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Research Article

Protective and therapeutic effects of hesperidin in an experimental colitis model

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Abstract: Ulcerative colitis is a gastrointestinal problem with increasing prevalence. In this study, the effect of hesperidin (commonly found in citrus white peel) on colitis was investigated. For this purpose, 28 Wistar albino female rats, 3–4 months old and weighing between 250–350 g, were used in the study. Rats were randomly divided into four groups: control (n=7), colitis (n=7), protective effect (n=7), and treatment (n=7) groups. Colitis was induced in rats with acetic acid (except control), and hesperidin was then administered at 150 mg/kg for 1 week before and after colitis. At the end of the study, IFN-γ and IL-6 values in blood, TAS, TOS, and OSI in tissue were evaluated. Intestinal tissues were assessed visually by haematoxylin and eosin staining. Our results showed that the levels of IFN-γ and IL-6 were highest in the colitis group and lowest in the treatment group, with statistical significance. The most histopathological damage was seen in the colitis group, while less prevalent in the treatment and control groups. The results of the study show that hesperidin had limited protective and therapeutic effects on experimental colitis mouse models.

1. INTRODUCTION

Inflammatory bowel disease is a chronic problem that begins with intestinal inflammation, progresses with impaired immune response, and significantly affects patients' quality of life (Nazlıkul, 2020). Although the exact cause is unknown, inflammatory bowel disease is reportedly affected by the immune system, genetic factors, and changing intestinal microbiota (Galipeau *et al*., 2020). These diseases affect millions of people worldwide (Tahvillian *et al*., 2020). Attacks and remission are characteristic of ulcerative colitis, while the aetiology is unknown. Aminosalicylates, antibiotics, systemic steroids, local steroids, immunomodulators, methotrexate, cyclosporine, biological agents, and mycophenolate mofetil are used medically for ulcerative colitis. Untreated ulcerative colitis leads to hospitalisation, surgery, and an increased risk of colorectal cancer.

Hesperidin is the most abundant flavonoid glycosides found in citrus peels. Its consumption is beneficial for health and helps prevent heart, brain and eye diseases. Hesperidin has analgesic, anti-inflammatory, anti-hypercholesterolemic, and anti-hypertensive properties (Victor *et al*., 2020). Crespo *et al*., (1999), showed that 10 mg/kg and 25 mg/kg doses of hesperidin reduced

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colon damage in rats. Furthermore, others have reported that administering 10, 40, and 80 mg/kg hesperidin doses had beneficial effects on ulcerative colitis in mice (Xu *et al*., 2009). There are studies on the therapeutic effect of hesperidin on colitis; however, its protective effects are unreported. This study aimed to examine the protective effects of hesperidin in a rat model of ulcerative colitis.

2. MATERIALS and METHODS

The experimental study began after receiving the local ethics committee's approval (Approval number: 64583101/2021/140), and special attention was paid to international ethical rules (2010/63/EU). 28 female Wistar albino rats were used in the study. Rats were kept in standard transparent plastic cages, paying attention to cleanliness and hygiene conditions. They had 24-hour access to drinking water and food and were kept in a temperature-controlled room (22±2°C) under a 12-hour light/dark cycle. Rats were separated into four groups: the control (n=7), colitis (n=7), protective effect (n=7), and treatment (n=7) groups. Hesperidin (Sigma Aldrich, Cat: H5254) was dissolved in 0.5% carboxymethyl cellulose as it does not fully dissolve in water. To the protective group, 150 mg/kg hesperidin was administered via gavage to the protective effect group, 7 days before colitis occurred. For the treatment group, after inducing colitis with acetic acid, hesperidin was administered orally at 150 mg/kg for 7 days, starting from day 0. To induce colitis in rats, 4% acetic acid solution (1 mL, pH 2.3) was slowly applied to the rectum via an intrarectal catheter under anaesthesia (Oruc *et al*., 2008). After colitis, the rats were kept in a head-down position. Once the treatments were completed, the rats were euthanised by the cervical dislocation method under ketamine (50 mg/kg) and xylazine (5 mg/kg) anaesthesia. Blood and intestinal tissues were taken and stored.

