Original Article

Effects of jujube fruit extract on acetic acid-induced colitis in adult male rats

Fardous Soror Karawya, El Sayed Aly Mohamed Metwally[1]

Departments of Histology and Cell Biology and [1]Anatomy and Embryology, Faculty of Medicine, Alexandria University, Alexandria, Egypt

Abstract

Background: Gut health is the most important factor for a healthy life. A large number of people are suffering from gut associated diseases. Inflammatory bowel disease is the general medical terminology for chronic inflammatory illness of unknown origin. Experimental colitis induced by acetic acid has been used extensively as a model for intestinal inflammatory disease. **Aim of the work:** This study aims to assess the effect of jujube fruit extract on the histological integrity of colon, in a rat model of acetic acid-induced colitis. **Materials and Methods:** Experimental colitis was induced under light ether anesthesia by intrarectal administration of 1 ml of 4% acetic acid (AA) in male albino rats, Control rats received an equal volume of saline intrarectally. Experimental rats were treated orally with jujube fruit extract (1 gm/kg body weight) for one month. **Results:** Myeloperoxidase and caspase-3 levels in the colonic tissues revealed significant increased level and morphological changes in the colonic mucosa were evaluated by Hematoxylin and Eosin, AB/PAS and Trichrome stains revealed alteration of the colonic mucosa as evident by crypt architecture disarray, mucin depletion from goblet cells, epithelial degeneration and necrosis accompanied by cellular infiltration in the lamina propria and submucosa. In our model, the administration of jujube fruit extract of 1 gm/kg body weight revealed, significant decrease in myeloperoxidase and caspase-3 levels as compared with the control group associated with mild acetic acid-induced lesions in the histological sections. **Conclusion:** These results indicated that jujube fruit extract acted as a treatment agent against inflammation in rat model of acetic acid-induced colitis.

Key words: Acetic acid, experimental colitis, jujube fruit extract, rats

INTRODUCTION

Inflammatory bowel diseases (IBD) is a group of chronic inflammatory gastrointestinal (GI) disorders, consisting of Crohn's disease (CD) and ulcerative colitis (UC) that may cause a substantial worsening of life quality (Ng, *et al*. 2013). UC, an idiopathic type of IBD in the colon, has become a clinical challenge, owing to the increasing incidence and poor prognosis. Despite extensive research, the etiology and pathogenesis of UC are still unknown and the therapeutic approach remains therefore empirical. Due to its unknown etiology, high risk of recurrence, and poor prognosis, UC has become a clinical challenge also in terms of treatment. Meanwhile, conventional therapies for UC have failed to successfully induce remission and prevent relapse, and also possibly caused various side effects (Thia, *et al*. 2008; Xavier and

Address for correspondence:
Dr. El Sayed Aly Mohamed Metwally,
Department of Anatomy and Embryology, Faculty of Medicine,
Alexandria University, Alexandria, Egypt.
E-mail: sayedmetwally2020@yahoo.com

Access this article online	
Quick Response Code:	Website: www.jecajournal.com
	DOI: 10.4103/1596-2393.190828

How to cite this article:
Karawya, F. S. & Metwally, A.S.A.M. (2016). Effects of jujube fruit extract on acetic acid?induced colitis in adult male rats. Journal of Experimental and Clinical Anatomy, 15 (1), 19-26.

Podolsky, 2005). Therefore, studies exploring alternative therapies for UC have become a topic of great interest. In recent years, herbal medicine, the most common modality of alternative and complementary treatment, has been established for the treatment of UC (Comar and Kirby, 2005; D'Inca *et al*. 2007).

Since ancient times, herbal medicines have been traditionally used to treat several diseases. The gastro-protective properties of these herbs and their active constituents have been experimentally demonstrated (Al Mofleh, 2010). Various natural products have been shown to safely suppress the pro-inflammatory pathway and control IBD. *In vivo* and/or *in vitro* studies have suggested that the anti-IBD effects exhibited by natural products are mainly caused by their ability to modulate cytokine production (Cho, *et al*. 2011; He, *et al*. 2012; Yuan, *et al*. 2009).

Ziziphus jujube fruit commonly known as jujube or Chinese date is used widely for the treatment of different diseases such as treatment of chronic fatigue, loss of appetite, diarrhea, anemia, irritability, and hysteria. The fruits are also believed to possess activities such as anodyne, anticancer, refrigerant, sedative, stomachic, styptic, and tonic. The principle chemical constituents of zizyphusjujuba fruit are flavonoids, saponins, tannins, Vitamins A, B2, and C, sugars (polysaccharides), mucilage, calcium, phosphorus, and iron. Zizyphusjujuba is reported for anti-complementary, anti-cancer, hypoglycemic, and anxiolytic activities. The fruits are also used in Chinese medicine to strengthen liver function (Huang, *et al*. 2007; Lee, *et al*. 2004).

