Original Article

The Protective Effect of the Gallic Acid Against TNBS-induced Ulcerative Colitis in Rats: Role of Inflammatory Parameters

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Abstract

Background: Ulcerative Colitis (UC) is an Inflammatory Bowel Disease (IBD) that causes long-lasting inflammation and ulcers in digestive tract. The current study aimed to evaluate the protective effects of gallic acid on the 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced UC in rats.

Methods: Forty-two adult Wistar rats were divided into seven groups (n=7) and UC was induced in six groups using TNBS solution. They received different daily doses of gallic acid (25, 50, 75 and 100 *mg/kg/day*, p.o). On the 11th day, the colon tissues were removed and examined regarding the macroscopic and histopathology lesions. Also, Disease Activity Index (DAI) and Myeloperoxidase (MPO) activity were measured in the colon homogenate.

Results: Pretreatment with this natural agent remarkably reduced the macroscopic scores of colon in rats with UC in comparison with the control group. DAI was also reduced by gallic acid significantly. Histopathological findings confirmed the beneficial effects of gallic acidon the animal model of UC. Gallic acid induced a significant decrease in the levels of inflammatory mediators like MPO.

Conclusion: We may conclude that gallic acid can be used as an effective medicine for treatment of UC in animal model, however it needs to be confirmed by human models.

Keywords: Gallic acid, inflammatory bowel disease, ulcerative colitis, natural product, oxidative stress

Introduction

Inflammatory Bowel Disease (IBD) is a chronic inflammatory condition affecting the gastrointestinal tract. Based on the pathological findings, the disease is classified into Ulcerative Colitis (UC) in which the most involved part of the bowel is the large intestine, and Crohn's Disease (CD) that may involve all parts of the gastrointestinal tract.

Diarrhea or constipation, blood discharge, visceral pain [1,2] as well as extra-intestinal manifestation, i.e. chronic fatigue, [3] psychological problems [4], dermatological and ocular complications [5,6] are the well-known symptoms of IBD. Epidemiological studies revealed an increasing trend in the prevalence of IBD across the world [7]. There are growing bodies of investigations on the pathophysiology of the disease; however, the exact etiology is not yet clearly understood. Environmental factors such as specific types of foods, stressful situations and depressed mood [8] can exacerbate the symptoms of IBD.

Oxidative stress plays a significant role in the development of the disease. Oxidative stress induces and aggravates IBD via two pathways, including oxidative damage to intestinal mucosal cells and up regulation of inflammatory cytokines. During inflammatory states, oxidative stress increases via stimulating ROS/RNS-generating systems, such as NADPH oxidases (NOXs) and inducible Nitric Oxide Synthase (iNOS), as well as the release of Myeloperoxidase (MPO) from inflammatory cells [9].

Current pharmacotherapy of IBD includes the use of corticosteroids, 5-aminosalycilates, immunomodulators and immunesuppresives, calcineurin inhibitors, and monoclonal antibodies against Tumor Necrosis Factor α (TNF-α) [10]. Despite the big steps of progress in the treatment of the disease, not all patients are completely satisfied with the results; thus, are seeking other choices to control their symptoms such as Complementary and Alternative Medicine (CAM) [11].

Medicinal plants and their isolated active components, as one of the main parts of CAM have been world widely used for the treatment of different gastrointestinal diseases like peptic ulcers [12], liver complications [13], Irritable Bowel Syndrome (IBS) [14] and IBD [2]. Polyphenolic compounds, as a category of phytochemicals, also showed beneficial effectson the management of gastrointestinal complications [15,16].

Gallic acid (3,4,5trihydroxybenzoic acid) is a phenolic acid, a subclass of polyphenolic compounds, which is isolated from several plant species including a large number of dietary fruits and vegetables [17]. The compound has demonstrated antioxidant, anti-inflammatory [18], anticancer [19] and cytoprotective [20] activities. Recently, demonstrated that gallic acid is capable of attenuating inflammatory conditions via suppression of pro-inflammatory cytokines and inflammatory mediators, such as iNOS and COX-2 [21].Considering the above-mentioned investigations, current study aimed to evaluate the pharmacological activity of orally administered gallic acid in animal model of IBD.

