Author's response to reviews

Title: Antioxidant and gastric cytoprotective prostaglandins properties of Cassia sieberiana roots bark extract as an anti-ulcerogenic agent.

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Author's response to reviews: see over
Reviewer 1-Illhami Gülçin

The reviewer accepted the revised edition of the manuscript
**Reviewer 2-Anderson Luiz-Ferreira**

**Comment 1:** When asked whether the antioxidant activity helps explaining the action against stomach disorder, gastric ulcer, stomach pain and indigestion, the authors did not answer. Indeed, compounds with antioxidant activities protect the gastric mucosa against injuries. What is the role of these substances in the pain and indigestion?

Response: The authors did respond on the role played by antioxidants in both gastric ulcer prevention and healing. The authors had previously investigated the antiulcer activities of the plant extract and therefore proceeded in this article to investigate its antioxidant properties. The background was therefore on the role of antioxidants in gastric ulcer healing and prevention. This has been adequately referenced in this revised manuscript [4-9, 29-32].

**Comment 2:** How do you correlate anti-inflammatory action with the increase in PGE$_2$ and PGI$_2$ levels? The supplied references by the authors do not correspond to the correlation between the increase in prostanoids and the anti inflammatory action and not simply take the term anti-inflammatory off. It is of extreme importance that this question is solved.

Response 2: The experimental work described in the manuscript was on the level of mucosal prostaglandins E$_2$ and I$_2$ in guinea pigs administered various concentrations of the plant extract. As previously answered, the role of PGE$_2$ and PGI$_2$ in gastric mucosal protection and healing has been extensively studied with the relevant references cited. The correlation between increase in mucosal PGE$_2$ and PGI$_2$ and their cytoprotective and ulcer healing properties has been extensively reviewed by several authors including John Wallace “**JOHN L. WALLACE: Prostaglandins, NSAIDs, and Gastric Mucosal Protection: Why Doesn’t the Stomach Digest Itself? Physiol Rev 88: 1547–1565, 2008**”. The term anti-inflammatory was taken off because the experimental work described in this manuscript did not involve the inducement of gastric ulcers and neither was any direct inflammatory study done.
Comment 3: The high antioxidant activity may be partly responsible for the observed stimulation of endogenous generation of PGE$_2$ and PGI$_2$, and the inhibition of PLA$_2$ activity and hence the therapeutic use as an anti-ulcer agent. This question must be revised by the authors, some metabolites of AA generate oxygen reactive species and thus may stimulate an antioxidant activity. The authors must discuss this matter.

Response 3: In this study, the experimental animals were not induced with gastric ulcers hence any significant increase in measured mucosal PGs might be due to the effect of the administered plant extract compared with the control and not the generation of free radicals. The text “The high antioxidant activity may be partly responsible for the observed stimulation of endogenous generation of PGE$_2$ and PGI$_2$, and the inhibition of PLA$_2$ activity and hence the therapeutic use as an anti-ulcer agent” has been revised accordingly. The authors however do admit the fact that as part of the inflammatory process, ulcerative stomachs generate ODRS which may stimulate the anti-oxidant activity of Catalase, Superoxide dismutase, Glutathione peroxidase and other anti-oxidative enzymes in a bid to repair and limit the effect of the ulceration but the activity of these anti-oxidative enzymes were not measured in vivo.

Comment 4: The authors must discuss how the extract inhibits PLA$_2$ (thus, acting as anti-inflammatory) and increases the production of PG, derived from AA, since the AA, precursor of PG, is released by the action of PLA$_2$. It is of extreme importance that this question is solved.

Response 4: The stimulation of endogenous generation of mucosal PGE$_2$ and PGI$_2$ despite the inhibition of serum sPLA$_2$ is interesting and puzzling. The authors have attempted to answer the question as part of the Discussion (page 15).

Comment 5: How can the authors correlate the anti oxidant assays performed in vivo with the main anti oxidant enzymes?

Response 5: The antioxidant assays described in the article were in vitro. No in vivo antioxidant assays were performed and hence the activity of antioxidant enzymes were not measured and
discussed.
Reviewer 3-Ciomar A Bersani-Amado

Comment 1: Animals, page 5. Why did authors use guinea pigs with such a wide range of weights (varying up to 100g). What was the maximum variation in the weights of animals for both the intra- and –intergroups?
Response 1: Their weight range has been replaced with their mean weight ± S.D. Their mean weight was 318 ± 30.3 g (mean ± S.D). This has been incorporated into the methods section of the manuscript.

Comment 2: Why were rats and not mice used in the experiments on acute toxicity? What was the weight of the rats used?
Response 2: Rats were used because of availability and appropriateness for the study (OECD-423 guidelines (acute oral toxicity-acute toxic class method). Their weight range has been replaced with their mean weight ± S.D. Their mean weight was 230.3 ± 14.5 g (mean±S.D). This has been incorporated into the methods section of the manuscript as recommended.

Comment 3: Blood sampling, p.7. Was anesthetic used? What type and dose of anesthetic were used?
Response 3: Yes, the anaesthetic sodium pentobarbitone (60 mg/kg body weight, i.p) was used.

Comment 4: Acute toxicity, p 8. The range of doses used is very wide (5-2000 mg / Kg). What doses were actually used?
Response 4: The dosage was gradually increased from 5mg/kg body weight to 2000mg/kg body weight with dosages such as 50, 300 and 600 up to 2000 mg/kg body weight. This has been incorporated into the methods section of the manuscript.

Comment 5: Describe the results in a more efficient way, because the present text is merely descriptive. Describe the results using tables or figures.
Response 5: Figures were presented in the first submission but were withdrawn and replaced with text in the revised manuscript as recommended by the reviewers. The authors find it appropriate to separate the results section of the article from the discussion in the present revised manuscript which also incorporates figures. This is to present the recommended incorporation of figures in the description of results.

Comment 6: The references must be modified in accordance with the journal norms. The number of references is very large and they could be selected more appropriately.

Response 7: Reference list have been modified accordingly. The number of references has been selected more appropriately and reduced from 50 to 40 as recommended.