On the benefit of galls of *Quercus brantii* Lindl. in murine colitis: the role of free gallic acid

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Abstract

Introduction: In this study we investigated the effect of gall of *Quercus brantii* Lindl., a traditional Iranian medicine, in a murine model of experimental colitis induced in male rats by rectal administration of 2,4,6-trinitrobenzene sulfonic acid (TNBS).
Material and methods: Quantification of the main active components was done for estimation of total phenolic content and free gallic acid. Gall of *Quercus brantii* Lindl. in two forms (gall powder and gall hydro alcoholic extract) was gavaged for 10 days (500 mg/kg). Ten days after induction of colitis, colonic status was examined by macroscopic, microscopic and biochemical analyses. Colonic tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) were analyzed as biomarkers of inflammatory condition. To determine the role of oxidative stress (OS) in colitis, the levels of cellular lipid peroxidation (LPO), total antioxidant power (TAP) and myeloperoxidase (MPO) were measured in colon tissues.
Results: TNBS-induced colitis exhibited a significant increase in colon MPO activity and concentrations of cellular LPO, TNF-α and IL-1β, while TAP was significantly reduced. Microscopic evaluations of the colonic damage in the colitis group revealed multifocal degenerative changes in the epithelial lining and areas of necrosis, extensive mucosal and sub-mucosal damage with congested blood vessels, edema and hemorrhages along with extensive infiltration of inflammatory cells. Parameters including macroscopic and microscopic scores, TNF-α, IL-1β, LPO, TAP and MPO improved by both gall extract and gall powder of *Quercus brantii* Lindl. and reached close to normal levels. The level of total phenols (GAE/100 g of sample) and free gallic acid were estimated to be 88.43 ±7.23 (mean ± SD) and 3.74% of dry weight, respectively.
Conclusions: The present study indicates that the gall of *Quercus brantii* Lindl. is able to exert antioxidative and anti-inflammatory effects on the biochemical and pathological parameters of colitis.

Key words: colitis, oxidative stress, *Quercus brantii* Lindl., animal.

Introduction

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is one of the chronic disorders of the gastrointestinal tract with an immuno-inflammatory origin. Recent studies have confirmed involvement of some pathological elements and beneficial effects of their inhibitors or activators such as growth factors and nitric oxide [1–3], inflammatory cytokines such as prostaglandins [4, 5] or tumor necrosis factor (TNF) [6–8], immunoregulators [9–11], oxidative stress [12], microbes [13–16], probiotics, potassium channel openers [17], adenosine triphosphate (ATP) donors [18], and phosphodiesterase inhibitors (PDEIs) [19–21] in IBD. Most of the synthetic drugs for management of IBD have serious adverse effects that led researchers to work on complementary and alternative remedies that can induce marginal remission in disease activity [22, 23].

Quercus brantii Lindl., belonging to the family of Fagaceae, is a small tree with galls arising on its young branches as a result of attack by the gall wasp *Adleria gallae-tinctoria* [24]. This herb is endemic in Lorestan and Kordestan provinces of Iran. The major components of galls are tannins, gallic acid, syringic acid, ellagic acid, β-sitosterol, amentoflavone, hexamethyl ether, isocryptomerin, methyl betulate, and hexagalloyl glucose [25].

Quercus brantii galls (locally called 'mazoo') are famous in traditional Iranian medicine for treating chronic diarrhea and many diseases, with or without inflammatory pathogenesis [26]. Regarding its great medicinal value, *Quercus* galls have been reported to have the following pharmacological actions: astringent, anti-pyretic, anti-parkinsonism [25], anti-tremorine, local anesthetic, central nervous system (CNS) depressant, analgesic [25, 27], anti-diabetic [25, 28], anti-bacterial [29–34], anti-oxidant [35, 36] and anti-inflammatory [25].

Therefore, the above knowledge led us to examine the beneficial effects of *Q. brantii* in a murine model of colitis to understand the mechanisms by which this herb might act. Quantification of the main active components was done for estimation of total phenolic content and free gallic acid.