Clinical studies with herbal therapy in IBD have shown promising efficacy data and an acceptable safety profile; however, these remain limited and heterogeneous. With this background in mind, we investigated the anti-inflammatory effects and mucosal healing of jujube on rats with acetic acid (AA)-induced experimental colitis - A condition that shares clinical and structural features with human UC.

MATERIALS AND METHODS

The study was carried out using 40 male albino rats weighing 150–200 g. The animals were purchased from the Medical Research Institute, Alexandria University and were kept in the animal house under standard conditions of light and temperature. They were housed in metal cages with free access to food and water.

The animals were randomly divided into two groups:

Group I (Control group): Twenty rats were further subdivided into two equal subgroups 10 animals each. Subgroup Ia received 1 ml saline intrarectally and Subgroup Ib given jujube fruit extract 1 g/kg body weight orally by gavage for 1 month (Choudhary. *et al*. 2001).

Group II (Experimental group): Twenty rats were further subdivided into two equal subgroups 10 animals each. Subgroup IIa, colitis was induced in these animals by AA, with a dose of 1 ml of 4% AA intrarectally, and Subgroup IIb received jujube fruit extract 1 g/kg body weight. 1 g of jujube powder was dissolved in 10 ml water and each rat received 1–1.5 ml orally by gavage. It was given 24 h after the induction of colitis.

Jujube fruit extract (jujube) used in the experiment was purchased as the commercially available "natural jujube," a product of lifelong company, Lebanon.

Induction of Colitis

The animals were fasted for 36 h before induction of colitis. Under light ether anesthesia, rats were positioned on their right side. Thereafter, 1 ml AA 4% was intrarectally administered, via a polyethylene catheter inserted 8 cm proximal to the anus. Rats were kept in the head-down position for 2–3 min to preclude immediate anal leakage of the instillate and then returned to their cages with free access to food and water (Choudhary, *et al*. 2001). Control rats were treated with 0.9% saline in the same way.

At the end of the experiment (1 month), rats were sacrificed under ether anesthesia. The distal colon (10 cm) was rapidly removed, opened longitudinally, and gently cleaned of fecal content using normal saline and examined for presence of ulcers. Then specimen was obtained 2 cm proximal to the anus.

One specimen was frozen in 0.05 M phosphate buffer, pH 6, containing 0.5% hexadecyle – trimethyl ammonium bromide. Tissue samples were weighed and stored at −20°C until used for:

- Myeloperoxidase (MPO) level in the colonic tissue (Bradley, *et al*. 1982)
- Caspase-3 analysis (Garcia-Calvo, *et al*. 1998).

Another specimen was fixed in 10% formal saline for preparation of paraffin blocks. Sections were cut 5 μm thickness and were subjected to the following staining procedures:

- Hematoxylin and eosin stain for routine histological examination (Suvarna, *et al* 2013)
- Alcian blue (AB)/periodic acid Schiff (PAS) stained section for mucus in goblet cells (Suvarna, *et al* 2013)
- Gomori's trichrome stain for demonstration of collagen fibers Gomori, 1950.

Statistical Analysis

It was done using Statistical Package of Social Sciences (SPSS program version 17). Test of normality was done using Kolmogrove–Smirnov test that revealed normally distributed data, so parametric tests were used. Quantitative continuous data described by mean as a measure of central tendency while the standard deviation was used as a measure of dispersion. Analysis of data was conducted using Kruskal–Wallis one-way ANOVA test to compare between the different groups.

In all statistical tests used, level of significance at 5% was considered, below which the results considered statistically significant.

RESULTS

Macroscopic Evaluation

The colon of the control group (Subgroup Ia and b) showed no evidence of macroscopic mucosal damage, adhesions or ulceration. On the other hand, examination of the colon of AA-induced colitis (Subgroup IIa) revealed the presence of multiple mucosal erosions and ulcerations but administration of jujube to AA-induced colitis animals (Subgroup IIb) resulted in amelioration of most of the macroscopic damage.

Biochemical Results

Determination of myeloperoxidase level in the colonic tissue

Tissue MPO activity was determined as an index of granulocyte infiltration. MPO is an enzyme found within the granules of neutrophils. It had been demonstrated that these levels reflect the state of inflammation in the mucosa of the intestine.

Administration of jujube 1 g/kg body weight for 1 month did not significantly alter the level of MPO in the colonic tissue in comparison to the normal control group (Subgroup Ia). The results of the present study revealed that intracolonic administration of AA was associated with a significant increase of MPO level in the colonic tissue but concomitant administration of jujube with AA-induced colitis for 1 month associated with a significant decrease in the mean value of MPO level [Table 1].

Determination of Caspase-3 Level in the Colonic Tissue

Caspase-3 activity was measured using caspase-3/CPP32 activity Colorimetric Assay Kit (Biovision Incorporated, USA) according to the procedure supplied by the manufacturer. Activation of ICE - family proteases/caspases initiates apoptosis in mammalian cells, the caspase-3/CPP32 colorimetric assay kit provides a convenient means for assaying the activity of caspases that recognize the caspase substrate. Administration of jujube 1 g/kg body weight for 1 month (Subgroup Ib) did not significantly alter the level of caspase-3 in the colonic tissue in comparison to the (Subgroup Ia) but intracolonic administration of AA revealed significant increase in caspase-3 in comparison to the normal control group. On the other hand, administration of jujube for 1 month to AA-induced colitis in rat associated with a significant decrease in the mean value of caspase-3 in the colon in comparison to AA-induced colitis [Table 2].