2.1. Biochemical Analysis

Blood taken from the rats was centrifuged at 3500 rpm for 10 minutes, and the supernatant for measurements was transferred to tubes. Samples were placed in a -20°C freezer and stored until the day of analysis. Total antioxidant level (TAS) and total oxidant level (TOS) were measured using the Rel Assay Diagnostic test kit according to the manufacturer's instructions. The method specified by Erel (2004) was used in the analyses. The results were calculated as mmolTrolox Equiv/L and μmolH_2O_2 Equiv/L. OSI (Oxidative stress index) was calculated using TAS and TOS values. Serum IFN-γ levels were measured with a kit purchased from Bioassay Technology Laboratory (Cat: E0103Ra). IFN-γ levels are expressed as ng/mL. For serum measurement, IL-6 levels were purchased from Bostonchem (Cat: BLS-1158Ra).

2.2. Histopathological Evaluation

Tissue samples were placed in 10% formaldehyde solution for fixing. 5 μm thick sections were cut and stained with haematoxylin and eosin. The samples were then examined with a trinocular research microscope (Pereira *et al*., 2010), and microphotographs were taken (Olympus BX51, Tucsen 5MP digital camera). When evaluating colitis lesions, mucosal epithelial degeneration and necrosis, inflammation, hyperaemia, oedema, necrosis, and haemorrhage criteria were used and classified according to the severity and extent of the lesion: (-) none, (+) light, (++) medium, (+++) severe, and a semiquantitative scoring was made as (++++) very Severe.

2.3. Statistical Evaluation

Compliance with normal distribution was evaluated with the Shapiro–Wilk test. All results were compared with each other statistically, and the changes were tested to determine for significance. For this purpose, the one-way ANOVA method was applied. Tukey's HSD was used as a post hoc test for within-group significance. A value of $p<0.05$ was considered statistically significant.

3. RESULTS

3.1. Biochemical Test Results

According to biochemical analyses, serum TAS values showed a significant difference in the control, colitis, protective effect, and treatment groups. In the intergroup evaluation, a significant difference was found between the control and colitis group ($p<0.001$). There was a decrease in TAS values between the control and protective effect and treatment groups, which was not significant ($p>0.05$, Table 1). In the TOS evaluation, a significant difference was observed between the groups ($p<0.001$). For intragroup TOS evaluation, a significant difference was determined between the protective effect ($p<0.05$) and treatment ($p<0.001$) groups. The TOS value, which increased in the colitis group, decreased in the treatment and protective effect groups; a significant difference was observed only in the treatment group. As a result of the ANOVA analysis performed with the data obtained in the oxidative stress index (OSI), These values showed a significant difference between the groups ($p<0.001$). When OSI values were examined, a significant difference was seen between the control and colitis groups (Table 1). OSI values were higher in the colitis group compared to the other groups. A significant difference was determined between the colitis group and the protective ($p<0.05$) and treatment ($p<0.05$) groups.

Table 1. TAS (total antioxidant status), TOS (total oxidant status), and OSI (oxidative stress index) values in colon tissue.

	Control	Colitis	Protective effect	Treatment	*p*
TAS (mmol Trolox Eq/L)	1.22±0.16	0.85±0.16	1.05±0.19	1.11±0.19	0.007
TOS (μmol H_2O_2 Eq/L)	9.98±1.49	16.43±2.14	13.57±2.20	14.37±1.82	0.001
OSI (index)	0.83±0.16	1.98±0.47	1.32±0.32	1.34±0.39	0.001

IFN-γ levels were significantly different between the groups ($p<0.001$). When the serum IFN-γ levels of the groups were examined, a significant difference was seen between the control, protective effect ($p<0.001$), and treatment ($p<0.001$) groups. An increase in IFN-γ levels was observed in the colitis, protective effect, and treatment groups compared with control. Serum IL-6 levels showed a statistical difference between groups ($p<0.001$). When the IL-6 levels within the groups were examined, a significant difference was observed between the control and protective effect ($p<0.001$) groups. IL-6 levels were not significantly different between the colitis group and the protective effect and treatment groups ($p>0.05$, Table 2).

Table 2. Blood IFN-γ (İnterferon gama) and IL-6 (Interleukin-6) levels.

	Control	Colitis	Protective effect	Treatment	*p*
IFN-γ (ng/mL)	25.08±2.01	43.87±5.57	36.41±4.84	33.35±4.28	0.001
IL-6 (pg/mL)	3.51±0.86^{c}	5.13±0.64^{a}	4.85±0.58^{b}	4.42±0.48^{b}	0.001

$^{a, b, c}$: different letters in the same column indicate a statistically significant difference.