Material and methods

Chemicals

2,4,6-Trinitrobenzene sulfonic acid (TNBS) and rhodanine from Sigma-Aldrich Chemie (GmbH, Munich, Germany), thiobarbituric acid (TBA), trichloroacetic acid (TCA), n-butanol, hexadecyl trimethyl ammonium bromide (HETAB), 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ), diphenyl-2-picryl hydrazyl (DPPH), methanol, butyl hydroxy anisol (BHA), hydrochloric acid (HCl), malondialdehyde (MDA), ethylenediamine tetra-acetic acid (EDTA), O-dianisidine hydrochloride, hydrogen peroxide, acetic acid, sodium acetate, Coomassie reagent, bovine serum albumin (BSA), ferric chloride ($FeCl_3 \cdot 6 H_2O$), sodium sulfate (Na_2SO_4), sulfuric acid (H_2SO_4), phosphoric acid (H_3PO_4), potassium dihydrogen phosphate (KH_2PO_4), potassium hydrogen diphosphate (K_2HPO_4), peroxide hydrogen (H_2O_2), sodium carbonate (Na_2CO_3), Na-K-tartrate, cupric sulfate ($CuSO_4 \cdot 5 H_2O$), Folin-Ciocalteu reagent and Gallic acid from Merck (Germany), rat-specific tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) ELISA kits from Bender MedSystems (Austria), and dexamethasone and vitamin E from the local Pharmaceutical Co. (Tehran) were used in this study.

Plant material

The galls of *Quercus brantii* Lindl. were collected from the Koohdasht region in the Lorestan province, Iran, in September 2010, and were ground well. A voucher specimen was preserved in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Science (TUMS), Tehran, Iran (No. 6730-TEH).

Extraction and preparation

The dried gall of *Q. brantii* was finely powdered by milling. The extract was prepared using 30 g of powder of the gall with a mixture of methanol/water (50 : 50) (21.8 g dry weight corresponding to 73% w/w).

In vitro diphenyl-2-picryl hydrazyl free radical scavenging activity

The free radical scavenging activity of galls was measured by a DPPH scavenging assay using a previously described method [37]. This test was done to estimate the best antioxidant dose of *Q. brantii* galls. In this regard the extract was prepared in four concentrations: 100, 300, 500 and 1000 µg/ml. A DPPH is one of a few stable and organic nitrogen radicals and has a maximum absorption at 517 nm. Upon reduction, the solution color changes from blue to yellow. The decrease in absorbance at 517 nm was determined after 5 min and then the free radical scavenging activity was calculated.

Estimation of total phenolic content

The Folin-Ciocalteu method [38] was used to determine total phenolic content. One ml of sample extract (100 µg/ml in methanol) was mixed with 1.5 ml of 0.2 N Folin-Ciocalteu reagent. Then, 1.5 ml of sodium bicarbonate solution (75 g/l) was added to the mixture, and after 30 min the absorbance was measured at 765 nm against a metha-

nol blank. The calibration curve was produced using standard gallic acid. The means of three tests in the 3 following days were reported as gallic acid equivalent (GAE)/100 g of the sample.

Quantification of free gallic acid

Rhodanine assay [39] was used for quantitation of free gallic acid. One hundred mg of sample was extracted by 10 ml of acetone (70%) in an ultrasonic water bath for 20 min at room temperature. After centrifugation for 10 min at 3000 g (the contents were cooled by keeping the tubes on ice before centrifugation), 200 µl of supernatant was pipetted in a test tube (4 tubes were prepared). Acetone was removed from samples and then 200 µl of 0.2 N sulfuric acid was added. To three tubes, 300 µl of the rhodanine solution (0.667% w/v in methanol) and to the fourth (blank) 300 µl of methanol were added. After 5 min, 200 µl of 0.5 N KOH solution was added to all the tubes and then, 2.5 min later, 4.3 ml of distilled water was added and the absorbance was read after 15 min at 520 nm against an appropriate blank. Four different concentrations of gallic acid (4–20 µg/5 ml) were prepared to produce the calibration curve. Each assay was done in triplicate and the data were expressed as mean ± SD.

In vivo study

Male Wistar-albino rats, weighing between 220 g and 230 g, were used in this study. Animals were maintained under standard conditions of temperature (23 ±1°C), relative humidity (55 ±10%), and a 12/12 h light/dark cycle, and fed with a standard pellet diet and water ad libitum. They were housed individually in standard polypropylene cages with a wire mesh top. All ethical themes of the studies on animals were considered carefully and the experimental protocol was approved by the Ethical Committee of TUMS with the code number 89-03-33-11232.

