

Amelioration of Alcohol-Induced Gastric Ulcer by Dietary Intervention with Green Algae(Chlorophyceae) in Experimental Rats

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ABSTRACT

Stomach ulcers (also called peptic or gastric ulcers) are sores in the stomach. They can be serious, but most people recover with treatment. Bioactive compounds from green algae can treat gastric ulcers by reducing oxidative stress and inflammation and enhancing gastric mucosal defense. Objective: Determine the effectiveness of different concentrations of green algae (Chlorophyceae) in alleviating the Symptoms of Alcohol-Induced in rats with stomach ulcers . Materials and methods: Thirty-five adult male rats were allocated into a negative control group (n=7 rat) and ulcer-induced groups (n=28 rat), which received a single oral dose of ethyl alcohol at a concentration of 10 ml/kg. The ulcer-induced groups were further categorized into four equal subgroups, each receiving distinct treatments: (50, 100 and 150) mg/kg of green algae(Chlorophyceae). Results: The group treated with oral administration of (green algae) 150 mg/kg treatment exhibited intermediate effects, with volume (3.99 ml) and acidity levels between those of green algae 50 and 100 mg/kg . These findings suggest that while (green algae) 50 mg/kg may exacerbate gastric acidity, both green algae 50 and 100 mg/kg can partially inhibit acid secretion and may have potential gastroprotective properties. Conclusion: Green algae (Chlorophyceae) can have a beneficial effect on gastric ulcer parameters by decreasing gastric volume and acidity (both total and titratable), and increasing gastric pH in rats. The effects are dose-dependent, with higher concentrations showing a more significant impact. This suggests potential therapeutic value for ulcer treatment, likely through a combination of gastroprotective and acid-reducing mechanisms. Further research is needed to determine the efficacy and safety of green algae in human subjects.

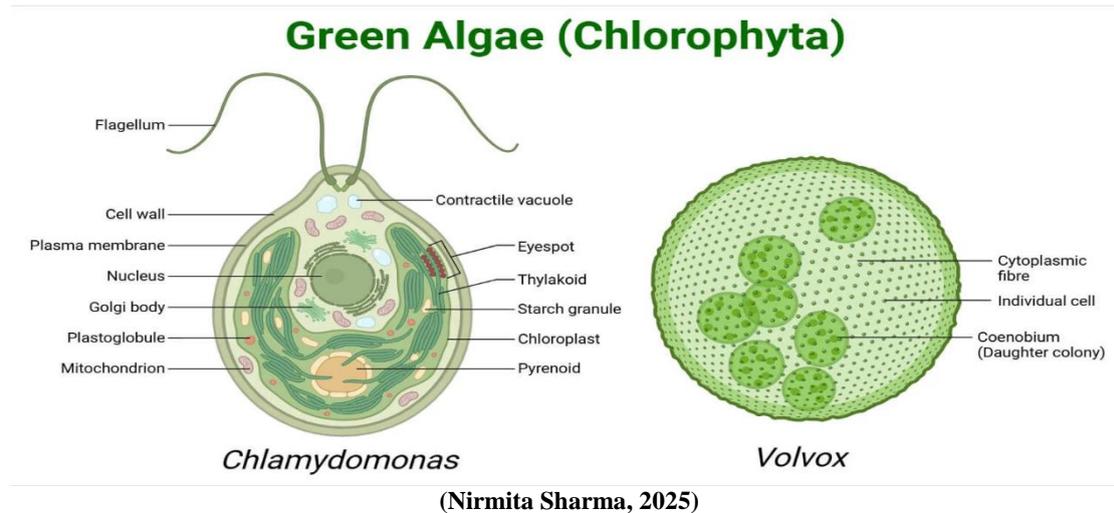
KEYWORDS: Green algae- Chlorophyceae – Gastric Ulcer - Experimental Rats.

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INTRODUCTION

Gastric ulcers, frequent gastrointestinal illnesses, influence persons of all ages globally. Under normal conditions, the integrity of the stomach mucosal barrier is maintained by a balance between irritation and defensive factors. Continuous exposure of the gastric mucosa to highly aggressive agents, including non-steroidal anti-inflammatory drugs (NSAIDs), nutritional deficiencies, smoking, stress, as well as excessive ethanol consumption, can disrupt this equilibrium and elevate the risk of gastric ulcer formation (Jackson *et al.*, 2015). Gastric ulcers, or peptic ulcers, form in the epithelial lining of the stomach as a result of decreased mucus production caused by increased secretion of acid and pepsin. The main factors contributing to gastric erosion include *Helicobacter pylori* infection, abuse of alcohol, smoking, and extended utilization of non-steroidal anti-inflammatory drugs (NSAIDs) (Butterfield *et al.*, 1988). The Chlorophyceae are a large and important group of freshwater green algae. They involve numerous frequent species, in addition to numerous members that are significant ecological and scientific importance (Fleming, 1989). Green Algae (Chlorophyta) consists of a diverse range of photosynthetic organisms in different habitats like freshwater and marine ecosystems. They display a distinctive green hue owing to the presence of chlorophyll a and b which are essential for photosynthesis. Green algae contribute to the production of oxygen in the aquatic ecosystem and act as the foundation

for the majority of aquatic food webs. (Lykov *et al.*,2023).



