

Reviewer #1:

Scientific Quality: Grade D (Fair)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: Authors investigated the effect of *Dendrobium officinale* in CAG animal models. The following issues should be resolved. Major issues: 1. Both the Methods and Results section is very repetitive. Further editing of the text, merging related paragraphs, etc. is needed. 2. "Its main function is to supplement Qi, nourish Yin and stomach to improve the production of body fluid, making it suitable for treating deficiency of both Qi and Yin". Please update the text that it would be understandable for western researchers as well. 3. "and so on." Please, indicate all ingredients. 4. Detailed description of the various staining methods are not necessary if they are routine ones and can be cited. 5. "Main reagents: Nitrosoguanidine (MNNG, Tokyo Chemical Industry, Japan). Antibody against gastric epithelial cell proliferating cell nuclear antigen (PCNA, NeoMarkers, USA) Anti- Bcl-2 antibody (Millipore, USA)." Instead of a plain listing, include the various chemicals in their corresponding paragraph. 6. Authors provided the sample files available on the WJG site for both Statistical and Language certificates. Those must be properly filed and updated. 7. Please explain, why the Ethical and Animal care documents have different dates. The first is from 2016 while the latter from 2019. Why the difference? 8. More detailed discussion of the results is needed? Eg., were any similar (animal) studies conducted to investigate traditional herbs of any countries over CAG? 9. Description of statistical methods must be more clear. I assume authors used ANOVA, however, for most readers the term ANOVA would be more understandable than F-test. Please include, which LSD test was used (Tukey??). Did authors use Kruskal-Wallis ANOVA for the non-parametric comparisons? 10. On figure 1, there is a minor weight loss for every animal from the 7th until the 8th week. Please explain. 11. ARRIVE checklist is empty. 12. Moderate English polishing is needed. Minor issues: 1. For better recognition of the various herbs please include the latin names for all of the plants mentioned throughout the text. 2. Pay attention to the correct spelling of binomial names: capital letter for genus and lowercase for species, both in italics. 3. Naming of study groups would be more clear as follows: CAG no-treatment, CAG DG low/medium/high, CAG YWS and healthy animals. 4. Do not merge Figures, use A/B/C/etc instead. 5. "Proliferating Cell Nuclear Antigen (PCNA) is an intranuclear protein that is expressed in a small amount during the quiescence phase of DNA synthesis. It is an important indicator of cell proliferation because its changes are consistent with intracellular DNA replication. The B-cell lymphoma-2 (Bcl-2) is a gene that inhibits apoptosis. Its overexpression can inhibit cell apoptosis and is indicative of a favorable precondition for the transformation of malignant cells." This should be moved from Results to Methods.

Reviewer #1:

Major issues: 1. Both the Methods and Results section is very repetitive. Further editing of the text, merging related paragraphs, etc. is needed.

The text has been further revised and edited, and repetitive paragraphs have been combined. Due to the long length of methods and results, please refer to the main text.

2. "Its main function is to supplement Qi, nourish Yin and stomach to improve the

production of body fluid, making it suitable for treating deficiency of both Qi and Yin". Please update the text that it would be understandable for western researchers as well.

We have changed it to the following:

"Based on the theory of TCM, this formula has the functions of enhancing energy metabolism of the stomach and restoring its nutrient supply to improve the production of body fluid, which makes it suitable for treating the type of CAG that has lower levels of energy production and nutrient supply."

3. "and so on." Please, indicate all ingredients.

We have added detailed information. The following is the revised paragraph:

"Herbal product as control: Yangweishu Granule (YWS, produced by Shenlu Shuanghe Pharmaceutical Co., Ltd, Hefei, China) 10 g per bag contained dangshen (*Radix Codonopsis*), chenpi (*Pericarpium Citri Reticulatae*), huangjing (*Polygonatum sibiricum*), shanyao (*Rhizoma Dioscoreae*), xuanshen (*Radix Scrophulariae*), wumei (*Fructus Mume*), shanzha (*Fructus Crataegi*), beishashen (*Glehnia littoralis*), ganjiang (*Rhizoma Zingiberis*), tusizi (*Cuscuta Chinensis*), baizhu (*Rhizoma Atractylodis Macrocephalae*), dextrin and saccharose. The dosage for clinical treatment was 20 g/ day."

4. Detailed description of the various staining methods are not necessary if they are routine ones and can be cited.

We have removed detailed staining methods. The revised methods are as following:

"The day after the last administration, all the rats were anesthetized and blood was taken from the abdominal aorta. The stomach was quickly removed and fixed in formalin. The longitudinal length and full layer of the anterior stomach to the duodenum was collected. A strip of tissue from the lateral wall was taken as the standard pathological sample. After paraffin embedding, the tissue was sliced into 4 µm sections and stained with Hematoxylin-Eosin (HE)^[6], Alcian Blue (AB)^[7], and immunohistochemistry against PCNA (antibody against gastric epithelial cell proliferating cell nuclear antigen, NeoMarkers, USA) and Bcl-2 (anti-Bcl-2 antibody, Millipore, USA).

