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Original article

Anti-inflammatory effect of *Pistacia atlantica* subsp. *kurdica* volatile oil and gum on acetic acid-induced acute colitis in rat

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Abstract

Background and objectives: Baneh tree or *Pistacia atlantica* subsp. *kurdica* is an endemic plant of Iran which belongs to *Anacardiacea*e family. It has various traditional uses including astringent and anti-diarrheal as well as improving some of the symptoms of gastrointestinal upsets. In this study we decided to investigate the effects of various fractions of baneh gum with different doses in an animal model of ulcerative colitis as one of the important chronic inflammatory bowel diseases of the gastrointestinal tract. **Methods**: The volatile oil and aqueous baneh gum suspensions were prepared and the constituents of the volatile oil were analyzed by GC/MS. They were used to treat colitis induced by acetic acid 4% in rats. Three doses of gum (100, 200 and 400 mg/kg) were administered both orally (*p.o.*) and intra-rectally (*i.r.*) while volatile oil was administered *p.o.* with doses 100, 200 and 400 µl/kg for four constitutive days. Anti-inflammatory effects of the test compounds were compared with oral prednisolone and hydrocortisone enema. Wet colon weight/ length ratio and tissue damage scores and area as well as indices of colitis and tissue myeloperoxidase activity were evaluated for each specimen. **Results**: Alpha-pinene was the main constituent of baneh volatile oil (41.23%). We observed therapeutic effects in applied doses of oral gum as well as volatile oil to reduce all indices of colitis and myeloperoxidase activity. Unlike the oral form of gum, its rectal administration was not significantly effective to improve colitis. **Conclusion:** This research has proved the anti-inflammatory potential of oral gum of *Pistacia atlantica* subsp. *kurdica* and its volatile oil in an experimentally induced colitis.

Keywords: Anacardiaceae, gum, *Pistacia atlantica*, ulcerative colitis, volatile oil

Introduction

Inflammatory bowel diseases (IBD) are one of the most important diseases of the digestive tract, which are progressive, chronic and mutable. The disease occurs in two forms of Crohn's disease and ulcerative colitis. The most important symptoms of Crohn's disease are diarrhea, abdominal pain and weight loss. In ulcerative colitis, symptoms include false membrane ulcers and polyps, diarrhea with blood and mucus, lethargy and anorexia, involvement of the anus to

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rectum and some other gastrointestinal manifestations. There are many factors that contribute to the IBD, although the etiology of the disease is still unknown. Immune system disorders, gastrointestinal system abnormalities and microbial contents, oxidative stress, atypical activities of cyclooxygenase-2, nitric oxide and leukotriene B are among those contributed factors [1,2]. Several treatments for IBD are considered and among them corticosteroids, amino salicylates, anti-tumor necrosis factors and immunomodulators are common although their high cost as well as several side effects have limited their clinical usage [1-4]. In recent years herbal medications and phytotherapeutics due to a variety of active constituents, possibility of long-term use and rational prices have been of interest to the clinicians [5]. Various pharmacological and animal based studies have been performed in the IBD area and some of them were focused on ulcerative colitis treatments by herbals. *Copaifera langsdorffii*, *Cydonia oblonga*, *Berberis vulgaris*, *Carum carvi*, *Moringa oleifera*, *Prunus armeniaca*, *Echium amoenum*, *Kelussia odoratissima* and *Zingiber officinale* are among medicinal herbs that their positive effects on induced ulcerative colitis of animals have been introduced [6-14].

Baneh tree or wild pistachio with scientific name of *Pistacia atlantica* subsp. *kurdica* is a plant from Anacardiaceae family. It is endemic in Iran, especially in the Auramanat area located in the Kurdistan province in western part of Iran [15,16]. Exudate gums of the tree contain resins and volatile oil and pinenes, sabinene and limonene are the main ingredients of its oil [17,18]. The traditional and folk uses of baneh indicate the positive effects of the whole plant and gums on gastrointestinal ailments and several digestive problems such as peptic ulcers, diarrhea, gastritis and intestinal upsets [16,19-21]**.** Anti-*Helicobacter pylori* effects of *P. atlantica* subsp. *kurdica* were examined and satisfactory results were obtained [17,18]. Other significant effects of baneh leaf or fruit extract include control of cutaneous leishmaniasis [22], alpha-amylase and alpha-glucosidase inhibitory effects [23,24], anti-tumor activity [25] and strong acetylcholinesterase (AChE) inhibition [26]. Different types of gums are achieved from other *Pistachia* species that mastic is one of the famous ones and has been used extensively for its anti-inflammatory effects [27]. Mastic can significantly decrease the plasma levels of interleukin 6 and C-reactive protein in patients with mild to moderate active Crohn's disease [28].

