Author's response to reviews

Title: Spirulina platensis versus silymarin in the treatment of chronic hepatitis C virus infection. A pilot randomized, comparative clinical trial.

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Author's response to reviews: see over
Dear Esteemed Editors,

I would like to thank dear editors and reviewers for their efforts and the fruitful comments. I have uploaded a revised version of the manuscript, where I made some modifications in the text (appeared in red color). I also added 2 figures (2 & 3) and I replaced 2 tables (5 & 6) with a text (red colored) and an Error Bar graph.

**Here are the replies to the reviewers' comments:**

**Reviewer 1:**

**Comment:** Liver related mortality/morbidity (stated as primary endpoints), the real hard endpoints, could not have been assessed in a study on 60 patients, for 6 months.

**Reply:** This comment is totally right, and we omitted this endpoint and rewrote the section under the subheading outcome measures.

**Comment:** The sample size calculation is wrong:

**Reply:** There was a typing mistake in the previous version this had been corrected and approved by the statistical reviewer 2 (To detect a 0.17 difference in proportion of responders between both comparison groups, with 80% power and two tailed 5% level of significance, the minimal sample size was calculated to be 30 for each group.)

**Comment:** The two scores (ASEX and CLDQ) are ordinal variables.

**Reply:** As the reviewer mentioned in his comment that in many journals are published articles where this kind of scales are treated as quantitative variables, with mean and SD, and compared by parametric tests; it’s up to the editors to decide this. We used the parametric statistical methods after ascertaining the normal distribution of these variables by Kolmogorov–Smirnov goodness of fit test.

**Comment:** In Table 1, there are not the baseline ASEX and CLDQ scores.

**Reply:** Yes, and this is because they had been written and emphasized in table 4.

**Comment:** Child-Pugh score is used for cirrhosis, and not for chronic hepatitis, for which it was not validated.

**Reply:** We did not use it here as an outcome measure, but we present it in the table of baseline characteristics to denote matching of both groups in the degree of severity of liver disease which might bias the treatment effect, that is why we only used as a covariate in the MANCOVA Model to adjust results of CLDQ & ASEX based on it.

**Comment:** The p should be written in table 4, on the corresponding rows, rather than in text; on the contrary, it’s not necessary to give both SD and SE in Table 4,5,6 (see also comment 3, above). In fact, results of Tables 5 and 6 could be written in one phrase of text.

**Reply:** We rewrote table 4 and included the p value instead of the SE. We deleted both table 5 & 6 as suggested and replaced it with a text and an Error Bar graph.

**Comment:** In Table 7 there is no need of rows with intercept, error, total, corrected total and columns 3,4,5,6,8.
**Reply:** We rewrote table 7 (that became table 5 after deleting 5&6) and we erased the suggested rows and columns leaving column 8 as it illustrates the relative effect size.

**Comment:** I don't think ASEX is a valid endpoint for the treatment of chronic hepatitis C.

**Reply:** This was explained in the discussion section. We included this outcome measure because we had had unintended frequently reported data coming from many of our patients who took Spirulina as nutritional supplement and reported to us marked improvement of the general wellbeing and sexual activity. From this probing experience, as well as from the results of Danoff A, et al.[44] and Soykan A, et al.[45] who reported an association of chronic HCV with depressed sexual functions independent of depression, we opted to compare the effect of both treatments on sexual functions beside the other efficacy parameters in such patients. We assumed that improvement in sexual appetite; frequency and performance are logical indicators for the improvement in the overall wellbeing.

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**Reply to comments of reviewer 2:**

**Comment:** I suggest showing a figure that displays individual measurements of the patients in both study groups at baseline, 3 months, and 6 months.

**Reply:** I added 2 graphs. Figure 1 which depicts the baseline, 3 and 6 months log transformed virus load in both groups in a readable and easy to understand Boxplot. I also added an Error Bar graph depicting CLDQ and ASEX mean (CI) differences from baseline. I found that plotting many different variables in one graph appeared to be unattractive and complicated with much noise due to the wide variations of values at the vertical axis.

**Comment:** Table 7 can be shown in an appendix (author choice), and the 2 measures (CDLQ, ASEX) should be shown separately.

**Reply:** Table 7 (table 5) had been relocated in appendix as the BMC format, and we did some improvement in the table and the text explaining it to be more easy to grasp for readers with no statistics background.

**Comment:** Was there a differential effect for M and F on the outcomes?

**Reply:** As this was a pilot hypothesis generating rather than confirmatory study, we recruited a marginally small sample size that is why we did not do subgroup analyses as comparing the 2 groups will be more powerful. Also in such studies, effect of sex is less important than many other covariables like severity of liver disease, baseline virus load,.. etc, that is why we usually do not compare it in such small studies.