A Dietary Supplement Containing Plant Sterols Exerts a Positive Effect on Inflammatory Markers in a Chronic Colitis Murine Model †

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Abstract: Plant sterols (PSs) have reported benefits in alleviating colitis in mice, but the mechanisms involved require further investigation. This study aimed to evaluate the effect of a dietary supplement containing PSs (PS-DS) on inflammation biomarkers in a mice model of chronic ulcerative colitis induced by dextran sulfate sodium (DSS). C57BL/6J mice (n = 34) were exposed to 1.5% DSS in drinking water for three 5-day periods, with 10-day rest intervals in between. The mice received daily PS-DS (35 mg PS/kg) or placebo by oral gavage, either simultaneously (treatment) or 30 days prior (pre-treatment) to DSS exposition. After euthanasia, myeloperoxidase (MPO) activity and the levels of pro- (TNF-α and IL-6) and anti-inflammatory cytokines (IL-10) in the colonic tissue were analyzed. PS-DS treatment reduced (vs. DSS + placebo) myeloperoxidase (MPO) activity (2.6 ± 0.2 vs. 2.1 ± 0.1-fold change) and levels of TNF-α (85 ± 11 vs. 39 ± 7 pg/mg protein) and IL-6 (214 ± 26 vs. 128 ± 18 pg/mg protein), increasing the levels of IL-10 (46 ± 5 vs. 136 ± 16 pg/mg protein). PS-DS pre-treatment provided a greater inhibition (vs. treatment) of MPO activity (2.1 ± 0.3 vs. 1.3 ± 0.1-fold change) and a greater increase in IL-10 levels (50 ± 9 vs. 178 ± 30 pg/mg protein). These findings suggest that PS-DS has the potential to alleviate colitis in mice by modulating the inflammatory response and reducing oxidative stress. However, studies in humans are required to validate and fully understand its anti-inflammatory effect.

Keywords: cytokine; myeloperoxidase; phytosterols; ulcerative colitis

1. Introduction

Ulcerative colitis (UC), a classic phenotype that falls under inflammatory bowel disease, represents a challenge for healthcare systems worldwide due to its high incidence [1]. Despite advances in medical management, a significant proportion of UC patients continue to experience inadequate symptom control, underscoring the need for innovative therapeutic approaches. In recent years, natural bioactive compounds have emerged as promising candidates to address the unmet clinical needs in chronic UC management. Among these, plant sterols (PSs), phytochemicals widely recognized for their cholesterol-lowering properties, stand out for their potential anti-inflammatory effects [2]. In this sense, PSs at doses between 10 and 150 mg/kg have shown positive results in animal models of colitis induced by oxazolone [3], trinitrobenzene sulfonic acid (TNBS) [3,4], dextran sulfate sodium (DSS) [5,6], or a high-fat diet [7,8], attenuating symptoms, colon shortening, and...
histopathological damage in the colon. However, it is unknown whether PS are effective in combating colitis when administered before its onset, which would help to elucidate its preventive capacity. From this perspective, it is reasonable to consider that bioactive compounds possessing anti-inflammatory potential might not only aid in the recovery from existing damage but could also supply defense mechanisms to a healthy intestine, safe-guarding it against the effects of inflammation-inducing agents. This exploration could offer information about the potential of PSs as valuable therapeutic adjuncts in UC, as well as to identify their role in the prevention of inflammatory disorders in the gastrointestinal tract.

The objective of this study was to investigate the anti-inflammatory effect of PSs in a mice model of DSS-induced chronic ulcerative colitis, exploring their suitability for both alleviating established colitis (treatment) as well as preventing the development of the inflammation in colon (pre-treatment). This study provides a quantitative comparison between therapeutic and preventive approaches for the first time, aiming to identify the most advantageous strategy for reducing inflammation. In addition, this preliminary research seeks to determine whether PSs could serve as a viable dietary approach for mitigating complications linked to UC, thereby laying the groundwork for a subsequent study involving patients in the remission phase of the condition.

