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Evaluation of the Gastroprotective and Therapeutic Effects of *Arthrospira platensis* Against Ethanol-Induced Gastric Ulceration and Associated Oxidative Damage in Rats

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Abstract

The therapeutic properties of *Arthrospira platensis* (*Spirulina platensis*) have attracted scientific interest. The present study discussed the protective and therapeutic effects of both locally cultured and organic *A. platensis* tablets USA on gastric ulcers in rats, as well as their possible antioxidant activity and effects on DNA fragmentation. Antioxidant defense enzymes (superoxide dismutase and glutathione peroxidase) were examined, together with lipid peroxidation (MDA) levels and DNA damage in the gastric mucosa using the comet assay to assess the effect of these treatments. The study also analyzed the biochemical composition of locally cultured *A. platensis* compared with commercial USA organic *Spirulina* tablets. Fifty-four rats were assigned to nine groups. First group acts as the control, whereas the remaining groups were treated with cultured *Spirulina platensis* powder or organic *Spirulina* tablets before or after ethanol administration. Intra-gastric administration of *Spirulina* (500 mg/kg body weight) before (protection) or after (treatment) ethanol administration significantly decreased the ulcer index relative to the ulcer control group ($p < 0.05$). Moreover, the ethanol-induced decrease in gastric antioxidant enzyme activity seen in the ulcer control group was prevented by *Spirulina* powder administered either before or after ethanol exposure. For addition, *Spirulina* reversed the ethanol-induced increase in lipid peroxidation and DNA fragmentation. The study also described the effects of these treatments on histopathological lesions induced by ethanol in the stomach of rats. In conclusion, *Spirulina* concluded as anti-ulcer, anti-inflammatory, and antioxidant activities and significantly reduced DNA damage. The results suggest that locally cultured *A. platensis* exerted gastro protective and therapeutic effects against ethanol-induced gastric ulceration. Its efficacy was comparable to that of commercial USA *Spirulina* tablets in most assessed parameters and exceeded that of famotidine in several biochemical, oxidative stress, and histopathological indices.

Keywords: *Arthrospira platensis*; Gastric ulcer; Ethanol-induced ulcer; Oxidative stress; DNA damage; Comet assay; Histopathology; Gastroprotection.

Introduction

Arthrospira platensis (Spirulina platensis) is a filamentous blue green alga recognized for its high nutritional value and biological activities. It contains high proteins concentration, essential amino acids, vitamins, minerals, carotenoids, essential fatty acids, and phycobiliproteins, particularly phycocyanin, which is considered one of its major bioactive constituents (Kumawat et al., 2025; Barros de Medeiros et al., 2022). Recent researche has shown that Spirulina contains several bioactive constituents, including phenolic compounds, flavonoids, carotenoids, and phycobiliproteins, which support its nutritional and therapeutic properties (Spinola et al., 2024), with biological activities, such as antioxidant, anti-inflammatory effects, immunomodulatory, antihyperlipidemic, and antigenotoxic effects These biological effects have been attributed to its ability to scavenge reactive oxygen species, reduce lipid peroxidation, and enhance endogenous antioxidant defence mechanisms (Abdel-Daim et al., 2015; Diniz et al., 2023;

Motamedzadeh et al., 2024). Moreover, Spirulina has shown protective effects against tissue injury caused by oxidative stress in various experimental models, suggesting its potential role in preventing cellular damage and promoting tissue recovery (Kamar et al., 2022; Guzmán-Gómez et al., 2023). A systematic review and meta-analysis further supported the beneficial effects of Spirulina supplementation on oxidative stress and inflammatory biomarkers, highlighting its potential role in modulating redox balance and inflammatory responses under different pathological conditions (Mousavi et al., 2025). More recent evidence has confirmed the antioxidant, anti-inflammatory, and cytoprotective activities of Spirulina and emphasized its potential application in preventing and managing oxidative stress-associated disorders (Behairy et al., 2024).

Gastric ulceration is a common gastrointestinal disorders and results from an imbalance between aggressive factors, including gastric acid secretion, pepsin activity, reactive oxygen species (ROS), and inflammatory mediators, and protective factors such as mucus secretion, bicarbonate production, prostaglandins, antioxidant defenses, and mucosal regeneration (Aboul Naser et al., 2020). Ethanol-induced gastric ulceration is a widely accepted experimental model because ethanol rapidly disrupts gastric mucosal integrity by increasing mucosal permeability, promoting hydrogen ion back-diffusion, and inducing oxidative stress and inflammatory responses that ultimately lead to tissue injury and ulcer formation. high level reactive oxygen species contribute to gastric mucosal damage through lipid peroxidation, cellular injury, and DNA damage, leading to poor tissue healing (Rahman et al., 2020).

Although several pharmacological agents, including histamine H_2-receptor antagonists such as famotidine, are commonly used to treat gastric ulcers, the search for natural therapeutic alternatives with antioxidant and cytoprotective properties remains an important area of investigation (Alazzouni et al., 2021)

Several studies have demonstrated the gastro protective of Spirulina against gastric ulceration. Kamar et al. (2022) reported that Spirulina supplementation attenuated histological and ultrastructural alterations in the gastric mucosa of rats through its antioxidant and cytoprotective activities. Similarly, Guzmán-Gómez et al. (2023) reported that phycobiliproteins isolated from *Arthrospira maxima* reduced the severity of indomethacin-induced gastric ulcers and improved gastric tissue integrity in rats**.**

Likewise, Aleid et al. (2021) reported that Spirulina extract improved antioxidant status, reduced gastric lesions, and improved the histological appearance of gastric tissue in rats with experimentally induced gastric ulcers. These beneficial effects are mainly related to the bioactive compounds in Spirulina, which help reduce oxidative stress, protect the gastric mucosa, and support tissue healing.

Therefore, this study aimed to evaluate the gastroprotective and therapeutic effects of locally cultured Arthrospira platensis produced at the National Institute of Oceanography and Fisheries (NIOF), Egypt, in comparison with commercially available organic Spirulina tablets. The assessment was based on gastric ulcer indices, inflammatory mediators, oxidative stress biomarkers, DNA damage, and histopathological alterations in gastric tissues.

