European Review for Medical and Pharmacological Sciences 2023; 27: 12029-12042

Protective effect of barbigerone against ethanol-induced ulcers *via* the interleukins/ICAM-1/Bcl-2 pathway

A.B. OMER[1], F.A. AL-ABBASI[2], S.A. ALGHAMDI[2,3], A.M. ALGHAMDI[2], R.A. SHEIKH[2,3], S.I. ALZAREA[4], N. SAYYED[5], M.S. NADEEM[1], I. KAZMI[1]

[1]Department of Basic Health Sciences, Foundation Year for the Health Colleges, Princess Nourah Bint Abdulrahman University, Riyadh, Saudi Arabia
[2]Department of Biochemistry, Faculty of Sciences, King Abdulaziz University, Jeddah, Saudi Arabia
[3]Experimental Biochemistry Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia
[4]Department of Pharmacology, College of Pharmacy, Jouf University, Aljouf, Sakaka, Saudi Arabia
[5]School of Pharmacy, Glocal University, Saharanpur, India

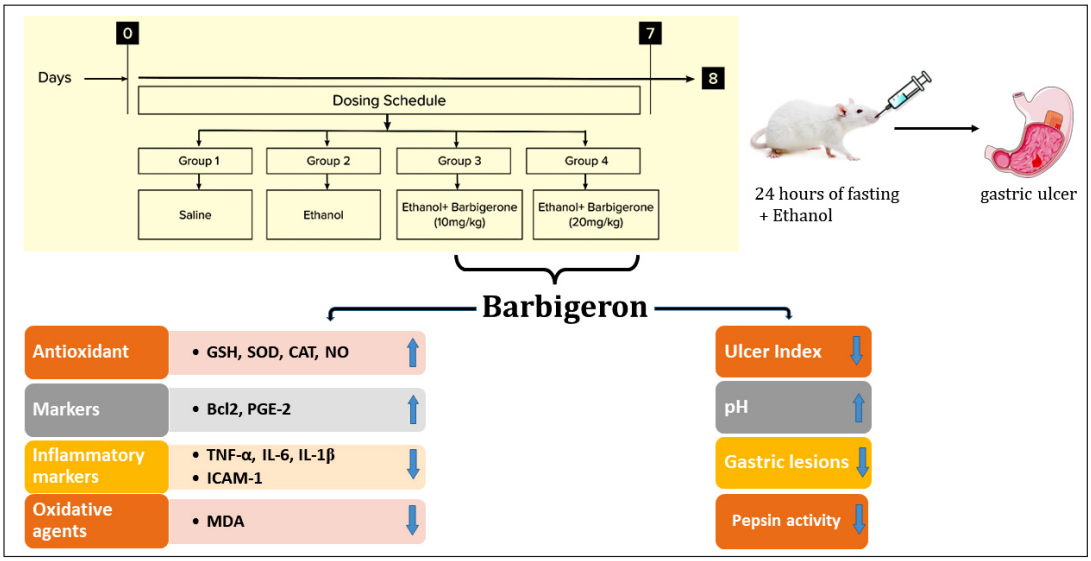
Abstract. – OBJECTIVE: The objective of the study was to assess the protective effects of barbigerone in ethanol-induced gastric ulcers in rats.

MATERIALS AND METHODS: Male Wistar rats (180±20 g) were used in the study (n=06). The rats were randomly divided into different groups, i.e., the normal group, ethanol control, and barbigerone 10 and 20 mg/kg group. Various biochemical parameters were assessed – total acidity and pH values, oxidative stress biomarkers such as superoxide dismutase (SOD), glutathione (GSH), malondialdehyde (MDA), and catalase (CAT) along with markers, i.e., tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-1β, intercellular adhesion molecule-1 (ICAM-1) and expression of B-Cell Leukemia/Lymphoma 2 (Bcl-2). Also, histopathology was performed.

RESULTS: Treatment with barbigerone in the ethanol-induced-ulcer rats restored the levels of biochemical parameters such as SOD, GSH, MDA, CAT, and markers expression, including TNF-α, IL-6, IL-1β, ICAM-1, and Bcl-2 with protected against cellular necrosis.

CONCLUSIONS: Barbigerone protective effects can be attributed to its ability to reduce oxidative stress and inflammation, as well as promote gastroprotection against ethanol-induced ulcers in rats.

Key Words:
Barbigerone, Ethanol, Gastric ulceration, Inflammation, Oxidative stress.



Graphical abstract. Bcl-2: B-Cell Leukemia/Lymphoma 2, CAT: Catalase, GSH: Glutathione, ICAM-1: Intercellular adhesion molecule-1, IL-1β: interleukin-1β, IL-6: interleukin-6, MDA: Malondialdehyde, NO: Nitric Oxide, SOD: Superoxide Dismutase, TNF-α: Tumor necrosis factor-α

Corresponding Author: Imran Kazmi, MD; e-mail: ikazmi@kau.edu.sa

Introduction

Alcohol consumption has been associated with approximately 60 distinct medical conditions. Consistent alcohol intake leads to erosive hemorrhagic gastritis and chronic gastric diseases. Gastric ulcer, a lesion that develops in the digestive tract, is attributed to excessive alcohol consumption, smoking, uncontrolled use of non-steroidal anti-inflammatory drugs (NSAIDs), and infection with pathogenic organisms like *Helicobacter pylori*[1,2]. Another significant factor in the formation of gastric ulcers is the disturbance of the balance between protective and aggressive physical, chemical, or psychological factors in the lining of the gastric mucosa. Among these, excessive alcohol consumption plays a pivotal role in the development of benign lesions in the gastric tract[3]. Consumption of ethanol leads to microvascular injuries by restricting blood flow and triggering immunological responses, including the rapid activation of neutrophils. This process induces oxidative stress by increasing the expression of reactive oxygen species (ROS) and pro-inflammatory cytokines, causing damage to the gastric mucosa. The regulation of alcohol-induced gastric ulcers involves pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and IL-1β, as well as oxidative stress molecules, including nitric oxide (NO), malondialdehyde (MDA), glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD). Ethanol disrupts the mucosal lining, rendering it susceptible to proteolytic and hydrolytic actions of pepsin and stomach acids. Ethanol-induced gastric ulcer rodent models share several similarities with human gastric ulcer conditions, making them valuable for experimental studies of anti-ulcer approaches drugs[4,5]. Gastric ulceration is one of the prominent causes of gastrointestinal surgeries worldwide, owing to its widespread prevalence and high morbidity and mortality rates. Patients with gastric ulcers often experience abdominal discomfort and nausea. Postmeal burning sensations and abdominal pain are characteristic symptoms of gastric ulcers. As a result, patients with gastric ulcer experience both a serious illness and emotional distress that interferes with their daily activities[6]. Available gastroprotection drugs mainly include inhibitors of the proton pump, antagonists of the histamine-2 receptor, and analogs of prostaglandin. These drugs restrict mucosal damage and stabilize gastrointestinal bleeding. However, these medications are complex, expensive, and have many side effects. The medical ecosystem faces significant challenges because of the low cure rates and high disease recurrence rates of current clinical therapies for gastric ulcers. Hence, it is necessary to develop novel alternative therapeutics for the management of gastric ulcer[6,7].

IL-6 stimulates T and B cells and regulates acute and chronic inflammation. In gastric ulcers, IL-6 activates macrophages, lymphocytes, and neutrophils, causing inflammation and triggering ROS and lysosomal activities, resulting in tissue injury to the mucosal layer. Pro-inflammatory cytokine (TNF-α) induces an acute inflammatory response and infiltration into the mucosal layer, which causes gastric ulcer[8]. Moreover, elevated TNF-α levels cause apoptosis in the mucosal lining *via* the caspase-3 pathway, which is accompanied by inhibition of gastric microcirculation and delayed ulcer healing. Furthermore, the synergistic effects of TNF-α and IFN-γ initiate pathophysiological actions on the gastric ulcer, such as neutrophil infiltration, apoptosis, cytoskeleton degradation, and proline and tyrosine kinase stimulation[9,10].

Current drugs used to treat gastric ulcers include anticholinergics, H2-receptor antagonists, proton pump inhibitors, anticholinergics, sucralfate, and bismuth, with some side effects. Additionally, these drugs have several detrimental effects, such as gynecomastia, alterations in hematopoiesis, arrhythmia, and hypersensitivity. Phytochemicals as potential drugs could be considered a better alternative therapy for managing ulcer disease. Therefore, the search for novel pharmaceutical compounds through the screening of various phytochemicals has led to the development of safe gastroprotective drugs[11]. Plant-derived compounds have been shown to exhibit gastroprotective properties by reversing the harm caused by ethanol administration in animal models. Previous studies have shown that these plant compounds prevent gastric injury by regulating the release of pro-inflammatory cytokines. Phytocompounds also promote antioxidant activities and reverse the effects of ROS, NO, GSH, and CAT activities[12,13].

