IJVS 2013; 8(2); Serial No:19

IRANIAN JOURNAL OF VETERINARY SURGERY (IJVS)
WWW.IVSA.IR

Protective effect of Fenugreek (*Trigonella foenum graecum*) seed extract on experimental reflux esophagitis in rat

Reza Kheirandish[1], Omid Azari[2], Saeedeh Shojaeepour[3]

Abstract

Objective- *Trigonella foenum graecum*, (Fenugreek) has anti-inflammation, antiseptic and antidiabetic activities in traditional medicine. This study was performed to investigate the protection effects of Fenugreek seed extract on the experimental reflux esophigitis (RE) in rats.
Design- Experimental study.
Animals- 24 male Wistar albino rats.
Procedures- Rats were randomly allocated into 4 groups including the sham-operated groups with normal saline (sham I) and extract (sham II) administration, the reflux esophagitis group (the reflux control group), and the reflux esophagitis group with extract administration (reflux extract group). Reflux esophagitis was induced by ligating the pylorus and the limiting ridge. Normal saline and Fenugreek extract were gavaged for one week before operation in the related groups. Then, the animals were euthanized and the esophagus was evaluated for gross and histopathologic features. Gross lesions in the esophagus were graded according to the scoring system of Oh and colleagues.
Results- Significant difference in the gross lesions was observed between the reflux control and reflux extract groups. The reflux control group showed severe mucosal erosions and ulcers in the esophagus. Microscopic findings in the extract treatment group varied from no lesions to mild damages including hydropic degeneration, epithelial clefts and vesicles formation.
Conclusion and clinical relevance- Based on the results, it can be concluded that the aqueous extract of *T. foenum graecum* seed have protective effects against RE and this extract can be considered as one of the therapeutic options in the clinical trial studies.
Key words- Reflux esophagitis, *Trigonella foenum graecum*, Rat.

Introduction

Reflux esophagitis (RE) is a common gastrointestinal disorder,[1, 2, 3] and affects approximately 40% of world population.[4] Reflux esophagitis can result in chronic esophagitis, esophageal strictures and Barrett's esophagus (intestinal or columnar metaplasia).[5, 6, 7] Failure of the preventive mechanisms of acid reflux leads to excessive exposure of the esophagus with gastric juice.[8] The gastric acid has principle role in the pathogenesis of reflux esophagitis.[1, 3, 6, 7] In patients with RE, relaxation of lower esophageal sphincter is a major etiology for refluxing acid into the esophagus.[3, 9, 10] It seems that the gastric contents contain different deleterious components that their reflux into the esophagus cause chemical injury.[6, 11]
Several studies have shown that the presence of oxygen-derived free radicals and pro-inflammatory mediators have some roles such as activated neutrophils and lipid peroxidation in the pathogenesis of RE.[2, 12, 13, 14] Therapeutic agents that scavenge free radicals and modulate inflammatory responses may be effective in attenuating tissue damages induced by gastric acid reflux.[13, 14, 15]
Now a days, proton pomp inhibitors such as omperazole or H_2 receptor antagonists are considered as conventional anti-reflux treatments to reduce gastric acidity. They exhibit various side effects such as impotence, gynaecomastia, hypergastrinemia and hemapoeitic hematopoietic changes, and also ulcer relapse after long-term treatment.[14, 16] In recent years,

[1] Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
[2] Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
[3] Graduated student, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
Address all correspondence to Dr. Reza Kheirandish (DVM, DVSc), E-mail: kheirandish@uk.ac.ir

developing of new drugs with herbal origin is considered for decreasing the side effects of chemical drugs.

Trigonella foenum graceum (Fenugreek) is an annual herb that is cultivated in Iran, India, China and Greece .[17] In traditional medicine, *T. foenum graceum* has been used for treatment of diabetes mellitus,[18] hypercholesterolemia, neoplasms,[19] and also for its antibacterial[20] and gastroprotective effects.[21, 22, 23]

The present study was purposed to evaluate the protective effect of Fenugreek seed extract on the experimental model of reflux esophagitis in rat.

Materials and methods

Plant extract prepration

Trigonella foenum graecum seeds were collected from local herbal markets of Kerman province, Iran. The seeds were dried in the room temperature (30±5) in the dark, and grounded into a fine powder. One g of milled seed was dissolved in 100 ml distilled water and mixed in a vortex cyclomixer. Then, the mixture was centrifuged at 3000 rpm for 10 minutes and the resultant supernatant was collected as the seed extract.

