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Healing Effects of *Elaeagnus angustifolia* Extract in Experimentally Induced Ulcerative Colitis in Rats

[1]A. Khodakarm-Tafti, [2]D. Mehrabani, [1]L. Homafar and [3]G. Farjanikish
[1]Department of Pathology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
[2]Department of Pathology, Stem Cell and Transgenic Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[3]Department of Pathobiology, School of Veterinary Medicine, Lorestan University, Khorram Abad, Iran

Corresponding Author: A. Khodakarm-Tafti, Department of Pathology, School of Veterinary Medicine, Shiraz University, P.O. Box 71345-1731, Shiraz, Iran Tel: 00987132286950

ABSTRACT

Ulcerative Colitis (UC) is a chronic idiopathic inflammatory bowel disease that involves mostly the mucosa of the colon and increases the possibility of colon cancer. *Elaeagnus angustifolia* is one of traditional herbal medicines widely used for treatment of digestive diseases. In the present study, 70 Sprague dawley rats were randomly divided into seven equal groups including negative control, positive control, enamel gel 10 and 20%, edible extract 300 and 600 mg and also pure base gel enema groups. Ulcerative colitis was induced by 3% acetic acid enema in all rats except negative control group. Positive control group received distilled water enema and all treatment groups received gel enema or edible extract daily during two weeks. Histopathological findings in the colon including regeneration and healing of mucosa, erosion and ulceration, cryptitis, cystic dilatation of the crypts and aggregation of inflammatory cells were compared in all control and experimental groups. The results showed edible extract 600 mg had the best effect on healing process. Edible extract 300 mg and 20% gel had lesser effects. In conclusion, 600 mg kg^{-1} edible extract of *Elaeagnus angustifolia* can be used for improvement of healing process in colon ulcers.

Key words: *Elaeagnus angustifolia*, colon ulcer, histopathology, healing effect, rat

INTRODUCTION

Ulcerative Colitis (UC) is a chronic idiopathic inflammatory bowel disease that involves the mucosa of the rectum and colon. Etiology of this complicated disease is unknown yet and recently believed to be multifactorial mostly due to genetic, immunological and environmental factors. Colon cancer happens due to side effects of medicine treatment particularly in long-term (Ho *et al*., 2006; Geboes, 2001). The therapeutic methods for UC are on basis of anti-inflammatory agents, glucocorticoids and salicylates as well as biological agents Against Tumor Necrosis Factor-α (TNF-α). Corticosteroids are potent inhibitors of T-cell activation and pro-inflammatory cytokines. However, a more secure and easier way is needed for treatment and prevention of this disease that minimizes the expenses and the side effects of the medical methods (Shepherd, 1991; Fiocchi, 1998).

Several experimental studies have used herbal extracts for treatment of the inflammatory bowel diseases. For example, Medhi *et al*. (2008) reported synergic effects of Manuka honey with sulfasalazine in enhancing antioxidant defense system in experimentally induced UC in rats. A decrease in antioxidant defense mechanisms have been shown in the mucosa of affected colon of UC patients (Hendriksen and Binder, 1980).

Elaeagnus angustifolia (Russian olive, Russian silverberry, Oleander) is one of the herbs applied mostly in Iran's traditional medicine (Farahbakhsh *et al*., 2011). Studies have revealed that *E. angustifolia* has anti-inflammatory, muscle relaxant activity, anti-ulcerogenic, antibacterial and antinociceptive effects (Hosseinzadeh and Rahimi, 1999; Ramezani *et al*., 2001; Hosseinzadeh *et al*., 2003; Gurbuz *et al*., 2003; Ahmadiani *et al*., 2000). Regarding to anti-inflammatory entities of *E. angustifolia* and lack of informations about its efficacy in treatment of UC, the present study was conducted to investigate the healing effect of *E. angustifolia* extract in experimentally induced UC in rat as a proper animal model.

MATERIALS AND METHODS

Preparation of *Elaeagnus angustifolia* extract: About 200 g of fresh *E. angustifolia* fruit were dried under ambient temperature and the dried fruits were milled. About 51 g extract was obtained from 100 g of the milled powder of the plant via hydroalcohol method. For preparation of 10 and 20% gel, 10 and 20 g of the extract were mixed with 90 and 80 g carbon oxide methyl cellulose gel, respectively. Also, in order to prepare the edible extract, 300 mg of extract was solved in 1cc of distilled water.

