Journal of Biology and Today's World
ISSN 2322-3308
Journal home page: http://journals.lexispublisher.com/jbtw/

Received: 17 June 2015 • Accepted: 01 August 2015

Research

doi:10.15412/J.JBTW. 01040402

The effect of Pomegranate juice, Date palm and Fig fruit in treatment and prevention of experimental acute colitis model on rat: Immunopathogenesis aspect

Farideh Zafari-Zangeneh[1]*, Mohammad Mehdi Naghizadeh[2], Fatemeh Amini[3]

[1] Vali-e-Asr Reproductive Health Research Center, Tehran University of Medical Sciences, Research Institute for Islamic and Complementary Medicine (RICM), Tehran, Iran

[2] Department of Biostatistics, Fasa University of Medical Sciences, Fasa, Iran

[3] Department of Veterinary, Tehran University, Tehran, Iran

*correspondence should be addressed to Farideh Zafari-Zangeneh, Vali-e-Asr Reproductive Health Research Center, Imam Hospital, Keshavarz Blvd, Tehran-1419733141-Iran; Tell: +982166581616; Fax: +982166581658; Email: Zangeneh14@gmail.com.

ABSTRACT

Inflammatory bowel disease (IBD) is the chronic inflammation of digestive tract. IBD primarily includes ulcerative colitis and Crohn's disease. Pathogenesis and etiology of IBD are still uncertain. The proinflammatory cytokines have a central role in the modulation of the intestinal immune system. In inflammatory bowel disease, (IBD) pro- and antiinflammatory cytokines are elevated in mucosal and systemic concentrations. Last decade researches showed that cytokines can be a good therapeutic target in IBD patients. Interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) exhibit a key role in the inflammatory process. Pretreatment and treatment effects of some modulators and inhibitors of these mediators which emphasize on different aspects of experimental colitis models mimicking IBD in human. The aim of this study was to investigate the therapeutic and prophylactic effects of pomegranate, date and fig fruits in daily regimen in experimental acute colitis model on rat. 48 rats were divided into four groups: Intact, Prevention, Treatment and Control. Every group was subdivided into two groups: 1) Food and water were provided ad libitum. 2) Food and water were provided ad libitum and food supplements (Pomegranate juice, dried Date palm and Fig fruits). Modeling of induction acute colitis method was done by 1 ml. acetic acid 4% (pH = 2.3) based on the techniques of MacPherson (1978) and Yamada (1991). The results of this study showed that pomegranate juice and palm date dried fruit induced prophylactic effect on colitis of rat model.

Key words: Pomegranate juice, Date palm fruit, Fig fruit, colitis, rat

Copyright © 2015 Farideh Zafari-Zangeneh et al. This is an open access article distributed under the Creative Commons Attribution License.

1. INTRODUCTION

Inflammation of the colon can occur for various reasons. The definitive treatment of colitis is dependent on upon the cause. A condition that causes inflammation of the intestines, such as ulcerative colitis or Crohn's disease is known as IBD (inflammatory bowel disease). IBD is one of the most common alimentary diseases. Etiology and pathogenesis of IBD remain obscure. In the last decade investigations were done on treatment and pretreatment effects of some modulators and inhibitors of cytokines which emphasize on different aspects of experimental colitis models mimicking IBD in human. Patients of IBD have an increased risk of colorectal cancer. A diet rich in fruits and vegetables can reduce the risk of cancer and may be useful in cancer prevention. For pomegranate (Punica granatum L; Lythraceae), both basic and clinical evidence of the benefits of particular classes of bioactive substances have been developed specifically for the juice and juice extracts. Since ancient times, pomegranate has been used for medicinal purposes. Traditionally it has been used for the treatment of various inflammatory diseases, including ulcerative colitis (UC). Because its fruits and extracts are rich in ellagitannins, which release ellagic acid when hydrolyzed, consumption of pomegranate products is currently being widely promoted for their potential health effects, including the prevention of inflammatory diseases and cancer (1). Hippocrates (400 bce) used pomegranate extract for a wide variety of ailments, such as a plaster to reduce skin and eye

