biology and life sciences forum

MDPI

Proceedings

Dietary supplement containing plant sterols exerts a positive effect on inflammatory markers in a chronic colitis murine model †

Mussa Makran[1], Rosa María Giner[2], Antonio Cilla[1,*], María Carmen Recio[2] and Guadalupe Garcia-Llatas[1]

[1] Nutrition and Food Science Area, Faculty of Pharmacy, University of Valencia, Avda. Vicente Andrés Estellés s/n, 46100 Burjassot (Valencia), Spain; Mussa.Makran@uv.es (M.M.); Antonio.cilla@uv.es (A.C.); Guadalupe.garcia@uv.es (G.G.-L.)

[2] Department of Pharmacology, Faculty of Pharmacy, University of Valencia, Avda. Vicente Andrés Estellés s/n, 46100 Burjassot (Valencia), Spain; Rosa.M.Giner@uv.es (R.M.G.); Maria.C.Recio@uv.es (M.C.R.)

* Correspondence: Antonio.cilla@uv.es; Tel.: +34-963-544-972

† Presented at the 4th International Electronic Conference on Foods: Focus on Sustainable Food Systems: Current Trends and Advances, 15-30 October 2023; Available online: https://foods2023.sciforum.net/ .

Abstract: Plant sterols (PS) have reported benefits in alleviating colitis in mice, but the mechanisms involved require further investigation. The study aimed to evaluate the effect of a dietary supplement containing PS (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

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Lastname

Published: date

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 (PS), phytochemicals widely recognized for their cholesterol-lowering properties, stand out for their potential anti-inflammatory effects [2]. In this sense, PS at doses between 10–150 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 high-fat diet [7,8], attenuating symptoms, colon shortening and histopathological damage in 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, safeguarding it against the effects of inflammation-inducing agents. This exploration could offer information about the potential of PS as valuable therapeutic adjuncts in UC, as well as to identify its role in the prevention of inflammatory disorders in the gastrointestinal tract.

The objective of this study was to investigate the anti-inflammatory effect of PS 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 PS 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 dietary supplements of plant sterols

Dietary supplement of PS (PS-DS) is a powdered product (Lipophytol® P Dispersable, Lipofoods) that contains PS 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; Δ^7-stigmastenol, 0.51 ± 0.02; stigmasterol, 0.48 ± 0.02; Δ^7-avenasterol, 0.33 ± 0.01; $\Delta^{5,24}$-stigmastadienol, 0.247 ± 0.003; Δ^5-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 a 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 recived daily via intragastric gavage placebo or PS-DS. 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 recived placebo or PS-DS either simultanously (treatment) or began 30 days prior to the DSS exposure (pre-treatment).

2.3. Myeloperoxidase activity

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

2.4. Cytokine determination

Cytokines were extracted from colon using the RIPA lysis buffer. Homogenates were clarified (15,000 × g, 4°C for 15 minutes) and total protein was quantified by Bradford´s

method. Cytokines (TNF-α, IL-6 and IL-10) were determined by ELISA kits (Invitrogen) and normalized to total protein content.

2.5. Stastical analysis

Results were expressed as mean ± standar desviation (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 colon mice

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

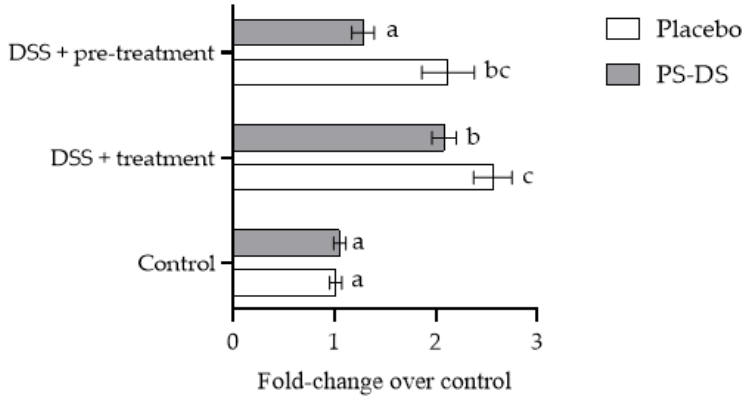

Figure 1. Fold-change over control (placebo) of myeloperoxidase activity (mean ± standard deviation) in colon tissue of control (*i.e.*, non-DSS exposed mice) (3 mice/group) and dextran sulfate sodium (DSS) groups (7 mice/group) receiving daily placebo or plant sterol dietary supplement (PS-DS, 35 mg 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 difference ($p < 0.05$) between the groups.

