World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i12.1901

World J Gastroenterol 2013 March 28; 19(12): 1901-1911
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Promising effect of Magliasa, a traditional Iranian formula, on experimental colitis on the basis of biochemical and cellular findings

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Supported by The Tehran University of Medical Sciences partly, as outcome of a PhD thesis and Iran National Science foundation
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Received: June 28, 2012 Revised: September 19, 2012
Accepted: October 30, 2012
Published online: March 28, 2013

Abstract

AIM: To investigate the efficacy of Magliasa, a traditional Iranian formula, on experimental colitis.

METHODS: After botanical authentication of herbal ingredients, formulation of Magliasa, quantitative determination of total glucosinolates and total phenolic content, and analysis of the thin layer chromatography profile were performed. Colitis was then induced in male rats by instillation of 2,4,6-trinitrobenzenesulfonic acid (TNBS) in all groups, aside from the Sham group. The experimental groups consisted of: the Sham group that received only normal saline; the Mag-50, Mag-100 and Mag-200 groups, which received 50, 100 and 200 mg/kg per day of Magliasa, respectively; the control group, which received vehicle water orally; the infliximab group, which received infliximab (5 mg/kg per day, subcutaneously); and the Dexa group, which received dexamethasone (1 mg/kg per day, orally). After completing the treatment period (2 wk), the rats were sacrificed, the colon was removed, its macroscopic and microscopic changes were recorded, and tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), total antioxidant capacity, myeloperoxidase (MPO), and lipid peroxidation (LPO) were assessed in colon homogenate.

RESULTS: The mean value of total glucosinolates in one gram of Magliasa was 19 ± 1 μmol. The mean value of the total phenolic content was 293.8 ± 17.6 mg gallic acid equivalents per 100 gram of Magliasa. Macroscopic scores were significantly decreased in Mag-100 (1.80 ± 0.58, P = 0.019) and Mag-200 (1.20 ± 0.20, P = 0.001) compared to the control group (3.40 ± 0.24), although some inflammation and hyperemia were evident. Treatment of rats by dexamethasone (0.33 ± 0.21, P < 0.001) and infliximab (0.83 ± 0.31, P < 0.001) remarkably attenuated scores where mild hyperemia was observed macroscopically. In comparison to the control group (4.00 ± 0.32), only Mag-200 (1.60 ± 0.40) showed a significant decrease in colonic histopathological scores (P = 0.005). Minimal mucosal inflammation was observed in the Dexa group (0.67

± 0.21, $P < 0.001$). The levels of TNF-α, IL-1β and MPO were significantly lower in all groups compared to the controls ($P < 0.05$). A significant decrease in LPO was seen in the Mag-200 (3.27 ± 0.77, $P = 0.01$) and Dexa (3.44 ± 0.22, $P = 0.011$) groups in comparison to the control group (6.43 ± 0.61). Only dexamethasone caused a significant increase in antioxidant power in comparison to the control group (346.73 ± 9.9 *vs* 228.33 ± 2.75, $P < 0.001$). Infliximab and different doses of Magliasa did not show any remarkable increase in antioxidant capacity ($P > 0.05$). The effect of Magliasa in all of mentioned parameters, except antioxidant capacity, was dose dependent.

CONCLUSION: The effects of Magliasa in TNBS-induced colitis are encouraging and warrant clinical trials for further confirmation.

Key words: Magliasa; Traditional Iranian medicine; Colitis; Neutrophil infiltration; Inflammatory cytokines; Oxidative stress

Rahimi R, Baghaei A, Baeeri M, Amin G, Shams-Ardekani MR, Khanavi M, Abdollahi M. Promising effect of Magliasa, a traditional Iranian formula, on experimental colitis on the basis of biochemical and cellular findings. *World J Gastroenterol* 2013; 19(12): 1901-1911 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i12/1901.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i12.1901

INTRODUCTION

Inflammatory bowel disease (IBD) includes two main types (Crohn's disease and ulcerative colitis), and is categorized as one of the chronic disorders of the gastrointestinal tract with an unclear etiology. Related to the involvement of possible pathological factors such as immunological abnormalities[1], oxidative stress[2], gut microflora[3], abnormal epithelial barrier[4], and inflammatory factors[5-9], various drugs are used for the management of IBD, including anti-tumor necrosis factor-alpha (TNF-α) drugs[10-12], immunosuppressants[13,14], antibiotics[15,16], probiotics[17,18], corticosteroids[19], aminosalicylates[20,21], selective cyclooxygenase-2 inhibitors[9], nicotine preparations[22], potassium channel openers[23], adenosine triphosphate donors[24], and phosphodiesterase inhibitors[25-27]. It cannot be ignored that most of the conventional treatments for the management of IBD have serious adverse effects that reduce compliance in patients[28-30], and therefore has led researchers to work on complementary and alternative medicines that can induce remission in disease activity with better safety and tolerability[31-33]. As recently reviewed by Rahimi *et al*[32], there are many plants in traditional Iranian medicine (TIM) that were historically used for the management of IBD. Magliasa is a TIM herbal prescription that has been used to treat tenesmus and diarrhea mixed with blood and mucus for a long time[34]. It consists of 6 components: the seeds of *Lepidium sativum*, *Linum usitatissimum*, and *Allium ampeloprasum* cv. Porrum, the fruit of *Bunium persicum* and *Terminalia chebula*, and the gum resin of *Pistacia lentiscus* (Table 1). Different mechanisms have been described in TIM for the usefulness of these plants in the treatment of colitis, including anti-inflammatory, antiulcer, wound healing, and anti-diarrheal effects[35,36]. Regarding the aforementioned knowledge, the present study was planned to investigate the effect of Magliasa in an experimental model of colitis to determine the involved mechanisms.

