

Protective Effect of *Trigonella foenum graecum* (fenugreek) Seed Extract on Experimental Intestinal Ischemia/Reperfusion Injury in Rats

Reza Kheirandish^{1*}, PhD
Omid Azari², DVSc
Hamed Samadieh³, DVM
Zohreh Rasa³, DVM

¹Department of Pathobiology, Faculty of Veterinary Medicine,
Shahid Bahonar University of Kerman, Kerman, Iran

²Department of Clinical Sciences, Faculty of Veterinary Medicine,
Shahid Bahonar University of Kerman, Kerman, Iran

³Graduated student, Faculty of Veterinary Medicine,
Shahid Bahonar University of Kerman, Kerman, Iran

Abstract

Objective- This study was performed to evaluate whether *Trigonella foenum graecum* (fenugreek) seeds extract has any protective activity in intestinal ischemia/reperfusion injury in rat.

Design- Experimental study.

Animals- 20 male Wistar albino rats.

Procedures- Four groups of rats were included in this study. Group I (I/R) underwent ischemia-reperfusion (I/R) of the intestine (45 min of ischemia followed by 1 h of reperfusion). Group II (fenugreek +I/R) was given fenugreek seeds extract via oral gavages for 2 wk before inducing I/R. Group III (vitamin C + I/R) and group IV (control) had sham I/R. After the experiments, the jejunum was removed and the tissues were processed for histopathologic examination.

Results- I/R group animals showed severe mucosal damage. The intestinal mucosa in the groups II and III was preserved in comparison with that in the group I. Significant decrease in histopathological scores was noted in the VitC and fenugreek groups in comparison with I/R group. There was no significant difference between the VitC and fenugreek groups ($p > 0.05$).

Conclusion and clinical relevance- According to the results of current study, administration of *Trigonella foenum graecum* (fenugreek) seeds extract before inducing I/R protects the intestinal mucosa from injury.

Key words- Ischemia/reperfusion injury, *Trigonella foenum graecum*, Intestine, Rat

* Corresponding author:

Reza Kheirandish, DVM, PhD

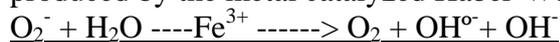
Department of Pathobiology, Faculty of Veterinary Medicine,
Shahid Bahonar University of Kerman, Iran.

E-mail address: E mail: kheirandish@uk.ac.ir

Introduction

Intestine injury resulting from ischemia/reperfusion (I/R) is an important clinical event and has a critical role in many diseases associated with high mortality and morbidity such as trauma, burns, septic or hypovolemic shock, strangulated hernias, neonatal necrotizing enterocolitis, mesenteric insufficiency, abdominal aortic aneurysm surgery, and cardiopulmonary bypass. It also plays an important role in the pathogenesis of systemic inflammation and multiple organ failure.^{1,2} Intestinal ischemia is a relatively uncommon cause of abdominal pain, with a reported incidence of 1% for all patients admitted with abdominal pain to a large metropolitan medical center.³ It can be a life-threatening condition, with mortality rates averaging about 63% across several studies.^{4,5}

One such mechanism is the production of oxygen free radicals such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and inflammatory cytokines that would surpass the natural defense mechanisms in the mucosa leading to cell damage.^{6,7} During ischemia, the hydrolysis of ATP via AMP leads to an accumulation of hypoxanthine.⁸ Increased intracellular calcium enhances the conversion of xanthine dehydrogenase (XD) to xanthine oxidase (XO). Upon reperfusion and reintroduction of oxygen, XO may produce superoxide and xanthine from hypoxanthine and oxygen.^{9, 10} Even more damaging, free radicals could conceivably be produced by the metal catalyzed Haber-Weiss reaction as follows:^{11,12}



In Iranian traditional medicine, fenugreek is commonly used for treatment of some conditions.^{13, 14} The seeds are reported to have nutritive properties and stimulating digestive processes. The seeds have been used to treat a number of gastrointestinal disorders.¹⁵

Many studies has been done on the beneficial effects of fenugreek seeds in diabetic and hypercholesterolaemic states.^{14, 16, 17, 18} In recent years, it has been stated that fenugreek seeds showed the anti-microbial,^{17, 19} anti-oxidant^{17, 19, 20, 21, 22} and anti-inflammatory properties²³. To nowdate, there is no documented data about the intestinal protective effects of fenugreek seeds in intestinal reperfusion injury. In the present study, effects of aqueous extract of fenugreek seeds in protection of the intestinal mucosa following experimental intestinal ischemia/reperfusion injury was evaluated in rats.

