Protective effect of L-carnitine against ethanol-induced gastric damage: Investigation of possible mechanisms of action

Research Article

ABSTRACT

The underlying mechanisms of L-carnitine's (L-CAR) protective effect against ethanol (EtOH)-induced gastric mucosal damage were investigated in this study. The rats were randomly divided into four groups: control (CON), EtOH, EtOH + L-CAR50, and EtOH + LCAR100. Control group was given saline (5 mL/kg) twice at 1-hour interval. EtOH group was given 5 mL/kg saline 1-hour before absolute EtOH administration (5 mL/kg). EtOH + LCAR50 group received 50 mg/kg LCAR 1-hour before absolute EtOH administration (5 mL/kg). EtOH + LCAR100 group received 100 mg/kg LCAR 1-hour before absolute EtOH administration (5 mL/kg). All the rats were euthanized 1 hour after the administration of EtOH. The gastric lesion area was grossly examined, and gastric lesions were histopathologically evaluated. Real-time PCR was used to examine the expression of cyclooxygenase 1 and 2 (COX-1 and COX-2), inducible- and endothelial- nitric oxide synthase (iNOS and eNOS), tumor necrosis factor alpha (TNF-α), heat shock protein 70 (HSP70), and trefoil factor 2 (TFF2) mRNA in the gastric mucosa. Histopathological examination revealed that L-CAR treatment reduced the severity and extent of gastric lesions caused by EtOH administration, such as shedding of the superficial epithelium, glandular gland necrosis, intralesional hemorrhage, submucosal edema, and neutrophil infiltration. L-CAR administration was found to significantly reduce the mRNA levels of COX-2, iNOS, eNOS, and TNF-α in the gastric mucosa compared to EtOH administration alone. It was determined that L-CAR administration further increased the gastric mucosal HSP70 mRNA expression than EtOH administration alone. L-CAR treatment increased TFF2 expression which was decreased after EtOH administration. Finally, L-CAR administration was thought to protect against EtOH-induced gastric mucosal damage by regulating the expression of gastric mucosal COX and NOS systems, reducing the inflammatory cytokine levels, inducing a cellular stress response, and stimulating the expression of factors associated with mucus secretion and gastric epithelium restitution.

Keywords: Cyclooxygenase, ethanol, heat shock protein, L-carnitine, nitric oxide synthetase, trefoil peptides

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INTRODUCTION

A mucus-bicarbonate-phospholipid barrier covering the surface of the gastric mucosa, epithelium, and gastric microcirculation, as well as gastroprotective factors (prostaglandins, nitric oxide, heat shock proteins, trefoil factor family peptides) secreted by epithelium and endothelial cells, maintain gastric mucosal integrity (Aihara et al., 2017; MacNaughton et al., 1989a; Martin and Wallace, 2006; Tsukimi and Okabe, 2001). Necrotizing agents, such as ethyl alcohol (EtOH), cause focal hemorrhagic lesions by destroying the gastric mucosal barrier via unknown mechanisms (Chen et al., 2021). Microscopically, EtOH-induced lesions show necrosis and exfoliation of the glandular glands and cells covering the gastric luminal surface and pits in addition to interstitial hemorrhage, capillary hyperemia, and thrombosis (Guth et al., 1984; Lacy and Ito, 1982).

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EtOH-induced gastric mucosal lesions are most likely associated with the microvascular lesions. Vascular changes are thought to cause gastric mucosal damage by decreasing regional blood flow (Oates and Hakkinen, 1988; Szabo et al., 1985). Gastric epithelium initiates a cellular stress pathway in response to EtOH-induced gastric damage. To reduce EtOH-related gastric damage in rodents, cyclooxygenase (COX) and nitric oxide synthetase (NOS) systems are activated, and heat shock protein 70 (HSP70) expression is increased (Bakalarz et al., 2021; Magierowska et al., 2015; Saika et al., 2000; Zhao et al., 2009).

L-carnitine (L-CAR), synthesized endogenously from lysine and methionine precursors, is essential for the transfer of long-chain fatty acids to mitochondria (Kart et al., 2006). L-CAR has been shown in animal studies to protect against gastric mucosal damage caused by various irritants. Studies indicate that L-CAR administration protects against EtOH-induced damage by decreasing gastric mucosal lipid peroxidation, and by increasing the glutathione, prostaglandin E2, and nitric oxide levels (Arafa and Sayed-Ahmed, 2003; Dokmeci et al., 2005; Erkin et al., 2006; Izgüt-Uysal et al., 2007; Madi and Al-Barr, 2014). However, only a few studies have investigated the underlying mechanisms to explain the gastroprotective effect of L-CAR, particularly those related to the gastric mucosal barrier (Izgüt-Uysal et al., 2007; Madi and Al-Barr, 2014). In this study, we investigated the underlying mechanisms of gastroprotective effect of L-CAR against EtOH-induced gastric mucosal damage in rats. To this end, COX-1, COX-2, inducible- and endothelial- NOS (iNOS and eNOS), HSP70, pro-inflammatory cytokine TNF-α, and TFF2 levels were evaluated to understand the potential role of L-CAR in gastroprotection.

MATERIALS AND METHODS

Animals

A total of thirty-two 8-weeks-old male Sprague-Dawley rats weighing 200–250 g obtained from Adnan Menderes University Veterinary Faculty Experimental Animals Production and Research Center were used in this study. Throughout the experiment, the rats were housed in transparent polycarbonate cages at 22°C ± 2°C, 50%–70% humidity, and 12/12 hours light/dark conditions, and were fed ad libitum with standard rat feed. All the experimental procedures were approved by the local ethics committee of Aydın Adnan Menderes University (Approval no. 64583101/2020/009).