Barbigerone is a flavonoid phytochemical primarily present in the leguminous species *Millettia ferruginea*. Flavonoids are natural polyphenols that are abundantly found in plants. These substances have several therapeutic advantages, such as antiviral, anti-inflammatory, antioxidative, and anti-tumorigenic properties. Furthermore, earlier

research[14,15] has shown that flavonoids help maintain intestinal barrier function, regulate gastric secretions, and modify the immune system, all of which help protect the intestinal epithelium. Flavonoids can reverse gastric ulcers by neutralizing acid secretion, increasing gastric mucus and bicarbonate secretion, and decreasing the pepsin effect. Moreover, flavonoids enhance the anti-inflammatory, antioxidative, and antibacterial defense mechanisms against gastric ulcer[14,15]. The present work was conducted to assess the anti-ulcer action of barbigerone in ethanol-induced gastric diseases in rats.

Materials and Methods

Animals

Male Wistar rats (180±20 g) were used in this study and allowed to adapt to standard laboratory settings. Animals were included in the investigation when they were 8 weeks old after being routinely examined and monitored for health. Animals were kept in polypropylene cages supported by stop-and-feed grills with nozzle orifices. These cages were equipped with autoclaved husks to protect the animals from common infections. During the experiment, the animals were subjected to 12 hours of light and darkness, with temperatures between 23-28°C and humidity levels between 45 and 65%. Animals were fed, and drinking water was provided *ad libitum* during the procedure. The current investigation was approved by the Institutional Animal Care and Use Committee (IACUC/TRS/PT/023/028), and research was performed as per the criteria mentioned in ARRIVE[16]. The animals used in the study had not undergone any previous procedures.

Chemicals

Ethyl alcohol and barbigerone (98% purity, gift sample) and kits for IL-1β, PGE2, IL-6, TNF-α, ICAM-1, and expression of Bcl-2 were determined using a rat enzyme-linked immune-sorbent examination kit (ELISA, MSW Pharma, Chandrapur, Maharashtra State, India).

Experimental Design

The current study's protocols were based on earlier research[17,18] and were modified to standardize the experiments.

A total of 24 rats were randomly segregated into four groups (n=6).

- Group 1 (normal group): treated with normal saline.
- Group 2 (ethanol-injected group): treated with a single dose of 1.5 ml/kg of ethanol.
- Group 3 (ethanol + barbigerone: low dose): treated with ethanol 1.5 ml/kg + 10 mg/kg of barbigerone.
- Group 4 (ethanol + barbigerone: high dose): treated with ethanol 1.5 ml/kg + 20 mg/kg of barbigerone.

All groups received the dose as per the schedule from the beginning to the seventh day. During the experiment, the treatment group was given barbigerone (10 and 20 mg/kg/day p.o.), and the normal group had been supplied only saline. After a 24-hour period of fasting, a single dose of ethyl alcohol (1.5 ml/animal) was administered orally on the eighth day to induce gastric ulcers (Figure 1). All experimental animals were sacrificed, their stomachs were excised, and the ulcer index was calculated, followed by collection of the gastric material for further analysis. The gastric contents from each animal were extracted for further analysis by excision of the stomach after

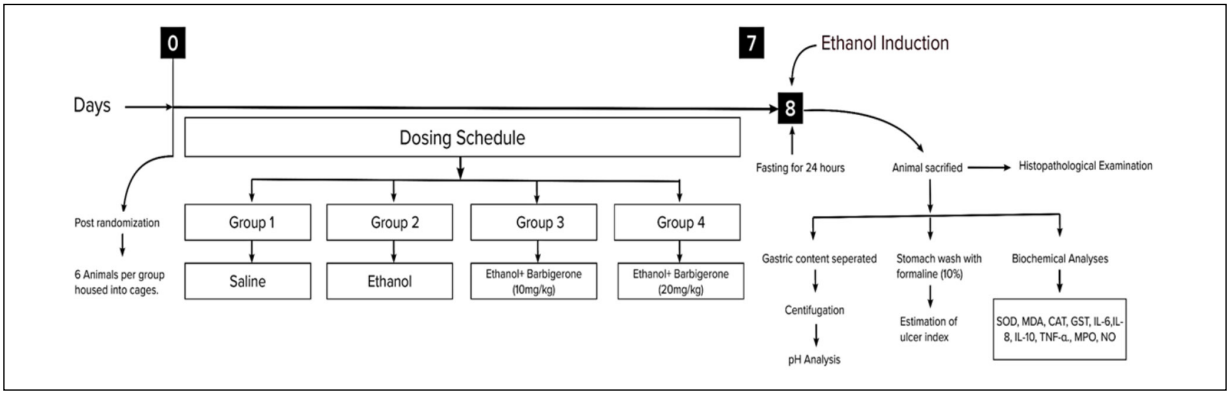


Figure 1. Experimental outline.

sacrificing the animals. The pH of the collected gastric matter was assessed after centrifugated at 1,000 rpm for 10 min. Biochemical parameters were obtained, followed by histopathological examination of gastric tissue.

Biochemical Examination

Ulcer index

The total ulcer area in the gastric tissue was estimated using the following mathematical expression[17]:

$$Ulcer\ Index = 10/x$$

Where X is a mucosal region with ulceration.

Ulcer scores were determined on the scale mentioned below:

0 = Normal, 1 for superficial mucosal erosion, 2 for deep ulcer or transmural necrosis, and 3 for ulcer with penetration or perforations

Estimation of pH

After centrifuging the gastric contents, the pH of the supernatant was analyzed by a digital pH analyzer.

Estimation of Total Acidity

To determine total acidity, 1,000 µL of gastric supernatant was added to distilled water in a 1:1 ratio and transferred to a conical flask, which was titrated with sodium hydroxide (0.01 N) using a phenolphthalein indicator[17].

Determination of Pepsin Activity

As previously described, pepsin activity was determined using the denatured hemoglobin hydrolysis method. P-Nitrophenyl sulfite (10 mg) was mixed with acetonitrile (2 mL) and dissolved to prepare a stock solution. The solution was then allowed to cool on ice for 3 hours. To achieve a quantity of 1.5 X 0-4 M, a 100 mL aliquot of the stock substrate solution was combined with glycine hydrochloride (0.01 M) at 26°C with the pH maintained at 1.9. A pH level of 1.8 to 2.0 was optimal for enzymatic hydrolysis, so a pH of 1.9 was maintained for the experiments. The minimal turbidity of the solution indicated that the substrate was not consumed and could be utilized for the assay. A duration of eight minutes was required for complete hydrolysis of the substrate.

Along with pepsin, the sample cuvette was quickly set in a double-beam spectrophotometer (25°C). A cuvette containing 1-10 units of pepsin (with a gastric content of 5-100 units) was used to measure the absorbance at 320 nm. Instrumentation used during the procedure included a Beckman DBG and a Haake thermostatic water bath[17].

Determination of Biochemical Markers of Gastric Ulcer

The excised stomach tissues from all experimental rodents were stored at -80°C and subjected to biochemical estimation as previously described. In this experiment, gastric tissue (~50 mg) was transformed into powder; later, it was homogenized with Phosphate buffered saline (PBS) buffer (500 µl). The prepared homogenate was used for several biochemical analyses, including Glutathione (GSH) estimation. The findings are presented in milligrams of GSH per gram of tissue. Malondialdehyde (MDA) present in the stomach was estimated as per the process described earlier, and the findings were measured as micrograms of MDA in one gram of tissue[19,20]. The Catalase (CAT) was determined as a reduction in H_2O_2 (hydrogen peroxide) and expressed in units of CAT in one mg of gastric tissue (U/mg tissue) SOD activity was assessed and expressed as units of SOD in one mg of tissue (U/mg tissue)[21].

Assessment of MPO Activity

MPO present in the gastric contents was assessed according to the protocols discussed in the literature. Gastric tissue pellets were initially suspended in a solution of PBS (50 mM), and 0.5% of C19H42BrN (hexadecyl trimethyl ammonium bromide), and the pH was maintained at 6.0. The gastric tissue content was solubilized by sonication and the heat produced during the procedure was stabilized by a cooling cycle, followed by centrifugation at 25,000 pm and 4°C for 10 min. MPO activity was estimated by recording the absorbance at 460 nm using H2O2 (0.005%) and o-dianisidine dihydrochloride.

Estimation of Nitric Oxide (NO)

In the current investigation, the activity of NO was determined. The concentration of nitrite produced by the oxidation of NO is calculated using the Griess method, which is based on a diazotization reaction.

Markers Estimation by ELISA

The activity of inflammatory biomarkers in the stomach tissue was estimated by ELISA, according to the manufacturer's guidelines. The activity of inflammatory molecules, such as IL-1β, IL-6, PGE2, TNF-α, ICAM-1, and Bcl-2 was measured.

