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Original Article

Antiulcer and hepatoprotective effects of aqueous extract of Plantago ovata seed on indomethacin-ulcerated rats

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ABSTRACT

Background: The objective of the study was to investigate the protective effects of aqueous extract of Plantago ovata seed (AEPOS) on peptic ulcer induced by indomethacin (IND) in rats.
Methods: Rats (250-300 g) were divided into three groups (5 rats in each group). Gastric ulcer was induced by a single oral gavage of 48 mg/kg IND. The first group received only 5% sodium bicarbonate orally (5 ml/kg) whereas the control (IND) group received only single oral dose of 48 mg/kg IND. The third group was pretreated with an extract (100 mg/kg) for 4 days. At the end of the 4th day, rats were kept fasted for 24 h before administration of IND 48 mg/kg. The rats were sacrificed 4 h after oral administration of IND and their stomach and liver were fixed in formalin (10%) and sections of 5 mm in diameter were prepared. Histological and morphological characteristics of stomach and liver were assessed using hematoxylin and eosin (H&E) staining.
Results: AEPOS (100 mg/kg) showed a significant ($p < 0.05$) reduction in microscopic and macroscopic ulcer index as compared to the IND group. Histological analysis indicated that AEPOS has hepatoprotective effect and can prevent mucosa damage in stomach.
Conclusion: Results revealed that AEPOS has anti-ulcer and hepatoprotective effects.

Nutrition supplements such as fibers have the potential to offer health benefits and to reduce the risk of developing coronary heart disease, stroke, hypertension, diabetes, obesity and certain gastrointestinal disorders [1]. Peptic ulcer is a gastrointestinal disorder resulting from the loss of the balance between aggressive and defensive factors of the gastric mucosa [2]. It is known that an increase in oxidative stress is linked to the aggressive factor-induced gastric mucosal damage [3]. Indomethacin is one the non-steroidal anti-inflammatory drugs (NSAIDs) with anti-inflammatory, anti-pyretic, and pain-relieving properties, and is known to produce erosions, ulcerative lesions and petechial bleeding in the stomach as its serious side effects [4]. Furthermore, development of the gastric mucosal lesions induced by

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At a glance commentary

Scientific background on the subject

Psyllium husk comes from the crushed seeds of the Plantago ovata plant, an herb native to parts of Asia, the Mediterranean, and North Africa. Psyllium is very rich in soluble fiber. In Iranian traditional medicine, *Plantago ovata* seed is introduced as a valuable remedy for gastrointestinal disorders such as diarrhea, ulcerative colitis hemorrhoids, constipation.

What this study adds to the field

In this research, we scientifically investigated the protective effects of *Plantago ovata* aqueous extract against peptic ulcer induced by indomethacin gastric ulcer in rats. Also we showed that aqueous *Plantago ovata* seed extract can hepatoprotective effect that these benefit effects may be related to mucilage properties of the seed.

indomethacin is mainly mediated by generation of oxygen free radicals [5] and depletion of endogenous prostaglandins via inhibition of the cyclooxygenase enzyme [6]. In recent years, a tendency to using herbal medicine has been increased people have recognized that many cultivated or wild plants. These products have less toxic effects than synthetic drugs and are a good source for novel therapeutic agents [7]. Plantago is the most important genus of Plantaginaceae family and long has been used in the traditional medicine for the treatment of wounds, bronchitis, haemorrhagia, cystitis with hematuria and diarrhea [8]. The husk and seed of *Plantago ovata* is commonly named psyllium that is widely used as a fiber supplement for the treatment of constipation [9]. Psyllium is classified as a mucilaginous fiber due to its powerful ability to form a gel in water. Clinical studies indicate that aqueous extract of Plantago ovate seed (AEPOS) can increase stool weight and reduce intestinal transit time in constipated patients [10,11]. It is also shown that psyllium can reduce glucose, total cholesterol and LDL levels in men with type2 diabetes [12]. Recent findings suggest that P. ovata husk has protective effect on intestinal mucosa against acetylsalicylic acid that is probably due to a reduction in the absorption of acetylsalicylic acid into epithelial cells [13]. Thus, the aim of this study was to evaluate the protective effect of the soluble fiber of P. ovata against stomach and liver histopathological damages induced by indomethacin for 4 days in rats.

Materials and methods

Animals

Male albino rats (250-300 g) 6e8 weeks old and bred in animal house of Medical School of Shahid Sadoughi University of Medical Sciences were selected. Animals were housed at a controlled temperature (22 ± 2 °C) with a 12 h-light/dark cycle and standard lab chow and tap water ad libitum. The experiments reported in this study were carried out in accordance with the current ethical guidelines for the investigation of experimental animals [14].