2. Materials and Methods

2.1. Preparation of the Dietary Supplements of Plant Sterols

A dietary supplement of PSs (PS-DS) is a powdered product (Lipophytol® P Dispersable, Lipofoods) that contains PSs extracted from tall oil to which excipients are added to promote its solubility in aqueous media. The product that contains only excipients (i.e., placebo) was used as a control. The PS profile was determined following the established protocols [9], yielding the subsequent composition (g/100 g): β-sitosterol, 60.7 ± 0.8; sitostanol, 15.9 ± 0.7; campesterol, 6.7 ± 0.3; campestanol, 2.0 ± 0.1; Δ7-stigmastenol, 0.51 ± 0.02; stigmasterol, 0.48 ± 0.02; Δ7-avenasterol, 0.33 ± 0.01; Δ5,24-stigmastadienol, 0.247 ± 0.003; Δ5-avenasterol, 0.085 ± 0.004; and total PS, 86.8 ± 1.8. An aqueous suspension of PS-DS was prepared to provide a daily dose of 35 mg PS/kg body weight following a previous study [10].

2.2. Animals and Chronic Ulcerative Colitis Model

Female C57BL/6j mice (8 weeks old, 18–20 g body weight) were placed in controlled conditions set at 22 °C, 60% relative humidity, and a 12/12 light/dark cycle. After the acclimatization period (7 days), mice were randomized into different groups. Controls groups (3 mice/group) were not exposed to DSS and received a placebo or PS-DS daily via intragastric gavage. The remaining groups (7 mice/group) were exposed to 1.5% (w/v) DSS in drinking water for three 5-day periods, with 10-day rest intervals in between, and received a placebo or PS-DS either simultaneously (treatment) or beginning 30 days prior to DSS exposure (pre-treatment).

2.3. Myeloperoxidase Activity

Neutrophil infiltration in the colon was indirectly assessed by measuring myeloperoxidase (MPO) activity. Colons were homogenized using 80 mM sodium phosphate buffer (pH 5.4) with 0.5% (w/v) hexadeyltrimethylammonium bromide. After the centrifugation of homogenates (12,000 × g, 4 °C for 15 min), MPO activity was measured by spectrophotometrically determining (630 nm) the oxidation of 3,3′,5,5′-tetramethylbenzidine, as previously described [10].

2.4. Cytokine Determination

Cytokines were extracted from the colon using the RIPA lysis buffer. Homogenates were clarified (15,000 × g, 4 °C for 15 min) and the total protein was quantified using
Bradford’s method. Cytokines (TNF-α, IL-6, and IL-10) were determined using ELISA kits (Invitrogen, Carlsbad, CA, USA) and normalized to total protein content.

2.5. Statistical Analysis

Results were expressed as mean ± standard deviation (n = 3 or 7, in control or DSS group, respectively). One-way analysis of variance (ANOVA), followed by post hoc HSD Tukey test, was applied to determine statistically significant differences (p < 0.05) between groups using Statgraphics Plus 5.1 software.

3. Results and Discussion

3.1. Plant Sterols Inhibit Neutrophil Infiltration in Mice Colon

As shown in Figure 1, DSS exposure induced neutrophil recruitment in the colon, as inferred from the significant (p < 0.05) increase in MPO activity compared to non-DSS exposed mice.

![Figure 1](image)

Figure 1. Fold-change over control (placebo) of myeloperoxidase activity (mean ± standard deviation) in colon tissue of control (i.e., non-DSS exposed mice) (3 mice/group) and dextran sulfate sodium (DSS) groups (7 mice/group) receiving daily placebo or plant sterol dietary supplement (PS-DS, 35 mg PS/kg body weight). DSS-exposed mice received placebo or PS-DS simultaneously (treatment) or 30 days before (pre-treatment) the exposure to DSS. Different letters (a–c) indicate statistically significant differences (p < 0.05) between the groups.

Although PS-DS treatment reduced MPO activity by 15%, pre-treatment resulted in the complete inhibition of neutrophil infiltration, as control values were reached. It was previously reported that the oral administration of β-sitosterol (10–20 mg/kg) reduced MPO activity (28–43%) in mouse models of intestinal inflammation induced by TNBS [4] or a high-fat diet [7], results that were confirmed in the present study. However, the novel finding of this study was that PSs, when administered before the disease was established, provided defense mechanisms that protected against the subsequent induction of inflammation, thus revealing the preventive role of PSs beyond the therapeutic one.

