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Gallic Acid Reduces Experimental Colitis in Rats by Downregulation of Cathepsin and Oxidative Stress

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ABSTRACT

Objective: Ulcerative colitis (UC) is an idiopathic inflammatory bowel disease (IBD) with common, repetitive inflammation of the colon and rectum, which is highly defined by loss of blood on colon mucosa, ulceration and acute inflammation. The present study aimed to investigate the potential protective effects of gallic acid (GA) through a 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis rat model, using biochemical and histopathological parameters.

Materials and Methods: The study consisted of four groups, each including seven rats, namely control group, colitis group, colitis-GA 50 mg/kg group and colitis-GA 100 mg/kg group. Colon tissue samples were analyzed for malondialdehyde (MDA), myeloperoxidase (MPO), cathepsin B and cathepsin L values.

Results: Tissue MDA, MPO, cathepsin L and cathepsin B values increased significantly in colitis group (p=0.028, p=0.038, p=0.024, p=0.019, respectively). However, MDA, MPO, cathepsin L and cathepsin B values showed a significant decrease in animals with GA (at a dose of 100 mg/kg) administration in TNBS-induced colitis in rats (p=0.021, p=0.026, p=0.019, p=0.031, respectively). Colitis group was defined by the severe detriment of surface epithelium, submucosal edema and inflammatory cell infiltration. Treatment with GA significantly decreased inflammatory cell infiltration.

Conclusion: GA can be used as an effective agent in the treatment of colitis due to its inhibitory properties in multiple pathways and its potent antioxidant effects.

Keywords: Cathepsin, colitis, gallic acid, myleperoxidase, oxidative stress

INTRODUCTION

Ulcerative colitis (UC) is accepted as inflammatory bowel disease (IBD), and this disease is chronic inflammatory cases impressive the gastrointestinal system (1). UC is an idiopathic IBD with common, repetitive inflammation of the colon and rectum, which is highly defined by loss of blood on colon mucosa, ulceration and acute inflammation (2). Severe diarrhea, abdominal pain, cramping, chronic fatigue, weight loss and dermatological complications are the well-known symptoms of UC (3, 4). Etiopathology of UC is complicated, including bacterial or viral infection, the difference of colon microbiota, extreme immune reply, and oxidative stress damage. Oxidative stress has well-known as an important pathogenic factor of UC (5, 6).

Lipid peroxidation is serious cellular damage and lipid peroxidation is formed by in the membrane, and attacking by reactive oxygen species (ROS) would lead to high structural and functional differences on membranes (6). Myeloperoxidase (MPO), which is among powerful oxidant sources in the living system, is an enzyme that is secreted by neutrophils at a high rate, and monocyte, macrophage and kupffer cells at a lower rate (7). MPO supports oxidative stress in many inflammatory situations, and it utilized the hydrogen peroxide to catalyze the generation of potent oxidants containing chlorine bleach and free radicals (8). Cathepsins also play a considerable role in physiological events, such as the antigen presentation in the immune system, wound healing, bone remodelling, and reproduction, apart from the proteolytic activities (9). Cathepsin B and cathepsin L that are the members of the cysteine cathepsin family act as a part of the proteolytic cascade and are present in an active and stable state in acidic cell structures such as lysosomes and endosomes (10).

Gallic acid (3,4,5 trihydroxybenzoic acid) (GA) is a product available in some plants. GA is accepted to display of bioactivities with the inclusion of anticancer, antimicrobial, antioxidant, and anti-inflammatory (11, 12). We investigated the putative protective role of two doses of gallic acid on 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced experimental colitis in rats.

MATERIALS AND METHODS

Chemicals

Gallic acid (97.5%), TNBS and all other chemicals of analytical grade were purchased from Sigma Chemical Co. (St. Louis, MO).

Table 1. Biochemical results of the groups

	MDA (nmol/mg protein)	MPO (U/mg protein)	Cathepsin B (ng/mg protein)	Cathepsin L (ng/mg protein)
Control	2.69±0.35	2.02±0.38	3.86±0.60	10.02±1.94
Colitis	4.29±0.74[a]	18.48±7.19[a]	7.47±1.12[a]	13.70±2.25[a]
Colitis-GA 50 mg/kg	4.03±1.19	16.76±6.58	5.13±2.77	12.39±5.41
Colitis-GA 100 mg/kg	3.02±0.49[b]	7.86±5.12[b]	4.99±1.48[b]	10.95±2.75[b]

Results are expressed as mean±SD. a: Significantly different when compared with the control group, ($p<0.05$); b: Significantly different when compared with colitis group, ($p<0.05$); MDA: Malondialdehyde; MPO: Myeloperoxidase

Animals

Twenty-eight adult male Sprague-Dawley rats (weighing between 230±20 g) were carried from TICAM (Medical and Surgical Experimental Research Centre, Eskisehir) and were proceeded in a light- and temperature-controlled room. The animals were fed the standard pellet diet and drinking water overnight before TNBS administration. The experimental method and application were confirmed by the Eskisehir Osmangazi University Institutional Ethical Committee for Animal Care (2009/21/127).

