Reviewer's report

Title: Fuzheng Huayu recipe alleviates hepatic fibrosis via inhibiting TNF-alpha induced hepatocyte apoptosis

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Reviewer: Ling Yang

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In this study, Dr. Cheng-hai Liu and his colleagues explored the effects of FZHY on hepatocyte apoptosis in acute and chronic liver injury induced by CCl4 in vivo and in vitro. The effects of FZHY on the activation of hepatic stellate cells was also observed in the chronic liver injury induced by CCl4. Hepatocytes apoptosis was assayed by TUNEL staining, flow cytometry and DNA ladder. In vitro study, they creatively employed serum containing FZHY to stimulate hepatocytes and stellate cells, which excluded the toxin effect of the impure components of FZHY. From their study, they found that FZHY attenuated hepatocyte apoptosis with the down-regulation of TNFR1 and bax, alleviated liver injury and hepatic fibrosis induced by CCl4. In vivo study they found that the DNA of TNF-alpha/Act D treated hepatocytes could stimulate HSCs activation and the DNA of FZHY treated hepatocyte suppressed HSCs activation. This work showed a new method to study the mechanism of traditional Chinese medicine. The data control was well setup. The method is also well described in detail. Here are still some points needed to further prove:

1. From the acute liver injury study, FZHY attenuated hepatocyte apoptosis, while the ALT,AST level (the serum marker of liver injury and apoptosis) had no decrease, whether FZHY had effects on liver inflammation? Or how about other inflammation markers (TNFa, IL-1b, etal) expression? Or CCl4 induced necrosis and FZHY could not prevent necrosis induced by CCl4? Cell death as the initial step for liver inflammation is important liver fibrosis. In the discussion the author also wrote “excessive hepatocyte apoptosis is thought to lead to liver dysfunction and damage in a variety of liver diseases ” and “FZHY could protect hepatocytes from apoptosis and necrosis in acute liver injury induced by LPS/D-GalN. So did FZHY has the same or different effect in the liver injury model induced by CCl4?

2. In vitro study, did the author measure the level of LDH or ALT in supernant? Did they have difference?

3. Apoptosis has two pathway: the mitochondrial pathway and the death receptor pathway. In this study the author measured the expression of TNFR,Bcl2,Bax. Whether FZHY has suppress both pathway? Other markers such as FAS/FASL,caspase-3,cleaved-caspase3,caspase8 should be measured to clarify these question.

4. The legends did not match the figures: there are only five legends but 12 figures.