Before starting the whole project, acute toxicity of *Q. brantii* galls was tested. For this, rats were fed with several doses of the gall extract and gall powder and observed for 48 h. It was found that up to 2000 mg/kg of gall extract and 2740 mg/kg of gall powder are not toxic and thus they are categorized as practically non-toxic [40].

At the next step, 30 male rats were randomly divided into five groups randomly containing 6 in each group. Colitis was induced by instillation of TNBS in 4 groups and the resting group was considered as a sham group that received normal saline instead of TNBS. The four groups receiving TNBS were: 1 – control group that received no treatment; 2 – dexamethasone treated group (Dexa) receiving dexamethasone as a positive standard (1 mg/kg); 3 – gall extract treated group (Extract) receiving extract of *Q. brantii* galls (500 mg/kg; the best antioxidant activity of galls of *Q. brantii* was achieved with concentration of 500 µg/ml according to the DPPH test result; Figure 1); and 4 – gall powder treated group (Powder) receiving gall powder in the form of a suspension (685 mg/kg; each 100 g of gall powder is equivalent to 73 g of extract).

Dexamethasone was administered intraperitoneally, and gall extract and gall powder were administered by gavage for 10 days. The day of induction of colitis was considered as the first day.

All the rats were fasted for 36 h before induction of colitis. They were deprived of food but were allowed free access to tap water. After this, rats were anesthetized with administration of 50 mg/kg pentobarbital sodium intraperitoneally. They were positioned on their right side, and 0.3 ml of a mixture containing 6 volumes of TNBS 5% w/v in water (equal to 15 mg TNBS) plus 4 volumes of ethanol (99%) was instilled through the rectum using a rubber cannula (8 cm long) [41]. Then, rats were maintained in a supine Trendelenburg position in order to prevent anal leakage of TNBS. Rats were treated in the next 10 days. On the 11th day, all the animals were sacrificed using an overdose of ether inhalation. The abdomen was rapidly dissected open and the colon was removed. The pieces of colon were cut open in an ice bath, cleaned gently using normal saline, and observed normally for macroscopic changes and scored as described later. Then samples were cut into two pieces, one piece for histopathology assessment (maintained in 5 ml formalin 10% as fixative) and one piece for measuring biomarkers. The pieces for analysis of biomarkers were weighed and maintained at –20°C for 24 h. Then, the colonic samples were homogenized in 10 volumes of ice cold potassium phosphate buffer (50 mM, pH 7.4), sonicated and centrifuged for 30 min at 3500 g. The superna-

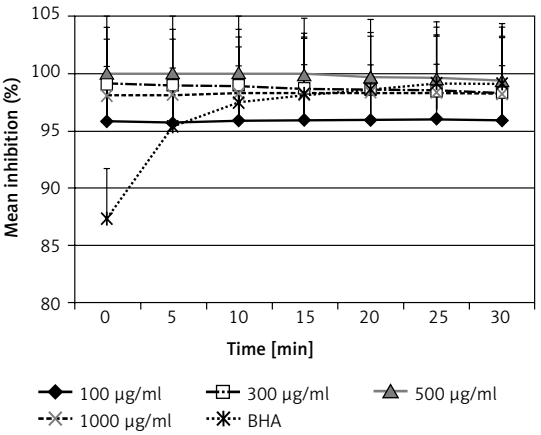

Figure 1. The DPPH radical scavenging activity of the *Q. brantii* extract. Each experiment was performed at least three times and data are expressed as averages. The BHA was used as a control

tants were transferred to several microtubes for separate biochemical assays and all were kept at –80°C until analyses.

Macroscopic and microscopic assessment of colonic damage

The severity of colonic damage was evaluated using the colon macroscopic scoring system, which takes into account the area of inflammation and presence/absence of ulcers: 0 (normal appearance with no damage), 1 (localized hyperemia without ulceration), 2 (linear ulceration without significant inflammation), 3 (linear ulceration with inflammation at one site), 4 (two or more sites of ulceration extending more than 1 cm along the length of the colon), and 5–8 (damage extending more than 2 cm along the length of colon and the score is enhanced by 1 for each increased cm of involvement) [42]. In addition, for microscopic analysis, the fixed segments in formalin 10% were embedded in paraffin and stained with hematoxylin and eosin. The scoring was performed by an expert cytohistopathologist blind to the treated groups. Microscopic scores were determined as follows: 0 (no damage), 1 (focal epithelial edema and necrosis), 2 (disperse swelling and necrosis of the villi), 3 (necrosis with neutrophil infiltration in submucosa), 4 (widespread necrosis with massive neutrophil infiltration and hemorrhage) as described previously [17].