Green algae (chlorophyceae) have significant therapeutic importance because of their rich content of bioactive compounds like antioxidants, carotenoids, and polysaccharides, which offer benefits for skin health, immune support, and disease management. (Ayswaria *et al.*, 2023). They are used in cosmetics for their anti-aging and moisturizing properties and in pharmaceuticals for their potential anticancer, anticoagulant, and anti-inflammatory effects, some compounds, such as β -carotene from *Dunaliella* sp. and polysaccharides from *Chlorella*, are being studied for their therapeutic potential in more serious conditions. (Lopez *et al.*, 2020). The green algae contains active compounds that may help inflammation of the alimentary tract, avoid or manage gastric ulcers and tumors produced by oxidative stress, and inhibit inflammatory activities by reducing the synthesis of inflammatory mediators (Del *et al.*, 2020). Algae illustrate potential promise in enhancing stomach health because of their rich nutritional profile and bioactive compounds. Examples of these bioactive compounds involve polysaccharides, terpenoids, proteins, sterols, polyphenols, as well as cyclic polysulfide compounds (Fucikova *et al.*, 2019). They showed that a high calcium algae supplement affected gastric ulceration in horses, potentially by buffering stomach acid and gradually decreasing pH without the use of drugs (Zhang *et al.*, 2018). Algae, particularly species such as *Spirulina* and certain brown and green algae, have been investigated for their potential to support or treat ulcers, especially peptic ulcers. Scientific interest largely centers on their bioactive compounds—including sulfated polysaccharides, phycocyanin, as well as antioxidants—which exhibit antimicrobial, anti-inflammatory, in addition mucosal protective effects in preclinical (animal and in vitro) studies. (Sanders *et al.* 2021). shown that extracts from green algae may reduce gastric ulceration in rats, possibly by decreasing gastric acid secretion and enhancing mucosal defense. Sulfated polysaccharides from brown algae have demonstrated the ability to inhibit *Helicobacter pylori* adhesion, which is a key factor in the development of peptic ulcers. Additionally, some algae-derived compounds appear to promote healing by stimulating the production of protective gastric mucus and reducing inflammation. (Arora *et al.*, 2021)

AIM OF STUDY

Determine the effectiveness of different concentrations of green algae (Chlorophyceae) in alleviating the symptoms of alcohol-induced in rats with stomach ulcers.

MATERIALS & METHODS

3.1- Materials:

green algae (*Chlorophyceae*): was obtained from health product and dietary supplement stores of Al-Baha City, Kingdom of Saudi Arabia.

Adult male white albino rats: were purchased from the Institute of Nutrition, Cairo, Egypt, Egypt.

Cellulose, casein, a blend of vitamins and minerals: were bought from Morgan Co. in Cairo, Egypt.

The other chemicals used in the experiment: including formalin, ethanol, and EDTA were supplied from El-Nasr Pharmaceutical Chemicals, El-Ameriea, Cairo, Egypt.

Preparation of aqueous green algae (*Chlorophyceae*) extract

The green algae (*Chlorophyceae*) stored at 4 °C in a refrigerator. The extract was then made in water with a solvent ratio of 1:50 and heated to 80 °C for 30 minutes. In the rotary vacuum evaporator, the extracted material was cooled, filtered, and concentrated at 60°C and 20 rpm until the volume was reduced to 4/5 of the total extract. The concentrated extract was kept in a dark, airtight plastic bottle at -18 °C. (Edward *et al.*, 2015).

Induction of gastric ulcer: For two hours, the rats in the experimental and positive control groups were given a single oral dosage of ethyl alcohol (10 ml/kg body weight) to cause stomach ulcers (Huang *et al.*, 2014).

Assessment of gastric mucosal injury: Histological sections were created by fixing stomach tissues in 10% formalin for a whole day in order to confirm the presence of peptic ulcers. Histological analysis of hemorrhagic regions in ulcer cases revealed that

the stomach pits and surface epithelium were either absent or severely damaged, which led to the emergence of subepithelial capillaries. (**Banchroft *et al.*, 1996**).

Experimental design

For seven days, 35 mature male white albino rats of the Sprague Dawley strain, weighing 150g±5 g. The rats were housed in wire cages that were cylindrical and had wire bottoms. In order to prevent food from being scattered, the diet was presented in special food containers. Additionally, the rats were given water by projecting a glass tube through the wire cage. The rats were lived in animal quarters with regulated humidity and temperature, a 12-hour light/dark cycle, and unrestricted access to food and water.

The experiment was carried out at the Biology Laboratory, Faculty of Home Economic, Menoufia University, Egypt. For seven days, all rats were fed the basal diet, as described by the American Institute of Nutrition (AIN , 1993). Rats were divided into two main groups,

The first group (n=? rats), known as the negative control group, consisted of seven normal rats who did not receive any intervention; and they were fed on basal diet and oral dose (5 ml/kg) of distilled water only.

The Second group (n=28 rats) is the gastric ulcer-induced rats that were randomly divided into four equal subgroups (7 rats):

- Positive control group (PCG): include ulcerative gastric rats that were infected with gastric ulcer and they were fed on basal diet and oral dose (5 ml/kg) of distilled water only.
- Gastric Ulcer rats group (GUG): include ulcerative gastric rats that were treated with oral administration of (green algae) 50 mg/kg
- Gastric Ulcer rats group (GUG): include ulcerative gastric rats that were treated with oral administration of (green algae) 100 mg/kg
- Gastric Ulcer rats group (GUG): include ulcerative gastric rats that were treated with oral administration of (green algae) 150 mg/kg.

