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Antioxidative Action of Alpha-Linolenic Acid during Its Gastroprotective Effect in an Indomethacin-Induced Gastric Injury Model

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ABSTRACT: Polyunsaturated fatty acids (PUFAs) are known to have beneficial effects. In particular, the consumption of omega-3 PUFAs has recently increased because of their effects on human health. Previous studies have investigated the activity of alpha-linolenic acid (ALA; C:18 omega-3) in metabolism and inflammation models. In a murine model of colitis, treatment with ALA effectively reduced inflammation. Previously, our research group identified the protective action of docosahexaenoic acid against gastric damage caused by nonsteroidal anti-inflammatory drugs. The present study aimed to examine the impact of ALA in an indomethacin-induced gastric injury model and to determine its antioxidant activity in gastric tissue. Female Wistar rats were administered ALA over 10 days (20 mg/kg, orally). Two hours after the final ALA administration, the rats were given indomethacin (30 mg/kg, orally) to induce gastric injury. After 3 h, the rats were euthanized, and each stomach lesion was measured to determine the total damage. Stomach tissue samples were collected for the analysis of various antioxidant indicators. The results show ALA's gastroprotective effect following 10-day administration. ALA treatment significantly reduced gastric reactive oxygen species and malondialdehyde levels in the indomethacin-induced injury group. Moreover, ALA treatment decreased the levels of nitric oxide, myeloperoxidase, leukotriene B_4, and increased glutathione following indomethacin administration. These results suggest that the gastroprotective effects of ALA are likely attributed to its role in the antioxidant pathway in indomethacin-induced gastric injury.

Keywords: alpha-linolenic acid, antioxidants, gastric injury, indomethacin, oxidative stress

INTRODUCTION

Omega-3 polyunsaturated fatty acids (omega-3 PUFAs) are essential nutrients that significantly impact human health and diseases. Among omega-3 PUFAs, alpha-linolenic acid (ALA; 18:3 n-3), eicosapentaenoic acid (EPA; 20:5 n-3), and docosahexaenoic acid (DHA; 22:6 n-3) are the three most clinically significant (Shahidi and Ambigaipalan, 2018). Humans cannot naturally produce omega-3 PUFAs because of the absence of desaturase enzymes, which are needed to insert a double bond in the n-3 position. Therefore, omega-3 PUFAs must be consumed as part of the diet. The primary sources of omega-3 PUFAs include fish and other seafood that are rich in EPA and DHA. Furthermore, flax, chia, and canola seeds are excellent sources of ALA (Cholewski et al., 2018; Goel et al., 2018).

Omega-3 PUFAs not only facilitate proper development in early life stages but also mitigate the risk and progression of metabolic disorders and chronic diseases later in life. As an important component of heart cells and the gray matter, DHA can be beneficial to patients with cardiovascular (Elagizi et al., 2021; Khan et al., 2021) and neurological disorders. For example, reduced levels of DHA have been identified in the brain cells of deceased individuals who had schizophrenia and other brain disor-

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ders (Lange, 2020). Furthermore, DHA supplementation has been associated with improved cognitive recovery in severely malnourished children in Malawi (Stephenson et al., 2022).

Although omega-3 PUFAs are most noted for their neuroprotective and cardioprotective properties, increasing evidence suggests that they exhibit effects on the gastrointestinal tract. Research has shown that low doses of DHA and ALA exert protective effects against ulcerative colitis in mice. These protective effects are believed to be associated with the antioxidative effects of DHA and ALA in the colon (Yum et al., 2017; Wen et al., 2019).

In addition, DHA reduces lipoperoxidation markers, and ALA prevents an increase in leukotriene B_4 (LTB_4) levels and a decrease in glutathione (GSH) levels in the colon (Hassan et al., 2010). Furthermore, a recent study found that marine-based omega-3 PUFAs can modulate the type and quantity of the gut microbiome (Fu et al., 2021). These fatty acids may also suppress the production of proinflammatory cytokines and promote the production of anti-inflammatory eicosanoids in intestinal cells (Lee et al., 2018).

