Emu Oil combined with Lyprinol™ reduces small intestinal damage in a rat model of chemotherapy-induced mucositis

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**Running Title:** Emu Oil and Lyprinol in a rat model of mucositis

**Keywords:** Dark Agouti rat, anti-metabolite, 5-Fluorouracil, experimental, anti-inflammatory agents, oils
ABSTRACT

Chemotherapy-induced mucositis is characterized by inflammation and ulcerating lesions lining the alimentary tract. Emu Oil and Lyprinol™ have independently demonstrated their therapeutic potential in intestinal inflammatory disorders, including mucositis. We investigated Emu Oil and Lyprinol™ in combination for their further potential to alleviate chemotherapy-induced mucositis in rats. Rats were gavaged with (1 mL) water, Olive Oil, Emu Oil+Olive Oil, Lyprinol™+Olive Oil or Emu Oil+Lyprinol™ from days 0-7, injected with saline (control) or 5-Fluorouracil (5-FU) on day 5 and euthanized on day 8. Myeloperoxidase (MPO) activity (indicative of acute inflammation), histological severity scores and intestinal architecture were quantified. Myeloperoxidase activity was significantly increased in the jejunum and ileum following 5-FU, compared to saline controls. Both Olive Oil and Emu Oil+Lyprinol™ significantly reduced jejunal MPO levels (1.8-fold and 1.7-fold, respectively); whereas only Emu Oil+Lyprinol™ significantly decreased ileal MPO levels, relative to 5-FU controls. All oil treatments decreased histological severity scores in the jejunum and ileum, and normalised crypt depth in the mid small intestine, relative to 5-FU controls. Emu Oil combined with Lyprinol™ partially reduced acute small intestinal inflammation. Isolating bioactive constituents of these naturally-sourced oils could provide a more targeted strategy to protect against intestinal mucositis.
INTRODUCTION

Chemotherapeutic drugs, such as the anti-metabolite 5-Fluorouracil (5-FU) are broadly effective at treating solid tumours and haematological malignancies\(^1\). However, these cytotoxic agents indiscriminately target both malignant and normal dividing cells of the gastrointestinal tract; often resulting in the serious condition known as mucositis\(^2\). Mucositis is characterised by ulceration and inflammation of the mucosa with clinical symptoms including severe pain, rectal bleeding, bloating, nausea, vomiting and diarrhoea\(^3,4\). In severe cases, mucositis may also limit the chemotherapeutic dose and delay scheduled treatment, thereby compromising therapeutic efficacy and hindering patient survival\(^5,6\).

The pathogenesis of mucositis involves a series of interactive biological processes which occur within the intestinal epithelium and submucosa\(^7-9\). 5-FU disrupts DNA synthesis by acting as a fluorinated pyrimidine to competitively inhibit thymidylate synthase\(^10,11\), subsequently inducing intestinal stem cell apoptosis. Furthermore, reactive oxygen species and pro-inflammatory cytokines, including interleukin-1\(\beta\), interleukin-2, interferon-\(\gamma\) and tumour necrosis factor-\(\alpha\), are indirectly generated which further amplify intestinal damage\(^7,12\). An increased understanding of the underlying pathobiology of mucositis has led to the identification of potential treatment approaches including keratinocyte growth factor-1\(^13\), grape seed extract\(^14\), prebiotics and probiotics\(^15\), and animal and marine derived oils\(^16-18\). Although these treatments have demonstrated therapeutic potential, they remain under experimental and/or clinical investigation.

Recent studies have indicated that marine oils such as Lyprinol\(\text{TM}\), an extract from the New Zealand green-lipped mussel (\textit{Perna canaliculus}; bivalve mollusc), have the potential to modulate inflammatory disorders including arthritis\(^19\), asthma\(^20\) and irritable bowel syndrome\(^21\). Torres \textit{et al.} (2008) demonstrated that Lyprinol\(\text{TM}\) partially reduced
myeloperoxidase activity, an indicator of inflammation, in a rat model of 5-FU-induced mucositis. It has been postulated that the high levels of long-chain omega-3 polyunsaturated fatty acids (n-3 PUFA) such as eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6) in Lyprinol™ were responsible for the therapeutic qualities.

Animal oils such as Emu Oil have recently been explored for their therapeutic application. Emu Oil is extracted from the subcutaneous and retroperitoneal fat of the emu (Dromaius novaehollandiae), a large flightless bird native to Australia. Emu Oil has been used in Australian Aboriginal medicine to treat wounds, alleviate pain and reduce joint inflammation. Moreover, recent studies have demonstrated the protective and reparative effects of orally-administered Emu Oil in experimental models of intestinal damage including chemotherapy-induced mucositis, dextran sulphate sodium (DSS)-induced colitis and non-steroidal anti-inflammatory drug (NSAID)-induced enteropathy. Mashtoub et al. (2013) reported that Emu Oil decreased jejunal and ileal inflammation and stimulated mucosal thickening of the small intestine in rats with 5-FU-induced mucositis. In a similar context, Abimosleh et al. (2012) demonstrated improved recovery following Emu Oil treatment in a rat model of DSS-induced colitis in rats, evidenced by increased colonic crypt lengthening and decreased damage severity. Furthermore, Abimosleh et al. (2013) highlighted the anti-inflammatory properties of Emu Oil in the small intestine of rats with NSAID-induced enteropathy.