MATERIALS AND METHODS

Materials

Plant materials (seeds of *Lepidium sativum*, *Linum usitatissimum*, and *Allium ampeloprasum* cv. Porrum, fruit of *Bunium persicum* and *Terminalia chebula*, and gum resin of *Pistacia lentiscus*), were obtained from the local market at the Tehran bazaar in 2010. After confirmation by a botanist, voucher samples were deposited at the herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences (Tehran, Iran). 2,4,6-trinitrobenzenesulfonic acid (TNBS, Sigma-Aldrich), ethanol, methanol, ethyl acetate, n-hexane, thiobarbituric acid, trichloroacetic acid, n-butanol, hexadecyl-trimethyl-ammonium bromide, 2,4,6-tri(2-pyridyl)-*S*-triazine (TPTZ), hydrochloric acid, anisaldehyde, malondialdehyde, ethylenediaminetetraacetic acid, Folin-Ciocalteu reagent, toluene, dichloromethane, o-dianisidine hydrochloride, hydrogen peroxide, acetic acid, sodium acetate, Coomassie reagent, bovine serum albumin (BSA), $FeCl_3$-$6H_2O$, Na_2SO_4, H_2SO_4, H_3PO_4, KH_2PO_4, K_2HPO_4, H_2O_2, Na_2CO_3, $NaHCO_3$, Na-K-tartrate, $CuSO_4$-$5H_2O$, Silica gel 60F254 (Merck, Germany), glucose kit (ZistChem, Iran), and rat-specific TNF-α and interleukin-1β (IL-1β) enzyme-linked immunosorbent assay (ELISA) kits (Bender Med Systems, Austria) were used in this study.

Botanical authentication

All 6 herbal ingredients were authenticated macroscopically and microscopically. Macroscopic examinations included measurements of appearance, size, shape, color, texture, odor, taste, fracture, and other characteristics according to pharmacopoeias[36,37]. Microscopic examinations determined the characteristic elements of each ingredient in powder form. For this purpose, each herbal material was mounted on a microscope slide after tissue disintegration with potassium hydroxide and cleared with sodium hypochlorite. The examination protocols followed the World Health Organization's quality control methods for medicinal plant materials[38]. Characteristic elements were photographed *via* a Leitz optical microscope.

Preparation of Magliasa

Bunium persicum fruit (22%), *Linum usitatissimum* seeds (8%), *Allium ampeloprasum* cv. Porrum seeds (8%), *Terminalia che-*

Table 1 Magliasa powder ingredient characteristics

Scientific name	Iranian name	Part	Major constituents	Pharmacological activities	Herbarium No.
Lepidium sativum	Taretizak	Seed	Glucosinolates, imidazole alkaloids, fatty acids, and sterols[60-63]	↓IL-2, TNF-α, leukotriene B4 and nitric oxide in immune cells; anti-inflammatory and analgesic in rats; prokinetic, and laxatives in mice; anti-diarrheal and spasmolytic in rats; anticholinergic and phosphodiesterase inhibitor[64-68]	PMP-716
Bunium persicum	Zire kermani	Fruit	Flavonoids, essential oils, and tannins[69,70]	Antioxidant and radical scavenger; antinociceptive and anti-inflammatory in rats[70-73]	PMP-627
Linum usitatissimum	Katan	Seed	Mucilage, cyanogenic glycoside, lignans, fatty acids, and phenylpropan derivatives[74]	Antiulcer, antioxidant , and protective against intestinal tumors in mice[75-77]	PMP-717
Allium ampeloprasum cv. Porrum	Tare	Seed	Saponins, flavonoids, carotenoids, and sulfur-containing compounds[78-80]	Antioxidant[78]	PMP-718
Terminalia chebula	Halile siah	Fruit	Tannins, anthraquinones, triterpene glycosides, and beta-Sitosterol[81,82]	Antioxidant, anti NF-κB, antiulcer, ↓TNF-α, IL-1β and IL-6, and antibacterial against intestinal bacteria[83-87]	PMP-606
Pistacia lentiscus	Mastaki	Gum resin	Triterpene acids and alcohols, and essential oils[81]	Antioxidant, ↓NO, prostaglandin E2, iNOS, and Cox-2 delayed the onset and progression of colitis and prevented weight loss in mice; ↓Intensity of gastric mucosal damage, ↓TNF-α, CD activity index, plasma IL-6, and ↑total antioxidant potential in CD patients; ulcer healing in patients with duodenal ulcer[88-93]	PMP-811

Cox-2: Cyclooxygenase-2; iNOS: Inducible nitric oxide synthase; CD: Crohn's disease; NO: Nitric oxide; TNF-α: Tumor necrosis factor-α; IL: interleukin; NF-κB: Nuclear factor κB.

bula fruit (8%), and *Pistacia lentiscus* gum resin (4%) were individually powdered by milling, and then mixed. Intact non-milled seed of *Lepidium sativum* (50%, w/w) was added to the powdered material and again mixed.