At the beginning of experiment, blood samples of the rats were tested to get the initial values before the dietary intervention. At the end of experimental periods the rats from each group were fasted for 12 hours, then slaughtered and blood samples were collected from hepatic portal vein into a dry clean centrifuge tube. Blood samples were centrifuged at (4000 rpm) for 10 minutes to separate blood serum, then kept in deep freezer until analysis.

Standard diet: The basal diet was made in accordance with (**AIN, 1993**).

Statistical Analysis: The mean ± SD was used to tabulate the results. A statistical analysis system was used to do an analysis of variance (ANOVA) on the experimental data for a completely randomized design (**Armitage *et al.*, 1987**). To ascertain mean differences at the 5% level, Duncan's multiple range tests were used.

- **Ethical Approval**

The study was approved by Al-Baha University's Research Ethics Committee (Ref. No. 46123022), Approval date 17 April 2025.

RESULTS & DISCUSSION

The study had shown the potency of dietary intervention with different concentrations of green algae (*Chlorophyceae*) in alleviating the symptoms of alcohol-induced in rats with stomach ulcers.

Effect of different concentrations of green algae (*Chlorophyceae*) on gastric juice parameters including volume, pH, total acidity, and titratable acidity in rats with stomach ulcers.

The table (1) and fig (1,2 and 3) Evaluated the effects of different treatments on gastric juice parameters involving volume, pH. The positive control group showed a significant rise in gastric juice volume (4.87 ml) and acidity, with the lowest pH (1.25) and highest total (260.02 Meq/L) and titratable acidity (13.33 Meq/L), indicating strong stimulation of acid secretion. Similarly, while group treated with oral administration of (green algae) 50 mg/kg resulted in high gastric juice volume (4.34 ml) and acidity (total acidity 244.47 Meq/L), though slightly less than the positive control, suggesting (green algae) 50 mg/kg potential to enhance gastric acid production. In contrast, the group treated with oral administration of (green algae) 100 mg/kg demonstrated a lower volume of gastric juice (3.23 ml) and significantly higher pH (3.10), along with reduced total (204.87 Meq/L) and titratable acidity (9.18 Meq/L), indicating a moderating or protective effect against excessive acid secretion. The group treated with oral administration of (green algae) 150 mg/kg treatment exhibited intermediate effects, with volume (3.99 ml) and acidity levels between those of green algae 50 and 100 mg/kg. These findings suggest that while (green algae) 50 mg/kg may exacerbate gastric acidity, both green algae 50 and 100 mg/kg can partially inhibit acid secretion and may have potential gastroprotective properties.

These results agree with (Porrás *et al.*, 2021) They showed the effect of green algae (*Chlorophyceae*) on gastric parameters in rats with ulcers shows that it can decrease gastric juice volume and total acidity, while raising gastric pH. These effects are dose-dependent, with higher concentrations leading to more significant changes. The mechanism is likely due to the algae's protective effects on the gastric mucosa and its ability to influence acid secretion. Also (**Patil *et al.*, 2019**) They found that green algae treatment can reduce gastric juice volume in rats with ulcers. This reduction is often statistically significant compared to control groups. While (**Gee *et al.*, 2020**) who green algae treatment can reduce gastric juice volume in rats with ulcers. This reduction is often statistically significant compared to control groups and Administration of green algae can increase gastric pH in ulcerated rats, indicating a more alkaline environment. The increase in pH is often dose-dependent, with higher concentrations having a more pronounced effect. In addition, (Santhakumaran *et al*

, 2020) Showed that green algae significantly decrease both total and titratable acidity in the gastric juice of ulcerated rats. This is a key factor in its gastroprotective effect, as it reduces the overall corrosive potential of the gastric fluid. (**Alghanmi *et al.*, 2020**) Reported that Green alga may protect the gastric mucosa from injury by enhancing protective factors and reducing the damage caused by aggressive factors .It can increase the activity of antioxidant enzymes and decrease oxidative stress in the

stomach mucosa, the effects may be due to the inhibition of acid-secreting mechanisms or enzymes like (H⁺,K⁺ ATPase) . The protective effect and the magnitude of the changes in gastric parameters are dependent on the concentration of the algae extract administered. (Riccio, and Lauritano, 2020). Also (Xia *et al.* , 2019) showed that algal extracts can lower damaging molecules

Groups	Volume of gastric juice (ml)	PH	Total acidity (Meq/1h)	titratable acidity Meq/L
Negative control	2.3267 ±0.07	3.55 ± 0.95	163.65 ± 2.91	8.41 ± 0.151
Positive control	4.867 ± 0.07	1.25 ±0.001	260.02 ± 6.45	13.33 ± 0.097
green algae 50 mg/kg	4.343 ± 0.095	1.99 ± 0.085	244.47 ± 3.56	13.31 ± 0.062
green algae 100 mg/kg	3.23 ± 0.096	3.1 ± 0.081	204.87 ± 2.46	9.18 ± 0.58
green algae 150 mg/kg	3.99 ± 0.15	2.66 ± 0.811	220.47 ± 3.059	11.39 ± 0.05
P value	P1<0.001 P2<0.001 P3<0.001 P4<0.001 P5<0.001 P6<0.001 P7<0.001 P8<0.001 P9<0.001 P10<0.001	P1<0.001 P2=0.0025 P3=0.27 P4=0.11 P5<0.001 P6<0.001 P7=0.0017 P8<0.001 P9=0.72 P10=0.22	P1<0.001 P2<0.001 P3<0.001 P4<0.001 P5<0.001 P6<0.001 P7<0.001 P8<0.001 P9<0.001 P10<0.001	P1<0.001 P2<0.001 P3=0.01 P4<0.001 P5=0.68 P6<0.001 P7<0.001 P8<0.001 P9<0.001 P10<0.001