Experimental Design

The rats were incidentally separated into four groups, each containing seven animals. The first group was control healthy (C), the second group was colitis-untreated (Colitis), the third (Colitis-GA 50 mg/kg) and the fourth groups (Colitis-GA 100 mg/kg) were colitis and GA treated at the doses of 50 and 100 mg/kg b.w., respectively (13).

Induction of Colitis and Treatments

Rats are often starved overnight and colitis was formed by colonic application of 1 ml solution of 2,4,6-trinitrobenzene sulfonic acid (TNBS, 30 mg/ml in 50% ethanol) with an 8-cm cannula under mild ether anaesthesia (14).

One day after following the created of colitis; GA (at the doses of 50 and 100 mg/kg b.w., dissolved with saline 2 ml/kg volume) or saline (2 ml/kg volume) was given orally one time a day for seven days. At the end of seventh-day, an overnight fasting all the test animals were decapitated. The last 8 cm of the colon was cut, opened longitudinally, and washed with saline solution. Samples of colon tissue were obtained from the distal colon area were preserved at −20°C for next analsis of malondialdehyde (MDA), MPO, cathepsin B and cathepsin L levels.

Biochemical Analysis

Colon tissue protein was assayed by the method of bradford protein assay (15), using serum bovine albumin as standard. The MPO activity was performed using the spectrophotometrically method by measuring the transformation of taurine totaurine mono-chloramine using the 2-nitro-5-thiobenzoic acid (TNB) analysis (16). TNB reagent was presented by dissolving 5,5′-Dithiobis-2-nitrobenzoic acid (DTNB) in aqueous 0.05 M sodium hydroxide solution to make a final concentration of 1 mM DTNB and then diluting 1:40 into 0.1 M phos-phate buffer (pH 7.4). The absorbances were read at 412 nm wavelength. MPO activitiy was presented as U/mg protein.

The MDA levels were analyzed using the spectrophotometric method of thiobarbituric acid (TBA) test. A total of 500 µl of the homogenate was reacted with 250 µl of trichloroacetic acid (TCA) 20% for deproteination. The samples were vortexed and all samples were centrifuged at a 5000x rpm for 10 minutes. The supernatants were taken, and 500 µl TBA 0.67% was added. The samples were vortexed and incubated in a water heater at 96°C, 10 minutes and cool at room temperature, then, read the absorbances at a wavelength of 530 nm (17). MDA levels were presented as nmol/mg protein.

Activities of cathepsin B and cathepsin L were analyzed with a general procedure utilizing methylcoumarylamide substrates. Fluorescence was determined on a Jasco fluorescence spectrometer with a caution wavelength set to 360 nm and distribution wavelength set to 460 nm provided the density/5 min using the maximum linear rate for both the test and blank (13). Cathepsin activities were stated as ng/mg protein.

Histological Evaluation of Colonic Damage

For light microscopic assay, tissue samples (1 cm^2 specimen at 8 cm from anus was provided from all rat) were fixed in 10% neutral formaldehyde solution and performed by usual methods before embedding in paraffin. Part of tissue was cut at 5 µm on a revolving microtome, affixed on slides, stained with standard H&E. Tissues were studied under an Olympus CX31 photomicroscope. Every tissue parts were investigated microscopically for description of histopathological differences.

Statistical Analysis

Analyses of all data were performed using Statistical Package for the Social Sciences (SPSS) for Windows 16.0 package program (SPSS Inc., Chicago, IL, USA). Kolmogorov-Smirnov and Shapiro-Wilk tests were used to evaluate the distribution of continuous variables. Continuous normally distributed measurements were compared across the groups using one-way analysis of variance (ANOVA) with the Tukey method and the Student–Newman–Keuls method multiple comparisons. The descriptive statistics demonstrated the mean and standard deviation (S.D) for continuous variables. All differences were accepted significant at $p<0.05$.