Quantitative determination of total glucosinolates and total phenols Magliasa

The amount of total glucosinolates as major constituents of *Lepidium sativum* and the amount of total phenolic compounds as major constituents of *Bunium persicum*, *Allium ampeloprasum* cv. Porrum, and *Terminalia chebula* were measured in Magliasa.

Total glucosinolates were determined by the measurement of enzymatically-released glucose[39]. For this purpose, four accurately weighed 1 g samples of Magliasa were transferred into separate loaded ball-mill cups. To three cups 1 mL of water was added (samples), while the last cup had 1 mL of acidified 40% v/v methanol/water added instead (sample blank). All cups were milled side by side for 2 min, allowed to stand for 5 min, and then had 19 mL of acidified 40% v/v methanol added to each cup. After recapping and shaking vigorously, the cup contents were filtered through charcoal-coated papers. Immediately prior to colorimetric assay, each of the filtrates was diluted ten-fold with water, and then 0.2 mL was poured into separate 10 mL tubes. About 0.2 mL of water was added into a fifth tube (water blank), with 0.2 mL of standard glucose solution (1 mg/mL) (ZistChem, Iran) added into a sixth tube. Five mL of buffer/enzyme/chromogen reagent (ZistChem, Iran) was added to all tubes, mixed, and then placed in a water bath at 37 ℃ and read within 10-15 min. The absorbance of each solution against the water blank was measured at 610 nm.

The total phenolic contents in the medicinal plants were determined spectrophotometrically according to the Folin-Ciocalteu method[40]. Gallic acid was used to set up the standard curve. The phenolic compound content of the samples was expressed as gallic acid equivalents (GAE) in mg per 100 g of Magliasa. All the samples were analyzed in triplicate.

Thin layer chromatography profile of Magliasa

Thin layer chromatography (TLC) was performed to obtain preliminary data from essential oils and lipophilic substances. For this purpose, 1 g of Magliasa was extracted by shaking for 15 min in 10 mL of dichloromethane at room temperature. The suspension was filtered, and the clear filtrate evaporated to dryness. The residue was dissolved in 1 mL of toluene. Samples were then applied to the plates, which were developed at room temperature in glass chambers previously saturated for 1 h. The development distance was 5 cm. The mobile phase was n-hexane-ethyl acetate 5:4 (v/v). The spray reagent was anisaldehyde- sulfuric acid[41].

Animals

Male Wistar-albino rats, weighing between 220 and 230 g, were maintained under standard conditions of temperature (23 ℃ ± 1 ℃), relative humidity (55% ± 10%), a 12-h dark and light period, and fed with a standard pellet diet and water *ad libitum*. All ethical themes of the animal studies were considered carefully, and the experimental protocol was approved by the ethical committee of Tehran University of Medical Sciences (code number of 88-04-33-10094).

Interventions

Rats were randomly divided into seven groups containing six individuals in each group. Colitis was induced

by the instillation of TNBS in all groups except group 1. The groups were: (1) Sham which received normal saline; (2) Mag-50 which received 50 mg/kg per day of Magliasa; (3) Mag-100 which received 10 mg/kg per day of Magliasa; (4) Mag-200 which received 200 mg/kg per day of Magliasa; (5) control which received vehicle water orally; (6) Infliximab which received infliximab (5 mg/kg per day, subcutaneously); and (7) Dexa which received dexamethasone (1 mg/kg per day, orally). Magliasa was dissolved in water and administered by gavage. The doses for Magliasa were selected after a pilot study. The effective doses of infliximab and dexamethasone were selected from our previous studies[42].

Induction of colitis

For induction of colitis, 36 h fasted rats were anesthetized with an intraperitoneal administration of 50 mg/kg of pentobarbital sodium, positioned on their right side, and then had 0.3 mL of a mixture containing six volumes of TNBS 5% w/v in H_2O (equal to 15 mg TNBS) plus four volumes of ethanol (99%) instilled *via* the rectum using a rubber cannula (8 cm long)[43]. Following instillation of TNBS, rats were maintained in a supine Trendelenburg position in order to prevent anal leakage of TNBS. Medications were then administered to the animals for 14 d as described above. On the 15th day, the animals were sacrificed by an overdose of ether inhalation. The abdomen was rapidly dissected open and the colon was removed. The colon was cut open in an ice bath, cleansed gently using normal saline, observed normally for macroscopic changes, and scored in a manner described later. Samples were then cut into two pieces; one piece for histopathology assessment (fixed in 5 mL formalin 10%) and one piece for measuring biomarkers weighed and maintained in -20 ℃ for 24 h. The colonic samples were then homogenized in 10 volume ice-cold potassium phosphate buffer (50 mmol/L, pH 7.4), sonicated, and centrifuged for 30 min at 3500 × *g*. The supernatants were transformed into several microtubes for separate biochemical assays, and all were kept at -80 ℃ until analyses[44].

Macroscopic and microscopic assessment of colonic damage

The macroscopic damage was assessed and scored according to criteria as described in our previous work[45,46]. For microscopic analysis, the fixed segments in formalin 10% were embedded in paraffin and stained with hematoxylin and eosin. The scoring was performed by one who was blind to the treated groups.

