ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Protective Effect of Tea Saponins on Alcohol-Induced Gastric Mucosal Injury in Mice

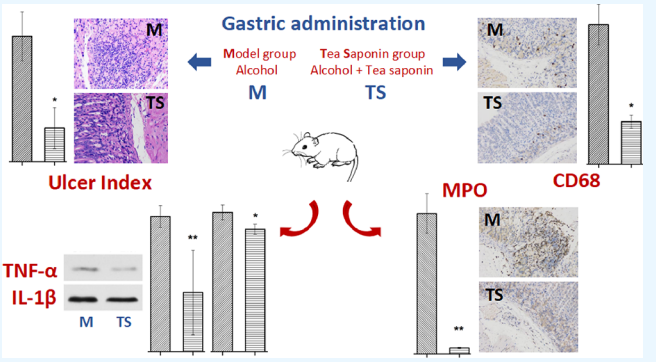
Zhaojuan Shi, Xue Long, Yan Li, Jing Jin, Jianfang Li, Chuanxun Yuan,* and Risheng Jin*

Cite This: *ACS Omega* 2023, 8, 673−681

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Excessive alcohol consumption harms the human body, particularly the digestive system, by causing damage to the gastric mucosa. Tea saponin is a natural active substance extracted from tea tree seeds that has gastroprotective potential against alcohol-induced mucosal damage. However, the protective mechanism of tea saponins is not fully understood. The current study aimed to explore the protective mechanism of tea saponins against alcohol-induced gastric mucosal injury in mice. Histopathological changes, immunohistochemistry, immunoblotting, and gastric mucosa-related cytokine levels were analyzed in three groups of male mice: model, control, and tea saponin-treated. Compared to the model group, the tea saponin group prominently ameliorated alcohol-induced gastric mucosal injury by improving cell necrosis, inflammatory cell infiltration, and edema. Downregulation of inflammation-related factors cluster of differentiation 68 (CD68), myeloperoxidase (MPO), tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β) was also found in the tea saponin group. These results suggest that tea saponins have a protective effect against alcohol-induced gastric mucosal damage in mice. Therefore, tea saponin may serve as a food additive for gastric mucosal protection.



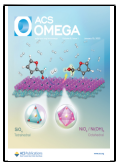
1. INTRODUCTION

Tea seed meal is generally considered useless after oil extraction, although it contains abundant triterpenoid saponins composed of glycosides, aglycones, and organic acid.[1] Tea saponins have been extensively studied as natural surfactants for their emulsifying, decentralization, and foaming properties.[2−4] In addition, their pharmacological functions, such as antibacterial, anti-oxidation, and antihypertension, have also been investigated.[5−7]

Acute alcoholism can cause damage to the nerves, digestive tract, liver, kidneys, and immune system. Among these, harm to the digestive system is most commonly documented.[8] A study on the emergency department of a university hospital in Germany reported that 30.7% of clinical cases of digestive system problems (the largest group) were related to alcohol abuse, among which 71.4% were diagnosed with alcohol intoxication, followed by alcohol dependency (12.5%).[9] As early as the 19th century, researchers discovered that short-term intake of alcohol in large amounts could cause acute gastric mucosal injury. Since then, research on the treatment of acute alcohol intoxication has expanded and diversified.[10−12] In addition to direct contact with alcohol, gastric mucosal injury is also associated with smoking, acid secretion, free radicals, *Helicobacter pylori* infection, and non-steroidal anti-inflammatory drugs.[13,14] Anti-ulcer therapy protects the gastric mucosa by inhibiting acid secretion, anti-oxidation, antibacterial, and other damaging effects. However, the side effects of anti-ulcer drugs are unpredictable.[15,16] Many researchers have sought safer and more effective natural ingredients to prevent and repair gastric mucosal injury. Lutein and two polysaccharides from *Hericium erinaceus* and *Trichodesma khasianum* Clarke leaves, which contain rosmarinic acid as the major component, are thought to positively affect protection against gastric mucosal damage.[13,17,18] Furthermore, *Punica granatum* fruit rind extract and polysaccharides extracted from *Dendrobium nobile* and *Phyllanthus niruri* leaves have shown potential as anti-ulcer drugs.[15,19,20]

The previously reported effect of the tea plant seed saponin fraction on alcohol metabolism suggests that saponin can play a protective role against alcohol-induced gastric mucosal lesions;[21−23] however, the current understanding of this role in mice is inadequate. In this study, we observed the effect of tea saponins on gastric ulcer lesions and pathological changes in alcohol-induced mice. The expression levels of cluster of differentiation 68 (CD68) and myeloperoxidase (MPO) were detected by immunohistochemistry, whereas variations in

Received: September 10, 2022
Accepted: December 14, 2022
Published: December 24, 2022



https://doi.org/10.1021/acsomega.2c05880
ACS Omega 2023, 8, 673−681

tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) levels were measured by Western blotting. Moreover, the levels of nitric oxide (NO), interleukin-8 (IL-8), prostaglandin E2 (PGE2), alcohol dehydrogenase (ADH), and aldehyde dehydrogenase (ALDH) in the stomach were assayed to further explore the protective mechanism of tea saponin against alcohol-induced gastric mucosal damage. This investigation aimed to provide basic theoretical parameters for researching gastric mucosal protection products and the possible application of tea saponin.

2. MATERIALS AND METHODS

2.1. Materials. Tea saponin (purity ≥ 96%, extracted from the tea seeds of *Camellia oleifera* Abel using alcohol extraction and belonging to the triterpenoid saponins) was obtained from Shanghai Fortune Biotechnology Co., Ltd. (Shanghai, China). This product is supplied as a yellow-brown powder that contains 10−25% sapogenin, according to the manufacturer. Baijiu was purchased from Hongxing Co. Ltd. (Beijing, China). Baijiu is brewed with high-quality red sorghum, barley, peas, and pure water using traditional brewing and modern microbial techniques. ADH, ALDH, and NO kits were purchased from Jiancheng Bioengineering Institute (Nanjing, China). IL-8 and PGE2 ELISA kits were acquired from Sigma-Aldrich Trading Co. Ltd. (Shanghai, China). Ethanol, xylene, citrate buffer, phosphate-buffered saline (PBS), 3% hydrogen peroxide, bovine serum albumin (BSA), paraformaldehyde, and 3,3-diaminobenzidine tetrahydrochloride (DAB) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