Previously, our research team examined the protective effects of DHA in a mouse model of gastric injury induced by nonsteroidal anti-inflammatory drugs (NSAIDs). The use of NSAIDs is often limited because of their gastric toxicity. Recently, a study revealed that the oral administration of DHA reduced gastric lesions in an indomethacin-induced gastric injury model. In addition, DHA administration decreased leukocyte recruitment and inflammatory markers, such as myeloperoxidase (MPO), LTB_4, and tumor necrosis factor-alpha, and increased antioxidant constituents, such as superoxide dismutase (SOD) and GSH (Pineda-Peña et al., 2018). However, the protective effects of ALA have not been evaluated in a gastric injury model. Although some studies have focused on gastric injury models induced by ethanol, ischemia/reperfusion, and water immersion stress, few have employed NSAIDs. Matsuba et al. (1998) administered linoleate (18:2 n-6) and alpha-linolenate (18:3 n-3) and found that an omega-6/omega-3 ratio close to two was beneficial for treating gastric injury. Recently, the protective effects of ALA have been investigated in models of colitis (Kim et al., 2020) and inflammatory bowel disease (Reifen et al., 2015). The present study aimed to assess the impact of ALA on an indomethacin-induced gastric injury model and to determine the antioxidant activity of ALA in gastric tissue. We hypothesized that ALA confers protective effects against indomethacin-induced gastric injury by reducing oxidative stress and enhancing antioxidant activity in gastric tissues.

MATERIALS AND METHODS

Drugs and reagents

ALA (L2376), indomethacin (I7378), and omeprazole (O104) were obtained from Sigma-Aldrich. Corn oil was used as the vehicle for ALA, whereas indomethacin and omeprazole were dissolved in 5% $NaHCO_3$ and 0.9% saline solution, respectively. All reagents were prepared directly before use.

Animals

Female Wistar rats weighing between 200 g and 250 g were sourced from the Center for Research and Advanced Studies (CINVESTAV) of the National Polytechnic Institute, Mexico City. All animal treatment, care, and surgical procedures were performed in accordance with the Mexican Official Norm for Animal Care and Handling (NOM-062-ZOO-1999). The study protocol was also approved by the Bioethics Committee of the National School of Medicine and Homeopathy of the National Polytechnic Institute, Mexico City, Mexico (registry number ENMH-CBE/007/2023) and adhered to international regulations for the care and use of laboratory animals.

Each test group comprised five to six rats. Moreover, the diet of rats consisted of standard laboratory food, and the rats had free access to tap water. To reduce coprophagy, the rats were housed in cages with wire-net floors. Before the tests, the rats were fasted for 12 h but continued to have unrestricted access to water.

Induction of gastric ulceration and assessment of gastric mucosal lesions

The rats were randomly separated into groups. They received an oral dose of indomethacin (30 mg/kg) to induce gastric injury. ALA (20 mg/kg) or omeprazole (10 mg/kg) were given daily for 10 days. On the final day, indomethacin (30 mg/kg) was administered 2 h or 30 min after ALA or omeprazole, respectively. The control group was given the corresponding vehicle mentioned previously. Three hours after the administration of indomethacin or vehicle, the rats were euthanized in a CO_2 chamber. Then, their stomachs were extracted, opened along the larger curve, and thoroughly cleaned with saline solution. To measure gastric damage, stomach images were captured and evaluated in a blinded manner. The length and width of each lesion were measured using ImageJ software (version 1.45) to calculate the total lesion area in the stomach (mm^2) for each rat. Samples from the stomach corpus were saved for subsequent analysis (Pineda-Peña et al., 2018). The doses of ALA were selected based on pilot studies performed in our laboratory (data not shown).

The percentage of gastroprotection produced by each drug regimen in each individual was calculated using the

following equation, as previously described (Pineda-Peña et al., 2018):

$$\text{Percent of protection} = (\text{IUC} - \text{IUT}) \times 100/\text{IUC}$$

where IUC is the Index of Ulcers for the Control group (mm^2) and IUT is the Index of Ulcers for the Treatment groups (mm^2).

Histological study

The gastric tissue was excised for histological evaluation and then preserved in a solution of 10% formaldehyde and phosphate-buffered saline (PBS) for 24 h. The samples were stained with hematoxylin and eosin in accordance with standard preparation procedures. The examination was performed using a high-resolution Nikon Eclipse Slog optical microscope equipped with a Nikon Digital Sight DS-2mv digital camera (Pineda-Peña et al., 2018). The pathological analysis of mucosal gastric injury and leucocyte infiltration was performed according to the following criteria (Table 1).

Determination of reactive oxygen species

Reactive oxygen species (ROS) detection was performed using 2,7-dichlorodihydrofluorescein diacetate in accordance with the method of Morales-Martínez et al. (2022) with modifications. We measured the fluorescence at an excitation wavelength of 488 nm and an emission wavelength of 525 nm. Then, the results were expressed in relative fluorescence units per milligram of protein.

Determination of gastric mucosal LTB_4 and nitric oxide

The gastric tissue was combined with PBS (10 mmol/L, pH 7.4) and treated in a circulatory water bath (37°C) for 20 min. Next, the samples were centrifuged at 5,000 *g* for 1 min, and the resulting supernatant was set aside at −70°C for subsequent analysis. The supernatant was used to determine LTB_4 levels using an enzyme-linked immunosorbent assay kit. We determined nitric oxide (NO) levels using a nitrate/nitrite colorimetric assay through the Griess reaction and expressed NO results in mM/g of tissue (Pineda-Peña et al., 2018).

Assessment of lipid peroxidation

Malondialdehyde (MDA) levels were measured using the thiobarbituric acid method (Galicia-Moreno et al., 2016; Pineda-Peña et al., 2018). The MDA levels were then calculated using an absorbance measurement taken at 532 nm, with an absorption coefficient of $1.56 \times 10^5\ cm^{-1}\ M^{-1}$. The results were expressed as nM/mg of tissue.

Measurement of gastric MPO levels

The MPO activity was determined in accordance with the method of Souza et al. (2008) with some modifications. The MPO activity, measured in U/mg of tissue, was identified at a wavelength of 450 nm.

Assessment of gastric SOD activity and reduced GSH levels

Gastric tissue was homogenized in accordance with the method of Pineda-Peña et al. (2018) to analyze SOD and GSH levels. The SOD activity was measured using the method of Sun et al. (1988) and Pineda-Peña et al. (2018) with slight modifications. The SOD activity, measured in U/mg of protein, was determined at a wavelength of 560 nm. Meanwhile, GSH levels were evaluated spectrophotometrically at 412 nm and compared against GSH standards. The GSH results were expressed as nM/g of tissue.

Fatty acid extraction and analysis

We extracted and methylated the total fatty acids from gastric tissues into fatty acid methyl esters (FAME) using an updated single-step method (Park et al., 2021). The FAME were quantified using a Hewlett Packard 5890 Series II gas chromatograph-flame ionization detector or a QP2010 instrument (Shimadzu). The setup comprised a BPX 70 column (Hewlett Packard) with H_2 as the carrier gas. The structural elements of FAME were identified through GC-covalent adduct chemical ionization tandem mass spectrometry. Occasionally, we applied an equal-weight mixture of FAME (GLC-462; Nu-Chek Prep, Inc.) to compute the response factors. Moreover, we nor-

Table 1. Histological scoring criteria

Pathological state	Score	Description
Gastric mucosal injury	0	Intact
	1	Desquamation of the epithelium
	2	Desquamation of the upper 1/3 of the mucosa
	3	Desquamation of 2/3 of the mucosa
	4	Desquamation of more than 2/3 of the mucosa
Leukocyte infiltration	0	Absent
	1	2–10/high-resolution field
	2	11–20/high-resolution field
	3	21–30/high-resolution field
	4	More than 31/high-resolution field

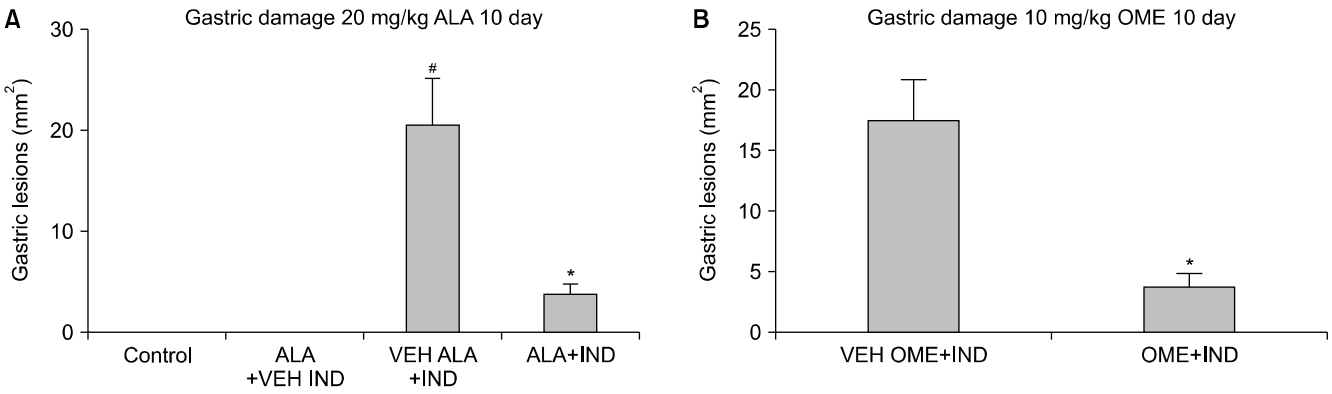

Fig. 1. Gastroprotective effects of ALA (A) and OME (B) administered for 10 days in an indomethacin-induced gastric injury model. The values are expressed as the mean±SEM (n=5–6). Statistical analysis was performed using one-way ANOVA followed by the Newman-Keuls test. $^{\#}P<0.05$ vs. control, $^{*}P<0.05$ vs. VEH ALA+IND. ALA, alpha-linolenic acid; OME, omeprazole; VEH, vehicle; IND, indomethacin.

malized the peak areas to 18:1 n-7. The fatty acid remains consistent between different treatments and does not impact the biological process.

Statistical analysis

The data are presented as the mean±standard error of the mean with a sample size of five to six. The control group was compared using one-way analysis of variance (ANOVA) followed by the Newman-Keuls test or a two-way ANOVA with Bonferroni's post-hoc test (proportion of fatty acid). A *P*-value of less than 0.05 indicates a statistically significant difference between the means. All statistical analyses were performed using GraphPad Prism 6 software.

RESULTS

Gastroprotective effects of ALA on the indomethacin-induced gastric injury model

Pretreatment with ALA for 10 days (20 mg/kg) significantly reduced the area of gastric hemorrhagic lesions (20.38±4.66 mm^2) induced by indomethacin (3.61±1.04 mm^2) (Fig. 1A). The gastric protection rate of ALA was 82.25%±5.10% (Table 2). Furthermore, ALA exhibited a protective effect on the gastric mucosa against indomethacin's ulcerative effect, which was comparable to that of the reference drug omeprazole (79.35%±6.97% at 10 mg/kg) (Fig. 1B and Table 2).

The vehicle-only control gastric mucosa and control group receiving only ALA treatment displayed no morphological changes (Fig. 2A and 2B). Comparatively, indomethacin-induced gastric ulcers were more extended (score criteria 3 for 67% of samples), with a larger necrotic area and a higher count of polymorphonuclear leukocytes (score criteria 3 for 50% of samples) (Fig. 2C and Table 3). Furthermore, concurrent histological preparations of a 10-day pretreatment with ALA yielded limited indomethacin-induced damage to the gastric mucosa. The gastric ulcer was largely confined to the upper third of the mucosa, exhibiting minimal cellular debris (score criteria 1 for 50% of samples) and only slight leukocyte infiltration (score criteria 1 for 84% of samples) (Table 3). The usual morphology of tissues surrounding the epithelium and the hidden structures beneath the epithelial disruption was maintained (Fig. 2D). Indomethacin-induced gastric injury (Fig. 3A) was compared with omeprazole as reference drug (Fig. 3B) and similar levels of protection and morphology were observed after omeprazole treatment.

Effects of the antioxidative activity of ALA in an indomethacin-induced gastric injury model

Indomethacin-induced gastric damage increases oxidative stress levels. However, pretreatment with ALA for 10 days significantly reduced ROS levels compared with the indomethacin-treated group (Fig. 4).

The increase in gastric NO levels induced by indomethacin was mitigated 10-day ALA pretreatment compared with that in the indomethacin-induced gastric in-

Table 2. Gastroprotective effects of ALA in an indomethacin-induced gastric injury model

Group	% Gastroprotection
	ALA 10-day pretreatment (20 mg/kg, p.o.)
Control	100±0.00
ALA	100±0.00
IND	0±22.93*
ALA + IND	82.25±5.10#
IND	0±19.74*
OME + IND	79.35±6.97#

The values are expressed as the mean±SEM (n=5–6). One-way ANOVA followed by the Newman-Keuls test. $^{*}P<0.05$ vs. control, $^{\#}P<0.05$ vs. IND.
ALA, alpha-linolenic acid; IND, indomethacin (30 mg/kg, p.o.); OME, omeprazole (10 mg/kg, p.o.).

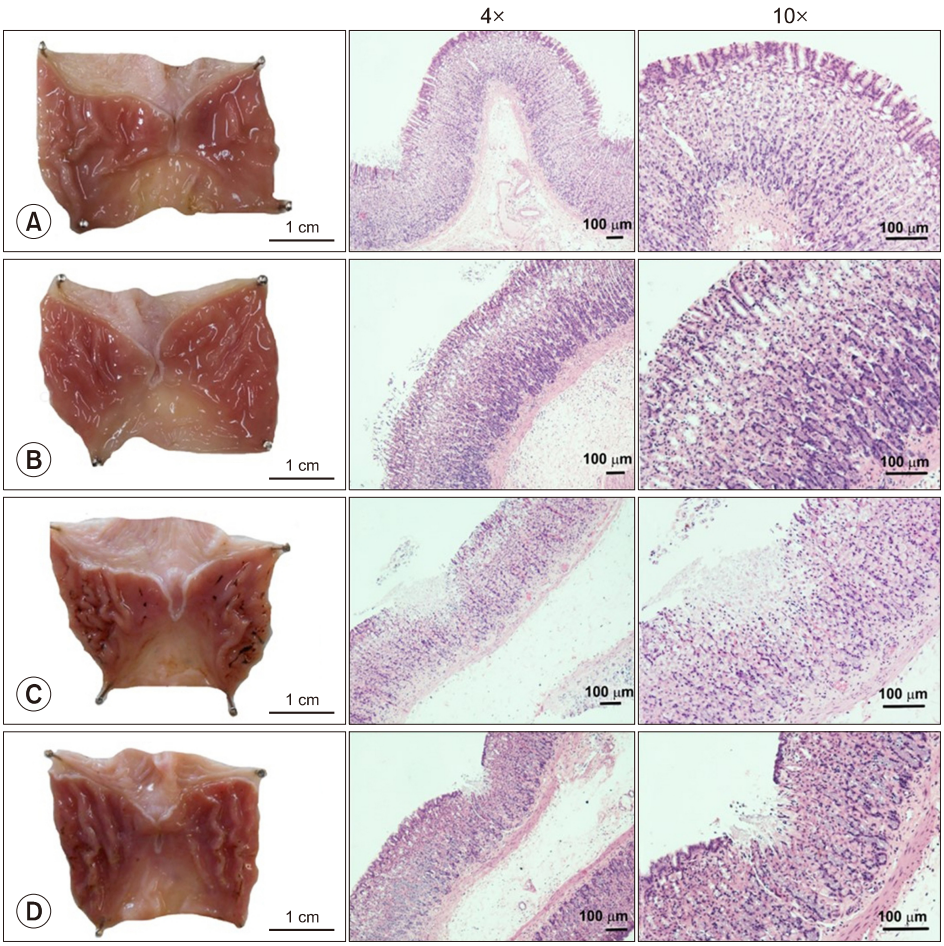

Fig. 2. Representative images of corpus stomach lesions (left) and histopathological sections of the gastric mucosa (right) following 10-day pretreatment with ALA. (A) Control, (B) ALA (20 mg/kg)+VEH IND, (C) VEH ALA+IND (30 mg/kg), and (D) ALA (20 mg/kg)+IND (30 mg/kg). ALA, alpha-linolenic acid; VEH, vehicle; IND, indomethacin.

Table 3. Pathological analysis of the effects of ALA on gastric mucosal injury and leukocyte infiltration from histological sections in an indomethacin-induced gastric injury model (n=5–6)

Group	ALA pretreatment for 10 days (20 mg/kg, p.o.)	
	Gastric mucosal injury	Leukocyte infiltration
Control	0 (100%)	0 (100%)
ALA	0 (100%)	0 (100%)
IND	3 (67%)	3 (50%)
ALA + IND	1 (50%)	1 (84%)
IND	3 (67%)	3 (50%)
OME + IND	0 (50%)	0 (50%)

ALA, alpha-linolenic acid; IND, indomethacin (30 mg/kg, p.o.); OME, omeprazole (10 mg/kg, p.o.).

jury group (Table 4). ALA pretreatment also reduced MDA levels compared with the indomethacin-treated group (Table 4).

Moreover, the MPO activity exhibited a significant reduction following 10-day ALA pretreatment. ALA pretreatment also significantly decreased LTB_4 gastric levels compared with that in the indomethacin-treated group (Table 4).

The administration of indomethacin significantly decreased gastric GSH levels compared with those in the control group. Conversely, ALA pretreatment significantly improved gastric GSH levels compared with those in the indomethacin-treated group. Furthermore, SOD activity was significantly decreased after indomethacin administration. However, despite an observed increase in SOD activity following 10 days of ALA administration, gastric SOD levels did not demonstrate significant variations across groups (Table 5).

Effects of ALA on the gastric fatty acid composition in an indomethacin-induced gastric injury model

We compared the effects of ALA pretreatment on the gastric fatty acid composition by determining the profiles of two omega-6 fatty acids [linoleic acid and arachidonic acid (AA)] and four omega-3 fatty acids (ALA, EPA, docosapentaenoic acid, and DHA). We observed a significant decrease in AA percentage in the group treated with indomethacin ($P \leq 0.0001$ vs. control, Table 6).