PCNA and Bcl-2 staining: After dewaxing, the sections were incubated with 3% H₂O₂ deionized water for 10 min, followed by incubation with primary antibodies at room temperature or 37°C for 1 to 2 h. After rinsing in PBS, secondary antibodies were added and incubated at room temperature or 37°C for 20 min. The sections were rinsed in PBS, and processed for color rendering dehydration, transparency and sealing."

6 **Jiang JY**, Liu DJ, Liu MX. The protective effect of NF-κB signaling pathway inhibitor PDTC on mice with chronic atrophic gastritis. *Scand J Gastroenterol* 2021; **56**: 1131-1139 [PMID: 34310252 DOI: 10.1080/00365521.2021.1953130]

7 **Qin R**, Wang NN, Chu J, Wang X. Expression and significance of homeodomain protein Cdx2 in gastric carcinoma and precancerous lesions. *World J Gastroenterol* 2012; **18**: 3296-3302 [PMID: 22783055 DOI: 10.3748/wjg.v18.i25.3296]

5. "Main reagents: Nitrosoguanidine (MNNG, Tokyo Chemical Industry, Japan).

Antibody against gastric epithelial cell proliferating cell nuclear antigen (PCNA, NeoMarkers, USA) Anti- Bcl-2 antibody (Millipore, USA). " Instead of a plain listing, include the various chemicals in their corresponding paragraph.

Thanks for the suggestion. They have been incorporated into the text.

6. Authors provided the sample files available on the WJG site for both Statistical and Language certificates. Those must be properly filed and updated.

Statistical and Language certificates have been uploaded to the attachment.

7. Please explain, why the Ethical and Animal care documents have different dates. The first is from 2016 while the latter from 2019. Why the difference?

Because the experiment began when the first grant was approved in 2016, and then another grant was approved in 2019. Animal experimental research can begin only after obtaining funding and animal ethical approval in China. The Institutional Review Board approval is not applicable in this study because it did not involve human beings. The two files were both about Animal Care and Ethics.

All animal experiments conformed to the internationally accepted principles for the care and use of laboratory animals. The study was reviewed and approved by the Zhejiang Academy of Traditional Chinese Medicine Research Ethics Committee.

The fund approval documents and animal ethical documents have been provided in the attachment.

8. More detailed discussion of the results is needed? Eg., were any similar (animal) studies conducted to investigate traditional herbs of any countries over CAG?

We added further discussion of the results as suggested. Below is a revised paragraph related to a discussion on similar studies.

"In recent years, treatment with traditional Chinese herbal medicine has been reported to improve CAG gastric mucosal atrophy and IM. In *Helicobacter pylori* positive CAG, a TCM herbal formula was shown to improve clinical symptoms and efficacy rate, and reverse atrophy in gastric mucosa in CAG patients^[22]. Fuzi Lizhong decoction can reverse IM due to CAG to a certain extent through a mechanism related to the regulation of shh gene expression of the diseased gastric mucosa^[23]. Recent research reported that notoginsenoside R1, an ingredient of *Panax notoginseng*, can improve CAG by increasing Bcl-2 expression and decreasing Bax expression in gastric tissue of rats with CAG induced by MNNG combined with an irregular diet^[24]. Morroniside, an extract from *Cornus officinalis*, was shown to relieve gastric mucosa injury due to CAG by preventing inflammation^[25]. Modified Sijunzi Decoction (MSD) relieved the symptoms of CAG, improved the pathologic changes in CAG including fatigue and tiredness symptoms in CAG patients^[26]. Berberine, an isoquinoline alkaloid from *Rhizoma coptidis*, has an inhibitory effect on gastritis^[27] and GC cells^[28], and significantly improves the pathological characteristics of gastric tissue, and alleviated serum biochemical indices^[29]. These studies show that in addition to the formula we used in this study (*Dendrobium Officinale* and *American ginseng*), fuzi Lizhong Decoction, notoginsenoside R1, morroniside, MSD, berberine, or TCM treatment aimed at invigorating spleen, soothing the liver, promoting blood circulation and detoxification, also have varying degrees of anti-inflammatory effects and reverse gastric mucosal atrophy and IM."

