



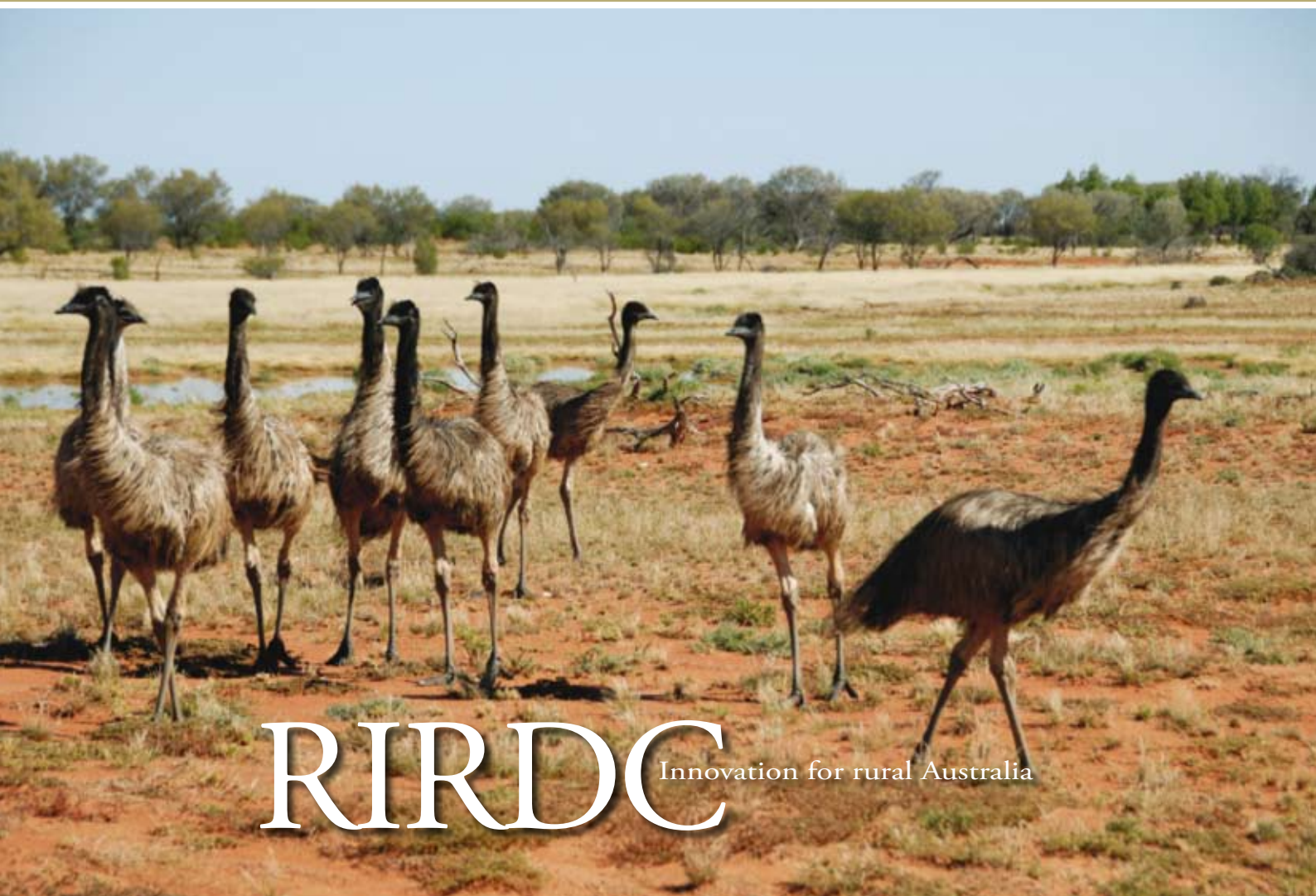
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# Effects of Bio-active Emu Oil on Chemotherapy-induced Mucositis

— *Emu Oil and Gastrointestinal Disease* —

RIRDC Publication No. 09/131



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**Rural Industries Research and  
Development Corporation**

# **Effects of Bio-active Emu Oil on Chemotherapy-induced Mucositis**

**Emu Oil and Gastrointestinal Disease**

by A/Prof Gordon S Howarth and A/Prof Ross N Butler

August 2009

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# Foreword

Intestinal mucositis is a serious disorder that results from chemotherapy for cancer whilst inflammatory bowel disease (IBD) is an incurable condition with uncontrolled bowel inflammation. The current study, utilising a Bio-active Emu Oil prepared by a novel rendering and filtration process, identifies Emu Oil as a product with the capacity to decrease the severity of intestinal injury from these conditions. This Emu Oil has also demonstrated the ability to improve growth of the damaged intestine, extending to effects in the inflamed colon (large intestine). These findings suggest a new mechanism of action for Emu Oil, expanding the spectrum of bowel disorders for which Emu Oil may have therapeutic application.

The Australian Emu Industry should benefit greatly from this research since there exists the opportunity to value-add significantly to the Emu Oil market. These findings could extend to international markets in which Emu Oil could be indicated as a simple dietary supplement for cancer patients undergoing chemotherapy and/or radiotherapy. The international market for cancer sufferers undergoing treatment is substantial.

The current study provides encouraging information to support expansion of the Australian Emu Industry in order to expand applications for Emu Oil. The new market for Emu Oil could include oncology patients and sufferers of inflammatory bowel disease.