Determination of TNF-α and IL-1β

Quantitive detection of TNF-α and IL-1β levels in colon tissues were performed using an ELISA kit. The absorbance of the final colored product was measured in 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 in our previous work[44].

Total ferric reducing antioxidant power assay

Total antioxidant power of the colon was evaluated by measuring the ability to reduce Fe^{3+} to Fe^{2+}. Interaction of TPTZ with Fe^{2+} results in the formation of a blue color, with a maximum absorbance at 593 nm. Data were expressed as mmol/L ferric ions reduced to ferrous per mg of protein, as described in our previous work[47].

Myeloperoxidase activity measurement

In this test, supernatant was combined with o-dianisidine and 0.0005% H_2O_2 that resulted in an absorbance at 460 nm that was measured for 3 min. One unit of myeloperoxidase (MPO) activity is described as the change in absorbance per min at room temperature in the final reaction. Details of the procedure have been described in our previous work[48].

Thiobarbituric acid-reactive substances assay

Levels of lipid peroxidation were assessed in colon tissue using thiobarbituric acid reactive substances (TBARS) assay as described in our previous work[49]. Data were reported as μg/mg of protein.

Total protein of colon homogenate

Total protein of the tissue was measured according to the Bradford method, using BSA as the standard[50]. Results were reported as mg of protein per mL of homogenized tissue.

Determination of LD50

In order to determine the acute toxicity (LD50) of Magliasa, doses of 5, 50, 300 and 2000 mg/kg per day were gavaged to rats. The animals were observed for 1 wk and any mortality was recorded at the end of this period[51].

Statistical analysis

Data were analyzed by StatsDirect ver. 2.7.8. One-way analysis of variance followed by a Newman-Keuls *post hoc* test for multiple comparisons were used. *P* values less than 0.05 were considered significant. Results are expressed as mean ± SE.

RESULTS

Botanical authentication

Microscopic characteristics of different herbal components of Magliasa are shown in Figure 1.

Quantitative determination of total glucosinolates and total phenols in Magliasa

The mean value of total glucosinolates in one gram of Magliasa was 19 ± 1 μmol. The mean value of total phenolic content was 293.8 ± 17.6 mg GAE per 100 g of Magliasa.

TLC analysis

Table 2 summarizes the retention value of spots visible in the TLC profile of Magliasa.

