Author's response to reviews

Title: Murraya koenigii leaf extract inhibits proteasome activity and induces cell death in breast cancer cells

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Author’s response to reviews: see over
To,  
Dr. Tom Rowles,  
Executive Editor,  
BMC Complementary & Alternative Medicine  

November 8th, 2012  

Subject: MS: 1602273541770919 - Murraya koenigii leaf extract inhibits proteasome activity and induces cell death in breast cancer cells  

Dear Dr. Rowles,  

I would like to thank you for considering our manuscript for publication in the journal BMC Complementary & Alternative Medicine.  

I am writing with regards to our above mentioned manuscript, which was returned for revision. Please find below point-by-point responses to the reviewers and editorial comments.  

Reviewer 1:  

Comment: There are several grammatical errors in the manuscript that need to be corrected.  

Answer: The manuscript has been checked for grammatical errors and corrected.  

Comment: In the conclusions, the authors indicate that the extracts involved in this study may be further developed for treatment of cancers that are resistant to conventional therapy but there is no evidence to support this claim. This claim needs to be removed.  

Answer: The sentence “treatment of cancers that are resistant to conventional therapy” is removed from the abstract and the final conclusion.  

Reviewer 2:  

Comment: In addition to whole extract some fractionation and potential narrow down of active compound/fraction is critical.  

Answer: In the current manuscript we tested the effect of a methanolic extract of curry leaf as an anticancer agent in breast cancer cell lines. We have just initiated studies on the isolation of the active compound/fraction, and this will be published later as a second manuscript, along with data on characterization and its anticancerous and proteasome inhibitory effects in vitro and in vivo.  

Comment: Comparison of extract with standard proteosome inhibitors as a control is missing.
**Answer:** Experiments using MG-132 (a standard proteasome inhibitor) as a control have been done and incorporated in the revised manuscript. Both MTT assays (to test cell viability) and inhibition of the endogenous 26S proteasome (in cell extracts) by MG-132 were done. Please refer to figure 1C and 1F for MTT assay data with MG-132 in the two breast cancer cell lines. Please refer to figure 11D and 12C for inhibition of 26S proteasome in cell extracts by MG-132 in both cell lines.

**Comment:** Inclusion of some Zymograph data would have strengthened this manuscript.

**Answer:** We thank the reviewer for a very good suggestion. However, we have performed proteasome inhibition experiments by the CLE using three different approaches – namely a purified 20S proteasome, inhibition of endogenous 26S proteasome in intact cells and also inhibition of the endogenous 26S proteasome in cell extracts. The proteasome inhibition experiments were performed using substrates specific to the three enzyme activities of the 20S proteasome (i.e Ch-L, T-L and Cp-L). Considering that the zymograph experiments are time consuming and needs to be standardized, we feel that its inclusion is beyond the scope of the present manuscript.

**Editorial Comments:**

**Comment:** Please move your methods section from its current location to after your Background section, in accordance with our formatting guidelines

**Answer:** As suggested the methods section has been shifted and now appears after the background section.

**Comment:** We recommend that you copyedit the paper to improve the style of written English. We would recommend that you ask a native English speaker to assist you with this

**Answer:** As recommended the manuscript has been copyedited and style of written English has been improved with the help of a professional editing service.

I hope you find the revised manuscript in order and suitable for publication in BMC-Complementary and Alternative Medicine. I look forward to your favourable response in this regard.

Best regards,

(Ayesha Ismail)
Corresponding Author