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ORIGINAL ARTICLE

Gastroprotective effect of vanillic acid against ethanol-induced gastric injury in rats: involvement of the *NF-κB* signalling and anti-apoptosis role

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Abstract

Background Vanillic acid (VA; 4-hydroxy-3-methoxybenzoic acid) is a flavouring agent found in various natural sources such as olives, fruits, and green tea. While VA exhibits numerous pharmacological effects, its potential protective effects against gastric injury warrants further investigation. Therefore, the primary objective of this study is to elucidate investigate the gastroprotective properties of VA against ethanol-induced gastric injury.

Methods and results Rats were orally administered either saline or VA at different doses (50, 100, and 200 mg/kg/day), with omeprazole (20 mg/kg) serving as a positive control, for fourteen consecutive days before ethanol administration. Blood and gastric tissue samples were collected one hour after ethanol administration for biochemical, molecular, and histological analyses. Pre-treatment with VA before ulcer induction alleviated both macroscopic and microscopic damage. It also increased antioxidant glutathione levels and decreased malondialdehyde and myeloperoxidase activity, along with reducing inflammatory markers such as tumour necrosis factor (TNF)-α, interleukin (IL)-6, and nuclear factor kappa B (NF-κB). Additionally, VA pre-treatment reversed the elevation of Bax mRNA expression and gastric caspase-3 levels induced by gastric damage. It also mitigated the reduction in Bcl-2 mRNA expression.

Conclusion These findings suggest that VA exerts protective effects against ethanol-induced gastric injury in rats. It achieves this by augmenting gastric antioxidant capacity and mitigating oxidative, inflammatory, and apoptotic damage.

Keywords Vanillic acid · Gastric ulcer · Oxidative damage · Apoptosis · Inflammation

Introduction

Gastric ulcer stands as a prevalent gastrointestinal disease affecting four million people annually worldwide, with a general prevalence of 5–10% [1, 2]. The aetiology and pathogenesis of gastric ulcer remain partially understood. However, they are primarily linked to an imbalance between aggressive and defensive factors, particularly on the luminal surface of epithelial cells [3].

Helicobacter pylori (H. pylori) infection, excessive intake of non-steroidal anti-inflammatory drugs (NSAIDs), excessive alcoholconsumption, smoking, and physiological stress are additional factors contributing to the onset of gastric ulcers [4].

Alcohol intake is a significant risk factor for inducing mucosal lesions, which are dose-dependent and often reversible under acute conditions [5]. Excessive alcohol consumption can lead to gastric mucosa damage as well as gastric ulcer and apoptosis of vascular endothelial cells due to heightened levels of oxidative stress and inflammatory cytokines [6, 7]. Animal models of ethanol-induced gastric ulcers share a pathogenesis similar to human ulcers [4]. These models, particularly in rats, are widely used to assess acute gastric ulcer treatments [8]. The lesions in the gastric mucosa in acute ethanol-induced gastric ulcers can be easily

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and reliably induced. This is achieved by administering varying concentrations of concentrated ethanol directly into the stomach [9].Following ethanol administration, rapid penetration of the gastrointestinal mucosa occurs, leading to direct and indirect damage to endothelial cells in both superficial and deep capillaries and venules. This ultimately results in haemorrhagic erosion and ulcer formation [10].

Various drugs, including antibiotics and proton pump inhibitors (such as omeprazole), are commonly employed in the treatment and prevention of gastric ulcers [11]. However, these pharmaceuticals may be associated with adverse effects. Both preclinical and clinical investigations have indicated that medicinal plants and their chemical constituents exhibit efficacy in preventing and treating gastric ulcers. They often have fewer adverse effects compared to conventional drugs [12].

Vanillic acid (VA; 4-hydroxy-3-methoxybenzoic acid) is an oxidized form of vanillin (4-hydroxy-3 methoxybenzaldehyde) and serves as an intermediate product in the conversion of ferulic acid to vanillin [13]. This phenolic compound is found in various plants and fruits and is utilized in the food industry as a flavouring agent. It can be synthesized and extracted from many natural sources such as olives, fruits, and green tea [14]. VA exhibits antimicrobial, anti-inflammatory, anti-apoptotic, and antioxidant properties by inhibiting free radical scavenging activity and lipid peroxidation [15–17]. Previous research by Kim et al. demonstrated that VA significantly attenuated chronic intestinal inflammation in an ulcerative colitis model [18]. The aim of the present study is to investigate gastro protective effects of VA and elucidate its potential underlying mechanism in rats with ethanol-induced gastric injury.

Materials and methods

Animals

Male Sprague-Dawley rats (250–310 g, 12 weeks old) were obtained from the Sakarya University Animal Centre (SÜDETAM). They were housed in a temperature-controlled room at 22 ± 2 °C with a humidity of 65–70% and a constant 12-hour light/dark cycle. Rats were fed with standard rat chow and water *ad libitum*. All experimental procedures were conducted in accordance with Turkish laws governing animal experimentation, following the guidelines outlined in the Care and Use of Laboratory Animals by the National Academy of Sciences, and approved by Sakarya University Animal Care and Use Committee (approval code: 27; date: 11.05.2022).