After two-weeks, the rats were randomly assigned to one of four groups: control (CON), EtOH, EtOH + LCAR50, and EtOH + LCAR100. Before the chemical application, all rats were subjected to fasting for 24 hours. The detailed applications for each group are given below:

1. Control group – rats were given saline (5 mL/kg) twice at 1-hour interval.
2. EtOH group – 5 mL/kg saline 1-hour before absolute EtOH administration (5 mL/kg).
3. EtOH + LCAR50 – 50 mg/kg LCAR 1-hour before absolute EtOH administration (5 mL/kg).
4. EtOH + LCAR100 – 100 mg/kg LCAR 1-hour before absolute EtOH administration (5 mL/kg).

The orogastric tube was used to administer all chemicals intragastrically. The L-CAR doses to be administered were determined based on studies that found protective effects against EtOH-induced gastric mucosal damage (Madi and Al-Barr, 2014). We obtained absolute EtOH (CAS no.: 64-17-5) from Merck KgaA (Darmstadt, Germany) and L-CAR (CAS no.: 541-15-1) from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were of analytical purity. All the rats were euthanized by cervical dislocation under general anesthesia induced by intraperitoneal injections of 50 mg/kg ketamine (Ketasol: Richter Pharma AG, Wels, Austria) and 5 mg/kg xylazine (Ksilazin: IPM Pharmaceuticals, İstanbul, Türkiye) 1-hour after the administration of absolute EtOH. Following systemic necropsy, all rats' stomachs were removed. An incision along the greater curvature of the stomach was made, and the gastric contents

were cleaned with physiological saline. After photographing the stomachs fixed on a flat surface with bulk needles, samples were taken for histopathological examination and gene expression analysis. The area of the gastric lesion (mm^2) was calculated by analyzing photographs with the Image J program (Wayne Rasband National Institutes of Health, USA).

Histopathological examination

Gastric tissue samples were fixed in 10% neutral-buffered formalin overnight. After dehydrating the gastric samples in increasing concentrations of ethanol, they were equilibrated in xylene and embedded in paraffin. Then, 4–5-µm-thick serial sections were cut with a microtome, stained with hematoxylin-eosin, and examined with a light microscope (BX51, Olympus) and a digital camera (SC180, Olympus). Gastric lesions were evaluated using a modified scoring system recommended by Magierowska et al. (2018). In terms of severity and extent, gastric lesions were graded as 0= no lesion, 1= mild, 2 = moderate, and 3= severe.

Real-time PCR

Quantitative real-time PCR was used to determine the expression of *COX-1*, *COX-2*, *iNOS*, *eNOS*, *TNF-α*, *HSP70*, and *TFF2* mRNA in the gastric mucosa. To summarize, total RNA was extracted using a Riboex GeneAll Biotechnology kit (South Korea) based on guanidium isothiocyanate / phenol chloroform method. Spectrophotometry was used to determine the concentration and purity of RNA. Then, using a cDNA Synthesis Kit with RNase Inh (High Capacity) from A.B.T.™ Laboratory Industry (Türkiye), 2-µg total RNA was reverse-transcribed into complementary DNA (cDNA). Table 1 shows the primer sequences used to amplify the specific mRNAs. cDNAs were amplified in the CFX Connect Real-Time PCR Detection System (Biorad, USA) using the A.B.T.™ 2X qPCR SYBR-Green MasterMix (with ROX) kit (A.B.T.™ Laboratory Industry, Türkiye) under the following cycling conditions: 95°C hold for 3 min, followed by 40 cycles of denaturation at 95°C for 10 seconds, annealing at corresponding temperatures (see Table 1) for 20 seconds, and extension at 60°C for 20 seconds were performed. Melt curves were used to test the specificity of amplicons. The mRNA expression of the genes was normalized against *β-actin*, and fold changes were calculated using the ΔΔCt method against the intact gastric mucosa as a reference (Livak and Schmittgen, 2001).

Table 1. Sequences of primers used to amplify specific mRNAs by quantitative real-time polymerase chain reaction

Target gene	Primer sequences (5'-3')	Annealing temperature (°C)	Accession number
COX-1	F: CTGCTCACAGATGCTGGG R: ATGAGTACTTCTCGGATGAAGGT	60	NM_017043.4
COX-2	F: TGTCAAAACCGTGGTGAATG R: CCGAAGGAAGGGAATGTTGT	60	NM_017232.3
eNOS	F: AGAACTCTTCACTCTGCCCC R: GGTCCCTCATGCCAATCTCT	60	NM_021838.2
iNOS	F: ACCACCCTCCTTGTTCAACT R: AGCCTCTTGTCTTTGACCCA	58	NM_012611.3
TNF-α	F: TTCATCCGTTCTCTACCCA R: TTCAGCGTCTCGTGTGTTTC	58	NM_012675.3
TFF2	F: TCTCTTGGTAGTGGTCCTTGTCT R: CAGGTTGGAAAAGCAGCA	58	NM_053844.2
HSP70	F: GACGACGGCATCTTCAAG R: GTTCTGGCTGATGTCCTTC	60	NM_031971.2
β-Actin	F: ATGGTGGGTATGGGTCAGAA R: GGTCATCTTTTCACGGTTGG	60	NM_031144.3

COX-1: Cyclooxygenase 1; *COX-2*: Cyclooxygenase 2; *eNOS*: Endothelial nitric oxide synthase; *iNOS*: Inducible nitric oxide synthase; *TNF-α*: Tumor Necrosis Factor-α; *TFF2*: Trefoil Factor 2; *HSP70*: Heat Shock Protein 70.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS version 22.0; IBM Corp., NY, USA) was used to apply one-way analysis of variance on the data. Normality of the data was checked, and non-normalized traits were normalized using the logarithmic or square root transformation method. Based on the homogeneity of variances, Tukey's, or Tamhane tests were chosen as post-hoc tests. Assumptions of 95% probability ($P < 0.05$) were made to separate significantly different means. The results are shown as mean ± standard errors.