Histological Results: Light microscopic examination

Group I examination

Hematoxylin and eosin (H and E) stained sections obtained from the colon of normal control rats (Subgroup Ia) revealed normal histological structure of the mucosa, submucosa, and musculosa. The mucosa is fully occupied by large number of tubular intestinal glands (crypt of leiberkuhn) lined by columnar cells alternating with clear goblet cells. The glands were straight and parallel with little amount of connective tissue lamina propria in between [Figure 1a]. Administration of jujube to the normal control rats (Subgroup Ib) revealed increased goblet cells and the length of the crypt [Figure 1b].

In AB/PAS-stained sections of the control colon, Subgroup Ia showed normal distribution of bluish goblet cells [Figure 1c] while positive control (received jujube) revealed increased goblet cells in the crypt of Leiberkuhn [Figure 1d].

Table 1: Myeloperoxidase level in the colonic tissue of different groups

Groups	Subgroup Ia	Subgroup Ib	Subgroup IIa	Subgroup IIb
Minimum value	4.79	3.45	32.63	4.53
Maximum value	8.57	8.53	39.82	8.87
Mean	7.07	6.44	37.47	6.54
SD	1.24	1.63	2.15	1.49
SE	0.39	0.51	0.68	0.47
χ^2	134.6	134.6	134.6	134.6
P	0.0001	0.0001	0.0001	0.0001

SD - Standard deviation, SE - Standard error

Table 2: Caspase-3 level in the colonic tissue of different groups

Groups	Subgroup Ia	Subgroup Ib	Subgroup IIa	Subgroup IIb
Minimum value	0.13	0.11	0.89	0.21
Maximum value	0.30	0.24	1.99	0.53
Mean	0.20	0.18	1.52	0.32
SD	0.04	0.04	0.37	0.09
SE	0.01	0.01	0.11	0.03
χ^2	15.574	15.574	49.29	49.29
P	0.01	0.01	<0.0001	<0.0001

SD - Standard deviation, SE - Standard error

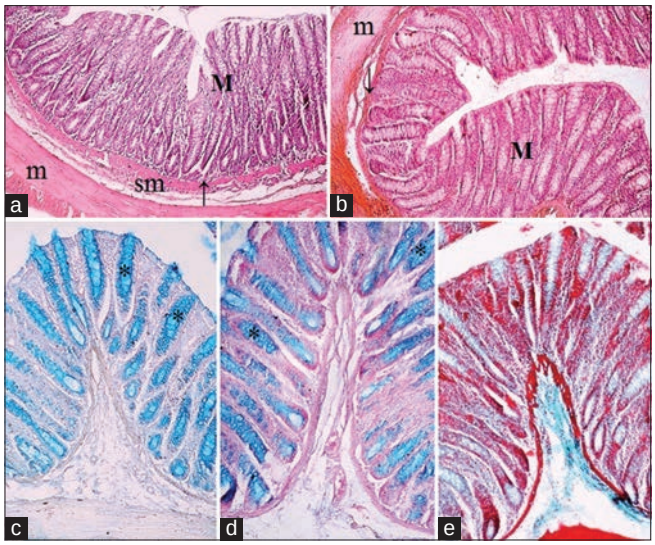

Figure 1: (a-e): Light micrographs of the control rat's colon: (a and b) – H and E stained section of rat colon Subgroup Ia and b showing, intact colonic mucosa (M), limited externally by muscularis mucosa (↑). Large number of tubular intestinal gland (crypts) lined with numerous goblet cells are seen separated by connective tissue lamina propria. Note: Submucosa (sm), musculosa (m). (c and d) Alcian blue/periodic acid Schiff-stained section of Subgroup Ia and b. The bluish goblet cells are frequently seen which increased with jujube in Subgroup Ib. (e) Gomori's trichrome-stained section of Subgroup Ia and b showing, normal distribution of collagen fibers in lamina propria and submucosa (Mic., X100)

Gomori's Trichrome-stained sections of the control colon (Subgroup Ia and b) revealed normal distribution of collagen fibers in the lamina propria and submucosa [Figure 1e].

Group II Examination

Light microscopic examination of H and E stained sections of Subgroup IIa (AA-induced colitis) sacrificed 1 month after administration of AA revealed disturbed architecture of the colonic mucosal glands, multiple mucosal erosions, evident glandular atrophy with short narrow destructed crypts and inflammatorycellular infiltration [Figure 2a and b].

In AB/PAS-stained sections of the colon, Subgroup IIa revealed evident mucosal atrophy where goblet cells were hardly seen in the atrophic crypt and were largely depleted around the ulcerated mucosa [Figure 2c].

