Author's response to reviews

Title: High fat diet fed obese rats responded well to Terminalia paniculata extract in reducing body weights

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Author's response to reviews: see over
To 
The Editor-in-Chief  
BMC Complimentary and alternative medicine

Dear Sir, 

I have carefully gone through the comments of the reviewers and accordingly revised our manuscript entitled “Evaluation of anti-obesity activities of ethanolic extract of *Terminalia paniculata* bark on high fat diet-induced obese rats”, thoroughly. I am submitting here with our revised manuscript for further processing and publication in your esteemed journal. I look forward for your early positive response.

Thanking you

Sincerely

Dr. BALAJI MERIGA

**Reviewer-1's Comments: And our clarifications**

**Minor comments**

**Comment:** Authors should justify in the Methods section why HFD rats received a 15g/rat daily instead of *ad libitum* access to food as control rats. There is no clear rationale to maintain same food intake during the whole 15 weeks experimental period.

**Response:** There was a typographical error and the reviewer has rightly pointed out. Actually food and water was provided *ad libitum*, but when calculated it was 35g/rat/day.

**Comment:** Triglycerides levels should be also reported.

**Response:** We have revised as per reviewers comments.

**Comment:** It could be illustrative to evaluate PPAR alpha expression.

**Response:** The PPAR alpha expression is generally seen in liver tissue. So in the present study, we did not concentrate on PPAR alpha. We focused on adipose tissue where PPAR-γ is the major transcriptional regulatory factor in obesity.
Reviewer-2’s comments: And our clarifications

Major comments and answers to them

Comment: Mention the age of the rats used for experimental purpose.
Response: For our study, we used 6-8 weeks old rats.

Comment: Administration of the extract is pre-treatment or post-treatment?
Response: After initial induction of obesity (confirmed by body weight and body composition), administration of plant extract was started from 10th week to 15th week.

Comment: Justify the relation between the selection of extract dose and body weight of obese rats.
Response: Based on oral toxicity studies, we calculated and administered 100, 150 and 200 mg/kg b.wt. Our maximum dose (i.e, 200 mg/kg b.wt) didn’t exceed 1/10th of the plant extract administered during the oral toxicity studies.

Comment: In extract, author reported the presence of Ellagic acid, Arjunolic acid, Galloylarjunolic acid, Termilignan and Butalinic acid. Among them which one is primarily responsible for therapeutic effect? Author should discuss in detail. The LC-MS/MS spectrum is not clear. Author should provide high resolution image.
Response: As per reviewer’s comment, LC-MS/MS spectrum is provided with high resolution. In the present work, based on the results of LC-MS/MS analysis of the extract, we explained that the above said compounds are present in the extract and therefore, we mentioned that they may be responsible for weight regulation. In our further studies, the actual bioactive components among them can be found out.

Comment: Page 6 line 150; How the anesthesia was performed. It should be clearly mentioned.
Response: As per reviewer’s comment, we have given clear procedure in revised manuscript.

Comment: In line 206; Experimental animals were anesthetized with isoflurane and sacrificed. What is the dose of isoflurane used?
Response: The given dose of isoflurane is 2%, and it is mentioned in the revised manuscript.

Comment: Provide reference for collection of plasma or serum by centrifugation at 2500 rpm for 15 min.
Response: As per reviewers comment, we have included the reference in revised manuscript.

Comment: In line 153; “Body composition of experimental animals was assessed” It was done under anesthetic condition or normal condition?
Response: We did the body composition under anesthetic condition and the same is mentioned in the revised manuscript.

Comment: Whether glucose tolerance test and blood collection were done on the same day i.e at the end of the experiment? Among the same group or different group? Compare and clarify with the previous paragraph also.

Response: At the end of the experiment, after overnight fasting blood sample was collected, then OGTT was performed and again blood samples were collected on the same day at specific intervals, in all the experimental groups, as mentioned in the methodology. As per reviewers comment clarifications are included in the revised version.

Comment: Provide reference for oral glucose tolerance test protocol as mentioned in the manuscript.

Response: Reference is included in revised manuscript.

Comment: There is no uniformity in sacrificing animals throughout the experiment. The procedure for collection of blood and volume of blood collected are also not clear. There is lack of information regarding repeated withdrawal of blood from animals which makes the manuscript complicated and difficult to understand.

Response: The experimental animals were sacrificed only at the end of the experiment, the procedure of blood collection is explained in the revised manuscript as per the reviewer’s comment.

Comment: Explain the parametric or non parametric method used for ANOVA statistical analyses in the foot note of table 2.

Response: We followed parametric method-ANOVA for statistical analysis and the same is mentioned in the foot notes of the table.

Comment: Author must present the daily food intake and weekly body weight in two separate graphs.

Response: Food intake is measured daily and body weights are measured weekly and they have been shown with different symbols for clarity and the same is mentioned in the revised table.

Comment: Data presented in table 2 are confusing. Some data are measured in weeks, some data at the end of the experiment period. Some data are after sacrificing the animals.
Kindly mention it clearly in the foot note. Author should present data accordingly to avoid confusion to the readers.

Response: As per reviewer’s suggestion, the data in table-2 are now clarified and the same is mentioned in the foot notes.

Comment: In the histopathology slide photographs author should mention the scale/magnification. Fat deposits are not marked properly.

Response: The magnification is 40X and fat deposits are specifically marked in the revised manuscript, as per the reviewer’s comment.

Minor comments:

Comment: In the title “Terminalia paniculata” will be in italic and uniformity should be maintained throughout the whole manuscript.

Response: As per reviewer’s suggestion plant name is italicized in the revised manuscript.

Comment: The title should be rewritten and precise.

Response: Evaluation of antiobesity activities of ethanolic extract of *Terminalia paniculata* bark on high fat diet induced obese rats.

Comment: Title correct the term “weights”.

Response: As per reviewer suggestions, we corrected the title in the revised manuscript.

Comment: In high fat diet composition mention the company details from where it was purchased.

Response: It is a well known fact that, National Center for Laboratory Animal Sciences (NCLAS), National Institute of Nutrition (NIN) (Indian Council of Medical Research) is known for its world class standards for laboratory animals, purified and semi purified diets for laboratory animals research. They follow AIN-93 guidelines during different formulation of diet. For reviewer kind perusal, we mentioned in the revised manuscript. (Ref: BrahmaNaidu P, Nemani H, Meriga B, Mehar SK, Potana S, Ramgopalrao S. Mitigating efficacy of piperine in the physiological derangements of high fat diet induced obesity in Sprague Dawley rats. Chem Biol Interact. 2014 Sep 25;221:42-51. doi: 10.1016/j.cbi.2014.07.008.)

Comment: In line 158; mention the number of days.

Response: In line OGTT is mentioned in the manuscript. This was done at the end of the experiment which means at the end of 15 weeks (105 days). As per reviewers’s comment, it is included in the revised manuscript.
Comment: Discussion part should be concise and relevant with the experimental results. It must be rewritten.
Response: The discussion part is improved and related to the experimental results in the revised manuscript.

Comment: There are many grammatical and typographical errors throughout the manuscript.
Response: The revised manuscript was thoroughly checked and corrected for typographical and grammatical errors by an English expert.