RESULTS

Macroscopic findings

Figure 1 depicts the macroscopic appearance of gastric lesions and the mean gastric lesion area of rats given L-CAR at doses of 50 mg/kg or 100 mg/kg in combination with absolute EtOH, absolute EtOH, and saline. There were no macroscopic lesions in the control group. Absolute EtOH administration was found to cause linear hemorrhagic lesions in rats' stomachs. The lesions were more visible in the gastric folds. Submucosa was quite thickened by gelatinous edema.

Figure 1 shows that 50 mg/kg L-CAR administration significantly reduced the gastric lesion area caused by EtOH administration. However, it was determined that L-CAR administration at a dose of 100 mg/kg had no effect on the gastric lesion size induced by EtOH.

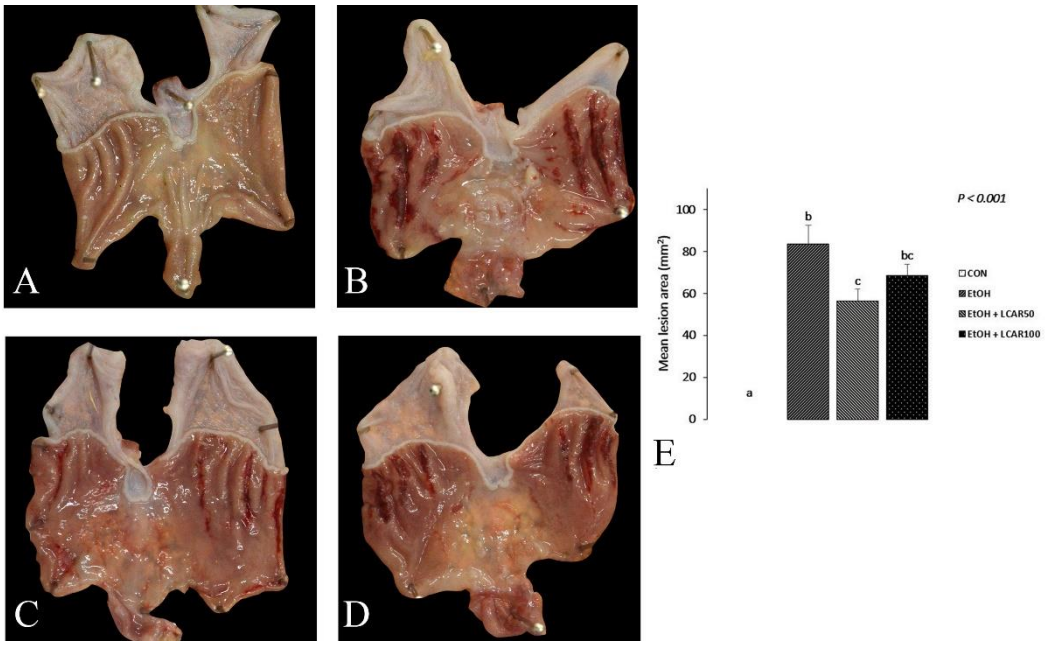

Figure 1. Gastric lesions in situ (A-D) and mean mucosal lesion area (E) in rats given saline (A), absolute ethanol (B), or L-CAR at 50 mg/kg (C) or 100 mg/kg (D) in combination with absolute EtOH. a, b, c Bars with different superscripts differed significantly

Histopathological findings

Figure 2 depicts the histopathological findings in rats given saline, absolute EtOH, or absolute EtOH in combination with L-CAR at doses of 50 mg/kg or 100 mg/kg. The control group showed no signs of pathological changes. Absolute EtOH administration resulted in wedge-shaped gastric erosions with multifocal distribution in rat gastric mucosa, characterized by exfoliation of the superficial epithelium and necrosis of the glandular glands. The lesions in the stomach extended deep into the mucosa. In the lesion areas, there was extensive hemorrhage with hyalinized vessel walls. Mild to moderate neutrophil infiltrations were seen around the vessels at the base of gastric mucosa. The submucosa was significantly enlarged with abundant edema accompanied by neutrophils. As shown in Table 2, gastric mucosal

damage was more superficial in rats given L-CAR before EtOH, and extent of gastric lesion was lower compared to rats administered EtOH. Furthermore, L-CAR administration reduced intralesional hemorrhage, submucosal edema, and neutrophil infiltrations. There was no statistically significant difference between L-CAR doses in terms of severity or lesion extent.