Materials and Methods

Chemicals

2,4,6-Trinitrobenzene sulphonic acid (TNBS) (Sigma-Aldrich, Steinheim, Germany), gallic acid, ethanol, methanol, hydrogen peroxide, O-dianisidine hydrochloride, Hexadecyltrimethyl Ammonium Bromide (HETAB), Ethylenediamine Tetra Acetic acid (EDTA) from Merck (Germany) were used in this study.

Animals

Forty-two male Albino rats of Wistar strain weighing 250-300 *g* were used in this study. Animals were kept individually under a standard vivarium condition with light/dark cycle 12:12 *hr* with free access to food and water. All experimental procedures were approved by the Ethical Committee of Tehran University of Medical Sciences and performed according to rules.

IBD induction and treatments

Animals has been kept fasting for 36 *hr* before the disease induction. Six rats were kept untreated as the sham (healthy control). TNBS was dissolved in 99% ethanol and were rectally administered to 36 remaining rats to induce inflammation within the cecal parts of the large intestine to produce a model of UC. These animals were divided into 6 groups of six rats, which received the following treatments separately: dexamethasone (1 *mg*/*kg*/*day* dissolved in water as a positive control), normal saline (negative control, p.o.), and gallic acid (25, 50, 75 and 100 *mg*/*kg*/*day*, p.o.).

Animals received the treatment in an interval of 24 *hr* for a period of 10 days [22,23].

Disease Activity Index (DAI) assessment

Multiple clinical parameters were measured throughout the experimental period. The body weight, stool consistency, and occult blood in the stool or at the anus were recorded daily. DAI was estimated based on the percentage of weight loss (0≤1%, 1=1-5%, 2=5-10%, 3=10-15%, 4≥15%), stool consistency (0= normal, 2= loose stool, 4= diarrhea) and presence/ absence of blood (0= negative, 2= positive, 4= gross bleeding) in the stool [24].

Sample preparation

In the last day of the experiment, all rats were sacrificed and the colonic tissue samples were obtained. The samples were cut in two parts. One part was fixed in 10% formalin for the preparation of tissue sections and stained with Hematoxylin and Eosin (H&E) for light microscopic examination. The other part was homogenized using a homogenizer device (Heidolph Silent Crusher M, Germany), then stored at -20 ^{o}C for 24 *hr*. The samples were centrifuged for 30 *min* at 3500 *g* and the supernatant fluid was transferred to a microtube. Then, the samples were kept at -80 ^{o}C until Myeloperoxidase (MPO) level was assessed as a marker of acute inflammation and neutrophil infiltration based on the previous studies [25].

Macroscopic and microscopic assessment

The macroscopic scoring system was used to evaluate the severity of colonic damage as previously described [23]. Tissues were scored from normal appearance with no damage; 1 - localized hyperemia without ulcer; 2 - localized hyperemia with an ulcer; 3 - a linear ulcer with inflammation at one site; 4 - two or more ulcers with damage extending 1-2 *cm* along the length of the colon; and 5 to 8 - damage extending more than 2 *cm* along the length of the colon and the score was increased by 1 for each increased cm of involvement. The microscopic scoring was done by an observer who was blinded to the treated groups. Microscopic scores were determined as follows: 0 - no damage; 1 - fecale pithelial edema and necrosis; 2 - disperse swelling and necrosis of the villi; 3 - necrosis with neutrophil infiltration in the submucosa; and 4 - widespread necrosis with massive neutrophil infiltration and hemorrhage.

Myeloperoxidase activity assessment

The colonic samples were homogenized in PBS (50 *mM*, pH 7.4), then sonicated and centrifuged for 30 *min* at 3500 *g*. The pellets were resuspended in 10 *ml* of 50 *mM* phosphate buffer (pH=6) containing trimethyl ammonium bromide hexadecyltrimethylammonium (HETAB) 0.5% and 10 *mM* EDTA. The lysate was centrifuged at 12,000 *g* for 20 *min*, with the addition of 0.1 *ml* of the supernatant of the samplesto 2.9 *ml*of 50 *mM* phosphate buffer solution (pH= 6) containing *O*-dianisidine hydrochloride (0.167 *mg/m*) and H_2O_2 (0.0005%). After the formation of orange color complex, which measured by monitoring absorbance at 460 *nm*. One unit of MPO activity is described as the change in absorbance per *min* at room temperature, in the final reaction [26].

