

Format for ANSWERING REVIEWERS



July 1, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 3541-revised.doc).

Title: Xiaotan Tongfu granules contribute to the prevention of stress ulcers

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 3541

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 (Page 7-31)**.

Reviewer 1

(1) A more detailed discussion of possible mechanisms for the effects seen, based on knowledge about the actions of the included components in the decoction, should be included.

RES: Thanks. We have tried to provide a more detailed discussion of the potential mechanisms for the components of the decoction, however, it may still far from clear when taking into consideration that the active ingredients of herbal medicine were very complex. Please see page12 para2: "...Previous studies indicated that some components in our decoction, for example, the *Magnoliae Officinalis Cortex*, *Coptidis Rhizoma* and *Glycyrrhizae Radix Et Rhizoma*, were effective in inhibiting gastric acid secretion by a potential mechanism of regulating the activity of various postsynaptic gastric receptors such as histamine H2^[48-49]"; page13, para1 "Additionally, it was notable that some traditional Chinese herbal medicines were effective in preventing inflammation by various mechanisms, such as the inhibition of NF-κB, TNF-α, and IL-17^[55-56]"; "Other components, such as *Coptidis Rhizoma*, could ameliorate acute inflammation by inhibiting NF-κB-mediated nitric oxide and pro-inflammatory cytokine production^[60]"; "Interestingly, *Glycyrrhizae Radix Et Rhizoma* was also demonstrated to be effective in protecting gastric mucosa via gastric mucin^[62]"; "Finally, the XTTF granule also inhibited cell apoptosis and promoted cell proliferation, which is related to the mucosal protection of some components such as *Magnoliae Officinalis Cortex*^[62] and *Aurantii Fructus Immaturus*^[63]. We speculate that all of these actions may contribute to tissue regeneration and reconstruction in the stomach^[64]".

Thanks.

(2) The rationale for using each should be presented in either the introduction or the materials and methods sections.

RES: Thanks. We have briefly summed up the rationale of the decoction in the materials and methods sections, please see page6, para3: "We established the granule under the guidance of TCM related to

stress ulcers^[21], and some of the components were previously shown to be effective in the management of stress-related symptoms. For example, the Xiao-ban-xia decoction could elevate gastric emptying^[22], which was delayed under stress conditions. In addition, the major component of the Xiao-cheng-qi decoction, *Rhei Radix Et Rhizoma*, was demonstrated to be effective in the prevention of stress ulcers via multiple mechanisms^[23-24]”.

Thanks.

(3) Table 1: It would be helpful for the Western reader if common names for the components were also included: rhubarb, bitter orange, magnolia bark, peony, snake-needle grass, citron fruit extract, liquorish root, etc.

RES: Thanks. We understand the comments as to provide the english name/common name of the components for the decoction in our paper. We have revised Table 1, please see page 24, Table 1.

(4) “Drug administration” paragraph: What is meant by “twice that of an adult dose”? Adult human? What is meant by “adaptive feeding”?

RES: Thanks. We have revised “twice that of an adult dose” into “twice that of an adult human dose”, please see page 7, para 1. Adaptive feeding means feeding the rats without any intervene, in order to avoid misunderstanding, we revised this sentence into: “The administration frequency was twice daily and sustained for 7 days”, please see page 7, para 1.

Thanks.

(5) “Induction of stress ulceration” paragraph: “model used in the present study”. Following onto the next page, why were all of these tests performed? (PCNA, MIF, etc.)

RES: Thanks. We have considered the following reasons for the markers determined in our study: 1. By literature review, we selected some of the most reported markers in stress ulcers, such as gastric pH, HSP70, MPO, etc. 2. Taking into consideration that herbal medicines are usually function with multiple possible mechanisms, we decided the markers reflecting the aggressive and protective factors in stress ulcer, such as PCNA vs apoptosis; HSP 70 vs inflammation, etc.

Thanks.

(6) “Measurement of apoptotic cells” para: What is meant by “terminated buffer”? Is “DNase” correct? “The apoptotic index was defined.....”

RES: Thanks. The “terminated buffer” used in our study refers to 3% H₂O₂ methanol solution, please see page 8, para 3. We confirmed that Dnase I in our paper was correct. We revised “The apoptotic index was defined” into “The apoptotic index was defined as the average number from 10 to 25 glands of each mucosal section”, please see page 9, para 1.

