Author's response to reviews

Title: Gallotannin-rich fraction from Caesalpinia spinosa (Molina) Kuntze exerts cytotoxic activity and increases sensitivity to doxorubicin treatment in a leukemia cell line.

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Gallotannin-rich fraction from Caesalpinia spinosa (Molina) Kuntze exerts cytotoxic activity and increases sensitivity to doxorubicin treatment in a leukemia cell line.

Diana M Castañeda DC, Luis M Pombo LMP, Claudia P Urueña CU, John F Hernandez JH and Susana Fiorentino SF

Dear Editor,

I would appreciate the general and specific comments of the reviewers, and I would send an answer to each one of the points. Additionally, the final version includes all of the changes.

Reviewer: Chin-Wen Chi.

1. “This paper used human PBMC and fibroblast in their experiments, an IRB approval is needed and should be indicated in their methods.

   The project supported this publication was previously approved by the ethics committee of the Faculty of Science of Pontificia Universidad Javeriana, together with the informed consent. This sentence was added on the paper.

2. The manuscript needs extensive editorial help with typos such as page 2, first line, caspasa 3 activation should be caspase 3 activation. Doxorubicine or doxorubicin, E2Et or E2ET, ...etc

   We already did the changes required.

3. It is not proper to cite 6 references after one sentence (page 2, 2nd paragraph, line 3, references 7-12.

   We correct this paragraph and added new references adequately.

Page 3. It is better to indicate the % of yield......

   Percentage of yield was already added on the text.

   2. In the results and discussion section, Shinoda test should be cited.....

   Description was done.
3. References 1 and 15 are not English. Reference 1 and 15 were eliminated.

4. Each figure or Table should be presented as an independent story. It was already corrected on the document.

5. It is hard for this reviewer to understand why the vehicle control Ethanol was used at different percentage for different experiments. Similarly, why a specific dose was selected for each experiment needs to be described.

P2Et fraction used in this paper was resuspended on EtOH, and subsequently diluted in culture medium or in the solvent used for each protocol (caspase 3, annexin, viability etc). Ethanol was used as negative control at the same dilutions than P2Et, taking in account that the activity of ethanol can change if there is more solvent on the culture. We clarify this idea on the document.

Regarding the different concentrations of P2Et used for each protocol, we made preliminary dose-response tests for each protocol and on the paper we only show some of the concentrations that correspond to the ranges of activity. The concentration shared in almost all of the protocol is 5.5 ug/ml. Dilutions used in the clonogenic test are not so different than the others, so we consider it is not so relevant.

6. Figure 1, what is the meaning of Uv, VS?. The resolution of 1E, 1F and 1G can be improved.

We added the meaning of Uv and VS, and corrected the resolution of the figures.

7. Figure 2, what kind of morphological changes was used for data on figure 2A needs photo or more detailed explanation. How many time was the experiment repeated with how many samples?. What is the meaning of Aqueous?. Is etoposide dissolved in ethanol and different concentration of etoposide treatment group has different levels of ethanol?

We added a supplementary figure shown morphological changes observed on K562 cells by optical microscopy.

The number of experiments was added at the figure legends.

Aqueous is the fraction obtained in water.

Etoposide is dissolved on ethanol and negative control has the same concentration of ethanol than on the dilution of the drug.
8. Figure 3, why different doses of Doxorubicin (0.004 ug/ml for figure 3B and 0.027 ug/ml for Figure 3C) were used. What is the number of experiments performed on figure 3E?

We try different concentrations of Doxorubicin including 0.004, however the concentration shows on the figure 0.027 is the clearest. In figure 3E we did 2 independent experiments, each one with duplicates.

9. Last but not the least, Figure 5, why the viability of P2Et (1.6) treatment groups had different viability in Figure 5A. It is suggested to include more leukemia cell lines.

We thanks for this observation. We are trying now with more cell lines, and studied in depth the molecular mechanisms implied in cell death. For the experiment in Figure 5A we calculated the average of viability after treatment with 1.6 ug of P2Et on the different experiments, and we added the standard deviation of this values on the figure to be clearest.

Reviewer: Vanessa Steenkamp

Major revisions
1. Was ethical approval obtained for collection of blood and fibroblasts? 
The project supported this publication was previously approved by the ethics committee of the Faculty of Science of Pontificia Universidad Javeriana, together with the informed consent. This sentence was added on the paper.

Minor revisions
1. Spelling mistakes: caspasa should be caspase, annexin has two "n's"
2. Background - replace the words "our country" with the country name
3. Provide unit for cells in Annexin V assay (?cells/ml)
We already did all of the changes.

4. Figures: Figure 1: E, F, G - include titles to axes. Figure 3: include magnification

We already change the figures.

5. Table 2: keep the amount of numericals after the decimal point constant
We already change the figures.

Reviewer: Min-Hsiung Pan

1. The authors should provide the purity of P2Et and its chemical characterization.

We already included the purity on the text.
2. Is the concentration (44.5 #g/kg) used relevant for those achieved in the human body?

Concentration we used were calculated for the in vitro assays. The evaluation of the dose relevant for humans is calculated after the evaluation of acute toxicity in vivo on animal models as mice or rats. Preliminary experiments carried out in our lab have allowed us to observe that a dose of 12 mg/Kg is well accepted and induce diminution of tumor size.

3. The authors should evaluate the cytotoxicity of P2Et in normal cells.

Evaluation of the activity on normal cells, are shown on table 2. On the document is clear that PBMC and fibroblast were obtained from normal donors.

**Reviewer:** Joseph Vedasiromoni

1. The authors have done cytotoxic studies in vitro. So in the biological experiments 'dose' has to be replaced with 'concentration'in all places.

   We already did the changes.

2. While mentioning references in the text, the number of the reference need to be given in brackets and not the authors and year as has been done for some references in the results and discussion section.

   We made the corrections on the document.

3. The reference of Sharma et al., 1998 is not found in the list of references.

   This reference was included on the paper.

4. Figure 3 legend: Why *** and ** and why not just *.

   The statistit program suggest that * is 0.05, ** <0.005 and *** is <0.001. The experiments on this figure were all significant above 0.005.

5. The manuscript is fraught with a lot of language mistakes like.....

   We send the paper to be edited by a native editor.