like malondialdehyde (MDA) and pro-inflammatory cytokines (like IL-6), while increasing protective factors including superoxide dismutase (SOD) and glutathione (GSH). These compounds can also stimulate mucosal healing by increasing mucus production and activating signaling pathways involved in tissue repair

Table (1): Effect of different concentrations of of green algae (*Chlorophyceae*) on gastric juice parameters including volume, pH, total acidity, and titratable acidity in rats with stomach ulcers.

P1: Negative control vs Positive control, P2: Negative control vs green algae 50 mg/kg , P3: Negative control vs green algae 100 mg/kg , P4: Negative control vs green algae 150 mg/kg , P5: Positive control vs green algae 50 mg/kg , P6: Positive control vs green algae 100 mg/kg , P7: Positive control vs green algae 150 mg/kg , P8: green algae 50 mg/kg vs green algae 100 mg/kg , P9: green algae 50 mg/kg vs green algae 150 mg/kg , P10: green algae 100 mg/kg vs green algae 150 mg/kg
 *: <0.05, **: <0.005, ***<0.001

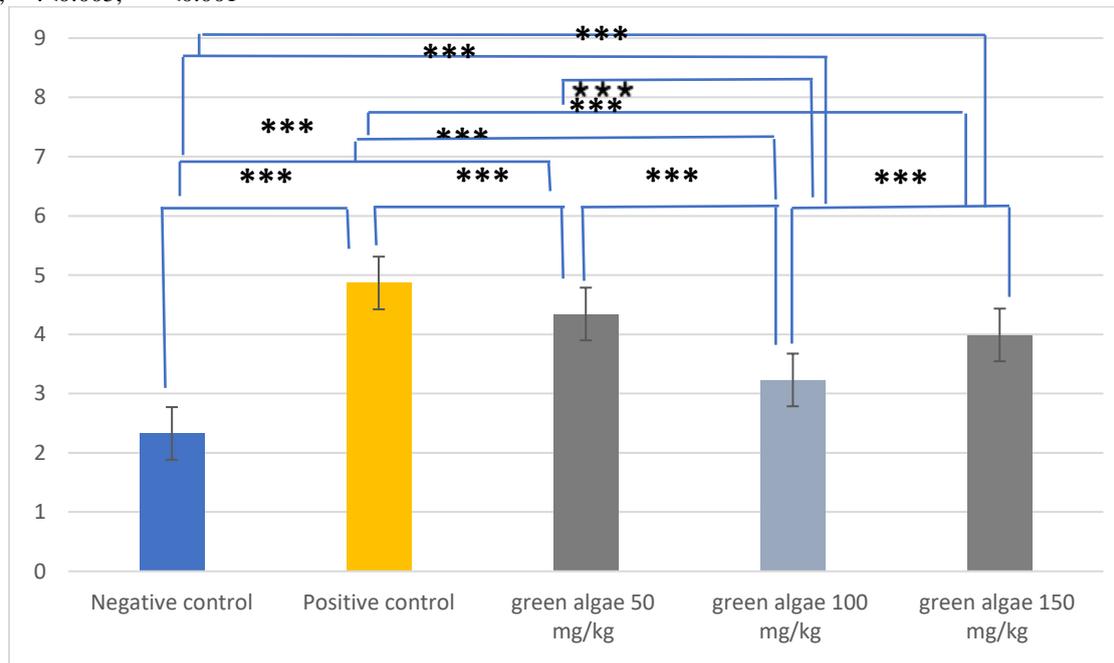


Figure (1): Effect of different concentrations of of green algae (*Chlorophyceae*) on Volume of gastric juice parameters in rats with stomach ulcers.