Experimental design

The rats were randomly divided into six groups, each containing eight rats. They were orally administered with either vehicle (physiological saline), vanillic acid (VA; Sigma-Aldrich, St Louis, MO, USA) at different doses (50, 100, and 200 mg/kg), and omeprazole (20 mg/kg) as a positive control [19] for 14 consecutive days. On day 15, ethanol was applied to all ulcer groups (Fig. 1). The control group received only physiological saline solution. VA was freshly prepared and dissolved in saline at room temperature. The doses of VA were selected based on previous studies [18, 20]. However, according to current literature, long-term use of vanillic acid in rats has been found not to exhibit toxic effects [21].

Gastric acute lesions induced by ethanol

After a 24-hour fasting period, gastric damage was induced in all groups except the control group by orally administering absolute ethanol (5 ml/kg; Sigma-Aldrich, St Louis, MO, USA) [22]. One hour later, intra-cardiac blood was collected from the rats while they were under anaesthesia induced by a combination of ketamine (100 mg/kg, intraperitoneal) and xylazine (10 mg/kg, intraperitoneal). The serum was then separated by centrifugation at 3000 g for 15 min and stored at -80 °C for subsequent biochemical analysis. Gastric tissues were immediately dissected and stored at -80 °C for molecular and biochemical analyses. Additionally, some gastric samples were fixed in 10% buffered p-formaldehyde for histological examination.

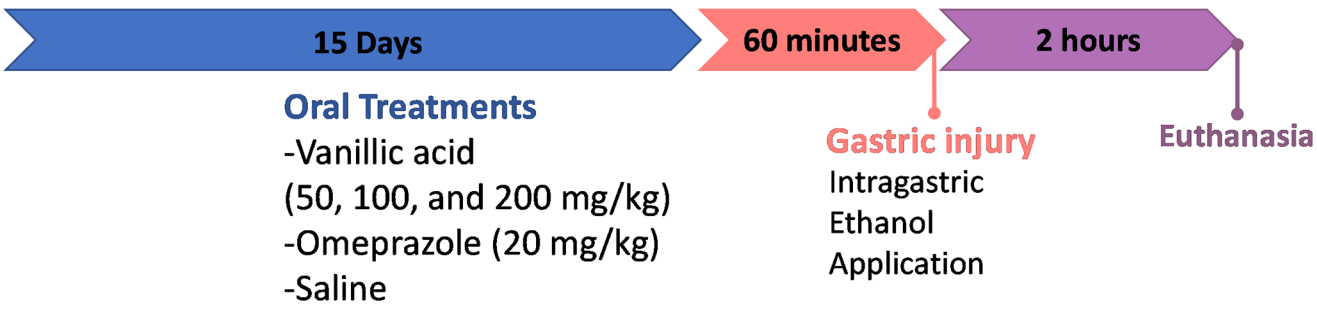

Fig. 1 The flow chart of experimental protocols

Macroscopic examination of gastric lesions

The stomach was opened along the greater curvature and rinsed with saline to eliminate any residues on the stomach surface. Stomach samples were evaluated in a double-blind manner by an expert researcher. The ulcer index score was determined based on the length of each lesion (in millimetres), with three petechiae counted as 1 mm [23]. Macroscopic damage scoring was performed according to the following arbitrary scale: 0 = no damage; 1 = blood in lumen; 2 = pin-point erosions; 3 = 1–5 small erosions (< 2 mm); 4 ≥ 5 small erosions; 5 = 1–3 large erosions (> 2 mm); 6 ≥ 3 large erosions [23].

Measurement of myeloperoxidase activity in the gastric tissues

Myeloperoxidase (MPO) is an enzyme typically present in the azurophilic granules of polymorphonuclear leukocytes and serves as an indicator of neutrophil accumulation in tissues. 2HCl was measured at 460 nm using a spectrophotometer. This was done to assess myeloperoxidase (MPO) activity in the tissue, which involves the H2O2-dependent oxidation of o-dianisidine.MPO activity was then expressed as units per gram of tissue [24].

Determination of malondialdehyde and glutathione levels in the gastric tissues

Malondialdehyde (MDA) levels were measured by observing the formation of thiobarbituric acid reagent to assess the level of lipid peroxidation products in gastric tissue samples of each animal. The results were then expressed as nanomoles of MDA per gram of tissue. Glutathione (GSH) levels were determined using the modified Ellman procedure, as outlined in previous studies. The results were presented in micromoles per gram of tissue [24]. .

Measurements of serum TNF-α and IL-6, and gastric caspase-3 levels

The levels of pro-inflammatory cytokines tumour necrosis factor (TNF)-α (E0764Ra, Bioassay Technology Laboratory, China), interleukin (IL)-6 (E0135Ra, Bioassay Technology Laboratory, China) were measured in the serum samples using rat ELISA kits following the manufacturer's instructions. To assess apoptosis, the gastric level of caspase-3 was determined using rat ELISA kits (E1648Ra, Bioassay Technology Laboratory, China).