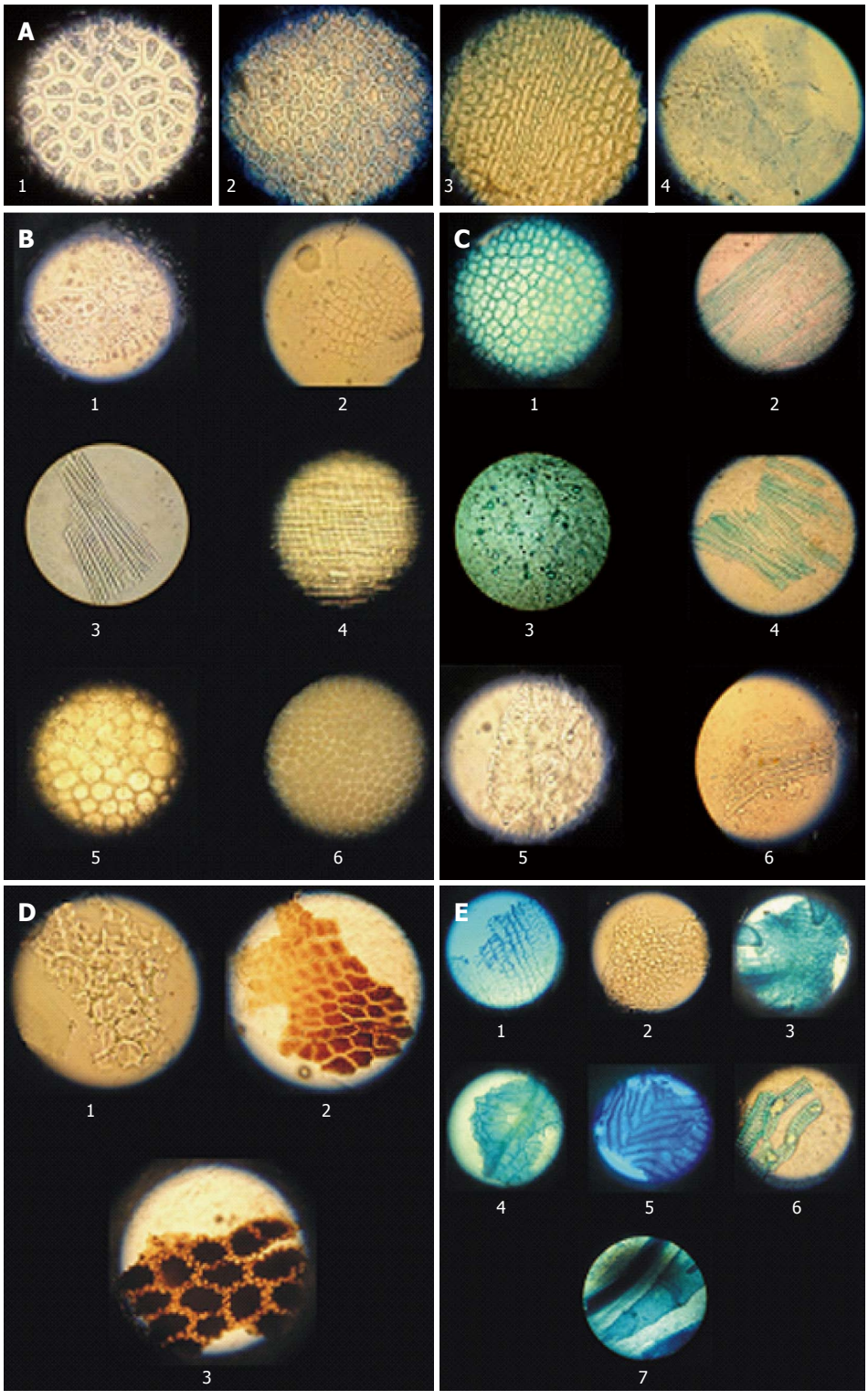

Figure 1 Microscopic characteristics of the herbal ingredients of Magliasa. A: Lepidium sativum seed. A1: Pericarp; A2 and A3: Sclereids of the mesocarp; A4: Parenchyma of the endosperm; B: Linum usitatissimum seed. B1: Endosperm; B2: Epidermis; B3: Fiber; B4: Sclerenchyma; B5: Parenchyma of the testa; B6: Pigment layer of testa; C: Terminalia chebula fruit. C1: Epidermis; C2: Fiber; C3: Parenchyma of the mesocarp; C4 and C5: Sclereids; C6: Vessels; D: Allium ampeloprasum cv. Porrum seed. D1: Endosperm; D2: Mesoderm; D3: Epidermis of the testa; E: Bunium persicum fruit. E1: Endocarp; E2 and E3: Endosperm; E4 and E5: Sclereids of the mesocarp; E6: Vessels; E7: Vittae. Magnification of all images was 40.

Macroscopic and microscopic assessment of colonic damage

Data are shown in Table 3. The colons of the Sham group appeared normal. In contrast, intracolonic administration of TNBS led to mucosal ulceration, inflammation, adhesion, and wall thickening in the control group. Treatment with Mag-50 did not significantly reduce macroscopic scores where linear ulceration and mesenteric inflammation were observed in some samples. Administration of Maglasia reduced the macroscopic score in a dose-dependent manner, and a significant effect was observed in the Mag-100 and Mag-200 groups, although some inflammation and hyperemia were evident. The median effective dose (ED50) value was 104.78 mg/kg. Treatment of rats by dexamethasone and infliximab remarkably attenuated scores where mild hyperemia was observed macroscopically.

Histopathological examination of the control group showed extensive severe transmural inflammation, dif-