Gomori's trichrome stained sections the colonic mucosa of Subgroup IIa revealed destruction of lamina propria and submucosa [Figure 2d].

Light microscopic examination of H and E stained sections of Subgroup IIb (received jujube fruit extract orally concomitant with AA intracolonic for 1 month) revealed that the mucosal crypts showed good cellularity of moderate length and width compared to the control sections. The regenerating surface columnar cells were observed covering the luminal surface of the mucosa

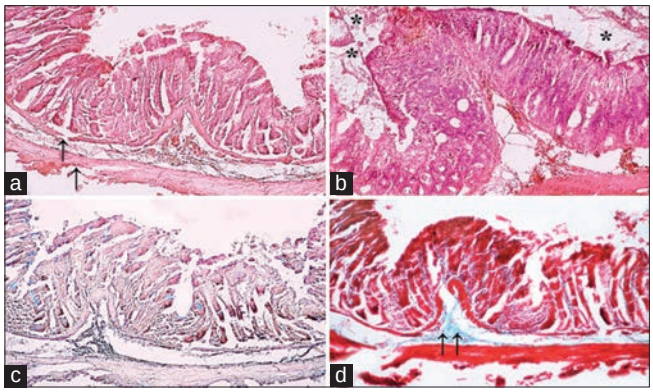

Figure 2: (a-d) Light micrographs of rat's colon Subgroup IIa (AA – induced colitis): (a and b) H and E stained section showing, features of inflammation and ulceration. The lumen contains sloughed necrotic fragments mixed with inflammatory debris (*), crypts with necrotic columnar and goblet cells, disruption of muscularis mucosa and musculosa (↑). (c) Alcian blue/periodic acid Schiff-stained section of the same subgroup showing, depletion of goblet cells. (d) Gomori's trichrome-stained section of the same subgroup showing, destruction of lamina propria and submucosa (↑↑) (Mic., X100)

associated with mild cellular infiltration in the connective tissue of the lamina propria and submucosa [Figure 3a].

In AB/PAS-stained sections of the colonic mucosa, Subgroup IIb showed intact crypt with adequate number of goblet cells and increased mucus secretion [Figure 3b].

Gomori's trichrome stained sections of the colon Subgroup IIb showed no more increased collagen fibers in the lamina propria and submucosa as compared with the control group [Figure 3c].

DISCUSSION

It is generally agreed that the initiation and pathogenesis of UC are multifactorial, involving interactions among environmental, genetic, microbial, and immune factors. These factors are all involved and functionally integrated into the generation of the chronic intestinal inflammatory reaction that characterizes UC (Scaldaferri and Fiocchi, 2007; Fiocchi, 1998).

The relationship between the environment and development of gut inflammation is complex. A variety of unrelated environmental factors are considered as risk factors for UC, including smoking, diet, hygiene, social status, and lifestyle (Scaldaferri and Fiocchi, 2007; Sartor, 2006).

Advances in the field of genetics open novel insight to investigate the relationship between genetic abnormalities and altered immune responses in IBD (Handschick, *et al*. 2014; Zhang, *et al*. 2008; Maloy and Powrie, 2011).

As regards the microbial flora, it is widely accepted that intestinal flora has a central role in the pathogenesis of

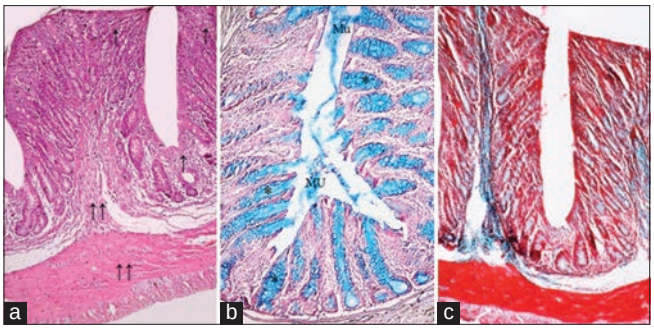

Figure 3: (a-c) Light micrographs of rat's colon Subgroup IIb (AA-induced colitis + jujube); (a) H and E stained section showing, intact crypt with adequate length and cellularity. Intact surface epithelial cells and goblet cells associated with mild cellular infiltration (↑), intact submucosa and musculosa (↑↑). (b) Alcian blue/periodic acid Schiff-stained section of the same subgroup showing, many bluish goblet cells with much mucus flowing in the lumen (Mu). (c) Gomori's trichrome-stained section of the same subgroup showing, no more increase in collagen fibers in the lamina propria and submucosa as compared with the control group (Mic., X100)

UC. It was suggested that loss of tolerance to the gut commensal flora in cases of UC may lead to a state of chronic intestinal inflammation (Maloy and Powrie, 2011; Swidsinski, *et al.* 2002; Hooper and Gordon 2001).