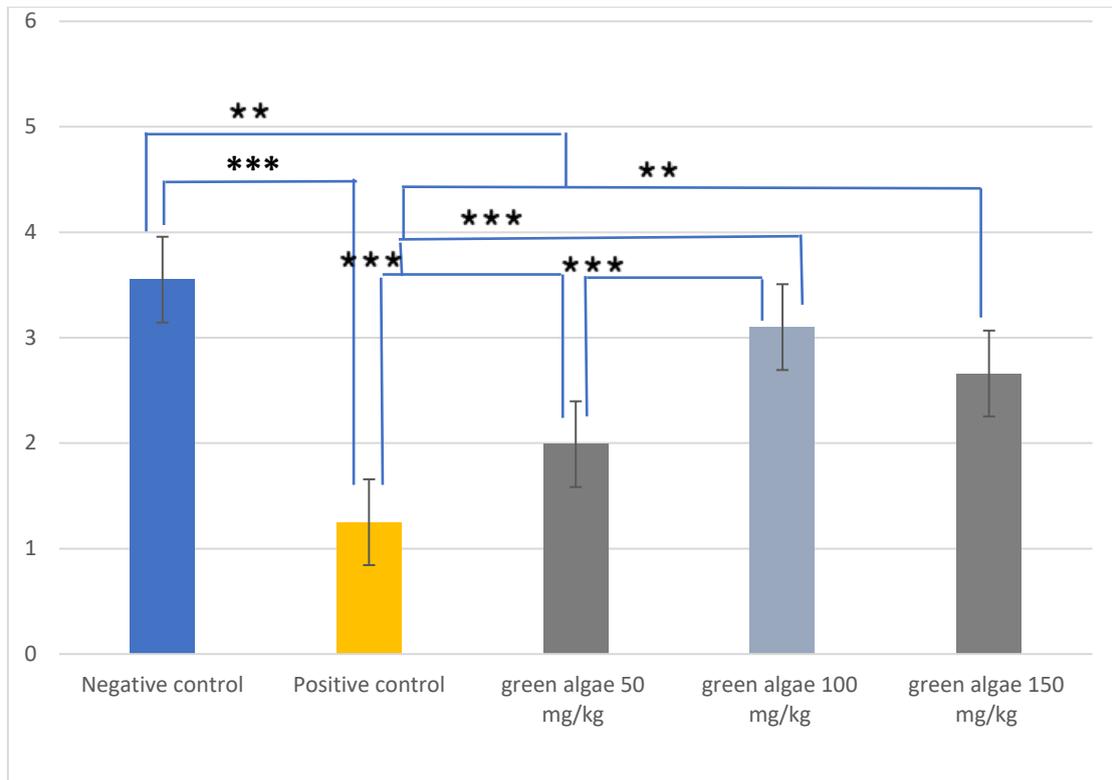


Figure (2): Effect of different concentrations of of green algae (*Chlorophyceae*) on PH of gastric juice parameters in rats with stomach ulcers.

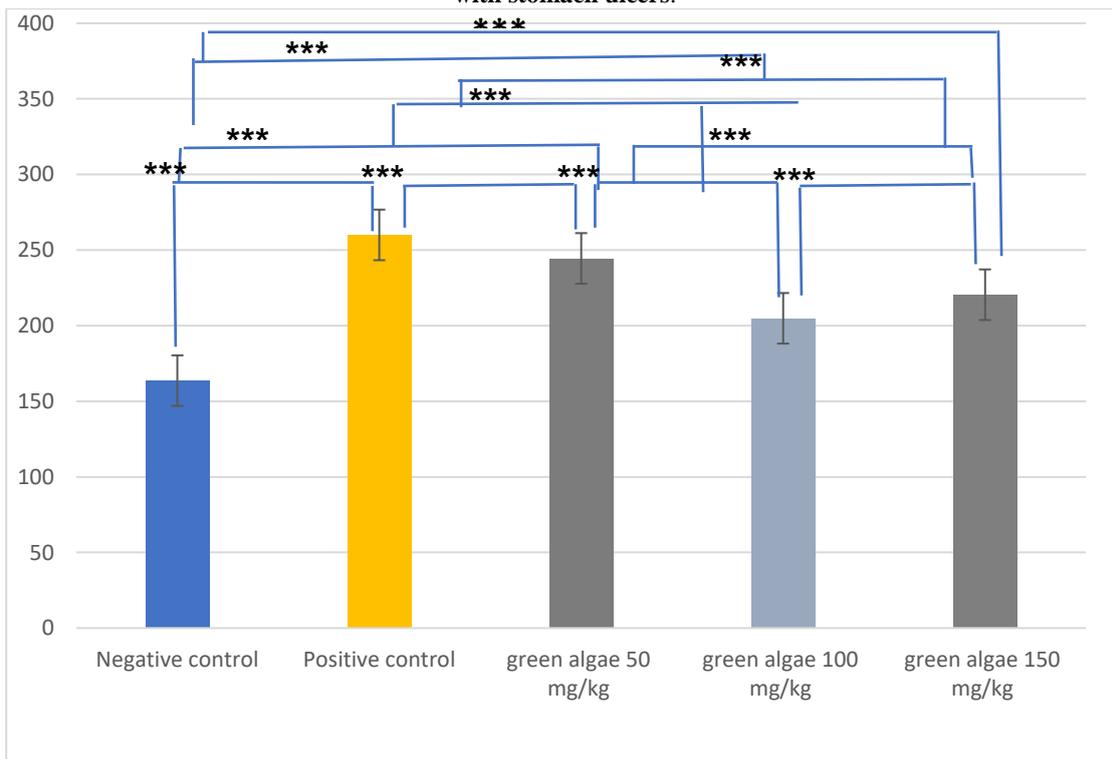


Figure (3): Effect of different concentrations of of green algae (*Chlorophyceae*) on total acidity of gastric juice parameters in rats with stomach ulcers.

