ANSWERING REVIEWERS

February 10, 2014

Dear Editor,

Happy Chinese New Year!

Please find enclosed the edited manuscript in Word format (file name: Format for original articles.doc).

Title: A Xiaotan Sanjie decoction attenuates tumor angiogenesis by manipulating Notch-1-regulated cell proliferation of gastric cancer stem-like cells

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Name of Journal: *World Journal of Gastroenterology*

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The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer.

The following are point-by-point responses (in blue) to address the reviewers' comments and concerns. All the changes are marked in red in the revised manuscript which was attached at the **Format for original articles**.

(1) First, the method of water extract preparation should be described in details.

RES: Thanks. We have revised the method of water extract preparation in our paper following the constructive comments, please see page 4, para 3: "After mixing the herbal components according to the scheduled proportions as listed in Table 1, the herbals were suspended in distilled water for 30 min and then boiled out in a stainless steel pot for 40 min. Then, the drug juice was cooled down to room temperature and filtered using sterile gauze"

Thanks.

(2) Since this extract contained several constituents, how the authors detected the presence of its components in sera from rats given the extract in different doses.

RES: Thanks. The detection of the drug metabolites in vivo is particularly important in pharmacology. However, for the majority of traditional Chinese herbal medicines, it is extremely difficult to identify and purify the potentially active components after digestion because a single herbal could contains thousands of such components, let alone for the more common Chinese herbal compounds which usually consist of a group of herbals. The XTSJ decoction was composed of 11 herbs, we are so sorry that we failed to identify the components in sera in our present study.

Thanks.

(3) What information the reader can get from Fig.1.

RES: Thanks. We tried to make a preliminary estimation of the quality control as shown in Figure 1 because the quality of traditional Chinese herbal medicines could differ from batch to batch. Using high-performance liquid chromatography, we could evaluate different batches of the herbals by comparing the peaks, as shown in Fig. 1. In addition, we also tried to estimate the active components of the decoction because previous reports have demonstrated that certain active components could be detected in our decoction, for example, polysaccharide from Rhizoma Arisaematis[1]; polysaccharide-protein from Scolopendra[2]; and phosphorylated (1→3)- β -D-glucan, phosphated (1→3)- α -D-glucan and polysaccharide from Poria cocos, etc [3-5]. Nevertheless, our results are preliminary, and numerous studies are needed if we want to definitively identify the active components in the future.

Thanks.

Ref.

1. Geng C, Jie X, Xia M, Yi H, Xiaogang L, Ying J, Xinsheng H. Characterization and antitumor activities of the water-soluble polysaccharide from Rhizoma Arisaematis. *Carbohydrate Polymers* 2012;90:67-72.

2. Zhao H, Li Y, Wang Y, Zhang J, Ouyang X, Peng R, Yang J. Antitumor and immunostimulatory activity of a polysaccharide-protein complex from Scolopendra subspinipes mutilans L. Koch in tumor-bearing mice. *Food Chem Toxicol* 2012;50:2648-2655.

3. Xiaoyu C, Xiaojuan X, Lina Z, Fanbo Z. Chain conformation and anti-tumor activities of phosphorylated phosphorylated (1 → 3)-β-D-glucan from Poria cocos. *Carbohydrate Polymers* 2009;78:581-587.

4. Qilin H, Lina Z. Preparation, chain conformation and anti-tumor activities of water-soluble phosphated phosphated (1 → 3)-α-D-glucan from Poria cocos mycelia. *Carbohydrate Polymers* 2011;83:1363-1369.

5. Chen YY, Chang HM. Antiproliferative and differentiating effects of polysaccharide fraction from fu-ling (Poria cocos) on human leukemic U937 and HL-60 cells. *Food Chem Toxicol* 2004;42:759-769.

(4) Why did the authors used serum from rats pretreated with the extract for their in vitro studies? Why not the extract itself was applied as in any other study?

RES: Thanks. We used the serum from rats pretreated with the extract for the in vitro study because of the following two reasons: 1) a literature review showed that a group of studies concerning traditional Chinese herbal medicines in vitro are using the serum from pretreated animals, as indicated in the attached references [1-2]; and 2) the method of developing serum from pretreated rats is long established in our lab. For the in vivo study, we used the crude extract itself (water extract) because the animal could digest the decoction orally. We further clarified the sentence in page 4, para 3: "…crude water extracts, which correspond to 1.46 g/ml, 2.92 g/ml and 5.84 g/ml, respectively.".