Thanks.

(7) Figures: Provide a clear explanation of what is meant by the various statistical comparison symbols used (*, #) – what is compared to what?

RES: Thanks. We further clarified the mean of the symbols in the figures according to the *Journal* requirements, after combined with the comments of other reviewers, we revised the figures meanwhile, please see Figure 1, 2, 3, 4A-C, 5A-B, 6A-B, 7A-B, and figure legends, page 22-23.

Thanks.

(8) “Gastric pH in the XP and RP groups” para: It is unclear what is meant by “complex pathological variations of body”. Since there is only one figure caption for figures 4-6, they should be renumbered as Figures 4A, 4B, and 4C. Then figures 7 and 8 would be Figures 5A and 5B, and figures 9 and 10 would be Figures 6A and 6B, and figures 11 and 12 would become Figures 7A and 7B.

RES: Thanks. In order to avoid misunderstanding, we deleted the sentence: “It was believed that gastric acid secretion during stress was attenuated under the complex pathological variations of body [29-31]”. We have revised the figures in our paper based on the comments, please see Figure 1, 2, 3,

4A-C, 5A-B, 6A-B, 7A-B.

Thanks.

(9) "Discussion", para 2: Suggest replacing "(article under review)" with "(unpublished observations)".

RES: Thanks. We completely agreed with the comments and replaced "article under review" with "unpublished observations", please see page12, para2.

Thanks.

(10) 2 para before "Conclusion": "Though previous studies....our study showed that the effect...." This sentence is unclear. What are potential drawbacks with this use of this ulcer model?

RES: Thanks. We are sorry for the obscure expression, we have revised this sentence into: "Except that previous studies...our study showed that the effect ...", please see page13, para3.

Thanks.

Reviewer 2

(1)In general, H. pylori infection relates with development of peptic ulcer. Therefore, authors should mention H. pylori infection.

RES: Thanks. We completely agreed with the comments and made some supplements concerning the H. pylori infection with stress ulcers: "The development of stress ulcers is largely determined by the balance between known aggressive factors and defense mechanisms. The former usually include gastric acid^[6], abnormal motility^[7], and H. pylori infection^[8-9], and the latter include heat shock protein^[10], cellular regeneration^[11], etc". please see page5, para2.

Thanks.

(2) Why authors select ranitidine, not PPI? Recently, most of cases with acid related diseases was dosed PPI, not histamine 2receptor antagonist.

RES: Thanks. We did have considered the use of PPIs in our study previously, however, by literature review (even including some papers published recently, ref. 1-3), we found out that H₂RA and PPI obtained equal support in most of the studies. In addition. we aimed to check the efficacy and potential mechanisms of a traditional Chinese herbal decoction in our study, which was composed as much as eleven herbals and may contained thousands of active ingredients with multiple mechanisms. Ranitidine was one of the most well-studied drugs with multiple mechanisms in stress ulcer (Except that...the comprehensive network of SU. Please see page13, para1), it could be regarded as an ideal positive control compared to PPIs in our study, taking into consideration of these factors, we selected ranitidine in our study.

Ref.

1. Fogas JF, Kiss KK, Gyura FG, Tóbiás ZT, Molnár ZM. Effects of proton pump inhibitor versus H₂-receptor antagonist stress ulcer prophylaxis on ventilator-associated pneumonia: a pilot study. Critical Care 2013; 17(Suppl 2): 402

2. Krag M., Perner A, Wetterslev J, MøLLER MH. Stress ulcer prophylaxis in the intensive care unit: is it indicated? A topical systematic review. Acta Anaesthesiologica Scandinavica Article first published online: 15 Mar 2013

3. Alhazzani W, Alshahrani M, Moayyedi P, Jaeschke R. Stress ulcer prophylaxis in critically ill patients: review of the evidence. Pol Arch Med Wewn 2012; 122: 107-114

Thanks.

(3) Reviewer cannot agree contents in 2nd paragraph in Introduction. First line treatment of stress ulcer related with H. pylori infection was PPI.

RES: Thanks. We have revised the second paragraph: "...SU prophylaxis (SUP) was thought to play a pivotal role in the care of critically ill patients, and it was reported that appropriate SUP could decrease

mortality. At present, although multiple protocols are available for SUP, there are no universally accepted regimens^[12]. Nevertheless, the evidence that the appropriate application of some pharmacologic agents, such as proton pump inhibitors (PPIs), histamine-2 receptor antagonists (H2RAs), and sucralfate^[13], could decrease the risk of bleeding has been long established” please see page5, para2.

Thanks.

(4) What was new finding in this study? Did you have difference of prevalence of H. pylori virulence factors for gastric cancer patients in Iranian previous study?

RES: Thanks. The purpose of this study was to investigate the efficacy and potential mechanism of a traditional Chinese herbal medicine in stress ulcer, to the best of our knowledge, although traditional Chinese medicine was widespreadly used in China to manage stress-related disorders, there were limited animal studies in stress ulcer. Our findings indicated that XTTF granules, a herbal formula which was composed of eleven herbals, could be applied in stress ulcer in future with multiple mechanisms that was different from the conventional pharmacologic agents. We used the model of cold-restraint stress in our study which usually lead to acute ulcers, we are sorry that we did not compared the difference of prevalence of H. pylori virulence factors for gastric cancer patients in Iranian previous study, but we have tried to cite these important papers. Please see page5, para2: “The development of...H. pylori infection^[8-9]...”.

Ref.

8. Kamali-Sarvestani E, Bazargani A, Masoudian M, Lankarani K, Taghavi AR, Saberifiroozi M. Association of H pylori cagA and vacA genotypes and IL-8 gene polymorphisms with clinical outcome of infection in Iranian patients with gastrointestinal diseases. *World J Gastroenterol* 2006; 12: 5205-5210
9. Siavoshi F, Malekzadeh R, Daneshmand M, Smoot DT, Ashktorab H. Association between Helicobacter pylori Infection in gastric cancer, ulcers and gastritis in Iranian patients. *Helicobacter* 2004; 9: 470

Thanks.

(5) There were many figures. Please summarize or delete any Figures.

RES: Thanks. We further clarified the mean of the symbols used in the figures in our paper according to the *Journal* requirements, after combined with the comments of other reviewers, we revised the figures meanwhile, please see Figure1, 2, 3, 4A-C, 5A-B, 6A-B, 7A-B, and figure legends, page22-23.

Thanks.

(6) Authors should discuss more how to inhibit gastric acid secretion by the XTTF granule.

RES: Thanks. Although the relevant studies are still limited, we have tried to elucidate some possible mechanisms of XTTF granule in inhibiting gastric acid secretion, please see page12, para2: “...Previous studies indicated that some components in our decoction, for example, the *Magnoliae Officinalis Cortex*, *Coptidis Rhizoma* and *Glycyrrhizae Radix Et Rhizoma*, were effective in inhibiting gastric acid secretion by a potential mechanism of regulating the activity of various postsynaptic gastric receptors such as histamine H₂^[48-49]”.

Thanks.

(7) Although author said as limitation, it will be required whether the XTTF granule acts in a dose-dependent manner in part of experiments, not all.

RES: Thanks. We admitted that it was of pivotal importance for us to demonstrate the efficacy of XTTF granule act in a dose-dependent manner, however, our study was aimed to test the scheduled markers at 4 different time endpoints, we have tried to maintain the minimum number of the animals in each group to ensure a statistical analysis, if we want to further obtain the data concerning to dose-dependent manner in any part of experiments, the number of the animals would be increased

sharply, which was an unaffordable situation for our group since we only get a limited financial support. Another problem maybe lies in the crude powder of the granule used in our study, a concentration of 4.9g/kg for the granule seems like to reach the maximum solubility, if we further elevate the concentration, the granule would become impossible to administrated to the rats, we think that we can solve this problem by extract and concentrate the crude powder of the granule in future, but it is hard for us to do so at present.

Thanks.

Reviewer 3

(1) Authors must include a chemical analysis of the formulation. Authors have to perform experiments with the main pharceutical components of the XTTF.