inflammation, and as an aid to digestion (2). Pomegranates have been shown to contain 124 different phytochemicals, and some of them act in concert to exert antioxidant, anti-inflammatory and anti-atherosclerotic properties effects on cancer cells. The most abundant type of polyphenols in pomegranate juice are ellagitannins that are hydrolysable tannins releasing ellagic acid on hydrolysis (3) and form urolithins such as urolithin a following metabolism by gut flora. Punicalagin is unique to pomegranate and is a part of a family of ellagitannins, which also include minor tannins such as punicalin and gallagic acid (structures not shown here). All these ellagitannins have in common ability to be hydrolyzed to ellagic acid, resulting in a prolonged release of ellagic acid into the blood following the ingestion of pomegranate juice. Pomegranate juice also contains other polyphenols, such as anthocyanins (cyanidin, delphinidin, and pelargonidin glycosides) and flavonols (quercetin, kaempferol, and luteolin glycosides) (3). Pomegranate fruit was extract and its purified ellagitannins inhibit the proliferation of human cancer cells and modulate the inflammatory subcellular signaling pathways and apoptosis (4, 5). As recent reports demonstrate that pomegranate juice and its extracts have potent anti-tumorigenic effects in prostate cancer. Hollebeeck and et al in 2012 evaluated the anti-inflammatory properties of a pomegranate fruit husk (PomH) polyphenolic extract, rich in punicalagin, using Caco-2 cells, an in vitro model of human intestinal epithelium. After 24 h incubation, 3 pro-inflammatory markers, i.e., interleukin (IL)-6, IL-8 and monocyte chemoattractant protein (MCP)-1, were assayed both at their gene transcription (qRT-PCR) and secretion (ELISA) levels. They suggest that pomegranate husk could be an interesting natural source contributing to prevent intestinal chronic inflammation (6). The purpose of this study was to evaluate the effects of pomegranate juice and date fruit regime in the rat model of colitis. In recent years, an explosion of interest in the numerous health benefits of dates had led to many in vitro and animal studies as well as the identification and quantification of various classes of phytochemicals (7). Dates are good sources of antioxidants, mainly carotenoids and phenolics. The fruits (dates) of the date palm (Phoenix dactylifera L.) contain a high percentage of carbohydrate (total sugars, 44-88%), fat (0.2-0.5%), 15 salts and minerals, protein (2.3-5.6%), vitamins and a high percentage of dietary fiber (6.4-11.5%). The flesh of dates contains 0.2-0.5% oil. There are at least 15 minerals in dates. The percentage of each mineral in dried dates varies from 0.1 to 916 mg/100 g is depending on the type of mineral. In many varieties, potassium can be found at a concentration as high as 0.9% in the flesh while it is as high as 0.5% in some seeds. Other minerals and salts that are found in various proportions include boron, calcium, cobalt, copper, fluorine, iron, magnesium, manganese, potassium, phosphorous, sodium and zinc. The elemental fluorine in Date is useful in protecting teeth against decay. Selenium, another element believed helping to prevent cancer and important in immune function; is also found in dates. Selenium is an essential element has antioxidant and immunomodulation function. Selenium is used to synthesize the amino acid, called selenocysteine, which is vital to the function of selenoprotein. At least, 25 selenoproteins are found in the human body and those are classified by the function into antioxidant enzymes (glutathione peroxidase, GPx), antioxidant proteins (selenoprotein P and W), other metabolic enzymes (thioredoxin reductase and iodine deiodinase) (8). Therapeutic benefits of selenium can be observed in oral buccal mucosa as well as small intestinal mucosa (9). Dates are good sources of antioxidants, mainly carotenoids, phenolics and the macro-elements measured are calcium, phosphorous, sodium, potassium and magnesium, while the essential micro-elements and the possibly essential micro-elements are iron, zinc, copper, manganese, cobalt, molybdenum and aluminum, arsenic, barium, cadmium, chromium, nickel, lead, strontium and vanadium, respectively (10). Fig fruit has a typical component in the health-promoting Mediterranean diet for millennia. L. Ficus Carica known, fig tree from Urticacea family that has passes more than five thousand years of culture in the Mediterranean. Fruits of the Mission variety contained the highest levels of polyphenols, flavonoids, and anthocyanins and exhibited the highest antioxidant capacity. The results indicate that properly dried figs can be used as a good source of phenolic compounds. In figs, monomer sugars predominate, which is important nutritional information, and the content of sugars as well as organic acids in fresh figs was lower than in dried fruits. However, the best sugar/organic acid ratio were measured after the sun-drying process. Analysis of individual phenolic compounds revealed a higher content of all phenolic groups determined after the oven-drying process, with the exception of cyanidin-3-O-rutinoside. Similarly, higher total phenolic content and antioxidant activity were detected after the drying process (11). The aim of this study is to investigate the therapeutic and prophylactic effects of pomegranate, fig and date in daily regimen in experimental acute colitis model on rat.

2. MATERIALS AND METHODS

2.1. Animals and care

Fortyeight adult male Wistar rats weighing 220–230 g (7–8 weeks of age) from the animal house of the Pasteur Institute were kept in a central animal care facility and housed: every six of them into a cage under typical condition (in a cycle of 12-hrs of illumination and 12-hr of darkness) and controlled ambient temperature (24 ± 0.5C°) for at least 1 week earlier. Food and water were provided ad libitum. All of the animal studies were also approved by a group from the Ethics Committee of Tehran University of Medical Sciences and experiments were conducted in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (publication No. 85-23, revised 2007).