Experimental animals

In this study, 24 male Wistar albino rats (200-250 g) were prepared from the animal house, Shahid Bahonar University of Kerman, Kerman, Iran. The animals were kept under standard conditions of 12 h dark-12 h light and temperature (21±1 ºC). They supplied with standard pellet food and tap water *ad libitum*.

Study design

The rats were randomly allocated into four equal groups including the sham-operated groups with normal saline (sham I) and extract (sham II), the reflux esophagitis group with normal saline administration (the reflux control group), and the reflux esophagitis group with extract administration (reflux extract group).

The reflux control and reflux extract groups rats were gavaged with normal saline and Fenugreek extract (3 ml/rat) for one week before operation. The animals were fasted for 24 h, but had free access to water. Then, they were anesthetized by intramuscular injection of xylazine hydrochloride (5 mg/kg BW) and ketamine hydrochloride (90 mg/kg BW). The abdomen was opened by a midline incision to expose the stomach. Subsequently, the pylorus and limiting ridge (the transitional region between the forestomach and the corpus) were simultaneously ligated for reflux of gastric juice into the esophagus (Fig. 1). Additionally, for decreasing the antireflux of cardiac sphincter and enhancement of the reflux of gastric juice, a longitudinal cardiomyotomy about 1 cm length was performed across the junction of the stomach and esophagus. In the sham groups (I and II), the abdomen was opened and only the visceral organs were manipulated without any ligation and cardiomyotomy. Then, the incisions and abdominal wall were sutured. Twenty four h post operation, the animals were sacrificed and the entire esophagus and stomach were removed.

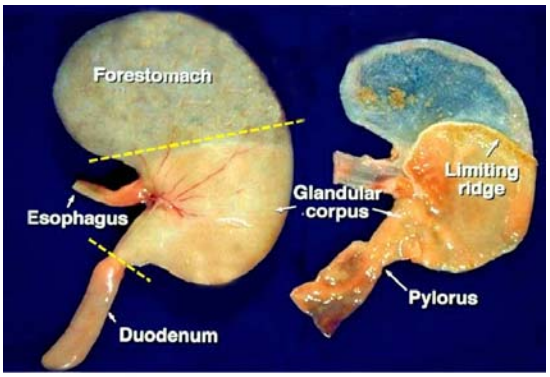

Figure1. Illustration of the reflux esophagitis experimental model. Dotted lines indicate the position of two ligations to induce reflux esophagitis

Gross and histopathological investigations

The removed esophagus was opened and washed with phosphate buffered saline. Gross lesions in the esophagus were graded according to the scoring system of Oh and colleagues.[14] Accordingly, the lesions were evaluated as follows: normal esophageal mucosa (score 0); edema with hemorrhagic spots in the mucosa (score 1); multiple erosions (score 2); linear ulcers (score 3); and hemorrhagic coalesced ulcers (score 4).

For histopathological examination, the esophageal samples were fixed in 10 % buffered formalin and processed by using standard procedure. Sections in 5 µm thickness were stained with Hematoxylin and Eosin. On microscopic study, some parameters such as the epithelial loss degrees (erosion and ulceration), regenerative changes of the epithelium, vascular changes (edema, congestion and hemorrhage) and infiltration of inflammatory cells were considered.

Statistical analysis

Obtained data were expressed as mean ± SEM for all groups. Scored values were analyzed using non-parametric Mann–Whitney U test due to the small sample size. The SPSS software version 16 (SPSS Inc., Chicago, IL, USA) was used in order to perform analyses. $P<0.05$ was considered to be statistically significant.