Thanks.

Ref.

1. Zhang YH, Liu JT, Wen BY, Xiao XH. In vitro inhibition of proliferation of vascular smooth muscle cells by serum of rats treated with Dahuang Zhechong pill. *J Ethnopharmacol* 2007;112:375-379.

2. Zhang YH, Liu JT, Wen BY, Liu N. Mechanisms of inhibiting proliferation of vascular smooth muscle cells by serum of rats treated with Dahuang Zhechong pill. *J Ethnopharmacol* 2009;124:125-129.

(5) The effect of the serum does not necessarily mean the effect of the decoction that was given to the rats. Other pro-inflammatory cytokines could be also involved.

RES: Thanks. We completely accept the critical comments. Numerous studies have indicated that some of the proinflammatory cytokines, such as IL-6, IL-8, and TNF-α, could affect cell proliferation. In our previous studies in animals, the XTSJ decoction could have played a role in inhibiting IL-6 and IL-8 in S180 engrafted tumors[1-2]. Although it is still unknown if it could affect serum pro-inflammatory

cytokines, this possibility cannot be ruled out in present studies, but additional studies are needed in the future; please see page 7, para 1: "However, these conclusions may not be exclusive because some pro-inflammatory cytokines contained in the serum could also affect cell proliferation.".

Thanks.

1. Ju DW, Wei PK, Lin HM, Sun DZ, Yu S, Xiu LJ. Effects of Xiaotan Sanjie decoction on expressions of interleukin-8 and its receptors in gastric tumor xenografts and gastric tissue adjacent to the tumor in mice. Journal of Chinese Integrative Medicine 2010;8:74-79.

2. Ju DW, Wei PK, Lin HM, Sun DZ, Yu S. Effect of Xiaotan Sanjie Decoction on the expression of IL-6 in mice with gastric tansplanted tumor and gastric tissues beside tumor. Chinese Journal of Integrated Traditional and Western Medicine on Digestion 2008;5:284-288.

(6) How tumour cells were injected into mice? What was the volume and number of cells used? Where these subcutaneous tumours?

RES: Thanks. We are so sorry for the negligence in our paper, we have supplemented the information; please see page 6, para 1: "For engraftment, both the CD44+ and CD44- cells were injected subcutaneously into the axilla of the mice using a handmade glass micropipette (1×10^5 cells per site)".

Thanks.

(7) The methods as well as the results could be better described. Also it will be useful to indicate and separate clearly the results obtained from in vitro in and in vivo experiments. It is also advisable to write the results in more details.

RES: Thanks. We completely agree with the constructive comments and have tried to rewrite and supplement the necessary information as well as correct the figures in the Methods and the Results sections from page 6 to 7. We re-edited the figure legends to conform to these revisions.

Thanks.

(8) Comparisons between different groups were evaluated by one-way ANOVA, followed by the Bonferroni test or Student's t-test.? Would the authors please clarify when and in which groups Bonferroni test or Student's t-test were used?

RES: Thanks. We specifically clarified this information in the figure legends in our paper, please see page 19-20.

Thanks.

(9) In the abstract section: "in the secretions"….." in the secretion" "than their counterparts"…which counterparts? Please clarify

RES: Thanks. We are sorry for these mistakes; however, we have rewritten the abstract according to the **Journal's Requirements**, please see page 1-2.

Thanks.

(10) Too many and unnecessary abbreviations e.g., (SD), (HPLC), other abbreviations were indicated several times e.g., (GCSCs), (VEGF)

RES: Thanks. We have revised our paper following the constructive comments; unnecessary and duplicated abbreviations were deleted throughout the paper after careful examination.

Thanks.

(11) In materials and methods: All mice were handled…. All rats and mice were handled

RES: Thanks. We have made changes according to the constructive comments, please see page 4, para 1: "All rats and mice were handled…".

Thanks.

3 References and typesetting were corrected

The original paper was edited by American Journal Experts, and we have sent the revision version to the company again, please see the attached certification.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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