



**SDI Review Form 1.6**

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| Journal Name:            | <a href="#">Journal of Cancer and Tumor International</a>                                 |
| Manuscript Number:       | 2014_JCTI_15519                                                                           |
| Title of the Manuscript: | Antitumour Activity of <i>Xanthium strumarium</i> L. on Human Cervical Cancer HeLa Cells. |
| Type of the Article      | Original Research Article                                                                 |

**General guideline for Peer Review process:**

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

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**PART 1: Review Comments**

|                                     | Reviewer's comment                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
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| <b>Compulsory</b> REVISION comments | <p>The manuscript is a proposal to use the <i>Xanthium strumarium</i> extract as an antitumor agent. Present aspects that need to be strongly justified, for instance, the title handle antitumor activity. Strictly, authors did not employ a right model to determine antitumor activity. They use an immortalized in vitro tumor cells and determine cell proliferation, viability, and DNA fragmentation in acute treatments.</p> <p>Authors forgot the relevance to show the <i>Xanthium strumarium</i> extract characterization at respect could be important answers; how characterize the extract in terms of xanthinin concentration? How the homogeneity of extracts components, as fiber, solvents and alcohol is? How reproducible is the extraction process? How author warranty sterility of extracts to be added to cell cultures?</p> <p>There are several aspects of methodology that need to be clarified; cancer cell culture needs indicate the use or not of antibiotics and CO<sub>2</sub> supplementation. It is not clear if number of cells, <math>2 \times 10^6</math> correspond to final number or is seeded</p> | <p>1. We have mentioned in the title itself that we have used the extract against Hela cell line.</p> <p>2. We have done the preliminary Screening for the crude <i>Xanthium</i> extract. Xanthitin was mentioned in the Introduction just for the information. However about the yield and sterility we have mentioned and highlighted the same.</p> <p>3. Antibiotics were used also the cultures were kept in a CO<sub>2</sub> incubator which has been mentioned and highlighted.</p> <p>4. <math>2 \times 10^6</math> cells/ml is the final number in all the assays before treatment which has also been mentioned and highlighted. The authors agree about the point of DMSO treatment and we have mentioned and highlighted the same in the paper. the results after treatment with 5% DMSO were almost similar to the normal untreated cells hence DMSO treatment in our previous studies hence was not considered further in this study. 24, 48 and 72 hours of treatment were taken into consideration as we were performing the preliminary</p> |



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|  | <p>amount. Indicate area of plated used.</p> <p>MTT assay did not indicate the amount of cells seeded. Authors need indicate if use control conditions of treatment in similar conditions of DMSO content. Justify treatment duration of 24, 48 and 72 h.</p> <p>It is not clear cell viability procedure, how many cells were seeded? How observe trypan blue stain? Light microscopy, indicate magnification? DNA fragmentation assay indicate that was determined in IC<sub>50</sub> condition, How determine IC<sub>50</sub>? Why IC<sub>50</sub> is better in terms of antitumor activity?</p> <p>Indicate % of agarose gel, electrophoresis duration, molecular weight marker, indicate if gel was stain after electrophoresis?</p> <p>How identify apoptotic cells vs other cells? This specific procedure is very sensible to pH; authors needs indicate buffers used and pH's (acridine oange/ethidium bromide).</p> <p>In protein estimation procedure is not clear of the estimation was for a pull or was determined independently.</p> <p>All results were adjusted to respect to control condition, however it is important to know if control was DMSO or not.</p> <p>Discussion is poor in terms of xantin, due to analytical data omitted. It is not at all appropriated make conclusion of antitumor activity with data obtained between 24 and 72 h of treatment. Coul</p> | <p>screening in this study. As we obtained the IC<sub>50</sub> value at 48 hours only that dose is considered further as our main focus was on the mechanism of action. Desired results were obtained at this dosage and taking into consideration all the results of all dosages at 24, 48 and 72 hours would be too much of the data. We have performed that but not included for this paper.</p> <p>5. As discussed in the 4<sup>th</sup> point the final cell count before treatment was adjusted to <math>2 \times 10^6</math> cells/ml. About light microscopy we have mentioned and highlighted the magnification in the paper. Further consideration of IC<sub>50</sub> concentration as dosage has been discussed in the previous point. It was by mistake not mentioned in assay in materials and methods which has been mentioned now.</p> <p>6. % of Agarose gel; staining and duration have been mentioned now. Gel was not stained after the run.</p> <p>7. Buffer and pH have been mentioned.</p> <p>8. Protein estimation assays were supprtive assays which supported our results further.</p> <p>9. By the control condition we considered untreated cells and the reasons for not using DMSO are discussed earlier.</p> <p>10. Our study was focused on the</p> |
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|  | <p>be more appropriate argue in terms of citotoxicity than viability. End of discussion is difficult to follow with manuscript data provide.</p> | <p>preliminary treatment of crude Xanthium extract. Further studies are going on for the detection of active components from this extract hence; we would be able to state the possible action of a particular component after further studies. Still we have tried to relate an active component with the anticancer activity which has been added and highlighted.</p> <p>We have tried our best to cover all the aspects in the discussion regarding the anticancer activity of the extract with respect to the assays performed as per facilities and funding we were provided.</p> |
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| <b><u>Minor</u></b> REVISION comments   | Introduction section reflects a simplistic definition about a complicate biological process as cancer is. Authors' definition (lines 35 and 36), only consider uncontrolled proliferation of cells. Actually there are definitions more appropriated as the National Cancer Institute or follow the hallmarks of cancer. | We have changed the definition and highlighted the same. |
| <b><u>Optional/General</u></b> comments |                                                                                                                                                                                                                                                                                                                          |                                                          |