With support from Technology Investment Corporation Pty Ltd (TIC) and Emu Tracks Australasia Pty Ltd (Emu Tracks), this project was funded from RIRDC Core Funds provided by the Australian Government.

This report, an addition to RIRDC's diverse range of over 1900 research publications, forms part of our New Animal Products R&D program, which aims to accelerate the development of viable new animal industries.

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**Peter O'Brien**  
Managing Director  
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# About the Author

A/Prof Gordon Howarth and A/Prof Ross Butler have amassed more than 20 years experience in the utilization of animal models of gastrointestinal disease for the therapeutic development of naturally sourced bioactives. These investigators are thus well-positioned to conduct efficacy investigations on animal sourced bioactives such as Bio-active Emu Oil, the subject of the current report.

# Acknowledgments

The investigators wish to thank Kerry Lymn, Ruth Lindsay and Suzanne Mashtoub who were instrumental in conducting the studies described in this report.

# Abbreviations

IBD	Inflammatory bowel disease
DA	Dark Agouti rat
MPO	Myeloperoxidase
SBT	Sucrose Breath Test

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# Executive Summary

## ***What the report is about***

At present there are no truly effective treatment strategies for two serious and potentially fatal, disorders of the digestive system known as *mucositis* and *inflammatory bowel disease (IBD)*. Mucositis affects the small bowel primarily, and occurs in almost all cancer patients undergoing high-dose chemotherapy. IBD is a chronic inflammatory disorder with no known cause which primarily affects the large intestine. New treatment strategies for these disorders are urgently required. Utilizing proven animal model systems, the current project investigated Emu Oil for its potential to treat or prevent mucositis.

## ***Who the report is targeted at***

The study confirmed many of the anecdotal claims of Emu Oil efficacy, specifically identifying Emu Oil as possessing therapeutic application to decrease intestinal injury in cancer patients undergoing chemotherapy. An aspect of the study suggests that Emu Oil holds promise as an adjunct to conventional therapy for the alleviation of large bowel inflammation, such as is evident in IBD. As such, this report has implications for cancer patients undergoing chemotherapy and also individuals afflicted by ulcerative colitis, one of the forms of IBD.

## ***Background***

Emu Oil is derived from the subcutaneous and retro-peritoneal fat of the Emu, a flightless bird (Ratite), native to Australia. Emu Oil has been used for therapeutic purposes by indigenous Australian peoples for hundreds of years, for its purported anti-inflammatory and wound-healing properties. Currently, Emu Oil has been commercialised, primarily for cosmetic and therapeutic related purposes, on the basis of this substantial anecdotal information. However, to date, Emu Oil has not been subjected to rigorous scientific investigations to identify its capacity to treat or prevent disorders of the digestive system.

## ***Aims and objectives***

To investigate the effects of orally-administered Emu Oil on:

1. Severity of mucositis
2. Level of intestinal inflammation
3. Re-growth of the damaged small intestine

### ***Who will benefit from the research***

This research greatly expands the medical applications of Emu Oil for disorders of the small and large bowel, value-adding to the existing Australian Emu Industry, particularly as this relates to Emu Oil and its applications for intestinal diseases and disorders, not necessarily restricted to the two disorders as described.

### ***Methods used***

The primary aims of the study were to investigate Emu Oil for its potential to alleviate intestinal injury in rat models of Chemotherapy-induced mucositis. Emu Oil was orally administered to rats with intestinal mucositis induced by injection of the common chemotherapy drug, 5-Fluorouracil (5-FU).

### ***Results and key findings***

The study revealed that Emu Oil had a profound beneficial effect on myeloperoxidase activity, an indicator of acute intestinal inflammation. The results indicated that the anti-inflammatory effects of Emu Oil were evident in all regions of the intestine. This important finding was accompanied by a surprising increase in the depth of the intestinal crypts following Emu Oil treatment, accompanied by a lesser effect on lengthening of the absorptive villi. Together this effect indicated an improved growth of the intestine. The effect was most impressive in the distal small intestine (ileum) and was still evident 96 hours after chemotherapy (the last time-point investigated). The mechanism of the crypt lengthening needs to be defined but could represent increased proliferation (and hence improved repair), decreased apoptosis (decreased cell death) or even hypertrophy (increased cell size). Further studies are indicated to define this mechanism. To conclude, Emu Oil decreased acute inflammation in the damaged intestine and improved intestinal re-growth after damage. Further studies could explore the effects of Emu Oil batches and dosing regimens, to ensure that normal bowel architecture is restored following Emu Oil ingestion. In summary, the current study recommends that Emu Oil be further pursued as a dietary supplement to augment conventional treatment approaches for inflammatory disorders of the digestive tract. Further studies, for example in models of NSAID-enteropathy and gastric ulceration, are suggested to expand biomedical applications for Emu Oil and determinations of batch effects should also be considered.

### ***Implications for relevant stakeholders***

The Australian Emu Industry will benefit substantially from this research as it provides scientific credibility for the use of emu oil as a therapy for gastro-intestinal conditions. In the past, anecdotal findings in relation to emu oil have lacked this validation. It will present the Emu Industry with the opportunity to market emu oil as a safe and natural treatment for conditions where there is currently little or no suitable medication. Furthermore, it will provide practitioners and sufferers, in particular cancer patients, with a natural treatment that is supported by sound, scientific evidence.

