Author's response to reviews

Title: Rectal gel application of Withania somnifera root extract denotes anti-inflammatory and muco-restorative activity in TNBS-induced Inflammatory Bowel Disease

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Version: 2 Date: 1 March 2011

Author's response to reviews: see over
To,
The Editor,
BMC Complementary & Alternative Medicine

Dear Sir,
Please find attached herewith a revised manuscript entitled, “Rectal gel application of *Withania somnifera* root extract denotes anti-inflammatory and muco-restorative activity in TNBS-induced Inflammatory Bowel Disease” for the publication in your journal.

The manuscript is now revised in light of the reviewer’s comments and a detailed report on action taken against each comment of every referee is included.

Thanking you and with warm regards.

Sincerely,

Abhay M. Harsulkar
Reviewer 1:

The reviewer has provided comments/questions in the form of notes directly on the pdf file, which was downloaded from the link provided.

Reviewer’s report
Title: Rectal gel application of Withania somnifera root extract denotes anti-inflammatory and muco-restorative activity in TNBS-induced Inflammatory Bowel Disease
Version: 1 Date: 17 August 2010
Reviewer: Mohammad Abdollahi

Reviewer’s report:
Major Compulsory Revisions
Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests: N/A

Title:
Comment 1: The world “denotes” is not good here in the title
Action: The title is now revised so as to remove the world “denotes”. The new title is now: Rectal gel application of Withania somnifera root extract expounds anti-inflammatory and muco-restorative activity in TNBS-induced Inflammatory Bowel Disease

Abstract:
Comment 2: Abstract line 2, oxidative is enough, you can omit nitrosative, nitrosative is kind of oxidative reaction.
Action: The word nitrosative is omitted.

Comment 3: Define all abbreviations
Action: Long form of TNBS is included.

Comment 4: IBD is once abbreviated before
Action: Long form of IBD at this placed is dropped.

Comment 5: Include ‘ferric reducing power” in place of reducing power assay
Action: Changed as indicated

Comment 6: Mesalamine is not a good candidate for comparison!!!! Because it is not used topically, something as gel must be compared
Action: Mesalamine is used in this study, as it is the commonly used therapeutic agent currently used in treatment of IBD. Moreover, no formulation exists for
topical application in IBD. However this is an important question and we will attempt to address this in our next experiment where we will compare same agent from oral and rectal route.

**Introduction:**
Comment 7: Reference 14 is of 1994 and too old for this purpose, replace it with the Rezaie et al 2007
Action: The older reference is replaced as suggested

Comment 8: The effective herbs with antioxidant activity in treatment of IBD has been already reviewed use the following paper- Rahimi et al 2009
Action: The suggested reference is incorporated in the text and in the bibliography

Comment 9: Bring a reference for W. somnifera water extract is prescribed for intestinal ulcers and rectal bleeding
Action: the reference is now incorporated

**Materials and Methods:**
Comment 10: By filtration some of active components might be missed, best way was to dissolve it
Action: Filtration was carried out to remove the undissolved particulate matter and to obtain a clear solution. Therefore should affect the active components.

Comment 11: More details of the procedure (HPTLC for quantification of Withanoloid D using Tolune:Ethylacetate:Formic acid
Action: HPTLC protocol is only used to standardization of the extract and estimating the withanolide content. There is no data presented in the current manuscript, hence deleted.

Comment 12: Anti oxidant assays-The criteria for selecting doses in different tests has not been noted
Action: Usually for biochemical assays, ranges of concentrations were taken for the assay for the primary experiment, and final concentrations were accordingly decided for the final graph.

Comment 13: Anti-lipid peroxidation activity- It is impossible to test LPO in a solution without having fatty acids? I doubt the procedure is correct, ferric chloride cannot produce peroxide in vitro without substrate
Action: Anti-Lipid peroxidation potential of WSRE was a measure of inhibition of thiobarbituric acid reactive species (TBARS) formation at different concentrations. The tissue homogenate serves as the lipid media. Lipid peroxidation was initiated by adding ferric chloride. The method is originally described by Ohkawa et al, Annal Biochem 1979, 95:351-358.
Comment 14: Nitric oxide scavenging activity- Again, the above mentioned problem exist
Action: In this procedure, sodium nitroprusside in phosphate buffer saline was mixed with various concentrations of WSRE and incubated with sulfanilic acid reagent and mixed with napthylethylenediamine dihydrochloride. The assay is described in Sumanont et al in Biol Pharm Bull 2004, 27(2): 170-173.

