Author's response to reviews

Title: Salvia libanotica improves glycemia and serum lipid profile in rats fed a high fat diet

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Author's response to reviews: see over
Responses to Reviewer 1

Abstract:

1. Abstract is not clear.

   Based on reviewers’ comments, additions and amendments were made to the abstract to improve its clarity.

2. Line no 19-20 they mentioned, body composition? I didn’t find any results related body composition? Body composition is nothing but body weight, lean mass, fat free mass, fat%, total body electrolytes (Na, K), bone mineral concentration and bone mineral density.

   We acknowledge this concern raised by the reviewer and thus “body composition” was replaced by “abdominal fat” in the abstract and the rest of the manuscript.

3. Why authors are choosing only water extract to evaluate the glycemia, lipemia and body composition lowering activities?

   We have chosen to test the effect of water extract on these parameters to simulate traditional human consumption, as mentioned in the introduction lines 59-60 and lines 82-83.

4. What is induction period of obesity with high fat diet?

   The present study did not aim to induce obesity in rats but rather to test the effect of the plant water extract on the prevention of high fat diet-induced metabolic abnormalities.

5. Line no 29, conclusion not clear, need to rewrite?
6. In conclusion authors stated that HFD induced type-2 diabetes complications are reverted. Statement is not clear.

   For both comments 5 and 6, the abstract conclusion was rewritten for further clarifications.

Key words:

1. Line no 32. Plant name should be always in italic.

   Plant name in keywords is now in italic.
Introduction

1. Line no 36-37. Insulin resistance……and cardiovascular diseases? What is the difference between insulin resistance and type-2 diabetes?

*Insulin resistance is a metabolic abnormality which occurs before the onset of type-2 diabetes. It is manifested by impaired insulin signaling in the peripheral tissues, muscles in specific, and in the liver. This is associated with a delayed insulin stimulated glucose uptake and hyperglycemia, observed mainly following meals (or glucose tolerance tests), an increased hepatic glucose and lipids production and a decreased hepatic glycogen synthesis (Shulman, 2000). Insulin resistance could potentially lead to type 2 diabetes as mentioned in lines 45-46.*


2. Lengthy introduction about plant? What is the rationale behind choosing the chronic dose?

*The second paragraph of the introduction (about the plant) was shortened. The chronic dose was used to reproduce and simulate usual human consumption, whereby the plant leaves are consumed as an infusion on a regular basis, as mentioned in the introduction lines 59-60.*

3. Mention how fat induces diabetes and its related complications?

*Two sentences (lines 47-51) were added in the introduction to elaborate on the dietary fat effect on diabetes-related metabolic abnormalities*

4. Is this extract/drug is suggestible to humans? In case what is the dose authors can suggest?

*At this stage, it is too early to recommend doses for human consumption due to the need to further purify, fractionate and extract the active ingredients of the plant responsible for the observed effects. Therefore, we need to conduct future studies to assess the latter as added in the conclusion lines 243-245.*
Results:

1. How the authors are choosing the three doses of drug?

*Only one study has previously described the hypoglycemic effects of the water extract of *Salvia libanotica*, in which a dose of 250mg/kg was administered (Perfumi et al., 1991). However, it was a short term study (7 weeks) that was conducted on rabbits fed a regular diet. We therefore aimed to test the effect of chronic intake of the plant extract at lower doses (50 and 150mg/kg) as well as a higher dose (450mg/kg) on rats fed high fat diet in order to better characterize the range of effectiveness of the plant. Moreover, similar doses are commonly used in the literature examining the effect of plant aqueous extracts on lipemia and glycemia in rats (Ngueguim et al., 2015; Zeeni et al., 2014; Daher et al., 2006; and Hage-Sleiman et al., 2011).