Determination of TNF-α and IL-1β

Quantitative detection of TNF-α and IL-1β levels in colon tissues were evaluated using an enzyme-linked immunosorbent assay rat-specific ELISA kit as instructed by the kit brochure. The absorbance of the final colored product was measured at 450 nm as the primary wavelength and 620 nm as the reference wavelength. TNF-α and IL-1β levels were expressed as pg/mg protein of tissue as described previously [43].

Total ferric reducing antioxidant power (FRAP) assay

Total antioxidant power of colon was evaluated by measuring the ability to reduce Fe^{3+} to Fe^{2+}. Interaction of TPTZ with Fe^{2+} results in formation of a blue color, with maximum absorbance at 593 nm. Data were expressed as µM ferric ions reduced to ferrous per mg protein. Details were described previously [44].

Myeloperoxidase (MPO) activity measurement

About 0.1 ml of supernatant was combined with 2.9 ml of 50 mM phosphate buffer containing 0.167 mg/ml o-dianisidine hydrochloride and 0.0005% H_2O_2. The change in absorbance was measured spectrophotometrically at 460 nm for 3 min. One unit of MPO activity is described as the change in absorbance per min at room temperature, in the final reaction. The details of the procedure have been previously described [45].

Lipid peroxide thiobarbituric acid-reactive substance (TBARS) assay

Level of lipid peroxidation was assessed in colon tissue using the thiobarbituric acid-reactive substance (TBARS) assay as described in detail previously [46]. Data were reported as µg/mg protein.

Total protein

Total protein (TP) of colon was measured according to the Bradford method. The BSA was used as a standard and results were reported as mg/ml of homogenized colon [47].

Statistical analysis

Data were analyzed by StatsDirect 2.7.8. One-way ANOVA followed by Tukey's post hoc test for multiple comparisons were used. Values of p less than 0.05 were considered significant. Results are expressed as mean ± standard error of the mean (SEM).

Results

DPPH

The *in vitro* evaluation of antioxidant effects of *Q. brantii* is shown in Figure 1.

The antioxidant power of *Q. brantii* did not change with time in 30 min in comparison to BHA, which increased from 87.3 to 99.08 in 30 min. Antioxidant level of the extract increased in a dose-dependent manner up to 500 µg/ml concentration, but this was not significant.

Total phenolic content

The level of total phenols (GAE/100 g of sample) was estimated to be 88.43 ±7.23 (mean ± SD) using the standard curve of gallic acid (Table I) (y = 0.0074x – 0.0756, R^2 = 0.9858). Therefore the total phenol content received in the 4th group (treated with gall powder) was 605.75 ±49.53 mg/kg GAE.

Free gallic acid content

The calibration curve of gallic acid was linear over the range (4–20 µg/5 ml) (R^2 = 0.9935). The result showed that the amount of free gallic acid in powdered galls was 3.74% of dry weight. Therefore the amount of free gallic acid received in the 4th group (treated with 685 mg/kg gall powder) was 25.62 mg/kg.

Table I. Total phenolic content of galls of *Quercus brantii* Lindl.

Day	Absorption [nm]			Phenolic content (GAE/100 g)		
1	0.584	0.608	0.651	89.14	92.38	98.18
2	0.490	0.583	0.634	76.43	89.00	95.89
3	0.509	0.553	0.597	79.00	84.95	90.89
Sum, mean ± SD	0.579 ±0.053			88.43 ±7.23		

Samples were tested in triplicate on three consecutive days.

Macroscopic and microscopic evaluation of colonic damage

Macroscopic and microscopic evaluations of the colonic damage are shown in Table II. Severe inflammation, ulceration, adhesion and wall thickening were induced by intracolonic administration of TNBS/ethanol in the control group in comparison to colons of the sham as normal group. Macroscopic scores were significantly improved by dexamethasone in colitic rats. Colonic damage decreased after administration of both gall extract and gall powder.

