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Original Article

Effect of *Tamarindus indica* L. fruit pulp and seed extracts on experimental ulcerative colitis in rats

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Abstract

Background and purpose: *Tamarindus indica* L. which has anti-inflammatory, radical scavenging, and ulcer healing effects can be useful for the alleviation of inflammatory bowel disease (IBD). Therefore, the effects of *T. indica* fruit pulp (TIPE) and seed extracts (TISE) were investigated on experimental colitis.
Experimental approach: TIPE and TISE (125, 250, and 500 mg/kg) were made by maceration (ethanol/water: 80/30) and administered to male Wistar rats with acetic acid-induced colitis. Prednisolone (4 mg/kg) and mesalazine (100 mg/kg) were used as reference drugs. The colon tissues were examined for macroscopic and pathologic parameters and myeloperoxidase (MPO) and malondialdehyde (MDA) values.
Findings/Results: The total phenols were 45.7 ± 1.1 and 453.0 ± 3.3 mg/g in terms of gallic acid for TIPE and TISE, respectively. Both of the extracts significantly improved most of the investigated parameters including body weight loss, the weight of colons, indices of ulcers, and total colitis. MPO activity and MDA in the treatment groups (except for TIPE at 125 mg/Kg) significantly decreased compared to the control.
Conclusion and implications: Both TIPE and TISE were effective in the treatment of colitis however it seems that the effective ingredients were more concentrated in seeds rather than pulp extract so the highest dose of seed extract had a competitive effect with reference drugs. More studies are needed to introduce *T. indica* as a suitable complementary medicine or food for patients with IBD.

Keywords: Acetic acid; Animal model; Colitis; *Tamarindus indica*; Plant extracts.

INTRODUCTION

Inflammatory bowel disease (IBD) is a common inflammatory disease of the gastrointestinal tract which is difficult to diagnose and treat. IBD comes in two forms: Crohn's disease and ulcerative colitis. The exact cause of IBD is unknown, but the most likely explanation involves a combination of one or more of the following: immune system disorders (caused by environmental or genetic factors), abnormal gastrointestinal factors (such as changes in the normal gut flora), and oxidative stress (1,2). IBD is associated with disorders in the gut mucosal barrier that allow luminal factors such as leukotriene B4, nuclear factor-κB (NF-κB), nitric oxide, and abnormal activity of cyclooxygenase-2 (COX-2) to penetrate the mucosa (3).

Corticosteroids, immunomodulatory drugs, including methotrexate, 6-mercaptopurine, and azathioprine, as well as anti-tumor necrosis factor alpha (TNF-α) drugs such as infliximab and adalimumab, are commonly used to treat IBD.

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Allergic reactions, bone loss, kidney disease, and bone marrow suppression are possible side effects of these drugs. So, despite being one of the most effective anti-inflammatory drugs, the use of corticosteroids is limited. Only about 60-70% of resistant cases respond to azathioprine and 6-mercaptopurine, and adverse effects such as severe liver damage, bone marrow depression, and pancreatitis might occur (4). Although non-steroidal anti-inflammatory drugs (NSAIDs) are among the drugs most often prescribed to treat various types of inflammation, they are rarely used to treat IBD due to their negative effects on leukotriene synthesis, sometimes even increasing it, which leads to gastrointestinal mucosal damage and aggravation of IBD (5).

The insufficient efficacy and safety of current drugs, as well as side effects and patient complaints, have created a strong motivation to use new and effective treatments including probiotics and herbal medicine, especially during the last 30 years. These drugs have attracted the curiosity of the public and researchers for a long time due to their potent and diverse compounds, low side effects, affordable price, and potential for long-term use (6,7).

The Indian tamarind (*Tamarindus indica* L.) is an evergreen tree of the Fabaceae family that is widely grown in India, Sri Lanka, Thailand, the South of Iran, and tropical parts of Asia (8). Pharmacological studies have shown the presence of abundant active ingredients including polyphenols, tannins, flavonoids, cardiac glycosides, organic acids, mucilage, pectin, and sugars (arabinose, xylose, galactose, fructose, and glucose) in this plant (8,9). Besides it has been proven that tamarind ethanolic extract contains fatty acids and several essential and trace elements including cadmium, calcium, magnesium, arsenic, copper, iron, sodium, manganese, potassium, phosphorus, and zinc (10).

Tamarind fruit pulp contains pyrazines (trans-2-hexanal), thiazoles (2-ethylthiazole, 2-methylthiazole), amino acids, inverted sugar (25-30%), pectin, protein, fat, and various organic acids including tartaric acid, acetic acid, citric acid, formic acid, malic acid, and benzoic acid for which antibacterial, anti-inflammatory, anti-diarrheal, anti-diabetic, spasmolytic, vasodilator, ulcer healing, antioxidant, and anticancer properties have demonstrated (10,11). In traditional Indian medicine (Ayurveda), the leaf decoction of tamarind is used for washing indolent and resistant ulcers and promotes healthy action (12). In addition, fruit seeds of *T. indica* contain protein, fatty acids, lipids with xylose (alpha-1,6), and specific keto acids (13).

Tamarind has traditionally been used to alleviate diarrhea and dysentery, parasitic infections, jaundice, and nausea during pregnancy (14). The beneficial effect of the drug on bloody diarrhea, which is usually caused by an intestinal infection, also indicates the antiseptic effect of this natural product (15). It is worth noting that according to toxicity studies, tamarind extract is considered almost non-toxic due to its lethal dose of 50% (LD50) of more than 5000 mg/kg in mice (16).