22 Zeng J, Ge Y, Guo H, Xia SJ. Treatment of pylori pyrolytic gastritis with the method of invigorating the spleen, soothing blood circulation and activating blood. *Jilin Zhongyiyao* 2017; **37**: 907-911 [DOI: 10.13463/j.cnki.jlzyy.2017.09.013]

- 23 **Liu XY**, Liu XM, Yang ZB. Clinical efficacy and mechanism of Fuzilizhong Decoction in the treatment of chronic atrophic gastritis intestinal metaplasia. *Yunnan Zhongyi Xueyuan Xuebao* 2016; **39**: 54-57
- 24 **Luo C**, Sun Z, Li Z, Zheng L, Zhu X. Notoginsenoside R1 (NGR1) attenuates chronic atrophic gastritis in rats. *Med Sci Monit* 2019; **25**: 1177-118 [PMID: 30757999 DOI: 10.12659/MSM.911512]
- 25 **Zhang J**, Wang, H. Morroniside protects against chronic atrophic gastritis in rat via inhibiting inflammation and apoptosis. *Am J Transl Res* 2019; **11**: 6016-6023 [PMID: 31632569]
- 26 **Tian G**, Wu C, Li J, Liang B, Zhang F, Fan X, Li Z, Wang Y, Li Z, Liu D, Lai-Han Leung E, Chen J. Network pharmacology based investigation into the effect and mechanism of Modified Sijunzi Decoction against the subtypes of chronic atrophic gastritis. *Pharmacol Res* 2019; **144**:158-166 [PMID: 30991106 DOI: 10.1016/j.phrs.2019.04.012]
- 27 **Wu X**, Li X, Dang Z, Jia Y. Berberine demonstrates anti-inflammatory properties in Helicobacter pylori-infected mice with chronic gastritis by attenuating the Th17 response triggered by the B cell-activating factor. *J. Cell. Biochem* 2018; **119**: 5373-5381 [PMID: 29345340 DOI: 10.1002/jcb.26681]
- 28 **Hu Q**, Li L, Zou X, Xu L, Yi P. Berberine attenuated proliferation, invasion and migration by targeting the AMPK/HNF4 α /WNT5A pathway in gastric carcinoma. *Front. Pharmacol* **2018**; 9: 1150 [PMID: 30405404 DOI: 10.3389/fphar.2018.01150]
- 29 **Tong Y**, Zhao X, Wang R, Li R, Zou W, Zhao Y. Therapeutic effect of berberine on chronic atrophic gastritis based on plasma and urine metabolisms. *Eur J Pharmacol* 2021; **908**: 174335 [PMID: 34265298 DOI: 10.1016/j.ejphar.2021.174335]

9. Description of statistical methods must be more clear. I assume authors used ANOVA, however, for most readers the term ANOVA would be more understandable than F-test. Please include, which LSD test was used (Tukey??). Did authors use Kuskal-Wallis ANOVA for the non-parametric comparisons?

We have clarified statistical methods, and addressed the reviewer's concerns. Please see the revised text in Methods.

10. On figure 1, there is a minor weight loss for every animal from the 7th until the 8th week. Please explain.

The weight loss may be related to the normal growth of animal body weight. The weight gain curve tends to plateau or slightly falls down after the animals reach adulthood.

11. ARRIVE checklist is empty.

ARRIVE checklist has been filled in and uploaded to the attachment.

12. Moderate English polishing is needed.

We have completed English polishing as suggested.

Minor issues: 1. For better recognition of the various herbs please include the latin names for all of the plants mentioned throughout the text.

We have added Latin name for all the plants mentioned in the text.

2. Pay attention to the correct spelling of binomial names: capital letter for genus and lowercase for species, both in italics.

We have corrected them as suggested.

3. Naming of study groups would be more clese as follows: CAG no-treatment, CAG DG low/medium/high, CAG YWS and healthy animals.

They have been modified as suggested.

4. Do not merge Figures, use A/B/C/etc instead.

The figures have been modified, please refer to the attachment.

5. "Proliferating Cell Nuclear Antigen (PCNA) is an intraconuclear protein that is expressed in a small amount during the quiescence phase of DNA synthesis. It is an important indicator of cell proliferation because its changes are consistent with intracellular DNA replication. The B-cell lymphoma-2 (Bcl-2) is a gene that inhibits apoptosis. Its overexpression can inhibit cell apoptosis and is indicative of a favorable precondition for the transformation of malignant cells." This should be moved from Results to Methods.

This section has been moved to the Methods.

Reviewer #2:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: Dear Editor, Thanks to the authors for this experimental study. There is limited studies in this topic. I think that it will contribute to the literature.

Reviewer #3:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: At first, this manuscript is too long for readers. I suggest that authors need to clear it up, especially the part of results. In discussion, there are a lot of repeated results, i suggest that authors need to add the speculations from their results in discussion.

We have shortened the manuscript by removing or combining several repeated sections. We have also moved the speculation text in Results to the Discussion.