Materials and Methods:

- **Cultivation of *Arthrospira platensis*.**

Arthrospira platensis was cultivated in modified Zarrouk medium at 28 ± 2°C under a natural light/dark photoperiod corresponding to the day–night cycle. The cultivation was carried out at the Algae Production Unit, Freshwater and Lakes Division, (NIOF), Egypt. Commercial organic *Spirulina* tablets were obtained from Micro Ingredients (California, USA).

- **Experimental Animals and Study Design**

Fifty-four female Wistar albino rats (120-140 g) were used as experimental animals in this study. The animals were kept in standard cages under controlled environmental conditions (25 ± 2°C) with free access to standard chow and water throughout the experimental period.

The 54 rats were randomly assigned to nine experimental groups, with six rats in each group, as follows:

- Group 1: control.
- Group 2: Received cultured *Arthrospira platensis* extract orally for 7 days.
- Group 3: Received organic *Arthrospira platensis* tablet extract orally for 7 days.
- Group 4: Ulcer control group. Rats were fasted for 24 h and administered absolute ethanol orally to induce gastric ulceration. The animals were euthanized 1 h after ethanol administration.
- Group 5: Protective cultured-extract group. Rats received cultured *A. platensis* extract orally for 7 days prior to ethanol-induced gastric ulceration.
- Group 6: Protective organic-tablet group. Rats received organic *A. platensis* tablet extract orally for 7 days prior to ethanol-induced gastric ulceration.
- Group 7: Therapeutic cultured-extract group. Rats received absolute ethanol to induce gastric ulceration, followed by daily oral administration of cultured *A. platensis* extract for 7 consecutive days.
- Group 8: Therapeutic organic-tablet group. Rats received absolute ethanol to induce gastric ulceration, followed by daily oral administration of organic *A. platensis* tablet extract for 7 consecutive days.
- Group 9: Reference treatment group. Rats received absolute ethanol to induce gastric ulceration, followed by daily oral administration of Antodine® for 7 consecutive days.

On the last day of the experimental, the animals were fasted for 24 h before being euthanized under diethyl ether anesthesia followed by decapitation. Samples were subsequently collected for biochemical, histopathological, and other analyses.

- **Doses and Route of Administration**

Gastric ulceration was induced following oral administration of absolute ethanol (0.5 mL/100 g weight) to rats fasted for 24 h, according to Mard et al. (2008). Cultured *Arthrospira platensis* extract and organic *A. platensis* tablet solution were given orally at 500 mg/kg weight/day for 7 consecutive days (Abdel-Daim et al., 2013). Antodine® (famotidine) was used as the reference anti-ulcer drug and was given orally 20 mg/kg body /day for 7 days (Karakaya et al., 2015).

- **Measurement of Gastric Volume and pH**

Following anesthesia, the stomach was excised, and the gastric contents were collected and centrifuged for 15 min. The volume of the supernatant was recorded, and its pH was determined using pH indicator strips.

- **Assessment of Gastric Lesions**

The stomachs were removed and opened along the greater curvature, rinsed gently with normal saline, and examined for gastric lesions. The number of gastric lesions was counted using a magnifying lens, and the lesion score was recorded.

- **Preparation of Tissue Homogenate**

Gastric tissue samples (0.5 g) were homogenized in 5 ml of phosphate buffered saline (PBS, PH 7.4). The homogenates were centrifuged at 4°C for 10 min, and the resulting supernatants were collected and stored at-20°C until biochemical analyses were performed.

Biochemical Analyses

Inflammatory biomarkers in gastric tissue homogenates were quantified using enzyme-linked immunosorbent assay (ELISA) kits. Tumor necrosis factor-alpha (TNF-α) was measured using a commercially available ELISA kit (BioLegend, San Diego, CA, USA) according to the manufacturer's instructions. Prostaglandin E_2 (PGE_2) levels were determined using a competitive ELISA kit (Abcam, Cambridge, UK; Cat. No. ab100785).

Lipid peroxidation was assessed by measuring malondialdehyde (MDA) levels according to Kei (1978). Catalase (CAT) activity was determined following the method of Aebi (1984), while glutathione peroxidase (GPx) activity was measured according to Paglia and Valentine (1967). Total protein content was estimated using the Bradford method (Bradford, 1976).

- **DNA Damage Assessment by Comet Assay**

DNA damage in gastric tissues was evaluated using the alkaline single-cell gel electrophoresis (Comet) assay. Parameters including tail length, tail moment, and the percentage of DNA in the tail were analyzed. Samples were stained with GelRed and examined under a fluorescence microscope equipped with an excitation filter of 420-490 nm and an emission filter of 510 nm. One hundred cells per sample were analyzed using Komet 5 image analysis software (Kinetic Imaging Ltd., Liverpool, UK).

- **Histopathological Examination**

Stomach tissues were fixed in 10% neutral buffered formalin, dehydrated through graded ethanol series, embedded in paraffin wax, sectioned at 5 µm thickness, and stained with hematoxylin and eosin (H&E) according to Toktay and Selli (2022). Histopathological changes were examined using a light microscope.

In addition, liver, kidney, and lung tissues were collected from all experimental groups, fixed in 10% neutral-buffered formalin, processed routinely for paraffin embedding, sectioned, stained with H&E, and examined microscopically for pathological alterations.

Ethical Statement:

All animal experimental procedures were performed in accordance with the guidelines of the National Institute of Oceanography and Fisheries (NIOF), Egypt, and were approved by the Institutional Medical Research Ethics Committee (Approval No. NIOF-AQ3O23R032).

Statistical Analysis:

All data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using one-way analysis of variance (ANOVA), followed by the Least Significant Difference (LSD) post hoc test for multiple comparisons among groups. Differences were considered statistically significant at $p < 0.05$.

Results

- **Biochemical Composition of Cultured and organic *A. platensis tablet***

Parameter	Unit	Cultured A. platensis	Organic A .Platensis tablet
Total protein %	%	62	65
Total carbohydrate %	%	15	< 1
Phycocyanine	%	18%	7.5
Viamin A	IU	11000	7500
Vitamin K	µg	200	133
Vitamin B12	µg	21.1	23
γ-linolenic acid	mg	300	106

Table 1: Comparison biochemical composition between cultured A. platensis and Organic commercial A. platensis Tablet

The biochemical composition of cultured *Arthrospira platensis* and commercial organic *Spirulina* tablets is presented in Table 1. Both products exhibited comparable protein and vitamin B12 contents, with protein levels of 62% and 65% and vitamin B12 concentrations of 21.1 and 23 µg, respectively. In contrast, the cultured *A. platensis* contained higher amounts of total carbohydrates (15% vs. <1%), phycocyanin (18% vs. 7.5%), vitamin A (11,000 vs. 7,500 IU), vitamin K (200 vs. 133 µg), and γ-linolenic acid (300 vs. 106 mg) than the commercial organic *Spirulina* tablets. These findings indicate that the locally cultured *A. platensis* represents a rich source of several bioactive compounds associated with antioxidant and nutritional properties.

- **Effect of *Arthrospira platensis* on Gastric Secretion, pH, and Gastric Lesions**

Groups	Gastric volume/ ml	Gastric PH	Ulcer lesion counts
Control	0.31^{*} ± 0.32	6.62* ± 0.28	---
Ulcer control	1.68^{a} ± 0.25	5.03^{a} ± 0.80	7.58^{a} ± 1.52
Spirulina powder	0.35* ± 0.07	6.36*** ± 0.28	---
Organic *Spirulina* T.	0.28* ± 0.05	6.20*** ± 0.28	---
Protected + *Spirulina* powder	1.50^{a} ± 0.05	5.06^{a} ± 0.28	3.60^{a*} ±1.00
Protected+ Organic *Spirulina* T	1.20^{a*} ± 0.10	5.40^{c} ± 0.54	1.46* ± 0.57
Treated + *Spirulina* powder	0.40* ± 0.10	5.45^{a} ± 0.64	2.85^{a*} ± 1.oo
Treated + Organic *Spirulina* T.	0.46* ± 0.07	5.50^{c} ± 0.50	1.80^{c*} ± 1.00
Treated + famotidine	0.48* ± 0.10	5.51^{c} ± 0.61	1.73^{c*} ± 0.57

Table 2: Therapeutic effects of *Spirulina platensis* powder and Organic *Spirulina* tablet on gastric ulcer index of ulcerogenic rats.

Data are expressed as mean ± SD of six rats in each group.

• Superscript letters are significant difference between groups.

Symbols *, **, ***, # are p levels at p<0.0001, 0.001,0.01 ,0.05 Respectively

• Values between brackets are % changes versus control group.

• Values between parentheses are % changes versus ulcer group.

The effects of cultured and commercial *A. platensis* on gastric volume, gastric pH, and lesion count are summarized in Table 2. Administration of either algal preparation to normal rats did not markedly affect gastric secretion parameters compared with the control group.Ethanol administration markedly increased gastric volume by 441.9% and decreased gastric pH by 24.01% compared with the control group. Pretreatment with cultured *A. platensis* reduced gastric volume by 10.71%, whereas pretreatment with commercial *Spirulina* tablets reduced it by 28.57% relative to the ulcer control group. Therapeutic administration of cultured *A. platensis*, commercial A. platensis tablets, and famotidine reduced gastric volume by 76.19%, 72.61%, and 71.42%, respectively. Similarly, ulcer lesion counts were markedly reduced in all treated groups, with the greatest reduction observed in the protected group receiving commercial *Spirulina* tablets (80.73%).

- **Therapeutic Effects of A. platensis and Organic *Spirulina* Tablets on Oxidative Stress Marker.**

Groups	SOD (U/gm tissue)	MDA (µmol/mg protein)	Glutathione peroxidase(GPx) (µg/gm tissue)
Control	151.54*±2.65	21.10* ± 1.64	31.62* ± 0.95
Ulcer control	80.32[a]± 1.09	94.82[a] ± 2.14	13.63[a] ± 1.39
Spirulina powder	140.42*±3.96	23.95* ± 1.83	29.80* ± 1.70
Organic *Spirulina* T.	146.54[a*]±4.98	22.84* ± 2.50	27.26[a*] ± 0.96
Protected + *Spirulina* powder	95.76[b*]±3.70	72.74[a*] ± 2.24	18.17[a*] ± 1.00
Protected+ Organic *Spirulina* T	102.34[a*]±1.56	78.51[a*] ± 1.44	19.49[a*] ± 0.85
Treated + *Spirulina* powder	121.53[b**]±2.93	48.07[a*] ± 1.95	21.26[a*] ± 0.84
Treated + Organic *Spirulina* T.	127.56[a*]±3.12	65.67[a*] ± 3.60	23.62[a*] ± 1.30
Treated + famotidine	131.87[a*]±2.13	52.50[a*] ± 2.19	25.24[a*] ± 1.10

Table 3. Therapeutic effects of *Spirulina platensis* powder and Organic *Spirulina* tablet on gastric ulcer index of Ulcerogenic rats.

• Data are expressed as mean ± SD of six rats in each group.
• Superscript letters are significant difference between groups
Symbols *, **, ***, # are p levels at p<0.0001, 0.001,0.01 ,0.05 Respectively
• Values between brackets are % changes versus control group.
• Values between parentheses are % changes versus ulcer group

The data presented in Table 3 showed that administration of cultured *Arthrospira platensis* powder or commercial organic *Spirulina* tablets to healthy rats did not significantly affect oxidative stress biomarkers compared with the control group. Ethanol-induced gastric ulceration resulted in a marked reduction in glutathione peroxidase (GPx) activity by 56.80%, accompanied by a significant decrease in superoxide dismutase (SOD) activity by 46.99%, compared with the normal control group. In contrast, malondialdehyde (MDA), a marker of lipid peroxidation, increased dramatically by 349.3%, indicating severe oxidative stress.

Pretreatment and post-treatment with cultured *A. platensis*, commercial organic *Spirulina* tablets, or famotidine significantly ameliorated these oxidative alterations. GPx activity increased by 33.30%, 42.99%, 55.97%, 73.29%, and 85.17% in the protected cultured, protected commercial, treated cultured, treated commercial, and famotidine groups, respectively, compared with the ulcer control group. Similarly, SOD activity increased by 19.22%, 27.41%, 51.30%, 58.81%, and 64.18%, respectively.

Conversely, MDA levels decreased by 23.28%, 17.20%, 49.30%, 30.74%, and 44.70%, respectively, demonstrating a marked reduction in lipid peroxidation.