2.2. Animals and Treatments. Thirty male SPF Kunming mice (20 ± 2 g, 5 weeks old) were obtained from the Anhui Medical University laboratory (Anhui, China). All animals were maintained in a pathogen-free environment and fed ad libitum. The procedures for the care and use of animals were approved by the Biomedical Ethics Committee of Hefei University of Technology (no. HFUT20211009001). All applicable institutional and governmental regulations concerning the ethical use of animals were followed. The mice were housed with free access to chow and water in stainless steel cages under a controlled environment at 24 ± 2 °C temperature with 55 ± 5% relative air humidity and 12 h light−dark cycles. In the pre-experiment, an intoxicated mouse model was established by selecting 14 mL/kg body weight (BW) of 56% (v/v) Baijiu. The effects of low (50 mg/kg BW), medium (100 mg/kg BW), and high (200 mg/kg BW) doses of tea saponin on sobriety promotion and blood alcohol concentration (BAC) of intoxicated mice were investigated (Table S1 and Figure S1). The results showed that high doses of tea saponin significantly reduced BAC and delayed the time to peak BAC in alcoholic mice. Therefore, 200 mg/kg BW was used as the experimental dose. The mice were adaptively fed for one week and randomly divided into three groups (n = 10): control, model, and tea saponin groups. The control and model groups were administered distilled water (10 mL/kg BW), and the tea saponin group was treated with tea saponin (200 mg/kg BW). After 30 min, 56% v/v alcohol was intragastrically administered to the model and tea saponin groups at a dose of 7 mL/kg BW twice a day. Alcohol administration continued for two weeks with a 1 mL/kg BW increment every two days. The control group was provided with distilled water at a dose equivalent to that of the control and treatment groups. After the last alcohol treatment, the mice were fasted for 12 h and sacrificed. The stomachs were excised and washed with a saline solution. A section of the stomach tissue was fixed (4% paraformaldehyde) for hematoxylin and eosin (H&E) staining and histochemical analysis, while another section was stored at −80 °C. All treatments were administered via oral gavage.

2.3. Evaluation of Gastric Mucosal Injury. The degree and gross morphology of gastric mucosal injury in mice were observed, including the integrity of the gastric tissue, surface smoothness, color, bleeding, erosion, and other abnormal conditions. The ulcer index (UI) was calculated according to Guth et al.,[24] and the score was as follows: 1 point for mucosal lesions of size ≤1 mm (punctiform bleeding included); 2 points for of size mucosal lesions <1 mm and ≤2 mm; 3 points for mucosal lesions of size <2 mm and ≤3 mm; 4 points for mucosal lesions of size <3 mm and ≤4 mm; 5 points for mucosal lesions >4 mm; if the damage width was greater than 1 mm, the score was doubled. The UI of the mice was calculated as the sum of all points in the entire gastric tissue.

Paraformaldehyde-fixed stomach tissues were embedded in paraffin, sectioned, stained with H&E, and observed for histopathological changes.

2.4. Immunohistochemical Assessment. Stomach tissue sections were deparaffinized three times in xylene, hydrated in graded descending ethanol, and rehydrated with distilled water. The dewaxed tissue was placed in a citrate buffer and heated in a microwave oven for repair. After cooling, the tissues were placed in PBS and rinsed three times. The sections were then incubated with 3% hydrogen peroxide (H_2O_2) for 25 min and rinsed thrice with PBS. Subsequently, the sections were blocked with 3% BSA for 30 min, incubated with the prepared primary antibodies (CD68 and MPO) (Sigma, USA), incubated overnight at 4 °C, and rinsed three times with PBS. The corresponding secondary antibody (HRP) (DAKO, Denmark) was added, incubated at room temperature for 50 min, and then rinsed three times with PBS. DAB was used after the tissue sections were gently shaken, and the reaction was observed under a microscope (XSP-C204, Chongqing Guangdian Instrument Co., Ltd.). The slides were counterstained with hematoxylin for 3 min, dehydrated with ethanol and xylene sequentially, sealed, and examined under a microscope.

2.5. Western Blotting. The gastric tissue homogenate was centrifuged and the supernatant was collected. The stomach proteins were separated from the supernatant by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. Membranes were blocked with 5% non-fat milk solution and incubated with a diluted primary antibody at 4 °C. They were then rinsed four times with tris-buffered saline (TBS) containing 5% BSA and 0.1% Tween 20 (TBST) and incubated with the corresponding secondary antibody (1:4000) for 30 min at room temperature. Protein expression was detected by enhanced chemiluminescence (ECL). The target protein bands were analyzed using AlphaEase FC software.

2.6. Determination of NO, IL-8, and PGE2 Content in Gastric Mucosa. Gastric tissue homogenate (10%) was prepared in an ice bath and centrifuged at 699g for 20 min at 4 °C. The NO, IL-8, and PGE2 levels in the supernatant were determined using the corresponding commercial kits.

2.7. Measurement of ADH and ALDH Activities. The gastric tissue homogenate was centrifuged at 1006g for 15 min,

https://doi.org/10.1021/acsomega.2c05880
ACS Omega 2023, 8, 673−681

and the supernatant was collected. ADH and ALDH activities were measured according to the manufacturer's instructions.

2.8. Statistical Analysis. The data of each group are expressed as the mean ± standard deviation (SD). Normality of the data was tested using the Shapiro−Wilk test. Multiple group comparisons were performed by one-way analysis of variance using SPSS 19.0, and couple comparisons were performed using the *t* test. Results were considered statistically significant and highly statistically significant at $P < 0.05$ and $P < 0.01$, respectively.

3. RESULTS

3.1. Effect of Tea Saponin on Alcohol-Induced Gastric Mucosal UI in Mice. The results of gastric mucosal UI in each group are shown in Figure 1. The model group had

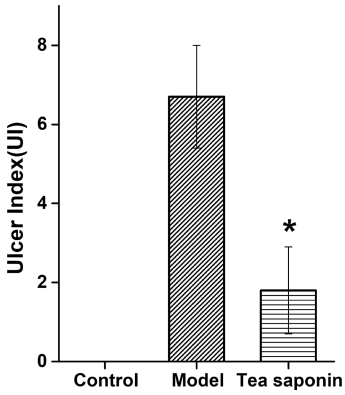


Figure 1. Effect of tea saponins on UI of gastric mucosa of alcohol-induced mice. Data were presented as mean value ± SD, $n = 10$. *$P < 0.05$ vs model group.

significantly increased UI ($P < 0.01$) compared to the control group, indicating alcohol-induced severe gastric mucosal damage. The structure of the gastric mucosa in the control group was complete and light red, with normal morphology. However, the gastric mucosa of the model group demonstrated an incomplete structure and non-smooth surface changes such as linear bleeding, erosion, and ulcer, denoted mottling. The tea saponin-treated alcohol-induced mice exhibited significantly reduced UI, which was lower than the model group ($P < 0.05$). Moreover, intact and smooth gastric mucosa was observed in tea saponin-treated mice, with scattered punctate erosion and linear bleeding. This indicates that tea saponins could effectively inhibit gastric mucosal damage caused by alcohol.