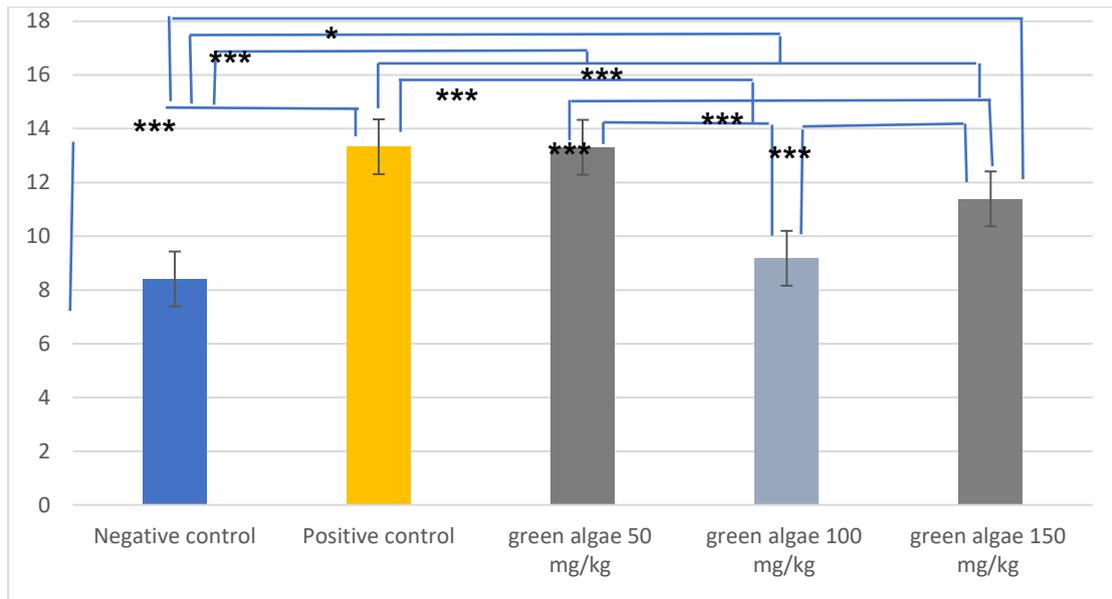


Figure (4): Effect of different concentrations of green algae (*Chlorophyceae*) on titratable acidity of gastric juice parameters in rats with stomach ulcers.

Histopathological examination of stomach:

Photo (1) illustrates the histological examination of stomach tissues in ulcer-induced rats subjected to different dietary treatments. In the negative control group, the gastric tissue appeared completely normal, with no histopathological alterations and well-preserved gastric layers. Conversely, the positive control group (ulcer-induced without treatment) showed severe damage, including necrosis of the gastric mucosa, hemorrhage, submucosal hemorrhage, inflammatory cell infiltration, focal necrosis, sloughing of the gastric mucosa, submucosal edema, and extensive inflammatory infiltration (photos 2 and 3). As shown in photo (4 and 5), rats with stomach ulcers treated with oral administration of (green algae) 50 mg/kg exhibited moderate histopathological changes, such as submucosal edema, hemorrhage, and inflammatory cell infiltration. In contrast, photo (8 and 9) shows that rats group treated with oral administration of (green algae) 100 mg/kg exhibited milder changes, including congestion of submucosal blood vessels, slight edema, and minimal inflammatory infiltration. Notably, photo (6 and 7) reveals that rats group treated with oral administration of (green algae) 100 mg/kg showed no histopathological changes, indicating a protective or healing effect on the gastric tissue.

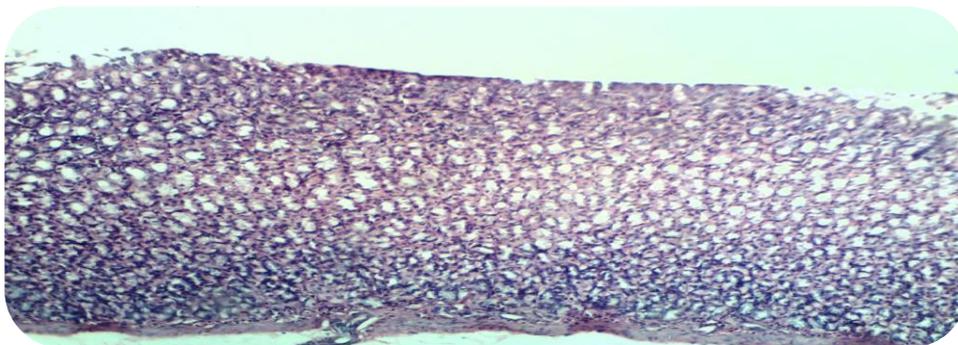


Photo (1): Stomach of rat from (C – ve group) (Hand E X100).

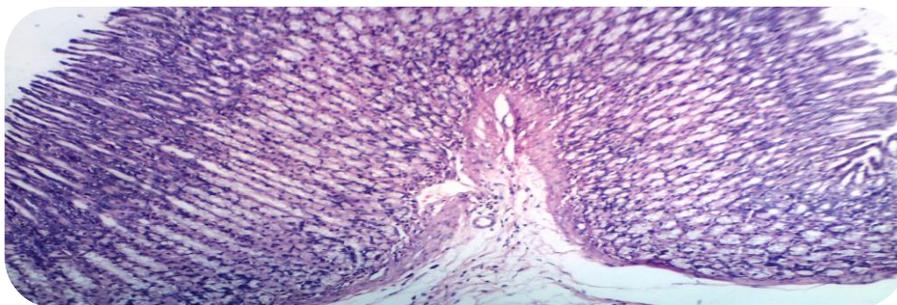


Photo (2): Stomach of rat from (positive control) (Hand E X100).