Results

In this study, the protective effect of *T. foenum graecum* was investigated on the experimental reflux esophagitis in rats. Statistically, significant difference in gross lesions was observed between the reflux control and reflux extract groups ($P<0.05$). In the reflux control group, the lower and middle parts of the esophagus showed various longitudinal erosions and ulcers that covered by hemorrhagic exudate. Severe hemorrhage, edema and thickening of esophageal wall were observed in the animals of this group. In the reflux extract group, the mean scores of gross lesions in the esophagus was significantly lower in comparison with the reflux control group ($P<0.05$). In two cases of treatment group, no lesion was seen (grade 0), while other cases showed scattered erosions and mild hemorrhages in the lower part of the esophagus. Histopathologically, in the reflux control group revealed large ulcers with complete necrosis of the mucosal epithelium that progressed to the tunica submucosa and muscularis. In this group, infiltration of inflammatory cells and severe hemorrhage were prominent in the lamina propria and submucosa. Microscopic findings in the extract treatment group varied from no lesions to mild damages including hydropic degeneration, epithelial clefts and vesicles formation. Sometimes, scattered erosions association with inflammatory cells and submucosal edema and hemorrhage were observed. The esophagus of sham groups (I and II) were histologically normal and no erosions were detected. The obtained results have been shown in Table 1.

Table 1. Comparison of histopatological scores in all groups*

Group	Score (mean ± SE)
I	0 ± 0^{a}
II	0 ± 0^{a}
III	4 ± 0^{b}
IV	2.5 ± 1.225^{c}

* Data are expressed as mean ± SE; Different alphabetic letters show significant differences between the groups ($P<0.05$). I: sham I; II: sham II; III: reflux control group; IV: reflux extract group

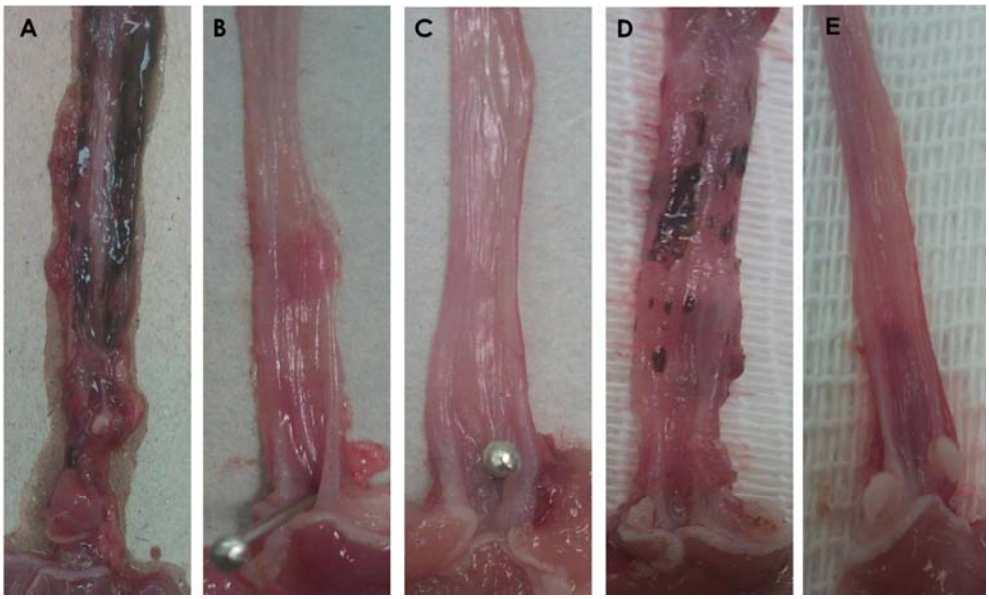

Figure 2. Esophagus. (A): Reflux control group. Hemorrhagic coalesced ulcers (score 4); (B) and (C): Normal esophageal mucosa in sham I and II groups; (D): Linear ulcers (score 3) and (E) normal esophageal mucosa in reflux extract group

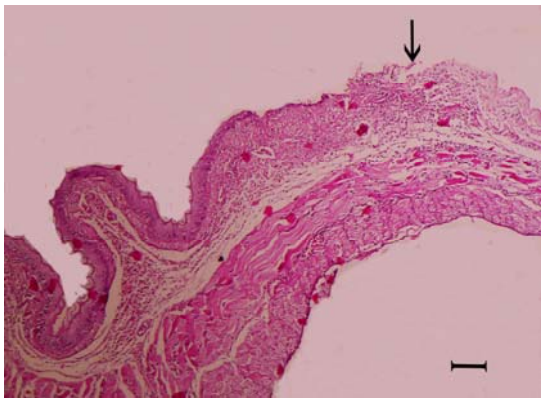

Figure 3. Reflux control group. Ulcer with complete necrosis of the mucosal epithelium that progressed to the tunica submucosa and muscularis (arrow). H&E. Bar=250µm.