Comment 15: Anova and then Tukey, These are two different tests
Action: Changed to Anova and Tukey tests.

Results:
Comment 16: MDA levels-Give detail of procedure, How did you get colon?
Action: The colon tissue was excised form animals after the experiment was completed. Details of the procedure is given on page 9 in ‘Assessment of severity of colitis’.

Comment 17: Discussion needs major improvement, please consider comments…
Action: We have attempted shaping the discussion according to the comments and the suggested references are incorporated.
Reviewer 2:

Reviewer's report
Title: Rectal gel application of Withania somnifera root extract denotes anti-inflammatory and muco-restorative activity in TNBS-induced Inflammatory Bowel Disease
Version: 1 Date: 23 November 2010
Reviewer: Jamil A Shilpi

Reviewer's report:
The authors are investigating the use of Withania somnifera root extract to treat Inflammatory Bowel Disease using rectal root. The results are quite exciting and a number of tests has been carried out to justify the mechanism.

In the DPPH assay, the concentration of DPPH solution has not been mentioned. The IC50 of ascorbic acid is too high and does not match with the value published in the literature. Therefore this experiment should be repeated. results paragraph of Abstract, as well as of some other parts (as mentioned in the attached file) of the manuscript should be rewritten to make the statements clear.

Codes used in the figure are not explained in the figure title. There are some typesetting errors that should be fixed.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interest

Comment 1: The authors are investigating the use of Withania somnifera root extract to treat Inflammatory Bowel Disease using rectal root. The results are quite exciting and a number of tests have been carried out to justify the mechanism.
Action: Thank you for the appreciation.

Comment 2: In the DPPH assay, the concentration of DPPH solution has not been mentioned.
Action: Deleted from the manuscript

Comment 3: The IC50 of ascorbic acid is too high and does not match with the value published in the literature.
Action: The assay was repeated with ascorbic acid at least 4-5 times now. However, the results are not consistent, may be since the extract stored even at –80 (now close to 2 years) has gone bad. Fresh extract was done but understandably, it is showing different values and thus may not be comparable with the other assays presented in this manuscript. The data on DPPH assays is
therefore dropped and removed from the Table 1 as well. The data is referred in
discussion but marked as ‘not shown’.

Comment 4: results paragraph of Abstract, as well as of some other parts (as
mentioned in the attached file) of the manuscript should be rewritten to make the
statements clear.
Action: many of the portions are now rewritten in the light of comments from
reviewers.

Comment 5: Codes used in the figure are not explained in the figure title.
Action: It is clarified now.

Comment 6: There are some typesetting errors that should be fixed.
Action: Many of the errors are now corrected

Action taken against each comment:

<table>
<thead>
<tr>
<th>Page no.</th>
<th>Para.</th>
<th>Line</th>
<th>As appeared</th>
<th>Change to/suggestion</th>
<th>Action taken</th>
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<td>Results: The extract showed maximum 95.4% DPPH scavenging, 95.6% inhibition</td>
<td>Results: The extract, at 500 µg/ml, the highest concentration tested, showed 95.4% DPPH scavenging, 95.6% inhibition of lipid peroxidation, 14.8% NO scavenging, and at 500 µg/ml concentration reducing capacity (0.80), comparable with standard antioxidants, ascorbic acid and curcumin. WSRE treatment positively scored on histopathological parameters like necrosis, edema, neutrophil infiltration. The post treatment intestinal features showed restoration at par with the healthy intestine. In view of these results, gel formulation containing an aqueous extract of \textit{W. somnifera}, prepared for rectal application was tested for</td>
<td>The sentences were reframed as suggested</td>
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its antiinflammatory activity along with commercially available anti-inflammatory drug Mesalamine in TNBS-induced rat models for IBD. prepared for rectal application was tested for its anti-inflammatory activity in TNBS-induced rat models for IBD. Commercially available anti-inflammatory drug Mesalamine was used as the standard in this assay.