RESULTS

Biochemical Results

The results of the experimental groups are presented in Table 1. When we compared our study groups, we observed that tissue

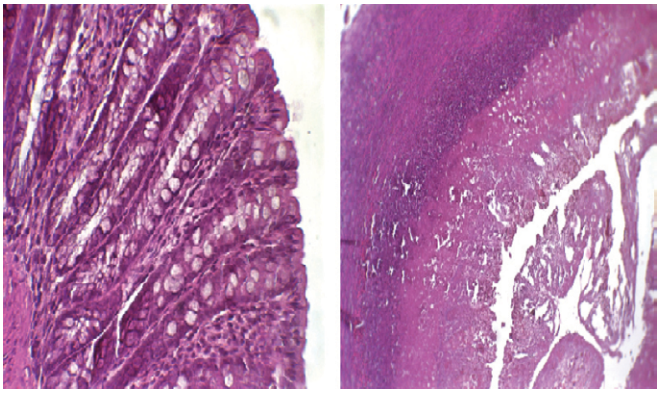

Figure 1. The control group was described as normal morphology. Colitis group; it was characterized by a massive loss of surface epithelium, submucosal edema and inflammatory cell infiltration

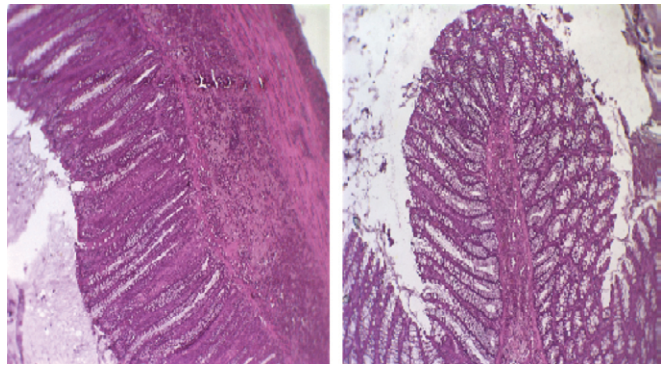

Figure 2. Gallic acid (50 mg/kg) colitis treated group: Mild inflammatory cell infiltration, regenerated glands and mild edema. Gallic acid (100 mg/kg) colitis treated group: regular surface epithelium, describe nearly a normal histological morphology

MDA (p=0.028), cathepsin L (p=0.024) and cathepsin B levels (p=0.019) with MPO (p=0.038) activity of the colitis group rose significantly in comparison to the control group. The dose of 100 mg/kg GA decreased tissue levels of MDA, cathepsin L and cathepsin B and activity of MPO enzyme concerning the colitis group, statistically significantly at p=0.021, p=0.019, p=0.031, p=0.026, respectively. However, the dose of 50 mg/kg GA decreased tissue levels of MDA, cathepsin L and cathepsin B and activity of MPO enzyme concerning the colitis group, but there were no statistically significant (p=0.299, p=0.169, p=0.431, p=0.634, respectively). The results displayed that post-treatment to colitis rats with GA, especially at a dose of 100 mg/kg, partially reduced the colonic damage induced TNBS.

Histological Results

The control group was described as normal morphology. TNBS caused significant macroscopic colonic tissue damage. The colitis group was defined by the severe detriment of surface epithelium, submucosal edema and inflammatory cell infiltration (Fig. 1). Treatment with 50 and 100 mg/kg of GA significantly decreased inflammatory cell infiltration. At a dose of 50 mg/kg of GA group, we observed mild inflammatory cell infiltration, regenerated glands and mild edema.

At a dose of 100 mg/kg of GA group, regular surface epithelium, described nearly a normal histological morphology, was observed. All doses of GA showed significantly positive effects on the TNBS-induced colitis model (Fig. 2).

DISCUSSION

Inflammatory bowel disease is a systemic disease, which involves the gastrointestinal system, of which etiology is not known completely and which affects the life quality of the patient significantly (18). Prenatal events, lactation, childhood infectious diseases, microbial factors, smoking, oral contraceptives, nutrition, cleaning, business, education, environment terms, stress, psychological factors and physical activity may play a role in the progress of ulcerative colitis (19, 20). In this study, to answer this question, we have now evaluated the effects of a TNBS induced colonic inflammation on colon cathepsin B/cathepsin L pathway, together with the lipid peroxidation and MPO activity, and the possible preventive effects of administration of gallic acid on these parameters.

TNBS-induced colitis is characterized by oxidative stress and upregulation of proinflammatory cytokines. The present study has demonstrated that TNBS causes an upregulation of levels of MDA, cathepsin B, cathepsin L and MPO activity. The levels of MDA, MPO and cathepsins were fundamentally reduced in rats treated with all the doses of gallic acid, but of all the doses, 100 mg/kg/day gallic acid dose was most effective and the results were statistically significant.

