Author's response to reviews

Title: Camel Milk Ameliorates Steatohepatitis, Insulin Resistance and Lipid Peroxidation in Experimental Non-Alcoholic Fatty Liver Disease

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Author's response to reviews: see over
Dear Editor in Chief of BMC Complementary & Alternative Medicine

It is my pleasure to submit the revised version of my manuscript titled: “Camel Milk Ameliorates Steatohepatitis, Insulin Resistance and Lipid Peroxidation in Experimental Non-Alcoholic Fatty Liver Disease”

All the reviewer’s comments were taken into consideration and the questions were answered.

In order to improve the quality of written English the manuscript was revised by a native English speaker specialist in scientific editing. Special care was directed to the abstract. The revised manuscript conforms to the journal style. Kindly find a list of the point-by-point response to the reviewer comments.

Yours sincerely,

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Point-by-point response to the Reviewer comments:

Please note that:
The red font text is the reviewer’s comments.
The black font text is the original text.
The blue font text is the author’s reply.
The deleted parts are highlighted grey and the newly added or the corrected parts are highlighted yellow.

Reviewer: Azin Nowrouzi
Reviewer’s report:

Dear editor

The manuscript entitled “Camel Milk Ameliorates Steatohepatitis, Insulin Resistance and Lipid Peroxidation in Experimental Non-Alcoholic Fatty Liver Disease” despite being interesting, contains so many mistakes that I’m afraid it cannot be accepted in the present condition. The article still needs extensive and careful revision.

The wrongs are numerous and I will mention just a few:

In response to the comments of the respected reviewer about the grammatical and typing errors the manuscript was subjected to English and grammar editing by a native English speaker specialized in scientific editing.

Methods:

Induction of hyperlipidemia:
Commercial rat chow …that contains (g/100 g) crude protein 20, fat 4, …. Perhaps it is better to omit units here because in the formula g/100g the gs cancel each other.

This was corrected to:
Commercial rat chow (Grain Silos & Flour Mills Organization Riyadh Branch, Riyadh, K.S.A) that contains (g/100 g) crude protein 20 g; fat 4 g; crude fiber 3.5 g; ash 6 g; salt 0.5 g; calcium 1 g; phosphorous 0.6 g; vitamin A 20 IU/g; vitamin D 20 IU/g; vitamin E 20 IU/kg, trace minerals of cobalt, copper, iodine, iron, manganese, selenium, and zinc was used in C and CCM Groups. 20% crude protein; 4% fat; 3.5% crude fiber; 6% ash, 0.5% salt, 1% calcium, 0.6% phosphorous, 20 IU/g vitamin A, 20 IU/g vitamin D, 20 IU/kg vitamin E, and trace amounts of cobalt, copper, iodine, iron, manganese, selenium, and zinc was used in the C and CCM Groups. A high-fat, cholesterol-rich diet (HCD), in which 42% of the energy is derived from fats, was prepared by the addition of 1.5% cholesterol (Sigma Aldrich, USA) and 8% coconut oil to the basal diet [5]. The HCD was prepared every 2 days, kept at 4°C, and given to rats in the Ch and ChM Groups for 8 weeks.

Results:
1. At the end of the first paragraph the sentence should probably be as follows:
   …..but the ChM group weight increase was comparable with the C Group (p>0.05). There was no significant difference ….

   This was corrected as advised by to:
   but that the ChM Group’s weight increase was comparable with that of the C Group (p > 0.05). There was no significant difference in weight gain between the CCM and C Groups (p > 0.05).

2. Page11: Glucose tolerance and serum insulin. Glucose tolerance may be better replaced by Serum glucose ….  
   This was corrected as advised by the reviewer to:

   **Glucose tolerance** **Fasting blood glucose** and serum insulin

3. What do you mean when you say CM abolished the HCD-induced increase in FBG? The FBG did not go to very high values in the first place. And in the caption for Fig. 2A you have both Ch and ChM groups similarly different from control. The where did you get this p value of 0.004 that you mention in the text.
   Actually there was significant increase in the FBG of the Ch Group (87.30 ± 2.51 mg/dl compared with 76.00± 1.22 mg/dl in the control Group ( p< 0.05).
   However, FBG in the CM- treated ChM Group (79.5± 1.71 mg/dl) was significantly lower ( p= 0.004) in comparison with to the Ch Group, but was comparable to the FBG level of the control Group (p>0.05). Regarding the Caption in Fig. 2 only the Ch Group is significantly different from the control
Group (has † strike) and the ChM is not significantly different from the control Group and the † strike was wrongly placed in the previous version of the figure and now we removed it.

**This part of the results is corrected in page 11 paragraph 2 as follows:**

Camel milk administration to the ChM Group abolished the HCD-induced increase in FBG in comparison with the Ch Group (p = 0.004). Camel milk administration inhibited HCD-induced hyperglycemia in the ChM Group in comparison with the Ch Group (p = 0.004), but there was no significant difference with respect to the C Group (p = 0.17)

4. There are so many semicolons that should be replaced by comma and you have misplaced periods.
   All the typing and grammatical errors were corrected after revision by a native English speaker.