References:


2. What is toxic dose? Is there any mortality?

*As mentioned in the discussion lines 235-237, toxicity was assessed by administering to a separate group of rats incremental doses of the plant extract over one week period, until the dose reached 8000 mg/Kg body weight, which is equivalent to 18 folds the highest dose used in the study. However, no toxicity or mortality was observed and thus we decided not to proceed further with higher doses.*

3. There was no effect on experimental group-1, entire this work???

*Indeed, plant extract administration at the dose of 50 mg/Kg body weight (GI) didn’t show any significant effect compared to the control group. Benefits started being observed at the 2nd higher dose (GII) of 150 mg/kg body weight. Future studies could*
clarify whether such effects could be seen with doses in between 50 and 150 mg/kg body weight

4. I didn’t found any intervals in Oral Glucose Tolerance test?

*During the glucose tolerance test that was performed on d17 and d36 of the study, serum glucose was only measured once; 45 min after IP injection of glucose solution, and thus we cannot draw intervals. To minimize confusion, these results were illustrated as a separate figure (Figure 2) in the revised manuscript to differentiate it from the other results of fasting serum glucose, insulin and liver glycogen (Figure 1) that were measured at the end of the experiment after animal sacrifice.*

5. What about insulin AUC?

*As mentioned in the response to comment 4, insulin was only measured at fasting after sacrificing the animals at the end of the experiment. We did not measure the change in insulin after glucose tolerance test, which should be explored in future studies.*

6. There was no boy composition paprameters?

*Only abdominal fat was collected, and as mentioned earlier, “body composition” was replaced by “abdominal fat” in the manuscript. We would like to note that abdominal fat is a well-established indicator of metabolic abnormalities associated with high fat intake (Riccardi et al, 2004).*


7. Why authors didn’t use any standard drug for comparision?

*In the present study, we could not use a standard drug for comparison since the plant extract was used as a prevention rather than treatment for metabolic abnormalities, whereby all rats were healthy at baseline.*

8. Just based on some lipid profile, how the authors can claim antiatherogenicity activity of the extract?

*Blood lipids are strong markers of atherosclerosis (Nicholls et al, 2007; Kastelein et al, 2008) and are widely used in clinical settings. Most importantly, the ratio of HDL-C to LDL-C that was improved with oral intake of the plant oral extract in GII and GIII in the study, was found to be stronger predictor of atherosclerotic burden than each of the parameters alone (Enomoto et al, 2011). This was emphasized in the revised manuscript discussion lines 227-228*
Moreover, since it is the first study that examined the effect of S.Libanotica on lipemia, it is a screening study by nature and the effect on other antherogenic parameters will be explored in future follow-up studies.

References:


Discussion:

Discussion is not clear, need to interrelate with results and aims of your study.

Discussion was revised and rewritten to be in line with the results and aims of the study.

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

The revised manuscript was proof-read by a Professor of English Language before submission.

Responses to Reviewer 2

1. In abstract in result section please the result of study mention with numbers according to tables.

   Numbers were added in the results section of the abstract

2. Please the mechanism of the Salvia libanotica effect mention briefly in introduction.

   Proposed mechanisms of action were added to the introduction lines 65-68 based on the only previous study conducted on S. Libanotica (Perfumi et al, 1991)


3. In methods the plant water extract preparing is incomplete. Please mention with extra details.

   Extra details about the plant preparation were added in the methods lines 92-96
4. Method of LDL-C estimation is not correct.

*Apologies for the mistake, LDL was calculated using the Friedwald equation (LDL = total cholesterol – HDL – (triglycerides/5)). This is now mentioned in the methods section of the revised manuscript.*

5. Did in statistical analysis, confounding factors consider?

*There was no need to control for confounding factors, since it is a well-controlled animal study, whereby groups were matched at baseline for all potential confounders like age, sex and weight.*

6. The mechanism of the Salvia libanotica effect on study parameters mention complete in discussion.

*Suggested mechanisms based on the study results were added in the revised manuscript discussion lines 202-205*

7. According to results, discussion section can be written better.

*Discussion was revised and rewritten to be in line with the results and aims of the study*

8. In statistic analysis, Tukey–Kramer’s post hoc test was mentioned but in results and tables comparison of the two groups according to tukey test was not present.

*In the revised manuscript results section, as well as in the legends of tables and figures, we added that ANOVA and Tukey post hoc test were used to test for significance*