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Original Article

Effects of Alpha-pinene on oxidative stress and inflammatory response in acute gastric ulcers in rats

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Abstract

Despite the many therapeutic advances, gastric ulcers continue to be prevalent. Natural compounds have been found to play a crucial role in preventing gastric ulcers in various phytochemical studies. The study aimed to investigate the protective effect of alpha-pinene against ethanol-induced gastric ulcers in rats by evaluating its impact on pro-inflammatory cytokines and oxidative stress markers. Male Wistar rats were orally administered alpha-pinene (50 and 100 mg/kg) prior to being induced with gastric ulceration using ethanol, and the gross morphological lesions, pro-inflammatory cytokine levels, and oxidative stress markers in gastric tissues were evaluated. Alpha-pinene treatment reduced gross morphological lesions in comparison to untreated animals. In ethanol-treated rats, alpha-pinene at 50 and 100 mg/kg also reduced oxidative stress, as verified by a decrease in tissue myeloperoxidase activity and malondialdehyde levels. In addition, alpha-pinene at both doses increased GSH and CAT levels compared to the untreated group. Alpha-pinene at both doses also lowered IL-1β and TNF-α production compared to the untreated group. Alpha-pinene may have a beneficial therapeutic role in gastric damage induced by ethanol as it reduces oxidative stress and pro-inflammatory factors.

Key words: Alpha-pinene, Inflammatory cytokines, Oxidative responses, Acute gastric injury

Introduction

Many diseases, including gastric ulcers and gastric carcinoma, are caused by oxidative stress (Tandon et al, 2004; Taheri Otaghsara et al, 2023). This results from high levels of reactive oxygen species production, leading to depletion of tissue antioxidant defense factors (Suzuki et al, 2012). Ethanol exposure disrupts the balance between protective and invasive factors of the gastric mucosa, stimulating pro-inflammatory cytokines and reactive oxygen species (ROS) (Pan et al, 2008), as well as myeloperoxidase (MPO) activity in neutrophils, causing gastric mucosal

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damage (Chatterjee et al, 2007). The previous studies have found that cytokines and oxidant/antioxidant mediators such as malondialdehyde (MDA), nitric oxide (NO), reduced glutathione (GSH), and superoxide dismutase (SOD) play an important role in acute gastrointestinal injury caused by ethanol (Almasaudi et al, 2017; J I Choi et al, 2010; Rozza et al, 2014).

Chemical anti-ulcer drugs have side effects (Koyyada, 2021), like omeprazole, a proton pump inhibitor (PPI) that inhibits gastric acid secretion (Toh et al, 2015). However, it may increase Clostridium difficile infection incidence (Trifan et al, 2017), induce hypomagnesemia, or attenuate anticoagulant drug efficiencies (Kenngott et al, 2010). Anti-inflammatory and antioxidant compounds can scavenge free radicals (Kinjo et al, 2008). Medicinal plant compounds have received attention for their anti-inflammatory and antioxidant effects in treating gastric ulcers (Danisman et al, 2023; Sumbul et al, 2011).

Several natural products have proved to have both protective and curative effects against gastric ulcer (Solmaz et al, 2009; Wang et al, 2011). Compounds such as *Apium graveolens* L, ginger, resveratrol, Quercus, Cirsium vulgare, and Falcaria vulgaris have beneficial effects in gastric ulcers due to their antioxidant effects (Basatinya et al, 2021; Solmaz et al, 2009; Wang et al, 2011). Alpha-pinene is a bicyclic monoterpene found in plants and acts as a repellent agent. It has many medicinal properties, including anti-inflammatory, antioxidant, and antibiotic activities (X Huang et al, 2013; Mercier et al, 2009). Alpha-pinene has been shown to improve the activity of SOD, CAT, and GPx, and reduce the concentration of MDA, NO, and IL-6 in the hippocampus and cortical tissue following stroke (Khoshnazar et al, 2019). Moreover, it can inhibit intracellular ROS production and significantly induce the expression of antioxidant enzymes such as SOD, CAT, glutathione peroxidase (GPx), glutathione reductase (GR), and heme-oxygenase 1 (HO-1) (Porres-Martínez et al, 2016). Additionally, it has anti-ulcer activity against ethanol-induced gastric ulcers and protects against gastric mucosal damage (Al-Juhaishi, 2014). However, detailed information about the mechanism of alpha-pinene effect on gastric ulcers is unavailable.

The animal model of ethanol-induced gastric injury is often used to evaluate the anti-ulcer activity of natural products and drugs (Abdelwahab et al, 2013; Brzozowski et al, 1998). Our study analyzed the impact of alpha-pinene on gastric tissue in rats with ethanol-induced gastric ulcers by scrutinizing the macroscopic changes and measuring levels of inflammatory and antioxidant factors.