As Emu Oil and Lyprinol™ have individually demonstrated therapeutic potential in previous experimental studies of intestinal disease, combining these agents could potentially offer increased therapeutic protection against intestinal ailments including chemotherapy-induced mucositis. Accordingly, it was hypothesised that the combination of Emu Oil and Lyprinol™ would decrease the severity of chemotherapy-induced mucositis to a greater extent than the individual agents.
MATERIALS AND METHODS

Animal study

Female Dark Agouti rats \( n=48; \) 6 weeks of age at starting weight \( 132\pm2 \) g) were sourced from Animal Resource Centre (Western Australia, Australia) and Laboratory Animal Services (The University of Adelaide, South Australia, Australia). Rats were individually housed in metabolism cages (Tecniplast Inc. Exton, Pennsylvania, USA) with a 12 hour light:dark cycle throughout acclimatization and the experimental period. Animals were provided \textit{ad libitum} access to water and an 18\% casein-based diet\textsuperscript{32} and were acclimatized for two days prior to experimentation. The animal trial was conducted in compliance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and was approved by The University of Adelaide Animal Ethics Committee.

Rats were randomly allocated to six treatment groups \( n=8/\)group): water+saline, water+5-FU, Olive Oil+5-FU, Emu Oil+Olive Oil+5-FU, Lyprinol\textsuperscript{TM}+Olive Oil+5-FU and Emu Oil+Lyprinol\textsuperscript{TM}+5-FU. Water, Olive Oil, Emu Oil+Olive Oil, Lyprinol\textsuperscript{TM}+Olive Oil or Emu Oil+Lyprinol\textsuperscript{TM} (1 mL) was administered daily via oro-gastric gavage from days 0-7 of the experimental period. On day 5, all rats were intraperitoneally injected with a single dose of either saline (control) or 5-FU (DBL\textsuperscript{®}, Mayne Pharma Pty. Ltd., Victoria, Australia) at a dose of 150 mg/kg. Metabolic data (body weight, food and water intake, and faecal and urine output) were monitored and recorded on a daily basis. Rats were sacrificed 72 hours post saline or 5-FU injection (day 8) via carbon dioxide asphyxiation.

Oil preparation

Emu Oil was sourced from emus farmed in north-eastern South Australia and manufactured utilizing specific methodologies developed by Emu Tracks Australia Pty Ltd (Marleston,
Adelaide, South Australia)). The daily dose (1 mL) of Emu Oil was a combination of 500 µL Emu Oil and 500 µL extra-virgin Olive Oil. Lyprinol™ double soft gel was sourced from New Zealand green-lipped mussels and processed utilizing specific methodologies unique to Pharmalink International Ltd (Queensland, Australia). These processes involved stabilization and extraction of lipids from the mussel meat. Lyprinol™ contained 50 mg PCSO-524 (active ingredient; New Zealand green-lipped mussel lipids), 100 mg natural monounsaturated Olive Oil and 0.255 mg vitamin E (d-α-tocopherol). The daily dose of Lyprinol™ was a combination of 500 µL of liquefied Lyprinol™ diluted with 500 µL extra-virgin Olive Oil. Each rat received a 1 mL gavage and the concentration of Lyprinol™ was 50 mg/mL. Commercially available extra-virgin Olive Oil (Moro, Conga Foods Pty Ltd, Spain) was selected as the control oil, consistent with previous studies. All stock solutions for oils were prepared and individually stored at 4°C in 100 mL opaque containers.

**Fatty acid analysis**

Fatty acid analysis of oil samples was conducted by the Waite Analytical Service at The University of Adelaide (Waite Campus, SA, Australia). Fatty acids were analysed via gas chromatography as previously described. The fatty acid composition of all oil treatments is presented in Table 1.

**Blood and Tissue collection**

After rats were euthanized via carbon dioxide asphyxiation, 2 mL of blood was collected via cardiac puncture for whole blood profile analysis (Cell-dyn® 3700, Abbott Diagnostics Division, Abbott Park, Illinois, USA). The lengths and weights of the gastrointestinal tract (duodenum, jejunum, jejunum-ileum junction (JI; mid small intestine), ileum and colon) were
recorded. Small intestinal tissues (4 cm) were snap-frozen in liquid nitrogen for biochemical analysis and 2 cm segments of the small intestinal tract were stored in 10% buffered formalin for histological analyses. The weights of the remaining organs, including the thymus, heart, lung, liver, spleen, kidneys, stomach and caecum were recorded.

**Biochemical analysis**

Myeloperoxidase (MPO) is an enzyme expressed in intracellular granules of neutrophils and therefore a marker of acute inflammation. MPO activity was measured in small intestinal homogenates (jejunum, JI and ileum). MPO assays were performed according to previously described protocols\(^\text{17, 18}\) using a spectrometer (Victor\(^\text{TM} \times 4\) Multilabel reader, Perkin Elmer, Singapore) at 450 nm at one minute intervals for 15 minutes. MPO activity was expressed as units of MPO activity per gram of tissue (U/g).

