Author’s response to reviews

Title: Potentiating and synergistic effect of grape fruit juice on the antioxidant and antiinflammatory activity of aripiprazole against hydrogen peroxide induced oxidative stress in mice

Authors:
Seema Zargar (szargar@ksu.edu.sa)
Tanveer Wani (twani@ksu.edu.sa)
Abdul-Rahman Al-Majed (tanykash@yahoo.co.in)

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Author’s response to reviews:

Reviewer 1:

Major points:

The work did not show any significant new finding, e.g. as shown in Figure 6 and 7. It cannot be concluded in any significant way.

Response: In Figure 6 the enzymes related to oxidative stress were correlated with total protein levels in various treatment groups and in figure 7 the cytokine levels were also compared to total protein levels because enzymes and cytokines are also proteins. The correlation was performed to check whether change in the total protein content was proportional to the change in cytokine/enzyme levels. The results indicate that the total protein content is not significantly changed with change in the cytokine and enzyme levels.
The chromatogram should be added with more standard compounds as shown in Table 1, to support the previous findings beside naringin. Since in the chromatogram shows many specific compounds.

Response: We agree with the esteemed reviewer’s suggestion but unfortunately the standards drugs for the other compounds are not available and due to funding constraint the new standards cannot be procured. Further, naringin being the most abundant flavonoid in the grapefruit juice and varies in its content depending on origin of grapefruit, therefore, only the values of naringin were determined in this study.

The aripiprazole is an anti-psychologic drug with the conventional mechanism of dopamine receptor signaling, which are not anti-oxidant and anti-inflammatory activities. This fact should be mentioned about the linkage to antioxidant and anti-inflammatory activities.

Response: We appreciate the esteemed reviewer’s concern that aripiprazole might lack anti-oxidant and anti-inflammatory activities however the beneficial effect of aripiprazole on oxidative stress parameters were studied in a previous studies. The same has been elaborated in this manuscript.


The manuscript has been accordingly corrected [Section: Background, Paragraph 4]

The Table 2 data are very complicated in interpretation, especially in page 9. In the Table 2, the significant marks should be *, compared to control, and #, when compared to H2O2. The
statistical significance value should be footnoted under the Table 2. The footnote under the Table 2, of these markers may help more understanding and the ns (not significant should be deleted). The authors concluded that the effect was synergistically in anti-oxidant and anti-inflammatory activities.

Response: The result section has been rewritten and table data has also been modified as per the reviewer’s suggestion. Further, the data in table 2 indicates that the combination of aripiprazole and grapefruit juice completely reversed the significant change in the enzyme, and cytokine levels in hydrogen peroxide treated group to those in the control group thus, acting synergistically with each other. Although the reversal in the enzyme and cytokine levels in hydrogen peroxide treated groups was by both aripiprazole and grapefruit juice individually, the levels didn’t reached to those in the control group.

The changes in the manuscript can be seen in the [Section: Results; Paragraph: 2 and 3 and Table: 2]

Cavaillon JM, (2001) reviews that cytokines amount, nature of target cells, nature of activating signal, nature of produced cytokines, timing, sequence of cytokine action, experimental model are parameters, which influence the cytokine properties. Inflammation is characterized by an interplay between pro- and anti-inflammatory cytokines, which are commonly classified in one or the other category: interleukin-1 (IL-1), tumor necrosis factor (TNF), gamma-interferon (IFN-gamma), IL-12, IL-18 and granulocyte-macrophage colony stimulating factor are well characterized as pro-inflammatory cytokines whereas IL4, IL-10, IL-13, IFN-alpha and transforming growth factor-beta are recognized as anti-inflammatory cytokines. However, one cytokines can act as pro-inflammatory and anti-inflammatory character, which depends on the mentioned factors. Nevertheless, the data in Figure 6 and 7 are bizarre in the R2 value, which are not concluded significantly in any specific relationship.

Response: We agree with the esteemed reviewer’s viewpoint that pro-inflammatory cytokines get increased in oxidative stress causing tissue damage. Hence we studied them on the basis of their pro-inflammatory character, however, one cytokines can act as pro-inflammatory and anti-inflammatory. In this study relationship between total protein concentrations with
cytokine/enzyme levels was observed and the same has been reported in figures 6 and 7. The correlation was performed to check whether change in the total protein content was proportional to the change in cytokine/enzyme levels. The results indicate that the total protein content is not significantly changed with change in the cytokine and enzyme levels. Whatever the correlation were obtained have been reported as such in the manuscript.