Light microscopic examination of the colon in the sham group showed normal histology and revealed a regular mucosal layer with intact epithelial surface (Figure 2 A).

Animals receiving TNBS showed multifocal degenerative changes in the epithelial lining and areas of necrosis, extensive mucosal and sub-mucosal damage with congested blood vessels, edema and hemorrhages along with extensive infiltration of inflammatory cells (Figures 2 B–D). Histological analysis of the colonic specimens from the gall powder group revealed a pronounced restoration of the epithelial cell layer in comparison to untreated rats. There was mild cellular infiltration in the sub-mucosa and only a maximum of 25% of the epithelium was affected in contrast to the comprehensive ulceration observed in non-treated animals (Figure 2 E). Treatment with extract orally showed a complete protective effect against TNBS-induced colonic injury (Figure 2 F). The mucosa showed intact epithelium, no ulceration, no congestion and no edema (Figure 2 F).

Colonic TNF-α level

The inflammatory condition caused a significant increase in TNF-α in the control group when compared to the sham group ($p < 0.01$). TNF-α significantly decreased in gall extract and gall powder groups in comparison to controls (Figure 3).

Colonic IL-1β levels

IL-1β level was higher in the control group as compared to shams ($p < 0.01$). The level of IL-1β in the extract group was lower as compared to controls. Values in extract were near the Dexa group and close to the sham group (Figure 4).

Table II. Extent of colonic damage according to macroscopic and microscopic scores

Groups	Macroscopic score mean ± SEM, median, min–max	Microscopic score mean ± SEM, median, min–max
Sham	0.0 ±0.0, 0, 0.0–0.0	0.0 ±0.0, 0, 0.0–0.0
Control	5.0 ±1.05, 6, 2.0–7.0	3.57 ±0.30[a], 4, 2.0–4.0
Dexa	0.66 ±0.33[b], 0.5, 0.0–2.0	0.83 ±0.30[b], 1.0, 0.0–2.0
Gall extract	1.0 ±0.32)[b], 1, 0.0–2.0	1.71 ±0.29[a,b], 2, 1.0–3.0
Gall powder	1.2 ±2.0[b], 1, 1.0–2.0	2.42 ±0.36[a,b,c], 2, 1.0–4.0

[a]Significantly different from Sham group at $p < 0.01$. [b]Significantly different from control group at $p < 0.01$. [c]Significantly different from Dexa group at $p < 0.01$.

Colonic total antioxidant power as FRAP

Colonic damage and inflammation caused a significant reduction in the FRAP value of the control group when compared to normal rats in the sham group ($p < 0.01$). The FRAP values were significantly elevated following administration of gall extract and gall powder (Figure 5).

Colonic lipid peroxidation level as TBARS

Induction of colitis caused a significant increase in the TBARS value ($p < 0.01$) as compared to shams. The TBARS decreased in the extract-treated animals in comparison to controls (Figure 6).

Colonic MPO activity

The MPO activity in the colitis group was significantly higher than the sham group ($p < 0.01$). Treatment with both gall extract and gall powder reduced MPO activity as compared to the control group (Figure 7).

Discussion

The outcome of the present study indicated that *Q. brantii* Lindl. has a protective effect on