**Histological analysis**

Segments of the small intestine (jejunum, JI and ileum) were processed and embedded in paraffin wax and stained with haematoxylin and eosin (Figure 1). Overall severity score of intestinal damage was assessed using a semi-quantitative analysis based on 11 parameters as described by Howarth \textit{et al.}\(^\text{34}\). A score from 0 (unaffected) to 3 (severe) was recorded to provide a maximum damage severity score of 33 for each intestinal region. Villus height and crypt depth measurements were determined (40 villi and crypts/ rat) using a Nikon Eclipse 50i light microscope (Nikon Corporation, Japan) with ProgRes C5 laser optik digital camera (Jenoptik, Germany) and Image-Pro Plus Software Package Version 5.1 (Media Cybernetics, Maryland, USA).
Statistical analysis

All statistical analyses were conducted using IBM SPSS version 19 for Windows (SPSS Inc. Chicago, IL, USA). Daily metabolic data, villus height and crypt depth, MPO activity, whole blood profile analysis and organ weights and lengths were analysed using a one-way ANOVA and Tukey’s post-hoc test. Histological severity scores were analysed using a Kruskal-Wallis test with Mann Whitney U tests. For all analyses, p<0.05 was considered significant.
RESULTS

*Daily metabolic data*

During the pre-injection period (days 1-5), food intake was significantly reduced (up to 20%, \( p<0.001 \)) following administration of all oils, compared to water controls (Table 2). Nevertheless, body weight gain, total water intake, urine and faecal output were not affected by any oil treatment during the pre-injection period, compared to water controls (\( p>0.05 \); Table 2). 5-FU resulted in significantly reduced food intake (39%; \( p<0.001 \)), relative to normal controls, however, did not impact other metabolic parameters (Table 3). None of the oil treatments prevented the reduction in food intake compared to 5-FU controls (\( p>0.05 \); Table 3).

*Visceral and gastrointestinal organ weights and lengths*

5-FU injection resulted in significantly decreased thymus (55%) and spleen weight (23%) compared to saline controls (\( p<0.001 \); Table 4). However, none of the oil treatments normalised thymus and spleen weights, compared to 5-FU controls. Interestingly, liver weights were significantly increased in 5-FU-injected rats treated with Lyprinol™+Olive Oil (13%) and Emu Oil+Lyprinol™ (6%) compared to 5-FU controls (\( p<0.01 \); Table 4). There were no significant differences in heart, lungs, kidneys, stomach or caecum relative weights among treatment groups (Table 4). Olive Oil treatment in 5-FU-injected rats resulted in a 6% decrease in small intestinal weight compared to 5-FU controls (\( p<0.01 \); Table 5). Moreover, 5-FU-injected rats treated with Emu Oil+Lyprinol™ resulted in significantly decreased small intestinal weight compared to Olive Oil treatment (7%; \( p<0.05 \)). Small intestinal length was significantly decreased in 5-FU-injected rats, relative to saline controls (\( \geq10\% \); \( p<0.01 \); Table 5). Duodenum and colon weights and lengths were not affected by oil or 5-FU treatment.
**Total leukocyte count**

Administration of 5-FU significantly decreased monocyte levels (54%; $p<0.05$) and white blood cell count (56%; $p<0.01$) compared to saline controls (Table 6). None of the oil treatments significantly impacted monocyte levels relative to 5-FU controls. Interestingly, only Emu Oil+Olive Oil treatment significantly increased white blood cell count relative to 5-FU controls (48%; $p<0.05$; Table 6). No significant difference in neutrophil granulocytes, lymphocyte concentration, eosinophil granulocytes or basophil granulocytes was observed between 5-FU and saline-injected rats. 5-FU-injected rats treated with Emu Oil+Olive Oil increased numbers of neutrophil granulocytes by 63% and decreased lymphocyte (34%) and eosinophil granulocyte count (59%), compared to 5-FU controls ($p<0.05$; Table 6).

**Histological assessment of the small intestine**

5-FU significantly increased disease severity scores in the jejunum (91%), JI (92%) and ileum (85%), compared to saline controls ($p<0.01$; Figure 2). 5-FU-injected rats treated with any oil treatment significantly decreased disease severity scores in both the jejunum (by 25%) and ileum (by 11%) relative to 5-FU controls ($p<0.05$; Figure 2). 5-FU resulted in significant blunting of the villi across all small intestinal sections, relative to saline controls ($\geq19$%; $p<0.05$; Figure 3). Only Olive Oil significantly increased villus height in the ileum of 5-FU-injected rats (21%), compared to 5-FU controls ($p<0.05$; Figure 3). Furthermore, 5-FU significantly reduced crypt depth in the jejunum (27%) and ileum (27%) compared to saline controls ($p<0.05$). 5-FU-injected rats treated with any oil treatment resulted in significant lengthening of JI crypts, compared with 5-FU controls ($\geq25$%; $p<0.05$). Moreover, only Olive Oil significantly increased crypt depth in the ileum of 5-FU-injected rats (24%), compared to 5-FU controls ($p<0.01$; Figure 3).
Myeloperoxidase activity (MPO)