2.2. Experimental design

2.2.1. Topical application of dilute acetic acid

Diffuse, topical application of dilute acetic acid to the serosal surface of rat colon, or standardized intraluminal (per rectum) instillation induced a reproducible, diffuse colitis in a dose-response manner. These lesions were reproduced with 100% reliability and were evaluated up to 60 days when healing occurred. Histopathological features of this chemically induced colitis were diffuse ulceration of the distal colon, occurrence of pseudopolyp-like structures, alterations in crypt depth and mucus secretion, and a transmural, nonspecific inflammatory response (12, 13).

2.3. Study procedure

After one week of acclimatization, 48 rats were divided into four groups: Intact, Prevention, Treatment and Control. Every group was subdivided into two groups: 1) Food and water were provided ad libitum. 2) Food and water were provided ad libitum and food supplements (Pomegranate juice, dried Date palm and Fig fruits).

1) Intact group
2) Prevention group: modeling colitis after two months by feeding nutrition desired
3) Treatment group: modeling colitis by feeding nutrition desired simultaneously

4) Control group: modeling (Colitis caused by acetic acid 3%) without feeding nutrition desired

In this study, feeding is down for two months by using of Yazd province pomegranate, Zahedi region dried dates and Estahbanate district dried Figure 10 ml of fresh pomegranate juice added to 90 ml of drinking water for each cage every day (14). One and half grams of dates and 2 g daily dried palm and fig for each rat (15). Modeling of induction acute colitis method was done by acetic acid based on the techniques of MacPherson (1978) and Yamada (1991), which after three days should be resulted to colitis**.** For colitis induction, 1 ml of acetic acid 4% (pH = 2.3) was slowly injected by catheter into 5 cm of the rectum. Immediately after thirty seconds, colon was washed by 1.5 ml physiology serum. Inflammatory model of acetic acid has a low cost, easy administration, and is readily available. Also, the pathological symptoms of acute colitis induced by acetic acid are closer to the human colitis.

2.4. Measurement of circulating levels of cytokines

Blood samples were collected from the heart. Serum interlukin6 and tumor necrosis factor alpha (TNFα) were determined by Elisa. Kits were used the measure Rat IL6, Rat TNF-α (Vainder med) from Austria Company.

2.5. Colon Histology

After opening the abdomen, two centimeters of the colon towards the rectum were removed and washed for pathologic assessment. Stripped of adherent connective tissue and fixed in 10% formaldehyde buffer for at least 24 h, then embedded in paraffin and sectioned. The sections were stained with hematoxylin and eosine (H&E) and the analyzing criteria used for qualitative analysis.

2.6. Statistical analysis

Data are presented as mean ± SD. To compare the increase or decrease the inflammation of colon Paired t-test was used. SPSS 13 was used for data analysis. $P<0.05$ was considered as significant level.

3. RESULTS AND DISCUSSION

3.1. Immunologic results

3.1.1. Acute colitis or modeling

The results of this study show that IL-6 as a result of the development of acute colitis compared to the intact group had a significant increase ($P = 0.002$) (Figure 1) (Table 1). But the inflammatory factor TNF-α in inflammation did not show significant differences with the group intact.