Histopathological Investigation

A histopathological study was performed using the techniques described in the literature. Tissue samples were prepared from glandular sections of the stomachs of all experimental groups, and tissue specimens were treated overnight with 10% buffer solution (neutral formalin) for histopathological analysis. Tissue specimens were prepared from glandular sections of the stomachs of all experimental groups, and all tissue specimens were treated overnight with neutral formalin (10%) buffer for histopathological analysis. The tissue samples were placed in paraffin using a microtome and sliced into 3 m tissue block slices, followed by staining of each segment with hematoxylin and eosin for histological observations.

Statistical Analysis

GraphPad Prism statistical tool (Version 8.0.2, GraphPad Software Inc., San Diego, CA, USA) was used to analyze the data obtained from all experimental procedures. The findings were subjected to standard error of the mean (SEM). The ulcer score was analyzed using a non-parametric test (Kruskal-Wallis) and the significance of the other findings, and a one-way analysis of variance (ANOVA) was used to compare the variables of each experimental animal group using a posthoc test (Tukey's post hoc test). Statistical significance was determined using a p-value <0.05.

Results

Determination of Ulcer Index

In this study, the control group (ethanol) demonstrated an elevated ulcer index compared with the normal group (p<0.01). Kruskal-Wallis test revealed that the treatment with barbigerone at 10 and 20 mg/kg led to a remarkable reduction in the ulceration index [F (3, 20)=248.3, (p<0.0001)] compared to ethanol control as depicted in Figure 2.

Estimation of pH

In this study, the ethanol control group showed a reduced pH on day 8 of the experiment, resulting in an acidic pH compared to the normal treatment groups (p<0.01). The animal group that was administered both doses of barbigerone showed results in terms of alkaline pH [F (3, 20)=35.18, (p<0.0001)], which were comparable to those of the control treatment group (Figure 3).

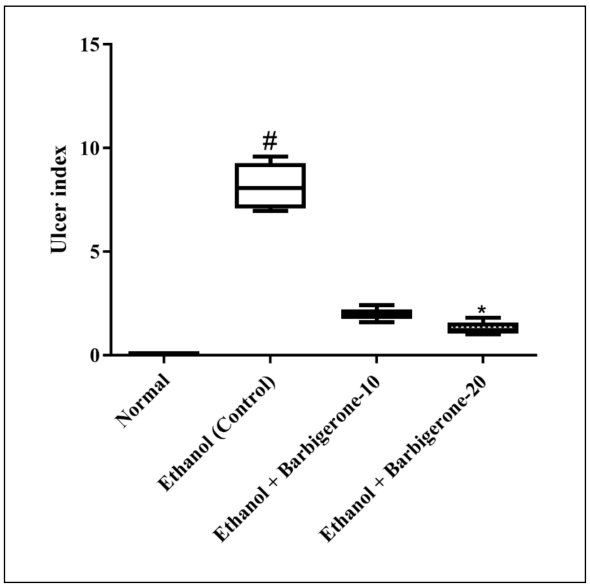


Figure 2. The consequence of barbigerone on the ulcer index. $^{\#}p$<0.001 *vs.* normal, *p<0.05 *vs.* ulcer control (Kruskal-Wallis test).

Total Acidity and Pepsin Assessment

The ethanol control group had notably higher total acid and pepsin activities than the control group (p<0.001). One-way ANOVA followed by Tukey's post hoc test revealed that the barbigerone-treated group (10 and 20 mg/kg) nota-

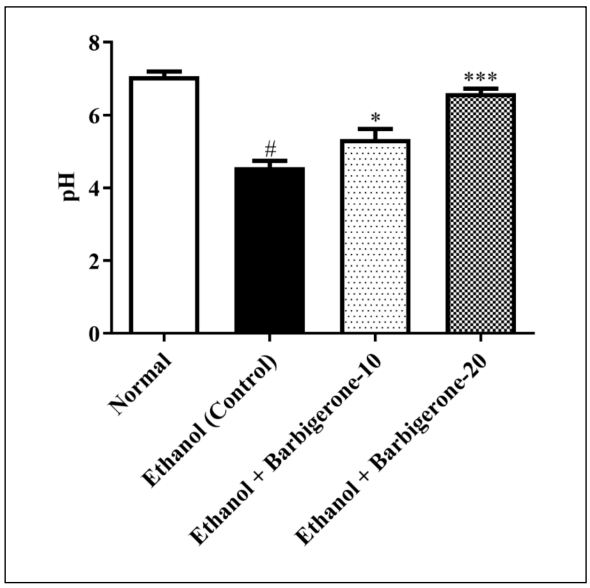


Figure 3. The consequence of barbigerone on pH. $^{\#}p$<0.001 *vs.* normal, *p<0.05 and ***p<0.0001 *vs.* ulcer control (One-way ANOVA followed by Tukey's test).

bly recovered the total acid [F (3, 20)=26.43, (p<0.0001)] and pepsin levels [F (3, 20)=49.59, (p<0.0001)] compared to the ethanol-control groups (Figures 4 and 5).

Estimation of Biochemical Markers

The effect of barbigerone treatment on GSH, MDA, SOD, and CAT activities in rodents with ethanol-induced gastric ulcers is shown in Figure 6 A-D. The ethanol control group showed a significant increase in MDA levels (p<0.001). SOD, GSH, and CAT expression markedly decreased (p<0.001) in the ethanol control group compared to that in the normal group. One-way ANOVA followed by Tukey's post hoc test revealed that both doses of barbigerone (10 and 20 mg/kg) resulted in a marginally maintained increase in GSH [F (3, 20)=40.52, (p<0.0001)], SOD [F (3, 20)=158.9, (p<0.0001)], and CAT [F (3, 20)=23.55, (p<0.0001)] levels in the treatment group and a decline in MDA expression [F (3, 20)=10.35, (p=0.0003)] compared to the ethanol control group (Figure 6A-D).

Assessment of Myeloperoxidase (MPO) Activity

The ethanol control group displayed a considerably elevated MPO level of MPO than the normal group (p<0.001). Animals that received barbigerone at 10 and 20 mg/kg demonstrated moderate MPO activity [F (3, 20)=12.82, (p<0.0001)] compared to the ethanol control groups. Figure 7A depicts the MPO activity in ethanol-induced gastric ulcers in rats.

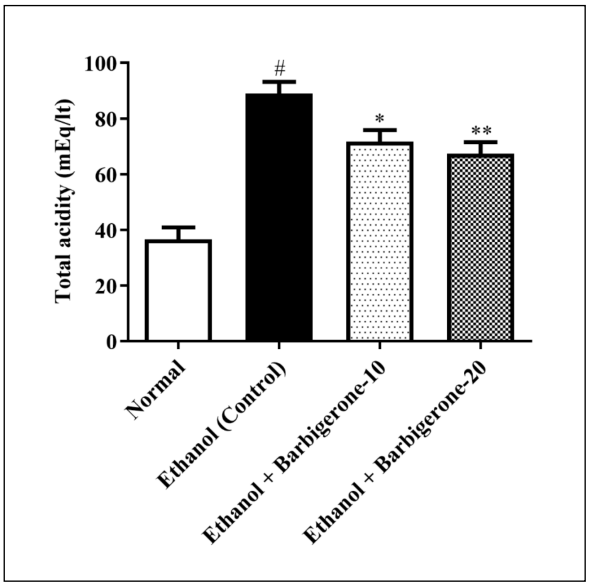


Figure 4. The consequence of barbigerone on total acidity. #p<0.001 *vs.* normal, *p<0.05 and **p<0.001 *vs.* ulcer control (One-way ANOVA followed by Tukey's test).

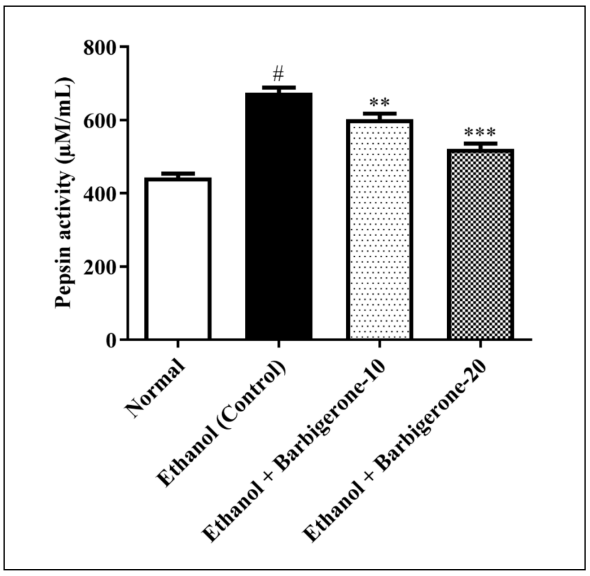


Figure 5. The consequence of barbigerone on pepsin activity. #p<0.001 *vs.* normal, **p<0.001 and ***p<0.0001 *vs.* ulcer control (One-way ANOVA followed by Tukey's test).