Among the tested treatments, the therapeutic administration of cultured *A. platensis* exhibited the greatest reduction in MDA levels (49.30%), whereas famotidine produced the highest recovery of antioxidant enzyme activities. Overall, both cultured and commercial *A. platensis* effectively restored the antioxidant defense system and attenuated ethanol-induced oxidative stress, with cultured *A. platensis* showing antioxidant efficacy comparable to, and in some parameters approaching, that of famotidine.

- **DNA Fragmentation Analysis in Rat Stomach Tissue**

Groups	% Tailed DNA	% Untailed DNA	Tail length (PX)	% DNA in tail	Tail moment
Control	11.4	88.6	9.57	5.75	0.47
Ulcer control	21.4	78.6	9.43	12.79	1.16
Spirulina powder	11.5	88.5	7.30	7.98	0.62
Organic *Spirulina* T.	11.8	88.2	3.50	7.84	0.27
Protected + *Spirulina* powder	14.9	85.1	11.50	5.85	0.61
Protected+ Organic *Spirulina* T	11.3	88.7	6.37	10.75	0.66
Treated + *Spirulina* powder	14.3	85.7	3.66	4.01	0.12
Treated + Organic *Spirulina* T.	16.9	83.1	4.83	6.61	0.35
Treated + famotidine	16	84	3.75	4.67	0.18

Table 4. Effects of cultured *Arthrospira platensis*, commercial organic *Spirulina* tablets, and famotidine on DNA fragmentation parameters in gastric tissue of ethanol-induced gastric ulcer rats.

The results presented in Table 4 revealed The effects of cultured *Arthrospira platensis*, commercial organic *Spirulina* tablets, and famotidine on DNA fragmentation parameters are presented in Table 4. Ethanol-induced gastric ulceration markedly increased DNA damage, as evidenced by a higher percentage of tailed DNA (21.4%), increased DNA in the tail (12.79%), and an elevated tail moment (1.16), accompanied by a reduction in untailed DNA (78.6%) compared with the control group. Pretreatment and post-treatment with cultured *A. platensis*, commercial organic *Spirulina* tablets, or famotidine markedly ameliorated these alterations, indicating improved DNA integrity and reduced genotoxic damage. Among the evaluated treatments, therapeutic administration of cultured *A. platensis* exhibited the lowest tail moment (0.12) and the lowest percentage of DNA in the tail (4.01%), whereas famotidine also demonstrated a marked protective effect, with corresponding values of 0.18 and 4.67%, respectively. Overall, both cultured and commercial *A. platensis* effectively reduced ethanol-induced DNA fragmentation, demonstrating pronounced antigenotoxic activity comparable to that of the reference drug.

- **Effect of *Arthrospira platensis* on Inflammatory Markers.**

TNF	PGE2 (ng/gm)	**Groups**
76.59* ± 0.83 ---	7.88* ± 0.54 ---	Control
237.28a ± 2.49 (+209.8)	2.54a ± 0.23 (-67.7)	Ulcer control
73.73* ± 0.45	7.44* ± 0.49	*Spirulina* powder

(+3.73)	(-5.50)	
71.83c* ± 0.75 (+6.21)	8.05* ± 0.17 (+2.1)	Organic *Spirulina* T.
164.72a* ± 1.92 [-30.57]	4.74a* ± 0.41 [+86.6]	Protected + *Spirulina* powder
155.48a* ± 2.36 [-34.47]	5.44a* ± 0.32 [+114.17]	Protected+ Organic *Spirulina* T
186.98a* ± 3.92 [-21.19]	4.99a* ± 0.27 [+96.45]	Treated + *Spirulina* powder
174.46a* ± 3.55 [-26.47]	3.74a* ± 0.20 [+47.24]	Treated + Organic *Spirulina* T.
125.04a* ± 3.84 [-47.30]	3.91a* ± 0.23 [+53.93]	Treated + famotidine

Table 5. Therapeutic effects of *Spirulina platensis* powder and Organic *Spirulina* tablet on inflammatory markers of ulcerogenic rats.

• Data are expressed as mean ± SD of six rats in each group.
• Superscript letters are significant differences between groups.
Symbols *, ** ,***, # are p levels at p<0.0001, 0.001,0.01 ,0.05, Respectively.
• Values between brackets are % changes versus the control group.
• Values between parentheses are % changes versus ulcer group.

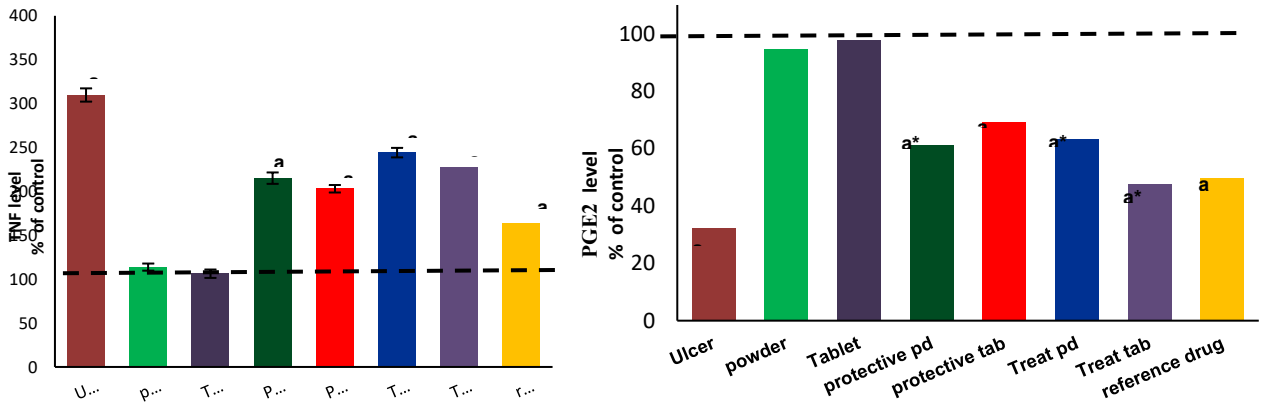
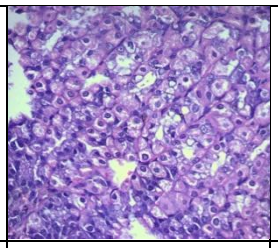
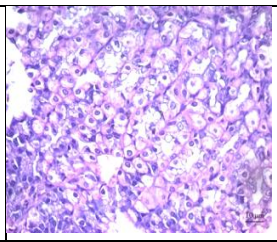
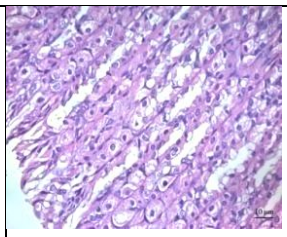
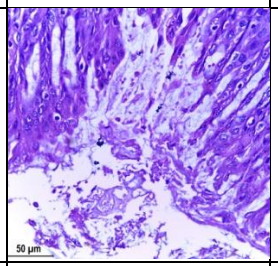
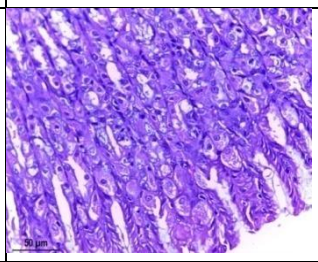
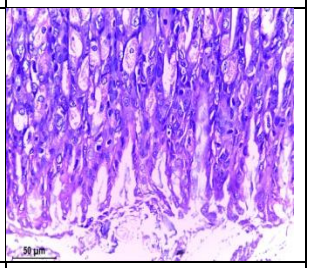
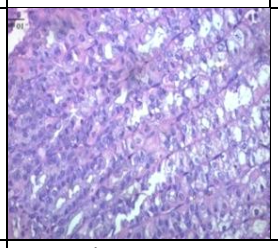
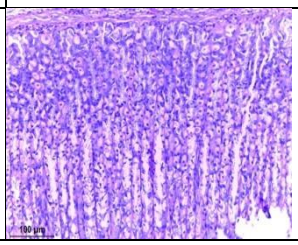
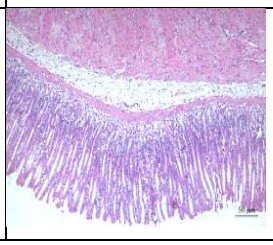