Materials and methods

Animals

Thirty male adult Wistar rats weighing 250±10 grams were obtained from the Animal Care Center of Shahid Chamran University of Ahvaz. They had free access to food and water and were kept under a 12-hour light/dark cycle at a temperature of 23±2°C. The animal use followed the Guidelines for the Care and Work of Laboratory Animals (NIH Publication No. 23-86). The animal work ethics committee of Shahid Chamran University of Ahvaz approved the study protocol (EE/1401.2.241.402.25/scu.ac.ir).

Experimental Design

Thirty Wistar rats were randomly divided into five groups, with each group comprising six rats. The study involved five groups of rats. The first group (control) received oral liquid paraffin (Chemicenter, Iran) for seven days followed by an intraperitoneal injection of normal saline on the day of the experiment. The second group (Eth) received oral liquid paraffin for seven days, followed by the induction of stomach ulcers through intragastric administration of ethanol (Merck, Germany) at a dose of 1ml/200gr bw. The third group received alpha-pinene (Sigma Alderich, St. Louis, MO, United States) (Zhang et al, 2020) at a dose of 50mg/kg for seven days, followed by the creation of gastric ulcers. The fourth group received alpha-pinene at a dose of 100mg/kg for seven days, followed by the creation of gastric ulcers. The fifth group received omeprazole (Irannajo Pharmaceutical Co, Iran) at a dose of 20mg/kg (Taheri Mirghaed et al, 9900) for seven days, followed by the induction of gastric ulcers. The rats were not allowed to eat but had access to water for 24 hours before receiving the ethanol. Rats were anesthetized with thiopental sodium (Loghman, Iran) (50 mg/kg, i.p.) and euthanized by decapitation 1.5 hours after Eth administration. The stomachs from each group were then isolated (Figure 1).

Evaluation of mean gastric ulcer index

The stomach tissue was cut from the dorsal surface, and then the area of ulcers (mm) was counted. When counting the ulcers, the names of the groups were blinded. The mean gastric ulcer index (MUI) was determined using the following formula:

MUI = total ulcer area/millimeter of rats ulcerated

% Inhibition Ulcer = (MUI of ethanol treated – MUI of rat pretreated)/MUI of ethanol treated * 100

Determination of Inflammatory Markers

The tissues were homogenized by pounding in a mortar. Pro-inflammatory cytokines such as IL-1β (IL-1β, Kiazist, Hamedan, Iran cat# E0119Ra), TNF-α (TNF-α, Kiazist, Hamedan, Iran cat# E0764Ra) were measured by enzyme-linked immunosorbent assay (ELISA) methods. The total protein content was measured by the Bradford method (Kiazist, Hamedan, Iran cat#KBRD96). To measure protein concentration, tissue samples were mixed with Bradford's reagent and distilled water. Absorbance was measured at 495 nm using a plate reader. A standard curve of bovine serum albumin protein was used to calculate the protein concentration of the samples.

Determination of oxidant/antioxidant factors

Oxidant/antioxidant factors such as MPO (MPO, Navand LAB Kit, Iran cat#KSOD96), MDA (MDA, Kiazist, Hamedan, Iran cat#KMDA96), GSH (GSH, Kiazist, Hamedan, Iran cat#KTHI96), and CAT (CAT, Kiazist, Hamedan, Iran) were analyzed by enzyme-linked immunosorbent assay (ELISA) methods.

Statistical Analysis

SPSS version 16 was utilized to analyze data. One-way ANOVA was conducted to compare the groups, followed by Tukey's statistical test for post-hoc comparison. *$P<0.05$, **$p<0.01$ and ***$p<0.001$ were considered statistically significant.

Results

Gastric Tissue Macroscopic changes

Our results revealed that the average ulcer size (measured in mm) was significantly greater in the ethanol, alpha-pinene 50 mg/kg, alpha-pinene 100 mg/kg, and omeprazole 20 mg/kg groups compared to the control group ($P<0.001$, $P<0.01$, $P<0.05$, and $P<0.05$ respectively). However, the average ulcer size was lower in alpha-pinene 50 mg/kg, alpha-pinene 100 mg/kg, and omeprazole 20 mg/kg groups when compared to the ethanol group ($P<0.05$, $p<0.01$, and $P<0.01$ respectively) (Figures 2 and Figure 3 A and C).

