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RESEARCH ARTICLE
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HISTOPATHOLOGICAL AND BIOCHEMICAL STUDY OF SILYMARIN AND VITAMIN C VERSUS OMEPRAZOLE IN THE TREATMENT OF GASTRIC ULCER INDUCED BY INDOMETHACIN IN ADULT MALE ALBINO RATS.

Hassan saad Hassan Elwakeel[1*], Manar A. Elkholy[2], Hammad Yahia Abdoh[3], Alaa AboSeif[4], Faten I. Mohammed[5], Ahmed Abdrabo Elshenawy Elsisi[6], Shaimaa fawzy Abdellatif Esmaeil[7], Noha O. Shawky[8]

[1*]Department of Anatomy and Embryology, Faculty of Medicine , Al-Azhar University, Damitta, Egypt.
[2]Department of Pharmacology, Faculty of Medicine, Benha University, Egypt.
[3]Department of Anatomy and Embryology, Faculty of Medicine , Al-Azhar University, Damitta, Egypt.
[4]Department of Pharmacology, Faculty of Medicine, Al Azhar University, Cairo, Egypt.
[5]Department of Physiology, Faculty of Medicine for Girls, Al-Azhar University , Cairo, Egypt.
[6]Department of Pharmacology , Faculty of Medicine , Al-Azher University, Asyut, Egypt.
[7]Department of physiology, Faculty of Medicine for Girls, Al-Azhar University, Asyut, Egypt.
[8]Department of Medical Physiology, Faculty of Medicine, Helwan University, Egypt.

***Corresponding Author:** Hassan saad Hassan Elwakeel
[1*]Department of Anatomy and Embryology, Faculty of Medicine , Al-Azhar University, Damitta, Egypt.

Abstract:

Objective: This study aims at investigating the role of each of Silymarin and Vitamin C in preventing gastric Ulcer induced by indomethacin in comparable with omeprazole.

Method:

A study was conducted on 40 adult male albino rats. The rats were divided into two groups: a control group, which received distilled water orally, and an experimental group, which received indomethacin intraperitoneally for two consecutive days to induce stomach ulcers. After inducing the gastric ulcer, the rats were divided into three groups: Silymarin, Vitamin C, and Omeprazole. Blood samples were collected for biochemical evaluation, and the rats were euthanized by cervical dislocation three hours after the duodenum was ligated. The gastric juice was examined for volume, titratable acidity, and total acid output. Histological examination of the stomach was performed after the opening was checked for ulcers.

Results:

The study compared three groups before and after treatment with indomethacin injection, followed by treatment with Silymarin and vitamin C. The results showed that Silymarin and vitamin C achieved the best results, followed by omeprazole. The average ulcer area, number of ulcers, and stomach size varied among the groups. After treatment, the ulcer area decreased by 63% in the first group treated with Silymarin, while the number of ulcers decreased by 27% in the second group treated with Vitamin C. The size of the stomach increased by 27.5% in the first group, 17% in the second group, and 67% in the third group.

Conclusion: This study illustrates the efficiency of Silymarin and Vitamin C in alleviating indomethacin-induced stomach ulcers in albino rats, both showing significant effectiveness relative to omeprazole. Silymarin exhibited a marked reduction in ulcer area, whereas Vitamin C indicated a notable decrease in ulcer quantity. Silymarin and Vitamin C produced a lesser increase in stomach size than omeprazole, indicating a potentially superior management of gastric distension.

Keywords: Silymarin, vitamin C indomethacin-induced stomach ulcers, male albino rats, Gastric ulcers.

Introduction:

Gastric ulcer (peptic ulcer, stomach ulcer) is a type of ulcer that forms on the lining of the stomach, resulting in excavation of the gastric mucosa **[1]**. This excavation occurs due to an imbalance between protective factors and aggressive factors in the gastric environment, resulting in degradation of the mucosa **[2]**. Protective factors include mucus secretion, bicarbonate, prostaglandins, growth factors, mucosal blood flow, and epithelium regeneration; while aggressive factors include hydrochloric acid, pepsin, non-steroidal anti-inflammatory drugs (NSAIDs), cigarette smoke, alcohol consumption, and stress **[3].** NSAIDs inhibit the synthesis of prostaglandins, which are protective in the stomach, resulting in ulcer formation. The most frequently consumed NSAID that induces gastric ulcer is indomethacin **[4, 5].** Current treatment options for gastric ulcers include: (1) reduce stomach acid, which can be achieved by either proton pump inhibitors (PPIs) or H2 receptor antagonists; (2) antibiotics for ulcer caused by Helicobacter pylori; (3) protect the lining of the stomach; (4) repairing the mucosal injury of stomach; and (5) lifestyle changes, such as diet modification and avoiding smoking and drinking alcohol **[6].** Of these options, PPIs are the first line choice due to their efficacy. However, there are concerns regarding the adverse effects of long-term PPI therapy, including rebound acid hypersecretion, increased risk of enteric infections, pneumonia, and osteoporosis **[7, 8].** Therefore, there is a growing interest in natural compounds that could be used to treat gastric ulcers. Silymarin is a natural compound with multiple bioactivities including hepatoprotective, nephroprotective, antioxidant, anti-inflammatory, and digestive system protection effects **[9]**. Silymarin has been reported to protect against gastric ulcer. Vitamin C is a water-soluble vitamin with antioxidant properties **[10].** It is a cofactor for prolyl hydroxylase and lysyl hydroxylase enzymes that stabilize collagen by catalyzing hydroxylation of proline and lysine residues during collagen synthesis. Sufficient collagen synthesis is essential for mucosal healing **[11]**. The mucosa of the stomach is rich in collagen. Vitamin C deficiency leads to scurvy, a disease associated with impaired collagen synthesis, causing weakened connective tissue and resulting in gastric ulcer **[12].** Interestingly, Vitamin C is also depleted in gastric ulcer **[13].** Therefore, Vitamin C has the potential to be developed as a candidate for gastric ulcer treatment as it could help in collagen stabilization and mucosal healing **[14].**

This study aims at investigating the role of each of Silymarin and Vitamin C in preventing gastric Ulcer induced by indomethacin in comparable with omeprazole.

