In this manuscript Chen et al. describe the effect of a metabolite of Rutin on the inflammatory responses induced by LPS in macrophage cell line. The effect of DHT at low concentrations was assessed on cytokine production, NO release and Cox2 expression. This effect is not mediated by MAPK and correlates with the decrease of NF-κB p65 nuclear translocation.

Major Compulsory Revisions

-The manuscript contains a myriad of errors, including incomplete sentences (ex: “In addition, the activation of mitogen-activated protein kinases (MAPKs) and NF-κB by western blotting.”). I also have some concerns about the data, largely which arise from the way it is presented and the lack of detail within the manuscript. Together, these concerns severely dampen the impact of this study. In general, to strengthen the manuscript and make the data sound, the abstract, introduction, results and discussion sections need to be restructured shortened and reformulated. The authors should in particular, include a more detailed background linking their present results with those of the literature. The English should also be improved.

- The authors should include a more adequate background and discussion linking their present results with those previously published in the literature.

While the authors largely report their previous finding concerning the SI-2 or rutin and general knowledge on inflammatory response to LPS they did not give informations about the effect of rutin on inflammatory response. If no results are available, how the authors can state in the results and discussion section that: “These results indicated that the DHT was the principal metabolite of rutin with anti-inflammatory activity.”

- The authors must clearly state from the beginning that their study is focusing on DHT metabolite and avoid sentences such as: “This research background has led us to examine the suppression of the inflammation effects of dietary supplements using rutin’s derivates, such as DHPAA, DHT, HPAA, and HVA on LPS-induced inflammation in macrophage cells.”

- In the first paragraph of “results and discussion”: the authors wrote: “The DHT also reduced the expression levels of TNF-a (Figure 2(b)) and IL-6 (Figure 2(c)). as well as IL 1β secretion, IL-1β precursor (proIL-1β) protein expression, and IL-1β mRNA expression (Figure 2(d)).” In the figure 2 however, only the IL-1β mRNA expression was shown. The authors have not presented data on IL-1β secretion. The authors claim that DHT reduce the expression of IL-6 and IL-1β. Their results however, clearly show that the incubation of the Raw264.7 cells with DHT has no significant effect on the production or the
mRNA expression of IL-6 and IL-1β respectively (figure 2b and 2d).

- Why did the authors measured the production of the cytokines 6 hours after LPS stimulation? This makes sense for the qRT-PCR experiment but less for the ELISA.
- The results show that LPS-stimulation led to a rapid phosphorylation and degradation of IκB and that DHT has an inhibitory effect on this phosphorylation. What about the effect of DHT alone on this phosphorylation? On the other hand, the authors should precise that DHT treatment has no obvious effect on IκB degradation.
The authors show an inhibition of p65 nuclear translocation that correlates with DHT treatment. No NF-κB reporter assay was reported, so there is no data that allow the authors to claim: “In this study, ....whereas p65 migrates from the cytoplasm to the nucleus to inhibit the COX-2 expression in response to (Figures 5(a) and 5(b)). (another incomplete sentence) These results indicated that the DHT could inhibit the activation of the NF-κB signaling cascades in LPS-activated macrophages.”

- The authors also claim that: “Combined, the results suggest that DHT might exert anti-inflammatory effects in vitro in LPS stimulated RAW 264.7 macrophages by inhibiting the NF-κB signal pathway activation.” this statement needs to be toned down as the effect of DHT could be mediated effectively, at least in part, by the inhibition of IκB phosphorylation, but the authors cannot rule out that other possible mechanisms may be involved.

- No results on lipid peroxidation have been presented in the manuscript so the sentence: “This study revealed that rutin and DHT inhibited the expression of COX-2 and decreased lipid peroxidation.” must be revised.

- Many repetitions in the text such: “The NF-κB, one of the critical transcription factors, regulates the inflammatory-related gene expression in the LPS-activated macrophages. In the resting macrophages, NF-κB is sequestered in the cytoplasm as an inactive precursor complex by its inhibitory protein, IκB. Upon the LPS stimulation, IκB is phosphorylated by IκB kinase, ubiquitinated, and rapidly degraded through proteasomes to release NF-κB. This results in the exposure of the nuclear localization signals (NLS) on the NF-κB subunits, p65, and the subsequent translocation of the molecule to the nucleus.” “Under basal conditions, NF-κB is inactive and prevented from binding with DNA and executing nuclear translocation because of the close association of the cytoplasm with inhibitory proteins. The cell activation that occurs through a variety of extracellular signals, such as oxidative stress, induces a cascade of events that lead to activating NF-κB and subsequently translocating it to the nucleus where it binds to DNA elements in the promoters of numerous proinflammatory gene families.” greatly reduce the fluidity of the manuscript.

- In the Methods section:
The viability assay used in the study should be detailed in the Methods section.

Minor Essential Revision
- In the Methods section:
  1) The authors need to clarify whether the 3 experiments (MTT, ELISA, NO) were in duplicate or triplicate determinations.
  2) “NO inhibitory assay” should be replaced by “NO Measurement” and the optical density used precised.