Reviewer's report

**Title:** 3,4-Dihydroxytoluene, a Metabolite of Rutin, Inhibits Inflammatory Responses in Lipopolysaccharide-Activated Macrophages by Reducing the Activation of NF-kappaB Signaling

**Version:** 2 Date: 11 September 2013

**Reviewer:** rabiaa manel sghaier

**Reviewer's report:**

This manuscript describes the anti-inflammatory activity of rutin derivates from Chinese Saussurea involucrate. The authors tested these effects with several in vitro tests. They showed that the component of rutin responsible of its anti-inflammatory activity. Although the study is well conducted, this manuscript shows major deficiencies and needs to be improved.

I would recommend the acceptance of the manuscript if the authors could revise major issues and address several points.

The manuscript needs a complete revision of English language by either a native English mother tong person or by a professional translator. It contains also a very high number of typos.

**Minor Essential Revisions**

1- The introduction and the conclusion sections should be completely reformulated.

2- The transition between traditional Chinese theories and in vitro cellular and molecular studies is not smooth!

3- Abbreviations should be cited correctly.

Saussurea involucrata should be cited for the first time as Saussurea (S.) involucrata.

Once cited, use only S. involucrata.

4- Some sentences are completely unintelligible, e.g.,

Page 10, 1st paragraph “Furthermore, the DHT reduced the phosphorylation levels of the exerted no influence on ERK1/2, p38 or JNK1/2 activation in LPS-activated macrophages (Figure 4).”

Page 10, 2nd paragraph “This results in the exposure of the nuclear localization signals (NLS) on the NF-#B subunits, p65, and the subsequent translocation of the molecule to the nucleus.”

or with wrong grammar, e.g.,

“Twenty grams of dried and powdered aerial parts, including flower, of S. involucrata was extracted with 100 mL of methanol three...”

Such expressions should be avoided: “showed excellent activity”
5- Figure 1 is poorly described in the legend. Authors are only talking about DHT but totally occulting the effect of other components.

6- They state in the first sentence that rutin and its derivatives are inducing cell toxicity. However, only DTH effect is shown. How many times the experience of figure 1, left panel A and figure 1 right panel were repeated? If three times as indicated, were are the error bars?

The results of cells treated with the whole rutin extract should be included to verify the anti inflammatory activity previously described.

7- It will be interesting to show the effect of DTH treatment on the transcriptomic levels of inhibited cytokines (i.e., TNF-alpha).

8- Authors stated that there is a reduction of IL-6 and IL-1 levels !!! Statistics are missing. There is no reduction at all for these two cytokines and their m-RNA. This statement is completely wrong.

9- Figure 2, panel D: It would be rather better to mix the two figures of panel D vertically instead of horizontally together.

Idem for Figures 3, 4 and 5.

10- What does mean “in the brain” within the following sentence?!?: “Figure 3 shows that the level of NO and iNOS in the brain of the LPS-treated RAW264.7 cells dramatically decreased after the DHT treatment.

11- The combined section Results and Discussion is rather a discussion and results are very poorly described. The authors have either to split the two sections separately or to further detail the results obtained.

12- Why talking about the effect of IL-1 and IL-6 blockade in other diseases when there is no effect on their levels in DTH treated cells?

13- Some references should be cited correctly and precisely. Indeed, authors say “In a report by Baek et al [59]., it was shown that the PI3K/AKT pathway regulates the TNF-a and IL-6 expression in human macrophage RAW264.7 cells as assessed by the inhibition of PI3K and AKT.” Does a raw human cell line exist??

14- Authors should better discuss the potential mechanisms of inhibition of p65 translocation and of accumulation of this transcriptional factor in the cytoplasm, which is directly related to their results rather than relating the literature published on PGE2 within a whole paragraph.

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: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:

I declare that I have no competing interests