In this research we decided to analyze the *P. atlantica* subsp. *kurdica* gum's volatile oil and examined the anti-inflammatory effects of oil and gum on acetic acid-induced acute colitis in rat.

Experimental

Plant material and preparation of extract

The gums of wild growing *P. atlantica* subsp. *kurdica* were collected from Nudsheh village, Kurdistan, Iran at the altitude of ca. 1600 meter in summer 2013. The plant specimen was identified by Dr. Hussein Maroufi (Department of Botany, University of Kurdistan, Iran) and its voucher herbarium specimen (No. 2829) was deposited at the Herbarium of Isfahan School of Pharmacy, Iran. The volatile oil of the fresh plant gums was isolated by hydrodistillation method. For preparation of gum solution, the gum was suspended in 0.2% (v/v) tween 80 in distilled water (vehicle), just before use [9,22,29,30]**.**

GC/MS Analysis

The constituents of baneh volatile oil were identified by gas chromatography followed by mass spectrometry. The analysis was performed on a Hewlett-Packard 5972A mass selective detector coupled with a Hewlett-Packard 6890 gas chromatograph, equipped with a HP-5MS capillary column (30 m × 0.25 mm; film thickness 0.25 μm). The oven temperature was programmed from 60-280 °C at the rate of 4 °C per min. Helium was used as the carrier gas at a flow rate of 2 mL/min. Injector and detector temperatures were 280 °C. The MS operating parameters were: ionization voltage, 70 eV; ion

source temperature, 230 °C; mass range, 35-425. The MSD ChemStation was used as operating software. Retention indices were calculated by using retention times of *n*-alkanes (C_8- C_{24}) that were injected after the oil at the same conditions. Components of the oil were identified by comparison of their retention indices (RI) with those reported in the literature and also by computer matching with Wiley275.L library. The fragmentation patterns of the mass spectra were also compared with those reported in the literature [29-33].

Chemicals

Prednisolone powder was purchased from Iran Hormone Pharmaceutical Company (Tehran, Iran). Hydrocortisone enema was purchased from Ani Pharmaceutical inc Company (USA). O-dianisidine HCl and Hexadecyltrimethylammonium bromide (HTAB) were purchased from Sigma (St. Louis, MO, USA).

Animals

Eighty-four male Wistar rats, in weight range 180-220 g, were obtained from the Isfahan School of Pharmacy, Iran. All rats were maintained in standard laboratory conditions of temperature, humidity, light, food and water. The rats were fasted for 24 h before induction of colitis but were allowed free access to water**.** All experiments were conducted according to the ethics guidelines for research on animals approved by the Research Committee of Isfahan School of Pharmacy for the update of the guide for the care and use of laboratory animals [11,12,34].

Animal groups

Fourteen groups of rats with 6 animals in each were studied.

Normal (Sham): Received vehicle (tween 80, 0.2% (v/v) in water) orally (*p.o.*), 2 h before instillation of normal saline intra-rectally (*i.r.*).

Negative controls: Received vehicle *p.o.* and/or *i.r.*, 2 h before colitis induction.

Positive controls (reference groups): Received prednisolone (4 mg/kg) *p.o.*, or hydrocortisone enema (20 mg/kg) *i.r.*, 2 h before colitis induction.

Test 1 (Baneh Gum): Doses (100, 200, 400 mg/kg) *p.o.*, 2 h before colitis induction.

Test 2 (Baneh Gum): Doses (100, 200, 400 mg/kg) *i.r.*, 2 h before colitis induction.

Test 3 (Volatile oil of Baneh): Doses (100, 200, 400 µL/kg) *p.*o., 2 h before colitis induction.

The treatments were continued daily for 4 days after colitis induction (total duration of treatment was 5 days).

Induction of colitis

Fasted rats were slightly anesthetized with inhalational ether and received enemas of 2 mL acetic acid (4%) in water [35]. All test drugs and compounds were prepared freshly before administration.

