Reviewer's report

Title: Polygonum viviparum L. induces vasorelaxation in the rat thoracic aorta via activation of nitric oxide synthase in endothelial cells

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Reviewer: José Eduardo da Silva-Santos

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Major Compulsory Revisions

This is a very interesting study investigating the vascular effects of an extract obtained from Polygonum viviparum in rat aortic rings. In the following lines I did describe some points that I hope may be helpful for the authors to improve their work and contribute with some different points of view in this field of vascular research.

# 1 My main criticism for this study is the method used to measure the effects of the extract of P. viviparum (PV) in rat aortic rings. Starting in the methods section, the description of incubation of PV in vessels is partially missing. The authors’ description includes a single concentration of PV (100 mcg/ml) as the only one tested in aortic rings when cGMP was measured (sorry, no page numbers in the submission to allow an easy location of sentences). However, nothing is described for the experiments used to evaluate the ability of the extract to induce vasorelaxation or how the results were analysed. With this lack of information and the short description of the ability of PV to cause relaxation, interpretation of figure 1 was a challenge. A closed inspection of the typical records provided in the submission revealed that the relaxation induced by Ach in aortic rings pre-contracted by phenylephrine was not greater than 90%, as expected in endothelium-intact rings (Fig. 1, A). Moreover, PV-induced relaxation (at 100 mcg/ml) showed in Fig. 1C was very small (perhaps 25%). How exactly the magnitude of PV-induced relaxation in endothelium-intact rat aortic rings was measured and analyzed? Figure 1C describes the relaxation obtained after PV incubation as a ratio of PV/Ach. In order to better describe these results, which are the main point for the rest of investigation, the authors must present the effects of PV as percentage of relaxation considering the maximum contraction elicited by phenylephrine in preparations with at least 80% of relaxation to Ach. These procedures must be described in details in the Methods Section.

#2 The authors justified their study using rat aortic rings and exploring the relaxation and effects of PV on the nitric oxide/guanylate cyclase pathway taken into account the popular usage of this plant to “boost blood circulation to dissipate blood stasis”. However, the model adopted is conventionally used to study anti-hypertensive effects. Is this plant used against other cardiovascular diseases such as hypertension? This relationship should be explored in the discussion and the disadvantages of the experimental model adopted clearly
considered.

#3 The process of preparation of the extract must be included in the article. Thinking about science the reader cannot be precluded of this information. Is it an alcoholic, aqueous, hydroalcoholic solution? Which parts of the plant are popularly used? Which parts were used in the extract? The source of plant also must be more detailed. For instance, was it collected in the winter or summer? Is there a voucher specimen?

#4 Clearly describe how the animals were killed. Were it anesthetized before exsanguination?

#5 The number of experiments performed for each experimental group was described as equal or greater than 3. A group of 3 samples is usually considered too small for functional studies for statistical purposes, although it is commonly accepted for biochemical or molecular approaches. However, to allow a better interpretation of the significance of the statistical tests adopted information such as “each experiment was repeated more than 3 times” is useless. The authors must revise this point and complete the groups for at least 5 experiments per group in the functional study.

# 6 The discussion must be improved. The significance of the results obtained can better explored. Since part of the results suggest the involvement of nuclear factor E2-related factor and HO in the effects of PV on vessels, the description of the function and relevance of this pathway is much more important than the description of the classical findings related to the discovery of nitric oxide.

Level of interest: An article whose findings are important to those with closely related research interests

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

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests