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Grape Seed Extract Reduces the Severity of Selected Disease Markers in the Proximal Colon of Dextran Sulphate Sodium-Induced Colitis in Rats

Ker Y Cheah¹, Susan EP Bastian¹, Thomas MV Acott², Suzanne M Abimosleh³⁴, Kerry A Lymn³³, & Gordon S Howarth²³*

¹School of Agriculture, Food and Wine, The University of Adelaide, South Australia, 5005
²School of Animal and Veterinary Sciences, Roseworthy Campus, The University of Adelaide, South Australia, 5371
³Centre for Paediatric and Adolescent Gastroenterology, Children, Youth and Women’s Health Service, 72 King William Road, North Adelaide, South Australia, 5006
⁴Discipline of Physiology, School of Medical Sciences, The University of Adelaide, South Australia 5001, Australia

*Corresponding Author:
Professor Gordon Howarth
School of Animal and Veterinary Sciences (Roseworthy campus),
University of Adelaide, 5371
South Australia.
Phone: +61 8 8303 7885
Fax: +61 8 8303 7972

Running Title: Grape seed extract and colitis
Professor Gordon Howarth is supported by the Sally Birch Cancer Council Australia Research Fellowship in Cancer Control.
Abstract

Background Grapeseed extract (GSE) constitutes a rich source of procyanidins. GSE has been demonstrated to exert encouraging anti-inflammatory and anti-ulcer properties in experimental settings, although its effects on inflammation of the colon remain undefined. Aim To determine the effects of GSE in a rat model of dextran sulphate sodium (DSS) for ulcerative colitis. Methods Male Sprague-Dawley rats were gavaged daily (days 0-10) with GSE (400 mg/kg). Ulcerative colitis was induced by substituting DSS (2% w/v) for drinking water from days 5-10. A sucrose breath test was performed on day 11 to determine small bowel function and intestinal tissues were collected for histological analyses. Statistical analysis was by one-way or repeated measures ANOVA, p<0.05 was considered significant. Results Compared to DSS-treated controls, GSE significantly decreased ileal villus height (14%; p<0.01) and mucosal thickness (13%; p<0.01) towards the values of normal controls. GSE reduced qualitative histological severity score (p<0.05) in the proximal colon, although no significant effect was evident in the distal colon. However, GSE failed to prevent DSS-induced damage to the crypts of both colonic regions. Administration of GSE did not impact negatively on metabolic parameters, nor did it induce any deleterious gastrointestinal side-effects in healthy animals. Conclusion GSE decreased the severity of selected markers of DSS-induced colitis in the distal ileum and proximal colon, suggesting potential as an adjuvant therapy for the treatment of ulcerative colitis. Future studies of GSE should investigate alternative delivery methods and treatment regimens, further seeking to identify the individual bioactive factors.

Keywords: Ulcerative colitis, colon, dextran sulphate sodium, procyanidins, rat model
Introduction

Inflammatory bowel disease (IBD) is the collective term for a group of idiopathic disorders which affect the gastrointestinal tract. These include ulcerative colitis (UC) and Crohn’s disease (CD), each characterized by varying degrees of inflammation and ulceration of the intestinal tract [1]. UC manifests as an idiopathic inflammation of the colonic mucosal layer with a chronically relapsing course [2]. UC affects the large bowel, involving the rectum (proctitis), and can extend proximally to include the sigmoid colon (proctosigmoiditis), descending colon (left-sided colitis), and the entire colon (pancolitis) [3]. Bloody diarrhoea, rectal urgency and tenesmus are hallmark symptoms of UC [4]. Fever, weight loss and signs of malnutrition are also common [5,6]. Moreover, 15% of UC patients require hospitalisation and intensive therapy [2]. The pathogenesis of UC is not well understood and, as yet, there is no known cause [7,8]. However, there is evidence to suggest that it is mediated immunologically and that expression of the disease is dependent on environmental and genetic factors [7,9,10]. Although the aetiology of UC is not entirely understood, recent advances in biotechnology have resulted in the development of novel nutraceutical treatment approaches for these diseases, to complement conventional treatments [2]. These ‘nutraceutical’ therapies include Lyprinol [11], certain probiotics [12], growth factors [13], and plant sourced extracts such as Iberogast™ [14] and more recently, Grape Seed Extract (GSE) [15].

GSE is produced as a by-product of the wine and grape juice industries. It contains high levels of phytochemicals such as flavonoids, which have been used for centuries to treat a wide range of ailments (digestive problem) without fully understanding their mode of action [16]. The proocyanidins in GSE are of particular interest as they have been shown to be potent...
antioxidants and free radical scavengers, further possessing anti-inflammatory, anti-viral, anti-carcinogenic, anti-bacterial, anti-allergic and vasodilatory properties [17,18]. Previously, we have reported GSE to partially ameliorate small intestinal damage induced by the chemotherapy drug, 5-Fluorouracil, in cell culture and in a rat model of mucositis [15]. However, it is unknown whether bioavailability of GSE persists beyond the small intestine.

Dextran Sulphate Sodium (DSS)-induced colitis is a reproducible model of large intestinal damage that closely resembles human UC. DSS-colitis can be induced in rats and mice [19-21], making this an appropriate model for the study of GSE. Accordingly, it was hypothesised that the antioxidant and anti-inflammatory properties of GSE and its constituent procyanidins would prevent colonic damage and attenuate DSS-colitis in the rat.
Materials and Methods

Grape Seed Extract (GSE) Preparation

Powdered GSE was obtained from Tarac Technologies (North Adelaide, South Australia) and stored in an air-tight and light sensitive package until being dissolved in MilliQ water prior to use. The GSE utilized in the current study was obtained from the same source with the same batch number as previous study (15).

Animals

This project was approved by the Animal Ethics Committees of The Children, Youth and Women’s Health Service and the University of Adelaide and followed the Australian Code of Practise for the Care and Use of Animals for Scientific Purposes. Male Sprague Dawley rats, 116 ± 4.5 g (mean ± SEM) starting weight (n = 33) were housed individually in metabolism cages (Tecniplast, Inc. Exton, PA, USA) under controlled conditions and a 12 hour light-dark cycle. Throughout the experimental period, rats were fed a standard 18% casein-based diet [22] and had continual access to water.

Rats were assigned to treatment groups as follows; Group 1: Water/Water (n = 8), Group 2: Water/GSE (n = 8), Group 3: DSS/Water (n = 8) and Group 4: DSS/GSE (n = 9). At day -2, the animals were transferred to metabolism cages and allowed two days to acclimatise to the new conditions. At day zero, and continuing until day ten, animals were gavaged orally once daily (approximately 8.30am) with either 1ml of water or GSE solution (400 mg/kg dose). At day five, 2% DSS w/v (ICN Biomedicals, Columbus, Ohio, USA) was substituted for drinking water for Groups 3 and 4, to induce colitis; this was continued until day 11 when the
animals were sacrificed. Groups 1 and 2 continued to drink tap water for the entire experimental period.

Daily data for body weight, feed and water/DSS consumption and urine and faecal outputs were recorded for all animals throughout the trial period. A disease activity index score was also recorded daily, from day six onwards, based on weight loss, stool consistency, rectal bleeding/blood in stool and the general body condition of the rat [23]. The system used was a 0-3 scoring system, whereby zero was normal and three reflected a severe colitis. On day 11, all animals were sacrificed by carbon dioxide overdose followed by cervical dislocation. The gastrointestinal tract was removed and the lengths of the intestinal sections were measured unstretched. The segments were then emptied of contents and weighed. Sections of the intestine (2 cm) were collected into 10% buffered formalin for histological analysis. Weights of all visceral organs were also recorded.

\textit{$^{13}$C-Sucrose Breath Test}

The sucrose breath test (SBT) was conducted on all animals on day 11, directly before sacrifice as described previously [24] to identify any potential effects on sucrase activity in the small bowel. Briefly, animals were sealed inside small perspex containers for two minutes and a sample of breath was collected for analysis of $^{13}$C content. A baseline sample was collected at time = 0, the animals were then gavaged with a $^{13}$C-sucrose solution and samples collected every 15 minutes for 120 minutes, before analysed using an Isotope Ratio Mass Spectrometer [24].
Histological analysis

Sections (2 cm) of colon (proximal and distal) and small intestine (distal ileum) were routinely processed and paraffin embedded. Sections were cut and stained with haematoxylin and eosin. Overall histological damage severity was graded in the colon in a blinded manner, based on the following parameters: crypt disruption, crypt cell disruption, surface enterocyte disruption, goblet cell numbers, polymorphonuclear cell infiltration in the mucosa submucosal thickening/oedema and muscularis externa thickening [21]. Crypt depth was determined using 40 crypts per rat [21]. Crypt depth and villus height (40 for each per animal) were the only parameters measured in the ileum. All analyses were performed using a light microscope (Olympus CX31 & BH-2), digital camera (crypt and villus scoring only) (Sony, Tokyo, Japan) and Image Pro-Plus Software Package Version 4.5.1.2.7 (crypt and villus scoring only) (Media Cybernetics, Silver Spring MD, USA).

Statistical analysis

All statistical analyses were conducted using SPSS version 15.0.1 for Windows (SPSS Inc. Chicago, Illinois, USA). Daily metabolic data and disease activity index scores were analysed using a repeated measures Analysis of Variance (ANOVA) and Holme’s post hoc test (p<0.05 considered significant). Organ weights and lengths, daily data totals, SBT scores and histological data were expressed using a one-way ANOVA and a Tukey’s post hoc test (p<0.05 considered significant).
Results

*Disease Activity Index (DAI) and Metabolism Data*

DSS ingestion increased DAI significantly compared to water controls (Water/Water) from day 6 onwards (Figure 1). The noticeable increase in DAI score for all groups on the final day was the result of fasting overnight in preparation for the SBT, resulting in a score of three for weight loss in all animals (Figure 1). GSE produced no statistically significant effect compared to either DSS controls or water controls over the six days (Figure 1). Administration of GSE from days 0-5 did not significantly alter food intake, urine output, food intake and faecal output compared to water controls (Table 1). DSS administration (day 6-11) significantly increased faecal and urine output compared to water controls, while DSS-treated rats receiving GSE exhibited similar faecal outputs to DSS controls (Table 1).

*Organ Weights and Lengths*

DSS treatment significantly (p< 0.05) increased colon weight, and DSS-treated rats receiving GSE exhibited similar colon weights, compared to DSS controls. There was no effect of GSE in healthy animals compared to water controls (Table 2). No statistically significant differences were observed in the weights of the stomach, duodenum, jejunum-ileum (JI) and caecum for any of the treatment groups (Table 2). Similarly, no statistically significant differences were observed in the visceral organ weights for any of the treatment groups (Table 2) and there was no GSE effect relative to either water controls or DSS controls (Table 2). Finally, no significant differences were apparent as a result of DSS or GSE on gastrointestinal organ lengths among any of the treatment groups compared to water controls (Table 3).
**13C-Sucrose Breath Test**

No significant differences in percentage cumulative dose of 13C for the SBT at 90 minutes (%CD90) were observed among the groups at day 11 (Fig. 2). There was no GSE effect compared to either water controls or DSS controls (Fig. 2).

**Qualitative Histological Severity Scoring**

DSS significantly increased histological severity score in the proximal and distal colon (p<0.05) compared to water controls (Fig. 3). There was no effect of GSE on histopathological severity score in healthy animals for either the proximal or distal colon. However, importantly, GSE significantly reduced histological severity score in the proximal colon of DSS-treated rats (p<0.05) (Figs. 3a, 4), although no effect of GSE was detected in the distal colon of DSS-treated rats (Fig. 3b).

**Quantitative Histological Analysis**

DSS significantly increased villus height (22%; p<0.01) in the distal ileum, when compared to water controls. In DSS-treated rats receiving GSE, the effect of DSS on villus height was reduced (p<0.01) by approximately 14% compared to DSS-treated rats (Fig. 5a). Similar effects were apparent for crypt depth. DSS increased crypt depth by 16% relative to water controls (Fig. 5). Mean crypt depth was reduced by 11% in DSS-treated rats receiving GSE compared to DSS controls, although this failed to achieve statistical significance (p=0.081) (Fig. 5). DSS treatment significantly increased mucosal thickness (20%; p<0.001) compared to water controls (Fig. 5b). Interestingly, GSE significantly decreased mucosal thickness (13%; p<0.01) compared to DSS-treated controls and resulted in similar mucosal thickness values to normal, healthy water controls in distal ileum (Fig. 5b). No GSE effect was apparent
on villus height nor crypt depth and mucosal thickness in normal animals compared to healthy controls (Fig. 5a, b).

GSE did not significantly affect colonic crypt depth in healthy animals (Fig. 6). DSS significantly increased crypt depth by 20% and 29% in both the proximal and distal colon (Fig. 6), respectively, compared to water controls. GSE tended to decrease crypt depth (32%) in the proximal colon compared to DSS controls, although this failed to achieve statistical significance (Fig. 6). There was no effect of GSE on crypt depth in the distal colon, compared to DSS controls, which was reflected by the qualitative severity scores (Fig. 6).
Discussion

Previously, GSE and its constituents have been demonstrated to exert anti-inflammatory and anti-ulcer activity in conditions such as hepatic ischemia-reperfusion injury and stomach ulceration [25-27]. Cheah et al. [15] have reported GSE to reduce inflammation, neutrophil infiltration and disease severity scores following small intestinal damage induced by the chemotherapy drug, 5-Fluorouracil (5-FU). These investigators further reported that the protective effects of GSE were less pronounced in the proximal small intestine (also the site of maximal injury), compared to the distal small intestine in regards to both villus height and mucosal thickness. The current study of DSS colitis employed a similar dose and duration of GSE treatment [15]. The colon is the major site of DSS-induced injury. However, recent studies have suggested that DSS also affects the distal small intestine (ileum) [28-30]. Recently, Geier et al. [30] reported that 2% DSS ingestion for 7 days altered distal ileum morphological structure, with an increased ileal crypt depth and crypt cell proliferation.

The intestine has the ability to compensate in the event that a segment is resected or injured [31]. These compensatory actions include increases in villus height, crypt depth (via cell hyperplasia) and enterocyte proliferation [31]. In the current study, it was likely that the increase in villus height and crypt depth detected in the distal ileum was an adaptive retrograde response to large intestinal damage, and not a direct result of DSS in the ileum, based on evidence displaying the ability of the small bowel to adapt to large bowel resection or damage [32]. This was also likely the case for the increase in crypt depth in the colon evident in colitic animals.
In the current study, GSE (400mg/kg) was effective at treating specific elements of DSS-induced colitis. GSE improved villus height and mucosal thickness, further returning both villus height and mucosal thickness back to normal values in the distal ileum. Moreover, GSE significantly reduced disease severity in the proximal colon, and histological analysis revealed that the numbers of immune cells (polymorphonuclear cells) were increased in the damaged tissue. It is possible that the basis for GSE efficacy was a consequence of its free-radical scavenging activity, particularly reactive oxygen species (ROS) and reactive nitrogen species (RNS) [33], and regulation of the release of proinflammatory cytokines [25]. ROS and RNS are released when immune cells are stimulated [33]. Release of these radicals is a hallmark of DSS-affected tissue. Increased levels of these species induce oxidative stress, which in turn results in an impairment of metabolism and eventually cell death and tissue destruction. Şehirli, et al. [25] suggested that potent GSE free-radical scavenging activity likely increased the availability of free Glutathione, an important constituent of protective intracellular mechanisms against noxious stimuli, which could then detoxify reactive intermediate oxidant species. Pro-inflammatory cytokines, such as tumour necrosis factor (TNF)-α, are released, in part, as a result of apoptosis (programmed cell death) [25]. GSE-sourced proanthocyanidins have also been shown to reduce cell apoptosis [26]. In the current study, GSE may have decreased certain indicators of inflammation in the intestinal wall as a result of an inhibition of cell apoptosis, thereby preventing the release of these agents. The mechanism by which GSE could prevent or ameliorate bowel damage therefore requires further investigation. Future studies will be performed either by ELISA or qRT PCR to identify the mechanism of GSE in reducing intestinal inflammation.
The current study revealed that the effects of GSE on parameters of colitis tended to decrease during passage of the extract through the bowel. Cheah et al. [15] reported beneficial effects of GSE in the distal small intestine. In the current study, there was a notable amelioration of DSS-effects in the distal ileum. As GSE entered the large bowel, partial protection from DSS damage was apparent in the proximal colon, as evidenced by histological analysis; however, with further passage of GSE into the distal colon, there was no demonstrable effect of GSE in this region. The decline in GSE bioactivity in the large bowel may have been due to its degradation by endogenous microflora. It is therefore possible that GSE could protect the distal colon if administered in greater quantities, by the intra-rectal route or if protected by micro-encapsulation. Targeted encapsulation, or an enema preparation, could potentially be used to deliver GSE directly to the inflamed areas, and maximise its bioavailability in these region.