DISCUSSION

Our findings revealed for the first time the efficacy of ALA in mitigating the gastric damage induced by indomethacin. After 10-day ALA pretreatment, the gastric protection rate was approximately 82.25%±5.10% under indomethacin-induced gastric injury. Interestingly, com-

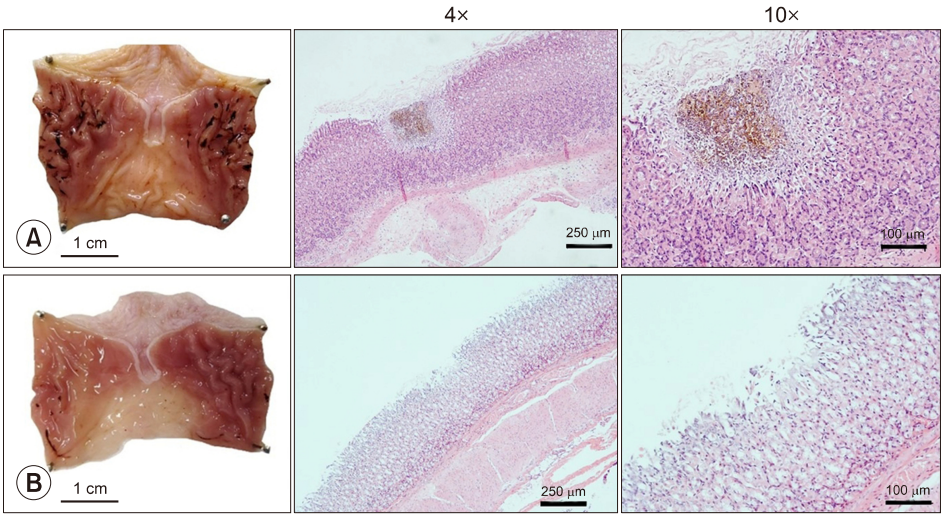

Fig. 3. Representative images of corpus stomach lesions (left) and histopathological sections of the gastric mucosa (right) following 10-day pretreatment with omeprazole. (A) VEH OME+IND (30 mg/kg) and (B) OME (10 mg/kg)+IND (30 mg/kg). OME, omeprazole; VEH, vehicle; IND, indomethacin.

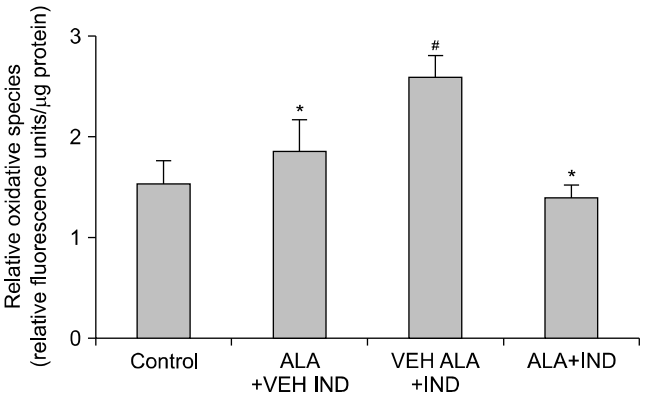

Fig. 4. Effects of ALA pretreatment (20 mg/kg) for 10 days on reactive oxygen species levels in an indomethacin-induced gastric injury model. The values are expressed as the mean±SEM (n=5–6). Statistical analysis was performed using one-way ANOVA followed by the Newman-Keuls test. $^{\#}P<0.05$ vs. control, $^{*}P<0.05$ vs. VEH ALA+IND. ALA, alpha-linolenic acid; VEH, vehicle; IND, indomethacin.

pared with the indomethacin-induced damage group, ALA pretreatment helped to restore the gastric levels of ROS and MDA. Moreover, ALA pretreatment prevented an increase in the levels of NO, MPO, and LTB_4, which are normally increased after indomethacin-induced gastric damage. ALA pretreatment also prevented the decrease in GSH observed in the gastric damage group. Based on these results, we concluded that the gastroprotective effects of ALA may be attributed to the activation of the antioxidant pathway.

Omega-3 PUFAs, specifically DHA and EPA, are known to exert anti-inflammatory effects (Harris et al., 2021). The anti-inflammatory properties of omega-3 PUFAs, which are often linked to cardiovascular benefits, mainly stem from marine omega-3 sources (Mori, 2017). Our current study highlighted the gastroprotective effects of ALA. Previous studies have explored the protective effects of fish oil (a marine omega-3) in aspirin, cold-restraint stress (CRS), alcohol, and pylorus ligation models (Bhattacharya et al., 2006) and Antarctic krill oil in ethanol-induced gastric injury (Huang et al., 2022). Previously, our team demonstrated the protective effects of DHA in gastric injury (Pineda-Peña et al., 2018) and small-intestine injury induced by indomethacin (Sánchez-Trigueros et al., 2021). The effects of ALA on the gastrointestinal tract were previously demonstrated only in a colitis model (Hassan et al., 2010; Reifen et al., 2015) but not in gastric tissues. To the best of our knowledge, the present study is the first to demonstrate the gastroprotective effects of ALA in an indomethacin-induced gastric injury model. Moreover, ALA was administered as a pure compound. We previously mentioned that a mixture of linoleate (18:2 n-6) and alpha-linolenate (18:3