Reverse transcriptase quantitative real-time PCR for gene expression analysis of NF-κB, Bcl-2, and Bax mRNA levels

Stomach tissue samples were homogenized using a tissue homogenizer (MTOPS SR30, Korea) to extract RNA with the PureLink RNA Mini Kit (Invitrogen, USA) according to the manufacturer's instructions. The concentration of RNAs was determined using the Qubit 4 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA). Real-time PCR was conducted in triplicate using TaqMan™ Gene Expression Assay to determine the mRNA levels of NF-κB, Bcl-2, Bax, and β-actin, employing The StepOnePlus™ Real-Time PCR system (Applied Biosystems, Foster City, CA). Data were presented as fold changes in each group relative to β-actin.

Histopathological examinations

For light microscopic investigation, gastric samples were fixed in a 10% formaldehyde solution processed by routine paraffin embedding technique. Paraffin Sect. (5 µm-thick) were stained by haematoxylin and eosin for histopathological evaluation. Each section examined at least five similar areas under a photomicroscope (Olympus BX51, Tokyo, Japan). Histopathological evaluation was made semi-quantitatively with a maximum score of 12 for gastric tissues (0, none; 1, mild; 2, moderate; 3, severe). Histopathological criteria for gastric tissues were desquamation of surface epithelium, haemorrhage, focal necrosis, and mucosal congestion, as well as degeneration of glandular cells and inflammatory cell infiltration [25].

Statistical analysis

Statistical analysis was conducted using GraphPad Prism 9.3.0 (GraphPad Software, San Diego, CA, USA). One-way ANOVA followed by the Bonferroni multiple comparisons test was performed to assess the statistical significance between experimental groups. All results were expressed as mean values with their standard error. A p-value of less than 0.05 was considered statistically significant.

Results

A significant increase in the ulcer index and macroscopic damage scores was noted in the ethanol-applied ulcer group compared to the control group ($p < 0.01 - 0.001$; Fig. 2). Furthermore, these gastric injury changes were attenuated

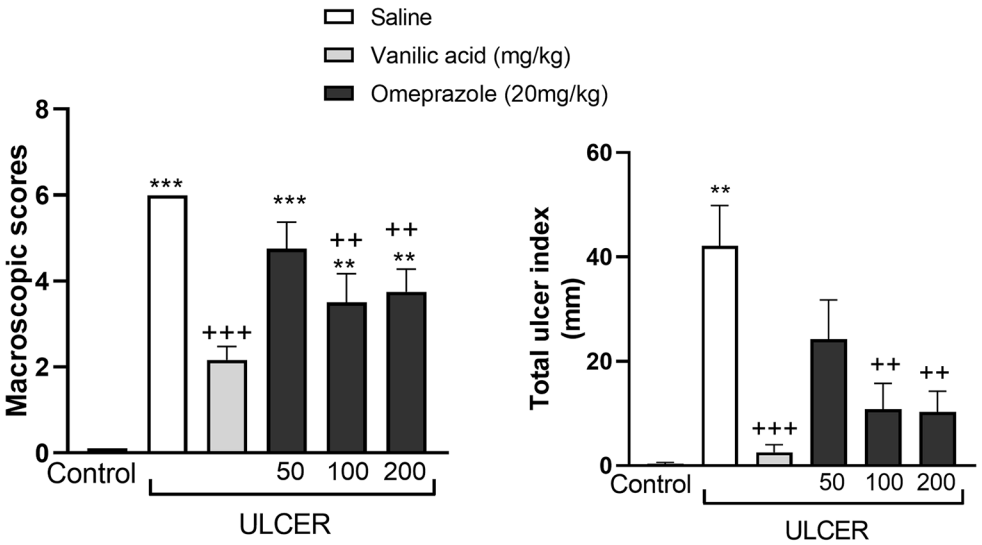

Fig. 2 Ulcer index and macroscopic damage scores of all the experimental groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared to control group; ++$p < 0.01$, +++$p < 0.001$ compared to ethanol-applied and saline-treated group

in the omeprazole-treated ulcer group ($p < 0.001$) compared to the saline-treated ulcer group. Although pre-treatment with the lower dose of VA (50 mg/kg) did not significantly affect ethanol-induced gastric injury, pre-treatment with both higher doses of VA (100 mg/kg and 200 mg/kg) significantly improved the microscopic and macroscopic damage scores ($p < 0.01 - 0.001$). This indicates that, like omeprazole, VA pre-treatment reduced gastric injury.

Histological examinations and microscopic scores revealed that the control group exhibited regular gastric mucosa and submucosa (Fig. 3). As compared to the regular gastric morphology observed in control rats, the saline-treated ulcer group displayed desquamated surface epithelium, severe degenerated glandular epithelium, mucosal haemorrhage, and inflammatory cell infiltration. Conversely, the ulcer group treated with omeprazole showed mild degeneration in mucous and glandular cells, along with inflammatory cell infiltration. Administration of VA as a pre-treatment in the ulcer group resulted in quite regular to mild degeneration in mucous and glandular cells, accompanied by mild inflammatory cell infiltration. These findings suggest that VA pre-treatment reduces inflammatory cell infiltration and mucosal degeneration .