Experimental design

To evaluate protective effect of the AEPOS, gastric ulcers were induced by a single oral dose of 48 mg/kg IND [5]. First group received only 5% sodium bicarbonate orally 5 ml/kg whereas the control (IND) group received only single oral dose of 48 mg/kg IND. Remaining group was pretreated with an extract (100 mg/kg) 4 days before induction of gastric ulcers. Rats were sacrificed 4 h after the oral administration of IND and their stomachs and livers were immediately removed for quantifying the lesions and histopathological analysis.

Histological studies

Stomach and liver tissues were removed and fixed in 10% neutral formalin and embedded in paraffin. 5-mm thick sections were prepared and stained with hematoxylin and eosin by standard procedures.

Assessment of gastric mucosal lesions

Long lesions were counted and measured along their greater length and petechial lesions were also counted. All five petechial lesions were taken as 1-mm of ulcer [15]. The sum of the total length long ulcers and petechial lesions in each group of rats was divided by its number to calculate the ulcer index (mm). Then, macroscopic and microscopic ulcer indexes and morphological characteristics of gastric mucosa were evaluated and a mean index was calculated. A mean microscopic ulcer index was calculated by formula [16].

Normal tissue 3 0.
Local damage to gastric pits cells 3 1.
Local damage to gastric glands 3 2.
Deep damage to gastric glands 3 3.
Microscopic ulcer index 3 (number of lesion 1)t(number of lesion 2) _ 2t(number of lesion3) _ 3.

Data analysis

All data are expressed as the mean ± standard error of the mean (S.E.M.). Graph pad prism 5 was used for data analysis. Statistical significant differences were determined using one-way ANOVA with the Tukey Kramer post-test for multiple comparisons. The values of $p < 0.05$ were regarded as statistically significant.

Results

Effect of AEPOS on macroscopic and microscopic ulcer indexes

Indomethacin induced gastric damage showed marked gross mucosal lesion, including long hemorrhage bands and petechial lesions. The results indicated that macroscopic and

microscopic ulcer indexes were significantly reduced in animals pretreated with AEPOS compared to indomethacin control group (Figs. 1 and 2, $p < 0.01$).

Histopathological findings of gastric tissue

The histopathological findings of the gastric specimens are shown in Fig. 3. The gastric mucosa of the sodium bicarbonate group demonstrated normal tissue and structure (Fig. 3A). The stomachs of the indomethacin-treated rats showed deep damage to gastric glands, mucosal hemorrhage, extensive edema and leucocytes infiltration of the submucosal layer. In this group, venule expansion in lamina propria was also seen, however muscularis mucosa was undamaged (Fig. 3B). Rats pretreated with AEPOS resulted in the maintenance of glandular organization and cellular architecture with no pathological changes (Fig. 3C).

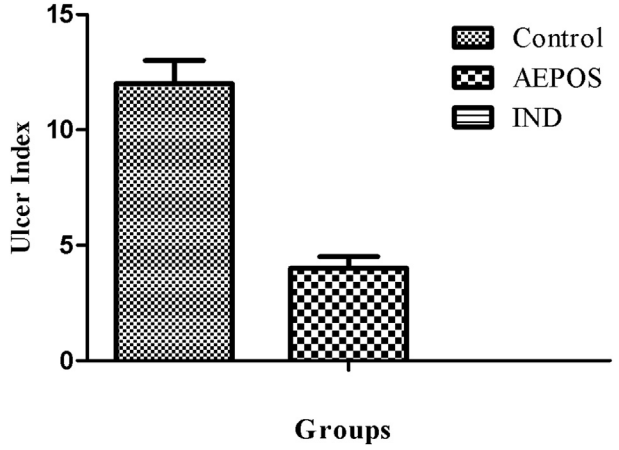

Fig. 1 Effect of AEPOS on macroscopic gastric ulcer index. Data expressed as mean ± SEM; *$p < 0.05$ compared with control group. Abbreviations used: AEPOS: aqueous extract of Plantago ovata seed; IND: indomethacin group.