Finally, the role of immune system in triggering and maintaining the inflammatory response in UC is of paramount importance. The immunity is mediated by different cell types, including neutrophils, monocytes and macrophages. There is a growing body of evidence that the behavior of these cells, the expression and function of their recognition receptors are altered in UC (Man, *et al*. 2004).

In IBD oxidative stress mediates disease progression by disrupting epithelial cell integrity. Reactive oxygen species (ROS) play an important role in the pathogenesis of UC. Moreover, the overproduction of ROS can overwhelm the protective antioxidant mechanisms resulting in oxidative damage to the cells (Grisham, *et al*. 2002).

A major conceptual advance in the understanding of the etiology of IBD has arisen from experimental studies. In this respect, one of the well-established models of induced colitis, AA model, was chosen in the present study to characterize the morphological changes associated with colitis. The model of induced colitis through AA enemas presents advantages over other experimental models of this disease. Such advantages include easy availability of the aggressor reagent, low cost, reproducibility and similarities to UC in humans. Certain pathophysiological features of human UC and CD involving weight loss and diarrhea accompanied by blood and/or mucus, fever, shortened colon, crypt abnormalities, gastric dysmotility and infiltration of inflammatory cells (Venkataranganna, *et al*. 2007). Most reports on animal models of colitis focused predominantly on the proinflammatory mediators that may initiate inflammatory process. The present study included biochemical estimation of MPO and caspase-3 in colonic tissues associated with histological examination of sections stained with H and E, AB/PAS for goblet cell mucus and trichrome stain for collagen fibers as indicator to fibrosis.

The present study demonstrated that AA-induced colitis was associated with a significant increase in the level of MPO. MPO has been used extensively as a biochemical marker of granulocyte (mainly neutrophil) infiltration into colonic tissue (Gonzalez and Sarna, 2001). Previous studies demonstrated similar increase in MPO level in trinitrobenzene sulfonic (TNBS) model and other models of induced colitis (AA-induced colitis and dextran sodium sulfate-induced colitis). Neutrophil infiltration characteristic of this inflammation was noticed (Zingarelli, *et al*. 1998; Ciccocioppo, *et al*. 2001).

Apoptosis is an essential physiological process required for the maintenance of tissue homeostasis. Under normal conditions in the intestine, the balance between cell proliferation and cell loss in crypt and villi is controlled by apoptosis (Kitazawa, *et al*. 2004; Levine, 2000). Dysregulated or accelerated apoptosis is usually seen in several forms of GI diseases. It may result in gut barrier failure and pathogenic microorganism infiltration (Jones, *et al*. 2000; Strater, *et al,* 1997). In the present study, there was a significant increase in colonic caspase-3 activity in the AA-induced colitis compared to the normal control group. Our results are in agreement with previous studies that demonstrated that the frequency of apoptosis in the colon cells in UC patients was increased (Strater, *et al,* 1997). Yue, *et al*. (2001), had demonstrated increased colonic epithelial cell apoptosis in TNBS-induced colitis in rats. Caspase-3 which is known to be expressed in enterocytes had been also demonstrated to be increased in animal models of induced colitis (Grossmann, *et al*. 1998; Rajilic-Stojanovic, *et al*. 2013).

In the present study, the histological sections of AA-induced colitis revealed disturbed architecture of colonic mucosa with multiple erosions, destruction of the crypt, mucus depletion from goblet cells as indicated by reduction of AB positive cells around the ulcerated mucosa and inflammatory cellular infiltrate. These findings are in accordance with previous reports in animal models of colitis which demonstrated similar changes (Wirtz and Neurath, 2007; Heller, *et al*. 2005; Campos, *et al*. 2002).

Glandular atrophy and depletion of goblet cells have been related to a decreased resistance of the mucosa and paralleled by alterations in the normal pattern of maturation of mucus in goblet cells. The protective

effect of mucus as an active barrier may be attributed largely to its viscous and gel-forming properties that are derived from mucin glycoprotein constituents (Torres, *et al*. 1999).

The results of the present study in the form of degeneration and ulcer formation were similar to that reported earlier on animal models of colitis which focused predominantly on excess ROS and proinflammatory mediators which are capable of causing extensive oxidative damage to cellular structures and neutrophil infiltration (Pravda, 2005; Rana, *et al*. 2014). The colon is more responsive to oxidative damage because of the relatively short amount of antioxidants accessible in the mucosa. The accumulation of ROS could cause damage to specific genes involved in cell growth or differentiation or could cause changes in antioxidant enzyme levels. Oxidative stress has been well documented in UC with increased ROS levels and decreased antioxidant levels in the inflamed mucosa. This could lead to severe damage to macromolecules which ultimately contribute to morphological and functional damage in the cell (Kanodia, *et al.* 2011; Ferguson, 2010; Loft and Poulsen, 1999; Caprillia, *et al*. 2009).