Animal model, control and experimental groups: In this study, 70 Sprague dawley rats (250±50 g), aged between 8 and 10 weeks were maintained under controlled environment at 22±2°C, with 55±5% of humidity and 12 h light-dark cycle. Standard laboratory chow and tap water were available *ad libitum*. All experiments, animal selection, subsequent care and the sacrifice procedure were identical and adhered to the guidelines of Animal Care Committee of Shiraz University, Iran. The animals were randomly divided into seven equal control and experimental groups including negative control, positive control, gel 10% enema, gel 20% enema, edible extract 300 mg kg^{-1}, edible extract 600 mg kg^{-1} and pure base gel enema groups. Ulcerative colitis was induced by 3% acetic acid (3 mg kg^{-1} $stat^{-1}$) enema in all rats except negative control group. Positive control group received 1cc distilled water enema and all treatment groups received gel enema or edible extract daily during two weeks.

Gross and histopathologic analysis: At the end of the experiment, all the animals were sacrificed with an overdose of anesthetics and after necropsy, the colon of each animal was removed and transferred in 10% neutral-buffered formalin, The specimens were embedded in paraffin and sections of 5 μm in thickness were provided and stained using hematoxylin and eosin (H and E) and studied by a routine light microscope In order to compare mean value of qualitative indicators of histopathology in different groups, non-parametric test of Mann-Whitney was applied. The data was analyzed using SPSS 11.5 edition. $p<0.05$ was measured as the significant level.

RESULTS

In the negative control group, no gross or microscopic lesions were observed in the colon. In rats of positive control group, 3 rats were died in 9, 10 and 11 days post induction of ulcerative colitis. Grossly, multifocal to diffuse ulcers were seen in the colon so that in two rats peritonitis with inflammation of serosal surface of colon was determined. Histopathological findings including degeneration and necrosis of the mucosa, cryptitis and cystic dilatation of the crypts, infiltration of neutrophils and mononuclear inflammatory cells in the mucosa and submucosa were observed (Fig. 1). The gross and histopathologic lesions in the colon of rats received pure gel without extract were to some extent similar to positive control group.

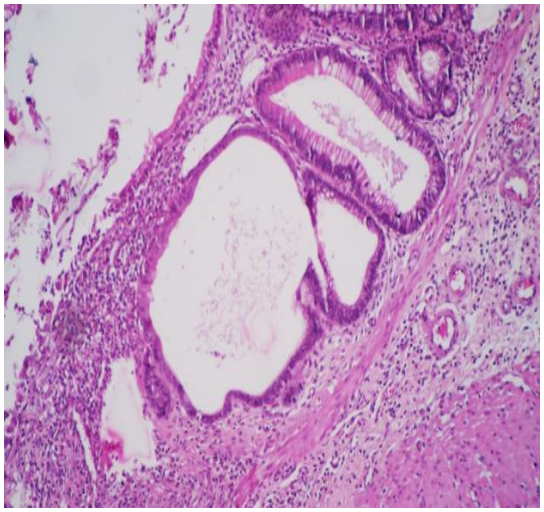

Fig. 1: Colon, rat of positive control group, chronic ulcerative colitis as fibrosis of the mucosa, cystic dilation of crypts and infiltration of inflammatory cells in the mucosa and submucosa are observed (H and E stain, ×80)

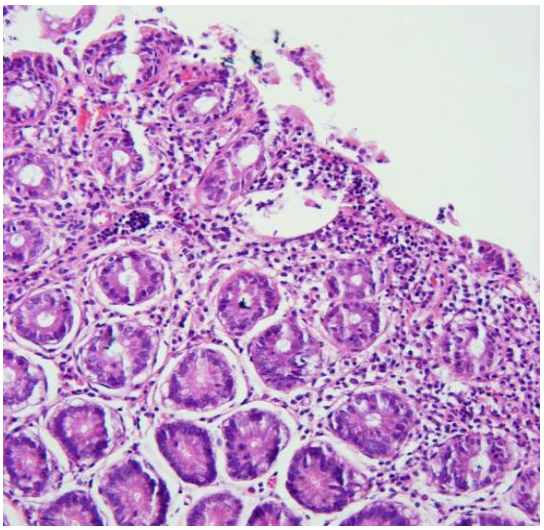

Fig. 2: Colon, rat received 10% extract gel via enema, minimal to moderate healing of ulcerative colitis associated with infiltration of inflammatory cells in the mucosa are seen (H and E stain, ×200)