Nitric oxide (NO) Assay

The effect of barbigerone treatment on NO levels in ethanol-induced gastric ulcers in rats is shown in Figure 7B. In the present study, the NO activity in the ethanol-induced control group was notably lower than that in the normal group (p<0.001). One-way ANOVA followed by Tukey's post hoc test revealed that treatment with barbigerone at 10 and 20 mg/kg significantly restored NO levels [F (3, 20)=25.08, (p<0.0001)] compared to the ethanol control group.

Estimation of Markers

In this study, animals treated with ethanol showed noticeably greater activities of TNF-α, IL-6, IL-1β, and intercellular adhesion molecule-1 (ICAM-1) (p<0.001). Additionally, decreased activities of B-Cell Leukemia/Lymphoma 2 (Bcl-2) expression and prostaglandin E-2 (PGE2) (p<0.001) were observed in the ethanol control group compared to those in the normal treatment group. Furthermore, treatment with barbigerone (10 and 20 mg/kg) significantly upregulated Bcl-2 expression [F (3, 20)=28.40, (p<0.0001)] and PGE2 levels [F (3, 20)=11.13, (p=0.0002)], as well as sig-

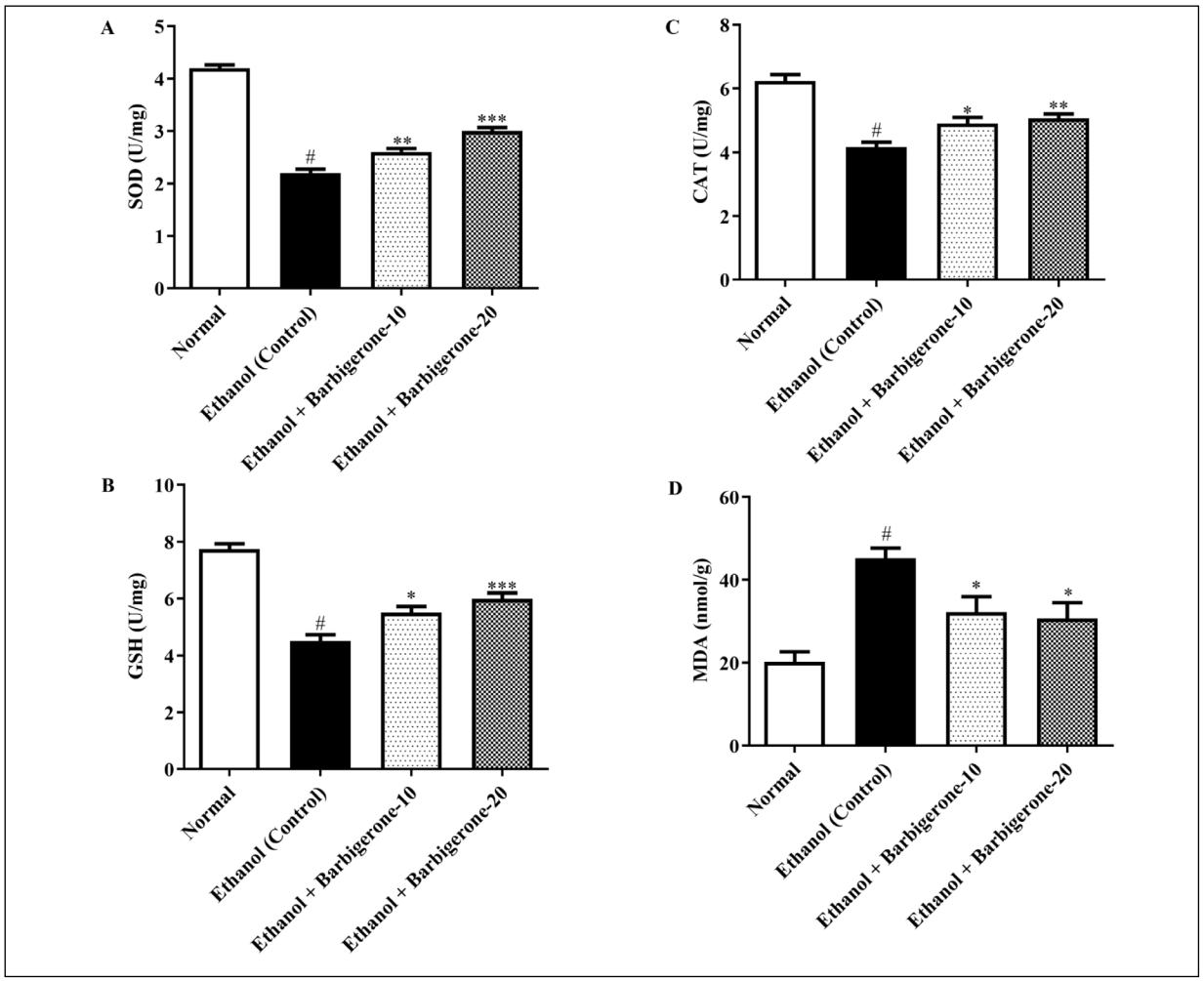


Figure 6. A-D, Effect of barbigerone on biochemical markers, i.e., (**A**) SOD, (**B**) GSH, (**C**) CAT, and (**D**) MDA. $^{\#}p<0.001$ *vs.* normal, $^{*}p<0.05$, $^{**}p<0.001$ and $^{***}p<0.0001$ *vs.* ulcer control (One-way ANOVA followed by Tukey's test).

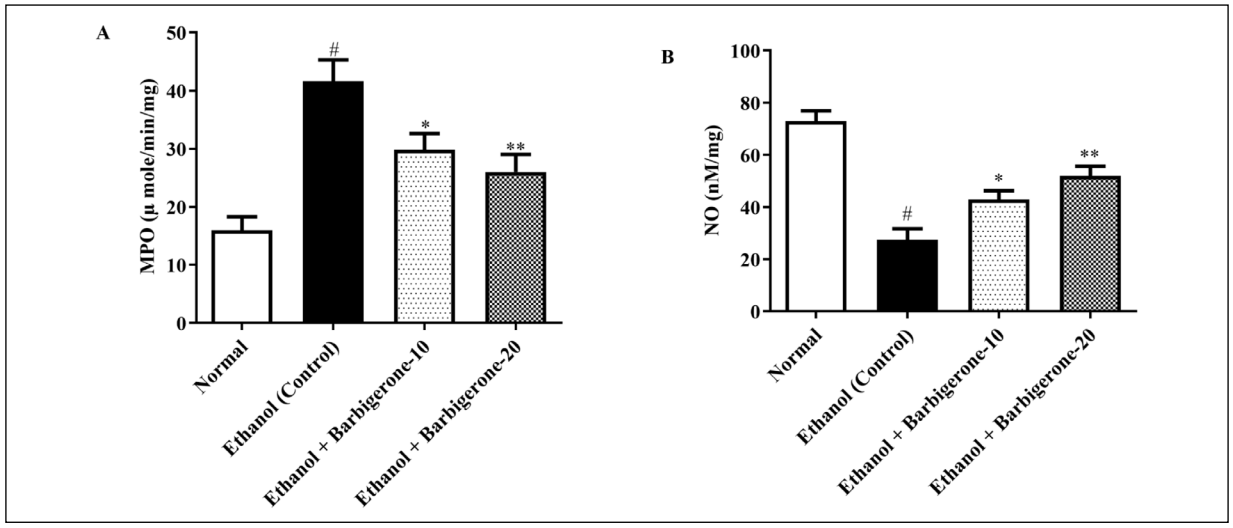


Figure 7. A-B, The consequence of barbigerone on (**A**) MPO activity and (**B**) NO assay. $^{\#}p<0.001$ *vs.* normal, $^{*}p<0.05$ and $^{**}p<0.0001$ *vs.* ulcer control (One-way ANOVA followed by Tukey's test).

nificantly downregulated TNF-α [F (3, 20)=34.95, (p<0.0001)], IL-6 [F (3, 20)=25.13, (p<0.0001)], IL-1β [F (3, 20)=81.39, (p<0.0001)], and ICAM-1 [F (3, 20)=75.25, (p<0.0001)] (Figure 8 A-F).

Histopathological Examinations

Normal histology and elongated villi were observed in the normal control group without exfoliation markings (Figure 9A). Villi in the ethanol control group exhibited histopathological alterations, including lesions, hemorrhages, and cellular necrosis (Figure 9B). Both doses of barbigerone (10 and 20 mg/kg) significantly protected against cellular necrosis caused by ethanol-induced ulcers in rats (Figure 9 C-D).