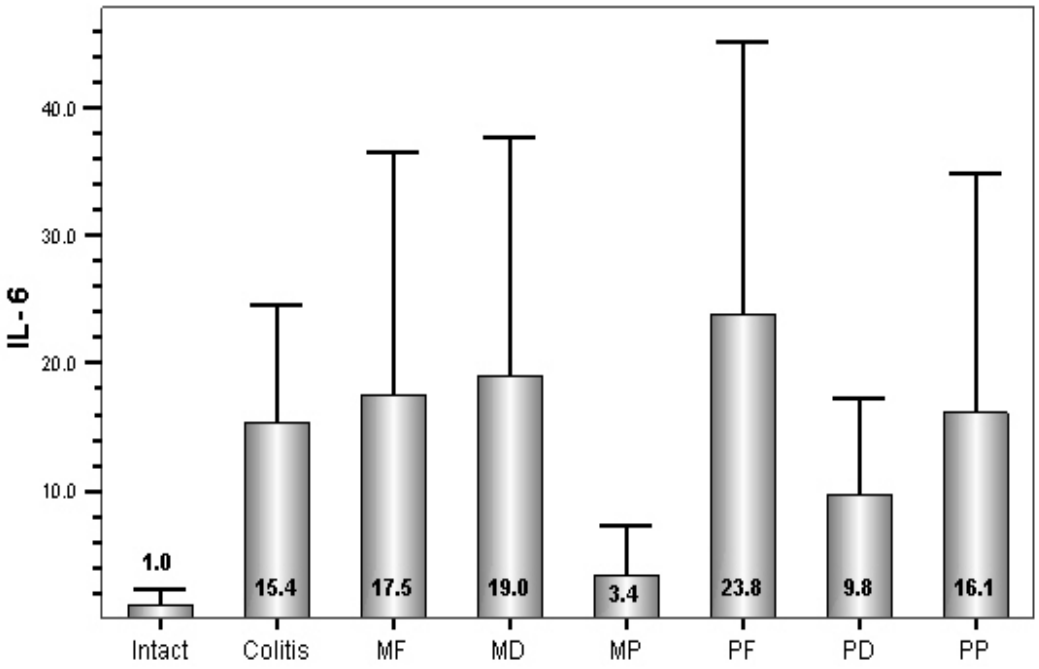

Figure 1. Comparison of serum Interleukin-6 in two study groups: treatment (M) and prevention (P) of Pomegranate juice, Date and Fig fruits regimen with control and intact groups

Groups treated with Pomegranate juice (MP), Date (MD) and Fig (MF)
Groups prevented with Pomegranate juice (PP), Date (PD) and Fig (PF)
M= TREATMENT (Rat Modeling)
P= PREVENTION
MP= TREATMNET WITH POMEGRANATE
MD= TREATMNET WITH DATE
MF= TREATMNET WITH FIG
PP= PREVENTION OF POMEGRANATE
PD= PREVENTION OF DATE
PF= PREVENTION OF FIG

Table 1. Comparison results of serum Interleukin-6 in two study groups: treatment (MF, MD and MP) and prevention (PF, PD and PP) with control and intact groups

Group	IL-6						P-value[1]	P-value[2]
	N	Median	Q_1	Q_3	Mean	SD		
Intact	6	0.4	0.0	2.6	1.00	1.28	---	---
Colitis	6	15.0	6.2	22.8	15.40	9.10	0.002	---
MF	6	9.4	3.6	39.2	17.53	18.99	0.026	0.818
MD	6	16.3	2.6	35.4	19.03	18.74	0.041	0.937
MP	4	3.1	0.0	6.7	3.35	3.89	0.610	0.067
PF	5	23.6	4.4	34.6	23.80	21.46	0.009	0.792
PD	4	10.4	5.0	14.5	9.75	7.48	0.114	0.476
PP	4	11.3	2.2	30.0	16.10	18.81	0.114	0.610

P-value[1]: Comparison with intact group (Mann-Whitney rank test)
P-value[2]: Comparison with Colitis (Mann-Whitney rank test)

3.1.2. Treatment of acute colitis

After inflammation, the group's study of acute colitis, three received the dietary intervention. As a result of this intervention in food only in MP (colitis Model group, and Pomegranate juice) levels of IL-6 compared to the group that after the induction of colitis model of conventional foods have decreased. However, this decrease is not significant ($P = 0.067$). The simultaneous reduction of TNF-α in this group ($P = 0.067$), the group may be a sign of inflammation, which must be examined.

3.1.3. Interventions to prevent the development of acute colitis

In three study groups, three different dietary interventions were conducted for two months. After this period, the three

groups were acute colitis model. The level of IL-6 in both groups: PD (Prevent Date) and PP (Prevent Pomegranate juice) compared to the control group were ($P = 0.476$) and ($P = 0.610$), although none of these changes were significant, but near the level of IL- 6 the two groups intact groups may indicate a resistance group against the mechanisms of IBD. The significant decreasing of TNF-α in PD group compared to the control group ($P = 0.010$) and also the significant lowering of inflammatory factors on the intact group ($P = 0.038$) indicate that dried date palm diet can prevent colitis in the rat intestinal (Figure 2) (Table 2).