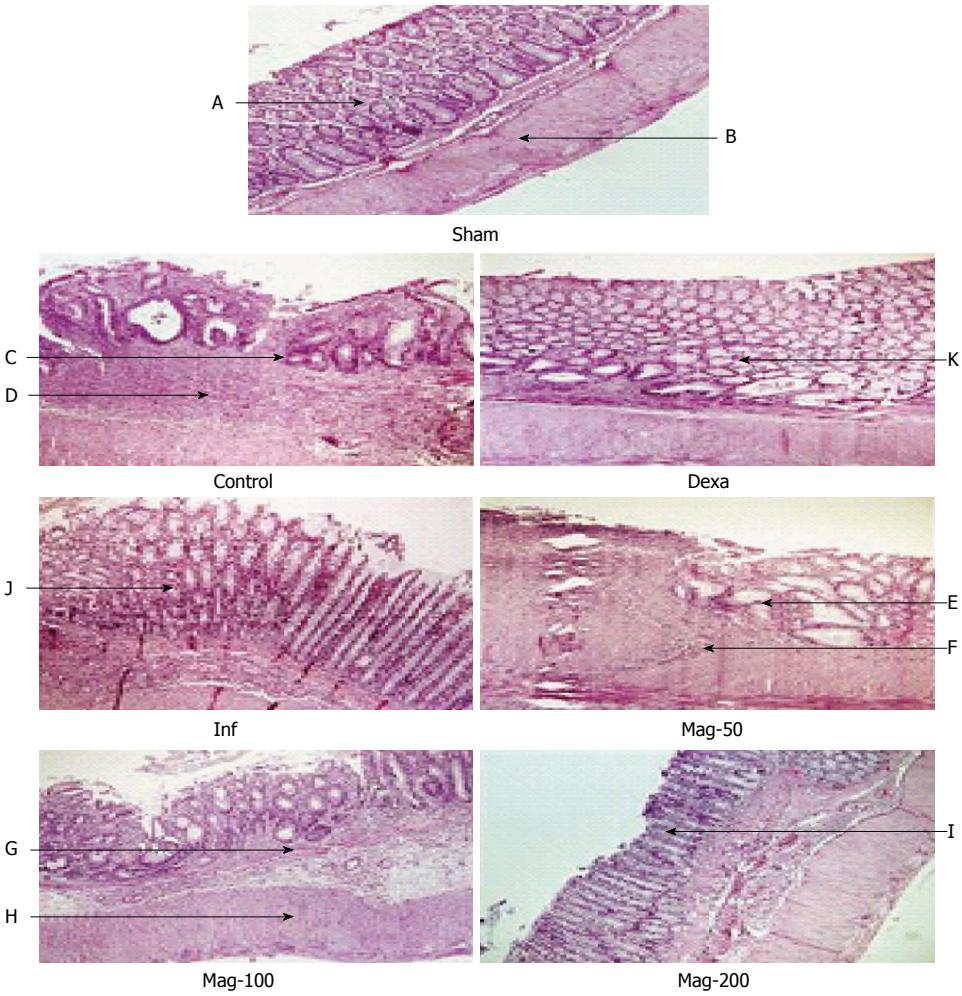

Figure 2 Histological images of colon samples. In the Sham group, colons were within normal limits including crypts (A) and submucosal tissue (B), but intense transmural inflammation (C), and severe crypt destruction (D) were observed in the control group. Moderate crypt distortion (E and G) and inflammatory cell infiltration (F and H) was seen in the Mag-5 and Mag-100 groups. Mild focal inflammation and crypt abscess were observed in the Mag-20 and infliximab groups (I and J). Mild focal inflammation was observed in the Dexa group (K). Magnification of all images was 100. Dexa: Dexamethasone; Inf: Infliximab; Mag-50: Magliasa at a dose of 50 mg/kg; Mag-100: Magliasa at a dose of 100 mg/kg; Mag-200: Magliasa at a dose of 200 mg/kg.

Table 2 Thin layer chromatography analysis of Magliasa

Type of extract	Solvent system	RF values	Intensity of spot
Dichloromethane	Hexane: ethyl acetate (5:4 v/v)	0.048	Moderately intense
		0.181	Faint
		0.238	Intense
		0.286	Faint
		0.333	Intense
		0.380	Intense
		0.430	Faint
		0.476	Faint
		0.524	Moderately intense
		0.571	Faint
		0.619	Faint
		0.670	Faint
		0.714	Faint
		0.762	Intense
		0.838	Faint
		0.876	Moderately intense

RF: Retention factor.

Table 3 Macroscopic and microscopic scores as criteria for assessing colonic damage

Group	Macroscopic score		Microscopic score	
	mean ± SE	Median (range)	mean ± SE	Median (range)
Sham	0.00 ± 0.00	0 (0-0)	0.00 ± 0.00	0 (0-0)
Control	3.40 ± 0.24[a]	3 (3-4)	4.00 ± 0.32[a]	4 (3-5)
Dexa	0.33 ± 0.21[c]	0 (0-1)	0.67 ± 0.21[c]	1 (0-1)
Inf	0.83 ± 0.31[c]	1 (0-2)	1.50 ± 0.34[c]	1 (1-3)
Mag-50	2.20 ± 0.37[a,e,g]	2 (1-3)	2.60 ± 0.51[a,e]	3 (1-4)
Mag-100	1.80 ± 0.58[a,c,e]	1 (1-4)	2.40 ± 0.75[a]	2 (1-5)
Mag-200	1.20 ± 0.20[c]	1 (1-2)	1.60 ± 0.40[c]	1 (1-3)

[a]$P < 0.05$ *vs* Sham group; [c]$P < 0.05$ *vs* the control group; [e]$P < 0.05$ *vs* the dexamethasone group; [g]$P < 0.05$ *vs* the infliximab group. Dexa: Dexamethasone; Inf: Infliximab; Mag-50: Magliasa at a dose of 50 mg/kg; Mag-100: Magliasa at a dose of 100 mg/kg; Mag-200: Magliasa at a dose of 200 mg/kg.

fused necrosis, mucosal and submucosal polymorphonuclear (PMN) leukocyte infiltration, and crypt destruction, whereas microscopic evaluation of the Sham group showed a normal situation. In the Mag-50 group, microscopic evaluation revealed moderate mucosal and submucosal inflammation, PMN infiltration, and extensive crypt distortion. In the Mag-100 group, moderate inflammation of the mucosa and submucosa, inflammatory cell infiltration, and some crypt abscess and destruction were observed. In the Mag-200 and infliximab groups, mild focal non-hemorrhagic edema and focal submucosal PMN infiltration were observed. Minimal mucosal inflammation was observed in the Dexa group (Figure 2). Administration of Maglasia reduced the microscopic score in a dose-dependent manner, and a significant effect was observed