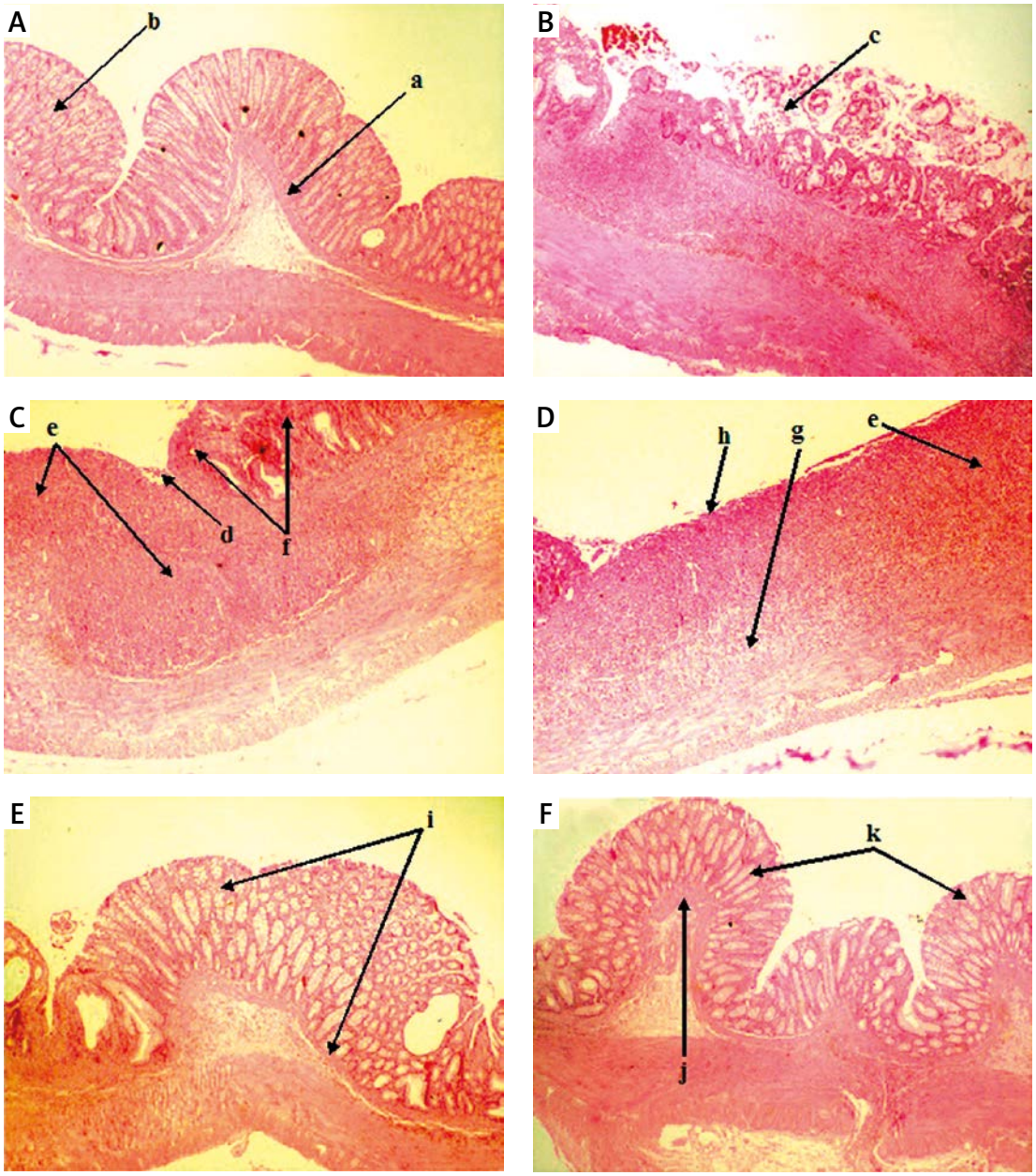

Figure 2. Photomicrograph of colon trans-mural histology in sham and experimental groups. H + E: hematoxylin and eosin stain. Original magnification: 100×. **A** – No histological modification is present in the sham animals and a regular mucosal layer (a) with intact epithelial surface (b) is seen. In the colitis group (**B**, **C** and **D**), multifocal degeneration and necrosis of epithelium (c, d), massive infiltration of inflammatory cells in the mucosa and sub-mucosa (e), obvious hemorrhages and congestion (f), edema (g), and crypt abscesses (h) are observed. Treatment with gall powder (**E**) reduced the morphological alteration showing protection of mucosal and epithelium architecture (i) with some focal areas of epithelial necrosis and mild inflammatory cell infiltration. No abnormality is seen in the extract-treated group (**F**). Treatment with *Quercus brantii* completely protected the morphological alteration associated with TNBS administration. Note the intact mucosal layer (j) and epithelial surface (k)

TNBS-induced colitis via suppression of free radicals, toxic stress and pro-inflammatory condition. This plant in combination with other plants has been used in a traditional Iranian medicine clinic located in the TUMS campus for IBD patients and had shown good effects. To the best of our knowledge, this is the first study showing the benefit of *Q. brantii* in a murine model of experimental colitis.