Assessment of colon macroscopic damage

On the fifth day after induction, the animals were euthanized by ether overdose inhalation, 24 h after the last dose. Then colon was excised from 2 cm proximal to the anus and 8 cm in length. Colon samples were cleaned up with normal saline; put on the table and photos were taken with camera. The pictures were analyzed subsequently by Fiji Image Processor Program to measure the surface that was ulcerated. Macroscopic mucosal damage was evaluated according to the Morris *et al.* method [36].

Scores were: 0= no ulcer, 1= mucosal erythema only, 2= mucosal edema, slight bleeding or erosions, 3= moderate edema, bleeding ulcers or erosions, 4= severe ulceration, erosions, edema and tissue necrosis and perforation.

The ulcer index was determined by summing the mean ulcer score and the mean ulcer area.

Assessment of colon histological damage

For further assessments, the tissue samples were divided into two parts of equal length, the weight of each part was measured, and one part was placed in10% formalin for pathologic studies and

was kept in the freezer (-18 °C). Other part of the colon was placed into the nitrogen tank for a minute, and then was kept in the refrigerator at -70 °C for assessing the myeloperoxidase activity (MPO).

Inflammation severity and extent as well as crypt damage were calculated on H&E stained and coded sections while a modification of a validated scoring system described by Cooper and Dieleman was used [37,38]. Total colitis index was measured by summing three sub-scores (inflammation extent, crypt damage and inflammation severity). Macroscopic and histological injuries were recorded by a blind pathologist using a Zeiss R microscope equipped with a Sony R color video camera for taking digital imaging.

Determination of MPO activity

The test solutions and reagents were prepared and MPO activity per g of tissue was determined and calculated by the following method:

Samples were removed from the freezer, and then 0.1 g was weighted from each sample and dissolved in 10 mM potassium phosphate buffer with pH 7 containing 0.5% hexadecyltrimethyl-ammonium bromide; then homogenized (homogenizer machine assistance). After sonication, the samples were centrifuged with 20,000 rpm for 30 min at 4 °C. To supernatant fluid that was centrifuged, (0.1 mM) H_2O_2 and (1.6 mM) O-dianisidine HCl were added, then the sample absorbance was measured at 450 nm [39].

Statistical analysis

To analyze the parametric data One-way ANOVA test with Tukey post hoc test was used. To compare the results of non-parametric data, Mann-Whitney U-test was used. Data was shown as mean±SEM. *P* value <0.05 was considered as significant level.

Results and Discussion

About 90% of the volatile oil compounds (38 compounds) were identified in the baneh gum. The most abundant compounds included α-pinene (41.23%), β-pinene (6.85%) and trans-verbenol (5.39%) which included about 54% of the volatile oil compounds. Other compounds comprised less than 5% of total volatile oil. The results are listed in table 1 with their retention times, retention indices and percentage shares.

Table 1. Constituents of the fresh gum volatile oil of *P. atlantica* subsp. *kurdica*

No.	RT (min)	RI	Compound	%
1	3.68	925	tricyclene	0.48
2	3.87	936	α-pinene	41.23
3	4.23	948	camphene	2.39
4	4.28	966	verbenone	0.68
5	4.62	971	sabinene	1.58
6	4.72	976	ß-pinene	6.85
7	4.94	988	ß-myrcene	1.18
8	5.15	1012	δ-3-carene	1.72
9	5.25	1015	α-terpinene	0.53
10	5.51	1020	α-phellandrene	0.31
11	5.63	1022	o-cymene	0.12
12	5.71	1026	ρ-cymene	1.19
13	5.82	1030	limonene	3.03
14	5.89	1033	1,8-cineole	1.37
15	6.01	1041	cis-ß-ocimene	0.20
16	6.54	1057	γ-terpinene	0.15
17	6.80	1066	cis-sabinene hydrate	0.28
18	7.37	1091	α-terpinolene	4.96
19	7.68	1100	linalool	0.72
20	7.97	1115	1,3,8-para-menthatriene	0.10
21	8.80	1140	cis-verbenol	1.25
22	8.90	1142	trans-pinocarveol	2.33
23	9.08	1146	trans-verbenol	5.39
24	9.49	1164	pinocarvone	0.18
25	9.94	1175	terpinene-4-ol	0.50
26	10.52	1195	myrtenal	0.58
27	10.57	1197	myrtenol	0.93
28	11.24	1217	trans-carveol	0.67
29	13.30	1285	bornyl acetate	0.75
30	13.37	1290	ρ-cymene-7-ol	1.48
31	15.26	1356	α-terpenyl acetate	1.24
32	15.64	1378	longicyclene	0.19
33	16.37	1391	isolongifolene	0.14
34	16.78	1408	longifolene	2.15
35	16.92	1481	germacrene-D	0.61
36	19.80	1508	α-muurolene	0.71
37	20.47	1530	cis-calamenene	0.18
38	32.30	1970	n-hexadecanoic acid	0.97
				Total 89.32%