3.2. Effect of Tea Saponin on Histological Observations of Alcohol-Induced Gastric Mucosal Injury in Mice. H&E-stained gastric tissues exhibited intact gastric mucosa in the control group with normal cell arrangement, mucosa, and muscularis mucosa devoid of obvious lesions, such as tissue hemorrhage and inflammatory cell infiltration (Figure 2a,b). In contrast, a disorganized cell arrangement and glandular structure, inflammatory cell infiltration, submucosal edema, local hemorrhage, and hydropic degeneration of epithelial cells were observed in the model group (Figure 2c,d). Interestingly, these symptoms improved significantly following pretreatment with tea saponin (Figure 2e,f). Morphological results demonstrated the protective effect of tea saponin on alcohol-induced gastric mucosal injury in mice.

3.3. Effect of Tea Saponin on the Expression of CD68 and MPO in Alcohol-Induced Gastric Mucosal Injury in Mice. The protein expression of CD68 indicated the invasion of macrophages, and the expression of CD68 resulted in a brown-yellow color in the cytoplasm. In the blank control group, only a small positive expression was observed in the lamina propria (Figure 3Ba). The model group demonstrated more positive brown-yellow expression (Figure 3Bb) and markedly higher (nearly 4-fold, $P < 0.05$) integrated optical density (IOD) of CD68 than the blank control group (Figure 3A). In the tea saponin group, there were fewer brown-yellow positive reaction cells in the mucosa muscular layer (Figure 3Bc), and the IOD of CD68 was noticeably lower than that in the model group ($P < 0.05$).

The positive expression of MPO presented with a brown-yellow color, which was not significant in the blank control group (Figure 4Ba). The IOD of MPO in the model group was significantly higher than that in the blank control group ($P < 0.01$). As shown in Figure 4Bb, the entire mucosal layer and part of the mouse submucosa of the model group exhibited many brown-yellow positive expressions. Conversely, the positive expression of MPO in the tea saponin group was significantly alleviated (Figure 4Bc). Concurrently, Figure 4A also confirms that the IOD of the tea saponin group was distinctly reduced compared to the model group ($P < 0.01$).

3.4. Effect of Tea Saponin on TNF-α and IL-1β Protein in Alcohol-Induced Gastric Mucosal Injury in Mice. To evaluate the impact of tea saponin on the inflammatory response of the gastric mucosa of alcohol-induced mice, the protein expression of TNF-α and IL-1β was measured (Figure 5). Compared to the control group, the protein expression of TNF-α and IL-1β was markedly increased in the model group ($P < 0.01$). Pretreatment with tea saponin dramatically downregulated the protein expression of TNF-α ($P < 0.01$) and IL-1β ($P < 0.05$) compared to the model group. These results signify that tea saponins ameliorated alcohol-induced gastric mucosal injury in mice by restraining the expression of inflammatory cytokines.

3.5. Effect of Tea Saponin on NO, IL-8, and PGE2 in Alcohol-Induced Gastric Mucosal Injury in Mice. The levels of NO, IL-8, and PGE2 reflect gastric mucosal injury. As shown in Figure 6a, the NO content in the alcohol-treated model group was remarkably reduced compared to that in the control group ($P < 0.05$). In contrast, the NO content in the gastric mucosa of the tea saponin group displayed a significant improvement compared to the model group ($P < 0.05$), implying tea saponin-mediated elevation of NO production. Figure 6b shows notably higher IL-8 levels as pro-inflammatory cytokines after alcohol intake than in the control group ($P < 0.01$). Although treatment with tea saponin brought ample reduction in the IL-8 levels ($P < 0.05$), it did not exert an apparent effect on PGE2 levels, which is one of the protective cytokines in the gastric mucosa (Figure 6c).

3.6. Effect of Tea Saponin on ADH and ALDH Activities in Alcohol-Induced Gastric Mucosal Injury in Mice. The activities of ADH and ALDH are used as references for gastric alcohol metabolism. Figure 7a illustrates the significantly superior ADH activity in the gastric tissue of the tea saponin-treated mice group compared to that in the model group ($P < 0.05$). Although the activity of ALDH in the tea saponin group was higher than that in the model group, the difference was statistically insignificant ($P > 0.05$). These results suggest that tea saponins can improve the first-pass