Considering the beneficial pharmacological effects (anti-ulcer, anti-diarrheal, anti-infective, and anti-inflammatory), ease of access, relatively reasonable price, and wide use as a seasoning and flavoring agent, this plant seems to have a good potential for treating and/or preventing the recurrence of the colitis. Therefore, this study was conducted to demonstrate the possible healing and therapeutic effects of *T. indica* fruit pulp (TIPE) and seed extract (TISE) in experimental colitis.

MATERIALS AND METHODS

Preparation of plant and its extracts

Indian tamarind with the brand Alfa® Food and Product Co. (Thailand) was procured from a trusted local market in Isfahan. It was approved by a botanist from Isfahan University. Fruit pulp (1 Kg) and seeds (422 g) were thoroughly and separately pulverized after drying. To make a hydroalcoholic extract, the crude materials were separately mixed with EtOH: water (80:20), and the mixture was shaken and filtered three times over three days. The result of each step was pooled and finally, it was dried in a rotary evaporator after that it was freeze-dried to produce a fully dried extract (17).

Measurement of yield values

When the final extract was achieved, the weight of each was freshly measured, and the overall yield value was measured for TIPE and TISE based on the primary crude material (18).

Determination of total phenol contents of plant extracts

The Folin-Ciocalteu method was conducted to measure the phenolic ingredients of TIPE and TISE overall (18). In this method, phenolic ingredients react with a color-making reagent, whose absorbance is measurable at 765 nm. In this method, gallic acid (0-500 mg/mL) was used for depicting of standard curve and biophenols were evaluated as gallic acid equivalents for each extract.

Drugs and chemicals

We bought powders of mesalazine and prednisolone from Iran Hormone Co. (Tehran, Iran). Ortodianisidine dihydrochloride and hexadecyl trimethyl ammonium bromide were also purchased from Sigma Co. (St. Louis, USA). All of the organic solvents and chemicals were procured from Merck Co. (Darmstadt, Germany).

Animals

Sixty male Wistar rats were purchased from the animal house of the School of Pharmacy, which was dedicated to the maintenance and breeding of laboratory animals. They were given a week to acclimatize to the laboratory conditions. They were housed in standard-sized polycarbonate cages with controlled light/dark photoperiods, temperature (21-23 °C), and humidity (20-50%), and provided with chow pellets and free access to drinking water. The study was conducted according to the national guidelines for animal experiments provided by the Ethics Committee of Isfahan University of Medical Sciences under Ethic No. IR.MUI.RESEARCH.REC.1399.025.

Animal grouping

The rats were randomly assigned to the following ten groups (6 each): (1) normal group (vehicle): normal saline, 2 mL/kg was administered orally (p.o.); (2) control group: colitis was induced and normal saline was administered (2 mL/kg, p.o.); (3-5, fruit pulp extract): rats with colitis were treated with TIPE (125, 250, 500 mg/kg, p.o.); (6-8, fruit seed extract): rats with colitis were treated with TISE (125, 250, 500 mg/kg, p.o.) (19,20); (9 and 10, reference): rats with colitis were treated with prednisolone (4 mg/kg, p.o.) or mesalazine (100 mg/kg, p.o.).

Administration of drugs and extracts was conducted by gavage starting 2 h before induction of colitis and repeated daily for 5 days thereafter.

Experimental protocol

Plant extracts and drugs were freshly made as suspensions or solutions, respectively. Rats were fasted for 24 h with free access to drinking water before colitis induction. Two mL of acetic acid (3%) was administered intra-rectally to induce acute colitis (21). Midazolam (5 mg/kg) was used to give the rats a favorable sedation, while a suitable tube with an inner diameter of 2 mm and a length of 8 cm was inserted into the anus. On the sixth day, the animals were weighed and euthanized by CO_2 inhalation, then their abdominal cavity was opened and the colon tissue was assessed both macroscopically and microscopically. In the end, myeloperoxidase (MPO) activity and malondialdehyde (MDA) levels were measured in the colon tissue and compared with the control group (22).

Evaluation of colon macroscopic damage

The distal colon was cleaned with normal saline, cut longitudinally, and its wet weight was measured. After mounting on a light and transparent sheet, macroscopic features of colitis were recorded. To measure ulcerated areas, pictures of colon sections were taken with a camera, downloaded to a computer, and analyzed using Fiji Win 32 software (22). Moreover, the following values were used to determine ulcer severity: 0, no wound; 1, inflammation and thickness; 2, hemorrhagic spots and bleeding; 3, necrosis and/or perforation. Ulcer score and ulcer area were added together and the ulcer index was obtained for each tissue sample. Tissue samples were cut lengthwise into three equal parts for further analysis. Two sections were immediately frozen (-20 °C) for analysis of biomarkers (MPO and MDA), while the other section was deposited in formalin (10%) for further evaluation (22).

Evaluation of colon histological damage

Fixed colon tissue was subjected to the following steps: dehydration, clearing, paraffin embedding, blocking, processing, cutting into 4-µm thick slices, and staining with hematoxylin and eosin (H&E). A valid scoring method provided by Dieleman *et al*. (23) and modified by Motavallian-Naeini *et al*. (24) was used to evaluate the intensity, extent, crypt damage, and leukocyte infiltration on H&E-stained tissue. Finally, for each sample, the total index for colitis; the sum of the four above-mentioned sub-scores was obtained. Digital photography and imaging were conducted by using a modern camera and optic microscope while pathological examination and scoring were performed by a blind pathologist.

