Author's response to reviews

Title: Protective effect of rutin on the antioxidant genes expression in hypercholesterolemic male Wistar rat.

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Version: 2 Date: 7 May 2013

Author's response to reviews: see over
Dear Editor In chief, Prof. BMC Complementary and Alternative Medicine

Thank you very much for reviewer’s comments and we appreciate the comments addressed by the peer reviewers which will improve the quality of our MS No MS: 1793849893902296. Titled: “Hepato-protective effect of rutin on the gene expression of sulfiredoxin-1, glutamate-cysteine ligase and paraoxonase-1 in hypercholesterolemic rats” Salem S. Al-Rejaie, Abdulaziz M. Aleisa, Mohamed M. Sayed-Ahmed, Othman A. AL-Shabanah, Hatem M. Abuohashish, Mohammed M. Ahmed, Khaled A. Al-Hosaini and Mohamed M. Hafez.

Thanks

Mohamed M. Hafez

Please find herewith our answers point to point to the comments. Also, you will find all the changes we have made in the manuscript text are clearly labeled in red.

Reviewer No.1

1- How the dose of rutin is (2%) selected? It is very high. A 300g rat eats ~30g rat chow per day. The dose is 2g/kg bw.

Thanks for your comment and I agree with you 2% of rutin is too high but the dose we used is 0.2 % as mentioned in material and methods and tables and 2% is corrected in the abstract.

2- In the section of "Dietary protocol", should cite reference for the diet.

I agree with you and two references have been added one for rutin protocol reference no. 36 and other one for high cholesterol diet protocol reference no. 37.

3- Figure 1 & 2 the bar of control with no error bar. The authors should not simple normalize the reading to 1 in control group. It is also important to let readers know the degree of variation in the control group. After doing that, please re-calculate the statistics.
The degree of variation in the control group has been added and re-calculates the statistics.

4- Since high cholesterol diet was used, the authors should discuss the possible pathway related to lipid and cholesterol metabolisms.

Pathway related to lipid and cholesterol has been added in the introduction section: Accumulation of lipid in hepatocytes may cause a dysfunction in the synthesis of fatty acids. Transcription factors such as sterol-regulatory-element-binding protein-1c (SREBP) and peroxisome proliferator-activated receptor alpha (PPARs) promote hepatic fatty acid synthesis. Long chain polyunsaturated fatty acids and acyl-CoAs, are metabolic regulators of many transcription factors that motivate the liver lipid metabolism. Fatty acids induce changes in the activity of four transcription factor families: PPARs, LXRs, hepatic nuclear factor 4, and SREBP [13, 14]. Downregulation of gene expression by fatty acids would be restricted to polyunsaturated fatty acids, but the upregulation would be independent of the saturation [15]. These Differences might involve differential metabolism (oxidative pathways, kinetics etc.) and selective transport of fatty acids to the nucleus. Polyunsaturated fatty acids regulates the genes involved in fatty acid oxidation such as PPARa target genes in which suppress SREBP-1c activity, leading to a reduction in liver triacylglycerol content [16]. The liver is a major source of newly synthesized cholesterol. Cholesterol can be derived from newly-absorbed cholesterol, peripheral tissues and cholesterol synthesized within liver. Cholesterol taken up by the liver is in the form of cholesterol esters [17] which can be either stored as esters or hydrolyzed to free cholesterol [18].

5- Running title should be shorted
The running title has been changed to: Protective effect of rutin on hypercholesterolemic

6- P. 5, line 7, don't use rpm but g. same comment also applicable to other sections, such as
   P. 5, section "Estimation of MDA in liver".....

All rpm overall the manuscript have been changed to g.

7- In the tables 2 & 3: Rutin (0.2%) is used.

Rutin 0.2% the dose we used in this study.

8- P. 8, last 5th line: "This may be explained on the basis that rutin has a strong ability to chelate multivalent metal ions, especially zinc, calcium and iron." Provide reference for it.
The statement of this may be explained on the basis that rutin has a strong ability to chelate multivalent metal ions, especially zinc, calcium and iron." has been provided by reference no. 33
**Reviewer 2:**

**Question 1:** The title could be changed to:

Protective effect of rutin (complementary antioxidant) on the gene expression of liver enzymes in hypercholesterolemic male Wistar rat.

The title has been changed to be: Protective effect of rutin on the antioxidant genes expression in hypercholesterolemic male Wistar rat.

The title cannot be changed to the reviewer recommendation as our study was not only on the liver enzymes but also our target is the antioxidant genes.

2- Key words: High-cholesterol diet, Rutin, oxidative stress, Glutathione peroxidase, Sulfiredoxin-1, Glutamate-cysteine ligase, Paraoxonase-1, Real time PCR

The key words have been changed to be: Hypercholesterolemic liver, Rutin, Oxidetive stress genes, Real time PCR.

Abstract: According to all the reviewer suggestions the abstract have been corrected and are in red color.

Introduction section: According to all the reviewer suggestions the introduction has been corrected and is in red color.

**Material and methods:**

Material and methods have been changed to materials and methods

and the subtitle animals  has been changed to animals used.

The first paragraph of materials and methods has been changed to : Twenty four young male Wistar albino rats six weeks old with average body weight 80-100 gms, were obtained from the Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The animals were acclimatized to laboratory condition prior ten days to the experiment.

According to all the reviewer suggestions in materials and methods, result and discussion have been corrected and are in red color.
The title of table 1 has been changed to: Table 1 Showing primers and probe used.

Figure Legend:

Figure 1 and 2 have been changed to: Figure 1: Showing the effect of HCD, rutin, and their combination on the expression levels of GPx (A) and GR (B) in rat liver. Data are presented as mean ± SEM (n = 6). * and # indicate significant change from control and HCD, respectively, at P < 0.05 using ANOVA followed by Tukey–Kramer as a post ANOVA test.

Figure 2: Showing the effect of HCD, rutin, and their combination on the expression levels of Glutathione S transferase α (A), paraoxonase-1 (B), sulfiredoxin (C) and glutamatecystein ligase (D) in rat liver tissues. Data are presented as mean ± SEM (n = 6). * and # indicate significant change from control and HCD, respectively, at P < 0.05 using ANOVA followed by Tukey–Kramer as a post ANOVA test.

- All tables should be done as histogram bars.

If all the tables change to histogram, it will be too much because in this case table 2 and 3 will be represented by 9 histograms.

- In all figures letter A & B & C & D should be put in a circle because it is not clear.

The letters A, B, C and D have been changed.