5-FU injection resulted in increased MPO activity in the jejunum (8.8-fold change), JI (4.1-fold change) and ileum (4.1-fold change), relative to saline controls ($p<0.001$; Figure 4). In 5-FU-injected rats, Olive Oil and Emu Oil+Lyprinol™ treatment significantly decreased jejunal MPO activity (1.8-fold and 1.7-fold change, respectively), compared to controls ($p<0.05$; Figure 4). JI MPO activity in 5-FU-injected rats was significantly reduced following all oil treatments ($\geq 1.9$-fold change), relative to 5-FU controls ($p<0.01$; Figure 4). Interestingly, only Emu Oil+Lyprinol™ significantly reduced ileal MPO activity compared to 5-FU controls (1.7-fold change; $p<0.05$; Figure 4). Additionally, 5-FU-injected rats treated with Olive Oil and Emu Oil+Lyprinol™ resulted in significantly reduced ileal MPO activity (1.2-fold and 1.4-fold change, respectively) relative to Emu Oil+Olive Oil treatment ($p<0.05$; Figure 4).
DISCUSSION

Both Lyprinol™ and Emu Oil have independently demonstrated therapeutic qualities in models of intestinal disorders including inflammatory bowel disease, NSAID-enteropathy and mucositis. As these oil-based agents are rich in anti-oxidant and anti-inflammatory-mediating constituents, believed to target different aspects of mucositis pathobiology, combining these two agents could potentially further decrease the severity of bowel inflammation. The current study revealed that the combination of Emu Oil and Lyprinol™ partially reduced certain parameters associated with small intestinal damage in a rat model of chemotherapy (5-FU)-induced mucositis; specifically indicated by reduced MPO activity (biomarker of neutrophil infiltration) and decreased histological severity scores.

Neutrophil infiltration is a key feature of acute inflammation in the small intestine. In the current study, the combination of Emu Oil and Lyprinol™ resulted in decreased jejunal, mid small intestinal and ileal MPO levels, however, the individual treatments decreased MPO activity only in the mid small intestine. The anti-inflammatory action of Emu Oil and Lyprinol™ in combination throughout the small intestinal tract may highlight the synergism between their constituents, thereby potentially offering greater protection against chemotherapy-induced damage. Interestingly however, Mashtoub et al. (2013) demonstrated that daily oral administration of 1ml of Emu Oil alone in a congruent experimental design decreased MPO activity in the jejunum and the ileum of 5-FU-injected rats on day 8; an effect not observed in the current study in which Emu Oil was administered in combination with Olive Oil (1:1). The enhanced anti-inflammatory activity of Emu Oil suggested that Emu Oil alone could improve intestinal protection in this model of mucositis, especially at a higher volume. Furthermore, Torres et al. (2008) demonstrated that daily 1ml gavage of low dose Lyprinol™ (1:1.5 [Lyprinol™:Olive Oil]) and high dose Lyprinol™ (4:1) decreased MPO activity on day 8 in the jejunum (high dose only) and the mid small intestine.
(both doses) in a rat model of 5-FU-induced mucositis. These results indicate a potential dose-response of Lyprinol™, as the lower dose (1:1) of Lyprinol™+Olive Oil administered in the current study did not exert a significant anti-inflammatory effect in the jejunum, as assessed by MPO activity.

Similar to the combination of Emu Oil and Lyprinol™, Olive Oil reduced jejunal and mid small intestinal MPO activity in 5-FU-injected rats. This may have been attributed to the high concentration of oleic acid (76%), a mono-unsaturated long-chain fatty acid, found in Olive Oil, which has been reported to modulate macrophage activation, decrease pro-inflammatory cytokine production and/or increase defences in scavenging reactive oxygen species. Olive Oil combined with Emu Oil or Lyprinol™ possessed similar oleic acid concentrations (63% and 65% respectively) as pure Olive Oil treatment; whereas, the combination of Emu Oil and Lyprinol™ had the lowest oleic acid concentration (52%). Nevertheless, in the ileum, only the combination of Emu Oil and Lyprinol™ resulted in decreased MPO activity compared to 5-FU controls. Further studies are required to investigate the impact of specific fatty acids on MPO activity and pro-inflammatory cytokine production, including interleukins-1β and -6, tumour necrosis factor-α and interferon-γ, in models of intestinal disorders.

In the current study, administration of 5-FU resulted in severe damage to the small intestine, indicated by an increase in histologically-assessed damage severity and blunted villi and crypts. All oils decreased jejunal and ileal damage severity, compared with 5-FU controls. Relative to 5-FU controls, oil treatment in 5-FU-injected rats improved villus architecture in the mid small intestine and ileum. 5-FU preferentially damages the jejunum due to its high cell turnover rate; an effect observed in the current study in which there was no improvement following any oil treatment. Interestingly, all oil treatments increased crypt length in the mid small intestine relative to untreated 5-FU controls, indicative of improved
mucosal architecture. Crypt lengthening may have resulted from increased crypt cell proliferation (hyperplasia), increased cell hypertrophy, reduced apoptosis or a combination of these factors. Interestingly, only Olive Oil improved villus and crypt structures in the ileum relative to 5-FU controls. In future studies, it would be important to assess proliferation and apoptosis utilizing well established techniques, for example, immunohistochemical quantification of Ki67 (G1-, S- and G2 phases) for proliferation and terminal deoxynucleotidyl transferase nick-end labelling for apoptosis.

