

## LETTER

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# Comments on the Article “[6]-Shogaol Induced Calcium Signal in Rat Insulinoma Cells”

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Dear Sir,

We read with a great deal of interest the study titled “[6]-shogaol induces Ca<sup>2+</sup> signals by activating the TRPV1 channels in the rat insulinoma INS-1E cells” by Rebellato *et al.* [1] in the January 2014 issue of JOP. Journal of the Pancreas (Online). At the outset, we would like to congratulate the authors for having conducted an exhaustive research with insights on the mechanism of action of [6]-shogaol. The manuscript is intelligently written with ample graphical representation of data leaving little room for criticism. However, it would be of enormous help if the authors could clarify certain doubts we had while reading the article.

The abstract mentions in its results section “... [Ca<sup>2+</sup>]<sub>i</sub> (*i.e.*, intracytoplasmic free calcium ion concentration) increase obtained by 1 micro mole [6]-shogaol was greater than that obtained by 10 mM glucose ...”. However, this observation is left for the reader to compare in two different graphs (1a and 4b) [1]. Furthermore, we did not find any mention of this observation in the main-text-result section nor any elaboration of the same was found in the discussion [1]. We would like to understand if this observation is incidental or the authors think it is an important finding. In the latter case, we believe that its interpretation should be elaborated in the discussion as well.

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**Abbreviations** [Ca<sup>2+</sup>]<sub>i</sub>: intracytoplasmic free calcium ion concentration

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Unpaired t-test is applied on experiments depicted in Figures 2, 3 and 4. However, there is no mention of test of significance for observation in Figure 1. Figure 1 elucidates increase in [Ca<sup>2+</sup>]<sub>i</sub> from baseline after administration of [6]-shogaol (the drug) and return of [Ca<sup>2+</sup>]<sub>i</sub> to baseline after the washout of the drug. We suggest that a paired test should have been applied in order to state that the increase was significant after [6]-shogaol administration. Moreover, pre-drug [Ca<sup>2+</sup>]<sub>i</sub> level and post-drug [Ca<sup>2+</sup>]<sub>i</sub> level also need to be statistically compared.

In our opinion, graphical representation of [Ca<sup>2+</sup>]<sub>i</sub> in Figure 1a, 2, 3, and 4a,b,c does not follow a fixed interval on the y-axis [1]. Although the graphical representation between the values is depicted equidistant, the difference of intervals between them is not fixed (e.g., 460-255=205; 255-75=180; and so on). Which kind of scale was shown needs to be better described.

We would also like to mention that in the results about “... increase of [Ca<sup>2+</sup>]<sub>i</sub> by [6]-shogaol was due to Ca<sup>2+</sup> entry across the plasma membrane ...”, description of mechanism of action of both carbachol and KCl is without a reference [1]. We suggest that the authors should provide references for mechanisms of action of carbachol and KCl.

Diabetes mellitus is a major public health problem and the present study would help in new drug development for treatment of diabetes mellitus. We believe that the clarification of the above mentioned concerns would make the study more robust.

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**Conflicts of interest** None

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### References

1. Rebellato P, Islam MS. [6]-Shogaol induces Ca<sup>2+</sup> signals by activating the TRPV1 channels in the rat insulinoma INS-1E cells. JOP. J Pancreas (Online) 2014; 15:33-7. [PMID: 24413781]
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## Editor Comments

*(The following Editor comments were sent to Drs. Rebellato and Islam together with the letter received from Dr. Aswani et al. in order to invite them to submit a reply and for highlighting the importance of reporting objective data and statistics in a scientific paper instead of showing representative examples only).*

The scientific debate that would be generated by publishing letters to editors is very important for increasing the scientific knowledge. In fact this allows the scientific community to assess and compare different and/or contrasting opinions of the other members of the community itself. Thus, the reply to comments is important even when the authors of the original paper consider some comments as not appropriate. If they do not agree with the comments, they have the opportunity in their reply of communicating to the scientific community their opinion by supporting their statements with more arguments as well as more evidences from literature. This would allow the scientific community to understand how much the authors who commented the paper were familiar with  $Ca^{2+}$  signaling and, in the same time, it also would allow the scientific community to better understand the previously published data.

On the other hand, it should be pointed out that in the present letter there are not only comments about  $Ca^{2+}$  signaling, but there are also some requests for reporting more methodological details of the analysis and of the data reporting that would likely require a reply. They can be summarized in the following three items.

1. A correct presentation of the description of the set of experiments the authors had made (i.e., mean $\pm$ SD increases) as well as a correct statistical analysis is reported in the original paper as far as data of Figure 4 only is concerned. On the contrary, in each of Figures 2 and 3, the authors had only shown the  $[Ca^{2+}]_i$  behavior in one example out of the three or four experiments they had made (probably it was the most representative). Some descriptive statistics summarizing the data of the peaks they had obtained in the three or four experiments they had made are needed also for the data described in the other figures. Therefore, in this way, the results of the entire set of experiments the authors had made remain hidden to the readers. Thus, according to the good publication practice, descriptive statistics of the study and control experiments - together with the P values resulting from their statistical comparison - should be accounted also for these figures in order to support the statements the authors had reported in the paper. In this way, by publishing the reply, the authors would have the occasion of adding this important information to that already published in their paper.

2. One comment of the letter referred to one statement reported in the abstract that was not explained and described in the Results section. The values the authors had compared, as well as, the results of the statistical analysis that supported such a statement, should be highlighted in the reply since they were not shown as results of the paper.

3. The knowledge of which kind of non-linear scale was used for drawing the data in the figures may be helpful for the readers and, certainly, would increase the readability also for researchers not familiar with  $Ca^{2+}$  signaling.