Figure1. TNF and PGE2 levels in the stomach of rats in the different studied groups.

The effects of the tested algal preparations on inflammatory biomarkers are presented in Table 5 and Figure 1. Administration of cultured or commercial *A. platensis* to normal rats did not significantly alter gastric TNF-α or PGE_2 levels compared with the control group.
Ethanol-induced gastric ulceration resulted in a significant elevation of TNF-α and a marked depletion of PGE_2 levels relative to normal rats. Treatment with cultured *A. platensis*, commercial organic tablets, or famotidine significantly ameliorated these inflammatory changes. All treated groups exhibited lower TNF-α concentrations and higher PGE_2 levels compared with the ulcer control group.
Among the tested treatments, cultured *A. platensis* demonstrated a pronounced anti-inflammatory effect, as reflected by the suppression of TNF-α and restoration of PGE_2 levels toward normal values. Similar protective effects were observed in the famotidine-treated group, confirming the gastroprotective potential of the algal preparations.

- **Histopathological Examination of the Stomach**

Photo (1) Microscopic Examination of gastric Tissues

G1		G 2		G 3	
	Normal gastric structure		Normal gastric structure with mild edema		Normal gastric structure with mild edema
G4		G 5		G6	
	Edema and vacuolar gastric glands		Edema and vacuolar gastric glands		Vacuolization of gastric glands and severe necrosis of mucosa
G7		G 8		G9	
	Gastric structure nearly normal with mild edema G7, G8 and G9				

Microscopic examination of the gastric tissues from the control group (G1), as well as rats cultured *Spirulina platensis* extract (G2) and organic *Spirulina* tablet extract (G3) for one week, revealed a normal gastric architecture with intact mucosal epithelium and well-organized gastric glands (photo. 1). In contrast, ethanol-induced ulcerative rats exhibited marked pathological alterations characterized by severe desquamation, loss of surface epithelial cells, and extensive mucosal necrosis (Fig. 2, G4). Additional lesions included cytoplasmic vacuolization, edema (Fig. 2, G5), dilated gastric glands, and inflammatory cell infiltration, predominantly neutrophils and eosinophils (Fig. 2, G6). Treatment with cultured *S. platensis* and organic *Spirulina* tablets (G7 and G8) markedly restored the gastric architecture, showing nearly normal mucosal structures with only mild edema. Similar histological improvements were observed in the famotidine-treated group (G9).

Discussion

Recent studies have focused on identifying natural sources of safe and effective compounds for preventing and treating of gastric ulcers (Prayoga et al., 2024; Aboul Naser et al., 2020). Rats were selected as the experimental model because the rat stomach is similar to the human stomach in both anatomy and function [Di Natale et al 2022]. Both tested Spirulina samples contained high crude protein levels (62-65%) and are high level of natural bioactive compounds (Thangaraj et al., 2022).

In addition, both samples were rich in phycocyanin, which has antioxidant and anti-inflammatory effects as well as antitumor, and immunomodulatory activities (Motamedzadeh et al., 2024).

The gastric protective effect of *Spirulina* showed in this study may also be related to its vitamin B12 content (21.1 and 23 μg in the cultured and commercial tablets, respectively), which has been reported to promote gastric ulcer healing (Anantharajappa et al., 2020).

Gastric ulcers develop when the balance between mucosal protective and aggressive factors is disturbed. Since current treatments do not always provide complete healing, there is increasing interest in finding safe and effective alternative therapies (Prayoga et al., 2024). The ethanol-induced gastric ulcer (EI-GU) model is widely used because it reproduces many of the pathological changes observed in human gastric ulcers. Compared with other experimental models, such as stress-, indomethacin-, and pyloric ligation-induced ulcers, the ethanol model is particularly useful for evaluating the gastroprotective effects of tested agents and for studying the roles of oxidative damage, inflammatory responses, and apoptosis processes in gastric ulcer development (Duran et al., 2020). In the present study, ethanol administration increased gastric volume and acidity. This effect is related to the direct damaging action of ethanol on the gastric mucosa, which stimulates gastric acid secretion and further aggravates mucosal injury (El-Naga, 2015).

The present findings demonstrated that *Spirulina* extract is a potent therapeutic agent with remarkable gastroprotective properties. Both the laboratory-cultured *Spirulina* and the commercial organic tablets effectively increased gastric pH while reducing gastric lesion counts and gastric acid secretion in ulcerated rats. Therefore, agents capable of reducing gastric acid secretion may promote the healing of gastric lesions and protect the gastric mucosa from further damage (Aboul Naser et al., 2020).