Chemotherapeutic agents target rapidly dividing cells including bone marrow, which acts as a reservoir for white blood cells. Consequently, in the current study, monocytes and white blood cell counts were significantly reduced by 5-FU. Treatment with Emu Oil+Olive Oil in 5-FU-injected rats resulted in normalisation of white blood cell count. However, in 5-FU-injected animals, Emu Oil+Olive Oil administration resulted in neutrophil granulocyte elevation and suppression of lymphocyte and eosinophil granulocytes, suggesting that components of Emu Oil were modulating the immune system. Further studies are warranted to measure other inflammatory biomarkers, both in the blood and tissue, to define the means by which animal-sourced oils impact immune responses in the context of mucositis.

During the pre-injection period (days 1-5) in the current study, all oil treatments in normal animals resulted in significantly decreased food (18% casein-based diet) intake; an effect observed by Mashtoub et al. (2013), not specific to Emu Oil or Olive Oil. This may have been attributed to the fulfilment of energy requirements following daily oil gavages, as there were no significant differences in body weight compared with water controls. Alternatively, the high volume of oil administered, combined with its viscosity, may have triggered satiety signals. Furthermore, a single dose of 5-FU (150 mg/kg) decreased food intake, however, did not impact water consumption or urine and faecal output, as per previous studies. In the current study, oil treatments did not normalise food intake in 5-FU-injected rats.
Nevertheless, neither 5-FU nor oil treatments significantly affected bodyweight change amongst experimental groups during the pre- and post-injection periods.

In the current study, 5-FU decreased small intestinal length; a characteristic response to epithelial damage\textsuperscript{41}. Decreased intestinal length may aid in maintaining mucosal integrity to protect against translocation of pathogens into the submucosa\textsuperscript{7,12}. Furthermore, 5-FU resulted in decreased thymus and spleen weights, similar to previous studies using 5-FU\textsuperscript{14,17,18,42}. Both Lyprinol\textsuperscript{TM}+Olive Oil and the combination of Emu Oil and Lyprinol\textsuperscript{TM} significantly increased liver weight, relative to body weight, in 5-FU-injected rats compared to 5-FU controls. The impact of Lyprinol\textsuperscript{TM} on liver weight is consistent with findings in a similar \textit{in vivo} study by Torres \textit{et al.} (2008)\textsuperscript{17}. Increased liver weight may have been due to the high volume of orally-administered oils, potentially causing an accumulation of fatty deposits within hepatocytes\textsuperscript{17}. Emu Oil+Olive Oil did not impact liver weight in the current study, consistent with findings using Emu Oil alone by Mashtoub \textit{et al.} (2013) in a model of 5-FU-induced mucositis\textsuperscript{18}.

In conclusion, Emu Oil and Lyprinol\textsuperscript{TM} in combination partially reduced selected indicators of damage associated with chemotherapy-induced mucositis, specifically, acute inflammation in the small intestine and histologically-assessed intestinal damage severity. Moreover, Olive Oil treatment was often comparable with the formulated Emu Oil and Lyprinol\textsuperscript{TM}. Future studies are warranted to investigate the bioactive constituents of Olive Oil, Emu Oil and Lyprinol\textsuperscript{TM} to further define their potential to ameliorate chemotherapy-induced mucositis. Furthermore, other biomarkers of inflammation, including pro-inflammatory cytokines, need to be measured throughout the progression of mucositis to provide information on the modulation of the immune system in response to oil therapies. Finally, it is important to define the optimal dosage and ratio of bioactive fatty acids and their impact in other small intestinal disorders including infective gastroenteritis, celiac disease and Crohn's disease.
ACKNOWLEDGEMENTS

S.M. is the recipient of the National Health and Medical Research Council Postdoctoral (Peter Doherty) Australian Biomedical Fellowship. G.S.H. was supported by a South Australian Cancer Research Collaborative Senior Research Fellowship. S.M. contributed to the intellectual development of the Emu Oil project, experimental design, analysis and interpretation of data and manuscript preparation. L.S.L. conducted animal trials, experiments, data analyses and manuscript preparation. G.L.E., K.Y.C. assisted with experimental planning and analysis of data. K.A.L. contributed to the experimental planning, organising and conducting animal trials. J.E.B. assisted with animal trials. G.S.H. contributed to the experimental design, analysis of data and manuscript preparation.
REFERENCES


Table 1. Fatty acid composition of the Olive Oil, Emu Oil+Olive Oil, Lyprinol™+Olive Oil, and Emu Oil+Lyprinol™ used in the current study.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Olive Oil (%)</th>
<th>Emu Oil + Olive Oil (%)</th>
<th>Lyprinol™ + Olive Oil (%)</th>
<th>Emu Oil + Lyprinol™ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td>14.0</td>
<td>24.6</td>
<td>17.5</td>
<td>28.1</td>
</tr>
<tr>
<td>Unsaturated/Trans</td>
<td>0</td>
<td>0.2</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>78.5</td>
<td>67.2</td>
<td>69.5</td>
<td>58.2</td>
</tr>
<tr>
<td>Total omega-3</td>
<td>0.7</td>
<td>0.6</td>
<td>6.4</td>
<td>6.3</td>
</tr>
<tr>
<td>Total omega-6</td>
<td>6.8</td>
<td>7.4</td>
<td>6.5</td>
<td>7.1</td>
</tr>
<tr>
<td>Total omega-7</td>
<td>2.3</td>
<td>4.2</td>
<td>3.7</td>
<td>5.6</td>
</tr>
<tr>
<td>Total omega-9</td>
<td>76.0</td>
<td>62.8</td>
<td>65.0</td>
<td>51.8</td>
</tr>
</tbody>
</table>