Given the current lack of suitable therapies for gastro-intestinal conditions and the scope of the market, this research will have a direct and positive impact on the growth of the Industry. The industry has already developed a marketable emu oil capsule so there is a readymade product for this market.

With consideration to the very positive outcomes from this current research, it will provide the Emu Industry and potential funding partners with the confidence to pursue further opportunities and applications for bio-active emu oil.

## **Recommendations**

The following issues are suggested as important steps in the optimisation and further expansion for Bio-active Emu Oil and its applications for diseases and disorders of the gastrointestinal tract:

1. Compare different batches of Emu Oil for efficacy against 5-FU induced mucositis
2. Investigate Bio-active Emu Oil for potential efficacy against other classes of chemotherapy drugs
3. On the basis of the distal small bowel effects of Emu Oil – investigate Emu Oil for its potential to treat disorders affecting this bowel region:
  - a. NSAID-enteropathy eg. non-steroidal anti-inflammatory drug induced ulceration
  - b. Crohn's disease
4. Investigate different doses of Bio-active Emu Oil and administration regimens
5. Consider investigations of Emu Oil on disorders of the stomach:
  - a. *Helicobacter pylori* gastric ulceration and gastritis
  - b. NSAID-induced ulceration (eg aspirin, indomethacin)

# 1. Introduction

Mucositis is a debilitating condition which occurs as a side effect of chemotherapy and/or radiation therapy which can drastically reduce the quality of life for cancer patients undergoing treatment <sup>1</sup>. It is one of the most important factors in morbidity and mortality of patients undergoing treatment for cancer <sup>2</sup>, as severe mucositis can lead to malnutrition and systemic infection. Currently, there are no effective treatments for gastrointestinal mucositis <sup>3,4</sup>. There is much anecdotal evidence to suggest that Emu Oil possesses some anti-inflammatory properties, however, many of these claims are yet to be scientifically tested. Recent research has focussed on the effect of the Oil on arthritic and dermal inflammation in animal models <sup>5-8</sup>. The topical application of Emu Oil to animals has been shown to reduce levels of tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), and other pro-inflammatory cytokines known to be involved in the development of mucositis, as well as swelling, another parameter of inflammation <sup>5-8</sup>.

## Emu Oil

The Emu (*Dromaius novae hollandiae*) is a large flightless bird native to Australia. It is both farmed and hunted for its meat, and more recently, its oil <sup>6</sup>. Emu Oil is extracted from both the subcutaneous and retroperitoneal fat by first rendering the macerated tissue, and then passing the liquefied fat through a series of filters to extract the oil (RIRDC Pub No 08/010). Some manufacturers also use centrifugation to separate the Oil from other extraneous components of the adipose tissue. Emu Oil is a product that is yet to be extensively tested with respect to its purported anti-inflammatory properties, with only a handful of scientific studies conducted. The majority of those studies have investigated the influence of topically applied Emu Oil on inflammatory conditions such as arthritis <sup>6</sup>. Despite the lack of scientific proof, there is much anecdotal evidence to suggest that Emu Oil has some therapeutic qualities when applied to inflammation <sup>5</sup>. It is a treatment that was first used by indigenous Australians, and then later by early European settlers to provide relief from pain and assist in wound healing <sup>6</sup>. It has also been claimed that Emu Oil has some analgesic properties <sup>6</sup>; however, there have been no rigorous laboratory studies to evaluate this claim. Emu Oil possesses excellent skin-permeation properties, as well as its own anti-inflammatory properties, and in this, it is relatively unique <sup>6</sup>. It is this feature that may make it particularly useful in a wide range of applications, in particular, trans-dermal delivery of other medications. Other advantages of Emu Oil, as defined by Whitehouse et al. (1998) are that it requires minimal refining, and presents a low health hazard, being readily metabolisable. Its source is also renewable and relatively in-expensive <sup>6</sup>.

## 2. Methodology

*Animal Studies:* Female Dark Agouti rats (n=80; 110-150g) were housed in individual metabolism cages, in a climate and light controlled room. All animals were given *ad libitum* access to water and food [18% casein diet; <sup>41</sup>]. Rats were randomly allocated to one of 10 groups (n=8); 5-Fluorouracil [(5-FU) 500mg in 10ml, supplied by Mayne Pharma Pty Ltd, Victoria, Australia] and water; 48, 72 and 96 hours; 5-FU and 0.5ml Emu Oil; 48, 72 and 96 hours; and 5-FU and 1ml Emu Oil; 48, 72 and 96 hours. A negative control group was gavaged with water, and received a saline injection instead of 5-FU. This experimental design was used to enable assessment of the likely mechanism of Emu Oil, where different time points could be used to determine the stage of the condition at which Emu Oil was having the most pronounced effect. A Bio-active Emu Oil was prepared utilising specific methodologies developed for Technology Investment Corporation by Emu Tracks (Marleston, Adelaide, South Australia). Briefly, these processes involved a novel method of rendering and filtration of Emu adipose tissue, with appropriate considerations for delivery of quality assurance and product consistency. All animal experiments adhered to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, and were approved by the Animal Ethics Committees of the University of Adelaide and the Children, Youth and Women's Health Service. Emu Oil or water was administered daily via oro-gastric gavage between days 1 and 8 of the experimental period. At day 5, all rats were intraperitoneally injected with a single dose of either saline (control group) or 5-FU (150mg/kg). Body weight, feed/water intake and faecal/urine output were monitored and recorded on a daily basis. Rats were sacrificed 48 hours, 72 hours or 96 hours post 5-FU injection, by CO<sub>2</sub> asphyxiation followed by cervical dislocation.