MDA is a product of the lipid peroxidation that comes about as a result of colonic damage. Lipid peroxidation causes cross-linking of nucleic acid molecules and protein. As a result, MDA is cytotoxic for the cell. Joo et al. (21) have indicated that TNBS-induced colitis is associated with increased MDA levels in rats, and diverse flavonoids used in the treatment of colitis disease can reduce levels of MDA in colon tissues. Another study, Yildiz et al. (22), induced colonic inflammation with intra-rectal administration of TNBS and MDA levels were increased in colonic tissue. Lee et al. (23) suggested that TNBS causes lipid peroxidation in the colon, which may cause colitis and ROS may substantially be related to the pathogenesis of inflammatory bowel diseases. In our study, TNBS was observed to increase the MDA levels, which was compatible with the literature.

MPO, a basic ingredient of neutrophil azurophilic granules, is a good parameter of inflammation and tissue damage. MPO is an important enzyme that ensures the formation of free oxygen radicals. The accumulation of neutrophils in the area where oxidative damage occurs causes an increase in tissue MPO activity (24, 25). Miroliaee et al. (26) suggested that among diverse parameters of oxidative stress, evaluation of MPO with lipid peroxidation and antioxidant power is sufficient in making a correlation with the seriousness of colitis. In another experimental study, Ghasemi-Pirbaluti et al. (27) used acetic acid for colitis model in rat and they observed to increase in MPO activity in colonic tissue. In this study, MPO activity in the colitis group was statistically higher than in the control group.

UC has a great prevalence global and is accepted as a risk factor of colon cancer. Hence, recent therapeutical agent and approaches for UC need to be improved. GA is a polyhydroxy phenolic compound that has been come across in multiple natural resources. GA is powerful antioxidant and it has anti-inflammatory properties (28). Our study has demonstrated that 100 mg/kg gallic acid dose significantly restrict experimental colitis-associated production of MPO and MDA.

Cathepsins can play a considerable mission in the regulation of apoptosis. The proteolytic and disruptive features of the lysosomal cathepsins have been indicated to a major role in chronic inflammatory diseases (29). The expressions of cathepsin B and cathepsin L increase in inflammatory bowel diseases and this increase occurs, particularly in the fields of tissue damage and mucosal ulceration. The researchers also put forth in their study that the inhibition of cathepsins is related to the level of inflammation, and cathepsin inhibitors may be effective in the treatment of inflammatory bowel diseases (30). In this study, a significant increase occurred in cathepsin B and cathepsin L levels in colonic inflammation induced by TNBS when compared to the control group. Our results also now showed to a pathophysiological role of cathepsins in TNBS-induced colonic inflammation. Additionally, a significant decrease was observed in the levels of cathepsins in a dose of 100 mg/kg GA that we used for treatment purposes. GA application was reduced extrication of lysosomal cathepsin B and L enzymes into the cytoplasmic piece and obstructed TNBS-induced colitis. Our histopathologic results indicated that TNBS induced colitis and we observed severe harm of surface epithelium, submucosal edema and inflammatory cell infiltration in colitis group. However, we evaluated to histological results; all doses of GA have positive effects of colonic tissue. In the dose of 50 mg/kg GA group, we observed mild inflammatory cell infiltration, regenerated glands and mild edema. In 100 mg/kg GA group, we observed regular surface epithelium, describe nearly a normal histological morphology in colon tissue.

CONCLUSION

Cathepsins are entirely identified as important diagnostic and therapeutic goal exceedingly for the UC. Their various functions pose a problem in the design of successful vehicles for their therapeutic targeting, as proved by the systematic failure of many cathepsin inhibitors in clinical tests (30). We found that treatment with GA significantly ameliorated TNBS-induced colitis generation of cathepsin activities and oxidative stress. The preventive effects of gallic acid might be effective therapeutic target on cathepsin activities in ulcerative colitis.

Ethics Committee Approval: The experimental method and application were confirmed by the Eskişehir Osmangazi University Institutional Ethical Committee for Animal Care (date: 19.08.2009, number: 127). All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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Author Contributions: Concept – GB, HŞ; Design – GK, MC; Supervision – MC, GB; Resource – GB, GK; Materials – GB, HŞ; Data Collection and/or Processing – ED, GB; Analysis and/or Interpretation – AB, ED; Literature Search – SE, ED Writing – ED, AB; Critical Reviews – GB, HŞ.

Conflict of Interest: The authors have no conflict of interest to declare.

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