Total omega-3 consists of α-linolenic acid (18:3 n-3), eicosatrienoic acid (20:3 n-3), eicosapentaenoic acid (20:5 n-3), docosapentaenoic acid (22:5 n-3) and docosahexaenoic acid (22:6 n-3). Total omega-6 mainly consists of linoleic acid (18:2 n-6) and small traces of eicosadienoic acid (20:2 n-6), arachidonic acid (20:4 n-6) and docosapentaenoic acid (22:5 n-6). Total omega-7 consists of palmitoleic acid (16:1 n-7) and Cis-Vaccenic acid (18:1 n-7). Total omega-9 consists of oleic acid (18:1 n-9) and gondoic acid (20:1 n-9).
Table 2. Body weight change, total water and food consumption, urine and faecal output in normal rats during the pre-injection period (days 1-5). Rats were administered water, Olive Oil, Emu Oil+Olive Oil, Lyprinol™+Olive Oil, or Emu Oil+Lyprinol™ daily (1 mL). Oil treatments significantly decreased food intake in normal animals.

|                          | Water  
| (n=16)                  | Olive Oil  
| (n=8)                   | Emu Oil + Olive Oil  
| (n=8)                   | Lyprinol™ + Olive Oil  
| (n=8)                   | Emu Oil + Lyprinol™  
| (n=7)                   |
|-------------------------|-----------------|
| **Body Weight Change (g)** | 1.2 ± 0.2 0.9 ± 0.9 0.1 ± 1.5 2.6 ± 1.5 -1.4 ± 1.6 |
| **Water Intake (mL)**    | 124.4 ± 8.3 137.5 ± 11.3 131.3 ± 13.6 120.6 ± 7.9 117.9 ± 8.9 |
| **Food Intake (g)**      | 50.5 ± 0.8 39.1 ± 1.0*** 36.9 ± 1.4*** 40.2 ± 1.1*** 38.1 ± 1.4*** |
| **Urine Output (mL)**    | 79.3 ± 5.6 96.3 ± 11.5 93.0 ± 10.5 83.4 ± 5.8 77.1 ± 7.4 |
| **Faecal Output (g)**    | 6.2 ± 0.3 5.5 ± 0.4 5.0 ± 0.5 5.4 ± 0.2 5.4 ± 0.4 |

Data are expressed as mean (g or mL) ± SEM. *** p<0.001 compared to water.
Table 3. Body weight change, total water and food consumption, urine and faecal output in rats during the post-injection period (days 6-8). Rats were administered water, Olive Oil, Emu Oil+Olive Oil, Lyprinol™+Olive Oil, or Emu Oil+Lyprinol™ daily (1 mL; days 0-7) via oro-gastric gavage and intraperitoneally injected with saline or 5-FU (150mg/kg) on day 5. 5-FU significantly decreased food intake compared to normal controls.

<table>
<thead>
<tr>
<th></th>
<th>Water + Saline</th>
<th>Water + 5-FU</th>
<th>Olive Oil + 5-FU</th>
<th>Emu Oil + Olive Oil + 5-FU</th>
<th>Lyprinol™ + Olive Oil + 5-FU</th>
<th>Emu Oil + Lyprinol™ + 5-FU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight Change (g)</td>
<td>0.2 ± 0.4</td>
<td>-3.7 ± 1.9</td>
<td>-3.6 ± 1.4</td>
<td>-5.8 ± 1.1</td>
<td>-4.7 ± 1.0</td>
<td>-4.5 ± 0.9</td>
</tr>
<tr>
<td>Water Intake (mL)</td>
<td>75.0 ± 4.3</td>
<td>94.4 ± 7.7</td>
<td>91.3 ± 7.8</td>
<td>93.1 ± 8.0</td>
<td>76.3 ± 3.4</td>
<td>72.9 ± 13.8</td>
</tr>
<tr>
<td>Food Intake (g)</td>
<td>29.1 ± 0.6</td>
<td>11.5 ± 1.4***</td>
<td>10.9 ± 2.0</td>
<td>7.7 ± 0.7</td>
<td>8.4 ± 0.4</td>
<td>8.4 ± 1.4</td>
</tr>
<tr>
<td>Urine Output (mL)</td>
<td>47.5 ± 4.7</td>
<td>64.5 ± 6.7</td>
<td>66.5 ± 6.3</td>
<td>64.8 ± 4.2</td>
<td>52.3 ± 3.5</td>
<td>48.0 ± 9.9</td>
</tr>
<tr>
<td>Faecal Output (g)</td>
<td>3.3 ± 0.2</td>
<td>3.2 ± 0.2</td>
<td>2.6 ± 0.2</td>
<td>3.1 ± 0.6</td>
<td>3.3 ± 0.4</td>
<td>2.2 ± 0.3</td>
</tr>
</tbody>
</table>

Data are expressed as mean (g or mL) ± SEM. *** p<0.001 compared to water+saline.
Table 4. Visceral organ weights relative to body weight of rats on day 8. Rats were administered water, Olive Oil, Emu Oil+Olive Oil, Lyprinol™+Olive Oil, or Emu Oil+Lyprinol™ daily (1 mL; days 0-7) via oro-gastric gavage and intraperitoneally injected with saline or 5-FU (150mg/kg) on day 5. 5-FU significantly decreased thymus and spleen weights compared to normal control.