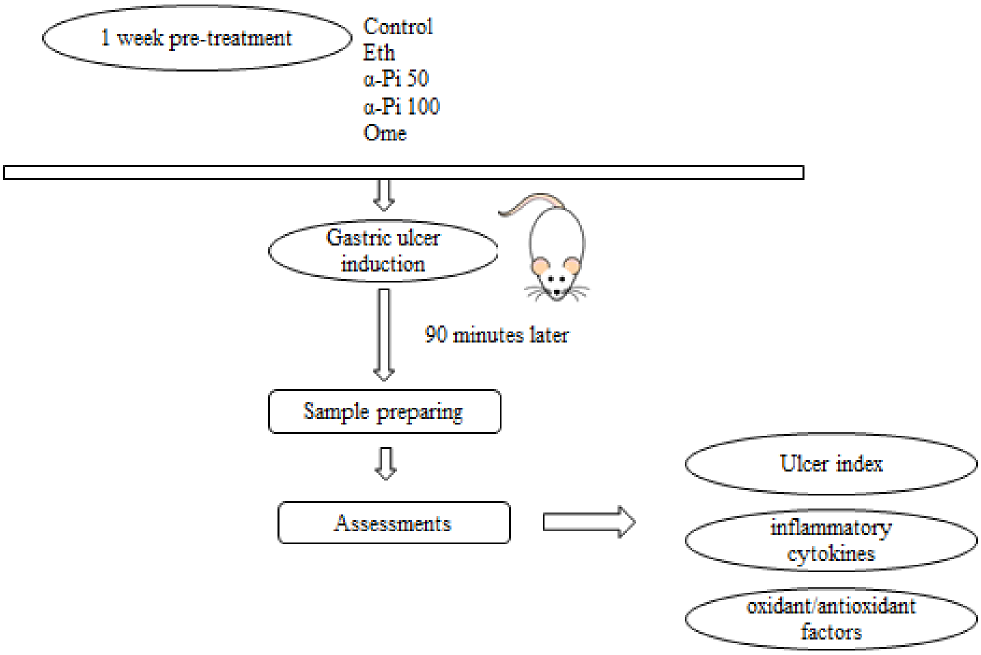

Figure 1: The schematic diagram

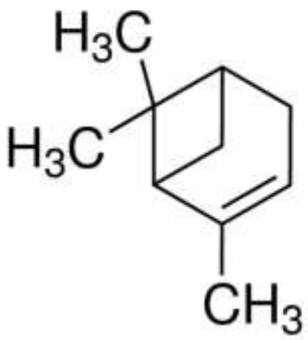

Figure 2: Chemical structures of α- pinene (C10H16)

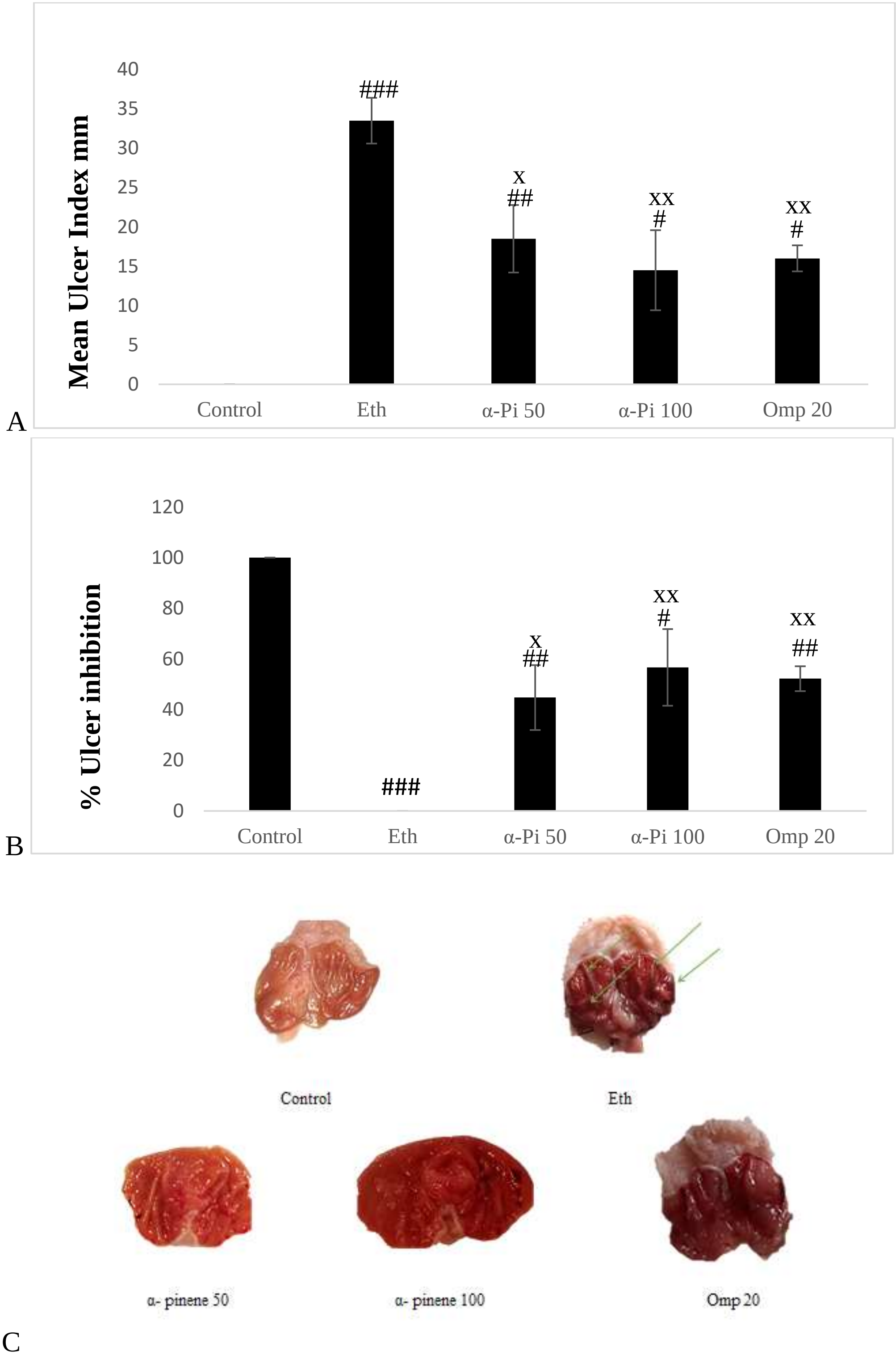

Figure 3: Ulcer index in study groups. (Mean±SEM) in control, ethanol (Eth), alpha-pinene 50 and 100 mg/kg groups + ethanol, and Omeprazole group with a dose of 20 mg/kg + ethanol (Omp 20). (A) Mean ulcer index. (B). %Ulcer inhibition. (C). Macroscopic image. # p< 0.05, ## p< 0.01, and ### p< 0.001 show a significant level difference compared to the control group. x p< 0.05, xx p< 0.01, and xxx p< 0.001 show a significant level difference compared to the ethanol group (n=6).

In the study, it was shown that the groups given ethanol, alpha-pinene 50 mg/kg, alpha-pinene 100 mg/kg, and omeprazole 20 mg/kg had a lower percentage of ulcer inhibition compared to the control group (P<0.001, P<0.01, P<0.05, P<0.01 respectively). Also, the groups given 50 mg/kg alpha-pinene, 100 mg/kg alpha-pinene, and 20 mg/kg omeprazole had a higher percentage of ulcer inhibition compared to the ethanol group (P<0.05, P<0.01, and P<0.01 respectively) (Figures 2 and 3B).

The concentrations of inflammatory cytokines in gastric tissue

TNF-α levels are significantly increased in ethanol, alpha-pinene 50 mg/kg, alpha-pinene 100 mg/kg, and omeprazole 20 mg/kg compared to the control group (P<0.001). However, TNF-α levels are significantly decreased in alpha-pinene 50 mg/kg, alpha-pinene 100 mg/kg, and omeprazole 20 mg/kg groups compared to the ethanol group (P<0.001) (Figure 4 A).

In addition, IL-1β levels are significantly increased in ethanol, alpha-pinene 50 mg/kg, alpha-pinene 100 mg/kg, and omeprazole 20 mg/kg compared to the control group (P<0.01). However IL-1β levels are significantly decreased in alpha-pinene 50 mg/kg, alpha-pinene 100 mg/kg, and omeprazole 20 mg/kg groups compared to the ethanol group (P<0.01) (Figure 4 B).

The levels of oxidant/antioxidant factors in gastric tissue

The groups treated with ethanol and alpha-pinene 50 mg/kg showed a significant increase in the MDA levels compared to the control group (P<0.001 and P<0.05, respectively). The levels of MDA in the groups treated with alpha-pinen 100 mg/kg and omeprazole 20 mg/kg did not differ from the control group. However, the levels of MDA in the groups treated with alpha-pinene 50 mg/kg, alpha-pinene 100 mg/kg, and omeprazole 20 mg/kg were significantly lower than the ethanol group (P<0.01, P<0.001, and P<0.001, respectively) (Figure 5 A).

The group treated with ethanol showed a significant increase in the MPO levels compared to the control group (P<0.01). The levels of MPO in the groups treated with alpha-pinene 50 mg/kg, alpha-pinen 100 mg/kg, and omeprazole 20 mg/kg did not differ from the control group. The levels of MPO in the groups treated with alpha-pinene 50 mg/kg, alpha-pinene 100 mg/kg, and omeprazole 20 mg/kg were significantly lower than the ethanol group (P<0.05) (Figure 5 B).

The groups treated with ethanol and alpha-pinene 50 mg/kg showed a significant decrease in the GSH levels compared to the control group (P<0.001 and P<0.01, respectively). The levels of GSH in the groups treated with alpha-pinen 100 mg/kg and omeprazole 20 mg/kg did not differ from the control group. However, the levels of GSH in the groups treated with alpha-pinene 50 mg/kg, alpha-pinene 100 mg/kg, and omeprazole 20 mg/kg were significantly higher than the ethanol group (P<0.05, P<0.0501, and P<0.0501, respectively). In addition, the levels of GSH in the groups treated with alpha-pinene 100 mg/kg, and omeprazole 20 mg/kg were significantly higher than the alpha-pinene 50 mg/kg (P<0.01) (Figure 5 C).

The group treated with ethanol showed a significant decrease in the CAT levels compared to the control group (P<0.001). The levels of CAT in the groups treated with alpha-pinene 50 mg/kg, alpha-pinen 100 mg/kg, and omeprazole 20 mg/kg did not differ from the control group. The levels of CAT in the groups treated with alpha-pinene 50 mg/kg, alpha-pinene 100 mg/kg, and omeprazole 20 mg/kg were significantly higher than the ethanol group (P<0.05) (Figure 5 D).

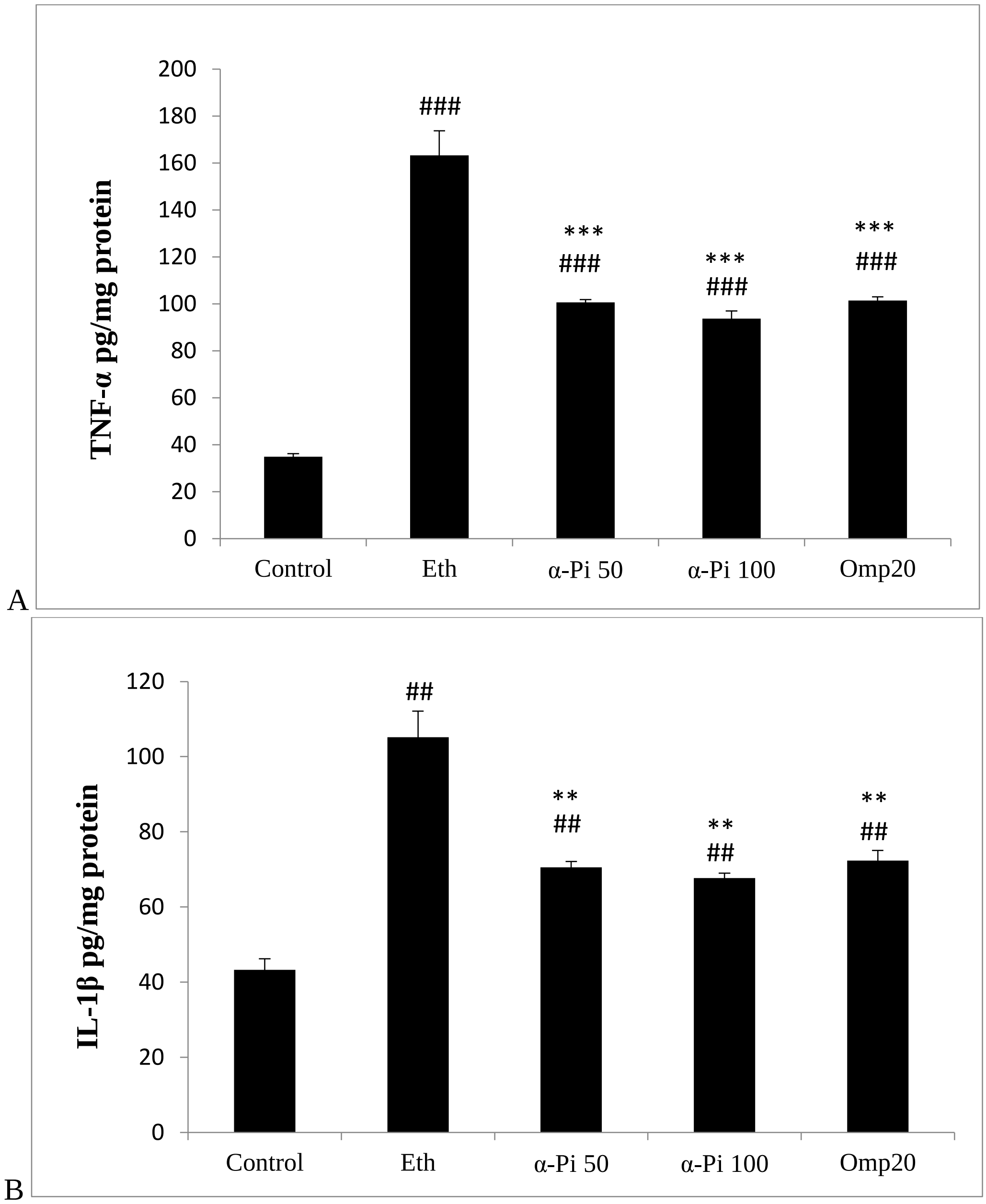

Figure 4: The effects of α- pinene on the levels of (A) TNF-α and (B) IL-1β. (Mean±SEM) in control, ethanol (Eth), alpha-pinene 50 and 100 mg/kg groups + ethanol, and Omeprazole group with a dose of 20 mg/kg + ethanol (Omp 20). # $p< 0.05$, ## $p< 0.01$, and ### $p< 0.001$ show a significant level difference compared to the control group. *$p< 0.05$, **$p< 0.01$, and ***$p< 0.001$ show a significant level difference compared to the ethanol group (n=6).