Gastric MDA levels and antioxidant GSH content were measured to assess the extent of oxidative injury, while gastric MPO activity was evaluated as indicative of neutrophil infiltration into gastric tissue. Elevated MPO activity and MDA levels were observed in ethanol-administered and saline-treated rats compared to saline-treated control rats, while gastric GSH content decreased($p < 0.001$, Fig. 4). However, compared to saline-treated rats with gastric damage, gastric MPO activity and MDA levels were reduced ($p < 0.05$ and $p < 0.001$), and gastric GSH content tended to increase in the omeprazole-treated ulcer group. Pre-treatment with the lowest dose of VA only caused a significant decrease in MDA levels ($p < 0.001$), while higher doses of VA (100 and 200 mg/kg) inhibited the ethanol-induced increase in MDA levels and MPO activity and replenished GSH content ($p < 0.05 - 0.001$). These findings suggest that pre-treatment with VA decreased ethanol-induced stomach oxidative damage and neutrophil infiltration, similar to observations in omeprazole-treated rats.

Levels of the pro-inflammatory cytokines TNF-α and IL-6 were significantly elevated in the serum samples of ethanol-applied rats compared to the control rats ($p < 0.01$; Fig. 5). These elevations were abolished following treatment with 100 and 200 mg/kg doses of VA and omeprazole. However, a statistically significant reduction in TNF-α was observed only in the 100 mg/kg dose of VA-treated group ($p < 0.05$). Conversely, the expression level of NF-κB, a mediator of pro-inflammatory gene activation, was upregulated in gastric tissues of saline-treated and ethanol-applied rats compared to the control group ($p < 0.001$, Fig. 5; Table 1). This increase in NF-κB mRNA expression was significantly suppressed by omeprazole and all doses of VA ($p < 0.05 - 0.001$). Particularly, pre-treatment with the two higher doses of VA (100 and 200 mg/kg) in ethanol-applied rats significantly caused the downregulation of NF-κB expression levels ($p < 0.001$).

The mRNA expression of the pro-apoptotic Bax and the level of caspase-3 were significantly upregulated in the gastric tissue of the saline-treated and ethanol-applied group compared with the control group($p < 0.05 - 0.001$; Fig. 6; Table 1). Additionally, the anti-apoptotic protein Bcl-2 expression was downregulated ($p < 0.01$). These results suggest that ethanol application induced apoptotic injuries in gastric tissue. Treatment with omeprazole reduced the Bax mRNA level compared to the saline-treated and ethanol-applied group ($p < 0.01$), although no significant changes were observed in the caspase-3 and Bcl-2 expression levels.