Materials and Methods

Plant material

Fenugreek seeds were purchased from the local market and the taxonomic identification of plant materials was confirmed by the Agricultural Research Centre of Shahid Bahonar University of Kerman and voucher specimens were deposited in the Herbarium of the School of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran (KUMS).

Preparation of the aqueous extract

The seeds were cleaned of extraneous matter, dried and were ground in to a fine powder. The powder was mixed with distilled water (1 g of seed powder per 100 ml of water). After thorough mixing in a vortex cyclomixer, the extract was centrifuged at 3000 rpm for 10 min. The supernatant was used as the aqueous extract for feeding the animals.²⁴

Animals

The experimental protocols were approved by the Research Ethic Committee of the Shahid Bahonar University of Kerman, Iran.

In this study, 20 male Wistar albino rats weighing about 280–300 g were prepared from animal house, Faculty of Medicine, Kerman University. The facility is air-condition and the temperature is maintained at 21–24°C, with controlled illumination (12-h light/dark cycle). Commercial pellet diet and water were given ad libitum. The animals were divided into four groups randomly, five animals in each group.

In group I (I/R), the animals were gavaged with normal saline (3 ml/rat) for two weeks before the commencement of surgery. On day 15, the animals were anesthetized with halothane and the abdomen was opened by a midline incision. The bowels were partly exteriorized outside the peritoneal cavity to the right side to expose the aorta. The bowels were kept warm by wet gauze with warm normal saline. The animals were kept warm at about 37°C by an overhead heating lamp during the experiments until euthanized. Intestinal ischemia was induced by clamping the aorta with a microclamp above the celiac artery. The abdomen was closed and after 45 min, the clamp was removed to reperfuse the gut, and the abdomen was closed. One hour after reperfusion, the animal's abdomen was reopened and 2 inches of proximal jejunum was removed. Finally the animals were euthanised.

In group II (I/R + extract), the animals were gavaged with the aqueous extract of *Trigonella foenum graecum* seeds (3 ml/rat). On day 15, the animals underwent abdominal operation same as group I (inducing I/R injury by clamping and declamping the abdominal aorta) and jejunum specimens were collected.

In group III (I/R + vitC), the animals were pretreated with 200 mg/kg via the left jugular vein before cross-clamping of the artery. Then, they underwent abdominal operation same as two previous groups and the jejunum samples were collected.

In group IV (sham) the animals were gavaged with the normal saline same as group I and the abdominal cavity was opened; the bowels and aorta were manipulated as in the other groups without applying clamps. The abdomen was closed and then reopened in the same time sequence as the other groups and the jejunum specimens collected at the end of the experiments.

The lumen of the intestine in all groups were immediately cleaned with phosphate buffered saline and the tissues were fixed by injecting 10% phosphate-buffered formalin through one end of the bowel using a 20-mL syringe, then intestine was engulfed in 10% phosphate-buffered formalin. The specimens were further fixed in the same fixative for 48 hours. The samples were embedded in paraffin wax afterwards. Five-micrometer serial sections of the samples were cut and stained with hematoxyline-eosin, examined in a blinded fashion under light microscope.

Tissue injury in the intestinal mucosa was graded from 0 to 5 according to the criteria described originally by Chiu et al (1970) as: G0, normal intestinal mucosal villi; G1, villous edema and vascular congestion; G2, fragmentation of tips of villi with hemorrhage; G3, fragmentation and loss of upper third of villi; G4, villi lost but crypts present; and G5, complete mucosal necrosis.^{25,26} Single-blind histopathological examination was performed by a pathologist.