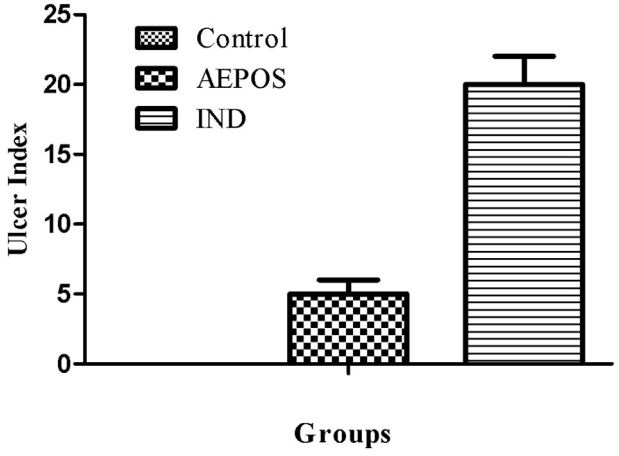

Fig. 2 Effect of AEPOS on microscopic gastric ulcer index. Data expressed as mean ± SEM; *$p < 0.01$ compared with indomethacin. Abbreviations used: AEPOS: aqueous extract of Plantago ovata seed; IND: Indomethacin.

Histopathological findings of the liver

The liver sections of the rats in sodium bicarbonate group revealed normal hepatic architecture and normal hepatocytes with prominent nucleus, central vein and portal areas with no signs of inflammation or necrosis (Fig. 4A). In indomethacin treated rats, hepatocytes were larger than normal size in comparison with the control group. Hypotrophied kupffer's cells were prominent and dilated hepatic sinusoids as well as blood vessels expansion in portal area were noticed. Leucocytes infiltration in hepatic sinusoids and portal vein were observed. In some parts, the general structures of the liver were destroyed (Fig. 4B). In pretreated rat with AEPOS, no signs of prominent pathological changes were identified. Leucocytes infiltration in hepatic sinusoids and portal vein were noticed. However, the general structures of lobules of liver were normal (Fig. 4C).

Discussion

The findings of present study demonstrated that AEPOS significantly decrease macroscopic and microscopic ulcer indexes in treated animals compared to control group. Histopathological studies have showed that the rats pretreated with AEPOS could be protected from adverse effects of indomethacin. The stomach showed normal glandular organization and cellular architecture. The AEPOS also protected the liver from damage and the tissue maintained normal architecture. Among the different models of experimentally induced gastrointestinal lesions, the NSAID models are frequently used [17]. Seed and husk of Plantago ovata are traditionally used as fiber supplements for the treatment of constipation and some other gastrointestinal disorders [9]. The aqueous extract of *P. ovata* seed contains a high proportion of hemicellulose, composed of anionic polysaccharide of L-arabinose, D-xylose and D-galactoronic acid [18]. The actual seed has a higher amount of fermentable fiber that is effective in preventing constipation, diarrhea, Crohn's disease, obesity, hypercholesterolemia, diabetes and atherosclerosis [13]. Because of slowed grades of this fiber, psyllium seed produces large amounts of butyrate and acetate. Butyric acid exhibits antineoplastic activity against colorectal cancer and may be helpful in the treatment of ulcerative colitis [19]. Several studies have suggested that a high fiber intake, especially soluble fibers, would have a mucosa-protective action, reducing the risk or promoting a faster healing of duodenal ulcers [13]. Satoh et al. have shown that diet supplementation with soluble fibers protects the small intestine against NSAID induced damage, but this study has been carried out over a short period of time [20]. Moreover, viscosity is also associated with prolonged gastric emptying, increasing the retention of indomethacin in stomach and, consequently, delaying the access of this drug to gastric mucosa [13]. In this study we also investigated hepatoprotective effect of AEPOS on histological and morphological property of the liver. It is demonstrated that indomethacin induces degenerative changes and cell necrosis in the liver's cells and decreases hepatic microsomal cytochrome P-450 and prostaglandin [21]. Our histopathological results showed that in the rat pretreated with AEPOS, gastric pathological changes