Although PS-DS treatment reduced MPO activity by 15%, pre-treatment resulted in complete inhibition of neutrophil infiltration, as control values were reached. It was previously reported that 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 high-fat diet [7], results that were confirmed in the present study. However, the novel finding of this study was that PS, when administered before the disease is established, provides defense mechanisms that protect against the subsequent induction of inflammation, thus revealing the preventive role of PS, beyond therapeutic one.

3.2. Effect of plant sterols on cytokine expression in colon mice

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 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.

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

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 difference ($p < 0.05$) 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 greater regulation of cytokine expression. This suggests that during those 30 days of pre-treatment, the PS 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 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. Conclusion

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 reduce the risk of inflammatory bowel disorders.

Author Contributions: Conceptualization, A.C., R.M.G., M.C.R., and G.G.-L.; formal analysis, M.M.; investigation, M.M., A.C., R.M.G., M.C.R., and G.G.-L.; data curation, M.M.; writing—original draft preparation, M.M.; writing—review and editing, M.M., A.C., R.M.G., M.C.R., and G.G.-L.; supervision, A.C., R.M.G., M.C.R., and G.G.-L.; funding acquisition, A.C., and G.G.-L. All authors have read and agreed to the published version of the manuscript.

Funding: This study is part of the project PID2019-104167RB-I00 funded by MCIN/AEI/10.13039/501100011033. Mussa Makran holds an FPU19/00156 grant from the Ministry of Universities (Spain).

Institutional Review Board Statement: The animal study protocol was approved by the Generalitat Valenciana, Spain (protocol code 2022/VSC/PEA/0256).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Burisch, J.; Pedersen, N.; Cukovic-Cavka, S.; Brinar, M.; Kaimakliotis, I.; Duricova, D.; Shonová, O.; Vind, I.; Avnstrøm, S.; Thorsgaard, N.; *et al*. East-West gradient in the incidence of inflammatory bowel disease in Europe: The ECCO-EpiCom inception cohort. *Gut* **2014**, *63*, 588–597.
2. Salehi, B.; Quispe, C.; Sharifi-Rad, J.; Cruz-Martins, N.; Nigam, M.; Mishra, A.P.; Konovalov, D.A.; Orobinskaya, V.; Abu-Reidah, I.M.; Zam, W.; *et al*. Phytosterols: From Preclinical Evidence to Potential Clinical Applications. *Front. Pharmacol.* **2021**, *11*, 599959.
3. Mencarelli, A.; Renga, B.; Palladino, G.; Distrutti, E.; Fiorucci, S. The plant sterol guggulsterone attenuates inflammation and immune dysfunction in murine models of inflammatory bowel disease. *Biochem. Pharmacol.* **2009**, *78*, 1214–1223.
4. Lee, I.A.; Kim, E.J.; Kim, D.H. Inhibitory effect of β-sitosterol on TNBS-induced colitis in mice. *Planta Med.* **2012**, *78*, 896–898.
5. Aldini, R.; Micucci, M.; Cevenini, M.; Fato, R.; Bergamini, C.; Nanni, C.; Cont, M.; Camborata, C.; Spinozzi, S.; Montagnani, M.; et al. Antiinflammatory effect of phytosterols in experimental murine colitis model: Prevention, induction, remission study. *PLoS ONE* **2014**, *9*, e108112.
6. Feng, S.; Dai, Z.; Liu, A.; Wang, H.; Chen, J.; Luo, Z.; Yang, C.S. β-Sitosterol and stigmasterol ameliorate dextran sulphate sodium-induced colitis in mice fed a high fat Western-style diet. *Food Funct.* **2017**, *8*, 4179–4186.
7. Kim, K.A.; Lee, I.A.; Gu,W.; Hyam, S.R.; Kim, D.H. β-Sitosterol attenuates high-fat diet-induced intestinal inflammation in mice by inhibiting the binding of lipopolysaccharide to toll-like receptor 4 in the NF-κB pathway. *Mol. Nutr. Food Res.* **2014**, *58*, 963–972.
8. Te Velde, A.A.; Brüll, F.; Heinsbroek, S.E.; Meijer, S.L.; Lütjohann, D.; Vreugdenhil, A.; Plat, J. Effects of dietary plant sterols and stanol esters with low-and high-fat diets in chronic and acute models for experimental colitis. *Nutrients* **2015**, *7*, 8518–8531.
9. González-Larena, M.; García-Llatas, G.; Vidal, M.C.; Sánchez-Siles, L.M.; Barberá, R.; Lagarda, M.J. Stability of plant sterols in ingredients used in functional foods. *J. Agric. Food Chem.* **2011**, *59*, 3624–3631.
10. López-García, G.; Cilla, A.; Barberá, R.; Alegría, A.; Recio, M.C. Effect of a milk-based fruit beverage enriched with plant sterols and/or galactooligosaccharides in a murine chronic colitis model. *Foods* **2019**, *8*, 114.