https://doi.org/10.1021/acsomega.2c05880
ACS Omega 2023, 8, 673−681

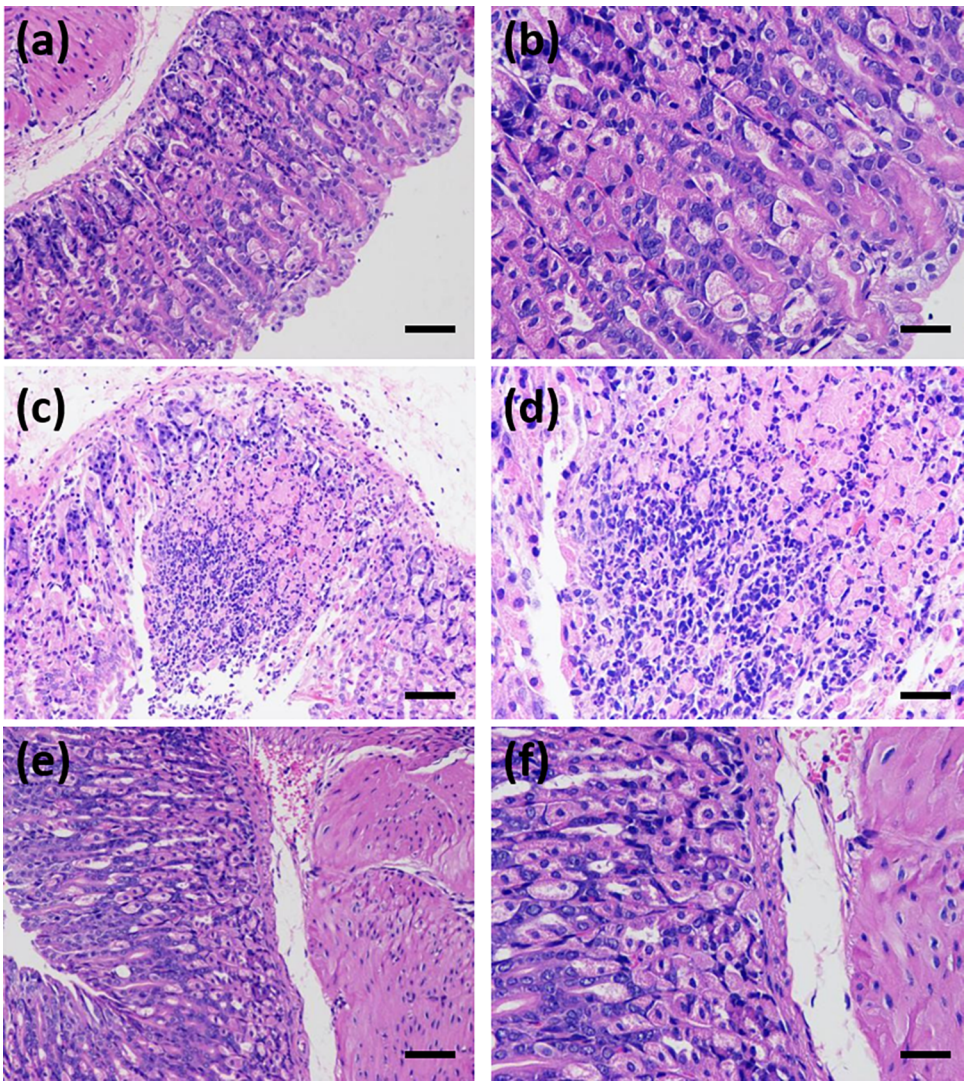


Figure 2. The morphology of gastric sections of (a, b) blank control, (c, d) model, and (e, f) tea saponin groups; scale bars of 100 μm (a, c, e) and 50 μm (b, d, f).

metabolism of alcohol in the stomach by enhancing the activity of ADH.

4. DISCUSSION

Tea saponin is a glycoside compound derived from tea seeds that is widely used as a surfactant owing to its amphiphilicity.[25] Various biological activities of saponins have been reported, including antibacterial, anti-inflammatory, antioxidant, anti-cancer, hepatoprotective, and anti-ulcer properties. These activities are related to the chemical structure of saponins.[26]

Excessive alcohol intake can damage the mucosal layer, disrupt the lipid−protein layer on the cell surface, and destroy the connections between cells, resulting in histological changes in the gastric mucosa.[27] Inflammatory reactions such as mucosal hyperemia, erosions, and ulcers have been observed after alcoholism. Tea saponin has been reported as early as the 1960s in reducing inflammation and resisting infiltration.[6] The seed saponin fraction from *Thea sinensis* L. administered pre- and post-ethanol administration decreased ethanol levels in the blood and liver by slowing gastric emptying and inhibiting absorption across the digestive tract membrane.[22] Recent studies have also shown that tea saponins have high potential to activate ADH.[28] Thus, tea saponins may provide a protective effect against alcohol-induced gastric mucosal damage in mice. The mechanism by which tea saponins protect the gastric mucosa from alcohol damage is poorly understood. In this experiment, 7 mL/kg BW of 56% v/v alcohol was administered to mice twice daily (12 h apart) for two weeks, incrementing the dose by 1 mL/kg BW. Since the alcohol concentration increased in the stomach after 2 weeks of alcohol administration, the gastric mucosa of the model mice displayed apparent damage, including changes in mucosal unevenness, erosion, and ulcers, and the highest UI (Figure 1). Furthermore, the absence of gastric mucosal abnormalities in the blank control group indicated a successful gastric injury.

The first-pass metabolism of alcohol was enhanced by the tea saponin-mediated elevation of ADH activity, minimizing alcohol damage to the gastric mucosa. Tsukamoto et al. reported a similar mechanism for tea seed saponins in mitigating alcohol-induced gastric mucosal injury.[22] Additionally, improvements in neutrophil infiltration, edema, and cellular disorganization were observed under a light microscope (Figure 2). Consequently, the UI in mice was

https://doi.org/10.1021/acsomega.2c05880
ACS Omega 2023, 8, 673−681

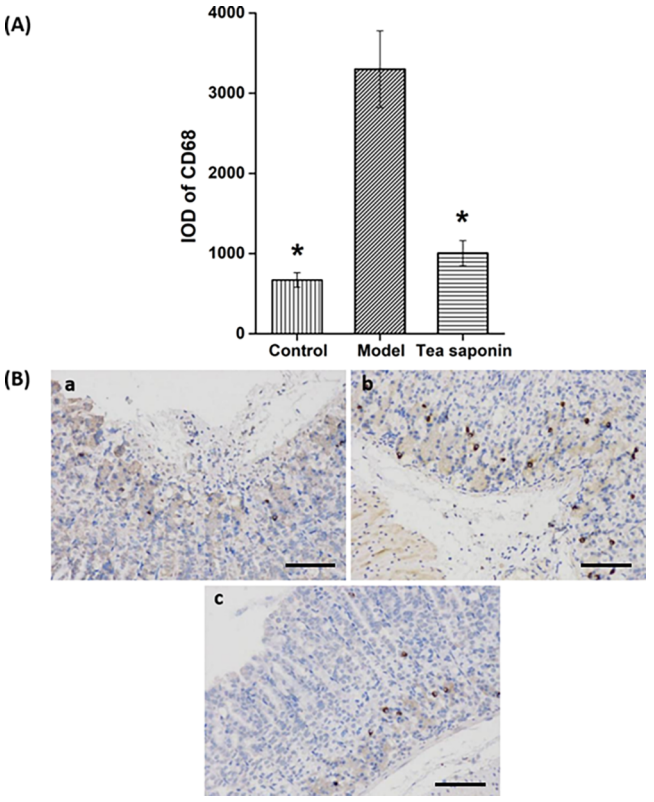


Figure 3. Effects of tea saponins on CD68 protein expression in stomach of alcohol-induced mice: results of (A) quantitative analysis and (B) immunohistochemical staining of (a) control, (b) model, and (c) tea saponin groups (scale bar: 100 μm). Data were presented as mean value ± SD, (n = 10). *P < 0.05 vs model group.

