**Author’s response to reviews**

**Title:** Effect of freeze-dried Carica papaya leaf juice on inflammatory cytokines production during dengue virus infection in AG129 mice

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**Version:** 1  **Date:** 24 Oct 2018

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Reviewer 1

1. Did author used any positive drug control for this study?

We didn’t used any positive drug for this study because there is no effective dengue anti-viral drug to be used as a control. Furthermore, the mechanism of action of FCPLJ is still unclear. The choice of positive drug should reflect the same mechanism of action as test item/drug used for treatment.
2. Did author find any major constituents of the Carica papaya extract which repress the viral infections?

C. papaya juice contains 2 major groups of compounds such as flavonoids and alkaloids as published in Afzan et al, 2012. From the literature review, only quercetin and fisetin have been reported to exhibit dengue anti-viral activity (Zandy et al, 2011). However, the quercetin and fisetin is found less abundance in FCPLJ.

3. In all figures except figure 2, statistically significant (*) P<0.05 value is missing.

We didn’t highlight the statistically significant (*) P<0.05 value in other figures because there are no significant changes between group observed. We have added “There was no significant different between group observed.” in the figure’s caption.

4. Supplementary figure S2 is typo error as figure S1.

The typo error has been corrected accordingly.

5. Does author perform PCR array analysis in duplicate or triplicate for each group?

The PCR array analysis was done in singleton reaction for each sample of 4 from each group. The statement has been added in the Method section, under subheading Real-time PCR and gene expression analysis.

6. PCR array analysis of 84 genes for both control and FCPLJ treatment groups should be represented in Venn diagram or Volcano plot for both up and down regulated genes. Heat map and graphic bar diagram can be shown up for mRNA expression levels of genes in comparison of both groups.
We have represented the up and down regulated genes in Volcano plot (Figure 3) in addition to the Table 1. The gene expression with significant different were indicated.

7. Since the author focused on liver cytokines does any changes seen in the section of liver H&E stains?

We did not perform the H&E stain for this study. We have addressed this limitation in the last paragraph of discussion.

8. Since author claimed that FCPLJ treatment significantly down regulated 8 genes: CCL6/MRP-1, CCL8/MCP-2, CCL12/MCP-5, CCL17/TARC, IL1R1, IL1RN/IL1Ra, NAMPT/PBEF1 and PF4/CXCL4. CCL6/MRP-1, CCL12/MCP-5, and IL1R1 after the viral infections in vitro or patients, can author further provide any evidence confirming any of these genes by Immunofluorescence or Immunohistochemistry study?

The 8 genes of the FCPLJ treated group were found significantly down-regulated by PCR Array. In this current study, we did not do include immunofluorescence or immunohistochemistry analysis. However, we have already proposed for the immunohistochemistry analysis in the future study. We have addressed this limitation in the last paragraph of discussion.

9. Does the author study any of liver protein expression of IL6, TNF, IL1, IL8, CCL2 and CCL8?

We did not perform protein expression of the liver. We have addressed this limitation in the last paragraph of discussion.

10. Any changes in macroscopic examinations of PBMC using GFB and Giemsa staining of post infection group and after 7 days of FCPLJ treatment.
We have added the differential count data (Table S1) but there is no significant different between experimental group on day 3, 5 and 7 of post infection. The changes on PBMC using giemsa staining of post infection + treated with FCPLJ after 7 days of treatment were not observed as the study was stopped at day 4 for liver gene expression study and on day 7 for cytokine analysis. We have included this result in the result section.

Reviewer 2

1. Write down the name of taxonomist who identified the Carica papaya which was deposited as voucher specimen.

The identification of C. papaya was done by using plant identification service provided by the Forest Research Institute Malaysia (FRIM) as service, therefore we could not provide the name of taxonomist.

2. Add a heading "acute toxicity analysis" before dose determination and put the data of acute toxicity analysis under this heading.

Acute Toxicity Analysis heading is not necessary for this paper because the toxicity analysis for C. papaya juice was done in previous study (not in current study) as mentioned in the Dose determination section. We have included the references of the toxicity analysis studies in the Dose determination section (Ref. 22-23).

3. Provide the gene expression data by Gel Doc System.

The gene expression was done by using PCR array. We do not run the western blot analysis on these samples. We have addressed this limitation in the last paragraph of discussion.
4. Rectify grammatically "The juice was then placed in containers and freeze at -50°C before subjected to freeze drying process"

The sentence was changed to “The juice was placed in containers and kept frozen at -50°C prior to freeze drying process”

5. Rectify "where no mortality nor adverse effect was observed"

The sentences were changed to “No mortality and adverse effect on the functions of the liver, kidney and bone marrow was observed in rats treated with up to 2000 mg/kg BW FCPLJ”

6. Cite the reference followed for the "propagation of viruses for inoculation"

The reference for propagation of viruses for inoculation was added.

7. Italicize the plant names throughout the manuscript including the bibliography

Plant names has been italicized throughout the manuscript.

8. Reference 12, remove the 2013 which is written twice and incorporate the volume and issue numbers.

Reference 12 was published in year 2013, in volume 2013 and issue 616737; therefore 2013 is written twice referring to year and issue.