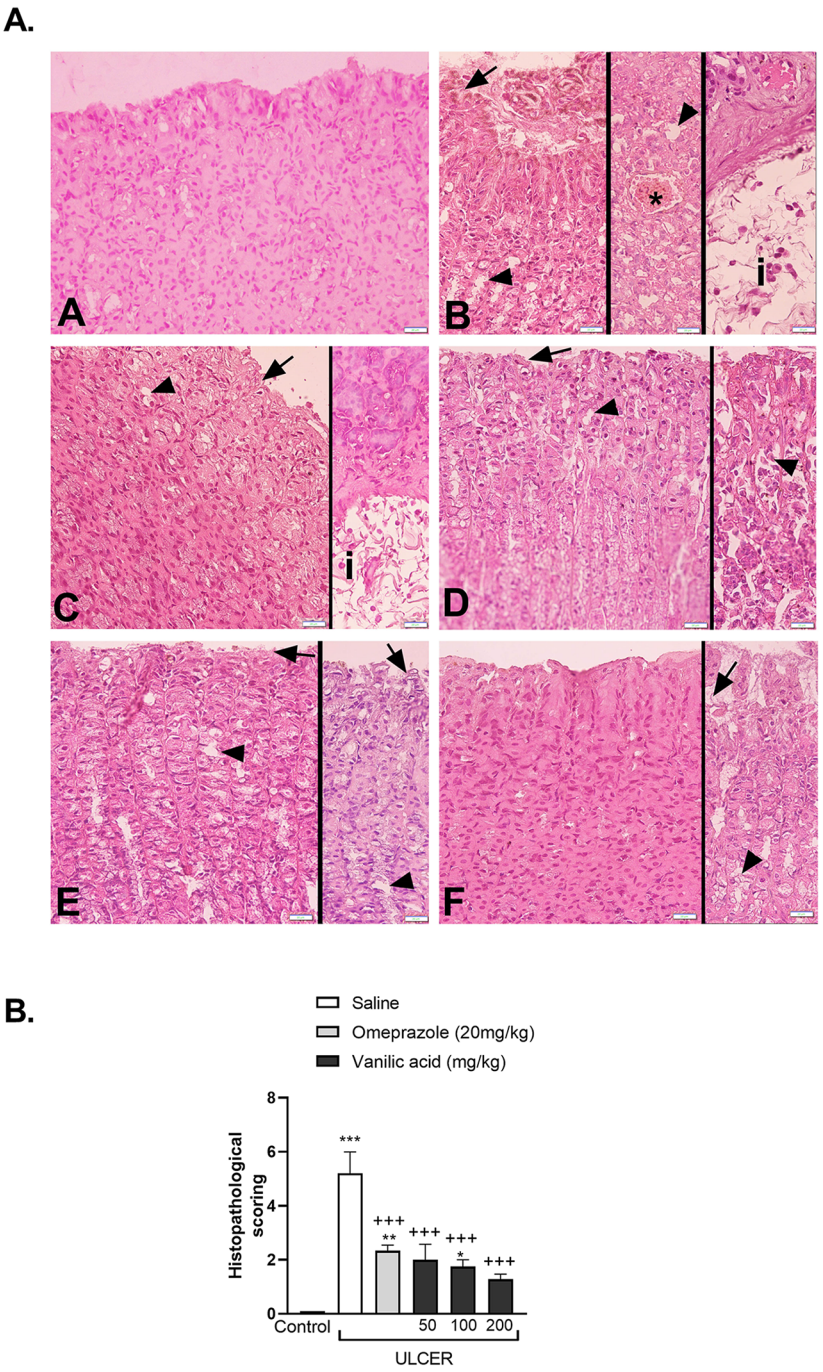

Fig. 3 Representative light micrographs (A) and microscopic scores (B) of experimental groups. Regular gastric mucosa with surface and glandular epithelium are seen in control group (**A**). Severe degeneration of surface (arrow) and glandular (arrowhead) epithelium, bleeding in mucosa (*) and inflammatory cell infiltration (i) are seen in ethanol-applied group (**B**). Mild degeneration of surface (arrow) and glandular epithelium (arrowhead) and inflammatory cell infiltration (i) are seen in ethanol-applied + Omeprazole (20 mg/kg) group (**C**). Quite regular to mild degeneration of surface (arrow) and glandular epithelium (arrowhead) are seen in ethanol-applied plus vanillic acid (VA) 50 $mg.kg^{-1}$ (**D**), ethanol-applied plus VA 100 $mg.kg^{-1}$ (**E**), and ethanol-applied plus VA 200 $mg.kg^{-1}$ (**F**) groups. Haematoxylin and eosin staining. Original magnification: 400x, scale bar: 20 µm

Pre-treatment with a 50 mg/kg dose of VA significantly suppressed the elevation of Bax expression ($p < 0.001$), whereas other doses of VA did not result in statistically significant changes. Gastric Bcl-2 mRNA expression was significantly upregulated by the two higher doses of VA. Furthermore, VA pre-treatment at all given doses reduced gastric caspase-3 levels ($p < 0.01 - 0.001$).

Discussion

The results of the current study suggest that ethanol application led to gastric lesions, increased oxidative and inflammatory damage, as evidenced by elevated lipid peroxidation, neutrophil infiltration, levels of pro-inflammatory cytokines, and reduced antioxidant GSH content, along with increased apoptosis in gastric tissue. Pre-treatment with VA before ethanol application in rats reduced lipid peroxidation and neutrophil infiltration in gastric tissue, while also

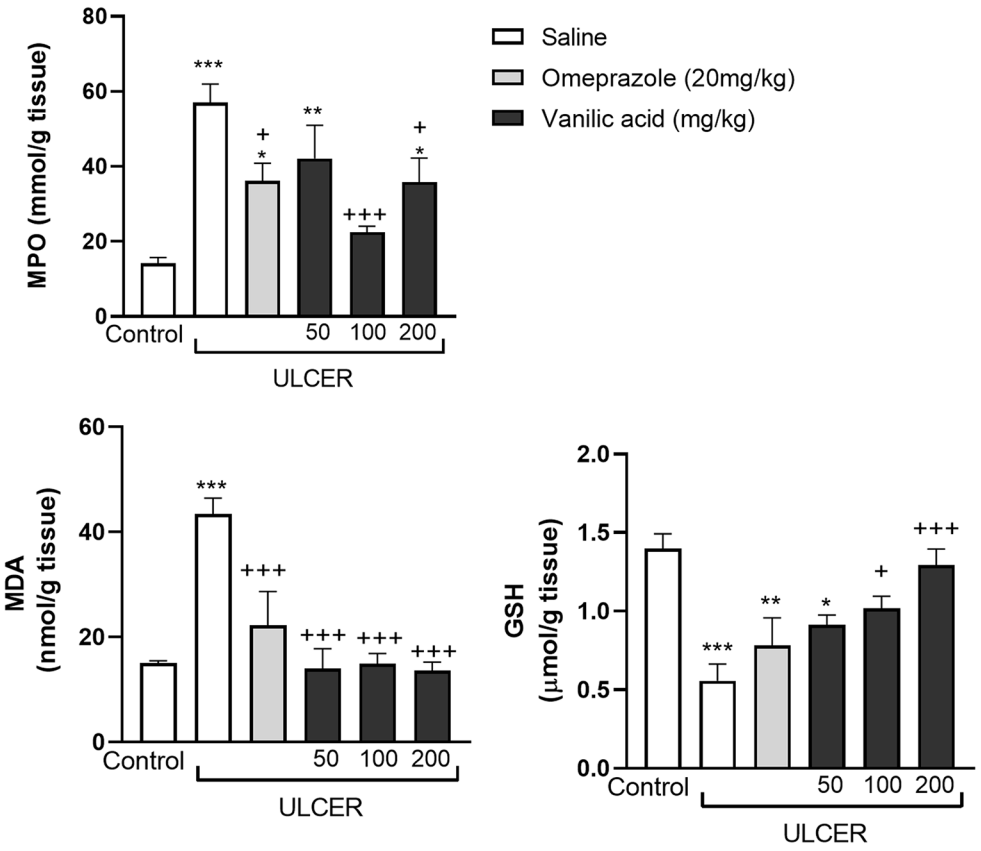

Fig. 4 Gastric levels of malondialdehyde (MDA), myeloperoxidase (MPO) activity and glutathione (GSH) levels of rats in all the experimental groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared to control group; +$p < 0.05$, +++$p < 0.001$ compared to ethanol-applied and saline-treated group