Method

1. Experimental animals:

A total of 40 adult male albino rats each of them weighed (150-180g) were obtained from the "National Organization for Drug Control and Research" farm at Giza, Egypt. The Rats were kept under the common laboratory conditions including the suitable temperature and humidity according to the ethics and guiding of animal experiments approved by the university.

2. Study protocol

The animals were deprived of food for forty-eight hours before to the start of the trials in order to ensure that their stomachs were completely empty according to Cho and Ogle **[15]**. They were allowed to drink as much water as they wanted as in the study of Ogletree ML **[16]**. Rats were

maintained in separate cages with a wide elevated mesh bottom during fasting to prevent coprophagy **[17].**

The 40 rats were divided into two main groups:

1. **Control group**: include 10 wester male albino rats which received distilled water orally.
2. **Experimental group**: Include 30 male albino rats of Western origin, all administered indomethacin intraperitoneally at a dosage of 20 mg/kg once daily for two consecutive days to induce stomach ulcers, followed by distilled water for a duration of 15 days **[18].** Indomethacin powder was provided in 50 mg vials. It was dissolved in 10 ml of distilled water to achieve a concentration of 5 mg/ml and then injected intraperitoneally.
3. After inducing the gastric ulcer those rats were divided into 3 groups as follow:

- **Group (1):** 10 rats orally received Silymarin at a dose of 50 mg/kg once a day for 15 days **[19].** Silymarin obtained as powder and was dissolved in distilled water to be delivered straight into the stomach using an oral gavage needle.
- **Group (2):** 10 rats orally received Vitamin C at a dose of 200 mg/kg once a day for 15 days **[20].** Vitamin C was obtained as powder and dissolved in distilled water and delivered straight into the stomach using an oral gavage needle.
- **Group (3):** 10 rats orally received Omeprazole at a dose of 3.6 mg/kg orally once a day for 15 days **[21].** Omeprazole was dissolved as powder in distilled water and delivered directly into the stomach using an oral gavage needle.

3. Collecting blood samples for biochemical evaluation:

At the end of the treatment period for rats in control and experimental groups blood samples were obtained while the rats still alive in order to determine the following parameters for oxidants and antioxidants:

- Glutathione (GSH)
- Malondialdehyde (MDA) activity
- Antioxidant enzymes: These include superoxide dismutase (SOD), catalase, and CAT (µmol/mg protein/min)
- pH of gastric acid
- The levels of inflammatory cytokines include TNF-α and IL-6 to evaluate inflammation.

4. Collection of gastric acid

Rats underwent pyloric ligation while under light ether anesthesia. The abdominal skin was excised, and a midline incision was performed, revealing the duodenum and elevating the pyloroduodenal junction. A silk suture was employed to prevent injury to blood vessels or gastric traction. The incision was sutured, sanitized, desiccated, and dressed with a colloidal solution. Anesthesia was terminated, and the rats were allowed to recuperate for 10 minutes.

5. Sacrifice of animals

The rats from were euthanized by cervical dislocation three hours after the duodenum has been ligated. The stomachs are exposed by making incisions in the abdominal cavities, and gastric secretions are then collected from them.

6. Histopathological examination:

After the opening stomach was checked for ulcers, pieces of the stomach that measured 1 by ½ cm were taken off and preserved in a 10% formalin solution. Paraffin slices were made and stained with hematoxylin, eosin for histological investigation.

7. Statistical analysis

Data obtained by measuring different parameters in this study were tabulated and organized to be analyzed using SPSS statistical package for assessing the results of the study.

8. Ethical consideration

The study was conducted according to the ethical approval obtained from …… and according to the ethics and guiding of the ethics committee regulating the research including animals.

Results

Table (1) shows that the study compared three groups before and after treatment with indomethacin injection, and between the control group. Silymarin (100 mg/kg) was found to be the most effective treatment, followed by Vitamin C (300 mg/kg) and Omeprazole (4 mg/kg). Before treatment, the average ulcer area was 18.7 mm2, with a standard deviation of 2.8. The number of ulcers varied among the groups, with the first group having an average of 1.8, the second group having an average of 1.8, and the third group having an average of 1.9. The stomach size varied between the groups, with the first group having an average of 2.84 ml, the second group having an average of 2.61 ml, and the third group having an average of 2.8 ml. After treatment, the average area of the ulcer increased to 3.57 mm2, the second group having an average of 6.68 mm2, and the third group having an average of 9.72 mm2.

The number of ulcers also varied among the groups, with the first group having an average of 1.1 and the second group having an average of 1.4. The stomach size varied between the groups, with the first group having an average of 4.2 ml and the second group having an average of 3.58 ml. The results were statistically significant, with all values less than 5% and a large coefficient of variation, indicating that the results can be analyzed comfortably.

Table 1: Comparison between the three studied groups according to Ulcer size (mm²), Number of ulcers and Stomach size ml.