*<sup>13</sup>C-Sucrose Breath Test:* The <sup>13</sup>C-sucrose breath test (<sup>13</sup>C-SBT) was performed as a non-invasive assessment of small intestinal brush border disaccharidase activity <sup>37,42</sup>. The <sup>13</sup>C-SBT was performed prior to commencement of the trial (-120 hours), prior to 5-FU injection (0 hours), and prior to sacrifice (48, 72, 96 hours post-injection). After an overnight fast, rats were gavaged with 1ml of 0.25g/ml sucrose solution, naturally labeled with <sup>13</sup>C. They were then placed in sealed plastic containers for 2 minutes, before a 10ml sample of breath was collected into an evacuated tube. Breath samples were collected at 0, 15, 30, 45, 60, 75, 90, 105 and 120 minutes after administration of the sucrose solution. Samples were analysed for <sup>13</sup>C by isotope ratio mass spectrometer, as described by Pelton (2004).

*Tissue Collection:* After sacrifice, the gastrointestinal tract of each animal was removed, measured, emptied of contents and weighed. Segments (2cm), were removed from the duodenum, jejunum, jejunum-ileum junction, ileum and colon, and placed in 10% buffered formalin for histological analyses. In addition, segments (4cm) directly adjacent to the corresponding histological samples were collected and snap-frozen in liquid nitrogen for biochemical analyses. The stomach, caecum, heart, thymus, spleen, lungs, kidneys and liver were weighed and discarded.

*Biochemical Analysis:* All samples were stored at -80°C, until required for the biochemical assays. All tissue was prepared for analysis by homogenization in 10mM phosphate buffer. Small intestinal sucrase activity (duodenum, jejunum and jejunum-ileum junction) was measured using a modification of the methods described by Dahlqvist (1968). Sucrase is an enzyme in the brush border of the small intestine, which is secreted by healthy, mature epithelial cells. Any damage which occurs to the brush border, such as that resulting from mucositis, (i.e. ulceration, and/or shortening and blunting of the villus) will impact on sucrase production; therefore, sucrase activity was used as an indirect indicator of intestinal integrity and maturity. Briefly, homogenized tissue was diluted (1/100, 1/80 for saline treated tissue, 1/50, 1/30 for 5-FU treated tissue), and 50µl of each dilution was added to a 96 well plate. 0.2mM sucrose was added to each well, and incubated for 30 minutes. Tris-glucose oxidase was then added to each well, before further incubation. Glucose production was measured colorimetrically by measuring the optical density of each well at 490nm. Myeloperoxidase (MPO) levels in the small intestine were determined as an indicator of inflammation, using the techniques described by Howarth et al., (2006). Samples were

centrifuged at 13000g for 10 minutes, after which the supernatant was discarded, and the tissue homogenate re-suspended in 0.5% hexadecyltrimethyl ammonium bromide (HTAB). After vortexing for 2 minutes, samples were re-centrifuged, at 5000g for 2 minutes. The supernatants were then aliquoted into 96 well plates. After the addition of an o-dianasidine reagent, the change in absorbance was measured at 450nm.

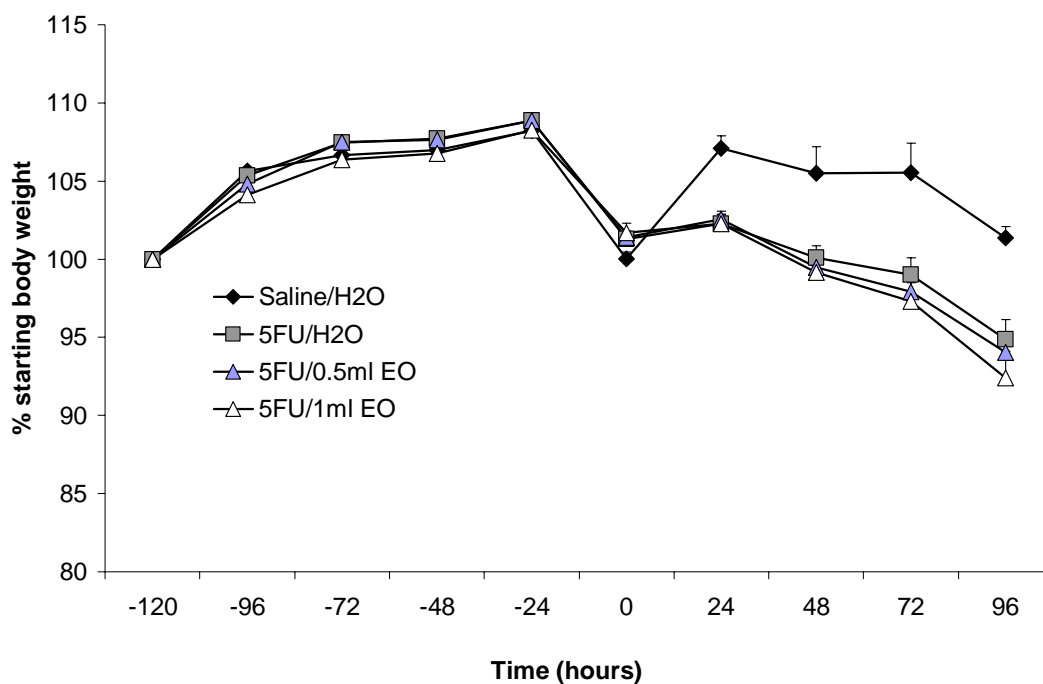
*Histology:* Samples of small intestine were transferred from 10% buffered formalin into 70% ethanol 24 hours after collection. Specimens were then routinely processed and embedded in paraffin wax, 4µm sections prepared and stained with haemotoxylin and eosin. Small intestinal crypt depth and villus height were measured in the jejunum, jejunum-ileum junction and ileum (40 villi and 40 crypts per section)<sup>43</sup>. Overall histological damage severity of intestinal sections was also assessed and scored semi-quantitatively according to parameters described by Howarth et al., (1996). The 8 parameters assessed were villus/crypt ratio, enterocyte disruption, reduction in goblet cell numbers, crypt disruption, crypt cell disruption, lymphocytic and polymorphonucleocyte infiltration, thickening/oedema of the submucosa and thickening of the muscularis externa<sup>43</sup>. All analyses were performed in a blinded fashion, using a light microscope (Olympus BH-2, Tokyo, Japan) and digital camera (Sony, Tokyo, Japan) and Image Pro-Plus Software Package Version 4.5.1.2.7 (Media Cybernetics, Silver Spring MD, USA)