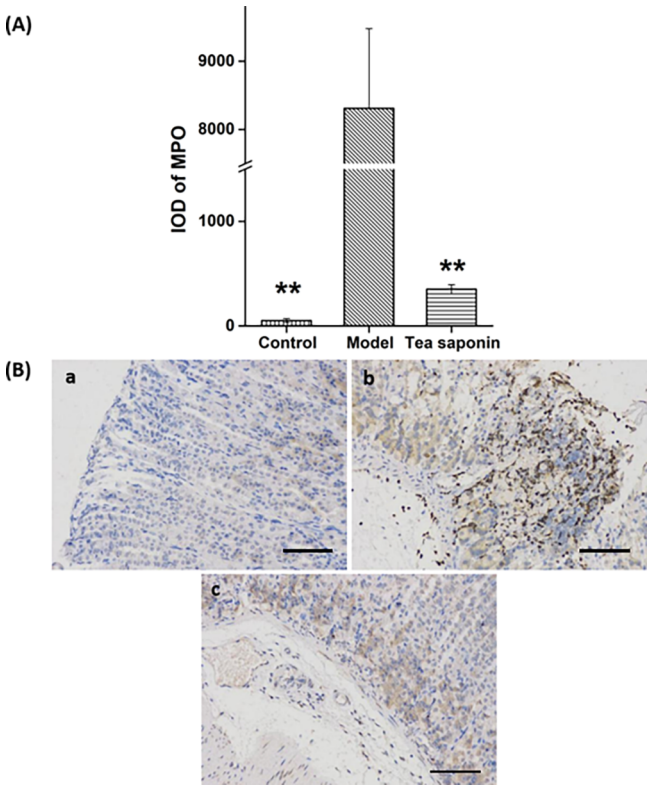


Figure 4. Effects of tea saponins on MPO expression in the stomach of alcohol-induced mice: results of (A) quantitative analysis and (B) immunohistochemical staining (scale bar: 100 μm) of (a) control, (b) model, and (c) tea saponin groups. Data were presented as mean value ± SD (n = 10). **P < 0.01 vs model group.

dramatically reduced, and gastric mucosal injury was alleviated by tea saponin pretreatment.

The inflammatory response of the gastric mucosa is caused by inflammatory factors that inhibit the secretion of gastric mucosal epithelial cells.[29] These could be one of the causes of alcohol-induced gastric mucosal damage in mice due to the important role of inflammatory cytokines in inflammation and gastric mucosal injury. CD68 is a transmembrane glycoprotein that is highly expressed by human monocytes and tissue macrophages and functions as a mediator, recruiter, and activator of macrophages.[30] MPO, an indicator of neutrophil infiltration, is most abundantly expressed in neutrophil granulocytes, which can induce tissue injury by releasing potent reactive oxygen species.[17,31] We measured the number of macrophages and neutrophils in the gastric mucosa of alcohol-induced mice using CD68 and MPO immunohistochemical staining. Severe damage to the gastric mucosa and a dramatic increase in macrophage and neutrophil infiltration were observed in the alcohol-treated model group. The IODs of CD68 and MPO in the model group were thousands of times higher than those in the control group. However, tea saponin treatment was remarkably associated with a decrease in CD68 and MPO expression, suggesting that tea saponin may reduce inflammatory response-related protein expression to alleviate gastric mucosal damage (Figure 4). Moreover, the expression of proinflammatory cytokines (TNF-α and IL-1β), which can mediate inflammation through interaction with the IL-1 receptor (IL-1R) and the TNF receptor (TNFR), was detected using Western blotting.[32] The proinflammatory chemokine IL-8, a critical index of inflammatory response determining the degree of gastric mucosal inflammation, can induce neutrophils to accumulate in the gastric mucosa, leading to severe gastric mucosal damage.[33] In the present study, we found that indicators related to inflammatory response were significantly affected by the excessive intake of alcohol. However, tea saponin treatment downregulated the expression of TNF-α, IL-1β, and IL-8 in the stomachs of alcohol-induced mice (Figures 5 and 6). Saponin from other sources, such as *Panax notoginseng* saponin, has also been reported to decelerate ethanol-induced gastric injury and accelerate gastric ulcer recovery by decreasing the expression of proinflammatory factors such as TNF-α, IL-1β, and IL-6.[34] Furthermore, histological data of the present study revealed an attenuating effect of tea saponin treatment on inflammation and gastric mucosal injury, suggesting a possible protective role against alcohol-induced gastric mucosal injury in mice by curbing the inflammatory response.

NO is a small molecule synthesized from the terminal guanidine nitrogen atom of L-arginine using nitric oxide synthase (NOS), which has three enzymatic sources: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS).[35] NO synthesized by constitutive NOS (nNOS and eNOS) regulates blood flow. It interacts with prostaglandins and neuropeptides to maintain gastric mucosal integrity and accelerate gastric ulcer healing, while high levels of NO produced by iNOS can trigger infection or inflammation.[36] The marked decrease in NO levels after alcohol administration implies the involvement of gastric mucosal protective and defense factors in the pathological process of alcohol-induced

https://doi.org/10.1021/acsomega.2c05880
ACS Omega 2023, 8, 673−681

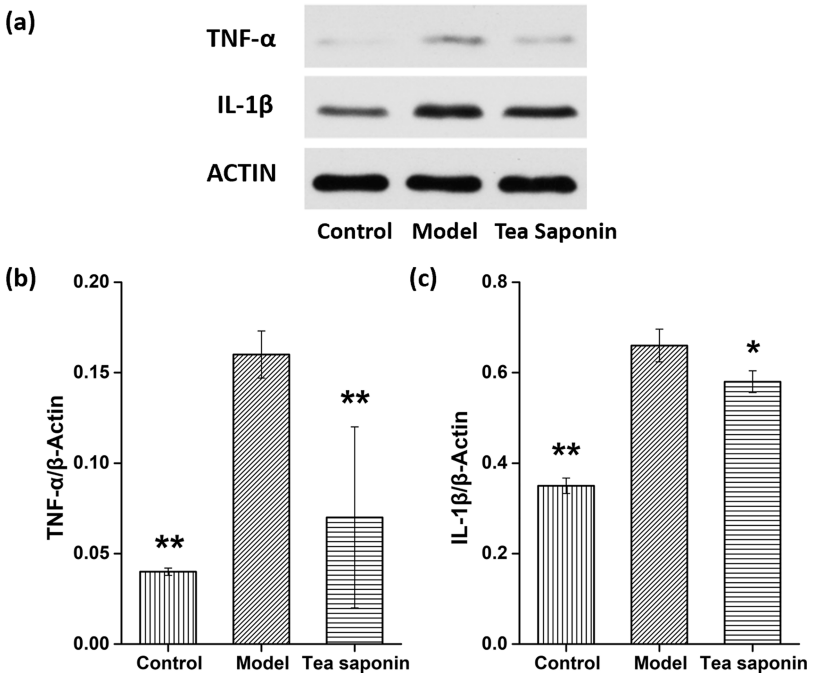


Figure 5. Effects of tea saponin on the protein expressions of TNF-α and IL-1β in gastric tissues of alcohol-induced mice: (a) immunoblot analysis, (b) TNF-α, and (c) IL-1β. Data were presented as mean value ± SD (n = 10). **P < 0.01, *P < 0.05 vs model group.