Composition (%), Retention times (RT,) Retention Indices (RI)

For Macroscopic presentation in control groups, inflammation, hemorrhage, ulcer, necrosis, and thickened colon were evident however, there was no visible damage in the sham (normal) groups (figure 1). The colitis caused by acetic acid was associated with an increase in MPO activity, and the MPO activity of control group showed significant increase compared with normal group (figure 2).

Table 2. Effect of gum (100, 200, 400 mg/kg) and volatile oil (100, 200, 400 µL/kg) of *P. atlantica* subsp. *kurdica* on macroscopic parameters of colitis induced by acetic acid in rats

Groups	Route	W/L ratio (mg/Cm)	Ulcer area (cm^2)	Ulcer severity (0-4)	Ulcer index (0-10)
Normal (Sham)	*p.o.*	105.1±9.4	0.0±0.0	0.0±0.0	0.0±0.0
Ctrl.	*p.o.*	212.9±36.0	6.0±1.0	4.0±0.0	10.0±1.0
Gum 100	*p.o.*	114.0±36.0***	2.1±1.4***	1.5±.0.8**	3.6±2.1***
Gum 200	*p.o.*	109.0±26.0***	2.1±1.1***	1.6±0.8**	3.7±1.5***
Gum 400	*p.o.*	96.1±46.0***	1.1±1.2***	1.3±0.5**	2.5±1.6***
VO 100	*p.o.*	143.1±20.0*	3.6±1.1*	2.3±0.5**	5.9±1.3
VO 200	*p.o.*	132.0±28.5**	2.6±2.3***	2.2±1.1**	4.7±3.4**
VO 400	*p.o.*	99.1±29.7***	1.6±1.0***	2.0±1.2**	3.5±2.1***
Pred. 4	*p.o.*	118.1±15.2***	0.6±0.1***	1.0±0.6**	1.5±0.6***
Ctrl.	*i.r.*	209.9±36.0	6.0±1.0	4.0±0.0	10.0±1.0
Gum 100	*i.r.*	184.6±42.2	4.9±1.4	3.4±0.8	8.2±2.1
Gum 200	*i.r.*	178.1±27.2	4.8±1.2	3.5±0.8	8.2±1.2
Gum 400	*i.r.*	149.1±23.2	3.9±1.5	3.6±0.8	7.4±2.1
Hydrocort. 40	*i.r.*	112.9±28.5***	1.8±1.2***	1.2±0.7**	3.0±2.4***

Data are expressed as means±SEM. Ctrl.: Control, VO: Volatile oil, Pred.: Prednisolone (4 mg/kg), Hydrocort.: Hydrocortisone (40 mg/kg), *p.o.*: oral, *i.r.*: intra-rectal, (n=6). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$