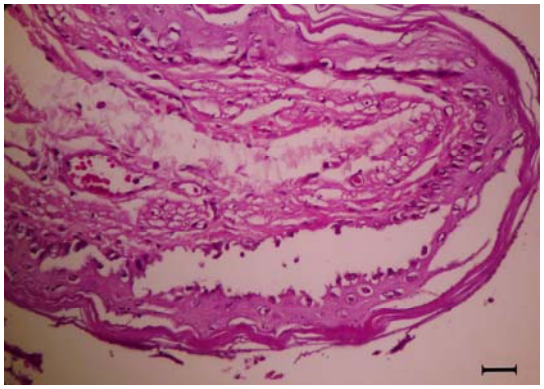

Figure 4. Reflux extract group. Epithelial clefts and vesicles formation. H&E. Bar=50µm.

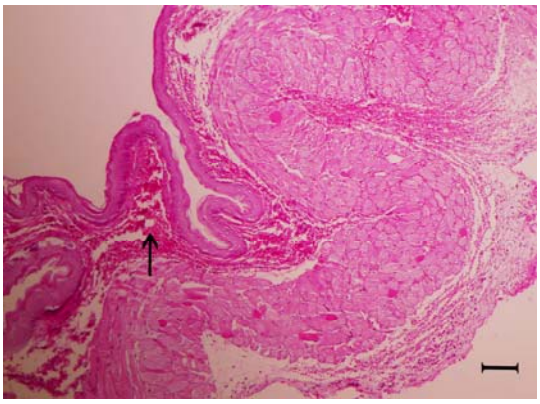

Figure 5. Reflux extract group. This photomicrograph shows submucosal hemorrhage (arrow). H&E. Bar=250µm.

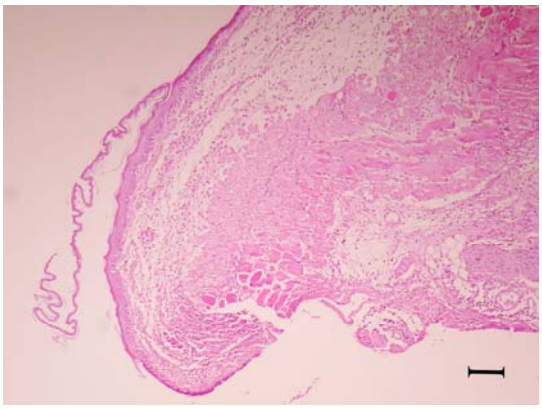

Figure 6. Reflux extract group. Edema and infiltration of inflammatory cells are seen. H&E. Bar=250µm.

Discussion

Under the physiologic condition, gastroesophageal reflux may occur immediately after eating, without damage to the esophageal mucosa. The esophagus is protected by effective defensive systems against reflux injury including the anti-reflux barriers, the luminal clearance mechanisms and the tissue resistance protecting esophageal epithelium.[6] If the gastroesophageal reflux takes a long time, esophagitis occurs.[5, 6] In most cases, reflux esophagitis heals by regeneration of epithelium. Prolonged exposure of acid with esophageal mucosa causes metaplasia in the epithelium named Barrett's esophagus and may develop into esophageal adenocarcinoma.[7] The refluxed materials (acid, bile or mixed reflux of gastric and duodenal juices are considered as one of the causal factor in occurrence of the reflux esophagitis.[24, 25] Also, it has been shown that oxygen derived free radicals are responsible in pathogenesis of RE, however free radicals can be blocked by various endogenous and exogenous scavengers.[14, 15] In human studies, positive correlation between the grade of esophagitis and levels of free radicals has been described thus patients with Barrett's esophagus have shown higher amount of free radical.[12] Chemical therapy with anti-secretory drugs like H_2 receptor antagonists or proton pump inhibitors can be effective approximately 90 % of RE cases.[14] These drugs is not effective to stop free radical generation or scavenging free radicals, which may be one explanation for the low treatment success in patients affected with RE.