Statistical analysis

One-way analysis of variances (ANOVA) and Tukey's post-hoc test were performed to compare the obtained data between the control and test groups. $P<0.05$ was considered as statistically significant difference.

Results

Disease activity index

Considering weight loss, stool consistency and presence/ absence of blood in the stool, DAI was calculated for each group (Figure 1). The sham group showed a zero DAI score as no disease was developed in this group. Negative control group (UC untreated animals) had the highest DAI score compared to sham group which was equal to 8.6 ($p<0.001$). Dexamethasone considerably decreased disease activity index in comparison with control group (TNBS) ($p<0.001$).Gallic acid treatment showed a relieving effect on DAI in a dose dependent manner so that the lowest DAI score was observed with 100 *mg/kg* of gallic acid (Figure 1). At this dose, the effect of gallic acid was the same as the gold standard drug, dexamethasone ($p> 0.05$).

Macroscopic and microscopic scores

Macroscopic and microscopic evaluations of tissues obtained at the end of the ten-day animal study as well as DAI scores revealed a significant and dose-dependent beneficial effect of gallic acid for the

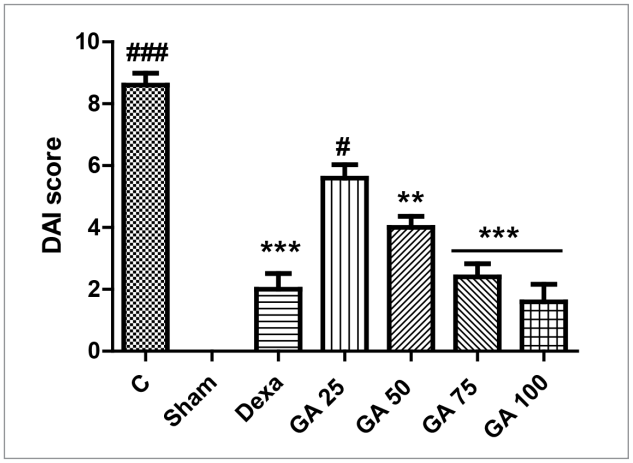

Figure 1. Disease Activity Index (DAI) activity in the colon. Values are mean±SEM. C, control (TNBS); Dexa, dexamethasone; GA 25; Gallic acid at dose of 25 *mg*/*kg*; GA 50, Gallic acid at dose of 50 *mg*/*kg*; GA 75, Gallic acid at dose of 75 *mg*/*kg*; GA 100, Gallic acid at dose of 100 *mg*/*kg*. ###Significantly different from the sham group at p< 0.001.#Significantly different from the Dexa group at p<0.05. ***Significantly different from the C group at p< 0.001. **Significantly different from the C group at p< 0.01.

Table 1. Macroscopic and microscopic scores as criteria for assessing colonic damage

Groups	Macroscopic score (mean ± SEM)	Microscopic score (mean ± SEM)
Sham	0.00±0.00	0.00±0.00[a]
Control	4.75±0.39[b,c]	3.75±0.25[b]
Dexa	1.35±0.13[a,c]	1.50±0.64[a]
GA 25	3.9±0.32[a,b,c]	3.25±0.47
GA 50	3.3±0.29[a,b,c]	2.25±0.47
GA 75	2.7±0.34[a,b,c]	1.25±0.62[a]
GA 100	1.7±0.39[a,c]	0.50±0.28[a]

Values are mean±SEM. Dexa, dexamethasone; GA 25; Gallic acid at dose of 25 *mg*/*kg*; GA 50, Gallic acid at dose of 50 *mg*/*kg*; GA 75, Gallic acid at dose of 75 *mg*/*kg*; GA 100, Gallic acid at dose of 100 *mg*/*kg*.[a]significantly different from Control group at p<0.05.[b]significantly different from Dexa group at p<0.05.[c]significantly different from Sham group at p<0.05.

treatment of IBD-related symptoms. Dexamethasone significantly reduced the adverse effects of TNBS (p< 0.001). Treatment with gallic acid represented improvements in all parameters of DAI score, including stool consistency, weight loss and absence/presence of blood in the stool (Table1).

TNBS caused considerable macroscopic tissue damage. Treatment with 50, 75 and 100 *mg/kg* of gallic acid significantly decreased macroscopic indices compared to the negative control group (p<0.05, Table 1). At a dose of 100 *mg/kg* of gallic acid, the therapeutic effect was statistically the same as dexamethasone treatment, in the positive control group.

Regarding the microscopic evaluations of the intestinal tissues, TNBS induced severe microscopic damages to the colonic tissue; however, all doses of gallic acid showed significantly decreased hemorrhage, infiltration of inflammatory cells and tissue necrosis compared to the negative control group (p<0.05). The dose of 100 *mg/kg* of gallic acid demonstrated similar effects similar to dexamethasone (Table 1), (Figure 2).

Myeloperoxidase activity

The MPO activity in the control group that received TNBS was significantly higher than the sham group (p<0.001). Dexamethasone effectively decreased the MPO value in comparison with the control group that received TNBS. As well, due to administration of gallic acid, MPO activity significantly reduced dose-dependently. The dose of 100 *mg/kg*, from the various concentrations of gallic acid was the most effective dose for reducing colonic damage (p<0.001), (Figure 3).

Discussion

In the present study, we have evaluated the protective effect of gallic acids in TNBS-induced IBD in a rat model. Our results indicated that gallic acid improved macroscopic and microscopic colonic damage in a dose-dependent manner, especially with a dose of 100 *mg/kg*. Histological alteration due to TNBS exposure indicated that severe ulceration, transmural inflammation and extensive necrosis in mucosa and submucosa with massive neutrophil infiltration, which was consistent with results of previous studies [16,26].

Until now, several methods for induction of colitis in animals have been introduced such as the use of genetically modified, infection and chemically induced colitis models [Dextran Sodium Sulfate (DSS), Trinitrobenzene sulfonic acid (TNBS)], and Dinitrobenzene sulfonic acid (DNBS) [27]. TNBS is one of the most common chemical induced methods inrodents,