Clinical variables		*group 3(Omeprazole: 4 mg/kg)*	*group 2(Vitamin C: 300 mg/kg)*	*group 1(Silymarin: 100 mg/kg)*	*Control*	*F*
Ulcer size (mm²)	***Pre***					
	Min. – Max.	*15.0-25.0*	*15.0-25.0*	*15.0-25.0*	*0.0-0.0*	
	Mean ± SD.	*18.64± 3.8*	*17.92± 3.04*	*18.7± 2.8*	*0.0± 0.0*	*15*
	Median (IQR)	*17(7.50*	*16.75(2.7)*	*20.4(3.3)*	*0 (0)*	
	Post					
	Min. – Max.	*8.0-12.0*	*5.0 – 10.0*	*3.0– 5.0*	*0-0*	
	Mean ± SD.	*9.72. ± 1.4*	*6.68. ±1.55*	*3.57± 0.6*	*0.0± 0.0*	*0.79*
	Median (IQR)	*10(2.8)*	*6.45(2.27)*	*3.1(0.7)*	*0 (0)*	
Number of ulcers	***Pre***					
	Min. – Max.	*1 .0– 3.0*	*1 .0– 3.0*	*1 .0– 3.0*	*0.0-0.0*	
	Mean ± SD.	*1.9± 0.8*	*1.8± 0.7*	*1.8 ± 0.6*	*0.0± 0.0*	*18.7*
	Median (IQR)	*2(1.75)*	*2(1)*	*1(1)*	*0 (0)*	
	Post					
	Min. – Max.	*1 .0–2.0*	*1 .0–2.0*	*1 .0–2.0*	*0.0-0.0*	
	Mean ± SD.	*1.4 ± 0.48*	*1.3± 0.8*	*1.1 ± 0.3*	*0.0± 0.0*	*1.2*

	Median (IQR)	*1(1)*	*1(0)*	*1(0)*	*0 (0)*	
Stomach size mL	***Pre***					
	Min. – Max.	*2.0 –4.0*	*2.0 –4.0*	*2.0 –4.0*	*3.0 – 5.0*	
	Mean ± SD.	*2.8± 0.6*	*2.61±0.5*	*2.84± 0.7*	*4.19 %± 0.6*	*15*
	Median (IQR)	*2.7 (0.8)*	*2.55 (0.2)*	*3.15(0.82)*	*4.25 (0.45)*	
	Post					
	Min. – Max.	*2.0 – 4.0*	*3.0 – 4.0*	*3.0 – 4.0*	*3.0 – 5.0*	
	Mean ± SD.	*2.3 ± 0.3*	*3.58±0.28*	*4.2 ± 0.29*	*4.19 %± 0.6*	*0.79*
	Median (IQR)	*2.15 (0.75)*	*3.5 (0.25)*	*4.3(0.35)*	*4.25 (0.45)*	

*IQR: Inter quartile range SD: standard deviation F: F for One way ANOVA test, p: p value for comparing between the three studied groups, *: Statistically significant at p ≤ 0.05*

The results showed a comparison between the three groups before and after treatment. It is clear from the comparison that the treatment Silymarin achieved the best results, followed by treatment with vitamin c, then followed by treatment with Omeprazole, especially with regard to the area of the ulcer that occurred, the number of ulcers, and the size of the stomach of the experimental rat. The first group that was treated with Silymarin, the ulcer area decreased with an improvement rate of 79%, while the ulcer area decreased in the second group. who received treatment with Vitamin C 63%, and group 3 with 51% while the ulcers shrank in the third group, which was treated with Omeprazole, the area of the ulcers decreased. As for the number of ulcers, it decreased in the first group by a rate of 27.5%, while in the second group, the number of ulcers decreased by a rate of 27% in group 2and group 3. As for the size of the stomach, it increased by 67% in the first group, increased by 27% in the second group, and increased by 17% in the third group.

Table 2: Comparison between the three studied groups according to Glutathione level (µmol/g), Malondialdehyde (MDA) level (nmol/g), and Gastric acidity (pH)

Biochemical variables:		***group 1(Silymarin:100 mg/kg) (n = 10)***	***group 2(Vitamin C: 300 mg/kg) (n = 10)***	***group 3(Omeprazole : 4 mg/kg) (n = 10)***	***Control (n = 10)***	***F***	***p***
Glutathione level (µmol/g)	***Pre***						
	Min. – Max.	*3.0-5.0*	*3.0-5.0*	*3.0-5.0*	*8.0-10.0*		
	Mean ± SD.	*4.39± 0.55*	*3.79± 0.73*	*4.31± 0.63*	*9.24±0.65*	*13.4*	*0.002*
	Median (IQR)	*4.55(0.4)*	*3.65(1.05)*	*4.55(0.7)*	*9.3 (0.7)*		
	Post						
	Min. – Max.	*6.0-8.0*	*5.0-7.0*	*7.0-9.0*	*8.0-10.0*		
	Mean ± SD.	*6.98. ±0.71*	*6.29. ±0.71*	*8.03± 0.9*	*9.24±0.65*	*0.8*	*0.031*
	Median (IQR)	*7.05(1.25)*	*6.45(0.9)*	*8(1.7)*	*9.3 (0.7)*		
Malondialdehyde (MDA) level (nmol/g)	***Pre***						
	Min. – Max.	*4 .0– 5.0*	*4 .0– 5.0*	4 .0– 5.0	*0.5-1.0*		
	Mean ± SD.	*4.4 ± 0.6*	*4.47± 0.3*	4.36± 0.32	*0.81± 0.15*	*16.7*	*0.002*
	Median (IQR)	*4.65(0.12)*	*4.5(0.32)*	4.3(0.4)	*0.8(0.12)*		
	Post						