Evaluation of colonic MPO activity

A previously published method was set up in this laboratory and MPO activity, a marker of polymorphonuclear aggregation, was assessed (25). Colon tissues were thawed and a part (0.1 g) was crushed in 5 mL of potassium phosphate buffer (pH 6) containing 0.5% w/v hexadecyl trimethyl ammonium bromide, transferred to a tube, and homogenized three times for 45 s with one-minute intervals. The homogenate was centrifuged at 4000 rpm for 10 min. Then, 0.1 mL of the solution was mixed with 2.9 mL of potassium buffer (pH 6) containing 0.167 mg/mL ortho-dianisidine dihydrochloride and 0.005% H_2O_2. Then, MPO activity was measured at 450 nm using a UV-VIS spectrophotometer (Unico, USA). MPO activity was measured in units (U) per 100 mg of wet colon and was defined as the amount of enzyme degrading 1 µmol H_2O_2 per minute at 25 °C (25).

Evaluation of MDA level

Evaluation of MDA (a lipid peroxidation marker) was done by adding KCl (1 mL, 1.15% w/v) to 10 mg of colon tissue. The homogenized mixture was centrifuged (7500 rpm for 10 min) and its absorbance was measured at 532 nm. The experiments were performed using its specific kit (Navand-Salam, Urmia) based on the instructions of the company (26).

Statistical analysis

The statistical software SPSS 16.0 was used to conduct the statistical analysis. Using Student's t. paired test and parametric one-way analysis of variance (ANOVA) followed by Turkey's HSD as a post hoc test, differences between groups were investigated. The Mann-Whitney U test was used to assess non-parametric (scoring) data. The mean ± SEM/SD or median (range) was used to express the data. The *P*-values < 0.05 were considered statistically significant.

RESULTS

Yield values and total phenolic contents of extracts

The yield values were 34.4% and 13.8% for TIPE and TISE, respectively. Also, the average percentage of dry material determined was 80.1 % and 92.3% for TIPE and TISE, respectively. The amount of total phenol in the extract in terms of gallic acid equivalent after thrice repeats equals 45.7 ± 1.1 and 453.0 ± 3.3 mg/g of dried TIPE and TISE, respectively.

Effect of T. indica extracts on body weight of rats

As shown in Table 1, the rats in the normal group gained weight in a significant manner. On the contrary, the rats in the control group had a significant weight loss, which indicates the induction of the disease condition. The results of other groups showed that treatment with different doses of extracts could stop the process of weight loss, although the weight gain that occurred was not significant. Administration of prednisolone was associated with weight loss in rats; however, it was not significant.

Effect of T. indica extracts on macroscopic parameters

As shown in Table 2 and Fig. 1, TIPE at 250 and 500 mg/kg and TISE at all examined doses (125, 250, and 500 mg/kg) significantly reduced the weight of colon tissue and related macroscopic colitis features like ulcer area, severity and index in overall in comparison with the control group. Similar and more clear results were obtained with reference drugs (prednisolone and mesalazine). No signs of inflammation, edema, ulcer, or necrosis were observed in the normal colon tissue, while the most extreme intensity of these parameters occurred in the tissue of the control group (Fig. 1).

Table 1. Weight changes of rats in experimental groups. Data are presented as mean ± SD, n = 6. Normal and control (colitis-induced) groups were treated with normal saline. *P < 0.05 and **P < 0.01 represent significant differences before and after treatment.

Groups	Before	After	*P*-value
Normal	207.5 ± 7.9	218.5 ± 10.3	*
Control	222.0 ±7.4	198.3 ± 6.8	**
TIPE (125 mg/kg)	205.6 ± 8.4	212.0 ± 8.5	NS
TIPE (250 mg/kg)	189.3 ± 5.3	198.3 ± 4.8	NS
TIPE (500 mg/kg)	222.3 ± 13.3	236.1 ± 10.1	NS
TISE (125 mg/kg)	220.5 ± 11.7	223.5 ± 6.2	NS
TISE (250 mg/kg)	214.3 ± 11.5	219.3 ± 13.8	NS
TISE (500 mg/kg)	220.8 ± 10.1	236.8 ± 11.9	NS
Prednisolone (4 mg/kg)	196.8 ± 8.8	187.5 ± 11.8	NS
Mesalazine (100 mg/kg)	198.5 ± 3.4	203.5 ± 3.0	NS

TIPE, *Tamarindus indica* pulp extract; TISE, *Tamarindus indica* seed extract; NS, non-significant.

Table 2. Macroscopic parameters of colitis in experimental groups of rats. Data are presented as mean ± SD, n = 6. Normal and control (colitis-induced) groups were treated with normal saline. *P < 0.05, **P < 0.01, and ***P < 0.001 represent significant differences compared to the control group, $^{###}P$ < 0.001 versus the normal group.

Groups	Ulcer area (cm^2)	Ulcer score (0-3)	Ulcer index (0-11)	Colon (mg/8 cm)
Normal	0.00 ± 0.00	0.0 (0-0)	0.0 ± 0.0	110.3 ± 15.0
Control	6.5 ± 0.4$^{###}$	3.0 (3-3)$^{###}$	9.5 ± 0.4$^{###}$	380.4 ± 18.0$^{###}$
TIPE (125 mg/kg)	5.3 ± 0.5	2 (2-3)	7.6 ± 0.6	368.6 ± 28
TIPE (250 mg/kg)	3.9 ± 0.4***	1.5 (1-3)*	5.6 ± 0.6***	254.4 ± 20***
TIPE (500 mg/kg)	2.6 ± 1.0***	2.0 (1-3)*	5.6 ± 1.4**	217.1 ± 30***
TISE (125 mg/kg)	2.9 ± 0.7***	1.5 (0-3)*	4.4 ± 1.0**	310.5 ± 30*
TISE (250 mg/kg)	1.1 ± 0.5***	1.0 (0-3)**	2.3 ± 0.9***	257.7 ± 10***
TISE (500 mg/kg)	0.5 ± 0.2***	1.5 (0-2)*	2.0 ± 0.5***	160.2 ± 10***
Prednisolone (4 mg/kg)	1.5 ± 0.6***	1.0 (0-3)***	2.6 ± 1.0***	127.2 ± 20***
Mesalazine (100 mg/kg)	1.6 ± 0.1***	1.0 (1-2)***	2.9 ± 0.3***	150.0 ± 17***

TIPE, *Tamarindus indica* pulp extract; TISE, *Tamarindus indica* seed extract.