Quercus has been used for centuries in Asian countries for treatment of inflammatory conditions and microbial infections [48]. Water-methanolic extract of *Quercus* galls has a large amount of tannins. Regarding the role of microbes and the oxidative inflammatory process in induction of colitis, the first thing that comes to mind is to relate the benefit of *Quercus* to its antibacterial [29–34], antioxidant [35, 36], and anti-inflammatory properties [24].

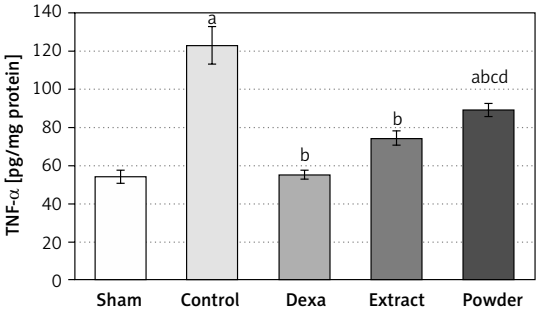

Figure 3. Tumor necrosis factor-α level in colon

Values are mean ± SEM. [a]Significantly different from sham group at p < 0.01. [b]Significantly different from control group at p < 0.01. [c]Significantly different from Dexa group at p < 0.01. [d]Significantly different from extract group at p < 0.01.

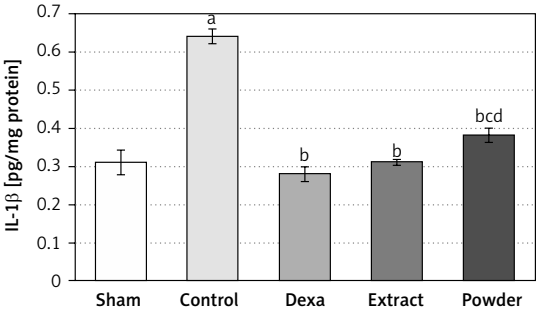

Figure 4. Interleukin-1β level in colon

Values are mean ± SEM. [a]Significantly different from sham group at p < 0.01. [b]Significantly different from control group at p < 0.01. [c]Significantly different from Dexa group at p < 0.01. [d]Significantly different from extract group at p < 0.01.

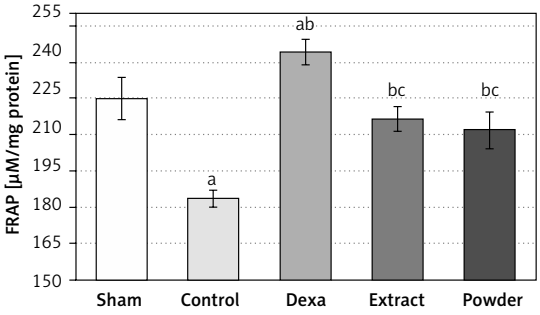

Figure 5. Ferric reducing antioxidant power of colon

Values are mean ± SEM. [a]Significantly different from sham group at p < 0.01. [b]Significantly different from control group at p < 0.01. [c]Significantly different from Dexa group at p < 0.01.

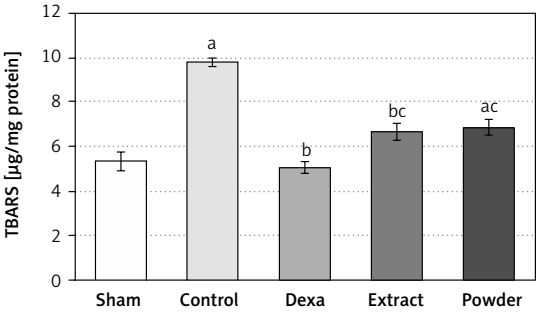

Figure 6. Lipid peroxidation as TBARS in colon

Values are mean ± SEM. [a]Significantly different from sham group at p < 0.01. [b]Significantly different from control group at p < 0.01. [c]Significantly different from Dexa group at p < 0.01.

Quercus brantii improved TNBS-induced colitis by inhibition of the synthesis or release of inflammatory mediators, reduction of oxidative stress and maintaining antioxidant power status. These results clearly indicate that administration of *Q. brantii* reduces LPO and MPO (markers of OS) and increases a marker of antioxidant potential in TNBS-induced colitis. All of the present protective effects of *Q. brantii* were consistent with histological evidence showing significant improvement in microscopic characteristics of the colon when compared to the colitis group.