	Min. – Max.	*7.0–9.0*	*5.0–7.0*	6.0–8.0	*0.5-1.0*		
	Mean ± SD.	*8.03 ± 0.9*	*6.89± 0.71*	6.28± 0.71	*0.81± 0.15*	*0.89*	*0.02*
	Median (IQR)	*8(1.70)*	*7.05(1.25)*	6.45(0.9)	*0.8(0.12)*		
Gastric acidity (pH)	***Pre***						
	Min. – Max.	*2.0 –4.0*	*2.0 –4.0*	2.0 –4.0	*4.0 – 5.0.0*		
	Mean ± SD.	*2.84± 0.7*	*2.45±0.35*	2.8± 0.6	*4.5 %± 0.72*	*9.8*	*0.003*
	Median (IQR)	*3.15(0.82)*	*2.5 (0.0.37)*	2.7 (0.8)	*4.5(0.62)*		
	Post						
	Min. – Max.	*4.0 – 4.5*	*3.5 – 4.5*	3.0 – 4.0	*4.0 – 5.0*		
	Mean ± SD.	*4.25 ± 0.5*	*4±0.38*	3.55 ± 0.41	*4.81 %± 0.3*	*0.17*	*0.033*
	Median (IQR)	*4.25(0.25)*	*4(0.75)*	3.5 (0.87)	*4.8(0.75)*		

IQR: Inter quartile range SD: Standard deviation F: F for One way ANOVA test, p: p value for comparing between the three studied groups, *: Statistically significant at p ≤ 0.05

Table 2 compares the results of three groups before and after treatment with a control group. The first group treated with Silymarin (100 mg/kg) showed better biochemical variables (Glutathione level, Gastric acidity (pH), and Malondialdehyde (MDA) level) compared to the third group treated with Omeprazole (4 mg/kg) and the second group treated with Vitamin C (300 mg/kg). Before treatment, the first group had an average of 4.39 µmol/g and a standard deviation of 0.55, while the second group had an average of 3.79 µmol/g and a standard deviation of 0.73.

The second group had an average of 4.31 µmol/g and a standard deviation of 0.63. In terms of MDA loss, the first group had an average of 4.47 µmol/g and a standard deviation of 0.3, while the second group had an average of 4.36 nmol/g and a standard deviation of 0.32. After treatment, the first group had an average of 6.98 µmol/g and a standard deviation of 0.71, while the second group had an average of 6.29 µmol/g and a standard deviation of 0.71. The third group had an average of 8.03 µmol/g and a standard deviation of 0.9.

The results are highly significant, with all values being less than 5% and a large coefficient of variation, indicating that the results are statistically significant and can be analyzed comfortably.

The results showed a comparison between the three groups before and after treatment. It is clear from the comparison that treatment with omeprazole achieved the best results Olney in Glutathione level (µmol/g), followed by treatment with silymarin, with regard to pH, then treatment with vitamin C, where the rate of change in pH for the first group was 33%, for the second group 22%, and for the third group, the rate of change was 21% Glutathione level (µmol/g):

The rate of change in the first group was 37%, while in the second group it was 39%, and in the third group the rate of change was 46%. As for Malondialdehyde (MDA) level (nmol/g), the rate of change for the first group was 40%, for the second group the rate of change was 30%, and for the third group the rate of change was 35%.

Table 3: Comparison between the three studied groups according to Inflammatory cytokines (TNF-α), SOD ((unit/mg protein), and CAT (µmol/mg protein/min):

Biochemical variables:		*group 1 (Silymarin: 100 mg/kg) (N=10)*	*group 2 (Vitamin C: 300 mg/kg) (N=10)*	*group 3 (Omeprazole: 4 mg/kg) (N=10)*	*Control (N=10)*	*F*	*p*
Inflammatory cytokines (TNF-α)	*Pre*						
	Min.– Max.	*169.0-176.0*	*170.0-177.0*	*170.0-175.0*	*34.0-42.0*		
	Mean ± SD.	*172± 2.5*	*172.9± 1.8*	*172± 2.3*	*37.8± 2.7*	*13.4*	*0.0021*
	Median (IQR)	*171(2.25)*	*173(3.5)*	*3.65(1.05)*	*38 (3.75)*		
	Post						
	Min.– Max.	*70.0-75.0*	*100.0-150.0*	*120.0-170.0*	*34.0-42.0*		
	Mean ± SD.	*72.09± 9*	*139.2 ±17.2*	*158. ±14.04*	*37.8± 2.7*	*0.8*	*0.031*
	Median (IQR)	*71.5(2.25)*	*145.5(17.75)*	*164.5(10.5)*	*38 (3.75)*		
SOD((unit/mg protein):	*Pre*						
	Min.– Max.	*90.0– 95.0*	*90.0– 95.0*	*90.0– 95.0*	*160.0-175.0*		
	Mean ± SD.	*93.9± 2.1*	*93.5± 1.9*	*93.3± 1.5*	*167.6± 4.6*	*16.7*	*0.002*
	Median (IQR)	*94.5(4)*	*94(3.25)*	*93.5(3)*	*168(3.75)*		
	Post						
	Min. – Max.	*130.0–170.0*	*120.0–160.0*	*100.0–140.0*	*160.0-175.0*		
	Mean ± SD.	*151 ± 10.8*	*147.2±12.7*	*129±9.6*	*167.6± 4.6*	*0.89*	*0.02*
	Median (IQR)	*150.5(13)*	*151(14)*	*129.5(15.5)*	*168(3.75)*		
(µmol/mg protein/min):	*Pre*						
	Min. – Max.	*90.0 –95.0*	*90.0 –95.0*	*90.0 –95.0*	*205.0– 226.0.0*		
	Mean ± SD.	*93.8±2.1*	*93.5±2.3*	*93± 2*	*217.2%± 6.8*	*9.8*	*0.003*
	Median (IQR)	*94.5(4)*	*2.5 (0.0.37)*	*94 (3)*	*218.5(4.5)*		
CAT	*Post*						
	Min. – Max.	*180.0 – 240*	*160.0–220*	*140.0 – 200.0*	*205.0– 226.0.0*		
	Mean ± SD.	*217.3± 15.5*	*206.03±16.3*	*183.7 ± 18.2*	*217.2%± 6.8*	*0.17*	*0.033*
	Median (IQR)	*215.5(11.5)*	*209.5(7.25)*	*194 (23)*	*218.5(4.5)*		