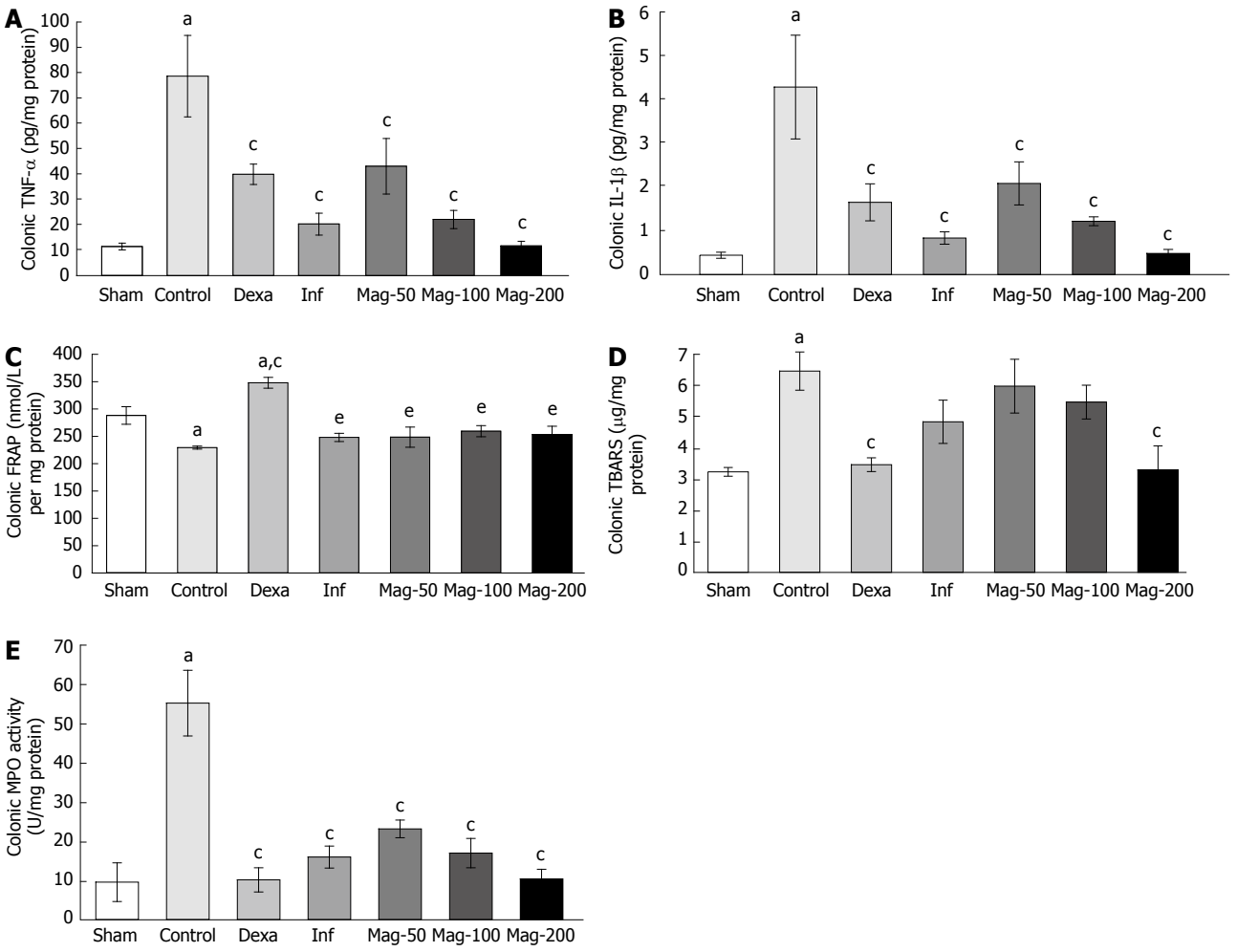

Figure 3 Levels of different biochemical parameters in the colon of rats after 2 wk of treatment. A: Tumor necrosis factor-alpha (TNF-α); B: Interleukin-1 beta (IL-1β); C: Total antioxidant capacity as a ferric reducing antioxidant power (FRAP) level; D: Lipid peroxidation as a thiobarbituric acid reactive substances (TBARS) level; E: Neutrophil infiltration as myeloperoxidase (MPO) activity. Values are mean ± SE. $^{a}P < 0.05$ *vs* the Sham group; $^{c}P < 0.05$ *vs* the control group; $^{e}P < 0.05$ *vs* the dexamethasone group. Dexa: Dexamethasone; Inf: Infliximab; Mag-50: Magliasa at a dose of 50 mg/kg; Mag-100: Magliasa at a dose of 100 mg/kg; Mag-200: Magliasa at a dose of 200 mg/kg.

in the Mag-200 group, with an ED50 of 132.29 mg/kg.

Colonic TNF-α

A significant difference was seen in TNF-α between the control and Sham groups ($P = 0.000$). TNF-α was significantly lower in all groups compared to the control, with an ED50 of 55.36 mg/kg. The level of TNF-α in the Mag-100 group (21.99 ± 3.54) was near to that of the infliximab group (20.18 ± 4.29), and both were lower than that of the Mag-50 (42.85 ± 10.87) and Dexa (39.72 ± 3.97) groups. TNF-α in the Mag-200 group (11.60 ± 1.83) was lower than that of the infliximab group, but the difference was not significant ($P = 0.98$, Figure 3A).

Colonic IL-1β levels

IL-1β was higher in the control group compared to the Sham ($P = 0.000$). IL-1β in all groups was lower than that of the control, with an ED50 of 48.78 mg/kg. IL-1β in the Mag-100 group (1.21 ± 0.10) was near to that of the Dexa group (1.64 ± 0.42), and both were higher than that of the infliximab (0.83 ± 0.14) and Mag-200 (0.48 ± 0.09) groups. IL-1β in the Mag-200 group was near to that of the Sham (0.44 ± 0.07), and was lower than infliximab, but the difference was not significant ($P = 0.998$, Figure 3B).

Colonic total antioxidant power as ferric reducing/antioxidant power

The ferric reducing/antioxidant power (FRAP) value was significantly lower in the control compared to the Sham ($P = 0.008$). Among interventions, only dexamethasone caused a significant increase in FRAP when compared to the control ($P = 0.000$). None of the Mag-50, Mag-100, Mag-200 or infliximab groups showed a significant difference to the control in FRAP (Figure 3C). The effect of Magliasa in FRAP was not dose dependent.