Statistical analysis

The results are expressed as the mean \pm the standard error of the mean (SE). Differences were tested by Mann-Whitney U-test. P value of <0.05 was considered significant.

Results

Histopathologic findings of the sham group revealed normal intestinal mucosa (grade 0) characterized by tall villi with equal thickness and normal crypt (Fig. 1A). Significant enhancement in histopathological scores was noted in I/R group compared to sham group. I/R group animals showed severe mucosal damage, denudation of villi, complete loss of villi, and eventually complete mucosal necrosis. In this group, the most sections were graded G3 to G5 (Fig. 1D, E, and F). Fenugreek and VitC groups showed less severe lesions in compared to I/R group and the major histopathological lesions were only increasing subepithelial (Gruenhagen's) space, epithelial lifting and villi denudation that ranged G1 to G2 and G1 to G3 in VitC group and fenugreek group, respectively (Fig. 1B, and C). Significant decreases in histopathological scores were noted in VitC and fenugreek groups in compared to I/R group ($p<0.05$). There were significant differences in histopathological scores of sham group compared to VitC and fenugreek groups ($p<0.05$). There was no significant difference between the VitC and fenugreek groups ($p>0.05$). Histopathological scores are summarized in Table 1.

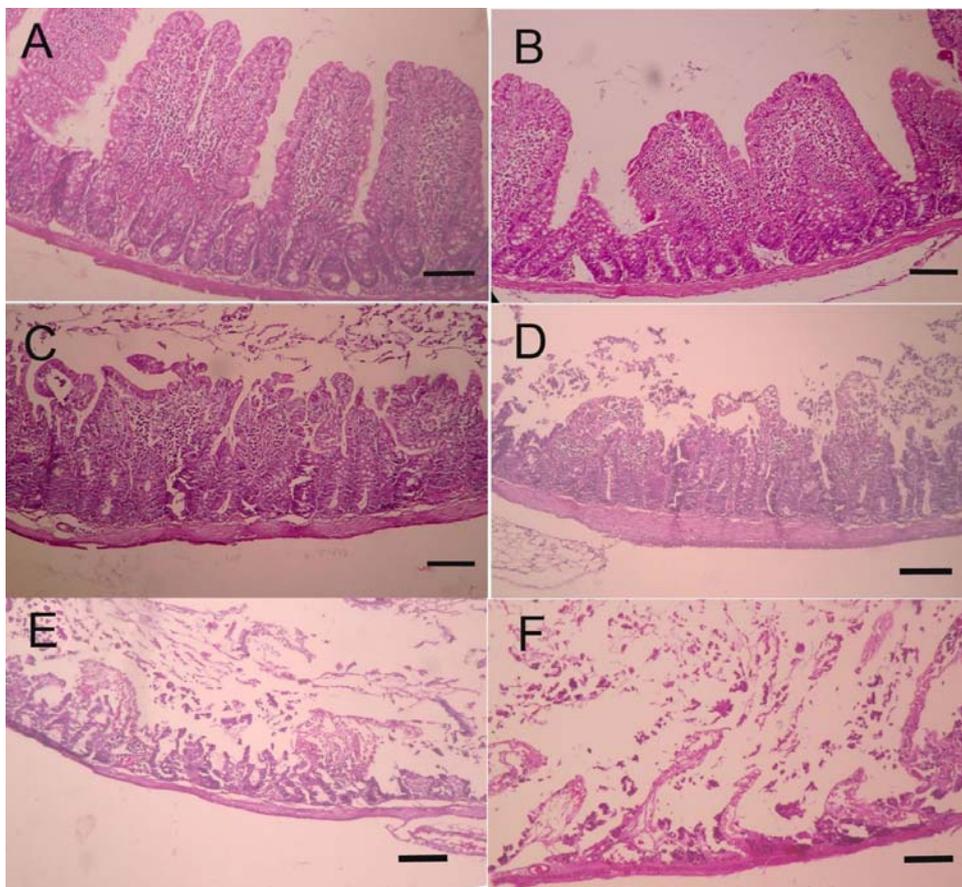


Figure 1: This photomicrograph shows histopathologic scores in intestinal injury. (A) Grade 0: normal intestinal mucosal villi. (B) Grade 1: villous edema and increasing subepithelial space. (C) Grade 2: necrosis of tips of intestinal villi. (D) Grade 3: fragmentation and loss of upper third of villi. (E) Grade 4: loss of complete villi and crypts. (F) Grade 5: complete mucosal necrosis. H&E. Bar=100µm

Table 1. Comparison of histopatological scores in all groups*

Group	Score (mean ± SE)
I	3.8 ± 0.84 ^a
II	1.8 ± 0.84 ^b
III	1.6 ± 0.55 ^b
IV	0 ± 0 ^c

* Data are expressed as mean ± SE; Different alphabetic letters show significant differences between the groups (p<0.05). I: I/R; II: I/R + fenugreek; III: I/R + vitamin C; IV: sham control

Discussion

Under physiological conditions, damaging effects of reactive oxygen species (ROS) are prevented by endogenous antioxidant enzymes such as SOD, which rapidly reduce superoxide radicals.²⁷ Ischemia and consecutive reperfusion causes oxidative stress, which is characterized by an imbalance between reactive oxygen species (ROS) and the anti-oxidative defence systems.⁶ Reperfusion of ischemic tissue, although is necessary for a compensatory mechanism, has been shown to worsen ischemic damage via the release of ROS.