IQR: Inter quartile range SD: Standard deviation F: F for One way ANOVA test, p: p value for comparing between the three studied groups, *: Statistically significant at p ≤ 0.05

Table 3 compares the biochemical variables of three groups before and after treatment with silymarin (100 mg/kg) and vitamin C (300 mg/kg). The first group showed superior improvement results in inflammatory cytokines (TNF-α), SOD (unit/mg protein), and CAT (µmol/mg protein/min). The average levels of TNF-α in the first group were 172.0 pg/mL, 139.2 pg/mL, and 158 pg/mL, respectively. The average levels of SOD (unit/mg protein) were 151 (unit/mg protein), 147.2 (unit/mg protein), and 129 (unit/mg protein). The average CAT (µmol/mg protein/min) was 217.3 (unit/mg protein/min), 206.3 (unit/mg protein/min), and 183.7 (µmol/mg protein/min). The results are highly significant, with all values less than 5% and a large coefficient of variation, indicating that the results are statistically significant and can be analyzed comfortably. The table also shows that the first group treated with silymarin had the highest improvement results.

Histopathological evaluation The gastric mucosa in adult male albino rats exhibited a velvety texture characterized by many longitudinal folds and gastric pits. Figure 3 (A). Indomethacin administration had an effect on gastric mucosa which exhibited visible characteristics such as many ulcers, deep ulcers that reach the submucosal layer, a complete loss of the surface mucus layer, considerable submucosal edema, congestion of the gastric mucosa, and dilated fundic glands in the stomach. Figure 3 (B).

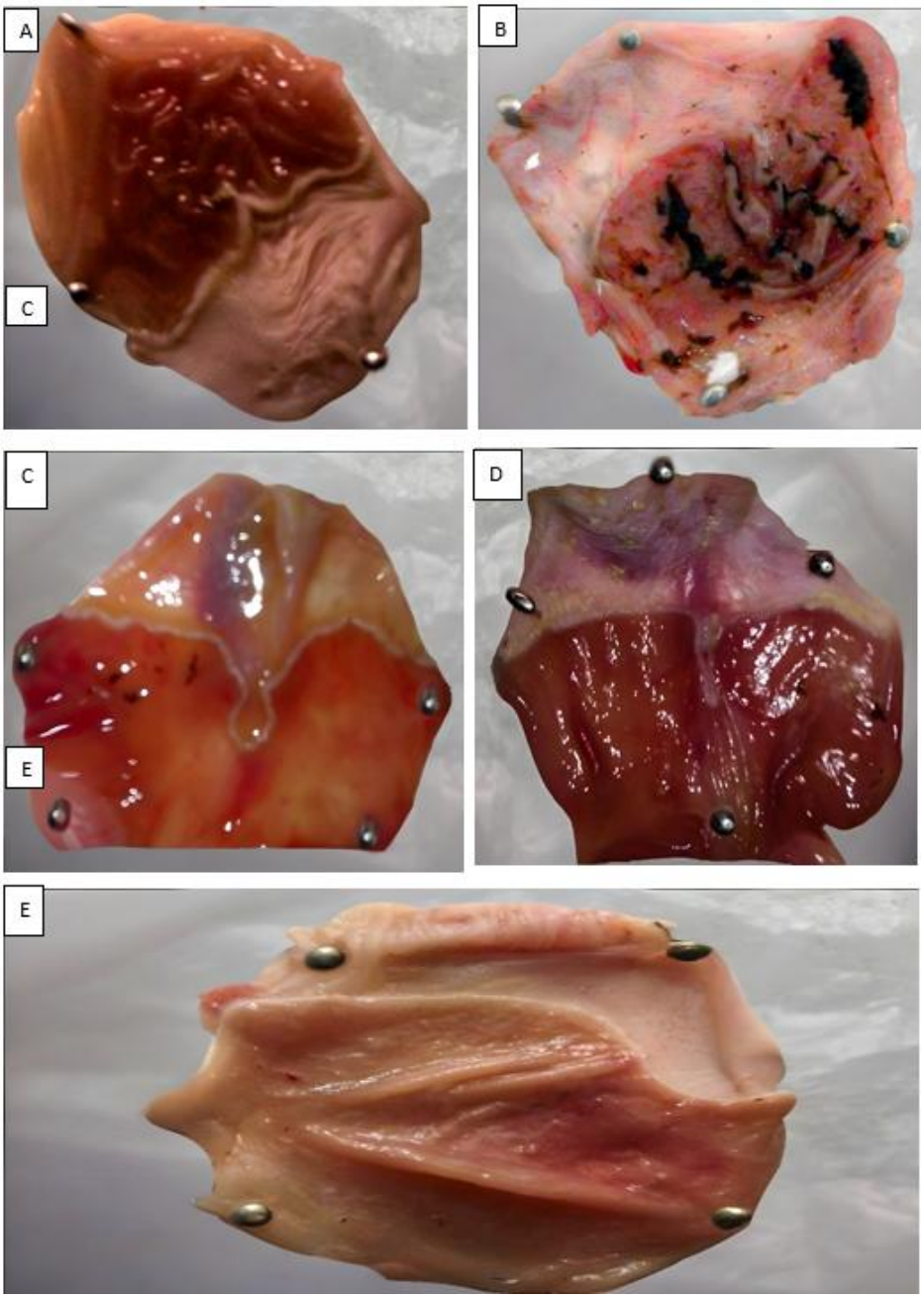

Figure 1: (A): Visual assessment of gastrointestinal mucosa in control vehicle rats. (B): Visual assessment of the impact of 20 mg/kg indomethacin administered intraperitoneally on gastric mucosa showing ulcerations, reddened appearance of gastric mucosa. (C): Visual assessment of the impact of 50 mg/kg silymarin treatment on indomethacin-induced stomach ulcers showing reduction in ulceration, diminished redness or indications of inflammation, implying a preventive effect against injury. (D): Visual assessment of the impact of 200 mg/kg vitamin C administration on indomethacin-induced stomach ulcers in male albino rats showing diminished reddening and decreased ulcer size. (E): Visual assessment of the impact of omeprazole treatment (3.6 mg/kg) on indomethacin-induced stomach ulcers in male albino rats showing improvement in the ulceration area and less reddening (H&E, X=200).

