Author's response to reviews

Title: Quality Assessment and Anti-obesity activity of Stellaria media (Linn.) Vill.

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Author's response to reviews:

I am sending you the revised manuscript and point to point clarification of the comments sent by the reviewers:

Justification to the Comments of First Reviewer

1-Major compulsory revisions
1.A) Experimental induction of obesity: Authors should provide references to support their methodology (i.e. 2 weeks of HFD) since many data from literature (Lee et al, Food Chem Toxicol 2006, 44(1); Okade et al, Phytotherapy Res 2011, 25(6); Kim et al, J Med Food, 2001, 14(6) etc) show that HFD-induced obesity is effective after at least 4 weeks.
Ans: The reference has been introduced in support of methodology
B. Dose of the plant extract used in the study
What is the main reason of using a single dose (900mg/kg b.w.) in the anti-obesity study?
Ans: The study was performed on two doses i.e. 400 mg/Kg b.w and 900mg/kg b.w. Since the second dose i.e 900mg/kg b.w gave significant results therefore the activity was reported on this dose. Now the results of 400 mg/Kg b.w are also being incorporated in the manuscript.

2. Minor Revisions
2. AThe running title should become the real title since “quality assessment” has no impact in the study.
Ans: Needful done
Background: lines13-15: these lines reported results from a study; a reference is therefore needed.
Ans: Needful corrections have been done, the statement has been modified
Line 25: change “under taken” into undertaken
Methods – Chemicals: line 1 Glycyrrhizic into glycyrrhizic
Preparation of lyophilized juice: line 3: cancer lyophilizer
Quality control parameters of LJ: line 2: change the physico-chemical parameters into the physico-chemical parameters.
Measurement of #-amylase inhibitory activity: line 9: change “by adding of 80µL of…” into “by adding 80µL of…”
Plasma triacylglycerol level after oral administration of lipid emulsion to mice: line 4 change “Food was withheld” into “Food was withdrawn”

High fat-diet induced obesity: As mentioned above, two weeks duration for HFD-induced obesity appeared insufficient. What is the Kcal percentage of the HFD? What is the composition of normal diet?

ANS: The composition of normal diet has been incorporated.
The “histopathological study” should be inserted before “statistical analysis”
Histopathological study: line 4; check the spelling of “Hematoxylen”
line 5: under compound trinocular microscope; what does that mean?

Results-effect of LJ on food consumption…
Authors found that there was no change in food consumption of HFD-induced obese mice.

Ans: The statement has been modified
It is likely difficult to approve such statement from which one important question rises: were the mice really obese? If yes, what were the initial criteria for considering those animals as obese? Were the mice hyperlipidemic rather than obese? Please specify.

Ans: The mice were obese as indicated by significant increase in body weight when compared to initial weight of the mice which was measured at the start of the experiment. The criterion for the obesity is in accordance to the reference cited in the article. Reference no 21.

Line 9: change “lowerd” into “lowered”

Discussion: this section should be written in the lights of the results obtained.
Specifically, comments on the contradictory results obtained for the food intake (no change), body weight (increase) and adipose tissue weight (increase) regarding the untreated HFD mice supported with recent references, if any.

ANS: Necessary changes in the discussion section done

References: ref 11; the Editor of the book is lacking  
Ref 16 Plantes Medicinale Phytotherapie should be read as "Plantes Médicinales Phytothérapie"

ANS: Needful done

Revision of article as per second Reviewer's report  
Title: Quality Assessment and Anti-obesity activity of Stellaria media (Linn.) Vill.  
Reviewer: kamal amin

1. Major Compulsory Revisions:

How Stellaria media. inhibiting pancreatic amylase and lipase activity. mechanism of action and the active principle responsible for that action

Ans: The article does not report the study on a particular phytoconstituent responsible for the anti-obesity activity, however the literature reports as mentioned in discussion section that triterpenoid saponins are responsible for anti-obesity activity. The LJ was quantitatively analysed for the presence of triterpenoid saponins by HPLC as reported in the present article hence this compound may be responsible as stated in the discussion section.

The in vitro alpha amylase and lipase inhibitory activity of the LJ has been incorporated in the results. The study is directed towards the mechanism of action of LJ for anti-obesity activity which is mediated by inhibition of digestive enzymes.

Methods:
- How many mice per cage need to be added?

ANS: Needful done

Also the initial and final body weight of each group

Ans: The increase in the body weight is mentioned in table 1.

-Symbol of * can not mark between the 3 groups by ANOVA

Ans: The study was performed on two doses i.e. 400 mg/Kg b.w and 900mg/kg b.w. Since the second dose i.e 900mg/kg b.w gave significant results therefore the activity was reported on this dose. Now the results of 400 mg/Kg b.w are also being incorporated in the manuscript.

Results:
The author reported that, The physico-chemical parameters viz; ash values, loss on drying, hemolytic activity; heavy metal analysis (Lead, Cadmium, Arsenic), microbial (E.coli, Salmonella Sp., S.aureus) and aflatoxin (B1 + B2 + G1 + G2) contamination were determined.

But this data not presented in table or figure in the result section.

ANS: The results are reported pairwise in the first paragraph of result section

- The author depends on measuring plasma TG as indirect evidence of the lipase activity.

Whey the activity itself not measure as it is the main cause of Stellaria media action as anti-obesity as the author’s hypothesis. It is critical.

Ans: As suggested the in vitro pancreatic lipase inhibitory activity has been incorporated in the article which supports the antiobesity activity.

- Blood glucose level should be done to support or not the activity of pancreatic amylase. Also pancreatic amylase activity inhibited by LJ invitro which may be different from invivo study and the author depend in this activity to evaluate its antiobesity in vivo. so the evident for the effect of LJ become faint and need more support.

Ans: The blood glucose level was not determined. Only in vitro alpha amylase inhibitory activity was performed. As suggested, now the invitro pancreatic lipase inhibitory activity has been done and incorporated in the article as a supportive evidence of antiobesity effect of LJ through enzyme inhibition.

- Concerning comparison between HFD group and normal animal control negative mice there were non significant changes in the food intake (Table 1) how the body weight increase and get the obesity condition. Markers of obesity seem not enough to document the obesity state.

Ans: The Kcal of HFD is more as compared to normal diet as mentioned in the revised methodology. The comparison was done on the quantity of diet, which did not significantly increase. The obesity was induced because of intake of high Kcal of HFD.