Table 4. Effects of ALA on oxidative stress in an indomethacin-induced gastric injury model

Group	ALA 10-day pretreatment (20 mg/kg, p.o.)			
	NO (mM/g tissue)	MDA (nM/mg tissue)	MPO (U/mg tissue)	LTB_4 (ng/g tissue)
Control	48.92±6.80	25.98±3.11	3.52±0.81	2.33±0.79
ALA	50.47±12.5$^{\#}$	32.04±1.80$^{\#}$	2.68±0.69$^{\#}$	3.82±0.86$^{\#}$
IND	83.88±7.09*	42.13±3.97*	8.24±1.29*	6.89±1.54*
ALA + IND	33.42±6.89$^{\#}$	30.61±2.79$^{\#}$	4.53±1.20$^{\#}$	1.96±0.46$^{\#}$

The values are expressed as the mean±SEM (n=5–6). Statistical analysis was performed using one-way ANOVA followed by the Newman-Keuls test. $^{*}P<0.05$ vs. control, $^{\#}P<0.05$ vs. IND.
ALA, alpha-linolenic acid; IND, indomethacin (30 mg/kg, p.o.); NO, nitric oxide; MDA, malondialdehyde; MPO, myeloperoxidase; LTB_4, leukotriene B_4.

Table 5. Effects of ALA on the antioxidant response in an indomethacin-induced gastric injury model

Group	ALA 10-day pretreatment (20 mg/kg, p.o.)	
	SOD (U/mg protein)	GSH (nM/g tissue)
Control	0.102±0.009	268.8±11.74
ALA	0.090±0.009	262.5±4.53#
IND	0.068±0.007*	202.3±9.41*
ALA + IND	0.074±0.007	268.2±22.03#

The values are expressed as the mean±SEM (n=5–6). Statistical analysis was performed using one-way ANOVA followed by the Newman-Keuls test. *P<0.05 vs. control, #P<0.05 vs. IND.
ALA, alpha-linolenic acid; IND, indomethacin (30 mg/kg, p.o.); SOD, superoxide dismutase; GSH, glutathione.

n-3) was administered to evaluate their gastroprotective effects in ethanol, ischemia/reperfusion, and water immersion stress murine models, but not in a model of gastric injury induced by NSAIDs. Furthermore, ALA was not administered as a pure compound (Matsuba et al., 1998).

The potential clinical relevance of ALA's effect on models involving the administration of NSAIDs should be noted. Although NSAIDs are frequently prescribed worldwide, their usage is restricted because of their severe side effects in the gastrointestinal tract (García-Rayado et al., 2018). Our team observed the gastroprotective effects of marine-derived omega-3 DHA in an indomethacin-induced gastric injury model (Pineda-Peña et al., 2018). In addition, studies have shown that the therapeutic efficacy of NSAIDs (e.g., naproxen or indomethacin) is not negatively affected after DHA administration (Arroyo-Lira et al., 2014, 2017). Unlike DHA, the administration of ALA does not produce a fishy taste or have adverse effects (e.g., flatulence), which could be considered advantageous (Belluzzi et al., 1996).

In this study, we measured the fatty acid profile of gastric tissues but found no differences in the levels of gastric omega-3 across the groups. Our study is somewhat limited as the changes in tissue fatty acid levels were previously observed only after a 30-day cycle with omega-3 treatment (James et al., 2022). Several studies have also found that the administration of omega-3 PUFAs may resolve colitis in fat-1 transgenic by suppressing the inflammation pathway of PUFA lipid mediators (Hudert et al., 2006). Remarkably, we found that gastric hemorrhagic lesions significantly decreased in the indomethacin-induced gastric injury model, despite having no discernible differences in stomach fatty acid levels after administering ALA after 10 days.

The protective effects of omega-3 PUFAs on the heart, brain, kidney, and gastrointestinal tract have been well established. Nonetheless, studies have emphasized the anti-inflammatory role of omega-3 PUFAs and the synthesis of lipid mediators such as resolvins (Ishihara et al., 2019). Comparatively, the antioxidant-related effects of omega-3 PUFAs have rarely been studied.