The present findings agree with those reported by Rahman et al. (2020) and Prayoga et al. (2024), who demonstrated that ethanol administration enhances inflammatory responses while lowering the anti-inflammatory cytokine IL-10. Prostaglandins are important for gastric mucosal protection by promoting mucus and bicarbonate secretion, maintaining gastric blood flow, and reducing gastric acid secretion (Sánchez-Mendoza et al., 2020). In addition, prostaglandins enhance epithelial cell resistance to injury and inhibit leukocyte recruitment (Aboul Naser et al., 2020). In agreement with previous studies by Liu et al. (2022) and Jabbar et al. (2023), ethanol administration significantly reduced PGE_2 levels. Oxidative stress further impairs prostaglandin synthesis and contributes to gastric mucosal injury through lipid peroxidation and inflammatory responses (Prayoga et al., 2024). Reduced PGE_2 levels impair gastric mucosal defense and delay ulcer healing by decreasing gastric mucosal blood flow, mucus secretion, and bicarbonate production (Prayoga et al., 2024). These findings are consistent with Aleid et al. (2021), who attributed the gastroprotective effect of *Spirulina* extracts to their potent antioxidant and anti-

inflammatory activities. The healing of gastric ulcers is associated with enhanced mucus production, epithelial regeneration, and pH regulation, processes that are further supported by prostaglandins (Aboul Naser et al., 2020). Furthermore, antioxidant supplementation helps maintain the cellular redox balance, thereby preserving prostaglandin-mediated gastroprotection and stabilizing the gastric mucus barrier (Prayoga et al., 2024; Mousavi et al., 2025). TNF-α is a key pro-inflammatory cytokine that impairs gastric microcirculation around ulcerated tissue, delays ulcer healing, and promotes NF-κB activation (Prayoga et al., 2024). Delayed gastric ulcer healing is associated with increased expression of pro-inflammatory cytokines, particularly TNF-α and IL-1β, which sustain the inflammatory response and impair mucosal repair (Rahman et al., 2020; Prayoga et al., 2024). Gastric inflammation is characterized by increased TNF-α expression together with macrophage and neutrophil infiltration into the gastric mucosa (Prayoga et al., 2024).

In the present study, administration of *Spirulina platensis* extract, either before or after ethanol-induced ulceration, markedly reduced gastric TNF-α levels, supporting its gastroprotective activity against gastric ulcers (Anantharajappa et al., 2020).

In this study, the histopathology examination of the gastric tissue from rats treated byethanol revealed marked disruption of the gastric mucosal architecture, including severe epithelial desquamation, vacuolization, loss of surface mucous epithelial cells, edema, necrosis, dilated gastric glands, and inflammatory cell infiltration (eosinophils and neutrophils). These results are agreeing with previous reports demonstrating that ethanol induces gastric tissue injury through excessive production of reactive oxygen species (ROS) and oxidative stress, which play central roles in the pathogenesis of ethanol-induced gastric ulceration (El-Naga, 2015). Neutrophil infiltration further aggravates oxidative stress, resulting in cellular damage and increased production of inflammatory mediators (Prayoga et al., 2024). Moreover, ethanol administration disrupts mitochondrial function by reducing the mitochondrial membrane potential and impairing electron transport, leading to excessive production of reactive oxygen species (Rahman et al., 2020; Prayoga et al., 2024). The overproduction of reactive oxygen species, together with reducing of enzymatic and non-enzymatic antioxidant defenses, results in oxidative stress that damages essential cellular components, including proteins, lipids, carbohydrates, and nucleic acids, thereby promoting lipid peroxidation (Rahman et al., 2020; Mousavi et al., 2025)

Following treatment with *Spirulina* extract, the gastric mucosa of ethanol-treated rats showed marked histological improvement compared with the untreated ulcer group and was comparable to that observed in the reference drug-treated group. Although mild loss of the superficial gastric epithelium was still evident, the gastric glands appeared nearly normal. These findings are consistent with previous studies demonstrating that *Spirulina platensis* supplementation protects the gastric mucosa against ethanol-induced injury by reducing oxidative stress and inflammation (Rahman et al., 2020; Prayoga et al., 2024). Similarly, other studies reported that *Spirulina platensis* supplementation attenuated gastric mucosal injury induced by non-steroidal anti-inflammatory drugs (NSAIDs), further supporting its gastroprotective potential (Prayoga et al., 2024; Mousavi et al., 2025).

The findings showed a marked reduction in gastric juice pH in the ulcer group in comparison with the control group. In contrast, both pre-treatment (protected) and post-treatment (treated) with locally produced or commercial organic *Spirulina platensis* extracts significantly improved gastric juice pH. Similar improvement was also observed following treatment with famotidine (20 mg/kg). Previous studies have reported that acute ethanol administration decreases gastric juice pH (Rahman et al., 2020; Prayoga et al., 2024). Ethanol administration also markedly increased gastric lesion formation, whereas treatment with *Spirulina platensis* extracts reduced the number of gastric lesions. Ethanol-induced gastric injury has

been characterized by extensive submucosal edema, linear hemorrhagic lesions, mucosal friability, and loss of gastric epithelial cells (Rahman et al., 2020; Prayoga et al., 2024). In general, an increase in the number of erosions is directly associated with greater gastric ulcer severity. Furthermore, the percentage inhibition of gastric ulcers was 52.5%, 88.73%, 62.40%, 76.25%, and 77.17% in the protected and treated groups receiving locally produced or commercial organic *Spirulina* extracts and famotidine (20 mg/kg), respectively (Prayoga et al., 2024).

In addition to its necrotizing effects, ethanol administration has been reported to increase oxidative stress by disrupting mitochondrial membrane integrity, thereby impairing mitochondrial electron transport and promoting excessive reactive oxygen species (ROS) production (Rahman et al., 2020).

The present findings indicated that ethanol administration induced oxidative damage in gastric tissue by suppressing antioxidant enzyme activities and increasing MDA levels (Aleid et al., 2021; Attiya, 2022). Several studies have demonstrated the high efficacy of *Spirulina* in the treatment of gastric ulcers (Kamar et al., 2022; Guzmán-Gómez et al., 2023). One of the major bioactive compounds responsible for its antioxidant activity is the biliprotein C-phycocyanin, which has strong antioxidant activity (Liu et al., 2022; Motamedzadeh et al., 2024).

