations are extremely high!. The flow cytometry of T lymphocyte subsets also need more detailed description by giving proper information for readers to repeat the experiments.

Since the mice were treated with 50g/kg liqi during the 12 days, which is great dose, however, authors could reduce the doses and increase the biological effect if they change the route of the of administration: apply intravenous, or subcutaneously given injection for comparison.

The evaluation procedure, including dose dependence and untreated controls
(called „models”) needs some re-evaluation by using the unpublished results of authors’ experiments if there are. The reason of my suggestion is that the tumor growth reduction was close to 30% or 40% in the liqi-treated animals, but there was no significant difference between the body weights of liqi-treated and non-treated groups.

Some data, probably measured by the authors are missing for comparison e.g. the preliminary toxicity or the suggested therapeutic window.

The effect of liqi on T-lymphocyte subpopulations, IL2 activity, NK cell activity, metastasis formation, platelet aggregation and thromboxane level give supporting evidences for the immune-modulatory effects of liqi on tumor bearing animals.

In case of the discussion of TNF interference with LPS induced TNF alpha, authors should take into consideration the direct electron charge transfer complex formation between LPS and flavonoids or terpenoids. From the convincing discussion, the same logic leads the reader the final evaluation of experimental results. However, the mentioned „systemic immune suppression” is more complex than changes in T subpopulations and NK cell activity.

Authors may find references, on the role of macrophages, cytokines and enzymes as mediators of invasion. The Figures need legends to explain briefly what is the difference between the columns. Instead of using the word „model”, authors should write untreated control of tumor transplanted animals. The word „Normal” used in some figures probably means healthy animal without tumor xenograft for the comparison.