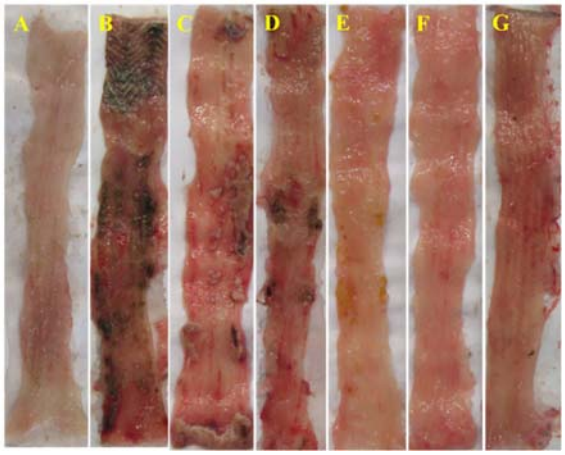

Fig. 1. Photos of colon tissue, 6 days after acetic acid-induced colitis in rats. (A) Normal colon treated with normal saline (5 mL/kg); (B) control colitis treated with normal saline (5 mL/kg); (C and D) colitis rats treated with TIPE at 125 and 500 mg/kg, respectively; (E) colitis rats treated with TISE at 500 mg/kg; (F and G) colitis rats treated with prednisolone at 4 mg/kg and mesalazine at 100 mg/kg, respectively. TIPE, *Tamarindus indica* pulp extract; TISE, *Tamarindus indica* seed extract.

Effect of T. indica extracts on microscopic parameters

As shown in Table 3 and Fig. 2, TIPE at 250 and 500 mg/kg and TISE at all examined doses (125, 250, and 500 mg/kg) decreased the total colitis index and involved pathologic features like inflammatory extent and severity, crypt damage, and leukocyte infiltration compared to the control group. Similar results were obtained with approved drugs. No signs of inflammation, infiltration of leukocytes, and crypt damage were occurred in the normal colon tissue, while the most obvious intensity of these parameters observed in the tissue of the control colitis group (Fig. 2).

Effect of T. indica extracts on MPO activity

MPO activity significantly decreased in the groups treated with TIPE (250, 500 mg/kg) and TISE (125, 250, 500 mg/kg) compared to the control. As expected, prednisolone and mesalazine were successful in decreasing MPO activity (Fig. 3A). MPO activity in normal tissue was negligible while it was exponentially increased (about 10 folds) after inducing colitis (Fig. 3).

Table 3. Pathologic parameters of colitis in experimental groups of rats. Data are presented as median (range). n = 6. Normal and control (colitis-induced) groups were treated with normal saline. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ represent significant differences compared to the control group, $^{###}P < 0.001$ versus the normal group.

Groups	Inflammatory severity (0-3)	Inflammatory extent (0-3)	Leukocyte infiltration (0-3)	Crypt damage (0-4)	Total colitis index (0-12)
Normal	0.0 (0-0)	0.0 (0-0)	0.0 (0-0)	0.0 (0-0)	0.0 (0-0)
Control	3.0 (3-3)###	3.0 (2-3)###	2.0 (2-3)###	4.0 (3-4)###	12.0 (11-13)###
TIPE (125 mg/kg)	2.0 (2-3)	2.0 (2-3)	2.0 (1-3)	3.0 (3-4)	9.0 (8-13)
TIPE (250 mg/kg)	2.0 (1-3)	1.5 (1-2)*	1.5 (1-2)	2.0 (2-4)*	7.0 (5-11)*
TIPE (500 mg/kg)	1.5 (0-3)*	1.5 (1-2)	1.5 (1-3)	2.0 (2-3)**	6.5 (4-11)**
TISE (125 mg/kg)	1.5 (1-3)*	1.5 (1-2)*	1.0 (1-2)**	1.5 (1-2)**	5.5 (4-9)***
TISE (250 mg/kg)	1.0 (1-1)**	0.5 (1-2)***	1.0 (0-1)***	2.0 (1-3)*	4.5 (3-7)***
TISE (500 mg/kg)	0.5 (1-2)***	1.0 (1-1)***	1.0 (0-2)**	1.5 (0-2)***	4.0 (2-7)***
Prednisolone (4 mg/kg)	0.5 (0-1)***	0.5 (0-1)***	1.0 (1-1)***	1.0 (1-2)***	3.0 (2-5)***
Mesalazine (100 mg/kg)	1.0 (0-1)**	1.0 (1-2)***	1.0 (1-2)**	1.5 (1-2)***	4.5 (3-6)***

TIPE, *Tamarindus indica* pulp extract; TISE, *Tamarindus indica* seed extract.