In ethnopharmocological knowledge, natural herbal products are studied for development of new drugs with less toxicity and more efficacies.[26] In folk medicine, *Trigonella foenum graecum* is a medical plant with various therapeutic properties certainly, gastroprotective activity.[22, 23] This study investigated the protective effects of *T. foenum graecum* on the experimental reflux esophagitis model in rats. In our study, gastric juice passed refluxed into the esophagus. This model

resembles the reflux esophagitis in the clinical state in human. In this study, significant difference in gross lesions was observed between the reflux control and reflux extract groups (P<0.05). We demonstrated that *T. foenum graecum* extract decreases the severity of ulcers and inflammation in the entire esophagus in comparing to reflux control group. In the extract reflux group, the infiltration of inflammatory cells especially neutrophils was decreased in consistent with reduced esophageal mucosal damages. Neutrophils are a potential source of oxygen metabolites and pro-inflammatory cytokines, [4, 27] and *T. foenum graecum* extract may act as scavenger for free radicals.[23]

Several herbal therapies with antioxidant activity have been already suggested for treatment of RE. Jang et al. (2012) determined the protective effect of *Rumex Aquaticus herba* extract containing quercetin-3-β-D-glucuronopyranoside (ECQ) on the experimental reflux esophagitis.[28] ECQ group reduced esophageal lesions significantly in comparision with omeprazole. Moreover, ECQ decreased the volume of gastric juice, increased the gastric pH similar to omeprazole and inhibited the acid output effectively in the reflux esophagitis. They suggested that the protective effect of ECQ may be attributed to its anti-oxidative and anti-inflammatory activities on the reflux esophagitis in rats. Mahattanadul et al. (2011) evaluated effects of *Morinda citrifolia* (Noni) fruit extract on the reflux esophagitis and gastric ulcer in rats. [16] They described that the extract of *M. citrifolia* fruit has inhibitory activity on free radicals and cytokine-mediated inflammation. Therefore, this extract can be used for treatment of gastro-esophageal diseases such as RE. Ku et al. (2012) examined the effects of anti-inflammatory of *Lonicerae flos* on RE induced by pylorus and forestomach ligation and compared with α-tocopherol, a well-known proton antioxidant.[4] The results of their study showed that *L. flos* extract has decreased the severity of esophageal mucosal damage of RE.

Trigonella foenum graecum used in this study has high concentrations of flavonoid and polyphenolic compounds playing an important role as free radicals scavengers. Flavonoids may be responsible in cytoprotective action of fenugreek and exert antiulcer effect [20, 29, 30] Fenugreek seeds have been shown to possess significant ulcer protective effects. The gastroprotective effect of the seeds seemed to be due their antisecretory action and effects on the mucosal glycoproteins.[21] In accordance with the previous studies, we showed that the application of extract of Fenugreek extract can be effective in attenuating the reflux esophagitis. This preliminary study should be followed by further experimental studies to understand the mechanism of protective effects of this extract in reflux esophagitis. It needs different components of Fenugreek seeds be detected and their therapeutic effects of each purified substance demonstrated.