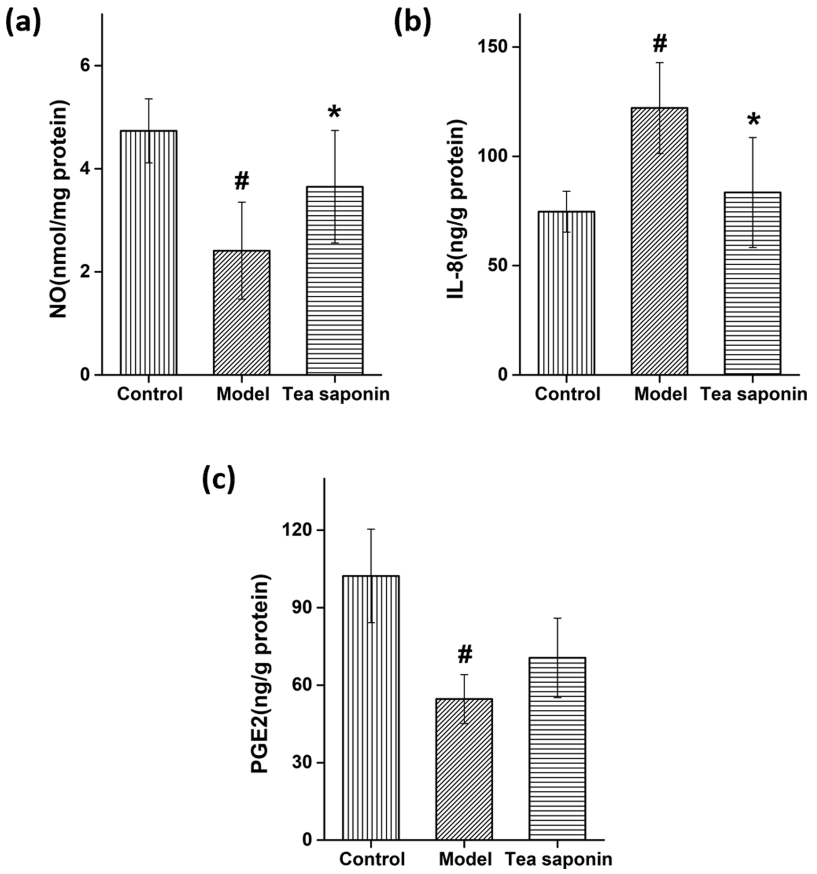


Figure 6. Effects of tea saponin on (a) NO, (b) IL-8, and (c) PGE2 in gastric tissues of alcohol-induced mice. Data were presented as mean value ± SD, n = 10. *P < 0.05 vs model group, $^{\#}P$ < 0.01 vs control group.

gastric mucosal injury. Our results showed that NO levels returned to normal after tea saponin treatment. This suggests that tea saponins may ameliorate alcohol-induced gastric mucosal injury in mice by regulating NO levels to modulate blood flow and maintain gastric mucosal integrity. The defense factor of the gastric mucosa can play a protective role when the

https://doi.org/10.1021/acsomega.2c05880
ACS Omega 2023, 8, 673−681

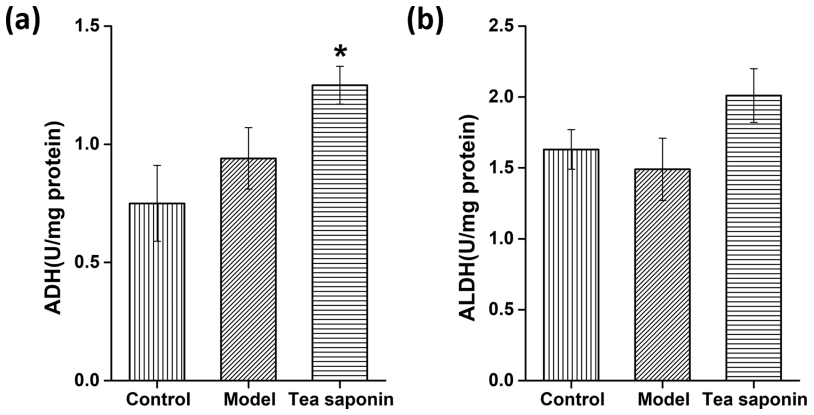


Figure 7. Effects of tea saponin on activities of (a) ADH and (b) ALDH in gastric tissues of alcohol-induced mice. Data were presented as mean value ± SD, $n = 10$. *$P < 0.05$ vs model group.

attack factor causes damage. PGE2 is a prostaglandin synthesized and secreted by the gastric mucosa, which can accelerate ulcer healing and increase the resistance of the gastric mucosa to injury via increasing the mucosal blood flow, reducing inflammation and stimulating the formation of protective factors in the mucosa.[37] Our study showed that PGE2 levels in mice were substantially decreased after excessive alcohol intake. PGE2 levels were elevated in the tea saponin treatment group, but the results were unremarkable, suggesting tea saponin-mediated protection against alcohol-induced gastric damage to a certain extent in mice. Therefore, the protective mechanism of tea saponin on the gastric mucosa might be unrelated to PGE2 content.

In this study, we investigated the protective effect of tea saponins on gastric mucosal injury in mice with alcoholism. We found that tea saponin participates in the metabolism of alcohol, promotes the first-pass metabolism of alcohol in the stomach, activates gastric mucosal protective factors, and reduces the activity of proinflammatory cytokines. The specific mechanisms of tea saponin detoxification should be further explored from the perspective of cells and genes.

5. CONCLUSIONS

In summary, the experimental results confirmed that tea saponin affected the enzymes associated with alcohol metabolism, regulated inflammation-related cytokines, and restored gastric mucosal integrity to protect the gastric mucosa from damage in alcohol-induced mice. Tea saponin may be a potential component of anti-ulcer drugs because it can reduce inflammation and accelerate alcohol metabolism in the stomach.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c05880.