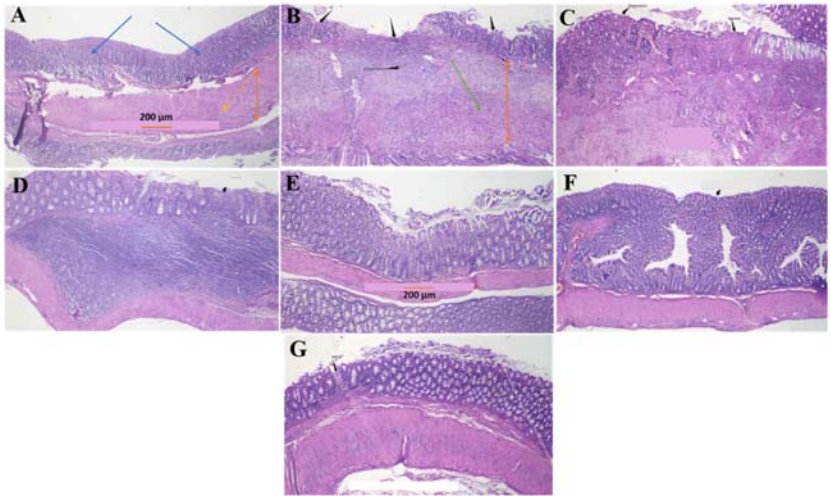

Fig. 2. Microscopic illustration of colonic tissue in rats by applying hematoxylin and eosin staining method. (A) Normal tissue treated with normal saline (5 mL/kg), the mucosal layer and the submucosa were intact and there was no sign of an ulcer or crypt damage (blue arrow); (B) control colitis treated with normal saline (5 mL/kg), the mucosal layer was completely damaged (black arrow) and the submucosa layer was severely swollen and inflamed (orange arrow). Crypts were severely damaged (black arrow) and leukocytes accumulated (green arrow); (C and D) colitis rats treated with TIPE at 125 and 500 mg/kg; (E) colitis rats treated with TISE at 500 mg/kg; (F and G) colitis rats treated with prednisolone (4 mg/kg) and mesalazine (100 mg/kg), respectively. Magnification: ×40. TIPE, *Tamarindus indica* pulp extract; TISE, *Tamarindus indica* seed extract.

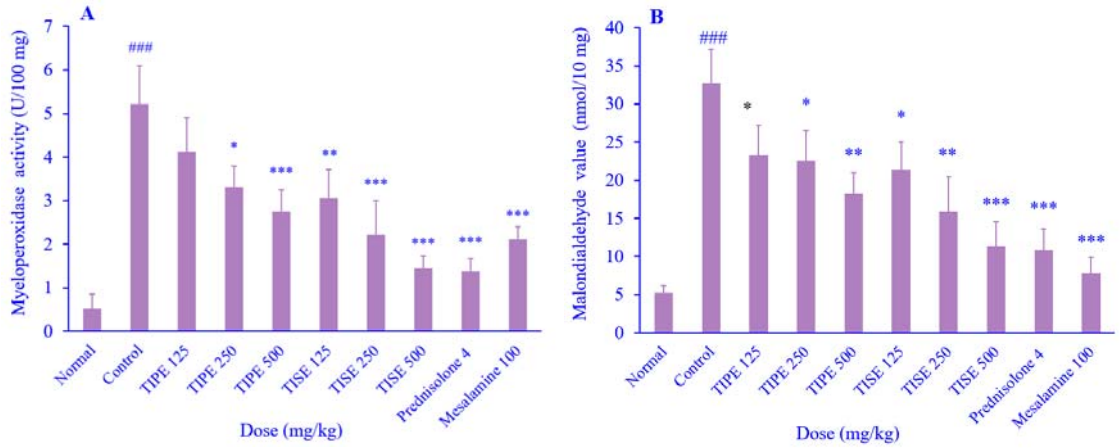

Fig. 3. (A) Myeloperoxidase activity and (B) malondialdehyde value in colonic tissue of rats treated with normal saline (control group, 5 mL/kg), TIPE and TISE, prednisolone, and mesalamine. Data are presented as mean ± SEM, n = 6. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ represent significant differences compared to the control group; $^{###}P < 0.001$ versus the normal group. TIPE, *Tamarindus indica* pulp extract; TISE, *Tamarindus indica* seed extract.

Effect of T. indica extracts on MDA value

MDA significantly decreased in all groups treated with TIPE (125, 250, 500 mg/kg) or TISE (125, 250, 500 mg/kg) compared to the control group. As expected, reference agents were successful in decreasing MDA activity (Fig. 3B). The amount of MDA in normal tissue was negligible while it exponentially increased (about 7 folds) after inducing colitis (Fig. 3B).

DISCUSSION

In this study, based on the general results and various evaluations at the tissue level and inflammatory biomarkers, it has been shown that tamarind hydroalcoholic seed and pulp extracts had a healing effect on experimental colitis.

Our results demonstrated obvious destruction of colon tissue in the control untreated group following the inoculation of acetic acid as an approved model of experimental colitis (27).

Weight loss in animals is one of the clinical symptoms of colitis, which can be caused by frequent bloody or watery diarrhea and anorexia caused by the disease condition (28). At the end of this study, animals in the control group showed significant weight loss compared to day one of the study, which could be caused by the complications of colitis. On the other hand, the cessation of weight loss and an increase in body weight, even though insignificant, in the treatment groups indicated the improvement of the disease (28). The only exception was the group treated with prednisolone, which showed weight loss despite the improvement of the disease features, and this can be attributed to its catabolic effects as a corticosteroid (29). In the current study, all the treatments were made by oral intake, so it can be concluded that there was a good bioavailability of the active ingredients. However, the active ingredients that were not absorbed probably reached the site of their action, the colon, through the bowel (30).

In addition, our results showed that the content of MPO and MDA of the colon tissue increased exponentially in the untreated colitis group indicating the activation and migration of macrophages and neutrophils, which leads to an increase in oxidative stress (26,27). In our study, almost all the doses of TIPE and TISE (except 125 mg/kg of TIPE) diminished MPO and MDA activity, which is due to the antioxidant and anti-inflammatory effects of the extracts. Interestingly, the least examined dose of TIPE (125 mg/kg) which did not affect most parameters of colitis, reduced MDA levels in colon tissue, indicating a significant antioxidant effect. This is a reason suggesting that the antioxidant effect alone is not enough for the treatment of acute colitis (31). The weight of the colon indicates the severity and level of inflammation and immune system response in the colon area. The higher the weight of the

colon, the more the accumulation of interstitial fluid indicates which is an important measure of edema and aggravation of colitis (32). The results of this study showed that TISE at all test doses and TISE at two larger doses were effective in reducing this parameter and this effect was directly related to the reduction of MPO level in the colon tissue. This result emphasizes the importance of MPO activity in evaluating the severity and extent of the disease in this model of colitis (27,32).