Previously, our research group identified the role of the antioxidative action of DHA in its gastroprotective effect in an indomethacin-induced gastric injury model (Pineda-Peña et al., 2018). Additionally, another study found that fish oil administration significantly enhanced the activity of the antioxidant enzymes catalase and GSH peroxidase in a CRS model (Bhattacharya et al., 2006). In a colitis model, GSH levels were restored following ALA pretreatment (Hassan et al., 2010).

In the present study, we discovered that the gastroprotective action induced by ALA is correlated with an increase in GSH production and the prevention of NO and leukocyte recruitment. This situation was supported by the corresponding decrease in MPO and LTB_4. Furthermore, a reduction in oxidative factors, such as ROS, and lipoperoxidation was observed after 10 days of ALA treatment.

The gastroprotective function of ALA had not been previously noted. Therefore, whether the antioxidative pathway participates in this effect remains to be elucidated. Although the correlation between the oxidative stress induced by indomethacin and gastric tissue injury is well known as a mechanism of induction of gastric hemorrhagic lesions (Pineda-Peña et al., 2018), the hypothesis of blocking prostaglandin synthesis is still valid (Wallace, 2008). Furthermore, the antioxidative impact of ALA has been assessed in other injury models unrelated to gastric damage, including colitis (Raj et al., 2014), nephrotoxicity (İstifli et al., 2019), and neurodegeneration (Alam et al.,

Table 6. Proportion of fatty acids in gastric tissue after treatment with ALA in an indomethacin-induced gastric injury model

Fatty acid (%)	ALA 10-day pretreatment (20 mg/kg, p.o.)					
	LA (18:2 n-6)	ALA (18:3 n-3)	AA (20:4 n-6)	EPA (20:5 n-3)	DPA (22:5 n-3)	DHA (22:6 n-3)
Control	15.44±0.81	0.99±0.17	12.90±2.06	0.44±0.12	1.05±0.30	0.62±0.19
ALA	15.91±1.06	0.68±0.16	17.74±1.08#	0.24±0.06	1.46±0.21	0.26±0.2
IND	19.94±5.84	0.63±0.22	7.26±2.16*	0.24±0.06	0.45±0.26	1.03±0.18
ALA + IND	13.58±2.74	0.45±0.16	15.38±1.62#	0.32±0.02	0.74±0.33	0.93±0.28

The values are expressed as the mean±SEM (n=5–6). Statistical analysis was performed using two-way ANOVA followed by Bonferroni test. *P<0.05 vs. control, #P<0.05 vs. IND.
ALA, alpha-linolenic acid; IND, indomethacin (30 mg/kg, p.o.); LA, linoleic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

2021). However, more data are needed to correlate the tissue levels of omega-3 PUFAs like ALA with their antioxidative influence.

Moreover, previous studies (Pineda-Peña et al., 2018) have reported an antioxidative effect following DHA supplementation. The way that DHA and EPA work is typically linked to their biosynthesis of resolvins. Some research has indicated the protective impact that resolvins have against colitis (Bento et al., 2011). However, data regarding the effect of resolvins during gastric injury are limited. Recently, PUFA-derived epoxides have been found to play a role in various diseases, including exerting a protective response to a diclofenac-induced gastric injury model (Goswami et al., 2017). This finding suggests that the antioxidative route is one of several pathways utilized by omega-3 PUFAs. In our study, we examined the role of ROS and oxidative markers and the antioxidant action of ALA in gastric tissues. However, the ALA-induced reduction in ROS production and NO synthase 2 was linked to an enhancement in the expression of nuclear factor erythroid 2-related factor 2 and heme oxygenase-1 in a mouse model of neurodegeneration. In the same study, ALA suppressed nuclear factor-kappa B and interleukin-1β in the mouse brain. These substances are more related to anti-inflammatory action than to an antioxidative effect (Alam et al., 2021). Additional studies related to antioxidative transcription factors are needed to obtain a better understanding of the gastroprotective mechanism of action of ALA.

In conclusion, to the best of our knowledge, this research is the first to report the gastroprotective effects of ALA in an indomethacin-induced gastric injury model. The observed gastroprotective effects of ALA appear to stem from its antioxidant properties. However, more studies are needed to uncover additional mechanisms at play in this action, and longer-duration administrations of ALA are warranted. Such work could shed light on alternative therapeutic strategies for treating gastric ulcers.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: CSN, NPH, AECP. Analysis and interpretation: NPH, JTB, HGP, EPH, AECP. Data collection: CNS, CASRL, SG. HGP, EPH. Writing the article: CSN, NPH, AECP, EPH. Critical revision of the article: CASRL, JTB, GCH. Final approval of the article: All authors. Statistical analysis: GCH, CSN, NPH, AECP. Obtained funding: NPH, AECP. Overall responsibility: AECP.

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