### 3. Statistical analysis

Statistical analysis was conducted using SPSS 15.0.1 for Windows (SPSS Inc. Chicago, Illinois, USA). Daily metabolic data were analysed using a repeated measures ANOVA with a Holme's *post hoc* test ( $p < 0.05$  significance) to compare the differences both between groups and within groups across the duration of the trial. Data from the SBT, sucrase and myeloperoxidase assays, and histology were expressed as mean $\pm$ SEM and analyzed using a one-way ANOVA, with a Tukey's *post-hoc* test where  $p < 0.05$  was considered significant.

## 4. Results

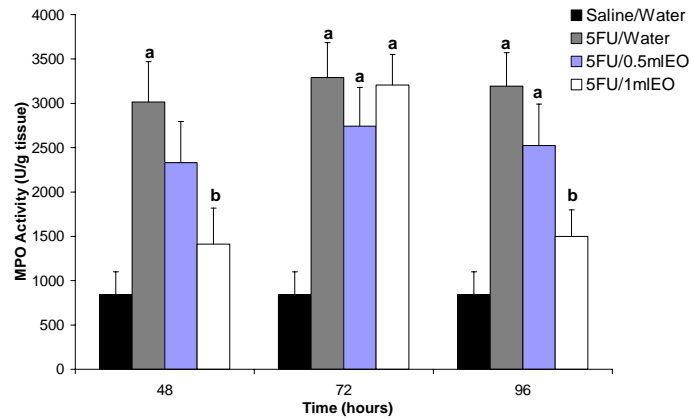
*Metabolic Data:* Prior to administration of 5-FU/saline, a constant increase in weight occurred, with no significant differences between rats receiving Emu Oil, and those receiving water. Following 5-FU/saline administration however, all 5-FU treated rats, including those treated with Emu Oil experienced significant weight loss, compared to saline treated controls. This decrease in body weight continued over the remainder of the trial, with no improvement apparent in any of the 5-FU treated groups (Figure 1).



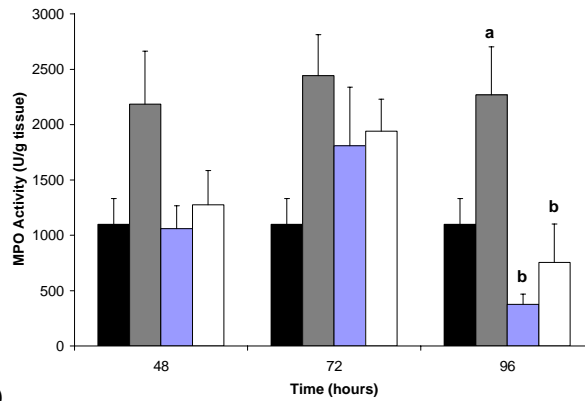
**Figure 1.** Bodyweight, from -120 hours to 96 hours. Data are expressed as (%starting BWT) mean  $\pm$ SEM.

*Myeloperoxidase Assay:* 5-FU induced a significant increase (275%) in MPO activity in the jejunum relative to saline control. 1ml Emu Oil significantly decreased MPO activity, compared to 5-FU control, at 48 hours and 96 hours. The lower dose exerted less of an effect, with no statistically significant difference between the 0.5ml Emu Oil and either saline, or 5-FU controls at 48 and 96 hours. At 72 hours, 5-FU significantly elevated levels of MPO, relative to saline. Emu Oil, at both doses had no effect (Figure 2a). In the mid intestine (JI), 5-FU did not induce a significant elevation in MPO relative to normal animals at 48 and 72 hours. Neither dose exhibited significantly different MPO levels relative to saline or 5-FU controls. At 96 hours, both Emu Oil doses significantly lowered levels of MPO compared to the 5-FU control (Figure 2b). In the ileum, no significant reduction in MPO levels with either of the Emu Oil doses occurred in the early 48 and 72 hour time points, relative to the 5-FU control, however, at 96 hours, MPO activity was decreased to levels comparable to that of the saline controls (Figure 2c).