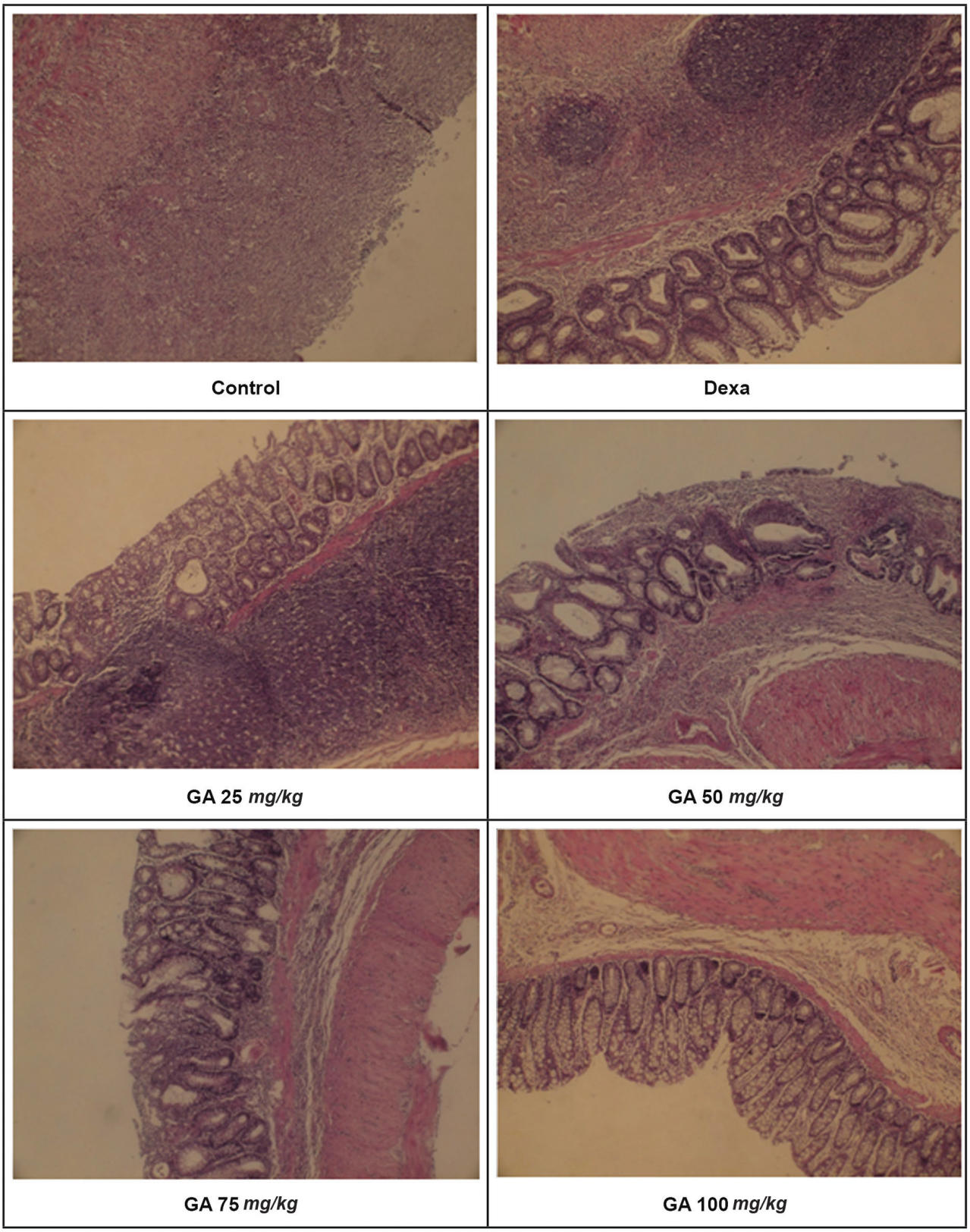

Figure 2. Histological images of colon tissues obtained from control and experimental groups.

Control group that received TNBS, Gallic acid at dose of 25 *mg*/*kg*, Gallic acid at dose of 50 *mg*/*kg*, Gallic acid at dose of 75 *mg*/*kg*, Gallic acid at dose of 100 *mg*/*kg* and dexamethasone. TNBS increased transmural inflammation and crypt destruction; treatment with Gallic acid significantly decreased histological changes caused by TNBS especially at dose of 100 *mg*/*kg*.

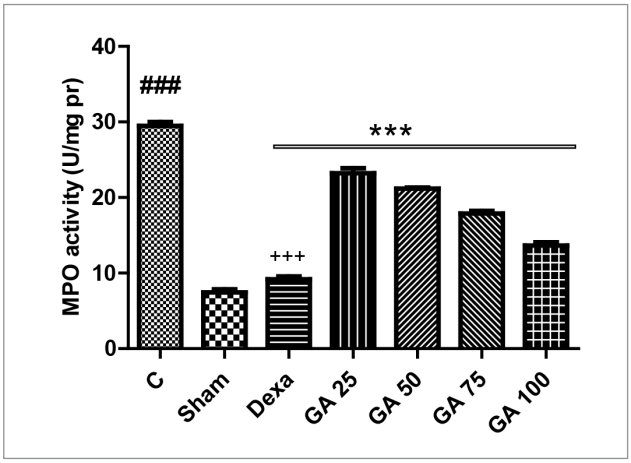

Figure 3. Myeloperoxidase (MPO) activity in the colon. Values are mean±SEM. C, control(TNBS); Dexa, dexamethasone; GA 25; Gallic acid at dose of 25 *mg/kg*; GA 50, Gallic acid at dose of 50*mg/kg*; GA 75, Gallic acid at dose of 75 *mg/kg*; GA 100, Gallic acid at dose of 100 *mg/kg*. ###Significantly different from the sham (normal) group at p< 0.001. ***Significantly different from the C group at p< 0.001.+++ Significantly different from GA groups at p<0.001.

which is used in combination with ethanol. The rational reason for the use of ethanol is to break the colonic mucosal barrier, allowing the penetration into the lamina propria [28].