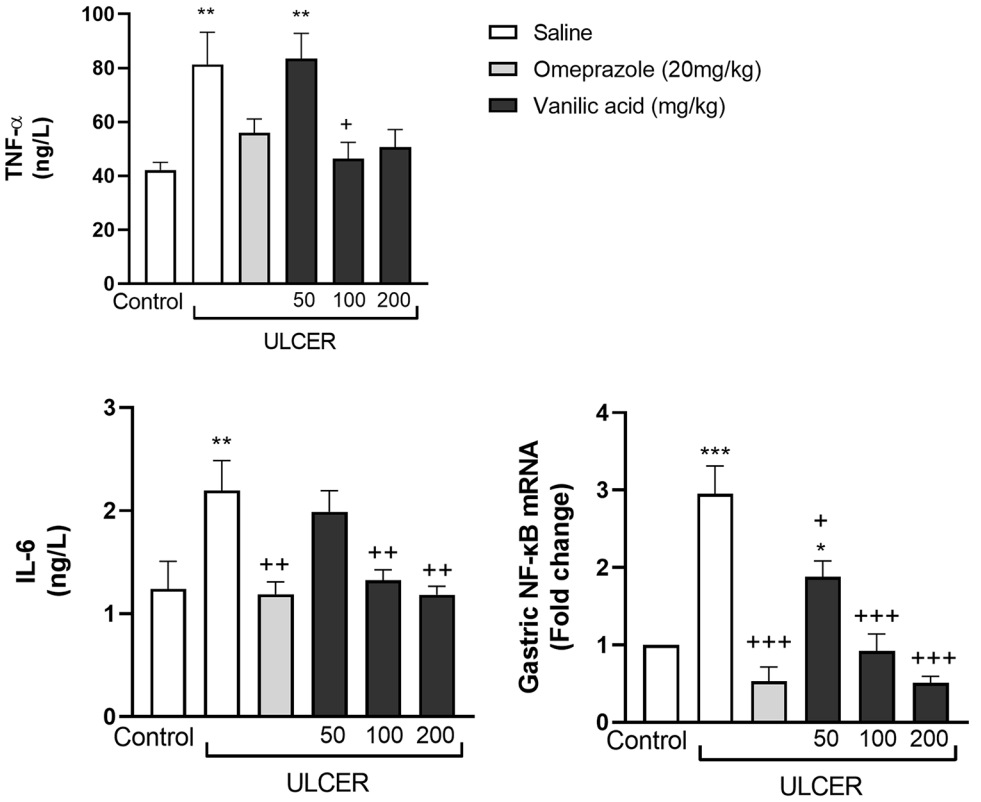

Fig. 5 Serum tumour necrosis factor (TNF)-α, interleukin-6 (IL-6), and gastric mRNA expression of nuclear factor kappa B (NF-κB) in all the experimental groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared to control group; +$p < 0.05$, ++$p < 0.01$, +++$p < 0.001$ compared to ethanol-applied and saline-treated group

Table 1 Comparison of mean $2^{-\Delta\Delta CT}$, fold change and p-value of ethanol-applied ulcer groups. VA: Vanillic acid

		Bax			Bcl-2			NF-κB		
		$2^{-\Delta\Delta CT}$	Fold Change	*p*	$2^{-\Delta\Delta CT}$	Fold Change	*p*	$2^{-\Delta\Delta CT}$	Fold Change	*p*
	Saline	0.564	2.23	0.014	0.001	0.48	0.669	0.005	2.88	0.001
	Omeprazole (20 mg/kg)	0.519	2.05	0.002	0.000	0.30	0.001	0.001	0.30	0.011
VA	50	0.210	0.83	0.662	0.001	0.40	0.017	0.003	1.84	0.008
mg/kg	100	0.603	2.38	0.003	0.001	2.76	0.003	0.002	0.94	0.554
	200	0.782	3.09	0.009	0.001	2.10	0.019	0.001	0.76	0.730