DNA damage was assessed using the comet assay, which is a reliable method for detecting DNA fragmentation, is considered an indicator of severe cellular injury in rats. Reactive oxygen species (ROS) can induce DNA fragmentation, as reflected by higher tail DNA (%), tail length, and tail moment in the ulcer group (Rahman et al., 2020; Mousavi et al., 2025). Post-treatment with *Spirulina* extract markedly reduced DNA damage in ulcerated rats. Therefore, *Spirulina* effectively inhibited DNA damage, an effect that may be related to the presence of the bio-active compounds C-phycocyanin and phycocyanobilin (Mousavi et al., 2025; Spínola et al., 2024).

Overall, the present study discussed that *Spirulina platensis* protected against ethanol-induced gastric ulcers and improved healing through its antioxidant, anti-inflammatory, and cytoprotective effects.

Conclusion and Recommendation

This study concluded that cultured *Spirulina platensis* has effects significant gastroprotective, anti-secretory, anti-ulcerative, antioxidant, and anti-inflammatory activities against ethanol-induced gastric ulcers. These findings were supported by histopathological observations, which confirmed marked improvement in gastric tissue architecture following *Spirulina* treatment. In addition, both cultured *Spirulina* and commercial organic tablets showed protective effects against ethanol-induced hepatic, renal, and pulmonary tissue injury, suggesting a broad cytoprotective potential. The beneficial effects of *Spirulina* are likely associated with its antioxidant and anti-inflammatory properties. However, further studies are required to clarify the underlying molecular mechanisms and to evaluate its efficacy and safety in clinical settings.

Based on the present findings, *Spirulina platensis* may represent a promising natural therapeutic candidate for the prevention and management of ethanol-induced gastric ulcers and their associated tissue damage. Further preclinical and clinical investigations are recommended before its routine medical application.

References

-Abdel-Daim, M. M., Abuzead, S. M., & Halawa, S. M. (2013). Protective role of Spirulina platensis against acute deltamethrin-induced toxicity in rats. PLoS ONE, 8(9), e72991. https://doi.org/10.1371/journal.pone.0072991

-Abdel-Daim, M. M., Farouk, S. M., Madkour, F. F., & Azab, S. S. (2015). Anti-inflammatory and immunomodulatory effects of Spirulina platensis in comparison to Dunaliella salina in acetic acid-induced rat experimental colitis. Immunopharmacology and Immunotoxicology, 37, 126–139. https://doi.org/10.3109/08923973.2014.998368

-Aboul Naser, A., Younis, E., El-Feky, A., Elbatanony, M., & Hamed, M. (2020). Management of Citrus sinensis peels for protection and treatment against gastric ulcer induced by ethanol in rats. Biomarkers, 25(4), 349–359. https://doi.org/10.1080/1354750X.2020.1759693

-Aebi, H. (1984). Catalase in vitro. Methods in Enzymology, 105, 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3

-Alazzouni, A. S., Daim, M. A., Gabri, M. S., Fathalla, A. S., Albrakati, A., Al-Hazani, T., & Hassan, B. N. (2021). Protective effect of pomegranate peels extracts against stomach peptic-ulcer induced by Brexin in albino rats. Research Square. https://doi.org/10.21203/rs.3.rs-474368/v1

-Aleid, I. S., Alfheeaid, H. A., Aljutaily, T., Alhomaid, R. M., Alharbi, H. F., Althwab, S. A., Abdel-Rahman, H. A., AlGeffari, M. A., & Barakat, H. (2021). Gastroprotective effects of Spirulina platensis, golden kiwifruit flesh, and golden kiwifruit peel extracts individually or in combination against indomethacin-induced gastric ulcer in rats. Nutrients, 13(10), 3499. https://doi.org/10.3390/nu13103499

-Anantharajappa, K., Dharmesh, S. M., & Ravi, S. (2020). Gastro-protective potentials of Spirulina: role of vitamin B12. Journal of Food Science and Technology, 57(2), 745–753. https://doi.org/10.1007/s13197-019-04107-8

-Attiya, H. (2022). Protective effect of Spirulina against aspartame-induced oxidative stress and molecular gene brain damage. Journal of the Hellenic Veterinary Medical Society, 73(2), 3689–3698. https://doi.org/10.12681/jhvms.25509

-Barros de Medeiros, V. P., da Costa, W. K. A., da Silva, R. T., Pimentel, T. C., & Magnani, M. (2022). Microalgae as source of functional ingredients in new-generation foods: challenges, technological effects, biological activity, and regulatory issues. Critical Reviews in Food Science and Nutrition, 62(18), 4929–4950. https://doi.org/10.1080/10408398.2021.1879729

-Behairy, A., Elkomy, A., Elsayed, F., Gaballa, M. M. S., Soliman, A., & Aboubakr, M. (2024). Antioxidant and anti-inflammatory potential of Spirulina and thymoquinone mitigate the methotrexate-induced neurotoxicity. Naunyn-Schmiedeberg's Archives of Pharmacology, 397(3), 1875–1888. https://doi.org/10.1007/s00210-023-02739-4

-Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72, 248–254. https://doi.org/10.1006/abio.1976.9999

-Di Natale, M. R., Patten, L., Molero, J. C., Stebbing, M. J., Hunne, B., Wang, X., Liu, Z., & Furness, J. B. (2022). Organisation of the musculature of the rat stomach. Journal of Anatomy, 240(4), 711–723. https://doi.org/10.1111/joa.13587

-Diniz, A. F. A., de Oliveira Claudino, B. F., Francelino, D. M. C., Alves Junior, E. B., Ferreira, P. B., Lacerda Júnior, F. F., Barros, B. C., Alves Arruda, R. R., Alves, A. F., Batista, L. M., & da Silva, B. A.