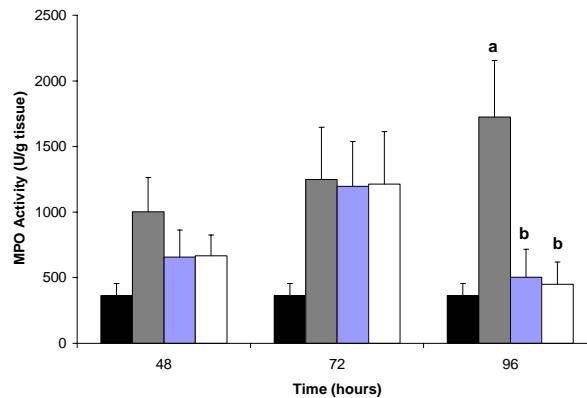
(a)



(b)



(c)

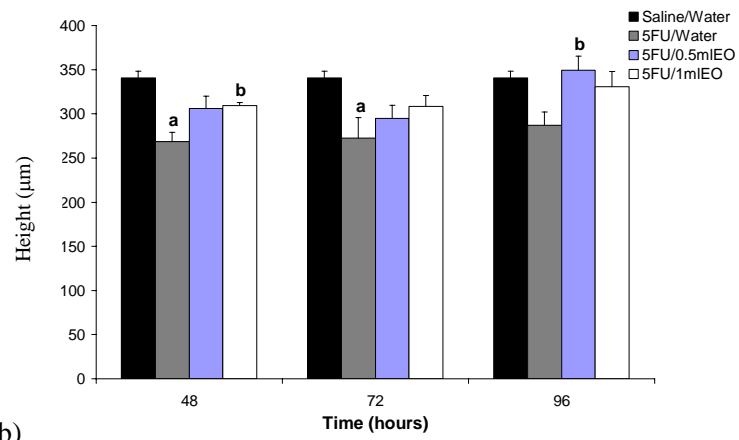


**Figure 2a-c.** Myeloperoxidase (MPO) activity in the jejunum (a), mid small intestine (b) and ileum (c) at 48, 72 and 96 hours. Data expressed as (MPO units gram tissue<sup>-1</sup>) mean±SEM. <sup>a</sup> indicates p<0.05 compared to saline, <sup>b</sup> indicates p<0.05 compared to 5-FU+water.

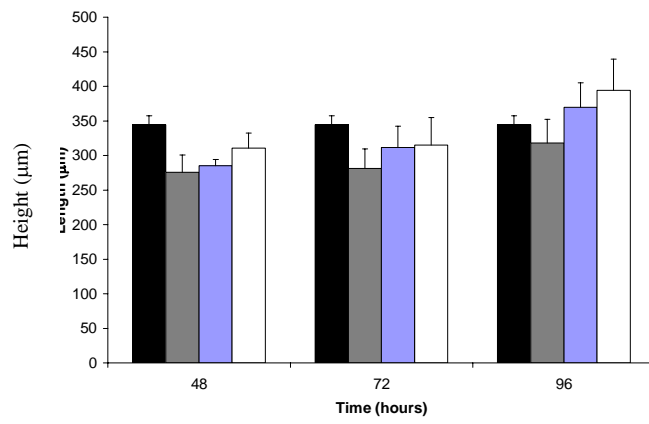
*Small Intestinal Histology:* Small intestinal sections, stained with haematoxylin and eosin, were examined for 5-FU induced damage, which manifests as shortened villi and crypts. In the recovery phase, it is usual for crypt depth to increase above normal, as cell proliferation is stimulated to repair damage sustained from administration of 5-FU. In the current study, Emu Oil slightly improved villus height in the proximal intestine 48 hours post 5-FU administration (Figure 3a), however, this improvement was not maintained in the more distal regions of the intestine, in the JI (Figure 3b) and ileum (Figure 3c). At 72 hours post 5-FU administration, when disease severity

had peaked, no effect was apparent with either dose of Emu Oil in any section of the intestine, relative to both controls. In the early stages of the induced mucositis, there were no significant differences in crypt depth, between treatments, with the exception of the jejunum, at 48 hours, where 5-FU significantly shortened crypts, relative to the saline control (Figure 4a). The greatest differences were at 96 hours, where, in the jejunum and ileum, the crypts in all 5-FU treated groups were significantly longer compared to the saline controls (Figures 4a and 4c). Further to those results, Emu Oil, at both doses, showed a non-statistically significant trend towards longer crypts. In the II, only the higher dose of Emu Oil was able to significantly lengthen crypts, relative to the saline control (Figure 4c).

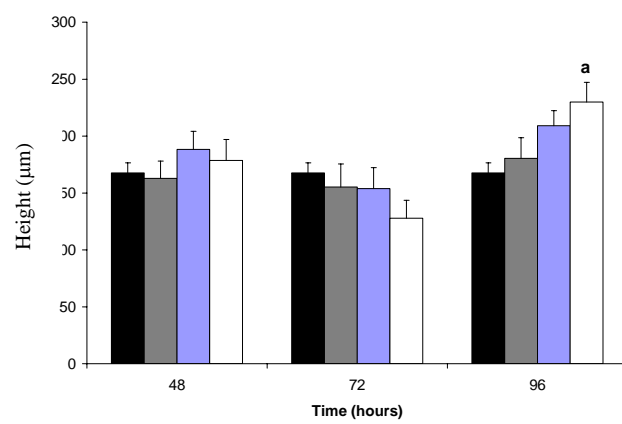
(a)