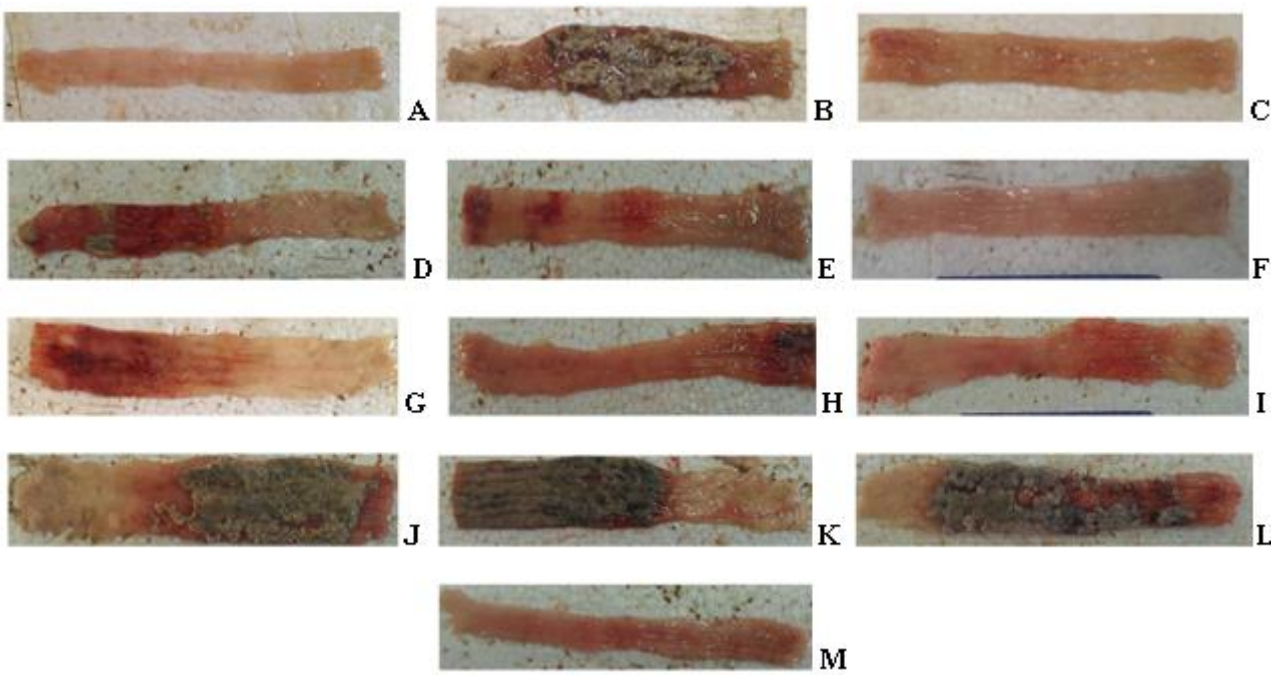

Figure 1. Macroscopic presentation of acetic acid-induced colitis in rats. Sham (A), control (B), prednisolone (4 mg/kg) (C), oral gum of baneh with doses 100 mg/kg (D), 200 mg/kg (E) 400 mg/kg (F), oral volatile oil of baneh with doses 100 µL/kg (G), 200 µL/kg (H), 400 µL/kg (I) and rectal administration of baneh gum with doses 100 mg/kg (J), 200 mg/kg (K) , 400 mg/kg (L) and rectal administration of hydrocortisone with dose of 40 mg/kg (M)

Treatment with prednisolone and hydrocortisone enema both reduced the damage score ($P<0.01$), ulcer area ($P<0.001$), ulcer index ($P<0.001$) and wet weight/length ratio ($P<0.001$) compared to respected controls (table 2).

The baneh gum and volatile oil by oral route significantly reduced the intensity of colitis parameters including ulcer severity (damage scores), ulcer area, ulcer index and wet weight/length ratio in examined doses compared to the control groups (table 2). In contrary with this, treatment with gum after intra-rectal