Creatine kinase isoform was measured, which isoform(s) since there are three of them? Why is CK an important factor, which needs to be determined?

Response: The serum creatinine kinase was estimated and the individual isoforms of CK were not determined in this study. Creatinine kinase was selected since it plays a key role in energy metabolism of nervous tissue and is sensitive to oxidative damage and might be one of the targets for ROS in brain of neurodegenerative disease.

Reference: Resveratrol protects C6 astrocyte cell line against hydrogen peroxide-induced oxidative stress through heme oxygenase1. PLOS one 2013; 8(5):E64372.

Minor point:

English language is needed to be corrected intensively, since it is in informal way and not suitable to report in a scientific journal, such as didn't, isn't. The word "GF", or "GF1" should be the same or consistent all through the manuscript. The references are not consistent with each other, such as the journal name, some are full names, and some are abbreviated forms.

Response: Corrections done as per the esteemed reviewer’s suggestion.
Siwen Chen (Reviewer 2): 1. The text itself contains various grammatical errors, extensively long sentences, confusing statements and disorganisation. A native English speaker should review the document to ensure this is remedied. I only point out few of these mistakes below.

For example, "……having high concentration of polyphenols might potentiate and synergize to the therapeutic effect of ARI, by increasing its bioavailability and inherent antioxidant potential of grape fruit juice (GFJ) decreasing the dosage of the drug." in "Background" of "Abstract", the correct sentence maybe "……having high concentration of polyphenols might potentiate and synergize the therapeutic effect of ARI by increasing its bioavailability and inherent antioxidant potential of grape fruit juice (GFJ) could decrease the dosage of the drug."

Response: The text has been corrected as per the esteemed reviewer suggestion [Section: Abstract; Paragraph 1].

For example, "To evaluate the protection against oxidative stress and inflammation by ARI and GFJ alone and in combination in H2O2 treated mice with respect to serum levels of certain biochemical markers (ALT, BUN, CK, creatinine and total protein) and pro-inflammatory cytokines (IL-1α, IL-2, IL-10 and TNF-α) were measured.", the correct sentence maybe "To evaluate the protection against oxidative stress and inflammation by ARI and GFJ alone and in combination in H2O2 treated mice, serum levels of certain biochemical markers (ALT, BUN, CK, creatinine and total protein) and pro-inflammatory cytokines (IL-1α, IL-2, IL-10 and TNF-α) were measured."

Response: The text has been corrected as per the esteemed reviewer suggestion [Section: Results; Paragraph: 2]

For example, what is the meaning of "BUN levels where significantly higher than H2O2 treated group compared to control group." in the 4th paragraph of Results? Please correct it.
Response: The results section has been rewritten and the table 2 updated for better understanding. [Section: Results; Paragraph: 2]

For example, what is the meaning of "……the difference between BUN levels of ARI+ H2O2 treated group and GFJ+H2O2 treated group were significant compared to control group. " in the 4th paragraph of Results? Please correct it.

Response: Correction done [Section: Results; Paragraph: 2]

2. In the 4th paragraph of Background, they state that "The anti-inflammatory response is demonstrated by the increased production of the pro-inflammatory cytokines,……", which confuse me. Do they mean "The inflammatory response is demonstrated by the increased production of the pro-inflammatory cytokines,……"?

Response: Correction done [Section: Background, Paragraph 4]

3. Are the "GF juice" in Experimental Protocol of Methods, "GF" in Table 2 and "GFJ" the same substance?

Response: GF has been replaced with GFJ in whole manuscript.

4. In the Biochemical Analysis of Methods, "Protein values are expressed as mg/ gram fresh tissue.", do they evaluate the protein values in fresh tissue? Please confirm.

Response: Fresh tissue is a typographical error and has been deleted. Total protein content has been evaluated in serum. Protein values were expressed as g/dL. [Section: Biochemical Analysis, Paragraph 4]
5. In the results, they always compared the data in the treatment group with that in the control group (fed with normal saline), do they believe that ARI, GFJ, and ARI+GFJ can bring down the data to be lower than normal? The comparison between the treatment group and the model group (fed with H2O2) maybe more appropriate. They must rewrite them in the Results, Discussion and Figure legend.