Discussion

Globally, excessive alcohol consumption has been linked to gastrointestinal disorders, such as gastric ulcers. Several *in vivo* preclinical experiments were performed in the current study using an animal model of alcohol-induced gastric ulceration. This study aimed to assess the applicability of ethanol-induced gastric ulcers in experimental animals. Recent studies[22] have also demonstrated the gastroprotective effects of plant-derived compounds as a novel therapeutic approach for treating gastric ulcers. Additionally, ethanol-induced experimental animal models have revealed detrimental impacts of alcohol intake on the gastrointestinal tract[23]. Several molecular mechanisms contribute to the pathophysiology of gastric ulcers that alter the mucosal membrane and harm the epithelial lining of the gastric mucosa[24,25]. Histopathological findings, such as decreased epithelial cells, migration of inflammatory cells, hemorrhage, and mucosal erosions, are defining characteristics of alcohol-induced gastric damage[26]. Alcohol also modifies the microscopic and

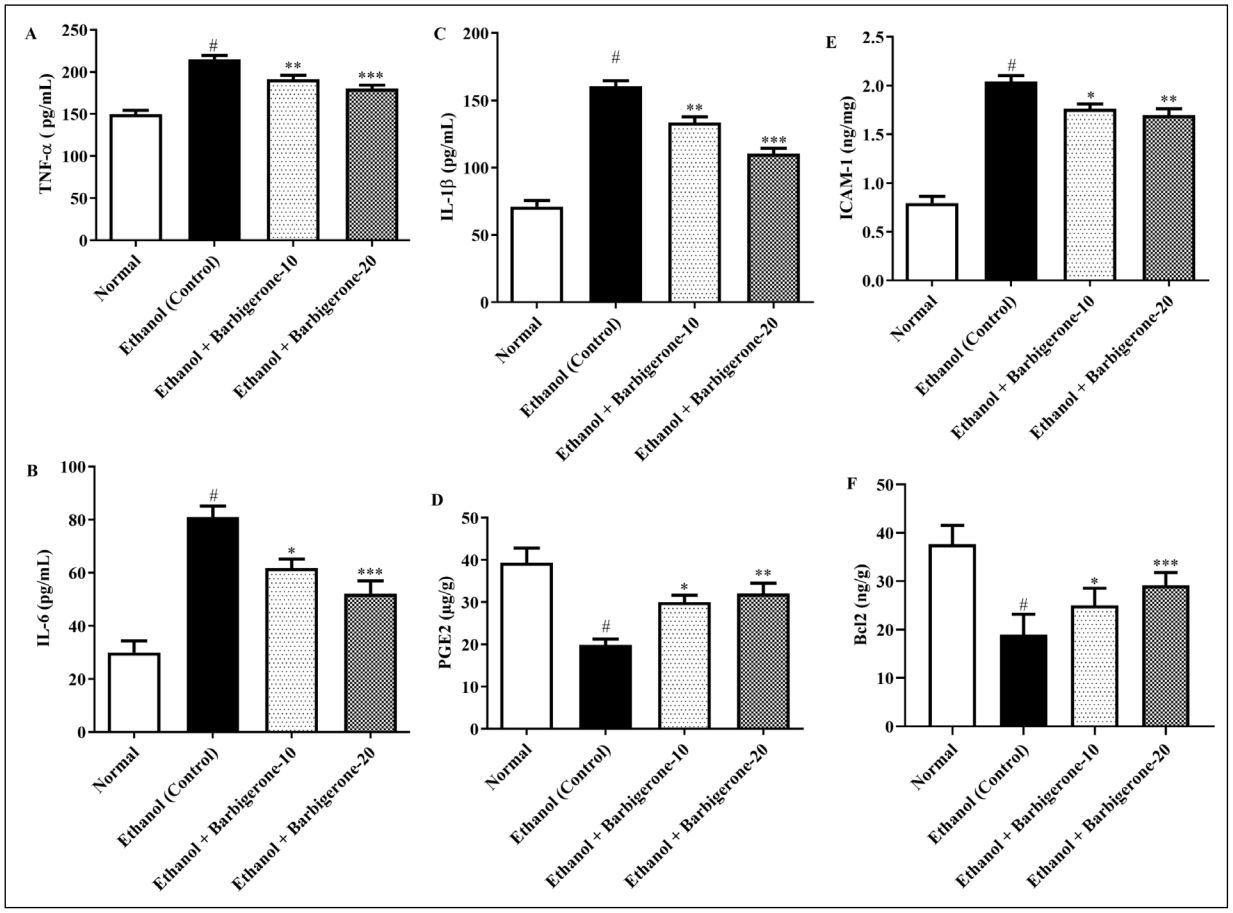


Figure 8. A-F, The consequence of barbigerone on (**A**) TNF-α, (**B**) IL-6, (**C**) IL-1β, (**D**) PGE-2, (**E**) ICAM-1, and (**F**) Bcl-2. #p<0.001 *vs.* normal, *p<0.05, **p<0.001 and ***p < 0.0001 *vs.* ulcer control (One-way ANOVA followed by Tukey's test).

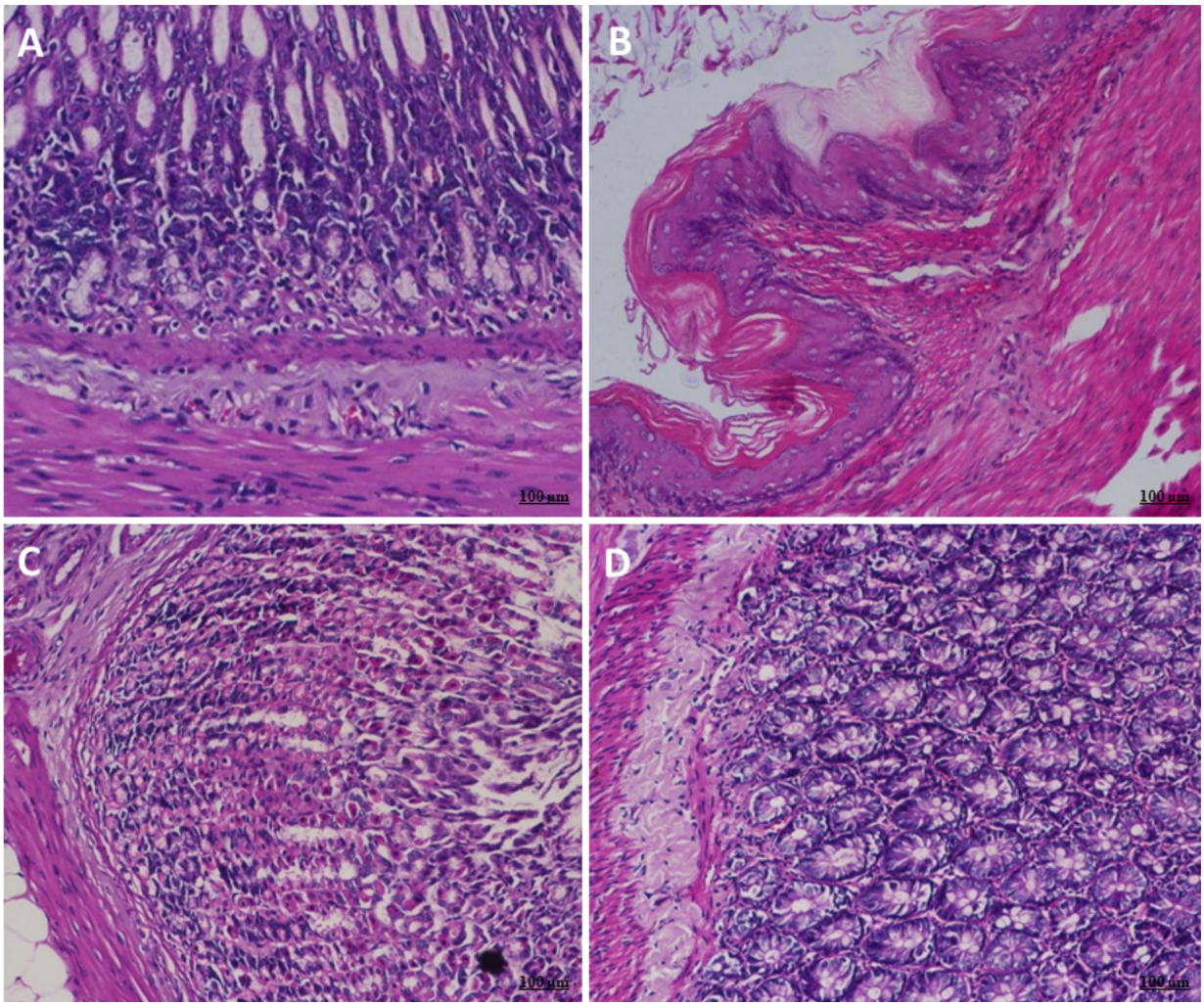


Figure 9. A-D, Histopathological changesin glandular stomach of rat stained with H&E 200x in ethanol-induced in gastric tissue; (**A**) Normal control- showing normal histoarchitecture (**B**) ethanol control- showing lesions and necrosis, histoarchitecture alterations (**C**) barbigerone 10 mg/kg - showing mild lesions and necrosis (**D**) barbigerone 20 mg/kg- showing reducing cellular necrosis and lesions.

macroscopic morphology of gastric tissue, resulting in submucosal edema and hyperemia, respectively[27].

This study examined barbigerone as a potential gastroprotective medication for ethanol-driven gastric ulcers. The present study included several comparative analyses between the animals of the normal control, ethanol control, and barbigerone-treated groups, which suggested that experimental rats treated with ethanol developed symptoms of gastric ulcers, such as an elevated ulcer index, increased MPO activity, and total acidic content. Additionally, biochemical analysis of experimental animals revealed abnormalities in the levels of biochemical indicators, such as SOD, GSH, MDA, and CAT, as well as inflammatory markers, such as TNF-α, ICAM-1, PGE2, IL-6, and IL-1β. Additionally, histopathological examination demonstrated that acute alcohol administration led to chronic mucosal injuries, characterized by lesions in the gastric villi. Earlier studies[28] have reported that oral ethanol administration in rats causes injury to the gastric mucosa by damaging the vascular and endothelial cells. Ethanol also triggers inflammatory pathways, microcirculatory disturbances, and hypoxia. Earlier studies[5] also showed that ethanol causes edema of submucosal cells and results in cellular necrosis in experimental animals. Furthermore, ethanol administration to lab animals elevates the expression of cytokines such as IL-6, IL-1β, and TNF-α, resulting in inflammation.

Ethanol overconsumption has also been reported to contribute to hemorrhagic injury in the gastric mucosal layer by impairing the mucosal barrier of the gastrointestinal tract[29].

Similarly, the results of another experiment[30,31] revealed that alcohol administration to animals noticeably elevated the ulcer index, indicating the applicability of ethanol-induced gastric ulcer disease models. The findings of preclinical experiments help the present work validate an experimental model to assess the gastroprotective properties of barbigerone against ethanol-induced gastric ulceration in rats. Earlier studies[14,32] postulated that flavonoids extracted from plants elevate the pH of gastric matter in ethanol administered to experimental animals, ultimately reversing ulceration in the stomach. The current investigation demonstrated the gastroprotective properties of barbigerone by balancing the pH of gastric juices in ethanol-induced gastric ulceration in rats. The results of this study support the gastroprotective properties of barbigerone against ethanol-induced gastric ulcers by modifying the pH of the gastric material in rats. Pepsin activity was elevated in the ethanol-treated experimental group. Previous research[33] has demonstrated that ethanol administration to animals increases pepsin activity. Moreover, it was previously reported[34,35] that plant-derived compounds reduce ulcer severity in experimental animals by increasing the pH and reducing the total acidity as well as pepsin activity. Alcohol consumption is crucial in the progression of gastric ulcers because inflammation in the stomach damages the mucosal layer by causing the migration of inflammatory cytokines in the gastric lining[36,37]. The results of this study support that ethanol dosing in animals elevates the effects of inflammatory cytokines in accordance with earlier studies. In contrast, the experimental group treated with barbigerone showed a decline in the activity of inflammatory cytokines. Earlier research[38,39] in ethanol-induced gastric ulcer animal models postulated that oxidative stress stimulates ROS and NO release. ROS activates pathways such as mitogen-activated protein kinase (MAPK) and redox-sensitive signal transduction, which regulate the expression of genes that code for pro-inflammatory cytokines. ROS and neutrophil infiltration were found to contribute to the pathogenesis of gastric ulceration by causing mucosal damage[38,39].

Previous studies[37] have shown that ethanol-induced rats show higher levels of lipid peroxidation as a result of ROS generation. Additionally, ethanol causes severe oxidative stress by significantly decreasing the levels of antioxidant enzymes such as SOD, CAT, and GSH. Furthermore, ethanol administration increases the expression of lipid peroxidation biomarker (MDA)[40,41]. Our study also showed that SOD, CAT, and GSH activities were reduced in the ethanol control group. Simultaneously, MDA levels were elevated in the ethanol-treated rats. These observations support the involvement of alcohol in the development of lipid peroxidation-mediated gastric injury in ethanol-driven gastric ulcerations. These results confirmed the involvement of alcohol in lipid peroxidation-mediated gastric injury, resulting in ethanol-induced gastric ulcerations. Similarly, barbigerone significantly reduced MDA levels and balanced the activities of GSH, SOD, and CAT in laboratory animals at both doses (10 and 20 mg/kg)[42]. Additionally, the observations of the current study imply that MPO is essential for neutrophil migration in gastric mucosa. Our findings also confirmed that ethanol administration led to an increase in MPO activity, which was balanced by barbigerone treatment in experimental animals. Another study[43] postulated that ethanol administration caused gastric damage by disturbing NO synthase. Additionally, NO synthase generates large amounts of NO, causing vascular microcirculation disturbances that lead to gastric injury[44]. In our study, animals treated with barbigerone showed a decline in NO activity compared to ethanol-induced rats. Several inflammatory markers, including TNF-α, ICAM-1, IL-6, and IL-1β, have been implicated in the pathogenesis of ethanol-triggered gastric ulcers by inducing apoptosis of gastric epithelial cells, accelerating the migration and activation of inflammatory cells and disrupting the gastric mucosal barrier[45,46]. Treatment with barbigerone (10 and 20 mg/kg) lowered gastric TNF-α, ICAM-1, IL-6, and IL-1β expression markedly compared with the ethanol control group. Treatment with barbigerone at both doses (10 and 20 mg/kg) significantly mitigated the inflammatory response by suppressing the levels of inflammatory cytokines, such as IL-6, IL-1β, and TNF-α. The findings of this study are in accordance with previously reported studies[42]. Barbigerone may indirectly influence the expression of TNF-α, ICAM-1, IL-6, and IL-1β by reducing oxidative stress, as these factors are often upregulated in response to oxidative stress. Barbigerone may exert its anti-inflammatory effects by targeting

multiple signaling pathways and molecular targets crucial in the pathogenesis of ethanol-triggered gastric ulcers[42,47,48].

According to previous literature, ethanol administration leads to abnormalities in the rat gastric tissue, such as necrosis of the mucosa, submucosal edema, and hemorrhage, which supported our results[49]. In the present study, histopathological analysis of rat gastric tissues demonstrated histopathological alterations, including lesions, hemorrhage, and cellular necrosis, in the control group. Our results showed that barbigerone treatment successfully reversed histopathological abnormalities in the gastric tissue. PGE2 exerts diverse effects on the gastrointestinal tract. A significant role of PGE2 in ulcer prevention and healing is that it regulates gastric acid secretion and the release of cytotoxic substances, as well as stabilizing the mast cell membrane and stimulating tissue repair. Gastric ulceration is caused by decreased PGE2 levels in the mucosa, which also aggravates pre-existing gastric ulcers[50]. The present study showed that the ethanol control group had reduced PGE2 levels, while barbigerone treatment moderated PGE2 levels in the gastric studies[51,52].

Bcl-2 plays a crucial role in the pathogenesis of ethanol-triggered gastric ulcers in rats. Bcl-2 expression was decreased in the gastric mucosa of animals with alcohol-triggered gastric ulcers. Decreased Bcl-2 expression contributes to gastric epithelial cell apoptosis, which leads to gastric ulcer development and progression[35]. Bcl-2 exerts its anti-apoptotic function by inhibiting mitochondrial outer membrane permeabilization, which leads to the release of cytochrome c and other proapoptotic factors from the mitochondrial intermembrane space into the cytoplasm. Thus, promoting the expression of Bcl-2 can downregulate apoptosis[53,54]. Treatment with barbigerone significantly upregulated Bcl-2 expression. By modulating Bcl-2 expression, barbigerone may promote cell survival and inhibit apoptosis in the gastric mucosa, thus potentially protecting against ethanol-induced ulcers. Anti-inflammatory, antioxidant, and anti-cancer properties of barbigerone have been reported[42,47,55,56].

Barbigerone, as a natural compound, may help in conditions such as gastric ulcers by preventing ethanol-triggered gastric ulcers in rats by restoring SOD, GSH, MDA, CAT, lipid peroxidation, and the expression of markers, including PGE2, TNF-α, IL-6, ICAM-1, IL-1β, and Bcl-2, with protection against cellular necrosis.

Limitations

The study's limitations include its short duration and small sample size. Further studies using cell-based assays, western blot analyses, and gene expression analyses are required to understand how barbigerone affects cellular processes and molecular mechanisms. Moreover, sex hormones can potentially influence the outcome of the study involving animal studies. Thus, further studies should be performed to estimate the effects of sex hormones on the observed effects.

Conclusions

The findings of the present study suggest that barbigerone has gastroprotective properties. Barbigerone demonstrated its effectiveness as an anti-ulcer agent and may be due to a reduction in ethanol-triggered gastric ulcers. The healing effect of barbigerone may be attributed to the activation of SOD, CAT, and GSH, as well as the inhibition of inflammatory molecules such as TNF-α, ICAM-1, PGE2, IL-6, IL-1β, and apoptosis by Bcl-2 regulation.

Conflict of Interest

The authors declare that they have no conflict of interests.

Funding

The Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, Saudi Arabia has funded this project, under grant No. (KEP-45-130-42).