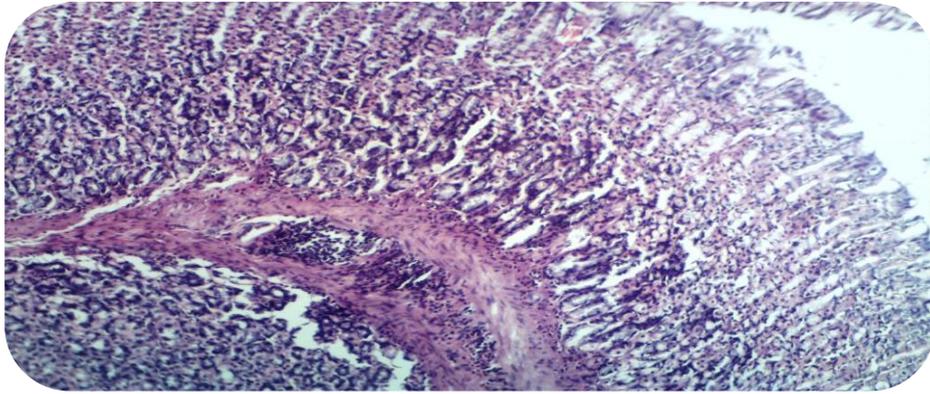


Photo (3): Stomach of rat from (positive control group) (Hand E X100).

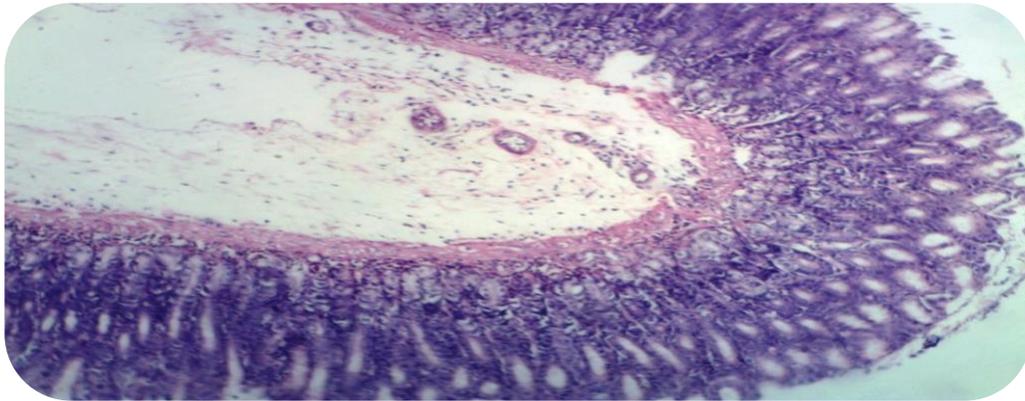


Photo (4): Stomach of rats treated with oral administration of (green algae) 50 mg/kg (Hand E X100).

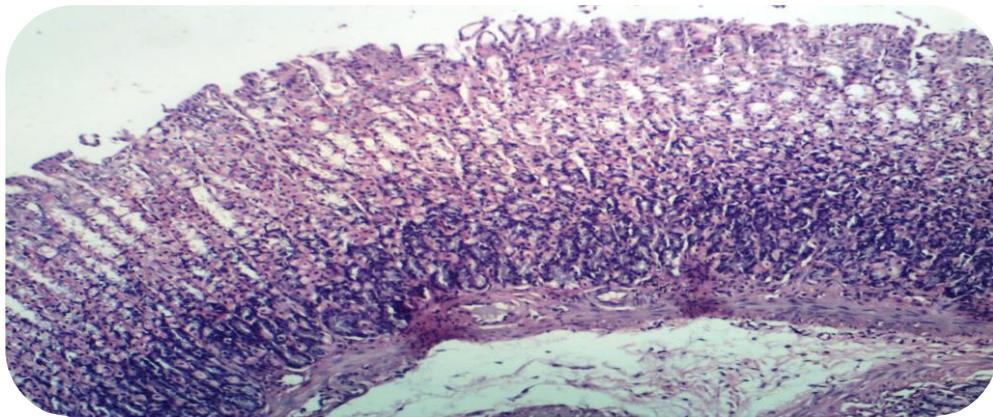


Photo (5): Stomach of rats treated with oral administration of (green algae) 50 mg/kg (Hand E X100).

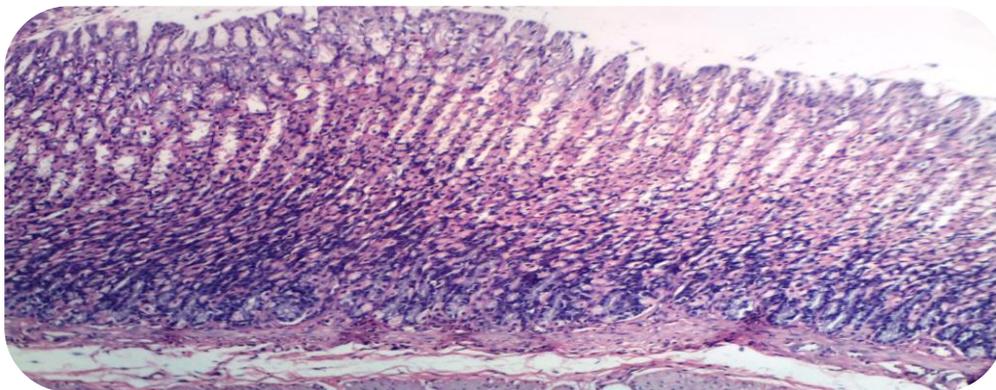


Photo (6): Stomach of rats treated with oral administration of (green algae) 100 mg/kg (Hand E X100).

