Manuscript No. 70101

Title: Baicalin provides protection against fluoxetine induced hepatotoxicity by modulation of oxidative stress and inflammation

Response to Reviewers

The authors of this manuscript express their sincere thanks to the Editor-in-Chief and Hon'ble Reviewers for their critical assessment of our work. The authors have acted upon the comments of the reviewers which have resulted in a significant enhancement of the quality of this manuscript. The modifications made in the manuscript are shown in red.

Comments of Reviewer 1.

Comment 1. Baicalin has exhibited an antidepressant property as shown in various reports. The authors used the combination of baicalin and fluoxetine which is also an antidepressant medication in this model. Is it possible for the synergistic action of both compounds in term of neuropsychological or CNS effects may occur? How can the authors assure the safety of those amplification effects, if any?

Response: The neuroprotective and antidepressant properties of baicalin and its derivatives have been widely studied. Baicalin facilitates stimulation of neurogenesis and production of neurotrophic factors, modulation of hypothalamic-pituitary-adrenal (HPA) axis, which may further counter oxidative stress, and inflammation. Similar to fluoxetine and its metabolite norfluoxetine, baicalin also elicits anti-depressant effect by regulation of the GABA neurotransmitter system, and upregulating the GABA receptors. (**Added on Page 11-12)**

Although synergistic action of both compounds in term of neuropsychological or CNS effects have not been reported so far, it is very likely that the combination of fluoxetine and baicalin produce synergistic neuroprotective and antidepressant effects. Moreover, baicalin being a natural compound eliminates the fluoxetine induced excess ROS that causes oxidative stress and inflammation. However, further studies are needed to deepen our understanding on both the safety profile and molecular

mechanisms underlying the beneficial effects of baicalin. The scope of current study was limited to hepatoprotective effect of baicalin during prolonged treatment with fluoxetine. The study demonstrated hepatoprotective action without causing any adverse effect suggesting that oral administration of baicalin is safe. Other studies on baicalin also substantiated the safety of baicalin intake at test doses in rat model.

Comment 2. The effect of treatment on Body Weight (BW)

Comment 2.1: The results of baicalin treated group (gr 6) and baicalin co-treatment with fluoxetine (gr 4) showed significant weight gain. However, there have been some reports for Baicalin to decrease appetite and reduce BW by modulating the orexigenic and anorexigenic signals, which is in disagreement with the results of this study. Please state this point in the discussion part.

Response: After careful examination of Fig 2 (weight vs period of treatment), it was observed there was no significant weight gain in gr 4 and gr 6. The weight remained almost consistent throughout the study period with minor changes. To a certain extent, coadministration of baicalin with fluoxetine (gr 3 & 4) prevented excessive weight loss observed in fluoxetine treated gr 2 rats. However, in gr 6 baicalin treatment did not produce weight loss in our study. (These details are incorporated in the discussion part **page no. 12)**

Comment 2.2: It is difficult to understand the results in the line graph of BW in Fig 2, and some seems to be questionable. For example, at day 21 and 28, no significant symbols represented on gr 2 when compared with the control as well as no symbols showed on the other treatments when compared with gr 2. Is it better to represent the BW results in another format than in the line graph?

Response: We have changed the line graph format of Fig 2 to grouped column graph for clarity of results as suggested. Significant symbols have also been added to show inter group comparison. Description of significance symbols have been incorporated in Fig 2 legend.

Comment 3: If fluoxetine can cause liver inflammation, would it be better to directly determine the inflammation cytokines in liver tissues? Serum levels of cytokines can represent the inflammation from other organs.

Response: I agree with the suggestion of reviewer. We did not determine the inflammatory cytokines in liver tissue. However, in current study we have reported the anomalies in the levels of blood-based liver function markers such as AST, ALT, ALP, bilirubin and total protein in serum along with changes in serum inflammatory cytokines. Hence alteration in serum cytokine levels reported in our study may find direct correlation with liver inflammation.

Comment 4: Overall results from gr 4 is not better than the results from gr 5 (fluoxetine+silymarin) which served as the positive control. It will make the manuscript more engaging if the authors discuss the benefit of using baicalin over silymarin in the discussion part.

Response: Silymarin is a standard hepatoprotective compound that has ameliorative potential against free radical induced oxidative stress. In this study, hepatoprotective efficacy of baicalin was compared with silymarin. The results suggested that the efficacy of baicalin at 100mg/kg was comparable to that of silymarin at the same dose. In addition, it has been reported by Xu et al. (2018) that baicalin possesses higher oral bioavailability than silymarin. **(Incorporated in the Discussion section on page 15-16. Reference has also been cited as Ref no. 60).**

Therefore, baicalin can be used over silymarin as an alternative hepatoprotective compound to prevent fluoxetine induced liver toxicity.

Comment 5: Please clearly state the axis unit labels in the figures whether they are from serum or liver homogenates. If they are from the tissues, the results should be normalized with the liver proteins or weight and showed in the unit labels.

Response: In figures 3, 4 & 5 only serum parameters have been shown and all the suggested changes (including the axis unit labels) have been incorporated while liver homogenate biomarkers are represented in Table 1. The units of biomarkers in liver homogenate were already normalized and now units are shown in the Table 1.

Comment 6: The histological sections of rat liver

6.1 Please replace the histological figures with the better-quality images.

Response: All the figures have been replaced with higher resolution images.

6.2 It would be great if the authors add liver figures in all group of the treatment, including the positive controls.

Response: Figures of all treatment groups including positive controls have been added.

Comment 7: Please take attention to the discussion part, no need to specify figures or tables or significant value at the end of the sentence. For example, Baicalin and silymarin administration did not produce significant change on IL-10 levels in groups 3, 4 and 5 and results were comparable to group 2 rats ($p > 0.005$).

Response: All the suggested changes have been made in the discussion section.

Reviewer 2.

Comment 1: The authors shall discuss that baicalin itself can act as an anti-depressive agent.

Response: The anti depressive potential of baicalin has been incorporated in discussion section (**page 12)**.

Comment 2: There many small incorrect forms e.g. 100mg instead of 100 mg - between a number and a unit is almost always a space! Also check: between a word and a bracket belongs a space! Please check In the paper you write 'wistar' it should be 'Wistar' it is a proper Name and must be written with a capital 'W'

Response: The suggested changes have been made in the manuscript. The space has been added between number and unit as well as between word and bracket throughout the manuscript. ‘wistar’ has been replaced with ‘Wistar’.

Comment 3: The sentence 'This is probably the first study that assessed the alleviating effects of baicalin against fluoxetine induced hepatotoxicity, inflammation and oxidative stress.' must be changed since similar work is dealing with the subject - see missing published paper: The paper by Limanaqi and co-workers (Antioxidants 2020, 9, 234; doi:10.3390/antiox9030234) dealing with the same subject has neither cited nor discussed. In the same line of evidence, the authors shall cite and discuss the paper by Yang et al. (Front. Pharmacol., 11 February 2020 | https://doi.org/10.3389/fphar.2019.01685).

Response: The sentence "'This is probably the first study that assessed the alleviating effects of baicalin against fluoxetine induced hepatotoxicity, inflammation and oxidative stress" has been deleted and both the suggested papers 'Yang et al., 2020 and Limanaqi et al., 2020' have been cited (**Refence No. 33 and 34)**.

In addition, two more citations have been incorporated in the manuscript in the discussion section. **(Ref. no. 35 and 60)**

Re-reviewer.

Comment: No further comments; the authors have largely addressed my remarks.

Response: Thanks for your comments.