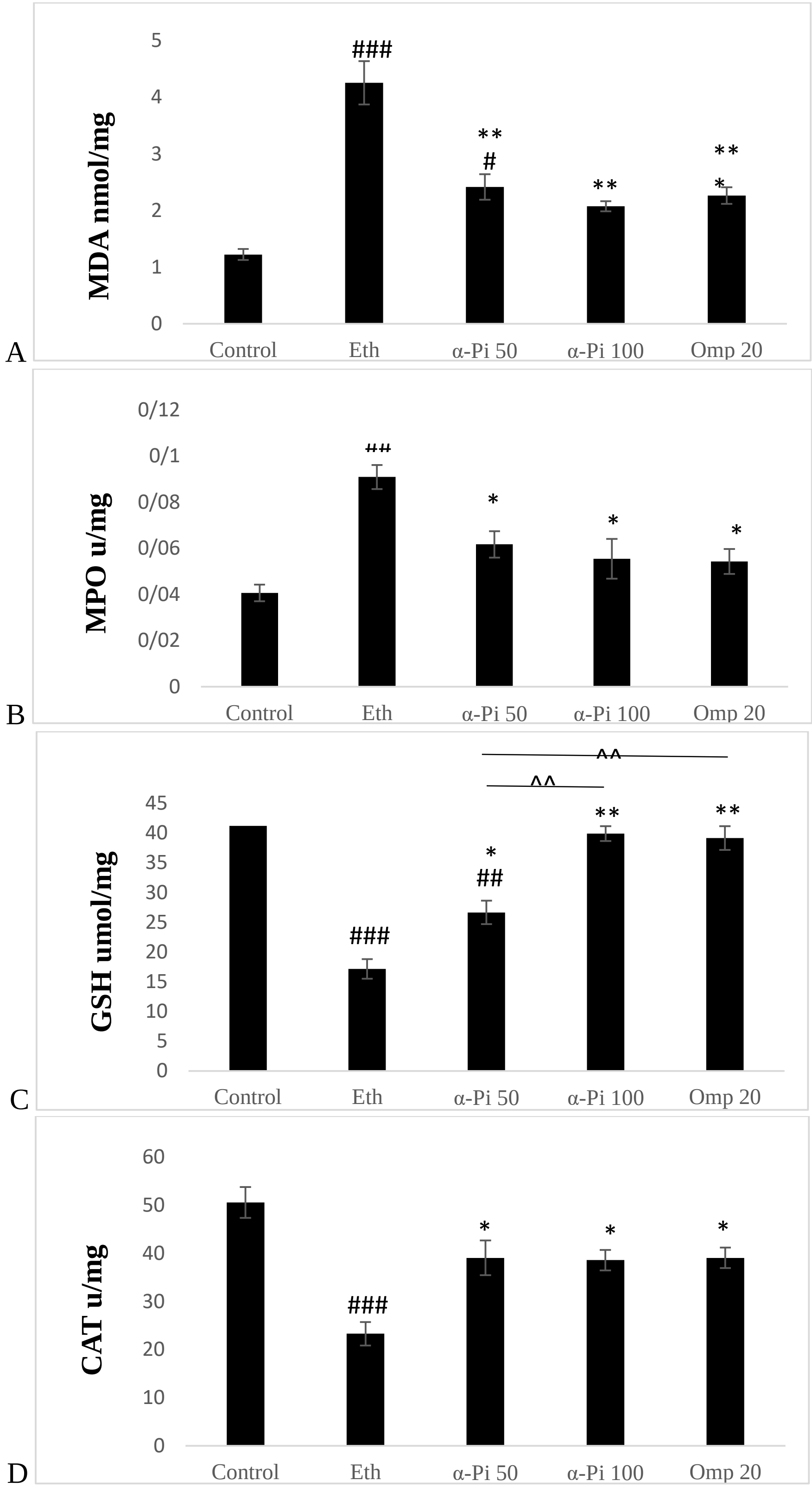

Figure 5: The effects of α- pinene on the levels of (A) MDA, (B) MPO, (C) GSH, and (D) CAT. (Mean±SEM) in control, ethanol (Eth), alpha-pinene 50 and 100 mg/kg groups + ethanol, and Omeprazole group with a dose of 20 mg/kg + ethanol (Omp 20). # $p< 0.05$, ## $p< 0.01$, and ### $p< 0.001$ show a significant level difference compared to the control group. *$p< 0.05$, **$p< 0.01$, and ***$p< 0.001$ show a significant level difference compared to the ethanol group. ^^$p< 0.01$ show a significant level difference alpha-pinene 50 + ethanol compared to the alpha-pinene 100 mg/kg + ethanol and Omeprazole group with a dose of 20 mg/kg + ethanol groups (n=6).

Discussion

The oral administration of ethanol induces a gastric ulcer model that is similar to the human gastric ulcer disorder. This model is useful for investigating the anti-ulcer effects of different drugs (Huang et al, 2013; Kim et al, 2005). Many studies have investigated the effect of medicinal plants and their substances on stomach ulcers, highlighting its significance (Bi et al, 2014). Our study evaluated the potential therapeutic effects of α- pinene in an ethanol-induced gastric ulceration model in rats. In our study, pretreatment with alpha-pinene (50 and 100 mg/kg) exhibited a gastroprotective effect against ethanol-induced gastric ulceration, reducing the gastric ulcer index.

The previous studies have shown that alpha-pinene has protective effects against inflammation and oxidative stress in vitro. For instance, in lipopolysaccharide (LPS)-stimulated macrophages, alpha-pinene reduces TNF-α, IL-6, and nitric oxide (NO) production by suppressing the nuclear factor kappa B (NF-κB) pathway (Kim et al, 2015; Kwak et al, 2019). Moreover, alpha-pinene has exhibited antioxidant and anti-inflammatory properties in ischemia and stroke experimental models (Choi et al, 2010). We studied the impact of alpha-pinene on the levels of pro-inflammatory cytokines in rat stomach tissue damaged by ethanol consumption (Abdelwahab, 2013; Badr et al, 2019; Li et al, 2018; Raish et al, 2021; Ren et al, 2020; Su et al, 2019). Our study showed that alpha-pinene can reduce inflammation in gastric tissue by suppressing TNFα and IL1β production at doses of 50 and 100 mg/kg.

Excessive production of reactive oxygen species (ROS) is a harmful factor in the progression of tissue damage (Corsini et al, 2018; Juránek et al, 2013; Nikitovic et al, 2013). Antioxidant systems in the body consist of various enzymes and biomolecules and defend against the adverse effects of ROS (Kıran et al, 2023). The first line of defense against oxidative stress involves enzymes such as CAT, GPX, and SOD. SOD neutralizes superoxide, producing hydrogen peroxide (H2O2), which is then eliminated by CAT (Ighodaro et al, 2018). Also, GPX is responsible for the reduction of H2O2 (Gebicka et al, 2019; Sharifi-Rad et al, 2020). Our study found that pretreatments with 50 and 100 mg/kg of alpha-pinene increased CAT levels compared to an ethanol-treated group. GSH reacts with ROS and non-enzymatic antioxidants, protecting cells against oxidative damage caused by H2O2, superoxide anion, hydroxyl radicals, and alkoxyl radicals. It also prevents inactivation of enzymes and proteins (Asantewaa et al, 2021). Our findings indicated that pretreatment with alpha-pinene and omeprazole led to higher levels of GSH when compared to a group treated with ethanol. Acute ethanol administration increases MDA and MPO activity (Li et al, 2011; Li et al, 2021). MPO promotes ROS production (Chen et al, 2020), which reacts with cell membranes causing, lipid peroxidation. This leads to oxidative damage in gastric tissue (Yu et al, 2017). Our study showed that pretreatment with alpha-pinene and omeprazole reduces levels of MDA and MPO compared to an ethanol-treated group. These results suggest that alpha-pinene could improve oxidative stress and inflammation in gastric tissue caused by ethanol exposure. Additionally, the previous research has found that alpha-pinene has antioxidant effects which could prevent UVA-induced aging by increasing antioxidant enzymes (CAT, GPX, and SOD) and reducing lipid peroxidation in an aging model caused by UVA (Karthikeyan et al, 2019).