Colonic lipid peroxidation level as TBARS

The TBARS value was significantly higher in the control compared to the Sham ($P = 0.005$), while TBARS in the Mag-200 (3.27 ± 0.77, $P = 0.010$) and Dexa (3.44 ± 0.22, $P = 0.011$) groups was significantly lower than that of the control (6.43 ± 0.61). Other groups did not show a signif-

icant difference from the control in TBARS (Figure 3D). Administration of Maglasia reduced TBARS in a dose-dependent manner, with an ED50 value of 216.4 mg/kg.

Colonic MPO activity

MPO in the control was significantly higher than that of the Sham ($P < 0.002$). Treatment with Magliasa in all groups significantly decreased MPO activity compared to the control. MPO in the Mag-200 group (10.65 ± 2.53) was lowest amongst the Mag groups, and close to the Dexa group (10.42 ± 3.18). MPO in the Mag-200 group was lower than that of the infliximab group (16.41 ± 2.89) (Figure 3E). The ED50 value was 34.38 mg/kg.

LD50

The acute toxicity test (LD50) demonstrated that Magliasa is not lethal up to a dose of 2000 mg/kg after oral administration. In the treated groups, no sign of toxicity was observed. It can therefore be considered as practically non-toxic.

DISCUSSION

There is a strong potential in the traditional and folkloric medicines of various countries, including Iran, for developing new and efficacious drugs for diseases that have a challenging treatment. One such disease is IBD. In this paper, Magliasa, one of the remedies recommended for colitis in TIM, was prepared, and its efficacy and possible mechanisms of action in different doses were evaluated in TNBS-induced colitis and compared with standard drugs. Macroscopic and microscopic scores, as criteria for colonic damage, improved by doses of 100 and 200 mg/kg per day with Magliasa. The microscopic score reduced only in the Mag-200 group, while the Mag-50 group showed no significant benefit against colonic damage. Colonic TNF-α, IL-1β and MPO activities were significantly decreased by all doses of Magliasa. TNF-α and IL-1β have been described as important mediators that contribute to intestinal inflammation in IBD patients[52-54]. Increased TNF-α has been found in the serum and mucosa of patients with IBD[55,56]. Moreover, inhibition of TNF-α by anti-TNF-α drugs, such as infliximab, has been an efficacious strategy in the management of IBD[10,57]. MPO is located in the granules of neutrophils and released upon stimulation by free radicals. The activity of MPO has been known as a marker of neutrophil penetration to the site of inflammation[58,59]. Magliasa did not affect oxidative stress as a factor involved in the pathophysiology of IBD[2]. Lipid peroxidation in the colon decreased only with a high dose of Magliasa (Mag-200). The effects of Magliasa in all investigated parameters were dose-dependent, except in total antioxidant power.

The total phenolic content of Magliasa was determined because phenolic compounds have pharmacological activities (antioxidant, anti-inflammatory, anti-diarrheal, and antimicrobial) that are all useful for the management of IBD, considering its pathogenesis. There is concern about the content uniformity of Magliasa, as intact non-milled seed of *Lepidium sativum* comprises 50% of the product. In addition to a reference marker for the standardization of Magliasa, total glucosinolates can be used for evaluating the content uniformity of the product.

There are some reports on the herbal ingredients of Magliasa that confirm their efficacy in IBD[32]. These reports are summarized in Table 1. Anti-inflammatory, antioxidant, analgesic, spasmolytic, antiulcer, ulcer healing, immunomodulatory, antibacterial, and anti-diarrheal activity are among the pharmacological properties of these ingredients that make them useful for IBD. It seems that the efficacy of Magliasa in IBD is due to the combination of the mentioned activities.

Overall, the results obtained from the efficacy of Magliasa on TNBS-induced colitis of rats are encouraging, although clinical trials are required for confirmation of these results.

COMMENTS

Background

Conventional treatments for the management of inflammatory bowel disease (IBD) have serious adverse effects that reduce patient compliance, and therefore investigators are trying to find useful compounds from complementary and alternative medicines with better safety and tolerability. There are many herbal preparations in traditional Iranian medicine (TIM) that were used for the management of IBD. Magliasa is one of them, and contains 6 components: seeds of *Lepidium sativum*, *Linum usitatissimum*, and *Allium ampeloprasum* cv. Porrum, fruit of *Bunium persicum* and *Terminalia chebula*, and gum resin of *Pistacia lentiscus*. Although, the efficacy of some herbal components of Magliasa in IBD have been confirmed by previous studies, no other study to date has investigated the beneficial effects of this preparation.

Research frontiers

In the present study, after formulation and explanation of the quality control methods of Magliasa, its effects were investigated in trinitrobenzenesulfonic acid-induced colitis of rats to determine the involved mechanisms.

Innovations and breakthroughs

Magliasa demonstrated a significant reduction in macroscopic colonic damage, tumor necrosis factor-alpha, interleukin-1 beta, and neutrophil infiltration. Determination of total glucosinolates and total phenolic contents, as well as performing thin layer chromatography, can be used successfully for quality control of this herbal preparation.

Applications

Since the effects of Magliasa in the experimental model of colitis were encouraging, it could potentially be used as an effective medicine for IBD after confirmation of obtained results by clinical trials. Moreover, this study is a step toward strengthening TIM evidence.

Peer review

Hopefully reviewers are positive to this article and believe that this TIM formula has enough support to go forward future clinical trials.

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