

**Response to reviewer comments for ‘Effects of Commercially Produced Almond By-products on Chemotherapy-induced Mucositis in Rats’**

**33967**

All amendments to the above manuscript have been highlighted in the revised version using the track change or highlight function of Microsoft Word. Specific responses to reviewers’ comments are detailed below.

**Reviewer 1:**

**Comment 1**

A second aspect of the work is the use of burrowing behaviour by the rats as a monitor of animal well-being and severity of mucositis. Indeed, almost half of the discussion relates to this aspect of the work. The paper thus falls between two stools, and does not deal comprehensively with either aspect. The linkage between burrowing behaviour and animal well-being during chemotherapy was well established in Whittaker et al, 2015. It should thus just be a tool or marker in the present study.

**Response 1**

We thank the reviewer for this comment and appreciate that the major aim of this study was to determine the effects of the almond extracts tested on mucositis in an animal model. However, we believe that the successful use of burrowing behaviour in this study is important. This is the first report of this technique being successfully applied in a mucositis model (the Whittaker et al. 2015 study failed to demonstrate a statistically significant change in burrowing activity). Since we refined the method considerably from our earlier study, we believe it is important to fully explain these changes to readers so they can understand the rationale for change, and reproduce the technique.

**Comment 2**

The extracts did contain phenolics and have anti-oxidant activity but these appear to account for only a small proportion of the material present. What was the rest? Could other components have interfered with action of the phenolics and anti-oxidant activity in vivo.

**Response 2**

Almond hulls also contain up to 37% dry weight water-soluble sugars and approximately 50% non-water soluble fibre. We do not believe that these components would have interfered with anti-oxidant capability.

**Comment 3**

Maximal gut disruption and damage due to 5FU occurs at around 2-3 days in rats. Crypt and villus regeneration and repair occurs thereafter. Some bioactive factors do not prevent this early damage to the gut but alter its nature, in particular the preservation of crypt stem cell microcolonies that facilitate rapid repair. This protective property would not be seen in the

absence of longer study. However, more detailed analysis of crypt cell numbers / type and crypt organisation would be helpful in defining whether the extracts had any or no potential ameliorative effect.

### **Response 3**

This is an excellent point which we concur with. Discussion to this effect has been included in the revised manuscript in the Discussion para 3.

### **Comment 4**

Pg. 5 para 3 What was the source of the almond hulls? Were they a certified stock? Was the blanched water extract from the same ones? Pg. 5 para 4 Phenolics and anti-oxidant appear to account for a small proportion of dry Pg. 10 para 1 weight. Any other analysis or indications what else could be present?

### **Response 4**

The source of the almond hulls was an almond processing plant in the Riverland region of South Australia. This has been added to the revised manuscript. There is unfortunately no certification process for almonds. The blanched water was from another processing plant which only dealt with kernels, not hulls. A comment to this effect has been added into the manuscript. No other analyses were performed since we were mainly interested in the phenolic and anti-oxidant content of the extracts.

### **Comment 5**

Pg. 11 para 4 It would be useful to give additional detail of the individual histological Pg. 28-29 parameters that were evaluated, rather than just the global index.

### **Response 5**

We agree that these data may be useful to the reader and have included a table (Table 4) with individual scores for each parameter.

### **Reviewer: 2**

#### **Comment 1**

Introduction – “The severe nature of the symptoms often leads to a patient-requested reduction of chemotherapy dose” – The word “often” is not adequate; I suggest “can lead”. In addition, it is important to add the information that intestinal mucositis can increase the frequency of peripheral parenteral nutrition prescription, which can predispose to high morbidity.

#### **Response 1**

The requested changes have been made with the word ‘often’ changed to ‘can lead’ and the additional sentence added in the introduction.

#### **Comment 2**

Results - The histological sections were not explored sufficiently. For example, the authors quantified the myeloperoxidase activity, which signalizes, among other effects, neutrophils activation. What are the characteristics of the inflammatory infiltrate observed in the histological sections? What are the differences and similarities between the groups? A detailed description of the histological finding is necessary, showing the scores for each tissue element individually. This is very important considering the discrepancies about the effect of 5-FU on intestinal mucosa (as the authors commented in the Discussion) and the contributions that the current study can offer to the literature in this area.

### **Response 2**

A table (Table 4) has been added with the scores for all individual histological parameters. There were no differences between treatment groups for any of the scored parameters. The 'Histological Severity Score' section now reads: 'Presence of mucositis with or without almond extracts led to increases in all individual severity scoring parameters (Table 4). Scores for these parameters were not significantly different between almond extract-treated and 5-FU control groups.'

Accordingly, there was no significant difference in the inflammatory infiltrate (lymphocyte and PMN) score between groups. This largely mirrors the MPO assay results where significant differences were not seen except in the groups receiving almond hull extract 1. However, the histology scoring method is semi-quantitative and did not involve individual identification or counting of specific populations of cells. It is therefore difficult to draw accurate comparisons between this method and the MPO analysis.

### **Comment 3**

Caption – Figure 3 – C – I believe that it is “5FU/PBS ileum” not “jejunum”. The resolution of the figures is not adequate, needing more sharpness. I suggest the replacement of the figures and the insertion of representative sections (two images) for almond groups.

### **Response 3**

We thank the reviewer for alerting us to this error in the caption to Figure 3. This has now been corrected to read 'ileum'. Figures have been replaced and two figures representing almond extract-treated groups have been included. The authors can provide the pictures as jpg files should the production team prefer. This may improve resolution in comparison to pictures embedded in a word document.

### **Comment 4**

Discussion –a commentary about the limitations of the study with regard to the effect of gavage on animals, mainly in relation to the low absorption episodes and alterations on the immune response that can be installed after repeated and chronic usage of this technique in rodents. Please insert the opinion of the authors about the influence of side effects of gavage on the results, mainly regarding the intestinal absorption and local effect of almond extracts.

### **Response 4**

We thank the reviewer for this suggestion. A comment on the effects of gavage on the stress response and bioavailability has been included in the revised manuscript in the final paragraph of the Discussion. It is our opinion that the stress-induced effects of gavage were minimal in the study. We have formed this opinion due to the researchers' considerable experience in the technique, and evidence from the literature on gavage in rats. However, we concede that the gavage method may have led to reduced bioavailability of the extracts in comparison to a dietary exposure route.