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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Version: 6 Date: 16 August 2013

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

Thanks for giving me the chance to revise my manuscript and re-submit it to your journal.

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

In the current study we reported and for the first time that camel milk is effective in inhibition of the steatohepatitis, insulin resistance and lipid peroxidation triggered by high-fat cholesterol-rich diet (HCD). This action would add an important strongly desired effect of camel milk in the prevention of fatty liver and insulin resistance associated with obesity. This in addition to the reported health promoting effects of camel milk is very promising and intensified the importance of this natural compound, with unknown major side effects, in achieving the goal of utilizing functional foods in treating one or more of the major health problems associated with the current unhealthy life style and feeding habits that leads to hyperlipidemia and insulin resistance with the consequent plethora of disorders constituting the metabolic syndrome.

The content of the manuscript is original and it has not been published or accepted for publication, either in whole or in part, in any form. No part of the manuscript is currently under consideration for publication elsewhere, and no conflict of interest is present. All authors have contributed to the work and agreed to submit it for consideration to BMC Complementary & Alternative Medicine

Kindly find a list of the point-by-point response to the reviewers’ comments.

Yours sincerely,

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Point-by-point response to Reviewers' comments:

The black color text is the reviewers comment, the blue one is the authors reply, the red highlighted text has been deleted and yellow highlighted text is the newly added parts. The page number has been changed in this version because of the added contents so the page number in the reviewer comment may be changed in the revised version.

Reviewer: nayef algharaibeh

7. The article was well written and the sequence of writing is understandable, the only mistake in the writing was in the third paragraph of introduction, line two: "it is known to helps" should be (It is known to help) Corrected as advised
and in Paragraph four line six “cellular ailments” should be (cellular elements). The word ailments means physical disorder or illness especially of a minor of chronic failure.

**Reviewer: Azin Nowrouzi**

**Reviewer's report:**

Minor Compulsory Revisions

**Introduction:**

- High fat-rich cholesterol diet should be replaced by high-fat, cholesterol-rich diet. Corrected as advised
- The content is fine but the organization and wording can improve.
- An example of grammar error “Camel milk ….its high energy and vitamin contents is known to helps immune deficient…” corrected to: camel milk (CM) is used in the hot and arid regions, as essential nutritional source, its high energy and vitamin contents is known to help immune deficient patients and those in convalescence from diseases

**Methods:**

- The method is fine.
- An example of grammar error “A commercial kit …..the antioxidant /cat activity … The method is based on the reaction…..” Corrected to: A commercial kit produced by Cayman Chemical, Ann Arbor, MI, USA measured the antioxidant CAT activity in serum and liver homogenate. The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H\textsubscript{2}O\textsubscript{2}.

**Results:**

- An example of grammar error under the title FBG and insulin levels when you finish explaining Fig. 2B, you say “However, CM….” Corrected to: However, CM-treated CCM Group showed no significant….”
- Under HOMA-IR last line you say “Notably, ….was resulted in …” Corrected to: Notably, administration of CM to the CCM Group, in addition to the normal rat chow, was resulted in no significant changes in the HOMA-IR as compared with the C Group (p > 0.05).
- Under liver function “Additionally, there serum protein….” Corrected to: Additionally, their serum protein and albumin levels were significantly low.
Discussion:

- The third paragraph starts with “Beside” which should be “Besides” meaning in addition to. The same in page 16. Corrected in both sites.
- “Not any more” can be replaced by “no longer”. Corrected as advised
- The observed loss of structural integrity ….., released excessive amounts….”
  Who is the subject? Who is releasing excessive amounts of …? This was corrected as follows: The observed loss of the structural integrity of the hepatic tissue, of the Ch Group animals, demonstrated hepatic cell injury that released excessive amounts of the intracellular hepatic transaminases…… (The subject is the hepatic cell injury).
- What do you mean by “functional affection”? “Affection” means fondness, love, liking… This was corrected to: functional impairment in the sentence (The structural distortion and the functional impairment of the hepatic cells……
- “This makes it an excellent source of … which exert a lot of the biological…” no need for” the”. The word the was deleted

