Reviewer's report

Title: Cytotoxicity effect of degraded and undegraded kappa and iota carrageenan in human intestine and liver cell lines

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Reviewer: Koji Higai

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Journal: BMC Complementary and Alternative Medicine
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Title: “Cytotoxicity effect of degraded and undegraded kappa and iota carrageenan in human intestine and liver cell lines”
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The manuscript “Cytotoxicity effect of degraded and undegraded kappa and iota carrageenan in human intestine and liver cell lines” by Shahrul Hisham Zainal Ariffin and colleagues described the mild acid hydrolysis products of FGKC, DKC, CGKC, FGIC and CGIC which showed moderate cytotoxic effects on the Caco-2, FHs 74 Int, HepG2 and Fa2N-4 cell line. Furthermore, the authors clarified that degraded polysaccharides induce the morphological apoptotic reaction, gene expression in the cells. The described results are interesting for understanding the role of degraded polysaccharide. But the results presented in the manuscript are too immature to be published in BMC Complementary and Alternative Medicine in this version. I, however, propose some correction for Major and minor points.

Major Points

#1) Although the cells viability decreased significantly in response to degraded FGKC, DKC and CGKC, non-degraded FGKC, DKC and CGKC don’t affect the cell viability.

Why the only degraded polysaccharides can induce apoptotic reaction in the cells?
I, therefore, recommend that the authors discuss in this point.

#2) In Gene Expression analysis section (p.14;line 8), Figure 13,14,15

“ In HepG2 cells, the RT-PCR result showed that the gene expression of PCNA (lane 6,10,14: Figure 13), Ki-67 (lane 7,11,15: Figure 13) and survivin (lane 8,12,16: Figure 13) gene were suppressed by the treatments of degraded FGKC, DKC and CGKC, respectively”.

But it's difficult to discuss the mRNA levels obtained from the results of RT-PCR analysis. So I recommend that the author should perform additional experiments using quantitative methods, i.e. real-time RT-PCR.

#3) Abstract
“The mod of cell death is suggested to be through apoptosis as the cells showed the characteristics of apoptosis”

But the evidence of apoptosis is poor in these experiments. So I recommend that the author should determine the caspace-3 activity, phosphatidylserine exposure on the cell surface, and other apoptotic reaction in the cells-treated with degraded carrageenan.

#4) Gel electrophoresis analysis (p.14;line 6)In Figure 11B;
“Figure 11b and 12b show DNA fragmentation of HepG2 and Caco-2 cells treated with TAM as positive control.”

This study used Tamoxifen as a positive control. Although the genomic DNA was prepared from Tamoxifen-induced apoptotic HepG2 cells, then why does not DNA ladder appear on gels of lane1 in Fig.11B? (In figure 10f, The expression of nuclear fragmentation and apoptotic body were induced by Tamoxifen in HepG2 cells). So I recommend that the authors add the additional description in this points.

Minor points
p.8;line 8
“... cells was stained with 10 µL of a mixture (1:1) of acridine orange (100 mg mL-1) and ethidium bromide (100 mg mL-1) solution in 1x PBS.”

The concentration of acridine orange and ethidium bromide are 100#g / mL.

p.14;line 3
“genomes (lane 1: Figure 11a). However, DNA fragment ladder was not seen in degraded k-carrageenan treated Caco-2 cells (Figure 12a).”

“genomes (lane 1: Figure 11A). However, DNA fragment ladder was not seen in degraded k-carrageenan treated Caco-2 cells (Figure 12A)

Legends of figure 1-4
“ and (d) tamoxifen at concentrations of 0.625- 20.000 µg mL-1 for 24 – 72 h.”

“ and (f) tamoxifen at concentrations of 0.625- 20.000 µg mL-1 for 24 – 72 h.”

Table 2
“IC50 values of FGKC, DKC, CGKC, FGIC, CGIC and Tamoxifen...”

“IC50 values of degraded FGKC, DKC, CGKC, FGIC, CGIC and Tamoxifen.”

Figure 5-8
I recommend that the authors merge the figure 5-8.
Figure 11,12
I recommend that the authors merge the figure 11 and 12.

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests.