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Effects of fish oil on ethanol-induced gastric ulcer in rats: inflammatory responses and oxidative stress

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Background: The prevalence of peptic ulcers is increasing due to lifestyle changes and harmful diets.
Objective: The aim of this study was to investigate the effect of fish oil (FO) on gastric ulcers induced by ethanol in rats.
Methods: The pharmacological efficacy of FO with doses of 5 and 10 mg/kg investigated using the gastric ulcer index, the acidity of gastric secretions, pro-inflammatory cytokine assessment, and oxidative stress examination.
Results: Ethanol-induced gastric ulcer improves with FO 5 or 10 mg/kg pretreatment ($P < 0.05$). FO did have acid-neutralizing activity. FO also increased the levels of glutathione and catalase and decreased the malondialdehyde levels ($P < 0.05$). Moreover, FO reduced the levels of tumour necrosis factor alpha (TNF-α) interleukin-6 (IL-6), through downregulation of nuclear factor kappa B (NF-κB) ($P < 0.05$). Pretreatment with FO attenuates ethanol-induced gastric ulceration.
Conclusion: The observed effects may be due to the role of FO in regulating gastric secretions, changes in the expression of NF-κB, and changes in the levels of oxidative stress factors.

Keywords: fish oil, gastric ulcer, ethanol

Introduction

Fish oil (FO) is rich in n-3 polyunsaturated fatty acids (PUFA), specifically docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). FO has been shown to have several health benefits, including improving symptoms of Crohn's disease, ulcerative colitis, rheumatoid arthritis, lupus erythematosus, psoriasis, multiple sclerosis, and migraine[1,2]. polyunsaturated fatty acids increased glutathione peroxidase (GPx) activity, total antioxidant capacity (TAC) and decreased malondialdehyde (MDA)[3]. Also, FO supplementation reduces have important roles as mediators and regulators of inflammation, so it has a high anti-inflammatory potential by reducing defensive inflammatory responses[4]. Most chemical drugs used to treat various diseases in humans have adverse side effects. As a result, there is an increasing interest in studying natural substances derived from animals and plants, and their impact on living organisms both in vitro and in vivo. The aim is to find safer alternatives to chemical drugs[5].

HIGHLIGHTS

- Ethanol-induced gastric ulcer improves with fish oil 5 or 10 mg/kg pretreatment.
- Fish oil did have acid-neutralizing activity.
- Fish oil also increased the levels of glutathione and catalase and decreased the malondialdehyde levels.
- Fish oil reduced the levels of tumour necrosis factor alpha interleukin-6.
- Pretreatment with fish oil attenuates ethanol-induced gastric ulceration.

Gastric ulcers (GUs) and duodenal ulcers (DUs) are common diseases of the human gastrointestinal tract[6]. The causes of GUs and DUs are controversial; however, they are thought to result from an imbalance between gastric mucosal invasive factors and gastric mucosal protective factors, which together lead to the breakdown of the protective mucosal barrier and ulcer formation[7,8]. The mucosal lining is often exposed to aggressive agents such as alcohol, NSAIDs, antibiotics, cigarette smoke, and chemical irritants; these factors can cause GUs due to the destruction of the protective mucous barrier[9,10]. Therefore, the treatment plan for anti-ulcer treatment includes increasing the protective barriers or reducing the aggressive factors of the gastric mucosa is essential[11].

Ethanol-induced gastric ulcer model in animals is used to study acute gastritis[12]. The pathophysiological changes caused by gastric ulcer in laboratory animals are similar to the changes observed in humans[13]. The accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) induces oxidative stress and inflammation in the gastric tissue, resulting in damage to the gastric mucosa[14–16]. The accumulation of ROS/RNS causes the oxidation of lipids and proteins. Therefore, the permeability of the intestinal mucosa increases. Also, macrophages stimulated and the release of inflammatory cytokines [interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α)]

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increases the activity of the NF-kB signalling pathway[14]. The objective of this study was to determine the gastro-protective effects of fish oil on ethanol-induced gastric ulcers through inflammatory responses and oxidative stress in rats.

Methods

Animals

The protocol of this study approved by the ethics committee of the place where it was conducted (Ethics number: IR.IUA.BEHBAHAN.REC.1401.024). All stages of this study has been reported in line with the ARRIVE criteria[17]. Wistar male rats (250 ± 10 g, age 6–8 weeks) randomly divided into seven groups ($n = 6$) under optimal conditions of 12 h of light and 12 h of darkness.

The first group received a placebo treatment where they were given sunflower oil as a drug carrier for 14 days, followed by oral administration of distilled water on the 14th day. The second and third groups were given FO (Sigma-Aldrich, USA, Product Number: PHR2979) 5 mg/kg and FO 10 mg/kg, respectively, dissolved in sunflower oil via gavage for 14 days. On the 14th day, they were given distilled water orally. The fourth group received sunflower oil as a drug carrier for 14 days, followed by oral administration of ethanol (Merck, Germany) (1 ml per 200 g of body weight) on the 14th day. The fifth and sixth groups were given FO 5 mg/kg and FO 10 mg/kg, respectively, dissolved in sunflower oil via gavage for 14 days. On the 14th day, they were given ethanol orally. The seventh group was given omeprazole (Omp) (Abidi Pharmaceuticals, Iran) 20 mg/kg via gavage for 14 days, and on the 14th day, they were given ethanol orally.

Stomach injury model

The induction of ulcer was achieved by oral administration of absolute ethanol at a dose of 1 ml/200 g body weight[18]. Twenty-four hours before ethanol administration, animals were food-restricted but had access to water. Ninety minutes after gastric ulcer induction, the animals euthanized under inhalation of CO_2.

The pH of stomach contents

The end of the oesophagus and the beginning of the duodenum were closed and the stomach was removed quickly. Stomach contents were collected with 5 ml of saline and then centrifuged. The pH of the supernatant was recorded with a pH metre.

Mean ulcer index

The severity of gastric mucosal erosions was assessed and scored using the ulcer score scale developed by Zhang *et al.*[19] (0): No erosion, (1): Pinpoint erosions, (2): Ulcer erosion size less than 1 mm, (4): Ulcer erosion between 1 and 5 small erosions, and (6): Ulcer erosion size greater than 2 mm. To calculate the percentage of inhibition of gastric erosion, the following formula was used: $[(UIcontrol - UItreated)/UIcontrol] \times 100$, where UI represents the ulcer index.

Assessment of biochemical changes

In this study, the levels of glutathione (GSH), catalase (CAT), and MDA were measured in the antrum region of the stomach. Tissue samples weighing 100 mg were taken and homogenized using a homogenizer with 500 microliters of RIPA buffer pH = 8. To prevent protein degradation, 10 µl of protease inhibitor mixture (Sigma, USA) was added to each ml of homogenizing buffer. The homogenized sample was then centrifuged for 15 min at 10 000 rpm in a four °C centrifuge. The supernatant was collected and stored in a −20°C freezer until the desired factors were measured. The amount of protein in the samples was measured using the Bradford method. The levels of GSH, CAT, and MDA were measured with an Elisa kit following the manufacturer's instructions (Kiazist, Hamedan, Iran).

Real-time PCR

The mRNA was extracted using an RNA Extraction Kit (Parstous, Iran). To convert RNA to cDNA, a cDNA synthesis kit (Parstos, Iran) was used. The PCR primers from Zistakhmir (Iran) used in this study are as follows: forward strand GAPDH 5′- AGTTCAACGGCACAGTCAAG - 3′, reverse strand GAPDH 5′- TACTCAGCACCAGCATCACC - 3′; forward strand NF-kB 5′- TCAACATGGCAGACGACGAT-3′, reverse strand NF-kB 5′-AATTAGGTGACCCTGTCGCT-3′. Real-time PCR was performed using real-time PCR Master Mix (SYBR Green - Parstos, Iran).

Assessment of pro-inflammatory cytokines

The ELISA kit was used to measure levels of IL-6 and TNF-α in gastric tissue, as per instructions from Red Biotechnology (Kiazist, Hamedan, Iran).

Statistical analysis

Data were analyzed using SPSS software (version 16). One-way ANOVA was followed by Tukey comparison tests. A P value of less than 5% was considered significant.

Results

Our study results indicate that the pH levels of gastric juice in the groups receiving FO 5 mg/kg and FO 10 mg/kg were not sig-

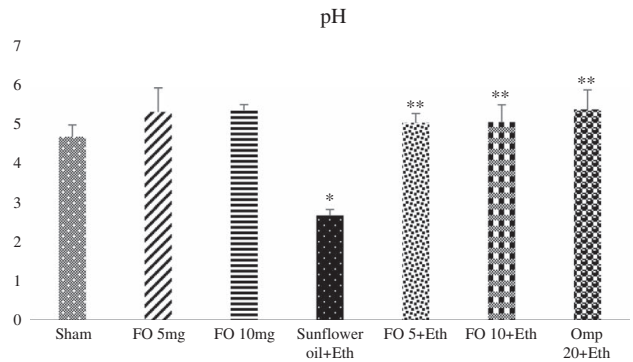

Figure 1. The pH of gastric juice in different study groups. The first group (Sham) received a placebo, the second and third groups were given fish oil (FO) 5 and FO 10 mg/kg, respectively, the fourth group was given ethanol (Eth), and the fifth and sixth groups were given FO 5 and FO 10 mg/kg, respectively, followed by ethanol. The seventh group was given omeprazole (Omp) 20 mg/kg followed by ethanol. A P value lower of 5% was considered statistically significant. *Significant difference between Sunflower oil + ethanol group and sham group. **Significant difference between FO 5 + ethanol, FO 10 + ethanol and Omp 20 + ethanol groups and Sunflower oil + ethanol.

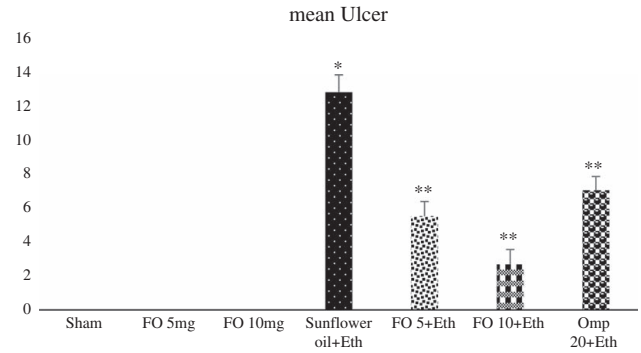

Figure 2. FO pretreatment effects on gastric ulcer scores in ethanol-induced ulcers in rats. The first group (Sham) received a placebo, the second and third groups were given fish oil (FO) 5 and FO 10 mg/kg, respectively, the fourth group was given ethanol (Eth), and the fifth and sixth groups were given FO 5 and FO 10 mg/kg, respectively, followed by ethanol. The seventh group was given omeprazole (Omp) 20 mg/kg followed by ethanol. A *P* value lower of 5% was considered statistically significant. *Significant difference between Sunflower oil + ethanol group and sham group. **Significant difference between FO 5 + ethanol, FO 10 + ethanol and Omp 20 + ethanol groups and Sunflower oil + ethanol.

nificantly different from those of the sham group. However, the pH levels of the gastric juice in the sham group were higher compared to the sunflower oil + Ethanol group ($P < 0.05$). Moreover, in the control group, the pH levels of gastric juice were lower than those in groups receiving FO 5 + Ethanol, FO 10 + Ethanol, and omeprazole 20 ($P < 0.05$) (as shown in Fig. 1).

Ethanol administration caused numerous gastric epithelium lesions along the long axis of the stomach compared to the sham group ($P < 0.05$). However, pretreatment with FO 5 and 10 mg/kg significantly reduced the number of gastric lesions, comparable to the ethanol group ($P < 0.05$) (Figs. 2 and 3).

The levels of MDA, CAT, and GSH in the FO 5 mg/kg and FO 10 mg/kg groups were not significantly different from the sham group. However, the levels of CAT and GSH were found to be higher in the sham group compared to the sunflower oil + Ethanol group ($P < 0.05$). Additionally, the levels of CAT and GSH in the control group showed a significant difference from the omeprazole (20 mg/kg) group ($P < 0.05$). The levels of GSH and CAT in the FO 5 + Ethanol and FO 10 + Ethanol groups were found to be higher than that of the sunflower oil + Ethanol group ($P < 0.05$). On the other hand, the levels of MDA decreased in the sham group compared to the sunflower oil + Ethanol group ($P < 0.05$). The levels of MDA in FO 5 + Ethanol, FO 10 + Ethanol, and omeprazole (20 mg/kg) groups were found to be significantly lower than the sunflower oil + Ethanol group ($P < 0.05$) (refer to Figs. 4–6 for details).

The study found that the expression of NF-kB in the FO 5 mg/kg and FO 10 mg/kg groups was not significantly different from the sham group. However, in the sunflower oil + Ethanol group, it was significantly elevated compared to the sham group ($P < 0.05$). On the other hand, the FO 5 + Ethanol, FO 10 + Ethanol, and omeprazole 20 mg/kg groups showed a lower expression of NF-kB than the sunflower oil + Ethanol group ($P < 0.05$) (as shown in Fig. 7).

The levels of IL-6 and TNF-α in the groups that received FO 5 mg/kg and FO 10 mg/kg were similar to the levels observed in the sham group. However, the sunflower oil + Ethanol

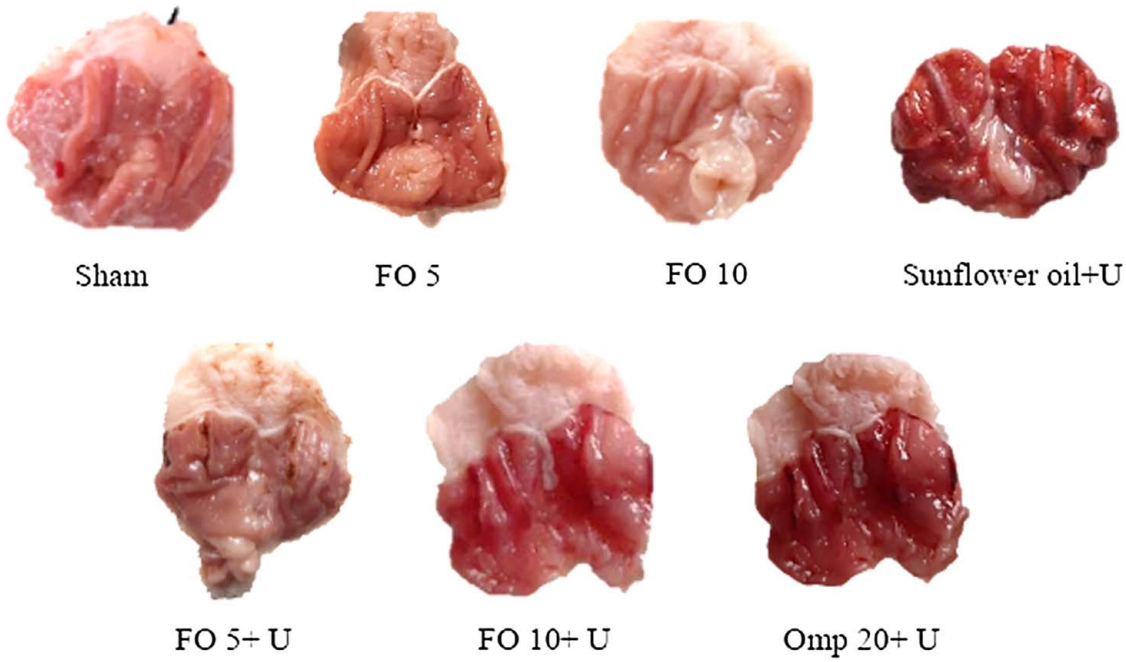

Figure 3. Gross macroscopic appearance of the gastric mucosa. The first group (Sham) received a placebo, the second and third groups were given fish oil (FO) 5 and FO 10 mg/kg, respectively, the fourth group was given ethanol (Eth), and the fifth and sixth groups were given FO 5 and FO 10 mg/kg, respectively, followed by ethanol. The seventh group was given omeprazole (Omp) 20 mg/kg followed by ethanol. A *P* value lower of 5% was considered statistically significant. The control group had a higher MUI compared to FO 5 + Ethanol and FO 10 + Ethanol groups, while the omeprazole 20 group had a lower MUI compared to the sunflower oil + Ethanol group.

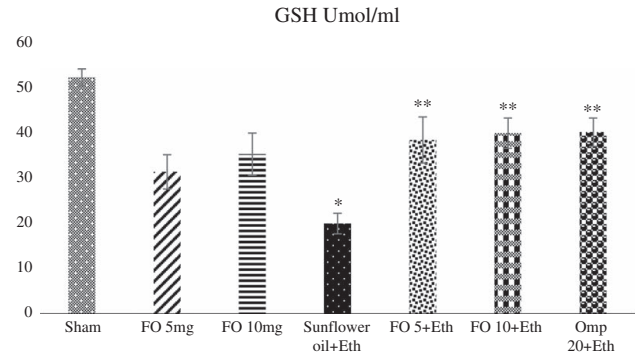

Figure 4. Fish oil (FO) effects on ethanol-produced changes on the levels of glutathione (GSH). The first group (Sham) received a placebo, the second and third groups were given FO 5 and FO 10 mg/kg, respectively, the fourth group was given ethanol (Eth), and the fifth and sixth groups were given FO 5 and FO 10 mg/kg, respectively, followed by ethanol. The seventh group was given omeprazole (Omp) 20 mg/kg followed by ethanol. A *P* value lower of 5% was considered statistically significant. *Significant difference between Sunflower oil + ethanol group and sham group. **Significant difference between FO 5 + ethanol, FO 10 + ethanol and Omp 20 + ethanol groups and Sunflower oil + ethanol.

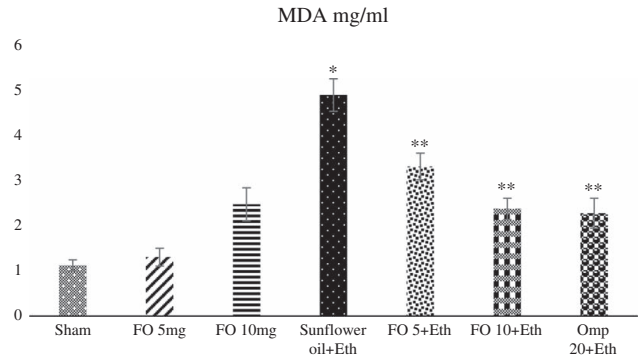

Figure 6. Fish oil (FO) effects on ethanol-produced changes on the levels of malondialdehyde (MDA). The first group (Sham) received a placebo, the second and third groups were given FO 5 and FO 10 mg/kg, respectively, the fourth group was given ethanol (Eth), and the fifth and sixth groups were given FO 5 and FO 10 mg/kg, respectively, followed by ethanol. The seventh group was given omeprazole (Omp) 20 mg/kg followed by ethanol. A *P* value lower of 5% was considered statistically significant. *Significant difference between Sunflower oil + ethanol group and sham group. **Significant difference between FO 5 + ethanol, FO 10 + ethanol and Omp 20 + ethanol groups and Sunflower oil + ethanol.

group showed significantly elevated levels of IL-6 and TNF-α compared to the sham group ($P < 0.05$). On the other hand, the groups that received FO 5 mg/kg and FO 10 mg/kg with ethanol, as well as omeprazole 20 mg/kg group, exhibited lower levels of IL-6 and TNF-α compared to the sunflower oil + Ethanol group ($P < 0.05$). You can also refer to Figures 8 and 9 for more details.

Discussion

The epithelial layer of the gastrointestinal tract, along with mucous secretions and intercellular connections, forms a protective barrier against harmful factors. However, external pathogens can activate leucocytes, cause oxidative stress and inflammation in the epithelium. Ethanol consumption enhances the production of ROS, such as hydroxyl and superoxide anions, and leads to lipid peroxidation in the gastric tissue. ROS can activate the NF-κB pathway, resulting in elevated levels of pro-inflammatory cytokines[20,21]. Previous studies demonstrated that oxidative homoeostasis, for example glutathione (GSH), MDA, and superoxide dismutase (SOD), play a role in ethanol-induced gastric ulcer[22,23]. Our study found that gastric damage caused by ethanol resulted in decreased levels of GSH and CAT, and increased levels of MDA. However, in the groups that were treated with FO 5 and 10 mg/kg or omeprazole 20 mg, there was a decrease in MDA levels and an increase in GSH and CAT levels.

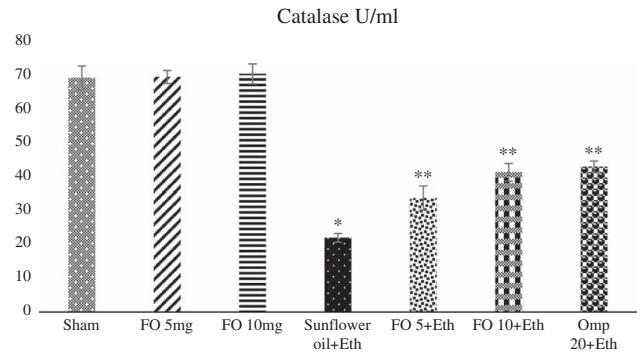

Figure 5. Fish oil (FO) effects on ethanol-produced changes on the levels of catalase (CAT). The first group (Sham) received a placebo, the second and third groups were given FO 5 and FO 10 mg/kg, respectively, the fourth group was given ethanol (Eth), and the fifth and sixth groups were given FO 5 and FO 10 mg/kg, respectively, followed by ethanol. The seventh group was given omeprazole (Omp) 20 mg/kg followed by ethanol. A *P* value lower of 5% was considered statistically significant. *Significant difference between Sunflower oil + ethanol group and sham group. ** Significant difference between FO 5 + ethanol, FO 10 + ethanol and Omp 20 + ethanol groups and Sunflower oil + ethanol.

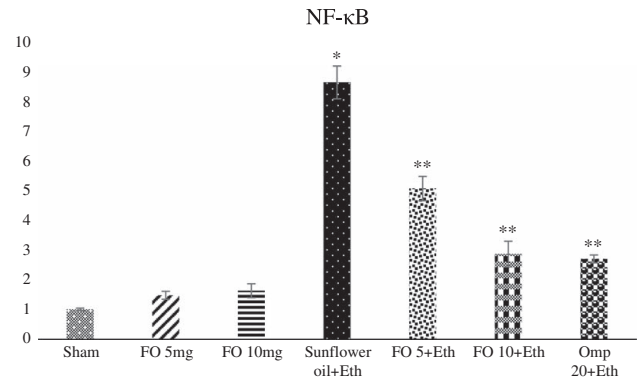

Figure 7. Fish oil (FO) effects on ethanol-produced changes on the expression of nuclear factor kappa B (NF-kB). The first group (Sham) received a placebo, the second and third groups were given FO 5 and FO 10 mg/kg, respectively, the fourth group was given ethanol (Eth), and the fifth and sixth groups were given FO 5 and FO 10 mg/kg, respectively, followed by ethanol. The seventh group was given omeprazole (Omp) 20 mg/kg followed by ethanol. A *P* value lower of 5% was considered statistically significant. *Significant difference between Sunflower oil + ethanol group and sham group. **Significant difference between FO 5 + ethanol, FO 10 + ethanol and Omp 20 + ethanol groups and Sunflower oil + ethanol.

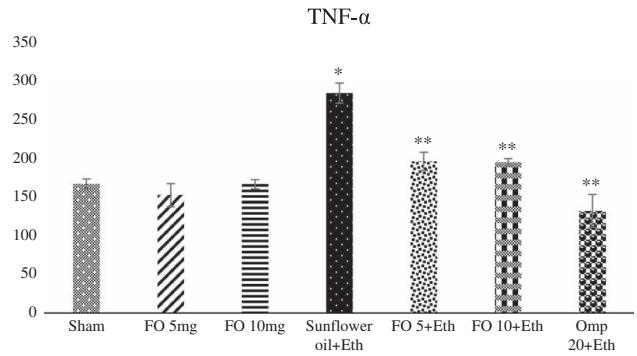

Figure 8. Fish oil (FO) effects on ethanol-produced changes on the levels of tumour necrosis factor alpha (TNF-α). The first group (Sham) received a placebo, the second and third groups were given FO 5 and FO 10 mg/kg, respectively, the fourth group was given ethanol (Eth), and the fifth and sixth groups were given FO 5 and FO 10 mg/kg, respectively, followed by ethanol. The seventh group was given omeprazole (Omp) 20 mg/kg followed by ethanol. A *P* value lower of 5% was considered statistically significant. *Significant difference between Sunflower oil + ethanol group and sham group. **Significant difference between FO 5 + ethanol, FO 10 + ethanol and Omp 20 + ethanol groups and Sunflower oil + ethanol.

NF-κB pathway has a vital role in inflammatory situations[24]. NF-κB enhances the expression of pro-inflammatory cytokines such as IL-6 and IL-8[25]. The p53, another stress response regulator, also has critical roles in inflammation[26,27]. P53 enhances the production of pro-inflammatory cytokines[28]. Similar stimuli activate both transcription factors. In addition, cross-talk between signalling p53 and NF-κB is antagonistic. However, there are several examples of cooperation between p53 and NF-κB[29–31]. A unique interaction between p53/NF-κB has identified in macrophages. Thus, activating p53 and NF-kB increases the expression of pro-inflammatory cytokines including CXCL1 and IL-6. Many external stimuli can activate p53 and NF-κB. This transcriptional regulatory mechanism may be involved in the response of macrophages to a wide range of tissue injuries[32]. In our study, gastric tissue damage following oral administration of ethanol increased the expression of NF-κB compared to healthy rats. NF-κB includes structurally related proteins including, c-Rel, p65 (Rel A) and p50 (NF-κB1). In resting cells, NF-κB is associated with inhibitor-κB (I-κB). In response to inflammatory stimuli, NF-κB activated. Therefore, compounds inhibiting NF-κB may play a vital role in healing gastric ulcers[33–36]. The findings of this study indicate that administration of FO at doses of 5 and 10 mg/kg in ethanol-treated groups significantly reduced the levels of NF-κB expression. In addition, the levels of pro-inflammatory cytokines IL-6 and TNF-α were found to be lower in the sunflower oil + ethanol group treated with FO at doses of 5 and 10 mg/kg.