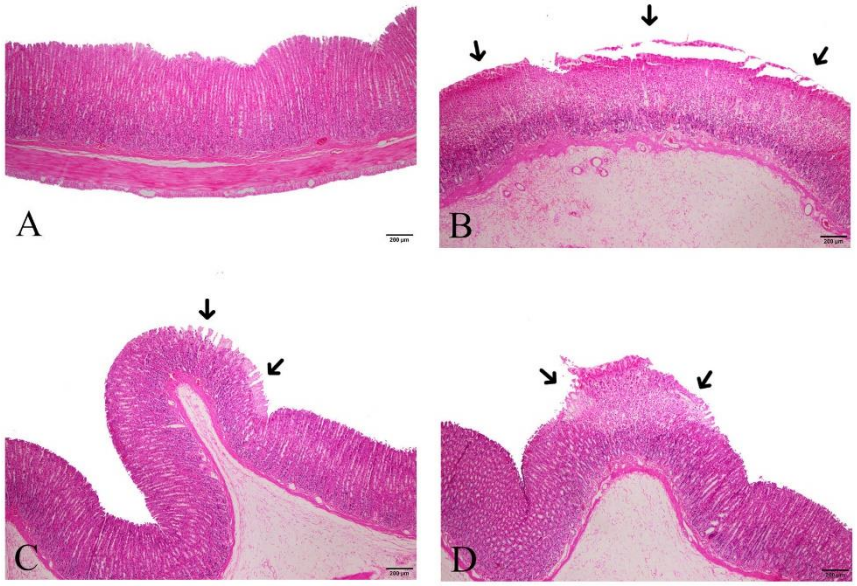

Figure 2. Microscopic images of gastric lesions in rats treated with saline (A), absolute ethanol (B) or L-CAR at a dose of 50 mg/kg (C) or 100 mg/kg (D) in combination with absolute EtOH. Arrows show the areas of gastric mucosal lesions

Table 2. Effects of L-carnitine treatment on gastric mucosal lesions induced by ethanol

Group	Necrosis	Hemorrhage	Neutrophil infiltration	Submucosal edema
CON	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
EtOH	2.63 ± 0.18^{b}	2.38 ± 0.18^{b}	1.63 ± 0.18^{b}	3.00 ± 0.0^{b}
EtOH + L-CAR50	1.50 ± 0.18^{c}	1.25 ± 0.25^{c}	0.75 ± 0.16^{c}	2.25 ± 0.25^{c}
EtOH + L-CAR100	1.88 ± 0.12^{c}	1.75 ± 0.31^{bc}	1.25 ± 0.25^{bc}	2.75 ± 0.16^{bc}
P value	<0.001	<0.001	<0.001	<0.001

CON: Control; EtOH: Ethanol; L-CAR: L-carnitine. $^{a-c}$ Means bearing different superscript within the same column differ significantly

Real-time PCR

Figure 3 shows that EtOH administration increased the gastric mucosal *COX-2*, *iNOS*, *eNOS*, *TNF-α*, and *HSP70* mRNA expression while decreasing *TFF-2* levels in comparison with control group. The administration of 50 mg/kg or 100 mg/kg of L-CAR with EtOH significantly reduced gastric mucosal *iNOS* and *TNF-α* mRNA levels compared to EtOH group. There was no statistically significant difference in *iNOS* and *TNF-α* mRNA levels between L-CAR doses. In addition, 50 mg/kg L-CAR treatment significantly reduced gastric mucosal *COX-2* and *eNOS* mRNA levels compared to EtOH administration only. *HSP70* mRNA expression in the gastric mucosa was found to increase even after L-CAR administration at a dose of 50 mg/kg. L-CAR at 50 mg/kg was found to significantly increase the expression of gastric mucosal *TFF-2* mRNA compared to EtOH-treated group. Neither EtOH nor L-CAR had any effect on gastric mucosal *COX-1* expression.

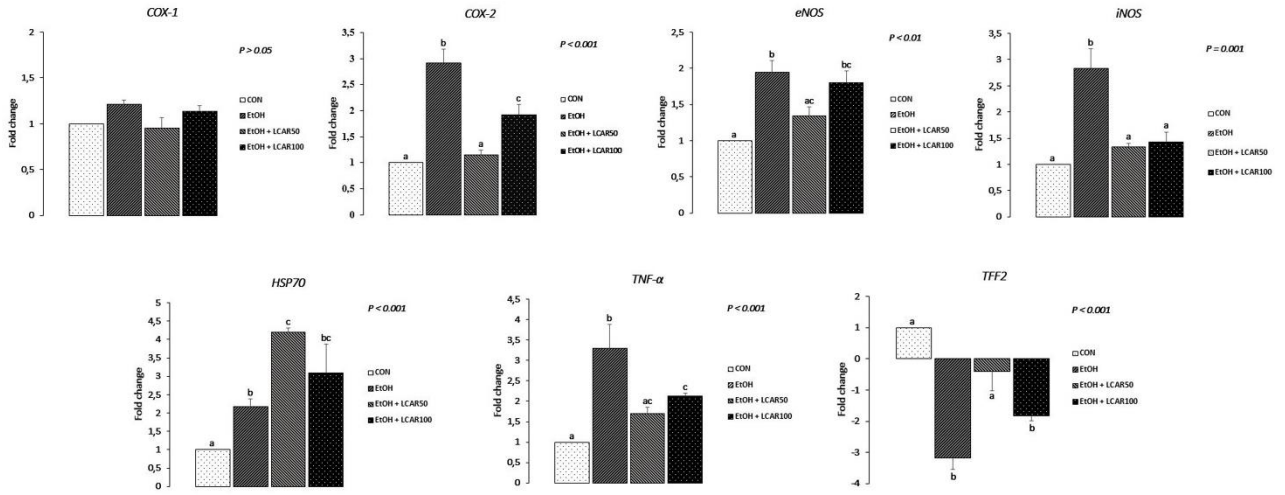

Figure 3: The effect of 50 mg/kg or 100 mg/kg L-CAR on gastric mucosal *COX-1*, *COX-2*, *iNOS*, *eNOS*, *TNF-α*, *HSP70*, and *TFF2* mRNA expression prior to EtOH administration. The data show fold change compared to control rats. [a-c] Bars with different superscripts differed significantly. *COX-1*: Cyclooxygenase 1; *COX-2*: Cyclooxygenase 2; *eNOS*: Endothelial nitric oxide synthase; *iNOS*: Inducible nitric oxide synthase; *HSP70*: Heat Shock Protein 70; *TNF-α*: Tumor Necrosis Factor-α; *TFF2*: Trefoil Factor 2.