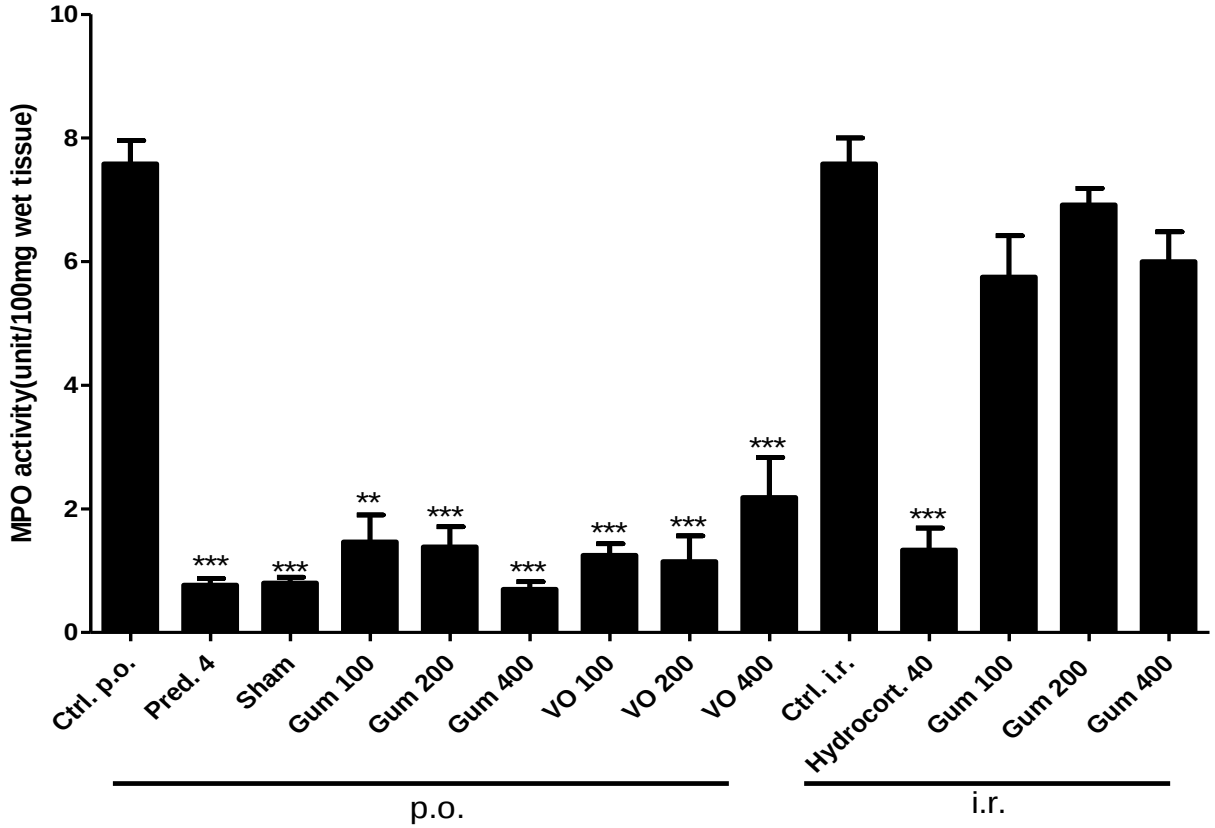

Figure 2. Effect of oral (*p.o.*) and rectal (*i.r.*) administration of gum (100, 200, 400 mg/kg) and volatile oil (VO, 100, 200, 400 µL/kg) of *P. atlantica* subsp. *kurdica* and prednisolone (Pred. 4 mg/kg) on myeloperoxidase (MPO) activity of colon tissue. The results are expressed as means±SEM, (n=6). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ denote significant difference versus control group. hydrocort.: hydrocortisone (40 mg/kg)

administration was ineffective even with the greatest test dose (400 mg/kg) (table 2). Treatment groups had no significant difference at any dose with prednisolone *p.o.* and hydrocortisone *i.r.* as references drugs.

Colonic MPO activity in the control groups was significantly higher than normal and reference groups ($P < 0.001$).

Oral gum of baneh and volatile oil were significantly effective in reducing the MPO level as compared to the control groups ($p< 0.001$), but there was no significant reduction in groups that were treated with baneh gum after rectal administration.

No significant difference was observed between oral gum and volatile oil considering MPO activity. Moreover, an increase in oral doses was not related to a more decrease in MPO activity (figure 2).

Transmural necrosis, edema and diffuse inflammatory cell infiltration in the mucosa, desquamated areas and loss of epithelium markers indicated the histopathological features of the control groups.

According to table 3 and figure 3 no histological damage was observed in normal (Sham) group. Microscopic assessments in the groups that were pretreated with prednisolone and hydrocortisone revealed significant decrease in pathologic parameters.

As shown in table 3**,** baneh gum, *i.r.* demonstrated no significant effects on

Table 3. Effect of gum (100, 200, 400 mg/kg) and volatile oil (100, 200, 400 μL/kg) of *P. atlantica* subsp. *kurdica* on histopathology parameters of colitis

Groups	Route	Crypt Damage	Inflam. Extent	Inflam. Severity	Total Colitis Index
Normal (Sham)	*p.o.*	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Ctrl.	*p.o.*	4.0±0.0	3.0±0.0	3.0±0.0	10±0.0
Gum 100	*p.o.*	2.1±1.7**	2.3±0.8	1.9±0.7**	6.3±3.1**
Gum 200	*p.o.*	1.6±1.8**	2.2±0.7*	2.0±.0.9*	5.8±3.3**
Gum 400	*p.o.*	0.5±1.2**	1.7±0.8**	1.4±0.8**	3.5±2.7**
VO 100	*p.o.*	2.0±1.5**	2.7±0.5	2.4±1.0	7.1±3.0**
VO 200	*p.o.*	1.2±1.6**	2.4±0.8	2.0±0.9*	6.6±3.2*
VO 400	*p.o.*	1.1±1.8**	1.5±0.8**	1.4±0.8**	4.0±3.3**
Pred. 4	*p.o.*	0.5±1.2**	1.9±0.9*	1.0±0.0**	4.8±3.1**
Ctrl.	*i.r.*	4.0±0.0	3.0±0.0	3.0±0.0	10±0.0
Gum 100	*i.r.*	3.8±0.4	3.0±0.0	2.7±0.8	9.5±0.8
Gum 200	*i.r.*	3.8±.0.4	3.0±0.0	2.9±0.4	9.7±0.8
Gum 400	*i.r.*	3.6±0.5	2.8±0.4	2.9±0.4	9.3±1.2
Hydrocort. 40	*i.r.*	2.3±1.8*	1.7±0.5**	1.7±0.8**	5.6±2.5**