<table>
<thead>
<tr>
<th></th>
<th>Water + Saline</th>
<th>Water + 5-FU</th>
<th>Olive Oil + 5-FU</th>
<th>Emu Oil + Olive Oil + 5-FU</th>
<th>Lyprinol™ + Olive Oil + 5-FU</th>
<th>Emu Oil + Lyprinol™ + 5-FU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus</td>
<td>15 ± 1</td>
<td>7 ± 1***</td>
<td>6 ± 1</td>
<td>5 ± 0</td>
<td>5 ± 1</td>
<td>5 ± 0</td>
</tr>
<tr>
<td>Heart</td>
<td>38 ± 1</td>
<td>39 ± 1</td>
<td>41 ± 1</td>
<td>40 ± 1</td>
<td>40 ± 1</td>
<td>40 ± 0</td>
</tr>
<tr>
<td>Lung</td>
<td>60 ± 2</td>
<td>63 ± 3</td>
<td>62 ± 1</td>
<td>67 ± 4</td>
<td>72 ± 4</td>
<td>70 ± 5</td>
</tr>
<tr>
<td>Liver</td>
<td>363 ± 6</td>
<td>363 ± 11</td>
<td>398 ± 7</td>
<td>387 ± 9</td>
<td>415 ± 10##</td>
<td>411 ± 9##</td>
</tr>
<tr>
<td>Spleen</td>
<td>20 ± 1</td>
<td>16 ± 0***</td>
<td>14 ± 1</td>
<td>13 ± 1</td>
<td>14 ± 1</td>
<td>15 ± 0</td>
</tr>
<tr>
<td>Kidneys</td>
<td>80 ± 2</td>
<td>87 ± 2</td>
<td>89 ± 3</td>
<td>84 ± 2</td>
<td>88 ± 2</td>
<td>88 ± 3</td>
</tr>
<tr>
<td>Stomach</td>
<td>57 ± 3</td>
<td>55 ± 1</td>
<td>54 ± 2</td>
<td>52 ± 1</td>
<td>55 ± 1</td>
<td>53 ± 1</td>
</tr>
<tr>
<td>Caecum</td>
<td>40 ± 1</td>
<td>44 ± 2</td>
<td>42 ± 2</td>
<td>41 ± 2</td>
<td>41 ± 2</td>
<td>44 ± 3</td>
</tr>
</tbody>
</table>

Data are expressed as mean (% relative to g/kg body weight) ± SEM. All values x10^2. *** $p<0.001$ compared to water+saline; ## $p<0.01$ compared to water+5-FU.
Table 5. Gastrointestinal organ weights (relative to body weight) and lengths of rats on day 8. Rats were administered water, Olive Oil, Emu Oil+Olive Oil, Lyprinol™+Olive Oil, or Emu Oil+Lyprinol™ daily (1 mL; days 0-7) via oro-gastric gavage and intraperitoneally injected with saline or 5-FU (150mg/kg) on day 5. 5-FU significantly decreased small intestinal length compared to normal controls.

<table>
<thead>
<tr>
<th></th>
<th>Water + Saline</th>
<th>Water + 5-FU</th>
<th>Olive Oil + 5-FU</th>
<th>Emu Oil + Olive Oil + 5-FU</th>
<th>Lyprinol™ + Olive Oil + 5-FU</th>
<th>Emu Oil + Lyprinol™ + 5-FU</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Duodenum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (g/kg)</td>
<td>211 ± 5</td>
<td>191 ± 5</td>
<td>241 ± 12</td>
<td>218 ± 9</td>
<td>209 ± 9</td>
<td>206 ± 5</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>5.4 ± 0.3</td>
<td>4.8 ± 0.2</td>
<td>5.2 ± 0.1</td>
<td>5.1 ± 0.1</td>
<td>5.0 ± 0.1</td>
<td>5.3 ± 0.2</td>
</tr>
<tr>
<td><strong>Small intestine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (g/kg)</td>
<td>15 ± 1</td>
<td>16 ± 1</td>
<td>15 ± 1##</td>
<td>16 ± 1</td>
<td>14 ± 1</td>
<td>14 ± 1^</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>72.3 ± 1.5</td>
<td>64.8 ± 0.9**</td>
<td>61.8 ± 1.9</td>
<td>62.9 ± 1.7</td>
<td>62.8 ± 1.3</td>
<td>63.3 ± 1.3</td>
</tr>
<tr>
<td><strong>Colon</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (g/kg)</td>
<td>47 ± 1</td>
<td>52 ± 2</td>
<td>55 ± 2</td>
<td>51 ± 3</td>
<td>55 ± 2</td>
<td>49 ± 2</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>10.9 ± 0.3</td>
<td>10.6 ± 0.4</td>
<td>11.4 ± 0.5</td>
<td>10.9 ± 0.4</td>
<td>10.9 ± 0.4</td>
<td>10.6 ± 0.4</td>
</tr>
</tbody>
</table>

Data are expressed as mean (% relative to g/kg body weight or cm) ± SEM. All values x10^-2. ** p<0.01 compared to water+saline; ## p<0.01 compared to water+5-FU; ^ p<0.05 compared to olive oil+5-FU.
Table 6. Total leukocyte count in rats on day 8. Rats were administered water, Olive Oil, Emu Oil+Olive Oil, Lyprinol™+Olive Oil, or Emu Oil+Lyprinol™ daily (1 mL; days 0-7) via oro-gastric gavage and intraperitoneally injected with saline or 5-FU (150mg/kg) on day 5. 5-FU significantly increased white blood cell count compared to normal controls, which was normalised by Emu Oil+Olive Oil.

