Author's response to reviews

**Title:** Banhabaekchulchunma-tang, a traditional herbal formula attenuates absolute ethanol-induced gastric injury by enhancing the antioxidant status

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**Author's response to reviews:** see over
Dear Editors

Thank you very much for your editorial decision letter, which also included the reviews of our manuscript by referees. We have made the changes as suggested by the reviewers. The changes are described in the revised text. We have made during the revision in a point-by-point response to each of the comments.

We hope the revisions made the responses provided are satisfactory, and our manuscript is now acceptable for publication in the **BMC complementary and Alternative Medicine**.

Please, let us know if further revisions are needed.

Once again, thank you for all your help. We look forward to hearing from you.

Sincerely yours

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Reviewers' comments

Reviewer #1:
Comment 1. In section Gross examination and histological analysis for gastric mucosa, authors write “compared with the EtOH group”, but quantitative or specific histological analyses were not presented. A more accurate analysis is requested.

We appreciate your comment. As commented by reviewer, the extent of mucosal injury was evaluated using light microscopy by an experienced histologist blinded to the treatment regimen. Quantitative analysis of gastric mucosal injury index was determined the representative photographs using an image analyzer (Molecular Devices Inc., CA, USA). These descriptions are inserted in Materials and Methods, and Results sections.
2. The rationale for choosing 400 mg/kg of this material in the present study may be explained. In addition, the authors used "omeprazole" as a positive control, while other studies usually utilize gastric mucus protective agents (e.g. rebamipide) as a positive in ethanol-induced gastric injury. The authors would explain why omeprazole is used as a positive control in this study.

We appreciated your comment. In preliminary study, we administrated BCT at doses of 400 and 600 mg/kg to rat before absolute ethanol intake. Mucosa damages and lipid peroxidation in stomach is reduced in 400 and 600 mg/kg groups of BCT compared with the absolute ethanol-induced gastric injury group (EtOH group). However, 600 mg/kg group reduced the protective effects in comparison to the 400 mg/kg. Based on these results, we determined the effective doses of BCT as 400 mg/kg. The drug treatment timing and absolute gastric injury induction induced by absolute ethanol were designed according to previous studies. In addition, omeprazole is a proton pump inhibitor and used in the treatment stomach ulcer. It is one of the most widely prescribed drugs. Many researchers used omeprazole as a positive control. According to previous studies, Omeprazole possesses anti-inflammatory and antioxidant activities [1,2]. Based on previous studies, we determined omeprazole as positive control.

Reference


3. Do the authors have any information that can be discussed about the active herbs and how it signals through the gastric mucosa to affect antioxidant system.

→ We appreciate your comment. We are hard to determine the active herbs of BCT because BCT is a herbal formula consisting 14 herbs. In oriental medicine, a mixture of several herbs is considered to enhance or prolong the pharmacological activities of any single component herb and decrease its toxic effects [1]. From the perspective of modern medicine, the production of formulation extracts is expected to result in chemical interactions between natural constituents existing in the component herbs of a herbal formula because the extracts are made using boiling water [2]. This may alter the extraction rates of the active ingredients or produce new molecules, which may exhibit additional pharmacological activities on various target systems in the body. These pharmacological actions are called “chemical combination effects”[3]. We consider these interactions produce the gastroprotective effect of BCT.


4. The author should explain or chemical analysis the active component of BCT. In addition, authors should be mentioned description for active component’s properties

→ We appreciate your comment. In our previous study [1], we analyzed the active component of BCT using high performance liquid chromatograph. We detected homogentisic
acid and hesperidin, as active components in BCT. BCT extract contained 1.1 mg of homogentisic acid and 5.4 mg of hesperidin in 1g BCT extract. In particular, hesperidin was proved its pharmacological properties by various in vitro and in vivo experiments [2-5]. Previous study demonstrated that hesperidin possessed antioxidant property [3,4,6]. Therefore, gastroprotective effect of BCT was considered to be closely related with the antioxidant property of hesperidin.


Reviewer #2:

Comment 1. In this study, authors evaluated the protective effects of BCT on gastric mucosal injury via its anti-oxidant property. Authors have used omeprazole as positive control drug. Omeprazole was considered as unsuitable drugs because it is proton pump inhibitor. The authors would demonstrate this issue.

-> We appreciated your comment. Omeprazole is a proton pump inhibitor and used in the treatment stomach ulcer. It is one of the most widely prescribed drugs. Many researchers used omeprazole as a positive control. According to previous studies, Omeprazole possesses anti-inflammatory and antioxidant activities (Lapenna et al., 1996; Sener et al., 2001). Based on previous studies, we determined omeprazole as positive control.

Reference


Comment 2. Authors have only explained the effects of BCT using figures for gross finding and histology of gastric mucus. However, authors need to quantitative analysis to investigate the effect of BCT.

We appreciate your comment. As commented by reviewer, the extent of mucosal injury was evaluated using light microscopy by an experienced histologist blinded to the treatment regimen. Quantitative analysis of gastric mucosal injury index were determine the
representative photographs using an image analyzer (Molecular Devices, Inc., CA, USA). These descriptions are inserted in Materials and Methods, and Results sections.

Comment 3. In gastric ulcer model, SOD activity is not significant between EtOH group and BCT group. This result should be explained in the manuscript.

→ We appreciate your comment. We inserted the description of SOD activity in discussion as follows:

In this study, oral administration of absolute ethanol notably decreased the activities of antioxidant enzymes, but administration of BCT significantly elevated the activities of enzymes in gastric tissue. Although BCT did not significantly increased SOD activity; however, the lack of an increase in SOD activity may reflect a lack of substrate for this enzyme [33].

Reference
Comment 4. In this study, the animals were received BCT at dose level of 400 mg/kg. Authors need to demonstrate the comparison between the dosage administrated to the animals in this study and clinical dose in human

→ We appreciate your comment. In human, the single dose of BCT is about 43.75 g dried herbs. Based on an average body weight of an adult of 60 kg, this dose for a 60 kg human is same as 729.1 mg /kg. This dosage is about 2-fold higher than experimental dose used in this study.

Comment 5. The authors described a lengthy explanation in discussion section. The authors need to reduce unnecessary or repeated sentences. In particular, authors need to make two paragraphs including MDA and GSH description into one paragraph in discussion section.

→ We appreciated your comment. We deleted the repeated and unnecessary states. In addition, in Discussion section, part of discussion about MDA and GSH was reconstructed to better understand.

Comment 6. Figures 2 and 3: Note magnification or scale bar

→ We appreciated your comment. We inserted the magnification.

Comment 7. Name of herbs should be italic

→ We appreciated your comment. We corrected as commented by reviewer
Comment 8. Reference:

- No. 21 : Title should be bold
- No. 26 and 27 : Page number check

→ We appreciated your comment. We corrected as commented by reviewer