3.2. Histopathological Findings

In the control group, there was normal colon histology with villi containing widespread goblet cells making papillary extensions towards the lumen between the intestinal mucosa surface epithelium and crypts. There were a small number of lymphoplasmacytic cells and a few eosinophil leukocytes in the lamina propria observed in a few cases (Figure 1). In the colitis group, widespread and advanced necrotic changes were observed, starting from the lamina epithelialis, including the lamina propria and submucosa, affecting the tunica muscularis to varying degrees and sometimes extending to the serosa. In the protective effect group,

widespread necrosis involving all intestinal layers was observed; however, histopathological changes were noted in the submucosal region, characterised by severe hyperaemia in the vessels, widespread oedema, intense inflammatory cell infiltrates, and haemorrhages of varying degrees. Although the tunica muscularis thickened with a homogeneous appearance and suffered necrosis in three cases, the areas above the submucosa were generally unaffected and there were no pathological changes in the epithelial layer or crypts. In the treatment group, while deep necrosis and haemorrhages involving the entire colon mucosa layer were detected in two cases, these changes were severe and affected the whole colon surface. In other instances, pathological changes were generally limited to the submucosa, characterised by hyperaemia, oedema, and severe inflammation, and the epithelial layer was not affected in these cases (Table 3 and Figures 1 - 2).

Table 3. Semiquantitative scoring of histopathological findings.

Groups	Epithelial degeneration	Inflammation	Hyperaemia	Oedema	Necrosis	Haemorrhage
Control	-	-	-	-	-	-
Colitis	+ + +	+ + + +	+ + +	+ + +	+ + +	+ + + +
Protective effect	+	++	+	+ +	+	+ +
Treatment	+ +	+ +	++	+ +	+ +	+ +

(-) normal, (+) mild, (++) moderate, (+++) severe, and (++++) high severe inflammation.

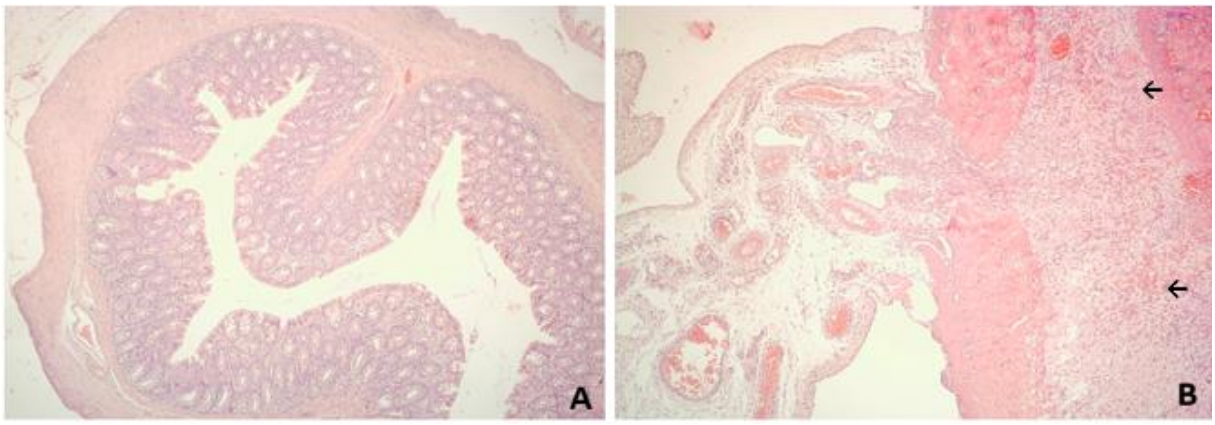

Figure 1. Histopathological images of colon tissue (H&E staining). **(A)** Control group: normal intestinal tissue appearance. **(B)** Colitis group: diffuse and advanced necrotic changes, bleeding, and inflammation in the intestinal tissue.