Among several models of experimental colitis, TNBS-induced colitis is one of the reliable models. This model is very useful in studying gut inflammation, cytokine secretion patterns, cell adhesion and immunotherapy. In this manner, ethanol breaks the mucosal barrier, and TNBS acts as a hapten, and its administration induces a delayed-type of hypersensitivity reaction similar to chronic colitis in humans [49].

It has been determined that pro-inflammatory cytokines such as IL-1β and TNF-α rise in primary stages of colitis [50]. IL-1β and TNF-α are produced by monocytes and tissue macrophages and

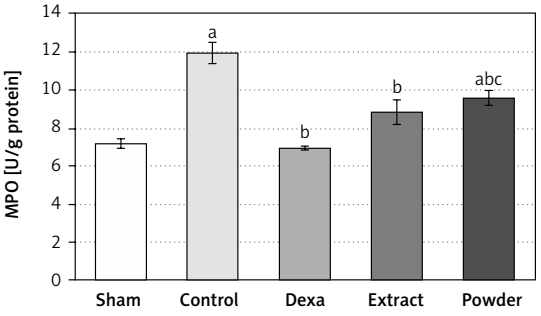

Figure 7. Myeloperoxidase (MPO) activity in colon

Values are mean ± SEM. [a]Significantly different from sham group at p < 0.01. [b]Significantly different from control group at p < 0.01. [c]Significantly different from Dexa group at p < 0.01.

have a regulatory effect on some inflammatory processes involving genes. TNF-α is a potent component of apoptosis that activates NF-kB too [51]. It has an important role in pathogenesis of IBD and a new strategy for colitis treatment is blocking TNF-α activity.

The MPO and LPO are markers of free radical damage to intestinal cells. Free radicals are released by phagocytes and to a lesser extent by

eosinophils, lymphocytes and fibroblasts during toxic stress. According to our results, MPO activity significantly decreased after consumption of *Q. brantii*. In this regard, it has been well documented that *Q. brantii* is able to reduce inflammation via secretion of destructive agents such as MPO resulting from penetration of neutrophils to the inflammation site [17]. Interestingly, the anti-inflammatory effect of most medicinal plants which have been used in traditional medicine relates to their antioxidant characteristics [6, 52]. The results of the TBARS assay in colonic tissue show that *Q. brantii* reduces colonic damage by down-regulating LPO. *Quercus* could increase the anti-oxidant power of colonic tissue in comparison to controls as expected; this property may relate to the potential of *Quercus* gall to scavenge free radicals and enhance the activity of anti-oxidant enzyme. Galls of *Q. infectoria* (another species) have been shown to contain a large amount of polyphenols. High performance thin layer chromatography (HPTLC) analysis of extract suggested that it contains 19.925% tannic acid (TA) and 8.75% gallic acid (GA) [35]. By measuring total phenolic content as the main components of galls of *Quercus* spp. [25], in our study, it was demonstrated that 88.43 ±7.23% of dry gall (Table I) was composed of phenolics, which are one of the main components responsible for radical scavenging activity of plants [53]. Quantification of free gallic acid as one of the present phenolic compounds in galls of *Quercus* spp. revealed that it constitutes one of the main components of the gall (3.74% of dry weight). In previous studies, the antioxidant and pro-oxidant activity of gallic acid, its derivatives and metabolites have been shown [54, 55].

Regarding the pathogenesis of colitis, two parameters are more important than others: microbes and inflammation [56–58]. Therefore, we can conclude that *Q. brantii* probably acts through its anti-inflammatory and antimicrobial effects. *Quercus brantii* induced antioxidants especially via inhibition of inflammatory cytokines such as TNF-α, IL-1β and MPO. It is notable that in macroscopic, microscopic and enzymatic evaluation, positive results with the gall extract surpassed those of gall powder. This might relate to better release of effective compounds of extract in comparison to crude plant powder, which needs more time. In the extract form, the treatment process is facilitated by decreasing the release stage of tannins.

Polyphenols including gallic acid are responsible for the main antioxidant and anti-inflammatory effect of galls of *Q. brantii*.

In view of the fact that antioxidants and anti-cytokines usually prevent complications or further progress of the disease, it is strongly recommended to examine useful medicinal herbs like *Q. brantii* in the clinic concurrent with standard therapies to determine their benefit in patients with colitis.

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