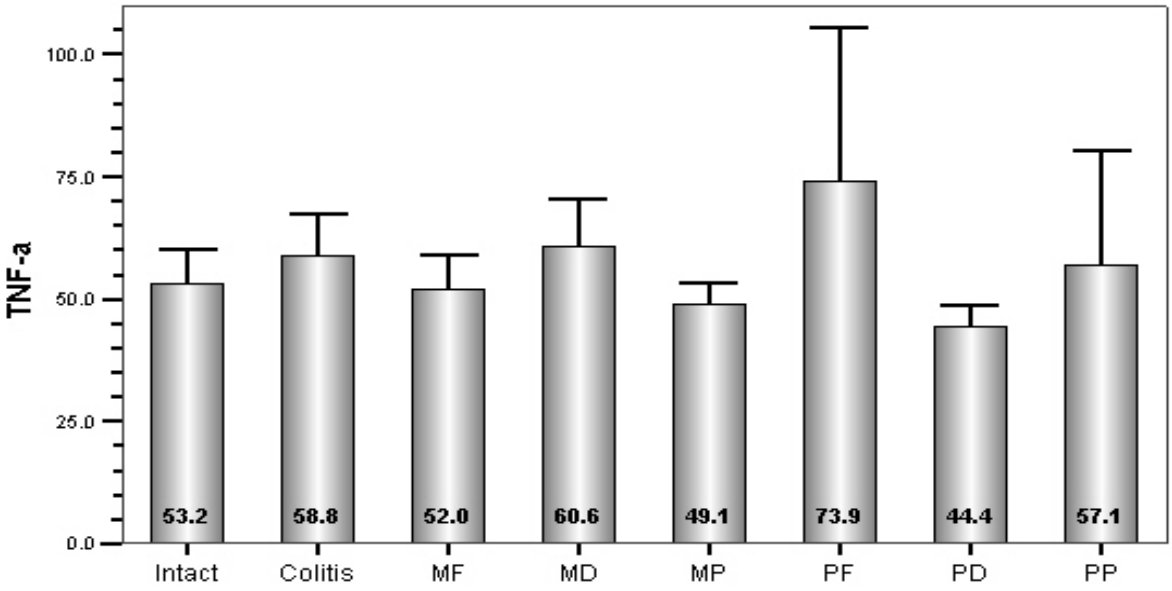

Figure 2. Comparison of serum TNF-α in two study groups: treatment (M) and prevention (P) Pomegranate juice, Date and Fig fruits with control and intact groups

Groups treated with Pomegranate juice (MP), Date (MD) and Fig (MF)
Groups prevented with Pomegranate juice (PP), Date (PD) and Fig (PF)

Table 2. Comparison results of serum TNF-alpha in two study groups: treatment (MF, MD and MP) and prevention (PF, PD and PP) with control and intact groups

Group	TNF-alpha						P-value[1]	P-value[2]
	N	Median	Q_1	Q_3	Mean	SD		
Intact	6	51.5	48.8	55.0	53.17	7.01	---	---
Colitis	6	56.9	52.0	64.0	58.77	8.66	0.240	---
MF	6	50.0	49.2	50.4	52.03	6.98	0.818	0.093
MD	6	56.1	53.0	69.8	60.63	9.97	0.132	0.699
MP	4	49.5	45.7	52.5	49.10	4.09	0.352	0.067
PF	5	57.6	52.6	84.8	73.92	31.54	0.177	0.662
PD	4	45.3	41.3	47.5	44.40	4.51	0.038	0.010
PP	4	50.0	43.1	71.0	57.05	23.29	0.762	0.352

P-value[1]: Comparison with intact group (Mann-Whitney rank test)
P-value[2]: Comparison with Colitis (Control) group (Mann-Whitney rank test)

3.2. Histologic results

3.2.1. Intact animal

Normal colon tissue in rat is shown in Figure 3 (a).

3.2.2. Acute colitis model

The histopat report in acute colitis model in rats (Figure 3) showed that there are in colon tissue: 1) the submucosal edema with dilated vessels, 2) blood cells and inflammatory cell infiltration single cell and 3) the formation of follicular lymphoid cells (Figure 3(b)).