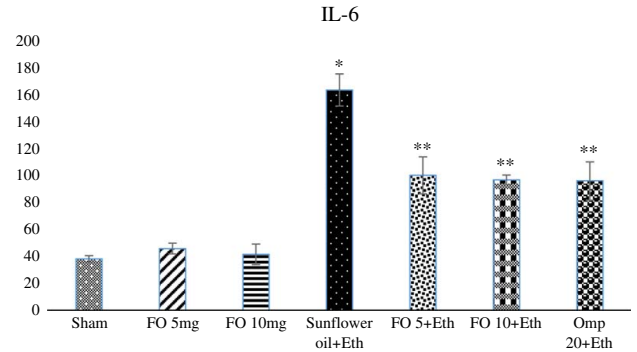

Figure 9. Fish oil (FO) effects on ethanol-produced changes on the levels of interleukin-6 (IL-6). The first group (Sham) received a placebo, the second and third groups were given FO 5 and FO 10 mg/kg, respectively, the fourth group was given ethanol (Eth), and the fifth and sixth groups were given FO 5 and FO 10 mg/kg, respectively, followed by ethanol. The seventh group was given omeprazole (Omp) 20 mg/kg followed by ethanol. A *P* value lower of 5% was considered statistically significant. *Significant difference between Sunflower oil + ethanol group and sham group. **Significant difference between FO 5 + ethanol, FO 10 + ethanol and Omp 20 + ethanol groups and Sunflower oil + ethanol.

The compounds that found in nature can be effective for the prevention and treatment of many pathological conditions[37]. The extract from Artemisia capillaries can inhibit acute gastric mucosal injury by inhibiting NF-kB and ROS[38]. Gastro-protective effect of bilobalide against gastric ulceration associated with NF-κB pathways[39]. Evidence shows that a diet with a high proportion of polyunsaturated fatty acids improves oxidative stress parameters such as SOD and GPx[40]. The positive impact of polyunsaturated fatty acids on reducing oxidative stress in brain tissue and enhancing neurogenesis has been demonstrated. Additionally, studies utilizing electron spin resonance have shown that polyunsaturated fatty acids have antioxidant effects against Helicobacter pylori infection by combating hydroxyl and superoxide radicals[41]. FO can inhibit NF-κB activity in the colonic mucosa[42]. The results of a study have shown that FO derived from Scomberoides commersonianus is involved in reducing ulcer severity in gastric ulcers. FO has reduced acid secretion and increased defense factors such as cellular mucus, mucin secretion, and mucosal cell longevity following pyloric closure[43]. Although interesting results were obtained in the animal phase, it is necessary to conduct similar studies in the human phase as well.

Conclusion

The present study suggests that pretreatment with FO effectively prevents ethanol-induced gastric ulcers. FO's protective effects were associated with regulating the gastric system.

Ethical approval

The protocol of this study approved by the ethics committee of Islamic Azad University, Behbahan Branch, Behbahan, Iran (Ethics number: IR.IUA.BEHBAHAN.REC.1401.024). 2/2/2023.

Consent

The experiments were performed in compliance with the Animal Research: Reporting of *in vivo* Experiments guidelines. The present study involved client-owned animals and demonstrated a high standard (best practice) of veterinary care and involved informed client consent.

Sources of funding

Shahid chamran university of ahvaz, iran.

Author contribution

M.T.M., S.F.M.N., U.A.G. and K.R. conceived and designed the project. M.T.and K.R. collected the data. M.T.M., S.F.M.N., U.A.G. and K.R. analyzed and interpreted the data. M.T.M., S.F.M.N., U.A.G. and K.R. drafted the manuscript. All authors read and approved the final manuscript."

Conflicts of interest disclosure

The authors declare that they have no competing interest.

Research registration unique identifying number (UIN)

Our research was animal study.

Guarantor

Kaveh rahimi is the person in charge of the publication of our manuscript.

Data availability statement

Data sharing is not applicable to this article.

Provenance and peer review

Our confirm that this study not commissioned, externally peer-reviewed.

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