Also, the ineffectiveness of the low dose of pulp extract (125 mg/kg) can probably be due to the low content of effective substances or their low availability by oral intake. Therefore, other routes of administration including parenteral or rectal for the pulp extract should be tried (33). Also, the highest examined dose of TISE (500 mg/kg) had clear anti-inflammatory and anti-ulcer effects, which in some cases was well comparable with the effect of reference drugs.

The difference in the effectiveness of the two parts of the *T. indica* (TISE and TIPE) could be attributed to the existence of different effective substances. The phenolic and flavonoid compounds obtained from the seed cover extract of this plant reduced nitric oxide production induced by lipopolysaccharide and interferon-gamma in RAW 264.7 mouse macrophage cells by 68% compared to the control group, and this effect was dose-related (20).

In another study carried out by Kalra *et al*. tamarind seed methanolic extract (100-200 mg/kg) has been shown to have dose-related protective effects on three models of experimental gastric ulcers including ibuprofen, alcohol, and pylorus-ligation-induced methods. This protective effect has been attributed to its polyphenolic compounds, mainly procyanidin, epicatechin, and seed polymer tannins, which have antioxidant and cytoprotective properties against free radicals. It seems tannins prevent ulcer formation through protein accumulation and stimulating the synthesis of antibodies (19).

Previous studies have reported that *T. indica* contains polyphenolic compounds mainly including polymeric tannins and pro-anthocyanidins in various forms such as apigenin, catechin, procyanidin derivatives, epicatechin, and taxifolinol (9). Procyanidin and its derivatives, which are abundantly found in tamarind seed and its coating, have great power in trapping oxygen free radicals and reducing oxidative stress in the target tissue (9,10). The same protective effect has been reported for apigenin and catechin to inhibit nitric oxide, TNF-α, NF-κB, interleukin (IL)-4, and COX-2 production. These substances have been shown to have cytoprotective properties and are associated with anti-ulcer activity (34-36). In many studies, the anti-ulcer and anti-inflammatory effects of polyphenolic compounds are attributed to their antioxidant properties. However, the antioxidant effect has many different components and cannot be fully evaluated by measuring one parameter such as MDA (31,37). In one study carried out by L. Roja *et al*., it was demonstrated that ethanol extract of tamarind leaves (200 and 400 mg/kg/d) caused protection against three models of gastric ulcer including cold and restraint, indomethacin-induced, and pylorus ligation and the effect was dose-related. They concluded that these effects were due to antioxidant properties, neutralization of free radicals, and prostaglandin mobilization in the gastric tissue. They also showed that the examined tamarind extract was quite safe up to 4000 mg/kg/day for two weeks and had no serious toxic effects on the main activities and vital organs of the animals (38). In another study conducted on xyloglucan (mucoadhesive abundant hemicellulose) isolated from tamarind seeds (100-300 mg/kg/day) on dextran sulfate sodium-induced colitis in mice, the authors found that this fraction could reduce the level of cytokines, especially IL-1β, IL-6, and NF-κB in the intestinal mucosa (39). In the previous study, no clinical, macroscopic, and pathologic evaluation was done on the affected tissue, and the study was limited to a polysaccharide fraction of the tamarind seeds. There are reports that flavonoids inhibit COX-2 activity. COX-2 catalyzes the synthesis of prostaglandin E2 playing an important role in inflammation and related diseases (40). Flavonoids inhibit inflammatory cytokines such as IL-6, IL-7, and TNF-α (38,41). Some flavonoids inhibit the NF-κB pathway, which

leads to a decrease in the production of TNF-α and IL-1β as one of the key enzymes in activating inflammatory cells. The reduction of IL-1β production by this group of compounds leads to the reduction of IL-2 and TNF-α production (41). Among the polyphenolic compounds, especially tannins, they are astringent and have a strong anti-diarrheal effect. This effect was well observed in the experimental groups and it was more evident in those who were treated with TISE. Intriguingly, the phenolic content of the TISE was much higher than that of the TIPE, which is consistent with the above-mentioned results. The astringent action can help deposit microproteins at the ulcerated area, thereby forming an impermeable layer on the lining that prevents intestinal secretions and protects the underlying mucosa from toxins and other irritants (19,38,41).

According to the pathology results (total colitis index), it is obvious that TISE and TIPE had a protective effect on colitis in rats, while the result of seed extract was better than pulp, especially at higher doses. It seems that seed extract can provide more impact on oxidative stress and inflammatory factors in colitis tissue.

CONCLUSION

Our results suggested that TIPE and TISE are effective in the colitis murine model. It seems the seeds of *T. indica* are more beneficial candidates due to better results obtained however, more complementary studies at analytical, toxicological, and clinical levels are required to explore the exact mechanism of *T. indica* action and its usefulness in IBD therapy.

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Conflict of interest statement

The authors declared conflicts of interest in this study.

Authors' contribution

M. Minaiyan presented the idea of research, designed, and supervised all of the parts related to the grouping of animals, determining the doses of drugs, arrangement of interventions, induction of colitis, and statistical analysis of data; S. Abolhasani carried out the experiments and interventions; S. Sima cooperated in the experiments related to MPO and MDA measurements, preparing pathology samples, drawing diagrams, and writing the manuscript; A. Yegdaneh designed and supervised all of the experiments related to the identification, preparation, and evaluation of herbal materials and extracts. All authors contributed to the writing, reviewing, and preparation of the manuscript. The finalized article was read and approved by all authors.