Until the early 20th century, the treatment of UC was empirical and ineffective with poor prognosis and high mortality rate. However, it appears that no currently available medical therapeutic modality is capable of addressing the fundamental disorder present and, therefore, unable to alter the natural history of the disease. So there is a real need for seeking more specific and safer approaches for treating UC. For example, mechanisms to limit proinflammatory cytokine expression, ROS generation as well as to block their effects may prove useful alternatives in the treatment of this condition (Ke, *et al*. 2012; Yue, *et al*. 2015).

The use of medicinal plants or their active components is becoming an increasingly attractive approach for treatment of various inflammatory disorders especially among patients unresponsive or developed toxicity to standard medicines. Zizyphusjujuba was reported for anti-cancer, hypoglycemic, and anxiolytic activities. The fruits are also used as hepatoprotective agent (Huang, *et al*. 2007; Lee, *et al*. 2004). Although herbal medicines are not devoid of risk, they could still be safer than synthetic drugs. The potential benefits of herbal medicine could lie in their high acceptance by patients, efficacy, relative safety, and relatively low cost (Yue, *et al*. 2015; Wang, *et al*. 2012).

The present study revealed that jujube fruit extract led to good healing of the ulcerated mucosa, with restoration of the general architecture of the colonic mucosal glands and mild cellular infiltration. These results are in agreement with Yue, *et al*. (2015) who demonstrated that jujube polysaccharides protect against experimental IBD by enabling enhanced intestinal barrier function. It is well known that intestinal inflammation increases the formation of harmful ROS and decreases the antioxidant defense system, thus causing oxidative stress. The colon is more vulnerable to oxidative stress than other organs due to its low antioxidant protection system (Man, *et al*. 2004). Because phenolic and flavonoid compounds are recognized as the bases of the antioxidant activity of plant extracts. Therefore, the jujube fruit extract was chosen for evaluation of the antioxidant activity using AA-induced colitis in rat model and it was found to have good protective effects evidenced by suppression of AA-induced oxidative stress in the colon of rats, and attenuating the morphological changes caused by AA. The positive effects of jujube could be explained by many mechanisms. It may be due to free radical-scavenging, antioxidant activity, immunomodulatory effect (Wang, *et al*. 2012). (Man, *et al*. 2004 assumed the good healing effect of jujube to its high content of Vitamin C). In animal experiments; jujube extract decreased GI transit time and increased fecal moisture content. Increased fatty acid concentration in the cecum and decreased fecal ammonia and bacterial enzyme activity in the feces were also measured (Huang, *et al*. 2008; Liu, *et al.* 2015).

CONCLUSION

The use of plants in treating diseases is a very old human tradition. Medicinal plants are the foundations for modern therapeutic agents. Healthcare professionals need to be aware of the pharmacology of these herbal medicines to provide well-informed advice to patients. The traditional herbal medicine field is very vast. Jujube fruit extract has the potential to treat UC. The evidence on herbal medicine are incomplete, complex, and confusing, and certainly associated with both risks and benefits, so there is a need for further controlled clinical trials of the potential efficacy of herbal medicine approaches in the treatment of UC, together with enhanced legislation to maximize their quality and safety.

Financial Support and Sponsorship
Nil.

Conflicts of Interest
There are no conflicts of interest.

REFERENCES

1. Al Mofleh I.A. (2010). Spices., herbal xenobiotics and the stomach: Friends or foes? World J Gastroenterol 16 (22):2710-9.
2. Bradley P.P., Priebat D.A., Christensen R.D., Rothstein G. (1982). Measurement of cutaneous inflammation: Estimation of neutrophil content with an enzyme marker. J Invest Dermatol 78 (3):206-9.
3. Campos F.G., Waitzberg D.L., Habr-Gama A., Logullo A.F.,