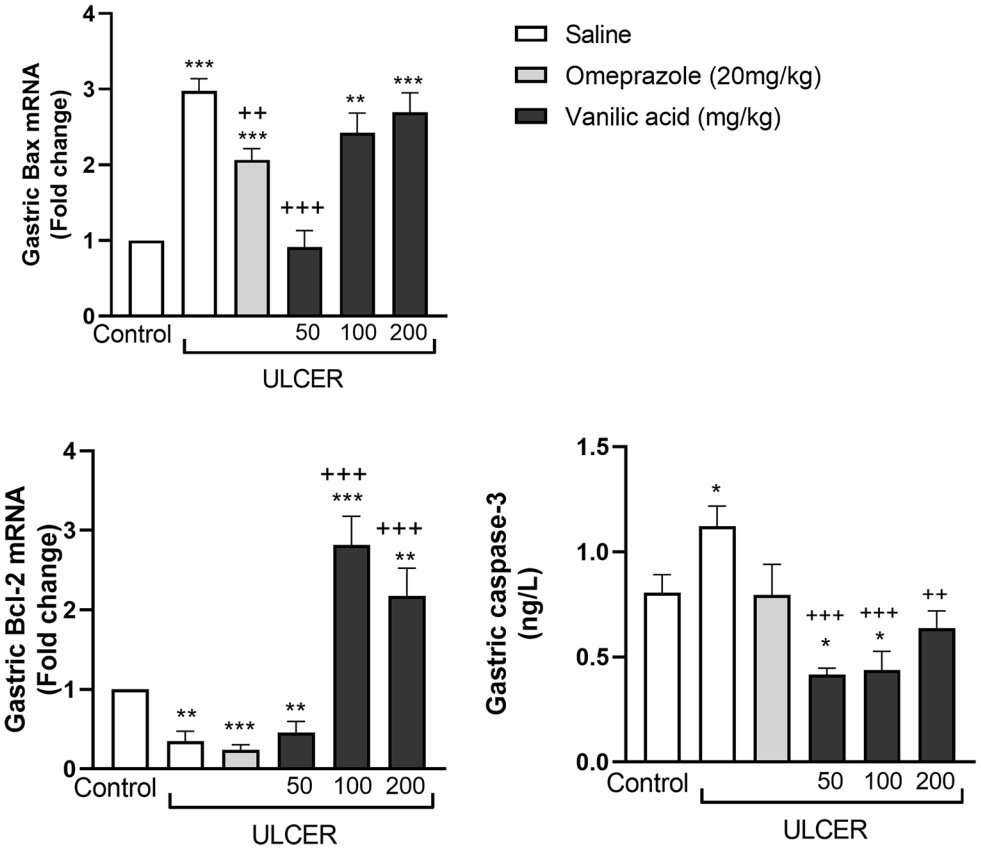

Fig. 6 Gastric mRNA expressions of Bax and Bcl-2, and gastric caspase-3 levels in all the experimental groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared to control group; ++$p < 0.01$, +++$p < 0.001$ compared to ethanol-applied and saline-treated group

replenishing depleted antioxidant GSH levels. Additionally, VA pre-treatment lowered the serum levels of pro-inflammatory cytokines IL-6 and TNF-α and downregulated the expression of NF-κB. Moreover, VA pre-treatment reversed the elevations in Bax mRNA expression and gastric caspase-3 levels induced by ethanol application, while also reversing the reduction in Bcl-2 expression. These results show that VA provides protective effects against ethanol-induced gastric injury in rats by enhancing gastric antioxidant capacity and mitigating oxidative, inflammatory, and apoptotic damage.

Excessive and prolonged alcohol consumption is associated with various gastrointestinal complications, including gastritis, stomach ulcers, and stomach bleeding [26]. The damage inflicted upon gastric tissue by ethanol arises from several factors, including the generation of reactive oxygen species (ROS), induction of apoptosis, inflammation, and a decrease in antioxidant capacity [27]. .

Oxidative stress plays a crucial role in ethanol-induced damage to the gastric mucosa by directly contributing to the production of ROS and depleting levels of GSH, consequently reducing antioxidant activity [28]. The present findings revealed that ethanol administration induces gastric damage by decreasing the antioxidant GSH content and increasing the level of MDA, an indicator of lipid peroxidation. Therefore, mitigating oxidative stress and augmenting antioxidant capacity are the main focus of the therapeutic approaches searching for the potential treatment of gastric ulcers.Previous studies have highlighted the antioxidant and free radical scavenging properties of VA in various experimental models [29, 30]. Previous studies found that VA application reduces lipid peroxidation and increases antioxidant enzyme levels in rats with testicular

damage or hypertension [30, 31]. However, VA suppressed oxidant damage in mitomycin C-induced genotoxic damage, suggesting that the hydroxyl group of VA is a possible factor contributing to this activity [32]. VA has also been described to decrease cyclooxygenase-2 (COX-2) and monocyte chemoattractant protein-1 levels, ameliorated insulin resistance, and suppressed phosphoinositide-3-kinase and glucose transporter-2 in high-fat diet-fed rats [33]. Moreover, it demonstrated the ability to scavenge hydroxyl superoxide anion and lipid radicals. Consistent with these previous studies, pre-treatment with VA in our study replenished depleted GSH content. It also reduced lipid peroxidation, demonstrating the inhibitory effect of VA on oxidative stress-induced gastric tissue injury caused by ethanol.

