Author’s response to reviews

Title: Ashwagandha Attenuates TNF-α- and LPS-Induced NF-κB Activation and Inflammatory Gene Expression in NRK-2E Cells

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Reviewer 1

1. Title. There is a mistake in the name of cells. Please, change NRK-2E by NRK-52E cells.

The title has been revised.

2. Results

A. In Figure 3A, Ash (Ash/Ash) diminished significantly the expression of CCL5 regarding basal gene expression. In Figure 3B, Ash/Ash did not produce changes in NF-kB activation. As you determined in the paper…”NF-kB regulates CCL2 and CCL5 expression"…then, please try to explain why there was not change in NF-kB activation but it was observed a significant CCL5 downregulation with Ash treatment. Does it mean that Ash downregulates CCL5 by an independent NF-kB pathway?

We have addressed the other CCL5 regulatory pathways in the Discussion.

B. All of your work regarding anti-inflammatory capacity of botanicals is based in the expression of 2 genes, CCL2 and CCL5.

We examined the expression of CCL17, 19 and 21, as well as COX-2. However, the dosing regimen for TNF-α did not significantly induce expression of any of these genes, therefore, we could not assess a potential effect of ashwagandha on these genes.

C. I would like you present a graph with data about cell viability.
We have not included a graph as there were no significant differences in cell viability (MTT assay) – with the exception of withaferin A, which caused a loss of viability.

D. Furthermore, have you determined in your model the effect of botanicals in important interleukins such as IL-1, IL-6 and IL-8?

We have not examined the expression of these interleukins, but focused on chemokines which have been demonstrated to promote fibrosis in the kidney. This information has been emphasized in the Introduction. IL-6 does not play a role in renal fibrosis (Yan et al, 2012; PLoS One), while IL-8 knockdown is associated with fibrosis (Hang et al, 2000; J Infect Dis). There is a potential role, however, for IL-1β in renal fibrosis (Burns 2002; Kidney Int) and future experiments will focus on a more complete battery of profibrogenic mediators in response to ashwaganda.

Reviewer 2

We thank Reviewer 2 for the positive comments.