5. After explaining Fig 2B you go on to explain Fig 3 A and B and then you come back to Fig 2b to explain HOMA-IR. It is better to finish with Fig. 2B and then go on to the next figure.
   We explained all Fig. 2 including the HOMA-IR in page 11 then we started to explain Fig.3 in page 12 paragraph 2 as advised.

6. Page 14: liver function. This paragraph is not well written. It is not clear.
   **This paragraph was re-written as follows:**

   After eight weeks of daily administration of HCD to the Ch Group there was significant alteration in the liver function as compared with the C Group (p < 0.001). Elevated AST, ALT, AP, GAMA-GT, and bilirubin levels manifested this. Additionally, their serum protein and albumin levels were significantly low (Table 2). Camel milk administration to the ChM Group abolished the HCD-induced changes in liver functions observed in the Ch Group (p < 0.001). Similarly, CM administration resulted in a significant increase in total serum protein and albumin levels in the CCM Group as compared with the C Group (p < 0.001), but the other liver function parameters were unchanged (p > 0.05).

   After eight weeks of The administration of HCD to the Ch Group for eight weeks resulted in significant increases in the AST, ALT, AP, GAMA-GT, and
bilirubin levels, but it decreased the serum protein and albumin levels compared with the C Group (p < 0.001) (Table 2). The alterations in liver functions observed in the Ch Group were abolished in the ChM Group after CM treatment (p < 0.001). Furthermore, the CCM Group showed significant elevation of the total serum protein and albumin levels compared with the C Group (p < 0.001), in the absence of any changes in the other liver function parameters (p > 0.05).

7. Figure 4 has been mislabeled as Fig. 4.

Figure 4 label was revised and corrected

8. In the figure captions you keep on repeating the statistical method, which is redundant.

The statistical method was removed from all the figures’ captions as advised.

9. In title of caption for Fig. 2 you have misplaced (B).

The caption of Fig. 2 was revised and corrected as follows:

Fig. 2: (A) Mean random blood glucose level in all groups during the eight weeks of the study (A), (B) fasting blood glucose, serum insulin and HOMA-IR in control and high-fat diet treated groups and the effect of camel’s milk intake at the end of the study (B). Statistical analysis was carried out by ANOVA, and when the results were significant, post hoc LSD test was performed to identify the significantly different groups. The results were considered significant at p < 0.05 † p < 0.05 versus Control, ‡p < 0.05 versus Control + camel milk, § p < 0.05 versus Cholesterol, ¶ p < 0.05 versus Cholesterol + camel milk.

10. Also in some figures you have used abbreviations for your groups and in some figures you mention the complete name.

We revised all the figures and used the abbreviations of the groups instead of the complete name.

11. In caption for Fig. 3 you do not mention anything about ITT.

The caption of Fig 3 was revised and ITT was added as follows:
Fig. 3: Intraperitoneal glucose tolerance test (i.p GTT) and insulin tolerance test (ITT) in rats fed standard and high fat diet with and without camel milk treatment for 8 weeks.

12. You did not explain what FP is in Table 1 and Table 2. F value of ANOVA test and P value (the probability of error) was shown in the tables’ captions. The results were considered significant at p < 0.05.

Discussion:

13. Paragraph 2 page 15: what do you mean by “…de-esterification of the abundant FFAs”?

Fatty acids are esterified to fatty acyl-COA and transported to the mitochondria to undergo beta oxidation, form phospholipids or triglycerides. De-esterification of free fatty acids from glycerolipids including phospholipids and triglycerides could lead to increased FFAs in plasma.


Saini-Chohan, H K et al., Delineating the role of alterations in lipid metabolism to the pathogenesis of inherited skeletal and cardiac muscle disorders. J Lipid Res. 2012. 53:4–27.

14. Another question: one problem with oral insulin is that it is degraded in the GI tract, then how do you think the insulin inside CM will be absorbed intact?

Camel milk was reported to resist acid digestion in the stomach and this makes it available for absorption in the intestine (Food and Agriculture Organization of the United Nations: Camel milk and cheese making. In: The Technology of Making Cheese from Camel Milk (Camelus dromedarius). FAO Animal Production and Health Paper 113, Rome, 2011).

Several possibilities were suggested by Malik et al 2012 to explain this:

i. Insulin in CM may have specific properties that helps absorption into the circulation and resist proteolysis.

ii. The insulin molecules in CM may be encapsulated in lipid nanoparticles that protect it during its passage in the stomach and entry into the circulation.

iii. CM may contain small yet unidentified small molecules of insulin-like regulatory values or protease inhibitory properties to prevent proteolysis.
15. There are many grammatical and punctuation errors throughout the manuscript and the recent corrections by the authors are not sufficient. The manuscript was subjected to English and grammar revision and the corrections were done as advised by a native English speaker specialist in scientific editing.

**Level of interest:** An article of importance in its field

**Quality of written English:** Not suitable for publication unless extensively edited.

The manuscript was subjected to English and grammar editing by a native English speaker specialized in scientific editing.

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
I have no competing interests