IBD, comprising UC and CD, is an inflammation within the gastrointestinal tract with severe disabling intestinal and extra intestinal complications which can dramatically affect patient's quality of life. Despite the current therapeutic approaches for the treatment of the disease, not all patients achieve a satisfying response to treatments; thus, researches toward the discovery of further pharmacotherpeutic options are still running ahead [15].

Plant-derived natural products have always been an inseparable part of treatment, especially in chronic diseases. Patient's perception of natural compounds as relatively safe and available treatment options along with the ancient history of their promising effects in the management of specific types of diseases have encouraged scientist to seek among medicinal plants for the identification of active components [2].

TNBS-induced rats had an increased DAI score compared to the sham group. As a result of gallic acid treatment, weight loss, blood in stool and stool consistency significantly decreased. The results of our study are consistent with results of previous studies. Kumar and colleagues indicated that DSS-induced experimental colitis in BalB/c mice considerably decreased after orally gallic acid [21]. They concluded that gallic acid significantly attenuated DAI and shortening of colon in mice. Various studies showed that protective effects of several phytochemicals in colitis [29-31].

In our previous studies, ameliorative effect of Tragopogongraminifolius and galls of Quercusbrantii Lindl in TNBS-induced colitis have been evaluated. Our previous results showed that 88.43 ±7.23% of dry gall composed of phenolics compound especially gallic acid. In another word, gallic acid is the main component in gall for attenuation of TNBS-induced colitis in rat, mostly by free radical scavenging activity [16,26].

MPO plays important role in inflammation and infection, by converting of hydrogen peroxide to chloride and HOCl. Several researchers reported that exposure to TNBS results in an increased MPO activity. In our study, MPO activity significantly increased due to TNBS exposure. Previous study demonstrated that gallic acid and its derivatives (n-alkyl ester) significantly inhibit the MPO activity [32]. Oxidative stress has a prominent role in the IBD development. Given that, these compounds lead to inhibition of the enzyme and reduced the production of free radicals, so have the potential to be used in the treatment of inflammatory diseases [33]. As well as the antioxidant effect of gallic acid, it has several biologic properties such as anti-inflammatory and anti-apoptotic effects [34].

Various studies have shown that during IBD, the balance of antioxidant system in the intestinal mucosa is impaired. Reduction of the level of reactive oxygen/nitrogen metabolites (ROM/RNM) plays an important role in improving the symptoms of inflammatory bowel disease. Plant-based compounds that can effectively eliminate free radicals can be considered as candidates for the treatment of this disease [35-37].

Several cytokines and molecules have been identified that are involved in the pathogenesis of inflammatory bowel disease. TNF-α is one of the most important cytokines. Regarding its inflammatory and proliferative role in inflammatory bowel disease, inhibition of this cytokine has been considered as a therapeutic approach [38,39].

Our results showed that gallic acid dose-dependently suppressed the MPO activity. In another study gallic acid significantly decreased colonic MPO and other inflammatory cytokines such as iNOS and COX-2 in DSS-exposed mice [21]. Other protective mechanisms of gallic acid against colitis induced by DSS are reduction in IL-21 and IL-23 expression and upregulation of Nrf2 gene and its downstream [21].In addition, some previous studies reported the beneficial effect of plant extracts rich in gallic acid, for the treatment of IBD [15]. Our study also proved the *in vivo* anti-inflammatory activity of gallic acid with respect to the MPO level of colonal tissues. These data were also previously confirmed in Pandurangan study [21].Thus, the positive role of gallic acid in UC, at least in part, is due to its anti-inflammatory activities.

Future studies are needed to evaluate the level of inflammatory cytokines as well as to assess its effect in other animal models of IBD to confirm the safety and efficacy of the gallic acid therapy and to discover the underlying molecular mechanisms of it in the treatment of IBD.

Conclusion

In conclusion, our study demonstrated the beneficial effects of oral gallic acid in an animal model of TNBS-induced UC. Gallic acid in a dose-dependent manner decreased DAI, macroscopic and microscopic colonic damage and MPO activity. These effects are due to antioxidant and anti-inflammatory properties of gallic acid. Further studies will be needed in future to assess safety and efficacy of gallic acid in order to introduce a compound to clinical trials.

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