The $^{13}$C-SBT was employed as a non-invasive indicator of small intestinal health. This technique is usually applied to detect small intestinal injury and assess novel therapeutic agents [24]. In the current study, the SBT did not detect any significant changes in intestinal sucrase activity following 11 days of GSE ingestion in healthy animals. In addition, there was no effect of GSE on any of the assessed parameters in healthy animals, indicating that there were no deleterious effects of GSE as it came into contact with the healthy bowel, strengthening its safety for potential human administration.

In conclusion, the current study revealed GSE to be a safe product capable of ameliorating selected parameters of experimentally-induced colitis.
Acknowledgements

The authors would like to acknowledge Ruth Lindsay, Roger Yazbeck, Mark Geier, Lynn Scarman and Jo Cool for their assistance with the animal experiments, data collection and data analysis. Professor Gordon Howarth is supported by the Sally Birch Cancer Council Australia Research Fellowship in Cancer Control. None of the authors had a personal or financial conflict of interest.


Table 1  Total water intake (mL), urine output (mL) feed intake (g) and faecal output (g) for the periods of day 0-day 5, day 6-day 11 and day 0- day 11 in male Sprague Dawley rats ingesting DSS or water and receiving GSE or water gavage.

<table>
<thead>
<tr>
<th>Time period</th>
<th>Water/Water</th>
<th>Water/GSE</th>
<th>DSS/Water</th>
<th>DSS/GSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Intake (mL)</td>
<td>0-5</td>
<td>6-11</td>
<td>0-11</td>
<td>0-5</td>
</tr>
<tr>
<td></td>
<td>123.0 (10.9)</td>
<td>148.7 (10.8)</td>
<td>271.7 (19.4)</td>
<td>123.4 (5.5)</td>
</tr>
<tr>
<td>Urine Output (mL)</td>
<td>0-5</td>
<td>6-11</td>
<td>0-11</td>
<td>0-5</td>
</tr>
<tr>
<td></td>
<td>62.3 (7.7)</td>
<td>83.5 (7.4)</td>
<td>145.7 (15.0)</td>
<td>68.4 (4.0)</td>
</tr>
<tr>
<td>Food Intake (g)</td>
<td>0-5</td>
<td>6-11</td>
<td>0-11</td>
<td>0-5</td>
</tr>
<tr>
<td></td>
<td>85.9 (6.6)</td>
<td>99.8 (6.2)</td>
<td>185.8 (12.6)</td>
<td>91.6 (3.1)</td>
</tr>
<tr>
<td>Faecal Output (g)</td>
<td>0-5</td>
<td>6-11</td>
<td>0-11</td>
<td>0-5</td>
</tr>
<tr>
<td></td>
<td>8.8 (1.0)</td>
<td>11.5 (1.3)</td>
<td>20.2 (2.0)</td>
<td>9.3 (0.5)</td>
</tr>
</tbody>
</table>

Water intake and urine output are expressed as mean (mL) ± (SEM). Feed intake and faecal output are expressed as mean (g) ± (SEM).

* indicates p<0.05 compared to Water/Water
Table 2  Effects of GSE on gastrointestinal organ weights (g) in male Sprague-Dawley rats on day of kill (trial day 11) following ingestion of 2% DSS in drinking water from day 5.

<table>
<thead>
<tr>
<th>Weight (g)</th>
<th>Water/Water</th>
<th>Water/GSE</th>
<th>DSS/Water</th>
<th>DSS/GSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>4.3 ± 0.1</td>
<td>4.4 ± 0.1</td>
<td>4.2 ± 0.1</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>Liver</td>
<td>36.8 ± 0.7</td>
<td>36.6 ± 0.9</td>
<td>39.7 ± 1.0</td>
<td>37.8 ± 2.1</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.4 ± 0.2</td>
<td>2.4 ± 0.1</td>
<td>2.5 ± 0.1</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>Thymus</td>
<td>2.9 ± 0.2</td>
<td>3.4 ± 0.2</td>
<td>3.5 ± 0.2</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>Lungs</td>
<td>7.1 ± 0.8</td>
<td>6.1 ± 0.2</td>
<td>5.7 ± 0.2</td>
<td>6.8 ± 0.6</td>
</tr>
<tr>
<td>Left Kidney</td>
<td>5.0 ± 0.1</td>
<td>4.9 ± 0.1</td>
<td>4.9 ± 0.0</td>
<td>4.9 ± 0.2</td>
</tr>
<tr>
<td>Right Kidney</td>
<td>5.1 ± 0.2</td>
<td>5.0 ± 0.1</td>
<td>5.5 ± 0.4</td>
<td>4.9 ± 0.2</td>
</tr>
<tr>
<td>Stomach</td>
<td>5.7 ± 0.3</td>
<td>5.8 ± 0.2</td>
<td>5.7 ± 0.1</td>
<td>6.1 ± 0.3</td>
</tr>
<tr>
<td>Duodenum</td>
<td>2.4 ± 0.1</td>
<td>2.3 ± 0.1</td>
<td>2.5 ± 0.1</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>Jejuno-Ileum</td>
<td>20.8 ± 0.7</td>
<td>20.8 ± 0.4</td>
<td>22.4 ± 0.5</td>
<td>21.4 ± 1.1</td>
</tr>
<tr>
<td>Caecum</td>
<td>3.9 ± 0.3</td>
<td>4.3 ± 0.2</td>
<td>4.5 ± 0.2</td>
<td>4.5 ± 0.3</td>
</tr>
<tr>
<td>Colon</td>
<td>4.2 ± 0.1</td>
<td>4.1 ± 0.3</td>
<td>5.3 ± 0.3*</td>
<td>5.9 ± 0.4*</td>
</tr>
</tbody>
</table>