References

1. Gaia Filho EV, Goldenberg A, Costa HO. Experimental model of gastroesophageal reflux in rats. *Acta Cir Bras* 2005; 20: 437-44.
2. Rieder F, Biancani P, Harnett K, et al. Inflammatory mediators in gastroesophageal reflux disease: impact on esophageal motility, fibrosis, and carcinogenesis. *Am J Physiol Gastrointest Liver Physiol* 2010; 298: G571-81.
3. Munster M, Horauf A, Lubke-Becker A, et al. Idiopathic esophagopathies resembling gastroesophageal reflux disease in dogs. *Tierarztl Prax Ausg K Kleintiere Heimtiere* 2013; 41: 173-9.
4. Ku SK, Kim JS, Seo YB, et al. Effect of *Curculigo orchioides* on reflux esophagitis by suppressing pro-inflammatory cytokines. *Am J Chin Med* 2012; 40: 1241-55.
5. Taha AS, Angerson WJ, Morran CG. Reflux and Barrett's oesophagitis after gastric surgery-long-term follow-up and implications for the roles of gastric acid and bile in oesophagitis. *Aliment Pharmacol Ther* 2003; 17: 547-52.
6. Li Y, Martin RC, 2nd. Reflux injury of esophageal mucosa: experimental studies in animal models of esophagitis, Barrett's esophagus and esophageal adenocarcinoma. *Dis Esophagus* 2007;20: 372-8.
7. Souza RF. The role of acid and bile reflux in oesophagitis and Barrett's metaplasia. *Biochem Soc Trans* 2010; 38: 348-52.
8. DeMeester TR, Wernly JA, Bryant GH, et al. Clinical and in vitro analysis of determinants of gastroesophagealcompetence: A study of the principles of antireflux surgery. *Am JSurg* 1979; 137: 39-46.
9. Tugtepe H, Tugay M, Bozkurt S, et al. Esophageal smooth muscle reactivity is impaired in chronic reflux esophagitis by both receptor- and nonreceptor-mediated mechanisms. *J Pediatr Surg* 2007; 42: 641-6.
10. Wasko-Czopnik D, Jozkow P, Dunajska K, et al. Associations between the lower esophageal sphincter function and the level of physical activity. *Adv Clin Exp Med* 2013; 22: 185-91.
11. Hamada H, Haruma K, Mihara M, et al. Protective effect of ammonia against reflux esophagitis in rats. *Dig Dis Sci* 2001; 46: 976-80.
12. Wetscher GJ, Hinder RA, Bagchi D, et al. Reflux esophagitis in humans is mediated by oxygen-derived free radicals. *Am J Surg* 1995a; 170: 552-6.
13. Wetscher GJ, Hinder PR, Bagchi D, et al. Free radical scavengers prevent reflux esophagitis in rats. *Dig Dis Sci* 1995b; 40: 1292-6.
14. Oh TY, Lee JS, Ahn BO, et al. Oxidative damages are critical in pathogenesis of reflux esophagitis: implication of antioxidants in its treatment. *Free Radic Biol Med* 2001; 30: 905-15.
15. Jimenez P, Piazuelo E, Sanchez MT, et al. Free radicals and antioxidant systems in reflux esophagitis and

Barrett's esophagus. *World J Gastroenterol* 2005; 11: 2697-703.
16. Mahattanadul S, Ridtitid W, Nima S, et al. Effects of *Morinda citrifolia* aqueous fruit extract and its biomarker scopoletin on reflux esophagitis and gastric ulcer in rats. *J Ethnopharmacol* 2011; 134: 243-50.
17. Hassanzadeh E CM, Mazaheri D, Rezazadeh S, et al. Physical and chemical variabilities among domestic Iranian fenugreek (*Trigonella foenum graecum*) seeds. *Asian J Plant Sci* 2011; 10(6): 323-30.
18. Hannan JM, Ali L, Rokeya B, et al. Soluble dietary fiber fraction of *Trigonella foenum-graecum* (fenugreek) seed improves glucose homeostasis in animal models of type 1 and type 2 diabetes by delaying carbohydrate digestion and absorption, and enhancing insulin action. *Br J Nutr* 2007; 97: 514-21.
19. Raju J, Patlolla JM, Swamy MV, et al. Diosgenin, a steroid saponin of *Trigonella foenum graecum* (Fenugreek), inhibits azoxymethane-induced aberrant crypt foci formation in F344 rats and induces apoptosis in HT-29 human colon cancer cells. *Cancer Epidemiol Biomarkers Prev* 2004; 13: 1392-8.
20. Premanath R SJ, Lakshmi Devi N, Aradhya SM. Antibacterial and anti-oxidant activities of fenugreek (*Trigonella foenum graecum L.*) leaves. *Res J Med Plant* 2011; 5(6): 695-705.
21. Pandian RS, Anuradha CV, Viswanathan P. Gastroprotective effect of fenugreek seeds (*Trigonella foenum graecum*) on experimental gastric ulcer in rats. *J Ethnopharmacol* 2002; 81: 393-7.
22. Yadav M, Lavania A, Tomar R, et al. Complementary and comparativestudy on hypoglycemic and antihyperglycemic activity of various extracts of *Eugenia jambolana* seed, *Momordica charantia* fruits, *Gymnema sylvestre*, and *Trigonella foenum graecum* seeds in rats. *Appl Biochem Biotechnol* 2010; 160: 2388-400.
23. Priya V JR, Vijayalakshmi K. studies on anti-oxidant activity of *Trigonella foenum graecum* seed using in vitro models. *Int J Pharmaceut Sci Res* 2011; 2(10): 2704-08.
24. Vaezi MF, Singh S, Richter JE. Role of acid and duodenogastric reflux in esophageal mucosal injury: a review of animal and human studies. *Gastroenterology* 1995; 108: 1897-907.
25. Nehra D, Howell P, Williams CP, et al. Toxic bile acids in gastro-oesophageal reflux disease: influence of gastric acidity. *Gut* 1999; 44: 598-602.
26. Borelli F, Izzo AA. The plant kingdom as a source of antiulcer Remedies. *Phytother Res* 2000; 14: 581-591.
27. Singh P, Singh N, Sengupta S, et al. Ameliorative effects of *Panax quinquefolium* on experimentally induced reflux oesophagitis in rats. *Indian J Med Res* 2012; 135: 407-13.
28. Jang HS, Han JH, Jeong JY, et al. Protective effect of ECQ on rat reflux esophagitis model. *Korean J Physiol Pharmacol* 2012; 16: 455-62.
29. Kaviarasan SNG, Gangabhagirathi R, Anuradha CV, et al. In vitro studies on antiradical and antioxidant activities of fenugreek (*Trigonella foenum graecum*) seeds. *Food Chem* 2007; 103: 31-7.
30. Xue W, Lei J, Li X, et al. *Trigonella foenum graecum* seed extract protects kidney function and morphology in diabetic rats via its antioxidant activity. *Nutr Res* 2011; 31: 555-62.

