ANSWERING REVIEWERS

July 20, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 3687-review.doc).

Title: Events associated with apoptotic effect of *p*-Coumaric acid in HCT-15 colon cancer cells

Author: Saravana Kumar Jaganathan, Eko Supriyanto, Mahitosh Mandal

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 3687

The manuscript has been improved according to the suggestions of reviewers:

1. Format has been updated according to the revision policies of BPG.

2. COMMENT 1:

The number of repetitions in each experiment is not very clear for me. In most of the experiments there is not any indication to the number of replicates. On the other hand, when authors say "in triplicate", does it refer to technical replicates or biological replicates? Why some of the experiments are only made using one cell line? Minor concerns: There are many typing errors and incorrect expressions: The manuscript should be revised. -Expressions that should be revised: -page 9 . 3.4 section: "..... ROS levels at various hours examined were significant" -Figure 2 legend "Means are significant at 12, 24 and 48 h ($p < 0.05$)". -Figure 3 legend: "....and the means are significant at 12, 24 and 48 h ($p< 0.05$)....." -Figure 4 legend: A. Means are significant at 6 h and 12 h ..... B. .....and means are significant at 3 h and 6 h compared with ..... -Figure 6 legend: "..... values of M2 were significant at 24 h and 48 h ($p < 0.05$) ...." -Table 1 ..."means are significant at $P < 0.05$." Significant are the differences, not the levels or the means. -Fig 1: X axis: treatment concentration (?icromolar) -Figure 5 B is not relevant. Could be eliminated

(1) In the subsection ***Statistical analyses,*** authors have clearly mentioned about the number of replicates.

All values are expressed as the mean ± standard error. Figures were plotted using Graphpad Prism software. All experiments were performed three times independently (biological triplicates). One-way ANOVA was performed to find statistical significance.

(2) Triplicates mentioned in all experiments are the biological triplicates and the same were mentioned in the text.

(3) Authors primary objective was to evaluate in HCT 15 cells as their current title of manuscript depicts. However some experiments were performed in both cell lines to extend their arguments in another cell line. However author may incorporate the reviewer suggestion in the coming research.

(4) Authors have carefully revised the manuscript according to reviewer suggestion. Following changes were made:

- In text: Moreover, the differences in the ROS levels at various hours examined were significant, compared to control with a p value of less than 0.05 (Figure 3).
- In fig 2: Mean differences are significant at 12, 24 and 48 h ($p < 0.05$).
- In fig 3: Data is representative of three independent experiments and the mean differences are significant at 12, 24 and 48 h ($p< 0.05$).
- In fig 4: Mean differences are significant at 6 h and 12 h compared with untreated control cells ($p < 0.05$). Data is representative of three independent experiments and mean differences are significant at 3 h and 6 h compared with untreated control cells ($p < 0.05$).
- In fig 6: Data is representative of three independent experiments and the differences in the values of M2 were significant at 24 h and 48 h ($p < 0.05$) compared to untreated control cells.
- In table 1: Mean differences are significant at $P < 0.05$.
- Fig 1: X axis: treatment concentration was included

Figure 5B is retained by the authors as it may add some value for the Figure 5A by providing some morphological changes in the treated cells. Hope reviewer may allow to use the figure 5B in the manuscript.

Revised sentences:

In our laboratory, experiments in studying the preventive effect of honey against colon cancer had been constantly done. Previous results depicted honey could inhibit the colon cancer cell proliferation. Antiproliferative effect was found to vary with the phenolic content present in the honey.

Since honey containing higher phenolic content was found to induce apoptosis significantly, the scope of this research was extended to study the apoptosis induced by one of the phenolic components of honey, *p*–Coumaric acid, against the colon cancer cells.

It showed that *p*-Coumaric acid could reduce the DOX-induced high serum levels of lactic dehydrogenase (LDH) and creatine phosphokinase

This current study, deals with the growth inhibitory effect of *p*-Coumaric acid in colon cancer cells. Further, an attempt has been made to explore the ROS and mitochondrial dependent mechanism in the apoptosis induced by the *p*-Coumaric acid.

Untreated control cells showed an average intensity of 229 after 12 h. From the results, it was observed that *p*-Coumaric acid treatment reduced the potential by 2.5 fold after 12 h

From our laboratory, it was showed that honey rich in phenolic content was able to induce apoptosis significantly in colon cancer cells.

Hence, it is believed that estimated IC_{50} values against these colon cancer cells are achievable internally.

COMMENT 2: The paper is interesting and very well written. In the results section, a brief summary of the method employed is reported at the beginning of each paragraph, I would suggest to delete all these repetitions.

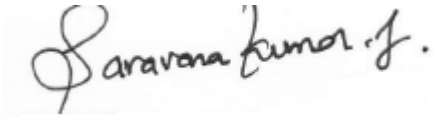
(1) Removed sentences according to reviewers suggestion:

- Colony formation is essential for cell survival. Colony forming assay was used to assess the colony forming ability of treated cells.
- Time-dependent cell cycle analysis of *p*-Coumaric acid treated cells was performed using propidium iodide staining.
- ROS levels were found altered during apoptosis. ROS generation was estimated using ROS-sensitive probe DCFH-DA.
- Mitochondrial membrane potential was assessed using Rh123 dye.
- Lipid layer breaks which are associated with the treated and untreated cells were examined using Merocyanine 540 dye.
- Apoptosis was assessed using YO-PRO-1 staining by flow cytometry.

3. All references were edited according to journal style and both PMID and DOI were provided.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



Dr Saravana Kumar Jaganathan
Visiting Research Fellow
Cardiovascular Engineering Center
Faculty of Bio-science and Medical Engineering
University Technology Malaysia, Malaysia
Email: jaganathaniitkgp@gmail.com;
Tel : 00607-5558548; Fax: 07-5558553