Nuclear factor kappa B (NF-κB), a transcription factor regulating various biological processes, is activated through phosphorylation of the inhibitors of κB (IκB) kinases (IKK) complex, leading to increased production of pro-inflammatory cytokines such as TNF-α and IL-6 [34]. Clinical and experimental studies have demonstrated significant NF-κB activation in gastric ulcers, suggesting its potential as a target for suppressing gastric inflammation [35, 36]. Previous experimental researches have shown that VA treatment exerts antioxidant and anti-inflammatory effects in brain and renal injury by suppressing NF-κB activity [16, 37]. Our findings revealed a significant upregulation of gastric NF-κB mRNA expression in ethanol-applied rats, which was suppressed by VA treatment, indicating that VA's protective effect against ethanol-induced gastric injury may involve the suppression of pro-inflammatory cytokines through the NF-κB signalling pathway. Elevated cytokine production can disrupt the mucosal barrier and activate inflammatory cells like neutrophils, lymphocytes, and macrophages at the site of inflammation [38]. In ethanol-induced gastric injury, inflammation was observed with increased TNF-α, IL-1β and IL-6 levels as well as reduced IL-10 levels in the serum and blood plasma samples [34, 39]. Consistent with previous studies, our results showed elevated TNF-α and IL-6 serum levels following ethanol application, which were suppressed by VA pre-treatment, confirming VA's anti-inflammatory action against ethanol-induced gastric damage. VA administration has previously been shown to reduce IL-6 levels and suppress cyclooxygenase-2 and NF-κB p65 expression in dextran sulphate sodium-induced ulcerative colitis [18]. Additionally, VA inhibited TNF-α and MPO elevation in cisplatin-induced ovarian toxicity [40]. Zhao and Yang reported that VA exhibits anti-inflammatory effects against lipopolysaccharide-induced human lung fibroblasts by inhibiting the mitogen-activated protein kinase and NF-κB pathways [41]. In our study, we provide the first evidence that VA exhibits gastroprotective effects against ethanol-induced gastric damage by exerting anti-inflammatory action through NF-κB mediation. However, further research is necessary to clarify the exact signalling mechanisms responsible for VA's beneficial effects on gastric injury.

Neutrophil infiltration into the gastric mucosa plays a key role in inflammation development and triggers the synthesis and release of various pro-inflammatory cytokines during ethanol-induced gastric ulcers [42]. MPO activity serves as a marker to assess neutrophil infiltration in tissues due to its strong correlation with tissue neutrophil content [43]. Our findings indicate that gastric MPO activity increased in ethanol-applied rats, confirming the involvement of neutrophil infiltration in gastric damage development. Consistent with these results, histopathological examination of gastric tissue sections from the saline-treated ulcer group revealed mucosal haemorrhage and inflammatory cell infiltration. Pre-treatment with VA significantly reduced MPO activity, inflammatory cell infiltration, and mucosal degeneration, suggesting that VA's gastroprotective effect may involve prevention of neutrophil infiltration. In line with our observations, previous studies have shown that VA attenuates inflammatory pain by inhibiting neutrophil recruitment, oxidative stress, cytokine levels, and NF-κB activation in mice [44]. Therefore, as seen in other inflammatory models, the protective effects of VA on gastric injury appear to include its antioxidant and anti-inflammatory actions.

Apoptosis, a form of cell death, significantly contributes to gastric mucosa damage induced by a variety of intrinsic and extrinsic factors [45]. In ethanol-induced apoptosis, upregulation of pro-apoptotic proteins such as caspase-3 and Bax, along with downregulation of the anti-apoptotic protein Bcl-2, leads to dysfunction of the gastric mucosa [46]. Potential gastroprotective agents aim to decrease the Bax/Bcl-2 ratio, thereby reducing caspase-3 expression and inhibiting apoptosis [47]. Our findings indicate that pre-treatment with VA suppressed ethanol-induced gastric apoptotic injury, evidenced by an increase in Bcl-2 mRNA levels and a decrease in caspase-3 levels in gastric tissue, highlighting the anti-apoptotic effect of VA on gastric injury. Consistent with these results, VA has been shown to exert anti-apoptotic effects by upregulating Bcl-2 mRNA expression and downregulating the expression of caspase-3 and Bax mRNA, thereby protecting the kidneys against methotrexate-induced nephrotoxicity [48]. Prince et al. reported that pre-treatment with VA reduced the expression of Bax and increased the expression of Bcl-2 in a myocardial infarction experimental rat model [49].

Conclusion

The results of this study reveal, for the first time, that pretreatment with VA offers gastroprotection against ethanol-induced gastric damage. This protection is achieved through enhanced antioxidant capacity, suppression of lipid peroxidation, inflammation, and apoptosis, as well as inhibition of NF-κB-related production of pro-inflammatory cytokines. However, additional molecular investigations are required to elucidate the specific molecular mechanisms underlying VA's protective effects on gastric damages.

Author contributions All persons designated as authors qualify for authorship. All persons who qualify for authorship are listed. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Data availability The data that support the findings of the present study are available from the corresponding author [SAT] upon request.

Declarations

Ethics approval Sakarya University Animal Care and Use Committee (approval code: 27; date: 11.05.2022).

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflicts of interest The authors have no relevant financial or non-financial interests to disclose.

Study conception & design of work Sevil Arabacı Tamer, Gamze Güney Eskiler, Feriha Ercan (all authors).

Data acquisition Sevil Arabacı Tamer.

Analysis and data interpretation All authors.

Drafting of the manuscript All authors.

Critical revision Sevil Arabacı Tamer.

Approval of the final version of the manuscript All authors.

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