In rats received 10% extract gel via enema, there was no any mortality during the experiment. Gross changes in the colon were including focal ulcers with moderate swelling and hyperemia of the mucosa in 6 cases and others had no remarkable gross changes. In microscopic examination, variable changes were observed. In 4 cases, mucosal atrophy, hyperemia and submucosal edema were diagnosed. In 2 cases, degeneration and necrosis of epithelium, dilatation of some crypts and cryptitis were observed (Fig. 2). In all rats of this group, infiltration and aggregation of mononuclear inflammatory cells were observed in the lamina propria of colon.

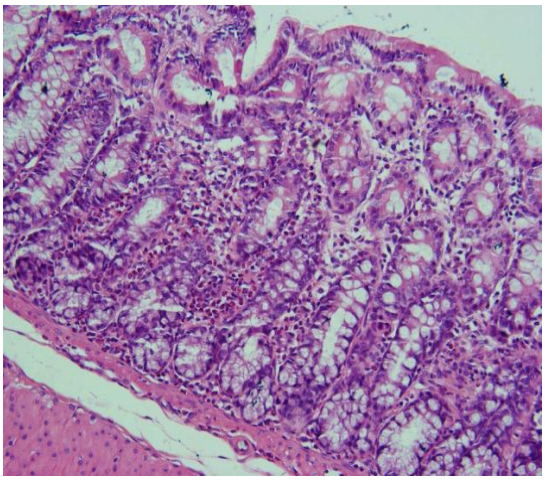

Fig. 3: Colon, rat received 20% extract gel via enema, moderate healing of colonic ulcer as regeneration of mucosal epithelium, infiltration of mononuclear inflammatory cells in the lamia propria between crypts are observed (H and E stain, ×200)

In group treated with 20% extract gel via enema, one rat died in day 5 of the experiment because of perforated ulcer of colon and peritonitis. In 6 rats, no gross change was observed in the colon. In 3 rats, hyperemia and linear healing of ulcers were seen in the colon. Histopathologic examination revealed regeneration of mucosal epithelium, edema of mucosa and submucosa, hyperemia and infiltration of a few neutrophils and a lot of mononuclear inflammatory cells in the lamia propria between crypts (Fig. 3).

In group treated with 300 mg kg^{-1} extract, one rat died in day 4 due to peritonitis. Other rats of this group had minor gross changes including focal linear ulcer healing associated with hyperemia and edema of the mucosa. Histologically, regeneration of epithelium and crypts, atrophy of mucosa, aggregation of number mononuclear inflammatory cells between crypts and submucosal edema were observed.

In group treated with 600 mg kg^{-1} extract, gross changes in the colon were not remarkable. Histopathological examination of colon revealed complete regeneration and healing of epithelium and crypts associated with aggregation of a few numbers of mononuclear inflammatory cells in the lamina propria. Atrophy of mucosa of the affected colon was observed only in 2 rats (Fig. 4).

Statistical analysis of obtained data from scoring the indices including wound improvement, mucosal cell depletion, crypt abscess, cystic dilation of glands, mucosal atrophy, sub-mucosal edema and aggregation of inflammatory cells indicated that maximum differences in terms of healing, comparing to control group, was created in experimental group under treatment of edible extract of 600 mg kg^{-1}. Significant differences have been observed in indices such as wound improvement ($p = 0.03$), aggregation of inflammatory cells ($p = 0.042$) and sub-mucosal edema ($p = 0.015$).