Authors' Contribution

Conceptualization and fund acquisition: Imran Kazmi; Methodology and the first draft of the manuscript: Asma B. Omer and Nadeem Sayyed; Critical Revision of manuscript: Shareefa A. AlGhamdi, Fahad A. Al-Abbasi, Muqtader Ahmed, Amira M. Alghamdi, Sami I. Alzarea, Nadeem Sayyed, Muhammad Shahid Nadeem.

Data Availability

Study data are included in the article; further inquiries may be directed to the corresponding author.

Ethics Approval

The Institutional Animal Ethics Committee (IAEC) of Trans-genica Lab, M.S., India (IAEC/TRS/PT/023/028) approved all experimental study.

Informed Consent
Not applicable.

ORCID ID
Fahad A. Al-Abbasi: 0000-0001-5609-4913
Shareefa A. AlGhamdi: 0000-0002-6603-6116
Sami I. Alzarea: 0000-0003-4007-4023
Ryan A. Sheikh: 0000-0003-3275-0861
Amira M. Alghamdi: 0009-0004-4538-5669
Nadeem Sayyed: 0000-0003-0517-4934
Muhammad S. Nadeem: 0000-0003-4112-0925
Imran Kazmi: 0000-0003-1881-5219

References

1) Ren S, Wei Y, Niu M, Li R, Wang R, Wei S, Wen J, Wang D, Yang T, Chen X, Wu S, Tong Y, Jing M, Li H, Wang M, Zhao Y. Mechanism of rutaecarpine on ethanol-induced acute gastric ulcer using integrated metabolomics and network pharmacology. Biomed Pharmacother 2021; 138: 111490.
2) Monteiro AFM, Viana JDO, Nayarisseri A, Zondegoumba EN, Mendonça Junior FJB, Scotti MT, Scotti L. Computational studies applied to flavonoids against Alzheimer's and Parkinson's diseases. Oxid Med Cell Longev 2018; 2018: 7912765.
3) Mousa AM, El-Sammad NM, Hassan SK, Madboli AENA, Hashim AN, Moustafa ES, Bakry SM, Elsayed EA. Antiulcerogenic effect of Cuphea ignea extract against ethanol-induced gastric ulcer in rats. BMC Complement Altern Med 2019; 19: 345.
4) Li WS, Lin SC, Chu CH, Chang YK, Zhang X, Lin CC, Tung YT. The gastroprotective effect of naringenin against ethanol-induced gastric ulcers in mice through inhibiting oxidative and inflammatory responses. Int J Mol Sci 2021; 11: 11985.
5) Rahman Z, Dwivedi D, Jena G. Ethanol-induced gastric ulcer in rats and intervention of tert-butylhydroquinone: Involvement of Nrf2/HO-1 signalling pathway. Hum Exp Toxicol 2020; 39: 547-562.
6) Beiranvand M. A review of the most common in vivo models of stomach ulcers and natural and synthetic anti-ulcer compounds: A comparative systematic study. Phytomed Plus 2022; 2: 100264.
7) Scally B, Emberson JR, Spata E, Reith C, Davies K, Halls H, Holland L, Wilson K, Bhala N, Hawkey C, Hochberg M, Hunt R, Laine L, Lanas A, Patrono C, Baigent C. Effects of gastroprotectant drugs for the prevention and treatment of peptic ulcer disease and its complications: a meta-analysis of randomised trials. Lancet Gastroenterol Hepatol 2018; 3: 231-241.
8) Aziz RS, Siddiqua A, Shahzad M, Shabbir A, Naseem N. Oxyresveratrol ameliorates ethanol-induced gastric ulcer via downregulation of IL-6, TNF-α, NF-κB, and COX-2 levels, and upregulation of TFF-2 levels. Biomed Pharmacother 2019; 110: 554-560.
9) Long X, Zhao X, Wang W, Zhang Y, Wang H, Liu X, Suo H. Protective effect of silkworm pupa oil on hydrochloric acid/ethanol-induced gastric ulcers. J Sci Food Agric 2019; 99: 2974-2986.
10) Serafim CADL, Araruna MEC, Alves Junior EB, Silva LMO, Silva AO, Da Silva MS, Alves AF, Araújo AA, Batista LM. (-)-Carveol prevents gastric ulcers via cytoprotective, antioxidant, antisecretory and immunoregulatory mechanisms in animal models. Front Pharmacol 2021; 12: 736829.
11) Kuna L, Jakab J, Smolic R, Raguz-Lucic N, Vcev A, Smolic M. Peptic Ulcer Disease: A Brief Review of Conventional Therapy and Herbal Treatment Options. J Clin Med 2019; 8: 179.
12) Liang J, Dou Y, Wu X, Li H, Wu J, Huang Q, Luo D, Yi T, Liu Y, Su Z. Prophylactic efficacy of patchoulene epoxide against ethanol-induced gastric ulcer in rats: Influence on oxidative stress, inflammation and apoptosis. Chem Biol Interact 2018; 283: 30-37.
13) Liu J, Lin H, Yuan L, Wang D, Wang C, Sun J, Zhang C, Chen J, Li H, Jing S. Protective effects of anwulignan against HCl/ethanol-induced acute gastric ulcer in mice. Evid Based Complement Alternat Med 2021; 2021: 9998982.
14) Zhang W, Lian Y, Li Q, Sun L, Chen R, Lai X, Lai Z, Yuan E, Sun S. Preventative and therapeutic potential of flavonoids in peptic ulcers. Molecules 2020; 25: 4626.
15) Vu TD, Nguyen XH, Le Vu TT, Do TL, Pham MQ. Evaluation of anti-inflammatory compounds isolated from Millettia dielsiana Harms ex Diels by molecular docking method. Vietnam J Sci Technol 2022; 60: 785-793.
16) Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. J Cereb Blood Flow Metab 2020; 40: 1769-1777.
17) Gilani SJ, Bin-Jumah MN, Al-Abbasi FA, Nadeem MS, Imam SS, Alshehri S, Ahmed MM, Ghoneim MM, Afzal M, Alzarea SI, Sayyed N, Kazmi I. Protective Effect of Fustin Against Ethanol-Activated Gastric Ulcer via Downregulation of Biochemical Parameters in Rats. ACS Omega 2022; 7: 23245-23254.
18) Alharbi KS, Al-Abbasi FA, Alzarea SI, Afzal O, Altamimi ASA, Almalki WH, Shahid Nadeem M, Afzal M, Sayyed N, Kazmi I. Effects of the Anthocyanin Hirsutidin on Gastric Ulcers: Improved Healing through Antioxidant Mechanisms. J Nat Prod 2022; 85: 2406-2412.
19) Dwivedi DK, Kumar D, Kwatra M, Pandey SN, Choubey P, Lahkar M, Jangra A. Voluntary alcohol consumption exacerbated high fat diet-induced cognitive deficits by NF-κB-calpain dependent apoptotic cell death in rat hippocampus: ameliorative effect of melatonin. Biomed Pharmacother 2018; 108: 1393-1403.

20) Trabadela C, Sánchez-Fidalgo S, Miño P, Berenguer B, Quilez A, De La Puerta R, Martín M. Gastroprotective effects of Piper carpunya against diclofenac-induced gastric lesions in rats. Pharm Biol 2008; 46: 829-837.

21) Lemos LMS, Martins TB, Tanajura GH, Gazoni VF, Bonaldo J, Strada CL, da Silva MG, Dall'Oglio EL, de Sousa Júnior PT, de Oliveira Martins DT. Evaluation of antiulcer activity of chromanone fraction from Calophyllum brasiliesnse Camb. J Ethnopharmacol 2012; 141: 432-439.

22) Alves Junior EB, de Oliveira Formiga R, de Lima Serafim CA, Cristina Araruna ME, de Souza Pessoa ML, Vasconcelos RC, de Carvalho TG, de Jesus TG, Araújo AA, de Araujo Junior RF, Vieira GC, Sobral MV, Batista LM. Estragole prevents gastric ulcers via cytoprotective, antioxidant and immunoregulatory mechanisms in animal models. Biomed Pharmacother 2020; 130: 110578.

23) Beiranvand M, Bahramikia S. Ameliorating and protective effects mesalazine on ethanol-induced gastric ulcers in experimental rats. Eur J Pharmacol 2020; 888: 173573.

24) Tarnawski AS, Ahluwalia A. The Critical Role of Growth Factors in Gastric Ulcer Healing: The Cellular and Molecular Mechanisms and Potential Clinical Implications. Cells 2021; 10: 1964.

25) Sun G, Lian T, Yang B, Gu Y, Li X. Ameliorative Effect of Sargassum fusiforme polysaccharides on Oxidative Stress and Inflammation in Ethanol-induced Gastric Ulcer. Pharmacogn Mag 2019; 15: 244-252.