Effect of tea saponin on BAC in alcoholic mice; effect of tea saponin on sobriety promotion in alcoholic mice (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Chuanxun Yuan − *School of Food and Biological Engineering, Hefei University of Technology, Hefei 230009, P. R. China; Engineering Research Center of Agricultural Product Biochemicals, Ministry of Education, Hefei 230009, P. R. China*; Email: ycx608@sohu.com

Risheng Jin − *School of Food and Biological Engineering, Hefei University of Technology, Hefei 230009, P. R. China; Engineering Research Center of Agricultural Product Biochemicals, Ministry of Education, Hefei 230009, P. R. China*; orcid.org/0000-0002-2140-6507; Email: jinrisheng@hfut.edu.cn

Authors

Zhaojuan Shi − *School of Food and Biological Engineering, Hefei University of Technology, Hefei 230009, P. R. China; Engineering Research Center of Agricultural Product Biochemicals, Ministry of Education, Hefei 230009, P. R. China*

Xue Long − *School of Food and Biological Engineering, Hefei University of Technology, Hefei 230009, P. R. China; Engineering Research Center of Agricultural Product Biochemicals, Ministry of Education, Hefei 230009, P. R. China*

Yan Li − *School of Food and Biological Engineering, Hefei University of Technology, Hefei 230009, P. R. China; Engineering Research Center of Agricultural Product Biochemicals, Ministry of Education, Hefei 230009, P. R. China*

Jing Jin − *School of Food and Biological Engineering, Hefei University of Technology, Hefei 230009, P. R. China; Engineering Research Center of Agricultural Product Biochemicals, Ministry of Education, Hefei 230009, P. R. China*

Jianfang Li − *School of Food and Biological Engineering, Hefei University of Technology, Hefei 230009, P. R. China; Engineering Research Center of Agricultural Product Biochemicals, Ministry of Education, Hefei 230009, P. R. China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c05880

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Science and Technology Major Project of the Anhui Province of China (202003a06020011 and 202103b06020025).

ABBREVIATIONS USED

MPO	myeloperoxidase
TNF-α	tumor necrosis factor- α
IL-1β	interleukin-1β
NO	nitric oxide
IL-8	interleukin-8
PGE2	prostaglandin-E(2)
ADH	alcohol dehydrogenase
ALDH	aldehyde dehydrogenase
H&E	hematoxylin and eosin
UI	ulcer index
IOD	integrated optical density
PBS	phosphate-buffered saline
BSA	bovine serum albumin
DAB	3,3-diaminobenzidine tetrahydrochloride.

REFERENCES

(1) Hao, W.; Li, H.; Hu, M.; Yang, L.; Rizwan-ul-Haq, M. Integrated control of citrus green and blue mold and sour rot by Bacillus amyloliquefaciens in combination with tea saponin. *Postharvest Biol. Technol.* **2011**, *59*, 316−323.

(2) Wei, Y.; Chen, X.; Yang, T.; Wan, J.; Cao, X. Partition of tea saponin with a novel recyclable thermo-pH aqueous two-phase systems. *Appl. Biochem. Biotechnol.* **2021**, *193*, 3062−3078.

(3) Liu, C.; Xu, B.; McClements, D. J.; Xu, X.; Cui, S.; Gao, L.; Zhou, L.; Xiong, L.; Sun, Q.; Dai, L. Properties of curcumin-loaded zein-tea saponin nanoparticles prepared by antisolvent co-precipitation and precipitation. *Food Chem.* **2022**, 133224.

(4) Yuan, Y.; Xiao, J.; Zhang, P.; Ma, M.; Wang, D.; Xu, Y. Development of pH-driven zein/tea saponin composite nanoparticles for encapsulation and oral delivery of curcumin. *Food Chem.* **2021**, *364*, 130401.

(5) Yu, Z.; Wu, X.; He, J. Study on the antifungal activity and mechanism of tea saponin from *Camellia oleifera* cake. *Eur. Food Res. Technol.* **2022**, *248*, 783−795.

(6) Vogel, G.; Marek, M. L.; Oertner, R. On the pharmacology of tea-seed-saponins of a saponin mixture from thea sinensis L. *Arzneim. Forsch.* **1968**, *18*, 1466−1467.

(7) Sur, P.; Chaudhuri, T.; Vedasiromoni, J. R.; Gomes, A.; Ganguly, D. K. Antiinflammatory and antioxidant property of saponins of tea [Camellia sinensis (L) O. Kuntze] root extract. *Phytother. Res* **2001**, *15*, 174−176.

(8) Vonghia, L.; Leggio, L.; Ferrulli, A.; Bertini, M.; Gasbarrini, G.; Addolorato, G. Acute alcohol intoxication. *Eur. J. Intern. Med.* **2008**, *19*, 561−567.

(9) Te Wildt, B. T.; Andreis, C.; Auffahrt, I.; Tettenborn, C.; Kropp, S.; Ohlmeier, M. Alcohol related conditions represent a major psychiatric problem in emergency departments. *Emerg. Med. J.* **2006**, *23*, 428−430.

(10) Robert, A.; Nezamis, J. E.; Lancaster, C. L.; Hanchar, A. J. Cytoprotection by prostaglandins in rats, prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl and thermal injury. *Gastroenterology* **1979**, *77*, 223−227.

(11) Keshavarzían, A.; Fields, J. Z.; Vaeth, J.; Holmes, E. W. The differing effects of acute and chronic alcohol on gastric and intestinal permeability. *Am. J. Gastroenterol.* **1994**, *89*, 2205−2211.

(12) Guth, P. H.; Paulsen, G.; Nagata, H. Histologic and microcirculatory changes in alcohol-induced gastric lesions in the rat: effect of prostaglandin cytoprotection. *Gastroenterology* **1984**, *87*, 1083−1090.

(13) Sindhu, E. R.; Kuttan, R. Carotenoid lutein protects rats from gastric ulcer induced by ethanol. *J. Basic Clin. Physiol. Pharmacol.* **2012**, *23*, 33−37.

(14) Park, H.; Cho, D.; Huang, E.; Seo, J. Y.; Kim, W. G.; Todorov, S. D.; Ji, Y.; Holzapfel, W. Amelioration of Alcohol Induced Gastric Ulcers Through the Administration of Lactobacillus plantarum APSulloc 331261 Isolated From Green Tea. *Front. Microbiol.* **2020**, *11*, 420.