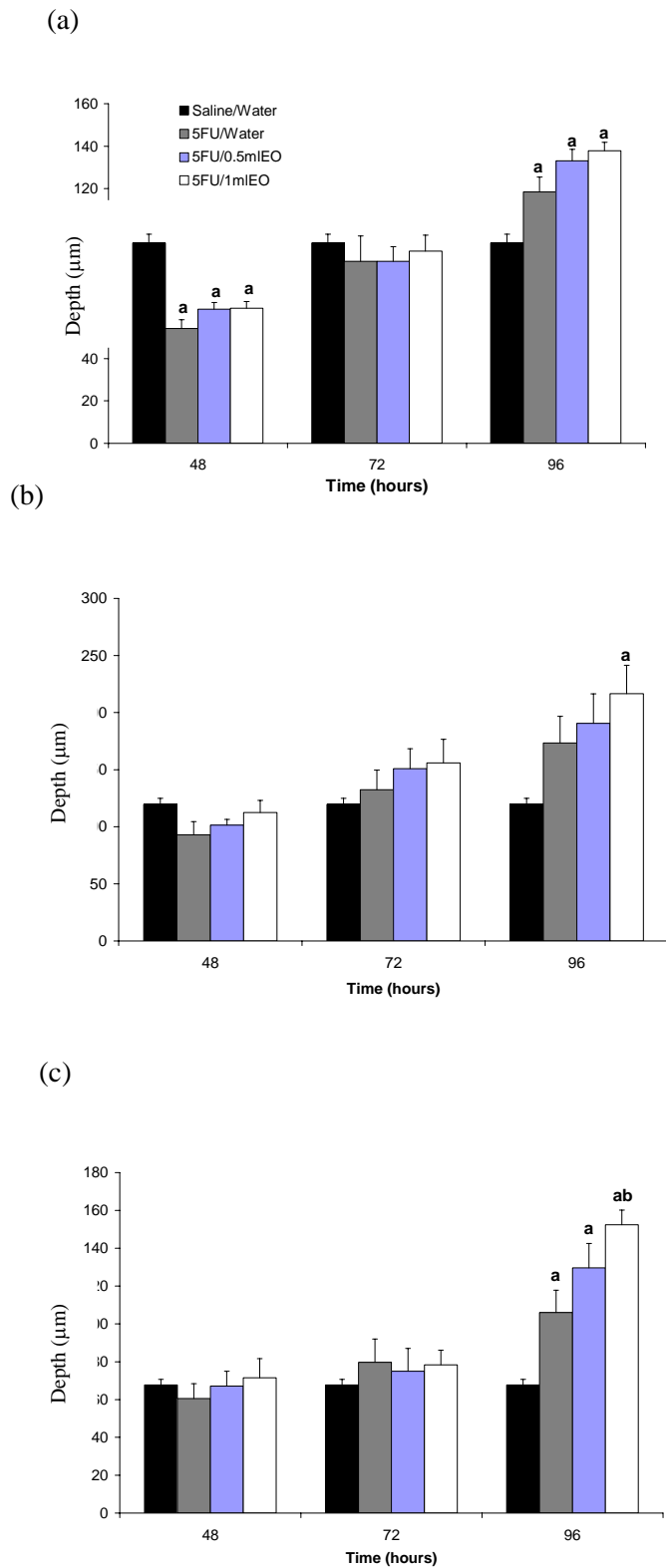


(b)



(c)





**Figures 3a-c and 4a-c.** Villus height (**Figure 3**) and Crypt depth (**Figure 4**) in the jejunum (a), JI (b) and ileum (c) at 48, 72 and 96 hours. Data expressed as ( $\mu\text{m}$ ) mean $\pm$ SEM. <sup>a</sup> indicates  $p < 0.05$  compared to saline. <sup>b</sup> indicates  $p < 0.05$  compared to 5-FU+water

## 5. Discussion of Method and Results

Mucositis is a serious side-effect of cytotoxic therapy, characterised by severe inflammation of the alimentary tract. Despite significant research, no effective treatments currently exist. In previous animal studies, Emu Oil has been shown to exert a beneficial effect in some inflammatory conditions<sup>7, 8, 44</sup>. It was postulated in the current study that Emu Oil would have a similar anti-inflammatory effect in gastrointestinal mucositis, and thereby decrease the severity of damage to the intestinal mucosa. In this study, Emu Oil, administered at two doses, consistently improved specific parameters associated with 5-FU induced damage in the recovery stage of the condition (96 hours). These parameters included a significant decrease in acute inflammation, an increase in small intestinal (SI) weight, and improvements in crypt depth and villus height. The current study represents the first report of decreased intestinal inflammation following the oral application of Emu Oil. The decrease in acute inflammation evident in this study was supported by findings from previous studies, in which Emu Oil significantly reduced inflammation in non-gut systems. A number of hypotheses relate to the potential mechanism of Emu Oil action, and the means by which it exerts its anti-inflammatory effects. It has been suggested that the n-3 and n-9 FAs present in Emu Oil may be involved<sup>7</sup>. Yoganathan et al. (2003) proposed that the anti-inflammatory properties of Emu Oil are not fully explained by the FA profile. Other components of Emu Oil, such as tocopherols, are known to exert their own anti-inflammatory<sup>45</sup> and anti-oxidant<sup>46</sup> effects. This combination could partially explain the anti-inflammatory effect exerted by Emu Oil in the current study. These anti-oxidants may also have impacted on levels of damaging reactive oxygen species which are produced during the development of mucositis<sup>1</sup>.

