Author's response to reviews

Title: Protective effects of Egyptian cloudy apple juice and apple peel extract on lipid peroxidation, antioxidant enzymes and inflammatory status in diabetic rat pancreas

Authors:

Samah M Fathy (samahfathy1972@gmail.com)
Ehab A Drees (ehab.kadry@gmail.com)

Version: 4 Date: 20 September 2015

Author's response to reviews: see over
Dear Editor

I noticed that some comments I made to the revised manuscript were not appear in the Pdf. Please accept this version of the revised manuscript.

I declare that I uploaded the revised manuscript for the article “Protective effects of Egyptian cloudy apple juice and apple peel extract on lipid peroxidation, antioxidant enzymes and inflammatory status in diabetic rat pancreas”. In addition, here are the answers for reviewer’s comments.

First, regarding the major and minor revisions that have been required by reviewer’s 1:

Major revisions

1. In Table 3 there is no value for the blood glucose level in normal controls 3 days after the STZ administration. There are no superscripts of a statistical significance of changes for this parameter between experimental and control groups 3 days after the STZ treatment, while in the Results section marked changes are reported. Moreover, on what basis did the Authors came to the conclusion described with the following sentence “After STZ injection, a significant increase in blood glucose was observed in diabetic control rats as compared with normal control rats (P<0.05)”, if the value in the normal control group is unknown?

We didn’t write blood glucose value despite we measured it because we are not comparing the level in diabetic groups until we started the treatment which is on the 4th day as we mentioned in Materials and methods (Induction of diabetes). Before that, it is already known that blood glucose level of 250 mg/dl or above were considered to be diabetic (Bagri et al., 2009). To avoid ambiguity, the 72 h was deleted in the revised manuscript for this part and it was a mistake to add (P<0.05) so it was deleted too (please refer to Effect of CAJ and APE supplementation on hyperglycemia in experimental animals, line 3, 4). Regarding the sentence “After STZ injection, a significant increase in blood glucose was observed in diabetic control rats as compared with normal control rats (P<0.05)”, it was referring to the increase from the first week till the third week compared with the normal control. If this will cause confusion, we can add the reading of blood glucose at 3 days with the superscript. The reading was (84.89± 8.12).

2. Page 16 lines 343-346. There is information that CAJ and APE could effect on enzymes engaged in carbohydrate’s metabolism. Did the Authors estimate activity or expression of any of these enzymes?

No, non of these enzymes were measured but this is a good point to be estimated in the future as we added in the revised manuscript (please refer to the correction in the revised manuscript).

Minor revisions:

1. In the description of the animal experiment there is wrong name of rat stock - Waster. Probably Wistar rats were used in the experiment.

Corrected

2. The description of treatments of experimental groups is unclear. There are no details on the time of the beginning of administration of the tested preparations and streptozotocin (STZ).

(Please refer to the revised manuscript).
3. The use of superscripts (a,b) in tables and figures is unclear. It would apply different superscripts for marking statistically significant differences observed versus normal control and diabetic control groups.

**Different superscripts were applied in tables and figures.**

4. Page 15 line 333. Epicatechins, catechins, and procyanidins are not “several classes of polyphenol antioxidants “they belong to the flavonoid class.

**Corrected (please refer to the manuscript).**

5. Page 17 lines 366-367. It is untrue to say that “LPO is an index of MDA production” because MDA serves as an index of lipid peroxidation (LPO).

**Corrected (please refer to the manuscript).**

**Quality of written English:** Needs some language corrections before being published

**It is carefully revised and corrected in the revised manuscript.**

Second, regarding the major and minor revisions that have been required by reviewer’s 2:

1. Many small errors happened throughout the text. For example:

   1) Background, paragraph 2, line 6, “beta cells” which was not a general writing.

   **Beta cells is written first in the revised manuscript as beta cells (β-cells) then it is adjusted as a general writing throughout the text as β-cells.**

   2) Background, paragraph 5, line 15, reference cite in text was not consistent with others.

   **It is corrected (please refer to the revised manuscript)**

   3) Methods, Preparation of CAJ and APE, paragraph 1, line 3, missing units. Same paragraph line 4, H₂O need subscript, and same paragraph line 8, seemed wrong writing of degree Celsius. And there were so many same errors in following paragraphs.

   **All errors are corrected (please refer to the revised manuscript).**

   4) Methods, Blood and tissue sampling, paragraph 1, line 3, wrong writing of unit.