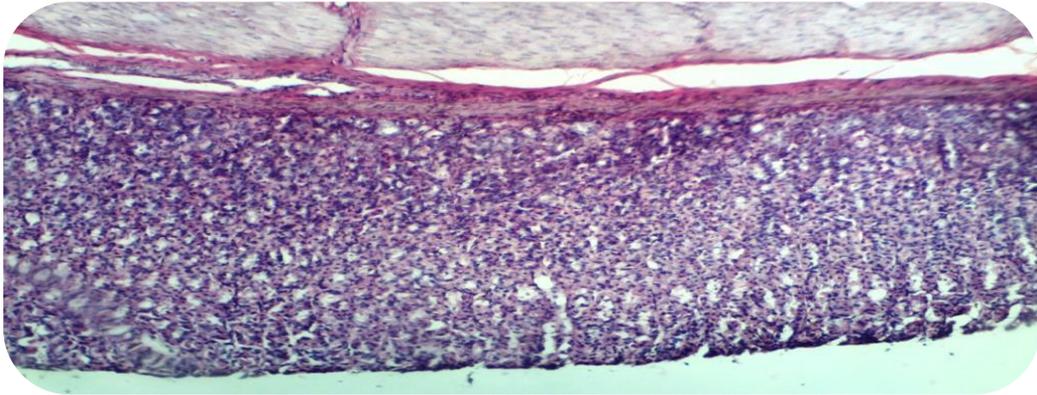


Photo (7): Stomach of rats treated with oral administration of (green algae) 100 mg/kg (Hand E X100).

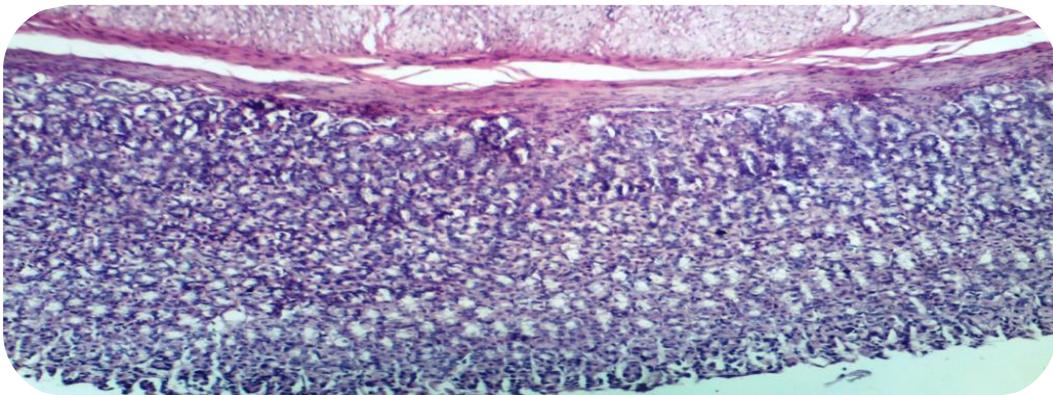


Photo (8): Stomach of rats treated with oral administration of (green algae) 150 mg/kg (Hand E X100).

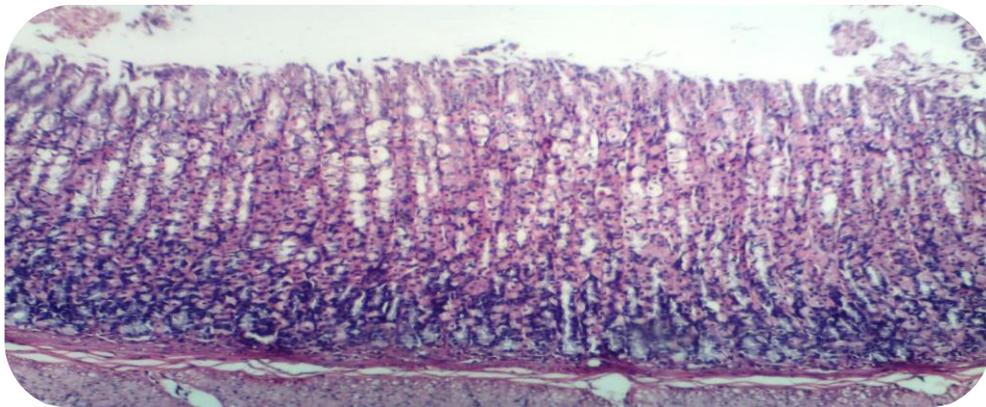


Photo (9): Stomach of rats treated with oral administration of (green algae) 150 mg/kg (Hand E X100).

CONCLUSION

In conclusion, green algae (*Chlorophyceae*) can have a beneficial effect on gastric ulcer parameters by decreasing gastric volume and acidity (both total and titratable), and increasing gastric pH in rats with stomach ulcers. The effects are dose-dependent, with higher concentrations showing a more significant impact. This suggests potential therapeutic value for ulcer treatment, likely through a combination of gastroprotective and acid-reducing mechanisms. Further research is needed to determine the efficacy and safety of green algae in human subjects. If proven effective, it could offer a natural alternative or complementary therapy for individuals suffering from gastric ulcers.

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