Visceral organ weights expressed as mean (organ %bodyweight) ± SEM. * indicates p<0.05 compared to Water/Water.
Table 3  Effects of GSE on gastrointestinal organ lengths (cm) in male Sprague-Dawley rats on day of kill (trial day 11) following ingestion of 2% DSS in drinking water from day 5.

<table>
<thead>
<tr>
<th>Length (cm)</th>
<th>Water/Water</th>
<th>Water/GSE</th>
<th>DSS/Water</th>
<th>DSS/GSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>6.7 ± 0.3</td>
<td>6.3 ± 0.2</td>
<td>6.6 ± 0.2</td>
<td>6.6 ± 0.3</td>
</tr>
<tr>
<td>Jejuno-Ileum</td>
<td>80.8 ± 1.7</td>
<td>81.3 ± 1.8</td>
<td>83.9 ± 1.3</td>
<td>80.9 ± 1.4</td>
</tr>
<tr>
<td>Colon</td>
<td>13.6 ± 0.4</td>
<td>13.7 ± 0.5</td>
<td>13.4 ± 0.4</td>
<td>13.7 ± 0.5</td>
</tr>
</tbody>
</table>

Visceral organ lengths expressed as mean ± SEM.
Figure Legends

**Fig. 1** Disease activity index scores after introduction of DSS into drinking water. Scores are expressed as mean ± SEM. * indicates p<0.05 compared to Water/Water.

**Fig. 2** Small intestinal sucrase activity assessed by the $^{13}$C-sucrose breath test on day 11. Data expressed as mean (% CD90) ± SEM.

**Fig. 3** Histological severity scores in the proximal (a) and distal (b) colon on day 11. * indicates p<0.05 compared to Water/Water. # indicates p<0.05 compared to DSS/Water.

**Fig. 4** Representative micrographs of histological sections from the proximal colon in rats treated with Water/water (a), Water/GSE (b), DSS/Water (c) and DSS/GSE (d) (original magnification x 40).

**Fig. 5** Villus height and crypt depth (a) and mucosal thickness (b) in the distal ileum on day 11. Data expressed as mean (μm) ± SEM. ** indicates p<0.01 and *** indicates p<0.001 compared to Water/Water. ## indicates p<0.01 compared to DSS/Water.

**Fig. 6** Crypt depth in the proximal colon (a) and distal colon (b) on day 11. Data expressed as mean (μm) ± SEM. * indicates p<0.05 compared to Water/Water.
Figure 1

Disease Activity Index Scores

- Water/Water
- Water/GSE
- DSS/Water
- DSS/GSE
Figure 2

![Graph showing % of CD90 in Water and DSS with GSE and Water comparison](image-url)
Figure 3

A
Figure 4
Figure 5
Figure 6

A

B

<table>
<thead>
<tr>
<th>Crypt Depth (m)</th>
<th>Water</th>
<th>GSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>300</td>
<td>330</td>
</tr>
<tr>
<td>DSS</td>
<td>300</td>
<td>330</td>
</tr>
</tbody>
</table>

Water

GSE

* * *

Crypt Depth (m)

<table>
<thead>
<tr>
<th>Water</th>
<th>DSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>300</td>
</tr>
<tr>
<td>DSS</td>
<td>330</td>
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</table>