Author's response to reviews

Title: Evaluating plants with ethno-pharmacological record from Darjeeling district of West Bengal, India, for cytotoxic activity in vitro against human cancer cell lines

Authors:

- Bipransh Kumar Tiwary (bipra.tiwary@gmail.com)
- Sony Bihani (crony_s@rediffmail.com)
- Anoop Kumar (anoopnibu@gmail.com)
- Ranadhir Chakraborty (rcnbusiliguri@gmail.com)
- Runu Ghosh (runughoshdey@gmail.com)

Version: 3 Date: 11 November 2014

Author's response to reviews:

To
The Editor
BMC Complementary and Alternative Medicine (Section: Basic research)

Re: Response to your Comments and Submission of Revised Manuscript MS: 2045626151440083 Research article: Evaluating plants with ethno-pharmacological record from Darjeeling district of West Bengal, India, for cytotoxic activity in vitro against human cancer cell lines - Bipransh Kumar Tiwary, Sony Bihani, Anoop Kumar, Ranadhir Chakraborty and Runu Ghosh

Dear Sir/Madam

At the onset, I would like to extend my gratitude for thoroughly assessing the MS. Please find below our response to each point brought up by you.

Reply to Editor's Comments:

Comment 1. There is some confusion about certain aspects of the MTT assay employed for the cytotoxicity testing. In line 135 it is stated that "After treatment, media was replaced with MTT solution (10 µl of 5mg/ml per well) prepared in PBS and incubated for 3hrs...". Can you clarify if the MTT dissolved in PBS was then diluted in medium so that the cells were exposed to medium as well as MTT during the three hour incubation period?

Response: Thank you for pointing out the confusion which certainly needs clarification. The MTT was dissolved in PBS at a concentration of 5 mg/ml and 10 µl from this “MTT in PBS” solution was placed in each well (from which the medium was completely removed) meaning that there was no medium during the 3 h incubation. The method followed was that of Denizot & Lang who observed that the background optical density was reduced in the absence of serum proteins and phenol red. Thus, we dissolved MTT in PBS and followed the protocol described by Denizot and Lang (Ref 18 in MS). The language in the MS...
has been changed to make the protocol clearer (lines 133 – 138).

Were the plant extracts washed completely from the cells before addition of MTT so that antioxidant components in the extracts did not react non-specifically to reduce the MTT?

Response: The media containing the plant extracts was removed completely from the wells following which 10 µl of MTT (5 mg/ml in PBS) was added.

The positive control should be mentioned in the Methods section. What was the IC50 of the positive control?

Response: We completely agree with the use of positive controls. However, in the present study, we did not use any positive control since this study involves screening the extracts (and not a purified compound) for their cytotoxicity on cancer cell lines. Thus, in agreement with you, we shall definitely use a positive control once we have purified the component with anticancer properties and this has been mentioned in the lines 287 - 289 in the MS. The present study demonstrates the probable cytotoxic property of extracts of folk medicinal plants of Darjeeling district of West Bengal, India. A similar observation was put forward by Fadeyi et al. who screened Nigerian medicinal plants (Ref 26 in MS).

The wavelengths tested need to be checked as 630 nm is usually the reference wavelength (if used) while the purple formazan absorbance should be read at absorbance 540 or 570 nm; 490 nm is too low. The fundamentals of the assay upon which the paper is based need to be scientifically accurate as this will affect the results.

Response: According to the protocol of Denizot & Lang, the suggested wavelength range for purple formazan absorbance is 560 - 690 nm. Thus, we measured the absorbance at 600 nm with 490 nm as reference (line 138 in MS). To further scientifically strengthen our claim, we also performed Trypan blue exclusion assay and also morphologically assessed the cells under phase contrast microscope.

2. It is essential that a non-cancerous cell line is included in the panel of cells used to test anticancer activity of the extracts. If the extracts are merely generally cytotoxic and kill all types of cells equally then they have limited value. Selectivity of toxic effects against cancerous cells needs to be demonstrated to draw the conclusions stated in this manuscript.

Response: This is a very pertinent question taking into fact that non-toxicity towards normal cell is the governing factor to define a candidate as possessing anticancer properties. However, since the plants used in this study are all medicinal plants being used by the locals, we assume that the extracts will be non – toxic towards normal cells. Agreeing with your valuable input, we have discussed this aspect in the revised MS (lines199 - 201, lines 268 -270 and line 288). Again, once we have the purified component, we shall test it on normal cell line before we render it as a probable candidate for being used as anticancer compound.

Instead of using the term anticancer, we have used ‘probable anticancer’ in line 61 and ‘cytotoxic properties of these plants on cancer cell lines’ in lines 98-99.
Overall response: The MS has vastly benefited from your comments. I have uploaded the revised version for your kind consideration for getting it published in your esteemed journal.

With sincere regards,
Runu Ghosh
Corresponding author