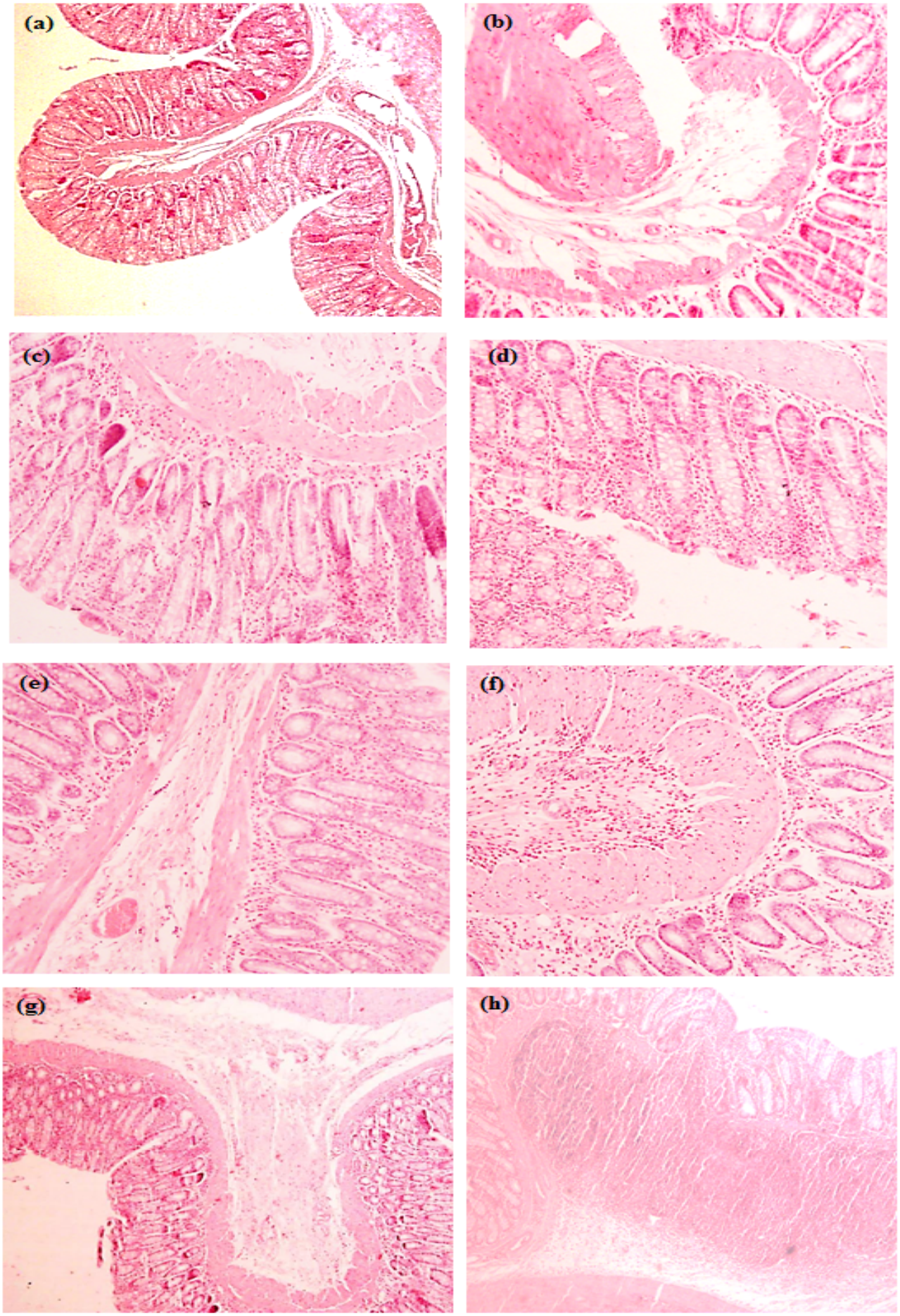

Figure 3. histopathology findings in rat colon tissue sections: **a.** control or normal tissue (intact), **b.** acute colitis model, **c.** Treatment of acute colitis (pomegranate juice), **d.** Treatment of acute colitis (date fruit), **e.** Treatment of acute colitis (fig), **f.** prevention of acute colitis (pomegranate juice), **g.** prevention of acute colitis (date fruit), **h.** prevention of acute colitis (fig fruit).

3.2.3. Treatment of acute colitis

The histological evaluation of the treatment of acute colitis by feeding two months in rats showed that pomegranate juice could eliminate edema and submucosal cell infiltration (Figure 3 (c)). Dried date and fig fruits dilated vessels and cellular infiltration existed but in the small allotment was visible and one follicle was observed in colon tissue (Figure 3(d), Figure 3(e)).