Response: The results and discussion has been rewritten [Section: Results, Discussion]

6. If the phrase appears more than once, full names should be accompanied by abbreviations for the first time, then abbreviations should be used from the second time. So the "GFJ" should be instead of "grape fruit juices" in the last paragraph of Background, and the "ROS" should be instead of "Reactive Oxygen Species" in the last paragraph of Discussion.

Response: Correction done [Section: Background, Paragraph 4]

7. They concluded that supplementing GFJ to ARI could decrease the potential side effects of ARI intake (in the Abstract). However in this manuscript, ARI had no influence on the liver function and kidney function. What was the potential side effects of ARI? How could they conclude that?

Response: The potential side effects of aripiprazole include vomiting, aggression, confusion, tremors, convulsions and all these side effects are dose dependent. Therefore decreasing the dose of drug might alleviate these potential side effects.

8. In this manuscript, results showed that supplementing GFJ to ARI treated mice could enhance the antioxidant and anti-inflammatory effects of ARI on hydrogen peroxide induced oxidative stress in mice. They conclude that "supplementing GFJ to psychotic patients treated with ARI might decrease the dosage of ARI" in the Abstract. I don't think this conclusion is rigorous,
because that the model used in this study was not "psychotic patients", but "oxidative stress in mice". Please correct it.

Response: Correction done [Section: discussion, Paragraph 4]

9. In this study, H2O2 treated mice was used as oxidative stress animal model, and the damage of liver and kidney function were considered as the results of oxidative stress, why not use malondialdehyde (MDA), the direct production of oxidative stress? They concluded that "supplementing GFJ to psychotic patients treated with ARI might decrease the dosage of ARI", why not detect the oxidative stress in the brain? If they did, it will be helpful to draw this conclusion.

Response: We agree with the esteemed reviewer’s suggestion and a future study will be designed to study the effect of combination in the brain.

10. In this study, decreased serum total protein was considered as the results of oxidative stress, but I think it was also the results of impaired liver function. They have better state it in this manuscript.

Response: The valuable suggestion has been added in discussion section.

11. The format of references must consolidate according to the guideline of the journal.

Response: Corrections done in the revised manuscript.
REVIEWER 3:

Alok Dash (Reviewer 3): Q1- Addition of graphical abstract may increase the strength of the paper.

Response: The graphical abstract is not supported by the journal format.

Q2- Give proper explanation along with citation of previously research grape fruit juice on the antioxidant and anti-inflammatory activity

Response: We would like to inform the esteemed reviewer that the references regarding the antioxidant and anti-inflammatory activity of Grapefruit have already been provided in the manuscript. (Refer to references 16-22)

Q3- The plant profile along with the image of the plant may be beneficial for the researchers.

Response: The details about the plant have been provided in the revised manuscript.

Q4- Give proper citation of extraction

Response: No specific procedure was undertaken to extract the grapefruit juice from the fruit. GFJ was extracted by a domestic squeezer (Braun Citromatic Pulp Control MPZ6) and is mentioned in the manuscript. [Section: Method, Subsection: Preparation of grape fruit juice]

Q5- Have you done acute toxic study, if not then how you calculate the effective dose?

Response: No toxicity study was performed and the dosage was selected on the basis of previous literature.


Q-6- In in vivo study what is your standard drug?

Response: Aripiprazole is itself a standard drug and was studied in combination with GFJ.

Q-7- Why not you done histopathology study?

Response: The aim of this study was to evaluate the synergistic effect of GFJ on aripiprazole using various biochemical and anti-inflammatory markers. Therefore no histopathological studies were performed.

Q-8- Before chromatography In pharmacognostical study the responsible phyto-constituent should be noted , have you done it?

Response: Naringin is the most abundant flavonoid present in GFJ and has antioxidant and anti-inflammatory potential.

Journal of Molecular Catalysis B: Enzymatic 2008, 52–53:13–18

Q-9- In HPLC where is your standard curve, So how you compare the peak of your extract.

Response: The calibration curve was prepared and the concentrations of calibration standards have been provided in the revised manuscript. We didn’t provided the standard curve as it would have unnecessarily increased the number of figures. [Section: Method, Subsection: Flavonoid analysis]

Q10- The author should properly arrange the table and figure Legends.

Response: Correction done
Q-11- The discussion and part should rewrite again.

Response: The discussion has been updated in the revised manuscript. [Section: Results and Discussion]

Q-12- Re-correct the typo error and arrange it according to the journal guidelines.

Response: Correction done

Q-13- The author should add latest reference in reference section.

Response: Old references replaced by more relevant References.