DISCUSSION

Consistent with previous findings (Dokmeci et al., 2005; Guth et al., 1984; Lacy and Ito, 1982), we determined that gastric hemorrhagic lesions formed quickly in rats given absolute EtOH intragastrically. Microscopic examination of these lesions revealed shedding of surface epithelium, necrosis and exfoliation of the glandular glands, intralesional hemorrhage, submucosal edema, vascular hyperemia, and thrombosis. Endothelial damage increased vascular permeability, and endothelial activation all play important roles in the pathophysiology of EtOH-induced gastric lesions. Gastric mucosal lesions caused by EtOH are most likely the result of gastric mucosal hypoxia caused by vascular lesions (Oates and Hakkinen, 1988; Szabo et al., 1985). Consistent with previous findings (Arafa and Sayed-Ahmed, 2003; Dokmeci et al., 2005; Madi and Al-Barr, 2014), we determined that administering L-CAR prior to EtOH reduced the severity and extent of EtOH-induced gastric mucosal lesions. Given that EtOH-induced gastric mucosal lesions are caused by microvascular lesions, it is possible that L-CAR has a gastroprotective effect by reducing endothelial cell damage.

Prostaglandins (PG), synthesized from arachidonic acid by the action of cyclooxygenases, strengthen the gastric mucosal barrier and exert a protective effect against acute gastric mucosal damage (Robert et al., 1979). Constitutional expression of COX-1 related PG is associated with gastric physiological functions. Unlike COX-1, COX-2-mediated PG induced in response to gastric mucosal damage protects against luminal irritants by increasing the regional blood flow, stimulating mucus and bicarbonate secretion, and by limiting the inflammatory response (Martin and Wallace, 2006). Our findings showed that EtOH administration increased *COX-2* expression while having no effect on gastric mucosal *COX-1* expression. Despite the fact that EtOH administration increases COX-2 expression, which helps to strengthen the gastric mucosal barrier, gastric mucosal PGE2 levels have been reported to decrease, contrary to expectations (Bakalarz et al., 2021; Magierowska et al., 2015; Zhao et al., 2009). Although COX-2 expression is induced, the decrease in gastric mucosal PGE2 level appears contradictory. This was explained by the fact that EtOH administration reduced PGE2 synthesis by inhibiting COX-2 enzyme activity (Zhao et al., 2009). The increase in *COX-2* mRNA expression after EtOH administration

is most likely due to decreased PGE-2 synthesis.

Experimental studies have shown that L-CAR administration protects the gastric mucosa from damage caused by various irritants (Arafa and Sayed-Ahmed, 2003; Dokmeci et al., 2005; Erkin et al., 2006; Izgüt-Uysal et al., 2007; Madi and Al-Barr, 2014). It has been established that intragastric L-CAR administration reduces EtOH-induced gastric mucosal damage and has a gastroprotective effect by reducing gastric lipid peroxidation with its antioxidant properties (Arafa and Sayed-Ahmed, 2003; Dokmeci et al., 2005). Madi and Al-Barr (2014) determined that administering 50 mg/kg of L-CAR for 7 days reduced EtOH-induced gastric mucosal damage by increasing gastric mucosal PGE2 expression. In our study, L-CAR treatment had no effect on gastric mucosal *COX-1* expression while decreasing *COX-2* expression. Concurrently, L-CAR administration reduced the severity of EtOH-induced gastric mucosal damage. These findings suggest that L-CAR downregulated *COX-2* expression by reducing EtOH-induced gastric mucosal damage with its protective effects.

Endothelium-derived relaxing (NO and prostacyclin) and vasoconstrictive (endothelin-1) factors regulate gastric microcirculation, which is directly related to gastric mucosal integrity (MacNaughton et al., 1989a). Vascular lesions have been shown in studies to play an important role in the pathogenesis of EtOH-induced gastric mucosal lesions (Oates and Hakkinen, 1988; Szabo et al., 1985). According to reports, these lesions form as a result of an imbalance between endothelium-derived factors (MacNaughton et al., 1989b). In response to EtOH-induced gastric mucosal damage, NO synthesized from L-arginine via a reaction catalyzed by the nitric oxide synthase enzyme (NOS) reduces the gastric lesions by increasing regional blood flow, decreasing leukocyte infiltration, and decreasing mast cell degranulation. NOS is divided into three isoforms: nNOS, eNOS, and iNOS. nNOS and eNOS are constitutionally expressed proteins found in enteric neurons and endothelium of the stomach, respectively. iNOS is a protein that is activated in the endothelium, epithelial cells, and immunocytes in response to gastric mucosal damage (Kubes and Wallace, 1995). We determined that EtOH administration increases the gastric mucosal *eNOS* and *iNOS* expression. Given its role in regulating gastric microcirculation, it was thought that the increased *eNOS* expression in response to EtOH administration occurred in order to regulate the impaired microcirculation. NO produced by iNOS is thought to have gastroprotective properties by reducing gastric leukocyte infiltration and preventing mast cell degranulation (Kubes and Wallace, 1995). Tepperman and Soper (1994) determined increased iNOS activity in the stomachs of rats given endotoxin and demonstrated that these rats were more resistant to EtOH-induced gastric mucosal damage. These findings confirms that iNOS expression is increased to protect the gastric mucosa. In our study, we discovered that EtOH administration increased gastric mucosal *iNOS* expression. Increased *iNOS* expression in rats exposed to EtOH suggested that this was a developing compensatory mechanism to reduce gastric mucosal damage. L-CAR treatment significantly reduced *eNOS* and *iNOS* levels in the gastric mucosa. Concurrently, it was determined that L-CAR administration reduced the severity of EtOH-induced gastric mucosal damage. The lower *eNOS* and *iNOS* expression in rats given L-CAR compared to those given EtOH suggest that rats given L-CAR had less gastric mucosal damage.