^{27, 28, 29, 30, 31} The intestine is a rich source of the xanthine dehydrogenase-oxidase enzyme system. An important factor that accelerates mucosal injury after reperfusion is the production of ROS derived from xanthine oxidase metabolism, and activated neutrophils.^{9, 10, 12} Hence, the intestinal mucosa is extremely sensitive to reactive oxygen species.^{30, 31} Neutrophils induce tissue injury through the production and release of ROS and cytotoxic proteins, such as proteases, MPO, and lactoferrin, into the extracellular fluid. These initiate inflammatory cascades that trigger the radical-induced I/R injury.^{27, 29, 32, 33} Free radical scavengers have been demonstrated to reduce intestinal I/R damages and can be beneficial for decreasing the pathophysiology related to I/R.³⁴

Fenugreek contains high concentrations of flavonoids and polyphenolic compounds such as dihydroxy phenylalanine (which increase antioxidant activity). These compounds are very important because of their antioxidant activities that are mainly due to their redox properties which play an important role as free radicals scavengers.^{18, 19, 20, 21, 23} The present study is the first investigation to demonstrate the effects of fenugreek in preventing I/R-induced intestinal injury.

Given the fact that histological assessment made using a microscopic scoring system has been accepted as a good standard method in the evaluation of I/R injury in the intestinal tissues.²⁶

The changes of the intestinal mucosa induced by I/R are directly related to the length of ischemia and reperfusion.^{27, 31, 35} On histopathological analysis, the intestinal tissue injury significantly decreased in the fenugreek-treated ischemic group, compared to the ischemic group (I/R). After 1 h of reperfusion, increases in the histological scores of the tissue lesions were observed in the I/R group, which is in agreement with the findings of other studies.^{28, 32, 33}

Histopathological grading of the small intestine has been used for evaluating determine intestinal IR injury in animal model studies of intestinal I/R. Chiu's classification for histological damage Were used in this study.^{25, 26, 30} Application of two test substances i.e. fenugreek and vitamin C, was significantly (p<0.01) effective in reducing the detrimental

effects of I/R. The results of present study showed that the application of fenugreek seeds extract was less effective than vitamin C; however, there was no significant difference in histopathological changes between two groups ($p > 0.01$). The intestinal mucosal integrity with fenugreek remained nearly to the normal group in this study. This conforms to the findings of others who used different antioxidant agents in the same animal model.^{25, 28, 30, 31, 32, 33, 35}