(2023). Arthrospira platensis prevents oxidative stress and suppresses IL-1β expression in the ileum of rats fed a hypercaloric diet. Journal of Functional Foods, 106, 105586. https://doi.org/10.1016/j.jff.2023.105586

-Duran, Y., Karaboğa, İ., Polat, F. R., Polat, E., Erboğa, Z. F., Ovalı, M. A., Öztopuz, R. Ö., Çelikkol, A., & Yılmaz, A. (2020). Royal jelly attenuates gastric mucosal injury in a rat ethanol-induced gastric injury model. Molecular Biology Reports, 47(11), 8867–8879. https://doi.org/10.1007/s11033-020-05939-w

-El-Naga, R. N. (2015). Apocynin protects against ethanol-induced gastric ulcer in rats by attenuating the upregulation of NADPH oxidases 1 and 4. Chemico-Biological Interactions, 242, 317–326. https://doi.org/10.1016/j.cbi.2015.10.018

-Guzmán-Gómez, O., García-Rodríguez, R. V., Pérez-Gutierrez, S., Rivero-Ramírez, N. L., García-Martínez, Y., Pablo-Pérez, S. S., Pérez-Pastén-Borja, R., Cristóbal-Luna, J. M., & Chamorro-Cevallos, G. (2023). Protective effect of the phycobiliproteins from Arthrospira maxima on indomethacin-induced gastric ulcer in a rat model. Plants, 12(8), 1586. https://doi.org/10.3390/plants12081586

-Jabbar, A. A. J., Mothana, R. A., Abdulla, M. A., Abdullah, F. O., Ahmed, K. A.-A., Hussen, R. R., Hawwal, M. F., Fantoukh, O. I., & Hasson, S. (2023). Mechanisms of anti-ulcer actions of Prangos pabularia (L.) in ethanol-induced gastric ulcer in rats. Saudi Pharmaceutical Journal, 31(12), 101850. https://doi.org/10.1016/j.jsps.2023.101850

-Kamar, S. A., Bayoumi, A. H., & Rady, H. Y. (2022). Spirulina supplements: an approach moderating aspirin persuaded histological and ultra-structural alterations in albino rats gastric mucosa. Ultrastructural Pathology, 46(2), 204–216. https://doi.org/10.1080/01913123.2022.2052779

-Karakaya, K., Barut, F., Hanci, V., Can, M., Comert, M., Ucan, H. B., Cakmak, G. K., Irkorucu, O., Tascilar, O., & Emre, A. U. (2015). Gastroprotective effects of CoQ10 on ethanol-induced acute gastric lesions. Bratislava Medical Journal, 116(1), 51–56. https://doi.org/10.4149/bll_2015_010

-Kei, S. (1978). Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. ***Clinica Chimica Acta***, 90(1), 37–43. https://doi.org/10.1016/0009-8981(78)90081-5.

- Kumawat, K. R., Pareek, A., & Singh, N. (2025). Algal nutraceuticals: a potential alternative to health supplementation for athletes. Sport Sciences for Health, 21(4), 2537–2563. https://doi.org/10.1007/s11332-025-01545-0

-Liu, Y.-C., Chang, C.-C., Matsui, H., & Chao, J. C.-J. (2022). C-Phycocyanin and Lycium barbarum polysaccharides protect against aspirin-induced inflammation and apoptosis in gastric RGM-1 cells. Nutrients, 14(23), 5113. https://doi.org/10.3390/nu14235113

-Mard, S. A., Bahari, Z., Eshaghi, N., & Farbood, Y. (2008). Antiulcerogenic effect of Securigera securidaca L. seed extract on various experimental gastric ulcer models in rats. Pakistan Journal of Biological Sciences, 11(23), 2619–2623. https://doi.org/10.3923/pjbs.2008.2619.2623

-Motamedzadeh, A., Rahmati-Dehkordi, F., Heydari, H., Behnam, M., Rashidi Noshabad, F. Z., Tamtaji, Z., Talebi Taheri, A., Nabavizadeh, F., Aschner, M., Mirzaei, H., & Tamtaji, O. R. (2024). Therapeutic potential of phycocyanin in gastrointestinal cancers and related disorders. Molecular Biology Reports, 51(1), 741. https://doi.org/10.1007/s11033-024-09675-3

-Mousavi, S., Yegdaneh, A., Shirani, M., Feizi, A., & Ghanadian, M. (2025). Spirulina supplementation and its effects on inflammation and oxidative stress: a systematic review and meta-analysis on randomized clinical trials. Journal of Functional Foods, 131, 106945. https://doi.org/10.1016/j.jff.2025.106945

-Paglia, D. E., & Valentine, W. N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. The Journal of Laboratory and Clinical Medicine, 70(1), 158–169.

-Prayoga, D. K., Aulifa, D. L., Budiman, A., & Levita, J. (2024). Plants with anti-ulcer activity and mechanism: a review of preclinical and clinical studies. Drug Design, Development and Therapy, 18, 193–213. https://doi.org/10.2147/DDDT.S446949

-Rahman, Z., Dwivedi, D. K., & Jena, G. B. (2020). Ethanol-induced gastric ulcer in rats and intervention of tert-butylhydroquinone: involvement of Nrf2/HO-1 signaling pathway. Human & Experimental Toxicology, 39(4), 547–562. https://doi.org/10.1177/0960327119895559

-Sánchez-Mendoza, M. E., López-Lorenzo, Y., Cruz-Antonio, L., Cruz-Oseguera, A., García-Machorro, J., & Arrieta, J. (2020). Gastroprotective effect of Juanislamin on ethanol-induced gastric lesions in rats: role of prostaglandins, nitric oxide and sulfhydryl groups in the mechanism of action. Molecules, 25(9), 2246. https://doi.org/10.3390/molecules25092246

-Spínola, M. P., Mendes, A. R., & Prates, J. A. M. (2024). Chemical composition, bioactivities, and applications of Spirulina (Limnospira platensis) in food, feed, and medicine. Foods, 13(22), 3656. https://doi.org/10.3390/foods13223656

-Thangaraj, M., Bhavan, P., Thanasekaran, J., Joen-Rong, S., Manjunath, M., & Pathakoti, K. (2022). Phytochemicals of algae, Arthrospira platensis (Spirulina), Chlorella vulgaris (Chlorella) and Azolla pinnata (Azolla). GSC Biological and Pharmaceutical Sciences, 19 (2) 023–043. https://doi.org/10.30574/gscbps.2022.19.2.0167

-Toktay, E., & Selli, J. (2023). Histopathological overview of experimental ulcer models. The Eurasian Journal of Medicine, 54(1), S120–S126. https://doi.org/10.5152/eurasianjmed.2022.22312