3.2. Effect of Plant Sterols on Cytokine Expression in Mice Colon

As expected, DSS induced a significant increase (vs. healthy groups, p < 0.05) in colonic levels of the pro-inflammatory cytokines (TNF-α and IL-6), in turn reducing IL-10 levels (Table 1). PS-DS treatment attenuated (vs. DSS + placebo, p < 0.05) TNF-α and IL-6 increase (55 and 40%, respectively), increasing IL-10 expression 2.9-fold. In addition, PS-DS pre-treatment provided greater benefits in the regulation of cytokine expression, since the increase in IL-10 levels (3.5-fold) was higher (p < 0.05) compared to PS-DS treatment. These results were consistent with previous studies in which the oral administration of β-sitosterol (20 mg/kg body weight) attenuated TNF-α (45–48%) or IL-6 (56–60%) increase induced by TNBS [4] or a high-fat diet [7]. In addition, stigmasterol (added to the basal diet
at 0.4%) was reported to inhibit the DSS-induced TNF-α increase (58%) without affecting colonic levels of IL-6, although β-sitosterol had no protective effect [6].

Table 1. Levels (pg/mg protein) of pro- (TNF-α and IL-6) and anti-inflammatory (IL-10) cytokines in mice colon.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TNF-α</th>
<th>IL-6</th>
<th>IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control + placebo</td>
<td>8.1 ± 1.1 ^a</td>
<td>18.6 ± 1.7 ^a</td>
<td>281.1 ± 45.7 ^a</td>
</tr>
<tr>
<td>Control + PS-DS</td>
<td>6.5 ± 0.6 ^a</td>
<td>21.2 ± 1.8 ^a</td>
<td>246.8 ± 19.5 ^a</td>
</tr>
<tr>
<td>DSS + placebo (treatment)</td>
<td>85.1 ± 11.1 ^b</td>
<td>213.7 ± 25.6 ^b</td>
<td>46.2 ± 5.0 ^b</td>
</tr>
<tr>
<td>DSS + PS-DS (treatment)</td>
<td>38.5 ± 6.8 ^c</td>
<td>127.9 ± 17.6 ^c</td>
<td>135.8 ± 15.7 ^c</td>
</tr>
<tr>
<td>DSS + placebo (pre-treatment)</td>
<td>55.7 ± 7.8 ^c</td>
<td>145.5 ± 19.6 ^c</td>
<td>50.3 ± 8.9 ^b</td>
</tr>
<tr>
<td>DSS + PS-DS (pre-treatment)</td>
<td>20.8 ± 0.7 ^d</td>
<td>83.2 ± 9.7 ^d</td>
<td>178.2 ± 30.1 ^c</td>
</tr>
</tbody>
</table>

Control (i.e., non-DSS exposed mice) (3 mice/group) and dextran sulfate sodium (DSS) groups (7 mice/group) receiving daily placebo or plant sterol dietary supplement (PS-DS, 35 mg PS/kg body weight). DSS-exposed mice received placebo or PS-DS simultaneously (treatment) or 30 days before (pre-treatment) the exposure to DSS. Different letters (a–d) indicate statistically significant differences (p < 0.05) between the groups in the same cytokine. Data are shown as mean ± standard deviation.

Again, the results of this study confirm that PS administration improves cytokine expression, as demonstrated by other investigations. However, the most relevant finding of the present study shows that PS administration before disease onset provides the greater regulation of cytokine expression. This suggests that, during those 30 days of pre-treatment, the PSs reinforce the antioxidant and anti-inflammatory defenses of the colonic tissue, making it more resistant to the colitogenic effect of DSS. The finding of the preventive role of PS-DS in the mice model of intestinal inflammation opens avenues for further research and paves the way for possible clinical applications, especially in people at risk of developing intestinal inflammation or with a history of such conditions, i.e., PS-DS could be considered as a prophylactic agent in such cases.

4. Conclusions

This study demonstrates the potential of PS-DS to alleviate inflammation in a mice model of chronic UC. The greater effect of pre-treatment, both in the inhibition of neutrophil infiltration and in the regulation of cytokine balance, suggests that PS-DS could not only hold promise as a therapeutic intervention for UC, but also provide a prophylactic effect, contributing to reducing the risk of inflammatory bowel disorders.


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