Previously, the effect of pretreatment with alpha-pinene on gastric ulceration induced by ethanol in male Swiss rats was investigated. Alpha-pinene reduced gastric juice volume and acidity while increasing gastric wall mucus (Pinheiro Mde et al, 2015). GC/MS analysis revealed alpha-pinene as the main component of Pistacia atlantica Deaf essential oil. The oil's protective impact against ethanol-induced gastric ulcer was evaluated in an animal model. Administering oral doses of 25, 50, and 100 mg/kg of Pistacia atlantica Deaf essential oil one hour before wound induction by ethanol led to a reduction in the destruction and necrosis of stomach tissue (Memariani et al, 2017). Studies suggest that Pistacia atlantica, Cymbopogon citratus, and Origanum vulgare L can protect against ethanol-induced gastric ulcer (Périco et al, 2020). Gastroprotective effects of alpha-pinene in ethanol-induced gastric ulcers have been demonstrated (Al-Juhaishi, 2014). However, the mechanism of pinene's effect on the digestive system is not well understood. According to its chemical structure, pinene has antioxidant and anti-inflammatory effects (Salehi et al, 2019). Thus, alpha-pinene may offer protection against gastric ulcers by reducing inflammation and oxidative stress.

Alpha-pinene pretreatment suppressed abnormal gastric changes caused by ethanol-induced gastric ulcers. Alpha-pinene had protective effects against gastric ulcers by reducing oxidative stress and inflammatory cytokine production. However, there are limitations to the experimental research methods. One limitation is that experiments may not reflect the real-world situations. Further research is needed to generalize the results from animal models to humans.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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اثرات آلفا پاینن بر استرس اکسیداتیو و پاسخ التهابی در زخم‌های حاد معده در موش‌های صحرایی

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چکیده

با وجود پیشرفت‌های درمانی فراوان، زخم معده همچنان شایع است. در مطالعات مختلف نشان داده شده است که ترکیبات طبیعی نقش مهمی در پیش‌گیری از زخم معده ایفا می‌کنند. هدف از این مطالعه بررسی اثر محافظتی آلفا پاینن در برابر زخم معده ناشی از اتانول در موش‌های صحرایی با ارزیابی تأثیر آن بر سایتوکاین‌های پیش التهابی و شاخص‌های استرس اکسیداتیو بود. موش‌های نر نژاد ویستار قبل از القا زخم معده با اتانول آلفا پاینن خوراکی (۵۰ و ۱۰۰ میلی‌گرم بر کیلوگرم) دریافت کردند و ضایعات مورفولوژیکی ناخالص، سطوح سیتوکین‌های پیش التهابی و شاخص‌های استرس اکسیداتیو در بافت‌های معده مورد ارزیابی قرار گرفتند. آلفا پاینن باعث کاهش ضایعات مورفولوژیکی در مقایسه با حیوانات گروه کنترل شد. در موش‌های صحرایی تیمار شده با اتانول، آلفا پاینن با دوزهای ۵۰ و ۱۰۰ میلی‌گرم بر کیلوگرم به واسطه کاهش فعالیت میلوپراکسیداز بافتی و سطوح مالون دی‌آلدئید استرس اکسیداتیو را کاهش داد. علاوه بر این، آلفا پاینن در هر دو دوز کاهش GSH و CAT ناشی از اتانول را بهبود بخشید. همچنین آلفا پاینن در هر دو دوز IL-1β و TNF-α را در مقایسه با گروه درمان نشده کاهش داد. آلفا پاینن ممکن است نقش درمانی مفیدی در آسیب معده ناشی از اتانول داشته باشد زیرا استرس اکسیداتیو و عوامل پیش التهابی را کاهش می‌دهد.

کلمات کلیدی: آلفا پاینن، سیتوکین‌های التهابی، پاسخ‌های اکسیداتیو، آسیب حاد معده

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