In the current study, Emu Oil maintained villus structure along the length of the small intestine, and at 96 hours, crypt depth was significantly increased in the ileum. Results indicated that Emu Oil had promoted mucosal growth in the recovery phase of mucositis. This may have been due to a number of effects, including increased cell proliferation, a decrease in apoptosis, cell hypertrophy, or a combination of these factors. The role of short chain fatty acids, in particular, butyrate, in cell proliferation and promotion of mucosal growth in the distal small intestine and colon is well documented<sup>47, 48</sup>, and in combination with glutamine and transforming growth factor-beta, has been shown to decrease mucosal injury due to methotrexate injury<sup>49</sup>. Despite this, there is no evidence to suggest that the long chain fatty acid component of Emu Oil would have a similar effect. However, this does not eliminate the possibility that one of the as yet unidentified constituents of Emu Oil may have exerted some effect, and this is a direction that should be pursued in future studies.

Several studies investigating the effect of other agents on the severity of mucositis have examined the crypt depth response. The improved crypt depth in the ileum at 96 hours shown in the current study exceeded any results for this parameter reported in several previous studies investigating the treatment of intestinal mucositis. These included Lyprinol<sup>40</sup>, insulin-like growth factor-1<sup>36</sup>, whey growth factor extract<sup>50</sup>, pentoxifylline and thalidomide<sup>21</sup> and keratinocyte growth factor (KGF-1)<sup>51</sup>. Indeed, KGF-1, when administered as a prophylactic treatment, has been shown to improve crypt length and survival, and mucosal thickness, prior to administration of chemotherapy<sup>51, 52</sup>, although KGF-1 was unable to improve crypt depth in the mucositis model, to the same degree as Emu Oil.

Previous studies have shown that the intestine has the ability to grow and adapt if one section is severely damaged or resected. This has been demonstrated in short bowel syndrome in which compensatory alterations include increased crypt depth and villus height as well as enterocyte proliferation<sup>53</sup>. In the current study, compensatory hyperplasia may have been occurring in the ileum, as 5-FU preferentially damages the upper small intestine. Haxhija (2007) reported that the adaptive response in the small intestine varied depending on the area of damage. Damage occurring predominantly in the proximal small intestine resulted in cells in the ileum becoming hypertrophic, however, in response to ileal damage, jejunal cells became hyperplastic<sup>54</sup>. This

hypertrophic response may serve to explain the effects on crypt depth observed in the ileum, in the current study.

On the basis of the SBT and sucrase assay results in the current investigation, there was no demonstrable protection of the intestinal lining by Emu Oil, although this effect had been described in a previous study in which the effect of another lipid based, animal extract (Lyprinol) was examined in the 5-FU mucositis model<sup>40</sup>. The Lyprinol study also demonstrated no improvement in sucrase activity, despite improvement in some other parameters<sup>40</sup>. The results from the histological analysis in the current study indicated that villus structure was maintained by Emu Oil. The greater crypt depth in the ileum at 96 hours evident following administration of 1ml Emu Oil may have been due to a rapid increase in cell proliferation in the crypts, in order to repair damage to the intestine. However, these cells may not have had sufficient time to mature and begin actively synthesising sucrase prior to migration up the villus. An increase in enterocyte proliferation may have been a mechanism for Emu Oil action in this study. Further analysis including the proliferating cell nuclear antigen technique (PCNA) would allow a more precise investigation into the likely mode of action. Future studies would benefit from the inclusion of other oils, such as olive oil and fish oil, to allow direct comparisons between the agents. In addition, identification of the bioactive factors would allow more targeted application of a potentially more potent agent. Examination of different doses, inter batch variation and the effect of Emu Oil on the healthy gastrointestinal tract also warrant further investigation.

In conclusion, in the current study, Emu Oil partially ameliorated intestinal mucositis and promoted recovery of the intestinal mucosa following 5-FU induced injury in the rat. Emu Oil has the potential to increase the rate of recovery following chemotherapy-induced damage and act as an adjunctive therapy in mucositis. Potentially, Emu Oil could be used in conjunction with other agents, such as KGF-1, which has previously demonstrated a protective effect. These results represent a new direction for research into treatments for mucositis, and provide hope for thousands of individuals suffering from the debilitating symptoms of mucositis, in the ongoing fight against cancer.

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# Effects of Bio-active Emu Oil on Chemotherapy-induced Mucositis

## — *Emu Oil and Gastrointestinal Disease* —

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By A/Prof Gordon S Howarth and A/Prof Ross N Butler

Intestinal mucositis is a serious disorder that results from chemotherapy for cancer whilst inflammatory bowel disease is an incurable condition with uncontrolled bowel inflammation. The current study, utilising a Bio-active Emu Oil prepared by a novel rendering and filtration process, identifies Emu Oil as a product with the capacity to decrease the severity of intestinal injury from these conditions. This Emu Oil has also demonstrated the ability to improve growth of the damaged intestine, extending to effects in the inflamed colon (large intestine).

These findings suggest a new mechanism of action for Emu Oil, expanding the spectrum of bowel disorders for which Emu Oil may have therapeutic application.

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