REFERENCES

1. Podolsky DK. The current future understanding of inflammatory bowel disease. Best Pract Res Clin Gastroenterol. 2002;1:16(6):933-943. DOI: 10.1053/bega.2002.0354.
2. Loftus Jr EV. Clinical epidemiology of inflammatory bowel disease: incidence, prevalence, and environmental influences. Gastroenterology. 2004;126(6):1504-1517. DOI: 10.1053/j.gastro.2004.01.063.
3. Guan Q. A Comprehensive review and update on the pathogenesis of inflammatory bowel disease. J Immunol Res. 2019;2019:1-17. DOI: 10.1155/2019/7247238.
4. Summers RW. Novel and future medical management of inflammatory bowel disease. Surg Clin North Am. 2007;87(3):727-741. DOI: 10.1016/j.suc.2007.03.004.
5. Klein A, Eliakim R. Non-steroidal anti-inflammatory drugs and inflammatory bowel disease. Pharmaceuticals. 2010;3(4):1084-1092. DOI: 10.3390/ph3041084.
6. Rahimi R, Mozaffari S, Abdollahi M. On the use of herbal medicines in management of inflammatory bowel diseases: a systematic review of animal and human studies. Dig Dis Sci. 2009;54(3):471-480. DOI: 10.1007/s10620-008-0368-x.
7. Rahimi R, Shams-Ardekani MR, Abdollahi M. A review of the efficacy of traditional Iranian medicine for inflammatory bowel disease. World J Gastroenterol. 2010;16(36):4504-5014. DOI: 10.3748/wjg.v16.i36.4504.
8. Rao YS, Mathew MK, Potty SN. Tamarindus indica. Ind J Arecanut Spices Med Plants.1999;1:127-145. DOI: 10.4103/0973-7847.79102.
9. Ibrahim NA, El-Gengaihi S, El-Hamidi A, Bashandy SAE. Chemical and biological evaluation of

Tamarindus indica L. growing in Sudan. Acta Hortic. 1995;390:51-57.
DOI: 10.17660/ActaHortic.1995.390.6.
10. Kabir Khanzada, Shaikh W, Shahzadi S, Kazi T, Usmanghani K, Kabir A, *et al*. Chemical constituents of *Tamarindus indica*. Medicinal plant in Sindh. Pak J Bot. 2008;40(6):2553-2559.
DOI: 4435/443543720011.
11. Wong Kc, Tan CP, Chow CH, Chee SG. Volatile constituents of the fruit of *Tamarindus indica L*. J Essent Oil Res. 1998;10:219-221.
DOI: 10.1080/10412905.1998.9700886.
12. Nadkarni KM. Indian Materia Medica, 1st ed. Mumbai: Bombay Popular Prakashan; 1976. pp. 1191-1193.
DOI: 10.4236/cm.2011.24024.
13. Kuru P. Tamarindus indica and its health related effects. Asian Pac J Trop Biomed. 2014;4(9):676-681.
DOI: 10.12980/APJTB.4.2014APJTB-2014-0173.
14. Havinga RM, Hartl A, Putscher J, Prehsler S, Buchmann C, Vogl CR. Tamarindus indica patterns of use in traditional African medicine. J Ethnopharmacol. 2010;127(3):573-588.
DOI: 10.1016/j.jep.2009.11.028.
15. Chhabra SC, Mahunnah RL, Mshiu EN. Plants used in traditional medicine in Eastern Tanzania, pteridophytes and angiosperms (Acanthaceae to Canellaceae). J Ethnopharmacol. 1987;21:253-277.
DOI: 10.1016/0378-8741(87)90103-6.
16. Abubakar M, Yerima M, Zahriya AG, Ukwuani AN. Acute toxicity and antifungal studies of ethanolic leaves, stem and pulp extract of *Tamarindus indica*. Res J Pharm Biol Chem Sci. 2010;4:104-111.
17. Handa SS, Khanuja SPS, Longo G, Rakesh DD. Extraction technologies for medicinal and aromatic plants. 1st ed. Trieste: ISC-UNDO Publication; 2008. pp. 131-150.
18. Chandra S, Khan S, Avula B, Lata H, Yang MH, El-Sohly MA, *et al*. Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: a comparative study. Evid Based Complement Alternat Med. 2014; 2014:1-10.
DOI: 10.1155/2014/253875.
19. Kalra P, Sharma S, Suman, Kumar S. Antiulcer effect of the methanolic extract of *Tamarindus indica* seeds in different experimental models. J Pharm Bioallied Sci. 2011;3(2):236-241.
DOI: 10.4103/0975-7406.80778.
20. Kumutarin T, Azadi S, Butterworth L, Keil D, Chitsomboon B, Suttajit M, *et al*. Extract of the seed coat of *Tamarindus indica* inhibits nitric oxide production by murine macrophages *in-vitro* and *in-vivo*. Food Chem Toxicol. 2004;42(4):649-658.
DOI: 10.1016/j.fct.2003.12.001.
21. Minaiyan M, Ghannadi A, Mahzouni P, Nabi-Meibodi M. Anti-ulcerogenic effect of ginger (rhizome of *Zingiber officinale* Roscoe) hydroalcoholic extract on acetic acid-induced acute colitis in rats. Res Pharm Sci. 2008;3(2):79-86.
22. Niknami E, Sajjadi SE, Talebi A, Minaiyan M. Protective effect of *Vitis vinifera* (black grape) seed extract and oil on acetic acid-induced colitis in rats. Int J Prev Med. 2020;11:102,1-7.
DOI: 10.4103/ijpvm.IJPVM_362_19.
23. Dieleman LA, Palmen MJ, Akol H, Bloemena E, Pena AS, Meuwissen SG, *et al*. Chronic experimental colitis induced by dextran sulfate sodium (DSS) is characterized by Th1 and Th2 cytokines. Clin Exp Immunol. 1998;114(3):385-391.
DOI: 10.1046/j.1365-2249.1998.00728.x.
24. Motavallian-Naeini A, Minaiyan M, Rabbani M, Mahzuni P. Anti-inflammatory effect of ondansetron through 5-HT3 receptors on TNBS-induced colitis in rat. EXCLI J. 2012;11:30-44.
PMCID: PMC4919924.
25. Mahdavi NS, Talebi A, Minaiyan M. Ameliorative effect of galantamine on acetic acid induced colitis in rats. Res Pharm Sci. 2019;14(5):391-399.
DOI: 10.4103/1735-5362.268199.
26. Khoramian L, Sajjadi SE, Minaiyan M. Anti-inflammatory effect of *Adiantum capillus-veneris* hydroalcoholic and aqueous extracts on acetic acid-induced colitis in rats. Avicenna J Phytomed. 2020;10(5):492-503.
PMCID: PMC7508316.
27. Tahan G, Aytac E, Aytekin H, Gunduz F, Dogusoy G, Aydin S, *et al*. Vitamin E has a dual effect of anti-inflammatory and antioxidant activities in acetic acid-induced ulcerative colitis in rats. Can J Surg. 2011;54(5):333-338.
DOI: 10.1503/cjs.013610.
28. Owusu G, Obiri DD, Ainooson GK, Osafo N, Antwi AO, Duduyemi BM, *et al*. Acetic acid-induced ulcerative colitis in Sprague Dawley rats is suppressed by hydroethanolic extract of *Cordia vignei* leaves through reduced serum levels of TNF-α and IL-6. Int J Chronic Dis. 2020;8785497:1-11.
DOI: 10.1155/2020/8785497.
29. Keyvanara AH, Yegdaneh A, Talebi A, Minaiyan M. Evaluating anti-inflammatory effect of hydroalcoholic extracts of *Citrus medica L*. pulp and peel on rat model of acute colitis. Res J Pharmacognosy. 2023:10(2):29-38.
DOI: 10.22127/RJP.2023.377466.2027.
30. Minaiyan M. Sajjadi SE, Naderi N, Taheri D. Anti-inflammatory effect of *Kelussia odoratissima* Mozaff. hydroalcoholic extract on acetic acid-induced acute colitis in rats. J Reports Pharm Sci. 2014;3(1):28-35.
31. Frankel EN, Meyer AS. The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. J Sci Food Agr. 2000;80(13):1925-1941.
DOI: 10.1002/1097-0010(200010)80:13<1925::AID-JSFA714>3.0.CO;2-4.
32. Yamada Y, Marshall S, Specian RD, Grisham MB. A comparative analysis of two model of colitis in rats. Gastroentrology. 1992;102(5):1524-1534.
DOI: 10.1016/0016-5085(92)91710-l.
33. Naini MA, Zargari-Samadnejad A, Mehrvarz S, Tanideh R, Ghorbani M, Dehghanian A, *et al*.