   **It is corrected (g instead of xg)**

   5) Methods, Estimation of pancreatic biochemical parameters, paragraph 2, line 2,

   **The compound should be named following IUPAC.**

   **It is written in IUPAC (see revised manuscript)**

   6) There are so many spacing errors throughout the manuscript. Please carefully check them.

   **They are checked and adjusted in the revised manuscript.**

2. GC/MS analysis section:

   1) It is too hard to recognize target peaks in Fig 1 and 2. Distinct total ion chromatograms of GC/MS should be provided.

   **The distinct total ion chromatograms were provided for both CAJ and APE (figures 1, 3) and GC/MS fragmentation patterns of polyphenolic compounds for CAJ and APE were also provided (figures 2, 4).**
2) In Fig 1 and 2, there were many other peaks except the target peaks that were not identified. Since these peaks account for chromatograms about more than half that cannot be ignored.

We focused on the peaks of compounds that are reported to have intimate relation to the parameters under investigation such as antihyperglycemic, anti-inflammatory, antioxidative, and hypolipidemic effects. To overcome this issue, we can provide the sheet which contains all compounds in the form of table as supplementary materials.

3) In Methods section, author mentioned that identified compounds were compared with authentic substances. However, there was not any other information about these “authentic substances”. The GC/MS chromatograms of authentic substances should be provided.

   We provided the name of the library which contains these authentic substances. To recognize compounds, we compare the ion chromatogram for each compound with ion chromatogram existing in the provided library (NIST and WILEY library).

4) To avoid confusion, the identified compounds of this manuscript should be named uniformly in Result and Discussion.

   They are consistently named in results, discussion and but to make it easier, we wrote both the common and IUPAC names in results, figures, tables while in the discussion, we named them by the common names only.

3. Four groups were set in this manuscript. However, positive control was not provided, and the antidiabetic effect of these apple juice and extract cannot be assessed.

   Actually, we had 2 positive control groups along with the rest of the groups and measured the blood glucose level in these groups too which were not significantly different from the normal control group. In order to reduce the costs, we didn’t measure the rest of the parameters for these groups and excluded them taking into account that several studies were carried out to investigate the antidiabetic effect of plant extract without providing positive control group for example:

1. Bagri et al., 2009 investigated “the antidiabetic effect of Punica granatum flower” without using positive control group (please refer to references in the revised manuscript). In addition,


4. There is no data regarding the content of polyphenolic compounds in apple juice and extract. Since the authors claim that this polyphenolic compounds is the important bioactive component. The percentage content of polyphenolic compounds should be provided (such as total phenolic content).

Total phenolic contents were analyzed in both apple juice and peel according to The Folin–Ciocalteu method (See Materials and Methods) and their results are added to the revised manuscript. The references’ numbers are adjusted accordingly.

5. The pharmacological effect of CAJ and APE were not significant different in this manuscript. However, the compositions of biomolecules were different between CAJ and APE. Author should be illustrate this contradiction.

This is not considered as a contradiction as all biomolecules detected in either CAJ or APE were phenolic compounds that were proven to exert antihyperglycemic, antihyperlipidemic, antioxidative and anti-inflammatory consequences as we explained in the last two paragraphs of the discussion part, even if these compounds are different.

1. In Discussion section, since NF-kB pathway is important to pharmacological effect of CAJ and APE. Some experiment should be done to exploit this pathway, such as Western bolt or qPCR.

Since so many analyses were carried out in this article, it will be difficult to explore this pathway at the moment and we hope that the measurement of the protein level will be enough for now. Meanwhile, in the future, we are planning to exploit NF-kB pathway in detail.

2. The main conclusions of this manuscript are ambiguous. Author should consider which aspect is the highlight of the manuscript, and made it clear and distinct.

We modified the conclusion part to make it obvious and distinct (Refer to the revised manuscript).
Third, for editorial requirements:

The paper was copyedited by a native English speaker. All corrections were highlighted in yellow or comments are added.

I hope my answers will be appropriate and satisfactory.

Thanks for the time you spend in reviewing my article and I am looking forward to hear from you.

Sincerely
Samah Fathy
Biology Department
Faculty of Science
Jazan University
Jazan
KSA

Permanent Address
Zoology Department
Faculty of Science
Fayoum University
Fayoum
Egypt