Author's response to reviews

Title: Brewers' rice induces apoptosis in azoxymethane-induced colon carcinogenesis in rats via suppression of cell proliferation and the Wnt signaling pathway

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Author's response to reviews: see over
Dear Tom Rowles,

Submission of Revised Research Article

With reference to the above matter, I am pleased to submit my revised research article to be considered for publication in BMC Complementary and Alternative Medicine. This manuscript presents on the potential of brewers’ rice, a by-product in the rice milling process, in the inhibition of cell proliferation, induce apoptosis, and suppress of COX-2 and β-catenin expression via the Wnt signaling pathway. This manuscript consists of 6 figures and 4 tables. The authors declare that they have no competing interests.

Your kind consideration regarding this matter is highly appreciated. Thank you.

Yours sincerely,

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# LIST OF CORRECTIONS

**Title:** Brewers’ rice induces apoptosis in azoxymethane-induced colon carcinogenesis in rats via suppression of cell proliferation and the Wnt signaling pathway

**Reviewer:** Ganapasam Sudhandiran

<table>
<thead>
<tr>
<th>No</th>
<th>Comments from the reviewer</th>
<th>Correction made</th>
<th>Page no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Injection period of azoxymethane should be specified. Once weekly for two weeks is not clear.</td>
<td>As explained in the paragraph</td>
<td>Page 5, line 106</td>
</tr>
<tr>
<td>2.</td>
<td>What is the nutritional value of Brewer’s rice? What component of this rice induces apoptosis? Are there any available reports?</td>
<td>As explained in the paragraph</td>
<td>Page 17, line 422-427</td>
</tr>
<tr>
<td>3.</td>
<td>The authors should mention how much quantity of Brewers rice they have fed the animals with (P.No.5).</td>
<td>As stated in Table 1</td>
<td>Page 27</td>
</tr>
<tr>
<td>4.</td>
<td>What did the authors mean by rTDT (P.N.8)?</td>
<td>As stated in the paragraph</td>
<td>Page 8, line 190</td>
</tr>
<tr>
<td>5.</td>
<td>What is the hypothesis of choosing 10-40% of brewer’s rice..?</td>
<td>As explained in the paragraph</td>
<td>Page 10, line 237-242</td>
</tr>
<tr>
<td>6.</td>
<td>Rectify the typographical errors (P&lt;0.05) throughout the manuscript.</td>
<td>All typographical errors were corrected</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>Grammatical errors need to be rectified. For eg: “Several studies reported by Norhaizan et al. [12] have also shown that rice germ can prevent AOM-induced colonic aberrant crypt foci (ACF) in rats” (P.No.3)</td>
<td>Grammatical mistakes were corrected</td>
<td>-</td>
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<tr>
<td>No</td>
<td>Comments from the reviewer</td>
<td>Correction made</td>
<td>Page no.</td>
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<tr>
<td>1</td>
<td>Tabulate the diet ingredients composition in control AIN-93G diet and treatments diet composition.</td>
<td>As stated in Table 1</td>
<td>Page 27</td>
</tr>
<tr>
<td>2</td>
<td>At what age of the rats carcinogen was injected?</td>
<td>As explained in the paragraph (at six weeks of age)</td>
<td>Page 5, line 106</td>
</tr>
<tr>
<td>3</td>
<td>A small figure showing the time line of experiments will help.</td>
<td>As shown in Figure 1</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Did the authors terminate the experiments after 20 weeks of treatment? When the authors started feeding the rats with brewer’s rice, before the AOM injections or after AOM injections?</td>
<td>Yes, the rats were sacrificed after 20 weeks of treatment. Two weeks after the second AOM injections, animals were fed with AIN-93G diet containing brewers’ rice.</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>At what age the rats were necropsied?</td>
<td>At 29 weeks of age, the rats were necropsied.</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>To observe adenomas and adenocarcinomas at least 40 weeks of experimental time is suggested by most of the published reports. Authors were able to see adenocarcinomas when</td>
<td>Our experimental procedures were based on a previous study by Norazalina et al. (2010), who reported that the incidence and multiplicity of total tumors in both of the adenoma and adenocarcinoma were reduced after 20 weeks administration of phytic acid. Another study by Nurul Husna et al. (2013)</td>
<td>-</td>
</tr>
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</table>
terminated after 20 weeks of treatment. That is as per the experimental design understood from the methods animals were sacrificed at ~25 weeks age. At this age after AOM injections probably, one should be able to observe ACFs and microadenomas and difficult to observe adenocarcinomas. Please clarify?

also showed that the incidence of adenoma and adenocarcinoma significant decreased after 16 weeks feeding with various concentration of inositol hexaphosphate in their drinking water (P<0.05). Most of the study need at least 40 weeks to observe adenoma and adenocarcinoma is because of the rats were given subcutaneous injections of azoxymethane (AOM) (Paul et al., 2010; Khatiwada et al., 2006; Takahashi et al., 2006; Zoran et al., 1997). However, in the present study, animals were induced with AOM intraperitoneally.

References


Takahashi M, Mutoh M, Shoji Y, Sato H, Kamanaka Y, Naka M, Maruyama T,
| 8 | Is COX-2 expression observed in normal appearing mucosa, ACFs, adenoma or adenocarcinoma, please clarify? | Yes, expression of cyclooxygenase-2 (COX-2) was observed in normal colonic mucosa, adenoma, and adenocarcinoma.

COX-2 is an inducible enzyme which catalyses the conversion of arachidonic acid into prostaglandins by up-regulated and boost the levels of inflammation-related molecules during inflammation and colorectal carcinogenesis (Kam and See, 2000; Church et al., 2003).

Up-regulation of COX-2 expression has been related to the human intestinal inflammation, colorectal cancer (Wang and Dubois, 2010), and worse survival among colorectal cancer patients (Ogino et al., 2008). In the present study, expression of COX-2 was observed in normal colonic mucosa. We speculated that this was due to the intestinal inflammation. This finding was consistent with the study reported by Kohno et al. (2005), who demonstrated the COX-2 expression in normal colon mucosa. However, high level of COX-2 expression was observed in colon carcinogen-treated rats. High COX-2 expression was associated to the formation of carcinogens, promotion of tumor, inhibition of apoptosis, and metastatic process (Meric et al., 2006).

References


Figure 4 and Figure 5, authors need to provide the better pictures showing the colon tumor histology. Most of the figures appear to be normal or near normal, with goblet cells, or else please provide the low magnification figures of colon tumors along with higher magnification in inlets for staining patterns. This will help in analyzing if the markers analyzed in the normal appearing like crypt or colon adenoma or adenocarcinoma. The figures were improved as suggested Figure 5 and Figure 6