

Dear Dr. Jia-Ping Yan,

We are pleased to submit the revised version of the manuscript (48308) entitled “**Mechanistic study on the efficacy of the herbal preparation, STW 5, against gastro-duodenal lesions induced by NSAIDs**”. We appreciate the constructive criticisms of the Editor and the reviewers which helped us to improve our manuscript. The comments have been appropriately addressed as shown below. We thank you for your kind help in handling this manuscript.

### REVIEWERS COMMENTS:

#### Reviewer #1:

In this manuscript the Authors compared the protective efficacy of the herbal preparation STW 5 and omeprazole against diclofenac-induced gastro-duodenal lesions in rats. They show that both STW5 and omeprazole are able to prevent diclofenac-induced changes in the levels of inflammatory mediators, specifically tumor necrosis factor  $\alpha$ , IL-1 $\beta$  and IL-10, and of the oxidative stress enzyme HO-1, as well as the diclofenac-induced histological damage. Moreover, they provide evidence supporting that the herbal preparation STW 5 could be more effective in protecting against histological damage, particularly in the duodenum. Based on these results They conclude sustaining the therapeutic utility of STW 5 as adjuvant therapy to long-term use of NSAIDs, for protecting patients from the possible occurrence of gastro-duodenal lesions. This is an interesting work that provide further evidences supporting the validity of STW 5 in preventing NSAIDs-induced damage of gastro-intestinal tract, reducing the risk of this side effect in NSAIDs long-term treated patients, also in light of the reported good tolerability of this herbal preparation(Ref 35). In this work the experiments are well designed, the

methodology used are appropriated, and the data obtained sustain the Authors conclusion. However, there are some minor points that must be addressed before acceptance for publication I listed these points following:

**1.** Even if the language is in principle correct, in some points the English is not fluent with some inaccuracies and should be carefully revised. Just some example:

- Pg. 3 lane 1: “.....irritable bowel syndrome is STW 5, has a favorable safety....”, should be: “..irritable bowel syndrome is STW 5, that has a favorable safety....”
- Pg. 5 lanes 8-9 :”... inflammatory cell infiltration in the mucosa and sub-mucosa, respectively.”: Respectively is not correct (is redundant); delete it
- Pg. 5, the last lane : “....stomach and duodenum as compared to normal controls”: “...as compared to untreated control” should be better.
- Pg. 9 lanes 7-8: “...Omeprazole was effective in preventing the changes in both tissues, whereas STW 5, however , was effective.....” “However” is redundant.
- Pg. 10, the last two lanes: “...in Fig.7, where STW 5 is clearly seen to supersede omeprazole in overcoming the damage induced by diclofenac.....” In this phrase the English is not fluent... for suggestion, it could be replace by: ...in Fig.7, where it appears evident that STW 5 is more efficient than omeprazolo in preventing the damage.....“
- Pg.14 lane 12: “...NF- $\kappa$ B, thereby helping to up-regulate....”: “...NF- $\kappa$ B, thereby contributing to up-regulate...” should be better.

The whole manuscript has been thoroughly revised and the required changes were made and highlighted in yellow.

**2. MATERIALS AND METHODS:** The description of MM is in some points defective:

- Paragraph “Experimental design”: for the treatments with diclofenac, omeprazole and STW, the modality of administration should be specified;

Sorry for that. All drugs were given orally and the mode of administration was mentioned and highlighted in the revised manuscript.

- the authors should specify on which kind of sample (tissue lysates,.. I suppose) the ELISA assays were performed, and should describe as they were obtained (for example: „tissue lysates were obtained by....“).

One segment of stomach and duodenum was homogenized in ice-cold normal saline to obtain a 10 % homogenate that was used for the ELISA assays. This has been specified and highlighted in the revised manuscript.

3. RESULTS: In the legends to Figs.1-4, which is the meaning of “ n= 7 – 10 rats“? Is the meaning “7 of 10 rats“? I think that “n=7” it’s enough.

Done.

4. RESULTS: In Figs. 5 and 6 the use of “short arrows“ and “long arrows“ could generate confusion... the use of two different kind of arrows (or different colors) should be better and less confusing for the reader.

Upon the revision of the above listed points, the manuscript will be acceptable for publication

Reviewer #2:

Title: “Mechanistic study on the efficacy of the herbal preparation, STW 5, against gastro-duodenal lesions induced by NSAIDs” Dear Editor, I read this manuscript and I think that:

- The results section of the abstract should be updated by including more numerical data. Please update.

The levels of B-cell lymphoma 2 (Bcl2) was measured as an indicator of apoptosis in the stomach and duodenum and the results were included and highlighted in the revised manuscript.

- Please revise the English of the text due to some typos.

Done.

- The authors should specify the diet of the rats.

Done and highlighted in the revised manuscript.

- The role of nutraceuticals on health conditions should be better discussed. Please consider and discuss the paper from Scicchitano P et al. Journal of Functional Foods 2014;6:11-32.

Thank you for the constructive comment. This has been taken in consideration and alluded to in the revised manuscript.

Reviewer #3:

Reviewer #4:

The author present “Mechanistic study on the efficacy of the herbal preparation, STW 5, against gastro-duodenal lesions induced by NSAIDs” in the current study.

1. Can you add more index about the bax, bcl-2 and the TUNNEL staining?  
Because the histological damage always involve the necrosis and apoptosis

The levels of B-cell lymphoma 2 (Bcl2) was measured as an indicator of apoptosis in the stomach and duodenum and the results were included and highlighted in the revised manuscript.

2. Author use ELISA in the current study, Can you also compare the four groups by RT-PCR or maybe western blotting (using the IL-10, HO-1, IL-1 $\beta$ , TNF- $\alpha$ )?

As ELISA method provides a means for the quantitative measurement of the desired parameters, the authors used ELISA to detect the changes of the measured parameters. The authors have examined literature and found that ELISA method is used alone without the necessity of carrying out Western Blotting. We sincerely hope that the reviewer will forgive us this time, and we shall keep this in mind in the future.

3. Can you show the morphology of the specimen?

Regretfully the morphology of the specimen was not examined at the time, and there is no way to show it at present.

Reviewer #4:

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