When proposing indirect mechanisms for the action of camel milk, no references has been given for (ii) item. I think you should be more specific mentioning for example, CM may act through adjusting the PPARalpha/SREBP1 ratio as mentioned in the recent paper by “Ziamajidi, N., et al. Amelioration by chicory seed extract of diabetes-and oleic acid-induced non alcoholic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH) via modulation of PPARa and SREBP-1. Food Chem.Toxicol.(2013), http://dx.doi.org/10.1016/ j.fct.2013.04.018” or other papers. This point was corrected as follows: Camel milk may act through adjusting the PPARalpha/SREBP1 ratio as mentioned in the recent work by Ziamajidi N, et al leading to enhanced activity of the fat metabolizing enzymes and hormones resulting in increased caloric loss or decreased fat storage is not unexpected hypothesis.
- In the last paragraph in page 16, it would be better to say increased peripheral response to insulin or something like that. Corrected to increased peripheral response to insulin.
- Again in this paragraph, you say that CM is distinctive oral natural compound that not only helps the glycemic control but also preserved the normal lipid profile ..” the two verbs should have the same tense. First one (helps) is present tense but “preserved “is in past tense. “Preserves” would be fine. Corrected to: identified it as distinctive an oral natural compound that not only helps the glycemic control but also
preserves the normal lipid profile in the CM treated animals in the current study.

- Again in this paragraph, in addition to CM, other natural products or medicinal herbs have been reported to have both glucose lowering and lipid-lowering capabilities; CM is not alone. Chicory seed extract for example. Actually Chicory seeds, This was corrected as follows: In addition to CM, other natural products or medicinal herbs including chicory leaves, barely grains, celery, lycium and barbarum have been reported to have both glucose lowering and lipid-lowering capabilities (57,58).

- “NAFLD induced by HCD’ can be replaced by “HCD-induced”; page 17. Corrected as advised

- “Mode of actions” should be replaced by “modes of action”; page 17. Corrected as advised

Conclusion:

- The second sentence “This in addition …..” is a very long sentence with meaning that is not clear. Please revise the sentence and be careful about the verb and subject relationship, and tenses. This sentence was rephrased as follows: This in addition to the reported health promoting effects of CM is very promising, and intensified the importance of this natural compound, with unknown major side effects, in achieving the goal of utilizing functional foods, in treating one or more of the major health problems, associated with the current unhealthy life style and feeding habits, that leads to hyperlipidemia, dyslipidemia, and the consequent plethora of metabolic disorders collectively known as the metabolic syndrome. These findings support the reported health promoting effects of CM and intensified its importance in treating the hyperlipidemia- associated chronic health problems resulting from the unhealthy life style and feeding habits.

List of abbreviations:

- Abbreviations should be in alphabetical order. Done already

- There are some in the test that are not mentioned here like H&E staining …: all abbreviations were included

References: all references were revised and the typing mistakes were corrected and spaces were adjusted

- Extra spaces seem to exist between the words sometimes. E.g., Ref. 2: corrected
Decreased survival. Corrected

• Ref. 17; j Saudi Chem, same for Ref. 19 Corrected

• Usually, when we send a manuscript to a journal, the whole text should not be justified.

Figures and Tables:

• Why did you provide two units for AST in Table 2? (U/L) and (IU/L). This was typing mistake only U/L was included

• In Fig. 4 caption Part I, when you mention cholesterol group and in the following parenthesis you put a C, this may be mistaken for your C group. To avoid this we replaced C by Part C

Abstract:

• The objective should be a complete sentence. Corrected to: The current study aimed to investigate the effect of CM on the hepatic biochemical and cellular alterations induced by high-fat, cholesterol-rich diet (HCD); namely the non-alcoholic fatty liver disease (NAFLD).

• The use of the word “amendment” here seems unusual. The word amendment was replaced by alterations

• Method and material and the results section in the abstract can be shorter and better. Revision was done

• In conclusion, you mention that there is insulin like proteins in CM, but I do not remember you mentioning that in the method or results section. This was reported previously by other researchers For example references number 13-15 in the current study and a recently published study by Malik A et al. A study of the anti-diabetic agents of camel milk. Int J Mol Med. 2012 Sep;30(3):585-92.

• Please check the number of key words allowed by the Journal. Done already

Reviewer: Jia Xiao

Major compulsory points:

1. Although consumption of camel milk exhibited remarkable ameliorative effects on NASH induced hepatic abnormalities. The exact improving mechanism (i.e. the effective monomer or interactions between different monomers) is unclear. I understand that to elucidate this point needs a lot of additional works. So, please at least state this limitation in DISCUSSION. This was added in the last paragraph of discussion
2. To demonstrate the alleviative effects of camel milk on insulin resistance and glucose metabolism, ITT and GTT experiments are needed. Figure 2 itself cannot draw such conclusion. Both intraperitoneal GTT and ITT were performed and results were included and the data was presented in Figure 3. To further examine the ameliorative effects of camel milk on lipid profile and metabolism, expression of key markers should be added (e.g. SREBP-1c and adiponectin).

4. It is stated in the DISCUSSION section that camel milk improved steatohepatitis. But they did not measure the markers of inflammation. Hepatic level of TNF-a and ILs should be added.

Although we totally agree with the Prof. Xiao about the importance of measuring the expression of SREBP-1c and adiponectin, TNF-a and ILs as an important markers of the amelioration of the lipid profile and inflammation by camel milk respectively, but actually it was difficult for us to estimate these markers in the current time because it was not planned in the study protocol and due to financial and technical reasons it is difficult to be done now. However, we believe that the question of the study about the effect of CM on the hepatic biochemical and cellular alterations induced by high-fat, cholesterol-rich diet (HCD); namely the non-alcoholic fatty liver disease (NAFLD) has been answered in a satisfactory was by the already undertaken biochemical and histopathological methods and we can consider further investigation of the anti-inflammatory effects of CM and the molecular basis of its hypolipidemic effect in another study.

Minor essential points:

1. Authors should make consistence of the word “non-alcoholic” instead of “non alcoholic”. The word Non-alcholic was used instead of non alcoholic.

2. In ABSTRACT, expand the abbreviations of HDL and LDL. Both abbreviations were expanded in the abstract.

3. Last sentence of the 3rd paragraph of INTRODUCTION “Conversely, certain food types and animal products are used in traditional and alternative medicine to treat some diseases.” Several examples with appropriate citations should be added. The following examples were added: Conversely, several plants such as ginger, sinamon, licorice, berries, plant leaves, and herbs roots in addition to animal products such as bones, hooves, skins, feathers, and milk have long been used
traditional and alternative medicine to treat some diseases and are increasingly valued as raw materials in preparation of modern medicine and herbal preparations.

4. Last paragraph of INTRODUCTION is not well-written. Authors should clearly state the research gap and the main finding of this study. The paragraph was corrected and the research gap was stated as follows: Recent studies showed that CM has antihypertensive, anti-cancerous, hepatoprotective, and hypocholesterolemic effects [19-22]. However the effects of CM on HCD-induced hepatic biochemical and structural changes, oxidative-antioxidative balance and glucose homeostasis has not been investigated. The reported health benefits of CM vindicated the great public concern and stimulated our interest to investigate its effects on NAFLD being one of the obesity associated disordered widely prevalent nowadays.

5. Body weight data of rats should be added in the first section of METHODS. The body weight data was added in the first paragraph.

6. Authors should justify the reason of choosing male rats instead of female gender or mixed genders. The male animals were selected to avoid the probable influence of cyclic changes in the female sex hormones on the body weight and lipid profile.

7. In METHODS, when introduce the recipe of HFD, authors should indicate the energy percentage from fat. (42% of the energy was provided from fats) This was included under the title of induction of hyperlipidemia.

8. For the analysis of histological data, the NAFLD activity score should be added (PMID: 21319198). This score was utilized in addition to the

9. Please change the dimension and direction of Figure 4. Its current form is difficult to read. The figure was edited and the labels were added to each group if pictures and its number became Fig.5.

10. In the first paragraph of DISCUSSION, authors stated that “Life style modification and acquiring healthy food habits are gold standard parameters to control the disease and minimize the progression to NASH and cirrhosis [29].” It is not correct. Lifestyle modification and maintaining good habit are most recognized treating method for NAFLD but not the “gold standard”. Please refer to some recent reviews for details (e.g. PMID: 23558065). After referral to the mentioned reference, this sentence was corrected to: Life style modification and acquiring healthy food habits are the gold standard parameters the most clinically
recognized and effective methods to control the disease and minimize the progression to NASH and cirrhosis [33]

11. There are a lot of grammatical and typing errors throughout the manuscript. Authors should thoroughly check the manuscript before re-submission. The manuscript was thoroughly revised for grammatical and typing mistakes and the appropriate corrections were undertaken.