RES: Thanks. We completely agreed with the constructive comments and believed that our paper would be remarkably polished if we could perform these experiments, however, based on our knowledge, it was difficult to carry out a chemical analysis of a formula which composed by as much as eleven herbals. We also consulted the experts in the College of Pharmacy of our university and found out it was also hard to confirm the main pharceutical components of our formula, let alone the substantial finacial support. Thus, we are really sorry that we are unable to perform these analysis at present.

Thanks.

(2) Furthermore, this study was done in a preventive base, i.e., first animals were treated with XTTF and then later they were subjected to stress. Consequently, the results must be interpreted in terms of prevention and not of treatment.

RES: Thanks. We completely agree with the pointed comments, and we removed the healing in the title of our paper and compress some parts in the discussion, please see page13, para1: "Except these effects, the XTTF granule has also been shown to play a role in the manipulation of HSP70 expression, and although the data are still limited, the previous study did indicate that some herbal medicine constituents, such as *Glycyrrhizae Radix Et Rhizoma* (an herbal component in the Xiao-cheng-qi decoction^[19]), could promote HSP expression^[61]. Interestingly, *Glycyrrhizae Radix Et Rhizoma* was also demonstrated to be effective in protecting gastric mucosa via gastric mucin^[62]. Finally, the XTTF granule also inhibited cell apoptosis and promoted cell proliferation, which is related to the mucosal protection of some components such as *Magnoliae Officinalis Cortex*^[62] and *Aurantii Fructus Immaturus*^[63]. We speculate that all of these actions may contribute to tissue regeneration and reconstruction in the stomach^[64]".

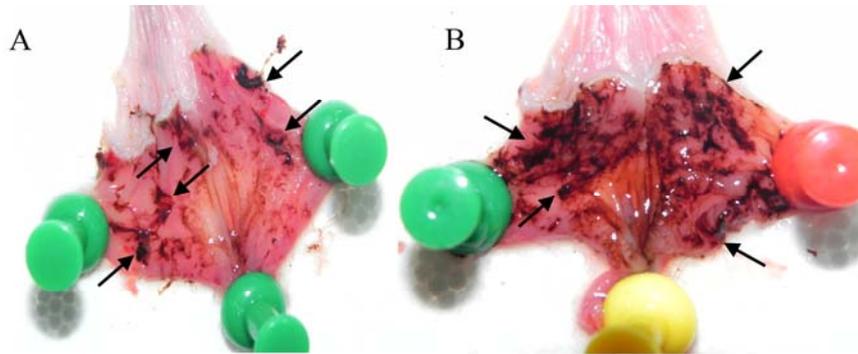
Thanks.

(3) Furthermore, authors have not discussed the transitory effect of the stress (i.e., after 24 the stressed animals were recovered, so what is the predictive value of the model in situations where a more permanent lesion is developed?

RES: Thanks. In our study, we used the classical cold-restraint stress model to induce acute ulcers, this model could produce an obvious gastric lesions accompanied by a series variation of pathological parameters. As we know, the hypothalamic-pituitary-adrenal (HPA) axis plays an essential role in the stress-related mucosal disease (SRMD), the characteristics of SMRD would be correlated to the accommodation of the HPA axis adjusted to the stress (ref. 1-2). In our study, although the cold-restraint stress successfully induced acute ulcers, all the lesions mainly recovered in 24 hours, as well as the tested pathological parameters. We thought maybe this could be expalined by the stimulus intensity of the model as in our previous study, an obvious gastrointestinal bleeding could be detected and not completely recovered in 24 hours when we extended the restraint time or further decreased the temperature (Supplement figure below). Based on these results, we speculated as for a more permanent lesion, the stress ulcers would also be emerged with a different feature, the pathological parameters tested in our study would fluctuate in a more board spectrum, however, additional studies are needed in future.

Ref.

1. Chrousos GP. Stress and disorders of the stress system. *Nat Rev Endocrinol* 2009; 5: 374-381
2. Metz DC. Preventing the gastrointestinal consequences of stress-related mucosal disease. *Curr Med Res Opin* 2005; 21: 11-8



A. Temperature 0°C for 3 hours B. Temperature -4°C for 3 hours
Black arrow: gastric bleeding

Thanks.

3 References and typesetting were corrected.

The original paper was edited by American Journal Experts, and we have send 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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