Oral administration of silymarin effectively prevented indomethacin-induced stomach ulcers. Silymarin reduced stomach lesions in a dose-dependent manner and prevented ulcer formation in experimentally created peptic ulcers. It also served a preventive function against indomethacin-induced stomach ulcers. Figure 3 (C). The study examined the impact of vitamin C administration on indomethacin-induced stomach ulcers in male albino rats found that it significantly reduced

ulceration and enhanced healing of the gastric mucosa. Figure 3 (D). Administration of omeprazole showed enhanced healing of the ulcers and reduction in the number and size of ulcers induced by Indomethacin Figure 3 (E).

A

B

Figure 2: (A): Microscopic appearance of rat stomach mucosa of a control marked by submucosa, inner epithelial surface, and muscularis layers. Most rats showed normal squamous gastric epithelial form, which is the characteristic of this layer. Little pits arranged methodically by columnar mucous cells, mucous neck cells, and a few parietal cells line the gastric glands. The stomach mucosa microvasculature shows a large lumen, smooth and uniform vessel walls, and no evidence of endothelial cell edema or protrusion.

Figure 4 (B): Microscopic appearance of rat stomach mucosa affected by Indomethacin 20 mg/kg for inducing Ulcer formation. Indomethacin at a 20 mg/kg dosage causes some distinct alterations in the rat stomach mucosa. These comprise stomach gland dilatation, erosions and ulcers suggesting injury to the epithelial layer, and cellular exfoliation (Orange arrow). Though erosions, the typical gastric gland architecture is sometimes intact with minor dilatation. The clear indication of ulcer development is a rupture of the muscularis mucosa.

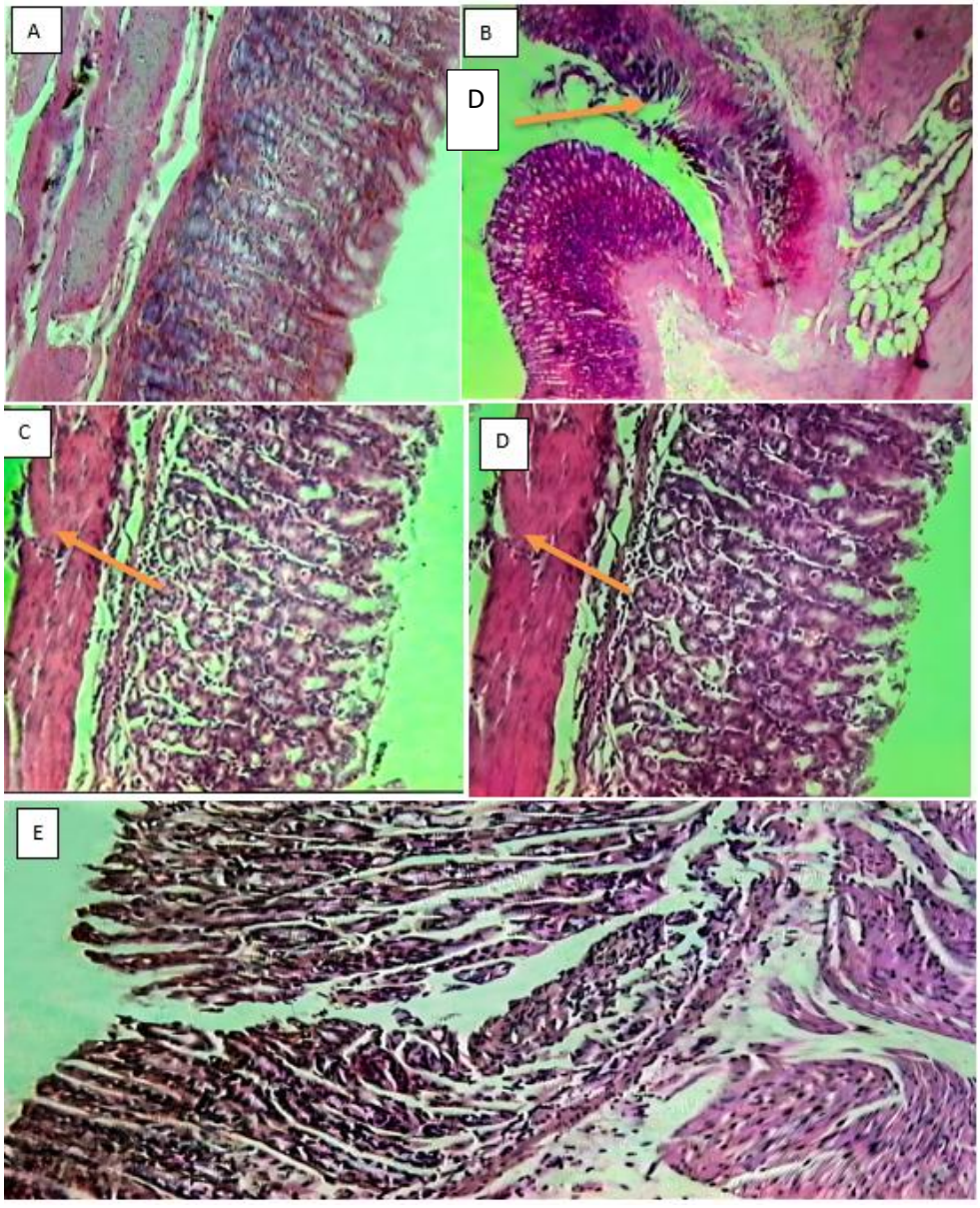

Figure 3: (A*): Microscopic appearance of rat stomach mucosa of a control vehicle. (B): Microscopic appearance of rat stomach mucosa affected by Indomethacin 20 mg/kg for showing Ulcer formation. (C): Rat stomach mucosa subjected to 50 mg/kg silymarin after

Indomethacin 20 mg/kg showing less ulcerative damage induced by indomethacin. (D): Rat stomach mucosa subjected to 200 mg/kg vit C after Indomethacin 20 mg/kg showing smaller and less severe erosions and ulcers. (E): Rat stomach mucosa subjected to (3.6mg/kg) Omeprazole after Indomethacin 20 mg/kg (H&E, X=200) showing reduction in the inflammatory cell infiltration. reduces.

Figure 4 (C): showed that 50 mg/kg silymarin considerably lessens ulcerative damage induced by indomethacin. It also has anti-inflammatory action since the submucosa's inflammatory cell infiltration reduces. It also maintains mucosal integrity since less disturbance of the epithelial layer and less evidence of cellular exfoliation results. The stomach glands keep their usual configuration with small pits, and cells lining them exhibit less damage than in the group including just indomethacin.

Figure 4 (D): showed that rat stomach mucosa treated with 200 mg/kg vitamin C following indomethacin treatment at 20 mg/kg displayed decreased ulcerative damage, anti-inflammatory effects. Comparing the mucosa to the group alone on indomethacin-only exhibited smaller and less severe erosions and ulcers. Vit C also lessened inflammatory cell invasion in the submucosa, so suggesting a lower inflammatory response to injury caused by indomethacin. With less disturbance of the epithelial layer and less indication of cellular exfoliation, the mucosa also shown improved preservation of its natural architecture. The stomach glands kept their usual configuration with small pits, and the cells lining them exhibited less damage than in the group just using indomethacin.

Figure 4(E): At a dosage of 3.6 mg/kg, omeprazole considerably lowered ulcerative damage produced by indomethacin. It also had anti-inflammatory action since the submucosa's inflammatory cell infiltration reduces. Omeprazole also maintained mucosal integrity since less disturbance of the epithelial layer and less evidence of cellular exfoliation results. The stomach glands kept their usual configuration with tiny pits, and the cells lining them exhibited less damage than in the group included only indomethacin.

Discussion

This study aims at investigating the role of each of Silymarin and Vitamin C in preventing gastric Ulcer induced by indomethacin in comparable with omeprazole. This research evaluates the effectiveness of Silymarin (100 mg/kg) versus Vitamin C (300 mg/kg) in the treatment of ulcers. The research indicates that Silymarin (100 mg/kg) exhibits greater efficiency in diminishing both the frequency and size of ulcers, exceeding that of Vitamin C (300 mg/kg). This exceptional efficacy necessitates additional exploration of its potential as a primary therapeutic agent for ulcer treatment.

Silymarin's enhanced ulcer reduction is evidenced by its direct comparison with Vitamin C, which also demonstrates ulcer-healing capabilities. Research has demonstrated Silymarin's capacity to neutralize free radicals, diminish lipid peroxidation, and decrease pro-inflammatory cytokines, all of which play a role in the onset and persistence of ulcers **[22]**.

Omeprazole, a proton pump inhibitor (PPI), efficiently diminishes stomach acid output, an essential element in ulcer formation **[23].** Although the research indicates that Silymarin is superior in diminishing the quantity and dimensions of ulcers, Omeprazole's function in acid suppression is essential, especially in instances of hyperacidity or when acid suppression is critical **[24].**

Research on stomach tissue has measured macroscopic ulcerative areas; silymarin at a 100 mg/kg dosage shows anti-ulcerative action **[25]**. Silymarin may work antiulcerally by reducing hydrochloric acid output, cytoprotective action, healing promotion, and enzymatic peroxidation inhibition **[26].** By means of the lipoxygenase pathway, silymarin's anti-ulcerogenic action could be connected to its inhibitory method of enzymatic peroxidation, therefore preventing leukotriene formation **[27, 28].**

Rats' gastrointestinal mucosal injury caused by indomethacin has been shown to be protected against by vitamin C **[29]**. It lowers lipid peroxidation, increases antioxidant action, and lessens

stomach damage **[30].** Indomethacin also lowers levels of antioxidant enzymes, which ascorbic acid raises.

The first group treated with Silymarin showed better biochemical variables (Glutathione level, Gastric acidity, and Malondialdehyde) compared to the third group treated with Omeprazole and the second group treated with Vitamin C.

Rats with indomethacin-induced stomach ulcers have seen silymarin raise glutathione levels and lower malondialdehyde levels. Silymarin (100 mg/kg) greatly raised GSH levels in stomach tissue injured by indomethacin, according a study employing Wistar albino rats **[25]**. The stomach's overall glutathione level likewise rose. Still, the search results don't specifically address how silymarin affects gastric acidity in the particular setting of ulcers brought on by indomethacin. While another study indicated that silymarin greatly lowered MDA levels in stomach tissue injured by indomethacin, one study noted that silymarin reduces hydrochloric acid output in pylorus-ligated rats **[25]**. In the framework of indomethacin-induced stomach ulcers in rats, silymarin shows antioxidant effects by raising glutathione levels and lowering malondialdehyde levels.