Anti-inflammatory, antioxidant, and healing-promoting effects of *Aloe vera* extract in the experimental colitis in rats. Evid Based Complement Alternat Med. 2021;2021.
DOI: 10.1155/2021/9945244.

34. Karaoglan ES, Bayir Y, Albayrak A, Toktay E, Ozgen U, Kazaz C, *et al*. Isolation of major compounds and gastroprotective activity of *Alchemilla caucasica* on indomethacin induced gastric ulcers in rats. Eurasian J Med. 2020;52(3):249-253.
DOI: 10.5152/eurasianjmed.2020.19243.
35. Sadraei H, Asghari G, Khanabadi M, Minaiyan M. Anti inflammatory effect of apigenin and hydroalcoholic extract of *Dracocephalum kotschyi* on acetic acid induced colitis in rats. Res Pharm Sci. 2017;12(4):322-329.
DOI: 10.4103/1735-5362.212050.
36. Kim JW, Kim CY, Kim JH, Jeong JS, Lim JO, Ko JW, *et al*. Prophylactic catechin-rich green tea extract treatment ameliorates pathogenic enterotoxic *Escherichia coli*-induced colitis. Pathogens. 2021;10(12):1573,1-12.
DOI: 10.3390/pathogens10121573.
37. Dangles O. Antioxidant activity of plant phenols: chemical mechanisms and biological significance. Curr Organ Chem. 2012;16(6):692-714.
DOI: 10.2174/138527212799957995.
38. Roja L, Jahan N, Wesley J. Antiulcerogenic activity of alcoholic extract of the leaves of *Tamarindus indica L*. on experimental ulcer models. Pharmacologyonline. 2008;3:85-92.
39. Periasamy S, Lin CH, Nagarajan B, Sankaranarayanan NV, Desai UR, Liu MY. Mucoadhesive role of tamarind xyloglucan on inflammation attenuates ulcerative colitis. J Funct Foods. 2018;47:1-10.
DOI: 10.1016/j.jff.2018.05.035.
40. O'Leary KA, de Pascual-Tereasa S, Needs PW, Bao YP, O'Brien NM, Williamson G. Effect of flavonoids and vitamin E on cyclooxygenase-2 (COX-2) transcription. Mutat Res. 2004;551(1-2):245-254.
DOI: 10.1016/j.mrfmmm.2004.01.015.
41. Gupta M, Mishra V, Gulati M, Kapoor B, Kaur A, Gupta R, *et al*. Natural compounds as safe therapeutic options for ulcerative colitis. Inflammopharmacol. 2022;30(2):397-434.
DOI: 10.1007/s10787-022-00931-1.