HSP family proteins are essential for normal cell growth and cell integrity during pathological conditions (Rokutan, 2000; Tsukimi and Okabe, 2001). In unstressed gastric epithelial cells,

HSP70 expression is either too low or undetectable. Experimental studies have shown that HSP70 expression is induced in the gastric epithelium in response to EtOH-induced gastric mucosal damage (Saika et al., 2000; Sun et al. 2022). It has been proposed that the HSP response is activated after EtOH administration to reduce the amount of unfolded and misfolded protein accumulated within the cell (Rokutan, 2000). In this study, we determined that EtOH administration significantly increased the gastric mucosal *HSP70* expression compared to the control group. Gastric mucosal HSP70 level was analyzed to determine whether the expression of HSP70 induced by EtOH administration was affected by L-CAR administration. In our study, L-CAR administration enhanced the HSP70 expression in rats exposed to EtOH. *In vitro* studies have shown that HSP70 overexpression induced by high temperature protects monolayers of guinea pig gastric mucous cells against EtOH-induced damage (Nakamura et al., 1991). Higher *HSP70* expression in rats treated with L-CAR in combination with EtOH compared to EtOH alone in this study suggests that L-CAR application made gastric mucosal cells more resistant to EtOH-induced damage by upregulating the *HSP7*0 expression.

An acute inflammatory response characterized by increased blood flow, plasma fluid exudation, and leukocyte infiltration is stimulated in response to gastric mucosal damage caused by exogenous and endogenous irritants to reduce tissue damage and facilitate regeneration of damaged tissue (Wallace, 2008). A number of mediators work together to coordinate the inflammatory process. Experiments have shown that the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κβ) signal is important in initiating the inflammatory response associated with EtOH (Yu et al., 2020). Damage-associated molecular patterns released from damaged or dying cells in response to EtOH administration stimulate the degradation of inhibitor subunit of NF-κβ that traps NF-κβ in the cytosol, resulting in the translocation of NF-κβ to the nucleus. TNF-α mRNA expression is then induced by NF-κβ, which is one of the primarily mediators of the inflammatory process triggered by EtOH (Kim et al., 2020). In this study, EtOH administration was found to increase gastric mucosal TNF-α mRNA expression when compared to control rats. L-CAR treatment significantly reduced the expression of TNF-α mRNA in the gastric mucosa, which was elevated by EtOH administration. Based on these findings, it is possible to argue that L-CAR treatment protects against EtOH-induced gastric mucosal damage by regulating the inflammatory response.

TFFs are a type of peptide with a low molecular weight that is found primarily in the gastrointestinal tract. TFF2 released from mucous neck cells in the gastric corpus and antrum has been shown to play an important role in maintaining the gastric mucosal integrity (Hanby et al. 1993). TFF2, secreted by epithelial cells in response to acute gastric damage caused by a variety of irritants, promotes the epithelial restitution by stimulating the migration of healthy cells near the denuded area and inhibiting the apoptosis of migrating cells (Xue et al., 2011). In addition to these effects, TFF2 also increases the viscosity of gastric mucin and stabilizes the mucus gel network (Thim et al., 2002). Oral administration of recombinant TFFs has been shown in rodent studies to protect against gastric mucosal damage caused by various irritants (McKenzie et al., 2000; Poulsen et al., 1999). As previously reported (Aziz et al., 2019), the gastric mucosal *TFF2* expression decreased with EtOH administration in this study. This is the first study showing the role of TFF2 in gastroprotective effect of L-CAR against EtOH-induced gastric mucosal damage. L-CAR treatment increased the gastric mucosal *TFF2* levels, which decreased when EtOH was administered, by upregulating the *TFF2* expression. Given the effects of TFF2 on the gastric mucosal defense system, it can be opined

that L-CAR administration prior to EtOH reduces the EtOH-induced gastric mucosal damage by inducing re-epithelialization and stabilizing the mucous barrier.

CONCLUSION

It was established that L-CAR treatment has a significant protective effect against EtOH-induced gastric mucosal damage. L-CAR treatment was regulated the expression of gastric mucosal cyclooxygenase and nitric oxide synthase systems, reduced the expression of inflammatory cytokines, activated the cellular stress response, and stimulated the expression of mucus secretion and gastric epithelium restitution factors. Therefore, it can be summarized that L-CAR treatment protects against EtOH-induced gastric mucosal damage by regulating the expression of factors important in maintaining the gastric mucosal integrity in addition to its previously suggested antioxidative effect.

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Conflict of interest: The authors declared that there is no conflict of interest.

Ethical statement: All the experimental procedures were approved by the local ethics committee of Aydın Adnan Menderes University (approval no. 64583101/2020/009).