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<td>DPPH was added</td>
<td>DPPH (Pls, mention the concentration of DPPH in %) was added</td>
<td>DPPH assay results are now dropped form the manuscript</td>
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</table>

Taking absorbance against 517 ascorbic acid (standard) methanol at 517 nm (blank) (b) DPPH (control), and extracts (test) percent radical scavenging activity was calculated by using the formula [22].

Malonyldialdehyde (MDA) generated by the oxidation of polyunsaturated fatty acids yielded a pink red complex, upon reaction with two molecules of thiobarbituric acid (TBA) was measured at 532 nm, from which % inhibition of lipid peroxidation by WSRE was calculated.

Rewrite the sentence. If necessary, split in sentences to express things clearly

Fe3+ to

extract

µg/ml

mg/kg

IC50

goat or rat?

Goat
| 11 | 4 | 1 | Text under the title ‘Evaluation of the reducing potential’ | Rewrite the whole Paragraph | Done |
| 15 | 3 | 7 | Non Steroidal Antiinflammatory Drugs | Non Steroidal Antiinflammatory Drugs (NSAID) | Done |
| 19 | | | References. | References | Done |
| **References** | | | | | Done |
| All | | | | No space between colon and page no. | Done |
| Selective | | | | Remove volume numbers | Done |
| **Figure 1** | | | Figure 1: a. Histopathological features of colon section of healthy control rat. b. Histopathological features of colon section of rat with TNBS treatment. c. Histopathological features of colon section of rat after TNBS +WSREF treatment. d. Histopathological features of colon section of rat after TNBS+ Mesalamine treatment. | Figure 1: Histopathological features of colon section of a. healthy control rat, b. rat with TNBS treatment, c. rat with TNBS +WSREF treatment, d. rat with TNBS+ Mesalamine treatment. | Done |
| **Figure 2 and Figure 3** | | | Include the meaning of the codes used in the figure | | Done |
| **Figure 2** | | | Figure does not represent the figure title. No need to put colon weight as title or legend inside the figure. Appearing with figure title is enough | | Done |
| **IC50 of ascorbic acid** | | | The IC50 of ascorbic acid in DPPH assay is not satisfactory. | Removed form the manuscript | |
| **Expression of** | | | All the temp units should be changed according to | | Done |
Reviewer 3:

Comment 1: The approach of therapeutic procedure with using plant extracts is worth to study in order to find the effective drug for IBD. The work has some biomedical practical significance.
Action: We thank reviewer for his appreciation

Comment 2: However, the manuscript needs thorough revision as it should be improved in several points, some of them are listed below. This manuscript is not acceptable in this form for publishing.
Action: the Manuscript is now revised considering several points suggested by the reviewers

Comment 3: P. 5, lines 2-4 down the page, please improve the style of this sentence, on the standard, test samples, also the next sentence, all should be more clear.
Action: Revised

Comment 4: P. 6, the paragraph on anti lipid peroxidation activity should be thoroughly reedited, especially the long sentence .In practice, (a) mixtures containing
Action: The text is reedited. The long sentence is made into simple short sentences.

Comment 5: P. 7, the same as above concerns the paragraph on NO scavenging activity, which should be written more clear for the reader.
Action: Similar as above, the entire paragraph is reedited with suitable sentence structure

Comment 6: P. 7, . was prepared in phosphate buffered saline (PBS).
Action: The short form ‘PBS’ is defined in the earlier paragraph and used here.

Comment 7: P. 10, please improve style of the paragraph on free radical scavenging activity. Tables should be inclued in manuscript also in such a way as figures.
Action: The section is redrafted

Comment 8: Legends to figures 2 and 3 are not complete, abbreviations HC, CC, WS, ASH, PC are not explained.
Action: It is explained now
Comment 9: Restoration of colon weight is convincing but the loss of body weight could be better discussed. The discussion part is too long and should be shortened.
Action: The discussion section is edited and reference to these data is now added.