Data are expressed as means±SEM. Ctrl.: Control, Inflam.: Inflammation, VO: Volatile oil, Pred.: Prednisolone (4 mg/kg), Hydrocort.: Hydrocortisone (40 mg/kg), *p.o.*: oral, *i.r.*: intra-rectal, (n=6). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ denote significant difference versus control group.

histological parameter; however, those groups that were treated by oral baneh gum and volatile oil, *p.o.* showed remarkable differences compared with the control group (table 3).

IBD is one of gastrointestinal diseases with its difficult treatment while its response to the treatments is usually uncertain. Recently, herbal medicines have gained a suitable and important position for IBD treatments [6-14,27]. Baneh or *Pistacia atlantica* subsp. *kurdica* is one of the most popular medicinal plants in Iran. The antioxidant and anti-inflammatory effects of baneh have been evaluated in several researches and it is used in Iranian traditional and folk medicine as well. The most important outcome of our present study was to examine the effects of baneh gum and volatile oil in improving the inflammation of colon induced by acetic acid in rats. It is the first study that was conducted on the effects of baneh gum and volatile oil on colitis that was based on the macroscopic and microscopic evaluations as well as tissue MPO assay. The method of ulcerative colitis induced by acetic acid in rat is one of the experimental methods which allows us to examine the effects of diverse candidate drugs on these conditions [13,14]. It is a model where inflammatory mediators such as reactive oxygen species, vasoactive amines and eicosanoids play important roles. Inflammation induced mechanism of acetic acid could be explained by delivery of proton into the epithelium which results in epithelial cell damage [36,40]. Acetic acid also produces some inflammatory mediators. The mediators that play fundamental roles in the inflammatory processes of IBD are IL-1 , IL-6 and TNF-α that play fundamental roles in the inflammatory processes of IBD [40,41].

In the present study we found that three applied doses of baneh gum and volatile oil were orally effective to reduce colitis parameters like ulcer index and total colitis index however there was not a direct relationship between the dose and the response. Rectal administration of baneh gum in contrast, could not be able to improve the colon tissue injuries. The results obtained by the reference groups, prednisolone and hydrocortisone enema, showed an effective protection in evaluated macroscopic and microscopic parameters which indicated the stability, effectiveness, and suitability of the method. MPO activity was another parameter that was measured in our treatment groups to evaluate the anti- inflammatory properties of baneh gum and volatile oil.

