Author’s response to reviews

Title: Silibinin Attenuates Adipose Tissue Inflammation and Reverses Obesity and Its Complications in Diet-induced Obesity Model in Mice

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Author’s response to reviews:

Dear Dr. Sridhar,

Please find the attached revised manuscript titled, “Silibinin Attenuates Adipose Tissue Inflammation and Reverses Obesity and Its Complications in Diet-induced Obesity Model In Mice”. In this draft, we revised our manuscript as per comments for revision noted by Editor and Reviewers. Below are point-to-point responses, and changes made accordingly. Changes are highlighted in red in the manuscript for your easier tracking.

I. Editor Comments.

1. Project approval number by Institutional Animal Care Committee.

Response: Project approval number has been added to the manuscript in “Declarations, Ethics Approval” part.

Change is shown on page 14, line 12.

2. Justification of selected dose of silibinin.

Response: The dose of silibinin (50 mg/kg) was selected based on previous studies showing ranges of doses sufficient to induce beneficial effects in relevant animal models. References to
these studies were cited in “Methods, Mice” part. A single dose was chosen because it was a proof of concept of beneficial use of silibinin in metabolic disorders. To the best of our knowledge, silibinin has never been used on human as a purified component. On the other hand, silymarin; a mixture of compounds including silibinin, has been used as a supplement for human, and it’s been demonstrated that silymarin is safe for human use even in doses up to 1 g/day. Change is shown on page 5 – lines 10,11.

3. Revision of statistical analysis for multiple comparison.

Response: Statistical analysis has been revised, and the part “Methods, Statistics” and Fig. 1 legend have been updated accordingly. Repeated measures ANOVA test was used. Also, Figure 1 has been updated.

Changes are shown in page 7 – line 2, page 18 – lines 4,5 and figure on page 20.

4. Addition of scale bar to histological pictures.

Response: Scale bars have added to histology pictures. Figure legends have been modified accordingly.

Changes: Figures 2 and 4 have been updated, along with figure legends on page 18, lines 12 and 24.

II. Reviewer Comments.

1. Reformulate abstract to broaden obesity definition beyond inflammatory disease.

Response: Abstract text has been updated to redefine obesity beyond a strict inflammation disease.

Changes are shown in page 2, lines 3-6.

2. Justification of animal number.

Response: The sample size was determined by statistical power, and based on numerous previously published studies of similar purpose and design. The number of animals was calculated using the equation:

\[ n = 1 + 2C \frac{s}{d^2} \]

(ref: https://www.ncbi.nlm.nih.gov/books/NBK43321/)
where C is dependent on values chosen for significance level (α, 0.05) and power (1-β, 0.90). d is the effect size (difference in body weight between control and treated groups, typically 10). s is the standard deviation, typically 3-5.

Using the equation: \[ n = 1 + 2 \times 10.51 \left( \frac{4}{10} \right)^2 = 4.36 \] (equivalent to 5 mice/group).

3. Justification of silibinin dose.

Response: Justification and references are discussed above (point I-2).

4. Rephrase of immune cell filtration sentence to better reflect evidence provided.

Response: The text has been rephrased to lower the tune of statement, and make it to the level of provided evidence (mRNA level instead of immunohistochemistry). Changes are shown in page 8-line 25, and page 9 lines 1,3.

We thank you for your valuable comments that helped making the manuscript being better.

Sincerely,

Mohammad Alsaggar, PhD