Reviewer's report

Title: Acute and Subchronic in-vivo effects of Ferula hermonis L. and Sambucus nigra L. and their Potential Active Isolates against Hyperglycemia, Tactile Allodynia and Thermal Hyperalgesia in a Diabetic Mouse Model of Neuropathic Pain

Version: Date: 6 July 2015

Reviewer: DAMIKI LALOO

Reviewer's report:

Major compulsory revision:
1. Authors have implemented a triple dose design, but the dose dependent relationship comparison was missing.
2. To support the mechanism of anti-diabetic activity of any tested extract/compounds, more parameters should be incorporated especially the estimation of triglycerides levels.
4. Material and methods part (Page 4, Section 2.4.2., Line 4): Why DMSO is used? Percentage of DMSO used for i.p. injection should be mentioned. As higher concentration is believe to be toxic. Did the authors check the solubility of glibenclamide in saline?
5. Material and Methods part (Page 4, Section 2.4.2., Line 6): If ethyl acetate is used as the solvent for extraction. Did the authors tested the solubility level of all the tested extract/components in saline for intra-peritoneal administration? For better absorption it is always recommended that the extract should be soluble in saline prior before testing.
6. Material and methods part (Page 4, Section 2.4.2., Line 7-27): Authors have selected the doses for Ferula extract (12.5, 25 and 50 mg/kg, i.p.), Elder extract (50, 100, 200 mg/kg, i.p.), Ft (0.4, 0.8, 1.6 mg/kg, i.p.) and KMF (16, 32, 64 mg/kg, p.o., i.p.). What is the criterion for selecting the doses of extracts? How do the authors decide the doses for isolated components?
7. Material and methods part (Page 5, Section 2.7., Line 16): Statistical analysis used is not appropriate for the study. Since the whole study is dealt with intra-day/hour and intra-group comparison, the authors should not used one-way ANOVA. In fact, the data can be represented in a more precise and reproducible manner using a two-way ANOVA followed by Bonferroni post hoc test. Authors need to change the statistical analysis.
8. Discussion part (Page 8, Section 4, Line 26-29): The authors report that the amelioration of DM and DN might be due to the synergistic activity of the active components present in the extract. Have the authors tested the actual synergistic
effect of the components to support the claims?

Minor essential revision:
1. Abstract part (Page 1, Line 17.): Alloxan is now a day not preferred for screening anti-diabetic, since it is having higher toxicity, mortality rate and less stable as compared to streptozotocin (STZ). The authors should have used streptozotocin for screening.
2. Introduction part (Page 2, Line 10): The sentence is incomplete.
3. Introduction part (Page 2, Line 25): What does the letter ‘b’ stands for as of elder b?
4. Material and methods part (Page 3, Section 2.4.1., Line 31): Range of mice body weight was missing.
5. Result part (Page 6, Section 3.1., Line 2): Check spelling of ‘isorhamntin’.
6. Result part (Page 7, Section 3.3., Line 4): Catalase is not the only parameter used to clarify the anti-oxidant activity of the tested samples. The authors should provide other parameters for supporting the antioxidant efficacy of the tested samples such as lipid peroxidises, superoxide dismutase, glutathione levels etc.
7. Discussion part (Page 8, Section 4, Line 21-23): Please provide the references as evidence for the folkloric usage of both the plants in the treatment of diabetes.
8. Figure 6: Please supply the calibration methods along with supportive data for HPLC determination of both the components as supplemental file.

Discretionary Revisions:
Note: The authors need to justify the following comments:
1. The anti-diabetic activity of the Ferrula harmonis has already been investigated clinically in human trials (Naguib YMA, 2004). The results represent in the present study indicates a controversial conflict in parameters such as weight loss which has been reported earlier. (Kindly refer the references below).
2. The anti-diabetic effect of Kaempferol has been investigated earlier (Zang et al., 2011). What is the rational of repeating the study? (Kindly refer the references below).

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:**

I declare that I have no competing interests.