Increased GSH levels in tissues follow from Vitamin C reversing the drop in glutathione levels brought on by indomethacin treatment **[31].** Glutathione's protective action is also seen in ulcer development since lower glutathione and mucus generation are adversely linked with higher acid back-diffusion **[32].**

In rats, omeprazole has been shown to raise gastric glutathione levels, a molecule that can help stop damage of the stomach mucosa **[33].** This is a result of its strong acid-suppressing properties, which lower stomach acidity and hence affect mucosal damage **[34].** Reducing levels of malondialdehyde (MDA), a measure of lipid peroxidation and oxidative stress, have also been linked to omeprazole treatment **[35].** Under several models of stomach damage, omeprazole treatment significantly lowered MDA levels, suggesting its possible function in reducing oxidative damage. These actions help omeprazole be therapeutively effective in encouraging ulcer healing and shielding stomach mucosa from oxidative damage. Ultimately, in rats with indomethacin-induced stomach ulcers, omeprazole seems to raise glutathione levels and lower malondialdehyde levels while simultaneously greatly lowering gastric acidity **[36].**

Histologically, the administration of indomethacin impacted the gastric mucosa, demonstrating notable features including numerous ulcers **[37]**, deep ulcers extending to the submucosal layer, a total absence of the surface mucus layer**,** significant submucosal edema, congestion of the gastric mucosa, and dilated fundic glands within the stomach **[37].**

Our finding showed that Silymarin preserved mucosal integrity due to reduced disruption of the epithelial layer and diminished indications of cellular exfoliation. The gastric glands maintain their typical structure with minor pits, and the cells that line them demonstrate less damage compared to the group treated just with indomethacin.

Sasu A, et al [38] found the same finding about Silymarin. The findings of the study revealed heightened expression of cytochrome P450 in all examined gastrointestinal tissues of the Epi group, signifying robust drug detoxification. Bax immunopositivity was pronounced in absorptive enterocytes, lamina propria cells, gastric surface epithelial cells, and colonic epithelium, accompanied by reduced Bcl-2 expression across all tissues. The gastrointestinal damage caused by epirubicin was confirmed via study of goblet cell numbers and shape. Bax immunopositivity was eliminated by the highest dose of silymarin, but p53 expression was present in all tissues and diminished with increased doses.

Our study found that Vit C also lessened inflammatory cell invasion in the submucosa, so suggesting a lower inflammatory response to injury caused by indomethacin. With less disturbance of the epithelial layer and less indication of cellular exfoliation, the mucosa also shown improved preservation of its natural architecture. The stomach glands kept their usual configuration with small pits, and the cells lining them exhibited less damage than in the group just using indomethacin. **Danielski LG, et al [39]** also found that preoperative vitamin C administration can enhance

intestinal anastomosis healing, metabolic changes, and extend survival in rats undergoing food restriction.

Omeprazole also maintained mucosal integrity since less disturbance of the epithelial layer and less evidence of cellular exfoliation results. The stomach glands kept their usual configuration with tiny pits, and the cells lining them exhibited less damage than in the group included only indomethacin

Lee M, et al [40] found that Omeprazole markedly diminished stomach ulcer development caused by indomethacin, notwithstanding a substantial (>80%) reduction of gastric mucosal prostaglandin E2 (PGE2) synthesis. Contradicting these findings; **Tomiyasu Arisawa et al [41]** found that Omeprazole facilitated epithelialization but failed to completely counteract the effects of indomethacin on granulation tissue maturation.

Silymarin, a natural antioxidant polyphenolic flavonoid, may surpass Vitamin C and Omeprazole in treating indomethacin-induced ulcers. Its antioxidant and anti-inflammatory effects include the reduction of malondialdehyde levels and myeloperoxidase activity, alongside the augmentation of superoxide dismutase activity and glutathione levels in stomach tissue **[42].** Silymarin had antiulcerative effects at a dosage of 100 mg/kg. Both it and Vitamin C shown significant therapeutic effects on indomethacin-induced gastrointestinal ulcers in male albino rats. A study indicated that silymarin exhibits significant antiulcer capabilities and may operate via diminishing hydrochloric acid secretion **[43].**

Rationale of our study:

The studies on Silymarin as anti-ulcerative agent are limited but to the best of our knowledge all studies have found that Silymarin is one of the most effective and protective agents against ulcers not just in stomach but also in the entire gastrointestinal tract. Silymarin, is a natural substance extracted from milk thistle, possesses antioxidant and anti-inflammatory characteristics that safeguard the gastric mucosa and facilitate ulcer healing.

Vitamin C is an antioxidant that aids in safeguarding the stomach lining and facilitating healing. Omeprazole, a proton pump inhibitor, diminishes gastric acid secretion, hence inhibiting ulcer development and facilitating ulcer recovery. Silymarin may be a superior option for indomethacin-induced ulcers owing to its capacity to safeguard the gastric mucosa by enhancing prostaglandin synthesis and shielding cells from reactive oxygen species (ROS). Omeprazole diminishes gastric acid secretion but fails to rectify the fundamental reasons of indomethacin-induced ulcers. Certain research indicate that PPIs may exacerbate indomethacin-induced ulcers. Silymarin seems to be a potential treatment for indomethacin-induced ulcers; however, additional research is required to validate these results.

Our study may be the spark for conducting more studies on Silymarin as anti-Ulcerative agent, either alone or in combination with Vitamin C since they proved their effectiveness with minimal side effects on the long term in contrast with Omeprazole which have side effects on the long term.

Conclusion

Ulcer is a very serious problem that face major sector of patients worldwide. Finding a safe treatment that can build the mucosal layer of the stomach and prevent the formations of more ulcers is very important especially in this era in which Patients are interested in natural products treatment. Silymarin has proved its effectiveness in treatment of ulcer surpassing Vitamin C and Omeprazole. More research is needed for exploring the most effective dose and the duration of treatment in humans. In addition, more research is needed for exploring the effect of combination of Vitamin C and Silymarin together in treatment of different types of induced ulcers either in stomach or any other parts of the gastrointestinal tract.

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