<table>
<thead>
<tr>
<th></th>
<th>Water + Saline</th>
<th>Water + 5-FU</th>
<th>Olive Oil + 5-FU</th>
<th>Emu Oil + Olive Oil + 5-FU</th>
<th>Lyprinol™ + Olive Oil + 5-FU</th>
<th>Emu Oil + Lyprinol™ + 5-FU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil Granulocytes (%)</td>
<td>12.9 ± 4.0</td>
<td>17.2 ± 3.0</td>
<td>24.3 ± 4.7</td>
<td>46.5 ± 10.5§</td>
<td>32.7 ± 8.0</td>
<td>26.0 ± 4.1</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>78.8 ± 4.0</td>
<td>77.5 ± 3.1</td>
<td>71.3 ± 4.1</td>
<td>50.8 ± 10.2§</td>
<td>62.5 ± 7.6</td>
<td>70.3 ± 3.9</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>3.8 ± 0.5</td>
<td>1.8 ± 0.2*</td>
<td>1.6 ± 0.7</td>
<td>0.8 ± 0.1</td>
<td>1.7 ± 0.6</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Eosinophil Granulocytes (%)</td>
<td>1.7 ± 0.2</td>
<td>1.2 ± 0.1</td>
<td>0.8 ± 0.3</td>
<td>0.5 ± 0.1§</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Basophil Granulocytes (%)</td>
<td>2.8 ± 0.4</td>
<td>2.4 ± 0.1</td>
<td>2.0 ± 0.3</td>
<td>1.4 ± 0.3</td>
<td>2.6 ± 0.6</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>White Blood Cell Count (10^{9}/L)</td>
<td>8.5 ± 0.9</td>
<td>3.8 ± 0.4**</td>
<td>5.1 ± 0.5</td>
<td>7.2 ± 0.8§</td>
<td>6.4 ± 1.0</td>
<td>5.5 ± 0.8</td>
</tr>
</tbody>
</table>

Data are expressed as mean (% or 10^{9}/L) ± SEM. * p<0.05, ** p<0.01 compared to water+saline; # p<0.05 compared to water+5-FU.
FIGURE LEGENDS

**Figure 1.** Representative photomicrographs of 4 µm rat ileum sections stained with haematoxylin and eosin (4x magnification). Groups include; Water+Saline, Water+5-FU, Olive Oil+5-FU, Emu Oil+Olive Oil+5-FU, Lyprinol™+Olive Oil+5-FU, Emu Oil+Lyprinol™+5-FU.

**Figure 2.** Histological disease severity scores in the jejunum, JI (jejunum-ileum junction) and ileum on day 8. Rats were administered water, Olive Oil, Emu Oil+Olive Oil, Lyprinol™+Olive Oil or Emu Oil+Lyprinol™ daily (1ml; days 0-7) via oro-gastric gavage and intraperitoneally injected with saline or 5-FU on day 5. Oil treatments significantly decreased jejunal and ileal damage severity compared to 5-FU controls. Data are expressed as median damage severity score (range). ** p<0.01 compared to water+saline; # p<0.05 compared to water+5-FU.

**Figure 3.** Villus height and crypt depth lengths (µm) in the jejunum, JI (jejunum-ileum junction) and ileum on day 8. Rats were administered water, Olive Oil, Emu Oil+Olive Oil, Lyprinol™+Olive Oil or Emu Oil+Lyprinol™ daily (1ml; days 0-7) via oro-gastric gavage and intraperitoneally injected with saline or 5-FU on day 5. Oil treatments significantly lengthened JI crypts compared to 5-FU controls. Data are expressed as mean ± SEM. * p<0.05, ** p<0.01, *** p<0.001 compared to water+saline; # p<0.05, ## p<0.01, ### p<0.001 compared to water+5-FU.

**Figure 4.** Myeloperoxidase (MPO) activity in the jejunum, JI (jejunum-ileum junction) and ileum on day 8. Rats were administered water, Olive Oil, Emu Oil+Olive Oil, Lyprinol™+Olive Oil or Emu Oil+Lyprinol™ daily (1ml; days 0-7) via oro-gastric gavage and intraperitoneally injected with saline or 5-FU on day 5. Emu Oil+Lyprinol™ significantly decreased small intestinal MPO levels compared to 5-FU controls. Data are
expressed as mean [units MPO activity per gram of tissue (U/g)] ± SEM. *** \( p<0.001 \) compared to water+saline; # \( p<0.05 \), ## \( p<0.01 \), ### \( p<0.001 \) compared to water+5-FU; † \( p<0.05 \), †† \( p<0.01 \) compared to Emu Oil+Olive Oil+5-FU.
Figure 2
Figure 3
Figure 4