Noronha I.L., Jancar S., *et al.* (2002). Impact of parenteral n-3 fatty acids on experimental acute colitis. Br J Nutr 87 Suppl 1:S83-8.
4. Caprillia R., Cesarinia M., Angeluccia E., Frierib G. (2009). The long journey of salicylates in ulcerative colitis: The past and future. J Crohns Colitis 3 (3):149-56.
5. Cho E.J., Shin J.S., Noh Y.S., Cho Y.W., Hong S.J., Park J.H., *et al.* (2011). Anti-inflammatory effects of methanol extract of Patrinia scabiosaefolia in mice with ulcerative colitis. J Ethnopharmacol 136 (3):428-35.
6. Choudhary S., Keshavarzian A., Yong S., Wade M., Boccekino S., Day B.J., *et al.* (2001). Novel antioxidants zolimid and AEOL11201 ameliorate colitis in rats. Dig Dis Sci 46 (10):2222-30.
7. Ciccocioppo R., Di Sabatino A., Parroni R., Muzi P., D'Alò S., Ventura T., *et al.* (2001). Increased enterocyte apoptosis and Fas-Fas ligand system in celiac disease. Am J Clin Pathol 115 (4):494-503.
8. Comar K.M., Kirby D.F. (2005). Herbal remedies in gastroenterology. J Clin Gastroenterol 39 (6):457-68.
9. D'Inca R., Garribba A.T., Vettorato M.G., Martin A., Martines D., Di Leo V., *et al.* (2007). Use of alternative and complementary therapies by inflammatory bowel disease patients in an Italian tertiary referral centre. Dig Liver Dis 39 (6):524-9.
10. Ferguson L.R. (2010). Chronic inflammation and mutagenesis. Mutat Res 690 (1-2):3-11.
11. Fiocchi C. (1998). Inflammatory bowel disease: Etiology and pathogenesis. Gastroenterology 115 (1):182-205.
12. Garcia-Calvo M., Peterson E.P., Leiting B., Ruel R., Nicholson D.W., Thornberry N.A. (1998). Inhibition of human caspases by peptide-based and macromolecular inhibitors. J Biol Chem 273 (49):32608-13.
13. Gonzalez A., Sarna S.K. (2001). Different types of contractions in rat colon and their modulation by oxidative stress. Am J Physiol Gastrointest Liver Physiol 280 (4):G546-54.
14. Grisham M.B., Pavlick K.P., Laroux F.S., Hoffman J., Bharwani S., Wolf R.E. (2002). Nitric oxide and chronic gut inflammation: Controversies in inflammatory bowel disease. J Investig Med 50 (4):272-83.
15. Grossmann J., Mohr S., Lapentina E.G., Fiocchi C., Levine A.D. (1998). Sequential and rapid activation of select caspases during apoptosis of normal intestinal epithelial cells. Am J Physiol 274 (6 Pt 1):G1117-24.
16. Handschick K., Beuerlein K., Jurida L., Bartkuhn M., Müller H., Soelch J., *et al.* (2014). Cyclin-dependent kinase 6 is a chromatin-bound cofactor for NF-κB-dependent gene expression. Mol Cell 53 (2):193-208.
17. He X., Shu J., Xu L., Lu C., Lu A. (2012). Inhibitory effect of *Astragalus polysaccharides* on lipopolysaccharide-induced TNF-a and IL-1ß production in THP-1 cells. Molecules 17 (3):3155-64.
18. Heller F., Florian P., Bojarski C., Richter J., Christ M., Hillenbrand B., *et al.* (2005). Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions., apoptosis., and cell restitution. Gastroenterology 129 (2):550-64.
19. Hooper L.V., Gordon J.I. (2001). Commensal host-bacterial relationships in the gut. Science 292 (5519):1115-8.
20. Huang X., Kojima-Yuasa A., Norikura T., Kennedy D.O., Hasuma T., Matsui-Yuasa I. (2007). Mechanism of the anti-cancer activity of Zizyphusjujuba in HepG2 cells. Am J Chin Med 35:517-23.
21. Huang Y.L., Yen G.C., Sheu F., Chau C.F. (2008). Effects of water-soluble carbohydrate concentrate from Chinese jujube on different intestinal and fecal indices. J Agric Food Chem 56 (5):1734-9.
22. Jones N.L., Islur A., Haq R., Mascarenhas M., Karmali M.A., Perdue M.H., *et al.* (2000). *Escherichia coli* Shiga toxins induce apoptosis in epithelial cells that is regulated by the Bcl-2 family. Am J Physiol Gastrointest Liver Physiol 278 (5):G811-9.
23. Kanodia L., Borgohain M., Das S. (2011). Effect of fruit extract of *Fragaria vesca* L. on experimentally induced inflammatory bowel disease in albino rats. Indian J Pharmacol 43 (1):18-21.
24. Ke F., Yadav P.K., Ju L.Z. (2012). Herbal medicine in the treatment of ulcerative colitis. Saudi J Gastroenterol 18 (1):3-10.
25. Kitazawa H., Nishihara T., Nambu T., Nishizawa H., Iwaki M., Fukuhara A., *et al.* (2004). Intectin., a novel small intestine-specific glycosylphosphatidylinositol-anchored protein., accelerates apoptosis of intestinal epithelial cells. J Biol Chem 279 (41):42867-74.
26. Lee S.M., Park J.G., Lee Y.H., Lee C.G., Min B.S., Kim J.H., *et al.* (2004). Anti-complementary activity of triterpenoides from fruits of *Zizyphus jujuba*. Biol Pharm Bull 27 (11):1883-6.
27. Levine A.D. (2000). Apoptosis: Implications for inflammatory bowel disease. Inflamm Bowel Dis 6 (3):191-205.
28. Lillie R.D, editor. (1977). Gomori G. A rapid one-step Trichrome stain. Am J ClinPathol 1950; 20:661.
29. Liu G., Liu X., Zhang Y., Zhang F., Wei T., Yang M., *et al.* (2015). Hepatoprotective effects of polysaccharides extracted from *Zizyphus jujube* cv. Huanghetanzao. Int J Biol Macromol 76:169-75.
30. Loft S., Poulsen H.E. (1999). Markers of oxidative damage to DNA: Antioxidants and molecular damage. Methods Enzymol 300 (3):166-84.
31. Maloy K.J., Powrie F. (2011). Intestinal homeostasis and its breakdown in inflammatory bowel disease. Nature 474 (7351):298-306.
32. Man A.L., Prieto-Garcia M.E., Nicoletti C. (2004). Improving M cell mediated transport across mucosal barriers: Do certain bacteria hold the keys? Immunology 113 (1):15-22.
33. Ng S.C., Lam Y.T., Tsoi K.K., Chan F.K., Sung J.J., Wu J.C. (2013). Systematic review: The efficacy of herbal therapy in inflammatory bowel disease. Aliment Pharmacol Ther 38 (8):854-63.
34. Pravda J. (2005). Radical induction theory of ulcerative colitis. World J Gastroenterol 11 (16):2371-84.
35. Rajilic-Stojanovic M., Shanahan F., Guarner F., de Vos W.M. (2013). Phylogenetic analysis of dysbiosis in ulcerative colitis during remission. Inflamm Bowel Dis 19 (3):481-8.
36. Rana S.V., Sharma S., Prasad K.K., Sinha S.K., Singh K. (2014). Role of oxidative stress and antioxidant defence in ulcerative colitis patients from North India. Indian J Med Res 139 (4):568-71.
37. Sartor R.B. (2006). Mechanisms of disease: Pathogenesis of Crohn's disease and ulcerative colitis. Nat Clin Pract Gastroenterol Hepatol 3 (7):390-407.
38. Scaldaferri F., Fiocchi C. (2007). Inflammatory bowel disease: Progress and current concepts of etiopathogenesis. J Dig Dis 8 (4):171-8.
39. Sträter J., Wellisch I., Riedl S., Walczak H., Koretz K., Tandara A., *et al.* (1197). CD95 (APO-1/Fas)-mediated apoptosis in colon epithelial cells: A possible role in ulcerative colitis. Gastroenterology 113 (1):160-7.
40. Suvarna KS, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques, 7th edition. Edinburgh: Churchill Livingstone; Elsevier; 2013.
41. Swidsinski A., Ladhoff A., Pernthaler A., Swidsinski S., Loening-Baucke V., Ortner M., *et al.* (2002). Mucosal flora in inflammatory bowel disease. Gastroenterology 122 (1):44-54.
42. Thia K.T., Loftus E.V Jr., Sandborn W.J., Yang S.K. (2008). An update on the epidemiology of inflammatory bowel disease in Asia. Am J Gastroenterol 103 (12):3167-82.
43. Torres M.I., García-Martin M., Fernández M.I., Nieto N., Gil A., Ríos A. (1999). Experimental colitis induced by trinitrobenzenesulfonic acid: An ultrastructural and histochemical study. Dig Dis Sci 44 (12):2523-9.
44. Venkataranganna M.V., Rafiq M., Gopumadhavan S., Peer G., Babu U.V., Mitra S.K. (2007). NCB-02 (standardized Curcumin preparation) protects dinitrochlorobenzene- induced colitis through down-regulation of NFkappa-B and iNOS. World J Gastroenterol 13 (7):1103-7.
45. Wang D., Zhao Y., Jiao Y., Yu L., Yang S., Yang X. (2012). Antioxidative and hepatoprotective effects of the polysaccharides from *Zizyphus jujube* cv. Shaanbeitanzao. Carbohydr Polym 88:1453-9.
46. Wirtz S., Neurath M.F. (2007). Mouse models of inflammatory bowel disease. Adv Drug Deliv Rev 59 (11):1073-83.