REFERENCES

Aihara, E., Engevik, K. A., & Montrose, M. H. (2017). Trefoil factor peptides and gastrointestinal function. *Annual Review of Physiology*, *79*, 357–380. https://doi.org/10.1146/annurev-physiol-021115-105447

Arafa, H. M., & Sayed-Ahmed, M. M. (2003). Protective role of carnitine esters against alcohol-induced gastric lesions in rats. *Pharmacological Research*, *48*(3), 285–290. https://doi.org/10.1016/s1043-6618(03)00154-3

Aziz, R. S., Siddiqua, A., Shahzad, M., Shabbir, A., & Naseem, N. (2019). 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. *Biomedicine & pharmacotherapy*, *110*, 554–560. https://doi.org/10.1016/j.biopha.2018.12.002

Bakalarz, D., Surmiak, M., Yang, X., Wójcik, D., Korbut, E., Śliwowski, Z., Ginter, G., Buszewicz, G., Brzozowski, T., Cieszkowski, J., Głowacka, U., Magierowska, K., Pan, Z., Wang, B., & Magierowski, M. (2021). Organic carbon monoxide prodrug, BW-CO-111, in protection against chemically-induced gastric mucosal damage. *Acta pharmaceutica Sinica B*, *11*(2), 456–475. https://doi.org/10.1016/j.apsb.2020.08.005

Chen, X., Zhao, Y., Liu, K., Li, Z., Tan, X., Wang, Y., Gao, N., Liu, C., Fang, X., & Wang, Y. (2021). Lycopene Aggravates Acute Gastric Injury Induced by Ethanol. *Frontiers in Nutrition*, *8*, 697879. https://doi.org/10.3389/fnut.2021.697879

Dokmeci, D., Akpolat, M., Aydogdu, N., Doganay, L., & Turan, F. N. (2005). L-carnitine inhibits ethanol-induced gastric mucosal injury in rats. *Pharmacological Reports*, *57*(4), 481–488.

Erkin, B., Dokmeci, D., Altaner, S., & Turan, F. N. (2006). Gastroprotective effect of L-carnitine on indomethacin-induced gastric mucosal injury in rats: A preliminary study. *Folia Medica*, *48*(3-4), 86–89.

Guth, P. H., Paulsen, G., & Nagata, H. (1984). Histologic and microcirculatory changes in alcohol-induced gastric lesions in the rat: effect of prostaglandin cytoprotection. *Gastroenterology*, *87*(5), 1083–1090. https://doi.org/10. 1016/S0016-5085(84)80068-2

Hanby, A. M., Poulsom, R., Singh, S., Elia, G., Jeffery, R. E., & Wright, N. A. (1993). Spasmolytic polypeptide is a major antral peptide: distribution of the trefoil peptides human spasmolytic polypeptide and pS2 in the stomach. *Gastroenterology*, *105*(4), 1110–1116. https://doi.org/10.1016/0016-5085(93)90956-d

Izgüt-Uysal, V. N., Bülbül, M., Tan, R., Derin, N., Ustünel, I., Ağar, A., & Yargiçoğlu, P. (2007). Effect of chronic stress and L-carnitine on rat stomach. *The Journal of Physiological Sciences*, *57*(3), 187–192. https://doi.org/10.2170/physiolsci.RP004707

Kart, A., Yapar, K., Karapehlivan, M., Tunca, R., Ogun, M., & Citil, M. (2006). Effects of L-carnitine on kidney histopathology, plasma and tissue total sialic acid, malondialdehyde and glutathione concentrations in response to gentamicin administration in Balb/C mice. *Revue de Médecine Vétérinaire, 157*(4), 179.

Kim, M. R., Kim, T. I., Choi, B. R., Kim, M. B., Cho, I. J., Lee, K. W., & Ku, S. K. (2020). Brassica oleracea Prevents HCl/Ethanol-Induced Gastric Damages in Mice. *Applied Sciences*, *11*(1), 16. https://doi.org/10.3390/app11010016

Kubes, P., & Wallace, J. L. (1995). Nitric oxide as a mediator of gastrointestinal mucosal injury?-Say it ain't so. *Mediators of Inflammation*, *4*(6), 397–405. https://doi.org/10.1155/S0962935195000640

Lacy, E. R., & Ito, S. (1982). Microscopic analysis of ethanol damage to rat gastric mucosa after treatment with a prostaglandin. *Gastroenterology*, *83*(3), 619–625. https://doi.org/10.1016/S0016-5085(82)80198-4

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods*, *25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

MacNaughton, W. K., Keenan, C. M., McKnight, G. W., & Wallace, J. L. (1989a). The modulation of gastric mucosal integrity by endothelin-1 and prostacyclin. *Journal of Cardiovascular Pharmacology*, *13*(Suppl 5), S118–S123. https://doi.org/10.1097/00005344-198900135-00029

MacNaughton, W. K., Cirino, G., & Wallace, J. L. (1989b). Endothelium-derived relaxing factor (nitric oxide) has protective actions in the stomach. *Life Sciences*, *45*(20), 1869–1876. https://doi.org/10.1016/0024-3205(89)90540-7

Madi, N., & Al-Barr, A. (2014). Comparative evaluation of the gastroprotective effect of L-Carnitine and Omeprazole on ethanol-induced gastric ulcer in male Albino Wister rats. *Bulletin of Egyptian Society for Physiological Sciences*, *34*(2), 293-309. https://doi.org/10.21608/BESPS.2014.34799

Magierowska, K., Wojcik, D., Chmura, A., Bakalarz, D., Wierdak, M., Kwiecien, S., Sliwowski, Z., Brzozowski, T., & Magierowski, M. (2018). Alterations in gastric mucosal expression of Calcitonin Gene-Related Peptides, Vanilloid Receptors, and Heme Oxygenase-1 mediate gastroprotective action of carbon monoxide against ethanol-induced gastric mucosal lesions. *International Journal of Molecular Sciences*, *19*(10), 2960. https://doi.org/10.3390/ijms19102960

