Author's response to reviews

Title: Biochemical characterization and 1H NMR based metabolomics revealed Melicope lunu-ankenda leaf extract a potent anti-diabetic agent

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Author’s response to reviews:

Response to Reviewers

Dear Editor

BMC Complementary and Alternative Medicines

We are pleased for the opportunity given us to improve our manuscript. Please see below the summary of changes as a response to your decision letter for the manuscript entitled “Biochemical characterization and 1H NMR based metabolomics revealed Melicope lunu-ankenda leaf extract a potent anti-diabetic agent in Rats”.

Summary of Changes

In improving the quality of our research output, we have carefully revised the manuscript by considering all the points brought up by Editor and Reviewers as well as their specific suggestions/questions. We acknowledged that the suggestions provided by the reviewers were very helpful and are fully considered in the revised version. Our detailed response to the referees is provided below, and response to the reviewers’ questions are included in the revised version. We would like to express our gratitude to the reviewers for their helpful and supportive comments.
1. Response to Editor Comments

Editor comment 1: Authors should be careful about the spelling of 1H, and 13C in the manuscript. 1 and 13 should be superscript.

Response: The manuscript is carefully rechecked and the spellings are corrected in the revised manuscript as suggested.

2. Responses to Comments from Reviewer 1

Reviewer reports:

Mohammad Mroueh (Reviewer 1): Dear authors,

Please find comments regarding the manuscript (BCAM-D-16-01529) entitled: Biochemical characterization and 1H NMR based metabolomics revealed Melicope lunu-ankenda leaf extract a potent anti-diabetic agent Mizher Hezam AL-Zuaidy; Muhammad Waseem Mumtaz; Azizah Abdul Hamid; Amin Ismail; Suhaila Mohamed; Ahmad Faizal Abdul Razis.

The manuscript is well written and has some originality.

Response: The authors are pleased for the acknowledgment of our work and valuable comments

Comment 1- The title should include any indication about the used model, for example: Biochemical characterization and 1H NMR based metabolomics revealed Melicope lunu-ankenda leaf extract a potent anti-diabetic agent in rats

Response: The suggestion is incorporated in the revised manuscript

Comment 2- In the Methods part of the Abstract, should indicate the model used: HFD STZ induced diabetes. You don't want a reader to read the whole paper to know the model you used in the manuscript

Response: The model used (HFD STZ induced diabetes) has been included in the Methods part of the Abstract as suggested.

Comment 3- In the Introduction part: Reference 2 is irrelevant??

Response: As per suggestion, reference 2 has been removed.

Comment 4- When you say "Rats fed a HFD …." needs more than one reference

Response: More references has been added as per suggestion

Comment 5- Line 73 "it helps building a profile …
Response: The suggestion has been incorporated

Comment 6- Line 75: "one of the most widely employed analytical tool" deserves more than one reference

Response: The suggestion has been incorporated with the addition of more references

Comment 7- Line 51-81: needs a reference

Response: Reference has been added as per suggestion

Comment 8- Methods: Line 108" remove "reduced pressure", when using a rotatory evaporator this means under reduced pressure.

Response: "reduced pressure" removed as suggested

Comment 9- Line 110: what was the percentage yield?

Response: The percentage yield was 20.8 ±1.9%

Comment 10- Don't you think 12 rats per group is too many? Excessive use of animals. 6 or 7 animals should be fine for statistics

Response: In our study, we have used 12 rats per group (it was expected that, some of the rats especially those belonging to untreated group (DG) may not survive during the course of study. However, for statistics 6 rats were used.

Comment 11- In 2.3 section: did all the animal treated with STZ developed diabetes? Response: Yes

Comment 12- Few typo errors for the micro symbol, in vitro should be italic, numbers in 1H13 should be superscript,

Response: The suggestion has been incorporated by removing the said typo errors.

Comment 13- Results: You only report your results. Therefore, the first paragraph is not results, it should be either in the Introduction or discussion parts.

Response: The suggestion has been incorporated in the revised manuscript

Comment 14- Line 228-229: remove sentence "the feeding ………………………parameters"

Response: The sentence "the feeding ………………………parameters" has been removed

Comment 15- induced obesity in all male sprague …
Response: The suggestion has been incorporated

Comment 16 - Line 252 - 257: rephrase

Response: Lines 252–257 rephrased as suggested

Comment 17 - Discussion is too long, almost 7 pages. Cut down into half.

Response: The discussion has been cut short by removing some paragraphs as suggested

Comment 18 - Typo error “Figure” should be capital.

Response: Typo error has been amended

Comment 19 - Line 554: end of the sentence "diabetic rats"

Response: Has been corrected as suggested

3. Responses to Comments from Reviewer 2

Reviewer reports:

Hsiu-Mei Chiang (Reviewer 2): The purpose of this study was to investigate anti-diabetic effect of Melicope lunu-ankenda (ML) leaves extract. The ML leaf extract significantly increased insulin level and insulin sensitivity of obese diabetic rats by decreasing glucose level and insulin resistance, as well as reducing hyperlipidemia.

This study is interesting and important in the benefits of herbs and the application metabolomic approach on serum biomarkers. However, there are some points must be clarified.

Response: The authors are pleased for the acknowledgment of our work and valuable comments

Comment 1 - The description of different groups of animal treatment in 2.2 animal experiments and 2.3 experimental design was confused, and it must describe clearly.

Response: The description of different groups of animal treatment in 2.2 animal experiments and 2.3 experimental design has been clarified in the revised manuscript as suggested.

Comment 2 - Why the food consumption was similar in all the groups? The diabetic mice may consume more food and water.

Response: The food consumption was similar in all the groups of our experiment. The increase in body weights however, may be due to the continuous feeding obese diabetic rats with HFD. However, the water consumption is different, where the diabetic rats are seen to consume more water as compared to that of normal and the treated rats.
Comment 3- The levels of ALP, ALT, AST and urea of normal diet group mice were significantly different before and after treatment (Table 1). It is unreasonable because the normal diet group was administrated with same treatment from beginning to end.

Response: Yes it is true, since the normal diet group was administrated with the same treatment from beginning to end, there should not be significant difference in levels of ALP, ALT, AST and urea of normal diet group before and after treatment. The same finding we have found during our experiments, in our study, we found non-significant difference regarding the levels of the said parameters for the normal group rats before and after treatment (Table 1). In Table 1, significance difference (p<0.05) among different groups within same period of treatment is shown by different letters, whereas * shows significance (p<0.05) difference within the group at different period of treatment.

Comment 4- The weight of spleen in DG and MG groups were decreased compared to control and ML groups (Table 2). Any explanation for these results?

Response: The weight of spleen in diabetic groups (DG, MG, LDG) was significantly reduced in comparison to that of normal rats. This is probably due to the disease state of the rats. However, interestingly, the spleen weight for the rats fed high dose of ML (HDG) improved and was not significantly different from that of normal group after 8 weeks of treatment.

Comment 5- The description about the different treated group mice was confused. Authors have to check their manuscript thoroughly.

Response: The manuscript thoroughly checked and description about the different treated group rats has now been clarified where required as suggested.