DISCUSSION

In traditional medicine, fruits and flowers of *E. angustifolia* are used as an aphrodisiac and anti-fever medicine. It is also used for treatment of nephropathies, stomach disorders, diarrhea, nausea, vomiting, jaundice, asthma and flatulence (Mirhydar, 1998; Gurbuz *et al*., 2003). In Iran's

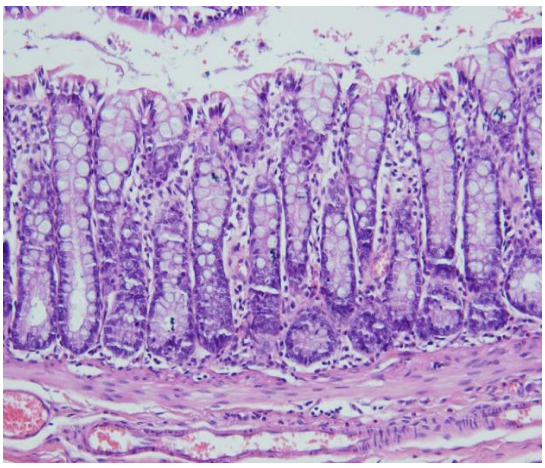

Fig. 4: Colon, rat received 600 mg kg^{-1} extract orally, perfect healing of the mucosa as complete regeneration of epithelium and crypts and mild chronic inflammatory reaction are observed (H and E stain, ×200)

traditional medicine, *E. angustifolia* is used for reduction of rheumatoid arthritis pain (Zargari, 1990). In Turkey's traditional medicine, fruits of *E. angustifolia* are used for stimulation of appetite (Baytop, 1984). Recently, it is shown aqueous extract of *E. angustifolia* L. leaf has obvious protective effects on myocardial/reperfussion injury which may be related to the improvement of myocardial oxidative stress states (Wang *et al*., 2014).

Elaeagnus angustifolia contains vitamins A, B and K as well as valuable compounds including olein, styrene, lanolin, 8% oil, sugar, organic acids, flavonoids and tannin. Tannins have cholesterol-lowering effects, cytotoxic effects on human cancer cells, cardio-protective properties and stimulation of angiogenesis in cutaneous wound healing without irritation of toxicological effects (Goncharova and Glushenkova, 1990). Vitamin A has different roles in wound healing such as: Antioxidant activity, increasing fibroblastic proliferation, modulating of cellular differentiation and proliferation, increasing collagen deposition and hyaluronate synthesis and decreasing matrix metalloproteinases (Burgess, 2008; Guo and Dipietro, 2010). Flavonoids are known to accelerate the wound healing process mainly due to their antimicrobial properties which appear to be responsible for wound closure and increased rate of epidermis regeneration (Nishino *et al*., 1987; Nayak *et al*., 2006).

For treatment of ulcerative colitis different medicines such as corticosteroids, salazosulfapyridine, azathioprine, mesalazine, methotrexate, mercaptopurine and cyclosporine have been applied. In the present study, *E. angustifolia* extract was used as two enamel gel and edible forms for treatment of experimental ulcerative colitis in rats. On basis of the results, both forms of *E. angustifolia* extract have positive effects on healing process of this disorder. The best results were seen in rats received 600 mg edible extract during two weeks and both 300 mg edible extract and 20% gel enema had same relatively good effects on healing process of colon ulcer.

Vaezi *et al*. (2011) have investigated effect of different doses of *E. angustifolia* water extract with and without morphine on analgesic ratio in rats. According to their results, using *E. angustifolia* extract along with morphine can enhance analgesic effects.

In folk medicine, *E. angustifolia* has been known as a wound healing accelerator (Rasekh *et al*., 1999). Based on histopathological examination and determination of the ulcer index (Gurbuz *et al*.,

2003) found potent gastroprotective effect for the methanolic fruit extract of *E. angustifolia* in ethanol induced gastric ulcer in rats. Natanzi *et al*. (2012) have studied the effect of *E. angustifolia* fruit on experimental cutaneous wound healing in rats, that their results demonstrate aqueous extract of *E. angustifolia* accelerates cutaneous wound healing.

CONCLUSION

Results of this study showed extract of *Elaeagnus angustifolia* can be used for improvement of healing process in colon ulcers and edible extract 600 mg has the best effect in comparison to edible 300 mg and 20% enema gel extracts.

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