نشریه جراحی دامپزشکی ایران
سال ۲۰۱۳، جلد ۸ (شماره ۲)، شماره پیاپی ۱۹

چکیده

اثر محافظتی عصاره دانه شنبلیله بر رفلاکس ازوفاژیت تجربی در موش

رضا خیراندیش*[۱]، امید آذری[۲]، سعیده شجاعی‌پور[۳]

[۱] گروه پاتوبیولوژی، دانشکده دامپزشکی، دانشگاه شهید باهنر کرمان، کرمان، ایران.
[۲] گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه شهید باهنر کرمان، کرمان، ایران.
[۳] دانش آموخته دامپزشکی، دانشکده دامپزشکی، دانشگاه شهید باهنر کرمان، کرمان، ایران.

هدف- عصاره دانه شنبلیله دارای خاصیت آنتی اکسیدانی، ضد التهابی و آنتی سپتیک است. بنابراین هدف از مطالعه حاضر، بررسی اثر محافظتی عصاره دانه شنبلیله بر مخاط مری متعاقب رفلاکس ازوفاژیت در موش است.

طرح مطالعه- مطالعه تجربی.

حیوانات- بیست و چهار سر موش نر نژاد ویستار آلبینو.

روش کار- در این مطالعه موش های صحرایی به چهار گروه تقسیم شدند. گروه های شم جراحی که با نرمال سالین (شم۱) و عصاره (شم۲) گاواژ شدند. گروه کنترل رفلاکس که بعد از گاواژ نرمال سالین، رفلاکس ازوفاژیت در آنها القا شد و گروه درمان که با عصاره گاواژ و رفلاکس در آنها القا شد. نرمال سالین و عصاره شنبلیله به مدت یک هفته قبل از جراحی در گروه های ذکر شده گاواژ گردید. القا رفلاکس ازوفاژیت با لیگاتور همزمان پیلور و ناحیه عرضی (بین کورپوس و پیش معده) صورت گرفت. ۲۴ ساعت پس از جراحی، موش ها به روش انسانی کشته و بافت مری برای بررسی های ماکروسکوپی و هیستوپاتولوژی اخذ گردید. درجه بندی ضایعات ماکروسکوپی بر اساس سیستم درجه بندی Oh و همکاران صورت گرفت.

نتایج- اختلاف معنی داری بین گروه های کنترل و درمان رفلاکس وجود داشت ($P<0.05$). در گروه کنترل ضایعات شدید مخاطی و زخم مشاهده گردید ولی در گروه درمان رفلاکس شدت ضایعات متفاوت بود و از حالت نرمال تا ضایعاتی خفیفی همانند دژنرسانس آبکی، ایجاد شکاف و وزیکول در بافت پوششی مشاهده شد.

نتیجه گیری- نتایج حاصل از این مطالعه به وضوح نشان می دهد که عصاره دانه شنبلیله قادر به کاهش آسیب های ناشی از رفلاکس ازوفاژیت تجربی در مری موش صحرایی می باشد.

کلید واژگان- رفلاکس ازوفاژیت، شنبلیله، موش صحرایی.