**Author’s response to reviews**

**Title:** Standardized ethanol extract of Tinospora crispa upregulates pro-inflammatory mediators release in LPS-primed U937 human macrophages through stimulation of MAPK, NF-κB and PI3K-Akt signaling networks

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Response to Reviewers’ comments (BCAM-D-20-00353)

Reviewer 1: The authors described that the extract from T. crispa augments the inflammatory response of macrophages when stimulated by LPS. However, the extract by itself is unable to increase these inflammatory mediators levels. The concept of immunostimulatory is interesting when your pharmacological tool can stimulate this inflammatory pathway. In the present study, T. crispa only increases the LPS-induced effect. Also, bands of Immunoblotting do not represent the graphs. The authors should consider to re-write this manuscript. Lack of control to demonstrated a possible immunostimulatory effect; immunoblotting should be revised. I do not recommend this manuscript for publication in this journal.

Authors’ response: The results of the present study are supported by many previous studies which showed that plant samples or pure compounds augmented the pro-inflammatory responses through augmenting the respective mediators in LPS-activated human macrophages. We have followed established procedures in this study to evaluate the stimulatory effect of Tinospora crispa on pro-inflammatory mediators release in LPS-primed U937 human macrophages. An example of previous studies to support the present study design is as follows:


We have presented the band and bands intensities by analyzing with the Image Lab™ software, which is a common procedure used in immunoblotting technique.

We have used specific inhibitors [NF-κB inhibitor (BAY 11-7082), Akt inhibitor (LY294002), JNK inhibitor (SP600125), ERK inhibitor (U0126) and p38 inhibitor (SB202190)] based on the mediator specificity and availability as no specific experimentally proven positive control can upregulate all cellular mediators. Therefore, we have further justified the outcomes by using
specific inhibitors (Page 9, lines 217-222). The use of specific inhibitors as positive control has been reported in many previous studies.

Reviewer 2: The study reports the underlying mechanisms involved in the stimulation of pro-inflammatory mediators by hydroethanolic extract of T. crispa stems. I found the topic interesting and I am in acceptance of the approach taken by the authors. Appropriate techniques: ELIZA, qPCR,HPLC and LC-MS/MS were enough to deliver the interesting results obtained in this study.

Authors’ response: Thank you.

Reviewer 3: The manuscript is well articulated and presented but the experiments have no positive controls in the most part.

Authors’ response: We have used specific inhibitors [NF-κB inhibitor (BAY 11-7082), Akt inhibitor (LY294002), JNK inhibitor (SP600125), ERK inhibitor (U0126) and p38 inhibitor (SB202190)] based on the mediator specificity and availability as no specific experimentally proven positive control can upregulate all cellular mediators. Therefore, we have further justified the outcomes by using specific inhibitors (Page 9, lines 217-222). The use of specific inhibitors as positive control has been reported in many previous studies.

Reviewer 4: The manuscript is well prepared and has good points to bring reader's attention. With various parameters measured, the manuscript has potential to be published in this journal. Thus, this manuscript is accepted with minor modifications. However, it is highly recommended that the author use the English proofread services to enhance the quality of the manuscript. Other than that, please refer the comments below:

Line 67: What do you mean by T. crispa

Authors’ response: T. crispa refers to Tinospora crispa. Its full scientific name is mentioned in full in line 67. Onwards the plant is mentioned as T. crispa.

Line 92: RAW 264.7 refers to?

Authors’ response: RAW 264.7 cells are a macrophage-like, Abelson leukemia virus transformed cell line derived from BALB/c mice. This cell line is commonly used as a mouse macrophages model for studying several cell signaling pathways.

Line 157: "synergi" change to "synergy"

Authors’ response: Corrected.