Histological damages which occurs after intestinal I/R injury, provides evidence of the high vulnerability of the small intestines. The shortening of the villous length and crypt depth, connected with denudation of the villi and disintegration of the villous tissue observed in our study and confirms previous studies.²⁸

The histopathological findings showed that the injury scores in the mucosa 1 h after reperfusion was significantly lower in the fenugreek group than in the I/R group and suggested that the small intestinal mucosa in the fenugreek and VitC group was better preserved than in the I/R group. In the current study, we demonstrated that I/R in the aorta artery is associated with significant histopathological damages in the mucosa of the jejunum. Yasar, et al. (2010) reported that doxycycline was associated with improved recovery from I/R injury through attenuating the response of cytokines after 1-hour reperfusion period.³¹ Sato, et al. (2011) showed that lutein, an antioxidant, significantly reduced oxidative injury.³⁵ Appropriate accumulation of antioxidants in tissues probably leads to protective effects against oxidative injury.

In conclusion, we have demonstrated that administration of the *Trigonella foenum graecum* (fenugreek) seeds extract protected the intestinal mucosa from ischemic damage induced by 45 min of transient intestinal ischemia.

Acknowledgement

This study was supported financially by the Research Council of Veterinary College, Shahid Bahonar University of Kerman.

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اثر محافظتی عصاره دانه شنبليله بر روی آسیب ناشی از ایسکمی-ریپرفیوژن تجربی در روده موش صحرائی

رضا خیراندیش^{*}، امید آذری^۱، حامد صمدیه^۲، زهره رسا^۳

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

هدف- ایسکمی-ریپرفیوژن می تواند اثرات پاتولوژیک وسیعی بر روی بافت روده داشته باشد. مهمترین استراتژی در برخورد با چنین رویدادی ممانعت از تولید رادیکال های آزاد با استفاده از تجویز آنتی اکسیدان ها است. بنابراین هدف از مطالعه حاضر بررسی تاثیر عصاره دانه شنبليله که دارای خواص آنتی اکسیدانی است بر تغییرات هیستوپاتولوژیک مربوط به ایسکمی-ریپرفیوژن تجربی در روده موش صحرائی نر می باشد.

طرح مطالعه - مطالعه تجربی.

حیوانات - بیست موش نر از نژاد ویستار آلبینو.

روش کار- در این مطالعه موش های صحرائی به چهار گروه تقسیم شدند. در گروه اول ایسکمی-ریپرفیوژن تجربی روده ایجاد شد (به مدت ۴۵ دقیقه ایسکمی و سپس یک ساعت خونرسانی مجدد). گروه دوم به مدت دو هفته عصاره دانه شنبليله از طریق گاواژ دریافت نمودند و سپس ایسکمی ریپرفیوژن تجربی ایجاد گردید. گروه سوم قبل از ایجاد ایسکمی-ریپرفیوژن تجربی ویتامین C دریافت نمودند. گروه چهارم به عنوان شم در نظر گرفته شد. سپس تمامی موش های صحرائی به روش انسانی کشته و از ژوژنوم نمونه های بافتی تهیه گردید.

نتایج- مخاط روده در گروه اول ضایعات ایسکمیک شدیدی را نشان داد. مخاط روده در گروه های دوم و سوم در مقابل آسیب های ناشی از ایسکمی ریپرفیوژن نسبت به گروه اول بهتر حفظ گردیده بود. کاهش معنی دار درجه آسیب بافتی در گروه های دوم و سوم نسبت به گروه اول وجود داشت ($P < 0.05$). درجه آسیب بافتی بین گروه های دوم و سوم تفاوت معنی داری را نشان نداد ($P > 0.05$).

نتیجه گیری- نتایج از این مطالعه به وضوح نشان می دهد که عصاره دانه شنبليله قادر به کاهش آسیب های ناشی از ایسکمی-ریپرفیوژن تجربی در روده موش صحرائی می باشد.

کلید واژگان - ایسکمی-ریپرفیوژن، شنبليله، روده، موش صحرائی