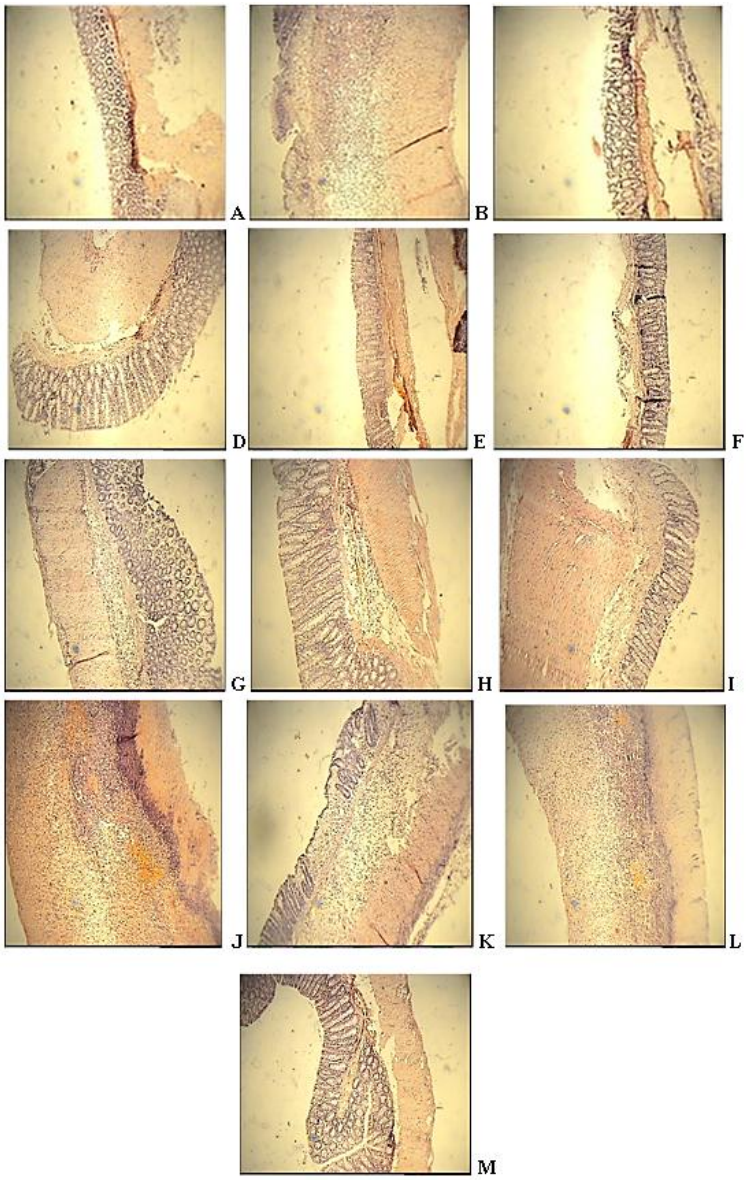

Figure 3. Microscopic presentation of acetic acid-induced colitis in rats. Sham (A) control (B) prednisolone (4 mg/kg) (C), oral gum of baneh with doses: 100 (D), 200 (E) and 400 mg/kg (F), oral volatile oil of baneh with doses 100 (G), 200 (H) and 400 µL/kg (I), and rectal administration of baneh gum with doses 100 (J), 200 (K) and 400 mg/kg (L), rectal administration of hydrocortisone with dose of 40 mg/kg (M)

The results showed that this enzyme activity was declined in those groups which were treated by oral plant fractions. Intra-rectal administration of gum was similarly not effective to diminish MPO activity in colon tissue in comparison to control vehicles. Therapeutic effects of baneh are probably due to intestinal absorption and systemic availability of active components that are beneficial in this model and/or may be due to a beneficial local effect represented by non-absorbable compounds that exist probably in gum and volatile oil reaching to distal colon. It is supposed that five days period of oral treatment of animals with baneh gum and volatile oil

provided a suitable condition for systemic absorption and/or local availability of active plant constituents throughout the GI tract. However in the case of rectal gum, it seemed that there was not enough time, area and/or specific site of absorption for the local beneficial effects. Many pharmacologic and bioactivity properties of baneh have been recently evaluated which some of them may explain these anti-ulcerogenic and anti-inflammatory effects. The anti-burn effect of baneh gum has been reported, that could be an evidence for local demulcent effects of gum and volatile oil on intestinal lumen [42]. Antioxidant and antimicrobial effects of baneh have been shown by another study [43]. The same activities were recently reported by another study on other *Pistachia* species such as mastic [27]. According to the reported pharmacological effects of isolated components of different species of *Pistacia*, terpenoids are associated with anti-inflammatory and antimicrobial effects and the antioxidant and anticancer activities are related to the high amounts of natural phenols and flavonoids. The antibacterial activity of baneh is also related to the compounds including α-pinene, verbenol and linalool [44]. Since antibiotics are used in patients with resistant ulcerative colitis, it is supposed that anti-infective effects of baneh gum may have essential effects in its anticolitis properties. The most dominant compound of baneh volatile oil in our study was α-pinene that is similar to other previous studies [45-47]. This compound have shown antioxidant activities [48,49]. Monoterpenoids such as α-pinene have also demonstrated anti-*Helicobacter pylori* effects [50,51].

Taken together, we have shown that baneh gum and volatile oil possessed beneficial anti-inflammatory activities and have prevented injuries due to the administration of acetic acid in the colon. These results confirm the traditional uses of baneh for treatment of some gastrointestinal disorders, although for clinical applications, more mechanistic and detailed toxicological examinations are needed.

Acknowledgements

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Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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