26) Yang Y, Yin B, Lv L, Wang Z, He J, Chen Z, Wen X, Zhang Y, Sun W, Li Y, Zhao Y. Gastroprotective effect of aucubin against ethanol-induced gastric mucosal injury in mice. Life Sci 2017; 189: 44-51.

27) Li W, Wang X, Zhi W, Zhang H, He Z, Wang Y, Liu F, Niu X, Zhang X. The gastroprotective effect of nobiletin against ethanol-induced acute gastric lesions in mice: impact on oxidative stress and inflammation. Immunopharmacol Immunotoxicol 2017; 39: 354-363.

28) Yu L, Li R, Liu W, Zhou Y, Li Y, Qin Y, Chen Y, Xu Y. Protective Effects of Wheat Peptides against Ethanol-Induced Gastric Mucosal Lesions in Rats: Vasodilation and Anti-Inflammation. Nutrients 2020; 12: 2355.

29) Park H, Cho D, Huang E, Seo JY, Kim WG, Todorov SD, Ji Y, Holzapfel WH. Amelioration of alcohol induced gastric ulcers through the administration of Lactobacillus plantarum APSulloc 331261 isolated from green tea. Front Microbiol 2020; 11: 420.

30) Memariani Z, Hajimahmoodi M, Minaee B, Khodagholi F, Yans A, Rahimi R, Amin G, Moghaddam G, Toliyat T, Sharifzadeh M. Protective effect of a polyherbal traditional formula consisting of Rosa damascena Mill., Glycyrrhiza glabra L. and Nardostachys jatamansi DC., against ethanol-induced gastric ulcer. Iran J Pharm Res 2017; 16: 694.

31) Hama Amin RR, Aziz TA. Gastroprotective effect of Azilsartan through ameliorating oxidative stress, inflammation, and restoring hydroxyproline, and gastrin levels in ethanol-induced gastric ulcer. J Inflamm Res 2022; 15: 2911-2923.

32) Serafim C, Araruna ME, Júnior EA, Diniz M, Hiruma-Lima C, Batista L. A review of the role of flavonoids in peptic ulcer (2010-2020). Molecules 2020; 25: 5431.

33) Wang XY, Yin JY, Zhao MM, Liu SY, Nie SP, Xie MY. Gastroprotective activity of polysaccharide from Hericium erinaceus against ethanol-induced gastric mucosal lesion and pylorus ligation-induced gastric ulcer, and its antioxidant activities. Carbohydr Polym 2018; 186: 100-109.

34) Minaiyan M, Sajjadi SE, Amini K. Antiulcer effects of Zataria multiflora Boiss. on indomethacin-induced gastric ulcer in rats. Avicenna J Phytomed 2018; 8: 408-415.

35) Shams SGE, Eissa RG. Amelioration of ethanol-induced gastric ulcer in rats by quercetin: implication of Nrf2/HO1 and HMGB1/TLR4/NF-κB pathways. Heliyon 2022; 8: e11159.

36) Li Q, Hu X, Xuan Y, Ying J, Fei Y, Rong J, Zhang Y, Zhang J, Liu C, Liu Z. Kaempferol protects ethanol-induced gastric ulcers in mice via pro-inflammatory cytokines and NO. Acta Biochim Biophys Sin (Shanghai) 2018; 50: 246-253.

37) Wu X, Huang Q, Xu N, Cai J, Luo D, Zhang Q, Su Z, Gao C, Liu Y. Antioxidative and anti-inflammatory effects of water extract of Acrostichum aureum Linn. against ethanol-induced gastric ulcer in rats. Evid Based Complement Alternat Med 2018; 2018: 3585394.

38) Akanda MR, Park BY. Involvement of MAPK/NF-kappaB signal transduction pathways: Camellia japonica mitigates inflammation and gastric ulcer. Biomed Pharmacother 2017; 95: 1139-1146.

39) Alzokaky AAm, Abdelkader EM, El-Dessouki AM, Khaleel SA, Raslan NA. C-phycocyanin protects against ethanol-induced gastric ulcers in rats: Role of HMGB1/NLRP3/NF-kappaB pathway. Basic Clin Pharmacol Toxicol 2020; 127: 265- 277.

40) Badr AM, El-Orabi NF, Ali RA. The implication of the crosstalk of Nrf2 with NOXs, and HMGB1 in ethanol-induced gastric ulcer: Potential protective effect is afforded by Raspberry Ketone. PLoS One 2019; 14: e0220548.

41) Selmi S, Rtibi K, Grami D, Sebai H, Marzouki L. Protective effects of orange (Citrus sinensis L.) peel aqueous extract and hesperidin on oxidative stress and peptic ulcer induced by alcohol in rat. Lipids Health Dis 2017; 16: 1-12.

42) Alharthy KM, Althurwi HN, Albaqami FF, Altharawi A, Alzarea SI, Al-Abbasi FA, Nadeem MS, Kazmi I. Barbigerone Potentially Alleviates Rotenone-Activated Parkinson's Disease in a Rodent Model by Reducing Oxidative Stress and Neuroinflammatory Cytokines. ACS Omega 2023; 8: 4608-4615.

43) Lebda MA, El-Far AH, Noreldin AE, Elewa YH, Al Jaouni SK, Mousa SA. Protective effects of miswak (Salvadora persica) against experimentally induced gastric ulcers in rats. Oxid Med Cell Longev 2018; 2018: 6703296.

44) Li WS, Lin SC, Chu CH, Chang YK, Zhang X, Lin CC, Tung YT. The gastroprotective effect of naringenin against ethanol-induced gastric ulcers in mice through inhibiting oxidative and inflammatory responses. Int J Mol Sci 2021; 22: 11985.

45) Eraslan E, Tanyeli A, Güler MC, Kurt N, Yetim Z. Agomelatine prevents indomethacin-induced gastric ulcer in rats. Pharmacol Rep 2020; 72: 984-991.

46) Lian YZ, Lin IH, Yang YC, Chao JCJ. Gastroprotective effect of Lycium barbarum polysaccharides and C-phycocyanin in rats with ethanol-induced gastric ulcer. Int J Biol Macromol 2020; 165: 1519-1528.

47) Al-Abbasi FA. Potential antidiabetic activity of barbigerone on glucose and inflammatory cytokine levels in streptozotocin-activated diabetic rats. J King Saud Univ Sci 2022; 34: 102249.

48) Alharthy KM, Althurwi HN, Albaqami FF, Altharawi A, Alzarea SI, Al-Abbasi FA, Nadeem MS, Kazmi I. Barbigerone Potentially Alleviates Rotenone-Activated Parkinson's Disease in a Rodent Model by Reducing Oxidative Stress and Neuroinflammatory Cytokines. ACS omega 2023; 8: 4608-4615.

49) Beiranvand M, Bahramikia S, Dezfoulian O. Evaluation of antioxidant and anti-ulcerogenic effects of Eremurus persicus (Jaub & Spach) Boiss leaf hydroalcoholic extract on ethanol-induced gastric ulcer in rats. Inflammopharmacology 2021; 29: 1503-1518.

50) Zhou D, Yang Q, Tian T, Chang Y, Li Y, Duan LR, Li H, Wang SW. Gastroprotective effect of gallic acid against ethanol-induced gastric ulcer in rats: Involvement of the Nrf2/HO-1 signaling and anti-apoptosis role. Biomed Pharmacother 2020; 126: 110075.

51) Fahmy NM, Al-Sayed E, Michel HE, El-Shazly M, Singab ANB. Gastroprotective effects of Erythrina speciosa (Fabaceae) leaves cultivated in Egypt against ethanol-induced gastric ulcer in rats. J Ethnopharmacol 2020; 248: 112297.

52) Zhou D, Yang Q, Tian T, Chang Y, Li Y, Duan LR, Li H, Wang SW. Gastroprotective effect of gallic acid against ethanol-induced gastric ulcer in rats: Involvement of the Nrf2/HO-1 signaling and anti-apoptosis role. Biomed Pharmacother 2020; 126: 110075.

53) Kale J, Osterlund EJ, Andrews DW. BCL-2 family proteins: changing partners in the dance towards death. Cell Death Differ 2018; 25: 65-80.

54) Tait SWG, Green DR. Mitochondria and cell death: outer membrane permeabilization and beyond. Nat Rev Mol Cell Biol 2010; 11: 621-632.

55) Li ZG, Zhao YL, Wu X, Ye HY, Peng A, Cao ZX, Mao YQ, Zheng YZ, Jiang PD, Zhao X, Chen LJ, Wei YQ. Barbigerone, a natural isoflavone, induces apoptosis in murine lung-cancer cells via the mitochondrial apoptotic pathway. Cell Physiol Biochem 2009; 24: 95-104.

56) Qiu N, Cai L, Wang W, Wang G, Cheng X, Xu Q, Wen J, Liu J, Wei Y, Chen L. Barbigerone-in-hydroxypropyl-β-cyclodextrin-liposomal nanoparticle: preparation, characterization and anti-cancer activities. J Incl Phenom Macrocycl Chem 2015; 82: 505-514.