47. Xavier R.J., Podolsky D.K. (2005). Unravelling the pathogenesis of inflammatory bowel disease. Nature 448 (7152):427-34.
48. Yuan Y., Sun M., Li K.S. (2009). *Astragalus mongholicus* polysaccharide inhibits lipopolysaccharide-induced production of TNF-alpha and interleukin-8. World J Gastroenterol 15 (29):3676-80.
49. Yue G., Lai P.S., Yin K., Sun F.F., Nagele R.G., Liu X, *et al.* (2001). Colon epithelial cell death in 2,4,6-trinitrobenzenesulfonic acid-induced colitis is associated with increased inducible nitric-oxide synthase expression and peroxynitrite production. J Pharmacol Exp Ther 297 (3):915-25.
50. Yue Y., Wu S., Li Z., Li J., Li X., Xiang J., *et al.* (2015). Wild jujube polysaccharides protect against experimental inflammatory bowel disease by enabling enhanced intestinal barrier function. Food Funct 6 (8):2568-77.
51. Zhang H., Massey D., Tremelling M., Parkes M. (2008). Genetics of inflammatory bowel disease: Clues to pathogenesis. Br Med Bull 87:17-30.
52. Zingarelli B., Cuzzocrea S., Szabó C., Salzman A.L. (1998). Mercaptoethylguanidine., a combined inhibitor of nitric oxide synthase and peroxynitrite scavenger., reduces trinitrobenzene sulfonic acid-induced colonic damage in rats. J Pharmacol Exp Ther 287 (3):1048-55.