Author's response to reviews

Title: Citrus aurantium L. essential oil exhibits anxiolytic-like activity mediated by 5-HT1A-receptors and reduces cholesterol after repeated oral treatment

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Title: *Citrus aurantium* L. essential oil exhibits anxiolytic-like activity mediated by 5-HT1A-receptors and reduces cholesterol after repeated oral treatment.

Dear editor,

We would like to thanks the reviewers for your critical analysis of the manuscript and say that we appreciate the pertinent comments and suggestions, which improve our paper. We have modified the manuscript accordingly and specific questions are answered separately for each referee as follows.

**Comments for Referee 1:**

However, the authors should carefully check the style of references.

We apologize for the inconvenience but due to a mistake, we sent the file without the Reference List. The full list, in accordance with the standards of the journal, is now added to the text.

**Comments for Referee 2:**

1. A major problem I have with the manuscript is that no clear dose-dependency of the anxiolytic action of *C. aurantium* essential oil can be seen. The dose of 5 mg/kg shows a significant effect similar in magnitude to that after a dose of 50 mg/kg, which however is not significant. On the other hand the dose of 10 exerts effect identical as the dose 1 mg/kg showing no efficacy. Perhaps doses of 2.5 and 7.5 mg/kg should be also investigated.

The monotonic dose-response curve was not observed in our results. To address this issue in manuscript, the text reproduced in the box was inserted as 5th and 6th paragraphs in the Discussion. Concerning the absence of significance with 50 mg/kg, in spite of the same magnitude of effect with 5 mg/kg, one of the more feasible explanations is the variability in data from both groups. Vertical lines in each bar enclose 50% of individual data, the remaining 50% are distributed upper (25%) and under (25%) the end of vertical lines. In group treated with 50 mg/kg the tendency is stronger in direction to
lower values (distant to median value and more similar to values from control group), while in the group treated with 5 mg/kg the values tend to stay closer to the median. The same reasoning is valid between results obtained with 1 and 10 mg/kg.

As previously reported for EO [23], the profile of dose-response curve is not monotonic as usually seen in classical pharmacology. In a monotonic curve, the sign (negative or positive) of the slope is maintained throughout the entire dose range. Conversely, in nonmonotonic dose-response curve the slope changes sign at some point along the range of doses, resulting in a U- or inverted U-shape. In more complexes cases, a nonmonotonic curve assumes a multiphasic shape, in which the slope changes the sign in multiple points along the curve. This complex situation emerges from our results given that the dose of 5 mg/kg shows a significant effect similar in magnitude to the dose of 50 mg/kg, with no effect at the lower (1mg/kg) and intermediate (10 mg/kg) doses.

This phenomenon has puzzled researchers for more than 50 years and recently a comprehensive overview discusses and contributes to its better understanding [46]. In spite of the main focus under endocrinology field, examples have also emerging in different areas of research such as toxicology, epidemiology and pharmacology [46, 47]. Among the several putative mechanisms which produce nonmonotonic responses (in cells, tissues, and animals) stand out the cytotoxicity, cell-specific and tissue-specific receptors and cofactors, receptor competition, superimposition of monotonic dose responses and other events related with responses of a biological system caused by products that have a complex mixture of substances [46, 48], including essential oils.

2. Concerning the FST- it is necessary to provide the locomotor activity data as an obvious control.

The Rotarod Test (RRT) procedure [33 in reference list] is recognized as suitable for detecting motor impairment due to pharmacological agents such as skeletal muscle relaxants or central nervous system depressants. Since EO was not able in modify the time of immobility in the FST, when compared with TW group, and since RRT data (Table 2 of manuscript) show absence locomotor impairment after treatment with EO (1, 5, 10 and 50 mg/kg) we find unnecessary a supplementary group to measure the same parameter.

3. References must be provided.
We apologize for the inconvenience but due to a mistake, we sent the file without the Reference List. The full list, in accordance with the standards of the journal, is now added to the text.

Essential minor revision:

1. The variability of some of the data shown in table 5 (e.g. the level of Dopamine is 722 +/- 744, and 1219 +/- 1199) is tremendous, is the assay working properly?

   We appreciate the pointed and actually the data show great variability. Alerted by comment, we did a thorough review in the raw data and we found some inconsistencies. The raw data were correctly treated and statistical analysis remade. The corrected Table 5 was added to the text.

Quality of written English: Needs some language corrections before being published

In order to avoid unsuitable use of language, the submitted manuscript has been checked for proper English usage by Elsevier Language Editing Services. In this way the text was sent back to the service of Elsevier to re-editing and returned with the message reproduced below. The corrections proposed were accepted and incorporated into the text.

De: “Elsevier Webshop Support (ELS)” <webshop_support@elsevier.com>
Para: “Mirtes Costa” <mcosta@ibb.unesp.br>
Enviadas: Terça-feira, 11 de Dezembro de 2012 3:55:56
Assunto: RE: Problem with the edited Document (Order No. 33516)

Dear Dr. Mirtes Costa,

Please find comments from the editor below:

Please accept my sincere apologies that the edited manuscript did not meet the customer's expectations. I have carefully re-edited the manuscript, which is attached. I hope that the manuscript is now of the high quality we promise and the customer deserves. Thank you for the opportunity to improve the editing of this manuscript. I am sorry for any inconvenience this may have caused.

Kind regards,

Nancy Jayaraj
Webshop Support