Magierowska, K., Magierowski, M., Hubalewska-Mazgaj, M., Adamski, J., Surmiak, M., Sliwowski, Z., Kwiecien, S., & Brzozowski, T. (2015). Carbon monoxide (CO) released from Tricarbonyldichlororuthenium (II) Dimer (CORM-2) in gastroprotection against experimental ethanol-induced gastric damage. *PloS One*, *10*(10), e0140493. https://doi.org/10.1371/journal.pone.0140493

Martin, G. R., & Wallace, J. L. (2006). Gastrointestinal inflammation: A central component of mucosal defense and repair. *Experimental Biology and Medicine*, *231*(2), 130–137. https://doi.org/10.1177/153537020623100202

McKenzie, C., Thim, L., & Parsons, M. E. (2000). Topical and intravenous administration of trefoil factors protect the gastric mucosa from ethanol-induced injury in the rat. *Alimentary Pharmacology & Therapeutics*, *14*(8), 1033–1040. https://doi.org/10.1046/j.1365-2036.2000.00796.x

Nakamura, K., Rokutan, K., Marui, N., Aoike, A., & Kawai, K. (1991). Induction of heat shock proteins and their implication in protection against ethanol-induced damage in cultured guinea pig gastric mucosal cells. *Gastroenterology*, *101*(1), 161–166. https://doi.org/10.1016/0016-5085(91)90473-x

Oates, P. J., & Hakkinen, J. P. (1988). Studies on the mechanism of ethanol-induced gastric damage in rats. *Gastroenterology*, *94*(1), 10–21. https://doi.org/10.1016/0016-5085(88)90604-x

Poulsen, S. S., Thulesen, J., Christensen, L., Nexo, E., & Thim, L. (1999). Metabolism of oral trefoil factor 2 (TFF2) and the effect of oral and parenteral TFF2 on gastric and duodenal ulcer healing in the rat. *Gut*, *45*(4), 516–522. https://doi.org/10.1136/gut.45.4.516

Robert, A., Nezamis, J. E., Lancaster, C., & Hanchar, A. J. (1979). Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl, and thermal injury. Gastroenterology, 77(3), 433–443. https://doi.org/10.1016/0016-5085(79)90002-7

Rokutan, K. (2000). Role of heat shock proteins in gastric mucosal protection. *Journal of Gastroenterology and Hepatology*, *15*, 12-19. https://doi.org/10.1046/j.1440-1746.2000.02144.x

Sun, Y., Zheng, J., Yi, J., & Cai, S. (2022). Investigation on the effects and mechanisms of alkaline natural mineral water and distilled water on ethanol-induced gastric ulcers in vivo and in vitro. *Processes*, *10*(3), 498. https://doi.org/10.3390/pr10030498

Saika, M., Ueyama, T., & Senba, E. (2000). Expression of immediate early genes, HSP70, and COX-2 mRNAs in rat stomach following ethanol ingestion. *Digestive Diseases and Sciences*, *45*(12), 2455–2462. https://doi.org/10.1023/a:1005615714451

Szabo, S., Trier, J. S., Brown, A., & Schnoor, J. (1985). Early vascular injury and increased vascular permeability in gastric mucosal injury caused by ethanol in the rat. *Gastroenterology*, *88*(1 Pt 2), 228–236. https://doi.org/10.1016/s0016-5085(85)80176-1

Tepperman, B. L., & Soper, B. D. (1994). Nitric oxide synthase induction and cytoprotection of rat gastric mucosa from injury by ethanol. *Canadian Journal of Physiology and Pharmacology*, *72*(11), 1308–1312. https://doi.org/10.1139/y94-188

Thim, L., Madsen, F., & Poulsen, S. S. (2002). Effect of trefoil factors on the viscoelastic properties of mucus gels. *European Journal of Clinical Investigation*, *32*(7), 519–527. https://doi.org/10.1046/j.1365-2362.2002.01014.x

Tsukimi, Y., & Okabe, S. (2001). Recent advances in gastrointestinal pathophysiology: Role of heat shock proteins in mucosal defense and ulcer healing. *Biological & Pharmaceutical Bulletin*, *24*(1), 1–9. https://doi.org/10.1248/bpb.24.1

Wallace J. L. (2008). Prostaglandins, NSAIDs, and gastric mucosal protection: why doesn't the stomach digest itself?. *Physiological Reviews*, *88*(4), 1547–1565. https://doi.org/10.1152/physrev.00004.2008

Xue, L., Aihara, E., Wang, T. C., & Montrose, M. H. (2011). Trefoil factor 2 requires Na/H exchanger 2 activity to enhance mouse gastric epithelial repair. *The Journal of Biological Chemistry*, *286*(44), 38375–38382. https://doi.org/10.1074/jbc.M111.268219

Yu, L., Li, R., Liu, W., Zhou, Y., Li, Y., Qin, Y., Chen, Y., & Xu, Y. (2020). Protective effects of wheat peptides against ethanol-induced gastric mucosal lesions in rats: Vasodilation and anti-inflammation. *Nutrients*, *12*(8), 2355. https://doi.org/10.3390/nu12082355

Zhao, W., Zhu, F., Shen, W., Fu, A., Zheng, L., Yan, Z., Zhao, L., & Fu, G. (2009). Protective effects of DIDS against ethanol-induced gastric mucosal injury in rats. *Acta Biochimica et Biophysica Sinica*, *41*(4), 301–308. https://doi.org/10.1093/abbs/gmp014