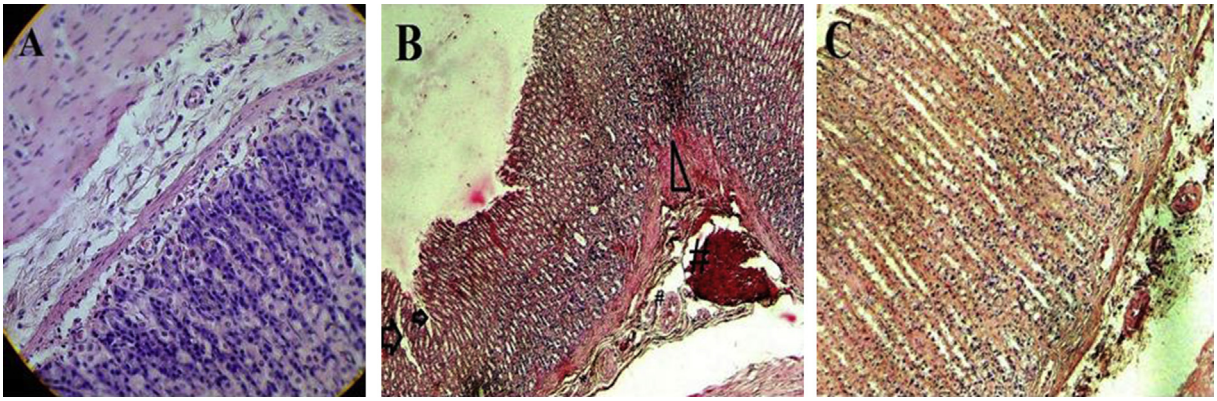

Fig. 3 Histologic evaluation of gastric mucosal lesions of the rats in different groups. H&E × 100. (A) In sodium bicarbonate group, normal structures of stomach tissue were seen. (B) Deep damage to gastric glands, sub-mucosal edema, venule expansion in lamina propria and leucocytes infiltration in the sub-mucosal layer were seen in indomethacin group. (C) In stomach of rats pretreated with AEPOS, formation of gastric lesions was reduced. Intact mucosa and muscularis mucose were seen.

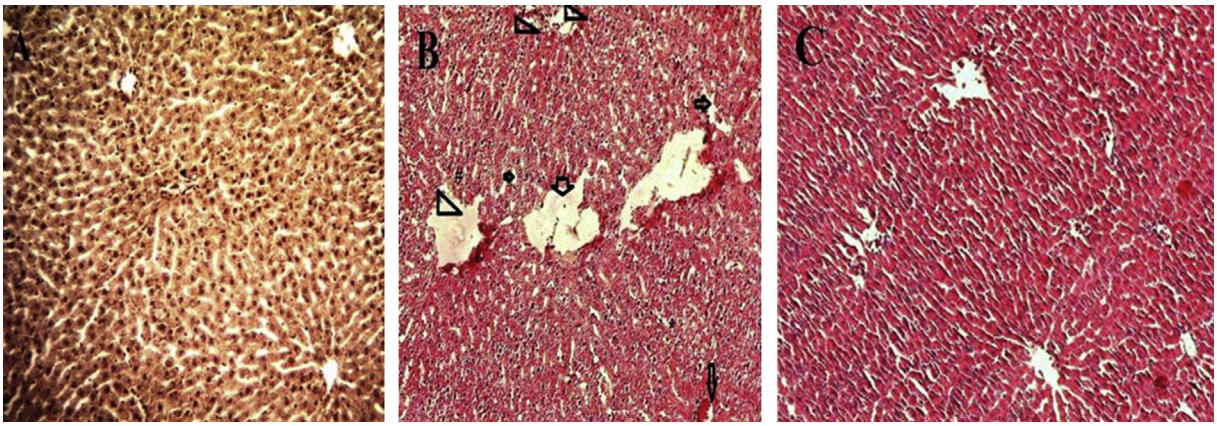

Fig. 4 Histologic evaluation of liver tissue of the rats in different groups. H&E × 100.The liver sections of sodium bicarbonate group showed normal appearance and there were no changes in central vein and portal areas with no signs of inflammation or necrosis (A). In indomethacin treated rats, dilated expansion of hepatic sinusoids (arrow) and blood vessels in portal area (arrow head) and leucocytes infiltration (*) in these parts were noticed. Mild periportal hepatic necrosis was also seen (#) (B). In AEPOS pretreated rats, liver tissue showed normal structures and there were no changes in central vein and portal areas with no sign of necrosis (C).

such as leucocytes infiltration dose not occur and the tissue maintains normal its architecture. These beneficial effects may be related to a highly hydrosoluble fiber of AEPOS that makes markedly viscous solutions and increases the thickness of the gastrointestinal diffusion barrier. This effect could help to maintain the morphology and functional integrity and morphologies of the liver and the stomach by preventing indomethacin penetration and trapping in the gastro mucosa layer.

Conclusions

According to the results obtained in this study, we can conclude that AEPOS could prevent from gastrointestinal lesions and liver damages. This could be due to the solubility and viscosity of the fiber or due to a reduction in the absorption of indomethacin. Further studies should be conducted to determine the active constituent of AEPOS that gives anti-ulcer and hepatoprotective properties to this extract.

Conflicts of interest

We certify that there are no actual or potential conflicts of interest related to this article exist.

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