3.2.4. Prevention of acute colitis

In colon tissue of pomegranate juice group there was a very small submucosal edema (Figure 3(f)). In modeling after feeding date fruit has been no damage in the colon tissue (Figure 3(g)). Fig fruit could not prevent of damage in the colon tissue and three pathological findings above were observed in acute colitis model in rat (Figure 3(h)). Colitis is an inflammatory disease in colonic mucosa with unknown aetiology. It seems to result from a complex series of interactions between susceptibility genes, environment and the immune system (16). Clinical features and histopathological findings and the therapeutic efficacy of immunosuppressive drugs indicate an involvement of the immune system in the pathogenesis of the disease. NF-κB is a protein transcription factor that can orchestrate complex biological processes, such as the inflammatory response. NF-κB is a collective term for members of the Rel family of DNA binding transcription factors that recognize and bind characteristic sequence motifs present in the promoters of many genes involved in immune and inflammatory responses. NF-κB regulates the expression of a wide variety of genes that play critical roles in innate immune responses (17). These NF-κB target genes include those encoding cytokines, such as IL-1, IL-2, IL-6, IL-12, TNF-α and so on (18, 19). Furthermore, some of these cytokine, such as IL-1 and TNF-α, which upregulate intestinal epithelial TLR4 expression in vitro have been found to play significant pathophysiological roles in triggering ulcerative colitis (20). These findings show that immunopathogenesis of colitis can be on axis of protein transcription factor NF-κB that regulates the expression and in this study we investigated serum interleukins IL6 and TNF-α. The acute inflammation or physiologic is a useful reaction of the tissue damage. But if the delay in resolving occurs, may lead to immune system-related diseases. Chronic inflammation can lead to cancer by some early changes through absorption of soluble mediators such as proinflammatory factor TNFα, interleukin 6 and 8, NF κB and other bioactive lipids are eicosanoids (21). The interpretation of these complex inflammatory mechanisms should be able to offer new preventive and therapeutic strategies in inflammatory diseases. In the past decade the use of pomegranate fruit extract in the treatment of inflammatory disease rheumatoid arthritis (22-24) as well as the cancer, inflammatory bowel disease (23, 24) is beneficial and recommended. Pomegranates, dates and figs (of the fruits mentioned in the scriptures) are strong and rich sources of anti-inflammatory ingredient. The strategy can be a useful application. Pomegranate, date and fig (of the fruits mentioned in the scriptures) are strong and rich sources of anti-inflammatory activity which can be useful in this application. Antioxidant, anti-carcinogenic and anti-inflammatory properties of pomegranate are known for the treatment and prevention of cancer, cardiovascular disease, diabetes, erectile dysfunction, bacterial infections, antibiotic resistance, as well as skin damage caused by UV rays (25). Antioxidant and atherosclerosis properties in pomegranates are due to polyphenols, including ellagic acid in the free form linked to glycosides, galutanins, anthocyanins (cyanidin, delphinidin, pelargonidin glycosides) and other flavonoids (quercetin, kaempferol and luteolin glycosides) (3, 14, 26-29). The most abundant phytochemicals in pomegranate juice are polyphenols, including the hydrolysable tannins called ellagitannins formed when ellagic acid and/or gallic acid binds with a carbohydrate to form pomegranate ellagitannins, also known as punicalagins (5). Fifty percent of the antioxidant propertie of pomegranate juice is the punicalagins. Condensed tannins in pomegranate juice are water soluble with anticancer property in two environments in vitro and in vivo (3). Pomegranate juice can inhibit the cells (30-100%) in colon cancer (antiproliferative) (5). In treatment of acute colitis in this study, using diet of pomegranate juice daily for two consecutive months decreased submucosal edema symptoms and stopped mononuclear inflammatory cell infiltration which confirms Larrosa et al., findings in 2010. They showed that pomegranate juice and its metabolites urolithin-A (UROA), both reduce inflammation in models of acute colitis in rats (30). Our results confirm it. The tissue healing response as a result of preventive, acute colitis model can be found in the pomegranate juice, as submucosal edema; cellular infiltration and lymphoid follicles which are too low. Also, the histological evaluation of the treatment of acute colitis by feeding two months in rats showed that only pomegranate juice could eliminate edema and submucosal cell infiltration (Figure 3 (c)). Date fruit was only effective in prevention of acute colitis. After modeling, TNF has significant reduction than the control group $P < 0.01$) and tissue damages submucosal edema, mononuclear cell infiltration and lymphoid follicles none were observed. Then modeling in rat was prevented by dried date fruit regimen. This data show that TNF-α probably contributed to the lack of modeling colitis in rat. Date consumption in the treatment of acute colitis showed no significant response that was similar to the response of the fig fruit. This study shows that daily consumption of pomegranate juice and dried dates is effective in prevention of acute colitis model in rat.

4. CONCLUSION

Phenolic content (TPC) and flavonoid content (TFC), the pomegranate, and palm date can be an important factor in prevention of acute colitis model in rat.

ACKNOWLEDGMENT

This research was supported by the Research Council of Tehran University of Medical Sciences and Research Institute for Islamic and Complementary Medicine (RICM), Tehran, Iran

AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

REFERENCES

1.Marin M, Maria Giner R, Rios JL, Recio MC. Intestinal anti-inflammatory activity of ellagic acid in the acute and chronic dextrane sulfate sodium models of mice colitis. Journal of ethnopharmacology. 2013 Dec 12;150(3):925-34. PubMed PMID: 24140585. Epub 2013/10/22. eng.
2.Adams F. The Genuine Works of Hippocrates: Translated from the Greek, with a Preliminary Discourse and Annotations: William Wood; 1929.
3.Ferreira D. Antioxidant, antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from Punica granatum L. L Planta Med. 2007;73(5):461.
4.Adams LS, Seeram NP, Aggarwal BB, Takada Y, Sand D, Heber D. Pomegranate juice, total pomegranate ellagitannins, and punicalagin suppress inflammatory cell signaling in colon cancer cells. Journal of Agricultural and Food Chemistry. 2006;54(3):980-5.
5.Seeram NP, Zhang Y, McKeever R, Henning SM, Lee R-p, Suchard MA, et al. Pomegranate juice and extracts provide similar levels of plasma and urinary ellagitannin metabolites in human subjects. Journal of medicinal food. 2008;11(2):390-4.
6.Hollebeeck S, Winand J, Hérent M-F, During A, Leclercq J, Larondelle Y, et al. Anti-inflammatory effects of pomegranate (Punica granatum L.) husk ellagitannins in Caco-2 cells, an in vitro model of human intestine. Food & function. 2012;3(8):875-85.
7.Vayalil PK. Date fruits (Phoenix dactylifera Linn): an emerging medicinal food. Critical reviews in food science and nutrition. 2012;52(3):249-71.
8.Shenkin A. Selenium in intravenous nutrition. Gastroenterology. 2009;137(5):S61-S9.
9.Kim Y, Kim DC, Cho E-S, Ko S-O, Kwon WY, Suh GJ, et al. Antioxidant and anti-inflammatory effects of selenium in oral buccal mucosa and small intestinal mucosa during intestinal ischemia-reperfusion injury. Journal of Inflammation. 2014;11(1):36.
10.Habib HM, Ibrahim WH. Nutritional quality of 18 date fruit varieties. International journal of food sciences and nutrition. 2011;62(5):544-51.
11.Slatnar A, Klancar U, Stampar F, Veberic R. Effect of drying of figs (Ficus carica L.) on the contents of sugars, organic acids, and phenolic compounds. Journal of agricultural and food chemistry. 2011;59(21):11696-702.
12.MacPherson B, Pfeiffer C. Experimental production of diffuse colitis in rats. Digestion. 1978;17(2):135-50.
13.Yamada T, Zimmerman BJ, Specian RD, Grisham MB. Role of neutrophils in acetic acid-induced colitis in rats. Inflammation. 1991;15(5):399-411.
14.Cerdá B, Cerón JJ, Tomás-Barberán FA, Espín JC. Repeated oral administration of high doses of the pomegranate ellagitannin punicalagin to rats for 37 days is not toxic. Journal of agricultural and food chemistry. 2003;51(11):3493-501.
15.FZ Z, L M, A A. The effect of diet containing fruits: dates, figs and olives on weight, pain threshold and memory of rats. Medicinal and Aromatic Plants. 2009;25(2):149-58.
16.Zhang S, Zhao X, Zhang D. Cellular and molecular immunopathogenesis of ulcerative colitis. Cell Mol Immunol. 2006;3(1):35-40.
17.Zhang G, Ghosh S. Toll-like receptor–mediated NF-κB activation: a phylogenetically conserved paradigm in innate immunity. Journal of Clinical Investigation. 2001;107(1):13.
18.Ghosh S, May MJ, Kopp EB. NF-κB and Rel proteins: evolutionarily conserved mediators of immune responses. Annual review of immunology. 1998;16(1):225-60.
19.Caamano J, Hunter CA. NF-κB family of transcription factors: central regulators of innate and adaptive immune functions. Clinical microbiology reviews. 2002;15(3):414-29.
20.Abreu MT, Arnold ET, Thomas LS, Gonsky R, Zhou Y, Hu B, et al. TLR4 and MD-2 expression is regulated by immune-mediated signals in human intestinal epithelial cells. Journal of Biological Chemistry. 2002;277(23):20431-7.
21.Lansky EP, Newman RA. Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. Journal of ethnopharmacology. 2007;109(2):177-206.
22.it Works H. MenoComplete™.
23.Balkwill F, Charles KA, Mantovani A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer cell. 2005;7(3):211-7.
24.Simmons DL, Buckley CD. Some new, and not so new, anti-inflammatory targets. Current opinion in pharmacology. 2005;5(4):394-7.
25.Jurenka J. Therapeutic applications of pomegranate (Punica granatum L.): a review. Alternative medicine review: a journal of clinical therapeutic. 2008;13(2):128-44.
26.Aviram M, Dornfeld L, Rosenblat M, Volkova N, Kaplan M, Coleman R, et al. Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E–deficient mice. The American journal of clinical nutrition. 2000;71(5):1062-76.
27.Nishigaki I, Rajendran P, Venugopal R, Ekambaram G, Sakthisekaran D, Nishigaki Y. Effect of extract of pomegranate (Punica granatum L.) on glycated protein-iron chelate-induced toxicity: An in vitro study on human umbilical-vein endothelial cells. Journal of health science. 2008;54(4):441-9.
28.Kim ND, Mehta R, Yu W, Neeman I, Livney T, Amichay A, et al. Chemopreventive and adjuvant therapeutic potential of pomegranate (Punica granatum) for human breast cancer. Breast cancer research and treatment. 2002;71(3):203-17.
29.Kroon PA, Clifford MN, Crozier A, Day AJ, Donovan JL, Manach C, et al. How should we assess the effects of exposure to dietary polyphenols in vitro? The American journal of clinical nutrition. 2004;80(1):15-21.
30.Larrosa M, González-Sarrías A, Yáñez-Gascón MJ, Selma MV, Azorín-Ortuño M, Toti S, et al. Anti-inflammatory properties of a pomegranate extract and its metabolite urolithin-A in a colitis rat model and the effect of colon inflammation on phenolic metabolism. The Journal of nutritional biochemistry. 2010;21(8):717-25.