(15) Zhang, Y.; Wang, H.; Mei, N.; Ma, C.; Lou, Z.; Lv, W.; He, G. Protective effects of polysaccharide from dendrobium nobile against ethanol-induced gastric damage in rats. *Int. J. Biol. Macromol.* **2018**, *107*, 230−235.

(16) Du, D.; Ma, X.; Zhang, J.; Zhang, Y.; Zhou, X.; Li, Y. Cellular and molecular mechanisms of 17β-estradiol postconditioning protection against gastric mucosal injury induced by ischemia/reperfusion in rats. *Life Sci.* **2010**, *86*, 30−38.

(17) Hou, C.; Liu, L.; Ren, J.; Huang, M.; Yuan, E. Structural characterization of two Hericium erinaceus polysaccharides and their protective effects on the alcohol-induced gastric mucosal injury. *Food Chem.* **2022**, *375*, 131896.

(18) Wang, G. Y.; Chen, S. Y.; Chen, Y. Y.; Hong, C. J.; Hsu, Y. H.; Yen, G. C. Protective effect of rosmarinic acid-rich trichodesma khasianum clarke leaves against ethanol-induced gastric mucosal injury in vitro and in vivo. *Phytomedicine* **2021**, *80*, 153382.

(19) Ajaikumar, K. B.; Asheef, M.; Babu, B. H.; Padikkala, J. The inhibition of gastric mucosal injury by punicagranatum l. (pomegranate) methanolic extract. *J. Ethnopharmacol.* **2005**, *96*, 171−176.

(20) Abdulla, M. A.; Ahmed, K. A.; AL-Bayaty, F. H.; Masood, Y. Gastroprotective effect of phyllanthus niruri leaf extract against ethanol-induced gastric mucosal injury in rats. *Afr. J. Pharm. Pharmacol.* **2010**, *4*, 226−230.

(21) Yoshikawa, M.; Murakami, T.; Matsuda, H.; Yamahara, J.; Murakami, N.; Kitagawa, I. Bioactive saponins and glycosides. iii. horse chestnut. (1): the structures, inhibitory effects on ethanol absorption, and hypoglycemic activity of escins Ia, Ib, IIa, IIb, and IIIa from the seeds of aesculus hippocastanum l. *Chem. Pharm. Bull.* **1996**, *44*, 1454−1464.

(22) Tsukamoto, S.; Kanegae, T.; Nagoya, T.; Shimamura, M.; Kato, T.; Watanabe, S.; Kawaguchi, M. Effects of seed saponins of Thea sinensis L (Ryokucha saponin) on alcohol absorption and metabolism. *Alcohol Alcohol.* **1993**, *28*, 687−692.

(23) Murakami, T.; Nakamura, J.; Matsuda, H.; Yoshikawa, M. Bioactive saponins and glycosides. XV. Saponin constituents with gastroprotective effect from the seeds of tea plant, *Camellia sinensis* L. var. assamica Pierre, cultivated in Sri Lanka: structures of assamsaponins A, B, C, D, and E. *Chem. Pharm. Bull.* **1999**, *47*, 1759−1764.

(24) Guth, P. H.; Aures, D.; Paulsen, G. Topical aspirin plus HCl gastric lesions in the rat. Cytoprotective effect of prosta-glandin, cimetidine, and probanthine. *Gastroenterology* **1979**, *76*, 88−93.

(25) Du, L.; Li, S.; Jiang, Q.; Tan, Y.; Liu, Y.; Meng, Z. Interfacial interaction of small molecular emulsifiers tea saponin and monoglyceride: Relationship to the formation and stabilization of emulsion gels. *Food Hydrocolloids* **2021**, *117*, 106737.

(26) Chey, W. Y. Alcohol and gastric mucosa. *Digestion* **2004**, *7*, 239−251.

(27) Julkunen, R. J. K.; Tannenbaum, L.; Baraona, E.; Lieber, C. S. First pass metabolism of ethanol: an important determinant of blood levels after alcohol consumption. *Alcohol* **1985**, *2*, 437−441.

(28) Chuanxun, Y.; Yan, L.; Qingchuan, L.; Risheng, J.; Lili, R. Purification of tea saponins and evaluation of its effect on alcohol dehydrogenase activity. *Open Life Sci.* **2018**, *13*, 56−63.

(29) Medzhitov, R. Inflammation 2010: New Adventures of an Old Flame. *Cell* **2010**, *140*, 771−776.

(30) Holness, C. L.; Simmons, D. L. Molecular cloning of CD68, a human macrophage marker related to lysosomal glycoproteins. *Blood* **1993**, *81*, 1607−1613.

(31) Linlin, C.; Huidan, D.; Hengmin, C.; Jing, F.; Zhicai, Z.; Junliang, D.; et al. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget.* **2018**, *9*, 7204−7218.

(32) Wallace, J. L. Gastric ulceration: critical events at the neutrophil–endothelium interface. *Can. J. Physiol. Pharmacol.* **1993**, *71*, 98−102.

https://doi.org/10.1021/acsomega.2c05880
ACS Omega 2023, 8, 673−681

(33) Palmer, R. M. J.; Ferrige, A. G.; Moncada, S. Nitric oxide release accounts for biological activity of endothelium-derived relaxing factor. *Nature* **1987**, *327*, 524−526.
(34) Bingqian, Z.; Wen, Z.; Yujia, W.; Ying, Y.; Na, W.; Junsong, L.; Tingming, F.; Lingchong, W.; Liuqing, D. Improved efficacy of *Panax notoginseng* saponin loaded into BSP/alginate microspheres for the treatment of alcoholic gastric ulcers. *Int. J. Pharm.* **2021**, *596*, 120218.
(35) Calatayud, S.; Barrachina, D.; Esplugues, J. V. Nitric oxide: relation to integrity, injury, and healing of the gastric mucosa. *Microsc. Res. Tech.* **2001**, *53*, 325−335.
(36) Masuda, E.; Kawano, S.; Nagano, K.; Tsuji, S.; Takei, Y.; Hayashi, N.; et al. Role of endogenous endothelin in pathogenesis of ethanol-induced gastric mucosal injury in rats. *Am J Physiol.* **1993**, *265*, G474−G481.
(37) Whittle, B. J. R.; Lopez-Belmonte, J.; Moncada, S. Regulation of gastric mucosal integrity by endogenous nitric oxide: interactions with prostanoids and sensory neuropeptides in the rat. *Br. J. Pharmacol.* **2012**, *99*, 607−611.

https://doi.org/10.1021/acsomega.2c05880
ACS Omega 2023, 8, 673−681