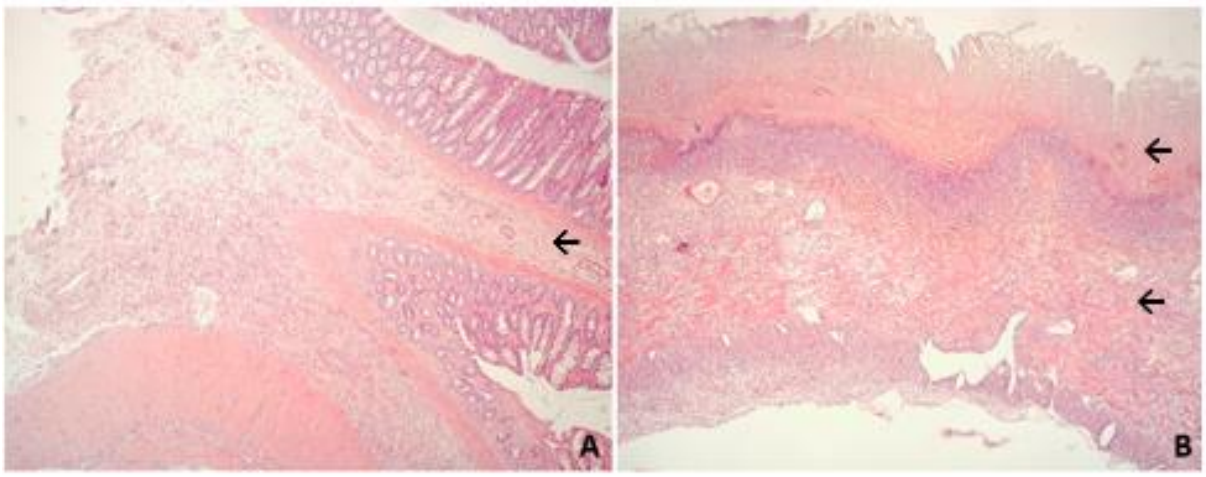

Figure 2. Tissue from hesperidin-treated rats. **(A)** Protective effect group: widespread necrosis involving all intestinal layers, pathological changes were shallow in three cases. **(B)** Treatment group: while deep necrosis and haemorrhages involving the entire colon mucosa layer were detected in two cases, pathological changes are generally limited to the submucosa in other cases characterised by hyperaemia, oedema and severe inflammation, and the epithelial layer was not affected.

4. DISCUSSION

Colitis is a highly prevalent inflammatory bowel disease. Although the exact cause is unknown, it is thought that genetic factors, the immune system, environmental factors, and changing intestinal microbiota might play a role in its aetiology. Immunological mechanisms play an active role in ulcerative colitis inflammation (Lu *et al*., 2022). Stimulation of immune cells results in the release of large amounts of cytokines and inflammatory mediators. Hesperidin is a flavonoid, is abundant in citrus fruits, and has a broad pharmacological effect (Xu *et al*., 2009). Hesperidin has anti-inflammatory, antioxidative, solid, and antimicrobial characteristics (Onal *et al*., 2022). In this study, there was a decrease in TAS values between the protective effect and treatment groups compared with the control group, and no significant difference was seen with these decreases ($p>0.05$). With TOS values, there was a significant difference between the groups ($p<0.001$). The decreases in the protective effect group were more significant than in the treatment group. The OSI values increased in the colitis group and decreased in the treatment and control groups, suggesting that hesperidin has a partial healing and protective effect on colitis by increasing the total antioxidant capacity and reducing the oxidant capacity. A study by Murata *et al*. (1995) showed increased levels of proinflammatory cytokines in ulcerative colitis. In another study by Funakoshi *et al*. (1998), they reported increased expression of IL-1β, IL-6, IL-8, and TNFα mRNA in patients with Crohn's disease and ulcerative colitis compared with the control group. In our study, there was a significant decreasing effect between the colitis group and the protective effect ($p<0.05$) and treatment ($p<0.001$) groups regarding serum IFN-γ levels. This finding shows that IFN-γ (essential in macrophage stimulation) is suppressed and has a reducing role in the severity of inflammation. There was a significant difference in IL-6 levels between the groups ($p<0.001$). In the same parameter, the protective effect and treatment groups showed statistically insignificant changes (Table 2).

In ulcerative colitis, significant pathological changes occur in the colon wall. The leading cause of this tissue damage is usually inflammation. As a result of inflammation, the immune system response is activated, and events such as the proliferation of lymphocytes, accumulation of neutrophils, and increased production of cytokines occur. In this study, normal colon histology was observed in the control group whereas in the colitis group, there was advanced colon epithelial degeneration and necrosis, inflammation, hyperaemia, oedema, necrosis, and haemorrhage (Figure 1). Lower scores were observed in the hesperidin protective effect and therapeutic groups compared with the colitis group. The lesions detected in the hesperidin

treatment group were observed to be more advanced than the protective effect group in severity and prevalence (Figure 2).

5. CONCLUSION

This experimental animal study concluded that hesperidin administered orally at 150 mg/kg/bw could partially reduce oxidative damage and tissue damage caused by colitis by exhibiting antioxidant and anti-inflammatory activity.

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Declaration of Conflicting Interests and Ethics

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Authorship Contribution Statement

Esra Güzel: Experimental study. **Serdar Aktaş:** Experimental study and experimental study. **Serdal Ogut:** Project manager.

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