March 20, 2020

Dear Drs. ***,

Thank you for considering our article, “Gan Shen Fu Fang ameliorates liver fibrosis via inhibiting the inflammatory response and reducing ERK phosphorylation in vitro and in vivo”. We appreciate the comments from both reviewers, and made appropriate changes in the manuscript. Below, please find a point-by-point response to the reviewers’ comments.

Please do not hesitate to contact me if you have any further comments.

With best regards,

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Reviewer #02549888

Question 1: Title: Needs to be modified.

Response: We agree the reviewer and have revised the title as “Gan Shen Fu Fang ameliorates liver fibrosis in vitro and in vivo by inhibiting the inflammatory response and ERK phosphorylation” on the updated manuscript with red font.

Question 2: there are quite a few language errors which need correction.

Response: Thanks a lot. We have polished the language in the context of the manuscript on any further.

Reviewer #00030389

Question 1: They should cite the manuscript "Du QH, Tang YB, Li WH, Xu Y, Han L, Jia X, Zhao T. The protective effect of Gan Shen Fu Fang on liver endothelial cells in common bile duct-ligated rats. World J Tradit Chin Med 2017; 3:21-5".

Response: Thanks for your suggestion, and we have cited this paper as reference 17 on the updated manuscript.

Question 2. Whether is GSFF cytotoxic for HSC-T6 cells or inhibitory of cell growth? Does GSFF inhibit the viability of cells other than HSC-T6 cell, such as hepatocytes or primary cultured hepatic stellate cells?

Response: In order to answer this question, we'd like to show some additional data. At the beginning of in vitro experiments, we have referred some references to determine the dose of GSFF and designed a wide range of dose, from 0.1～100μM (Table 1). The CCK-8 assay was performed to test cell viability. HSC-T6 cells were seeded in 96-well plates at 5×10^4 cells. ml^{-1}. After 24 h, the cells were treated with different concentrations of GSFF for 24 h. Subsequently, 10 μl of CCK-8 reagent was added to each well, followed by incubation at 37°C for 2 h. The original data is in Table1. The result is also showed in scatter diagram (Figure 1).

As showed in figure 1, we can see that more than 50μM (such as 100μM) of GSFF inhibits cell viability, which means such concentration of GSFF is cytotoxic for HSC-T6 cells. However, 1-50μM of GSFF increases cell viability. Less than 1μM of GSFF (such as 0.5, 0.1, 0.01) also inhibits cell viability. This is not because of cytotoxicity, but due to reduced ERK phosphorylation or other mechanisms. Regarding, we select 0.5, 0.25, 0.125μM to evaluate the effect of GSFF on HSC-T6 in the presented study. Moreover, to ensure the safety of such dose, we also check some related papers. For example, Zhang *et al.* examine the effect of salvianolic acid B (SA-B) on primary rat hepatic stellate cells and the dose they used is 1μM. Glycyrrhizin has similar effect as

diammonium glycyrrhizinate (DG)[1]. Yang *et al.* test the effect of Glycyrrhizin on hepatic stellate cells proliferation and the dose they used is 1-1000μM[2]. Therefore, based on our experiment and other researcher's study, the dose we use in this study is safe.

In conclusion，GSFF is cytotoxic for HSC-T6 cells when the dose is more than 50μM. The dose we use in this study is safe. Because of the limit of time, we have not examined the effect of GSFF on other cells.

Table 1：The original data of CCK8 assay

GSFF (μM)	100	50	10	5	1	0.5	0.1	0.01	0
OD	1.5165	1.3013	1.6277	1.3843	1.7693	0.9477	1.5677	1.4078	1.5272
OD	1.3990	1.6261	1.7619	1.7674	1.5825	0.8974	1.4130	1.3429	1.3145
OD	0.9119	1.4038	2.2261	1.7017	1.4364	1.4882	1.4671	1.4311	1.4223
OD	1.0457	1.6231	1.9180	1.8960	1.3038	1.4362	1.2818	1.1911	1.3235
OD	0.9106	1.3793	2.0546	2.1748	1.3171	1.1900	1.3484	1.6900	1.4330
OD	1.1440	1.4898	1.8570	1.7521	1.7398	1.1925	1.3709	1.5573	1.4546
Average	1.1546	1.4706	1.9076	1.7794	1.5248	1.1920	1.4082	1.4367	1.4125
Cell viability	79.08382	104.708	140.1481	129.7536	109.1077	82.11568	99.64586	101.9613	100.00

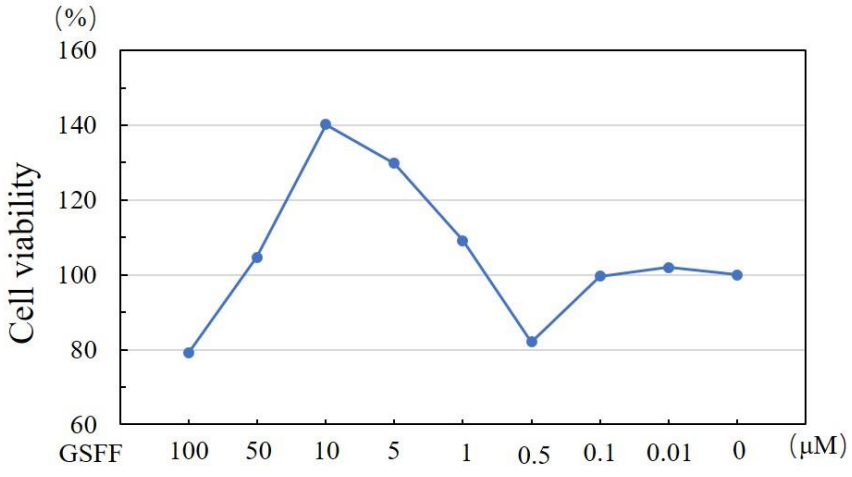

Figure 1： Different dose of GSFF on HSC-T6 cell viability

Minor comments. #1. Fig. 4H. The 2nd bar and 3rd bar must not be “GSFF+”, but probably “GSFF-“. #2. Figure legends. Fig. 1. There is no description for “***”. #3. Figure legends. Fig. 2. There are no descriptions for “△”,”**”, and “***”.

Response: I am sorry it is our fault and thank you for your serious manner. we have corrected all of the errors on the updated manuscript.

References:

[1] **Zhang W**, Ping J, Zhou Y, Chen G, Xu L. Salvianolic Acid B Inhibits Activation of Human Primary Hepatic Stellate Cells Through Downregulation of the Myocyte Enhancer Factor 2 Signaling Pathway. *Front Pharmacol* 2019; **10**: 322 [PMID: 31031620 DOI: 10.3389/fphar.2019.00322]

[2] **Yang SH**, Sun FY. Preliminary study on inhibitory effect of glycyrrhizin on hepatic stellate cell proliferation. *Journal of Guangdong Medical College* 2008; **26**: 237-242