Therapeutic Effects of Esomeprazole, Curcumin, Chitosan, and Curcumin-Chitosan Mixture on Ethanol-Induced Gastric Ulcer in Female Rats

Jenan Mahdi Jawad Al-kawaz [1]*, Fakhir Magtoof Al-Zubaidy [2ψ], Hussein Jassim Obaid Al -Harbi [1τ]

Abstract

Introduction: Gastric ulcer (GU) is a prevalent health issue linked to alcohol consumption, smoking, and physiological stress. This study aims to assess the therapeutic effects of esomeprazole (40 mg), curcumin (40 mg/kg), chitosan (150 mg/kg), and a mixture of curcumin (40 mg/kg) and chitosan (150 mg/kg) on ethanol-induced gastric ulcers in female rats. Method: The study involved 30 female rats with an average weight of 190-200gm. Rats were divided into two control groups and four treated groups (esomeprazole, curcumin, chitosan, and mixture) containing 5 rats. Gastric ulcers were induced by orally administering 2 ml of absolute ethanol to All groups, except the negative control, after a 19-hour fasting period. Therapeutic effects were evaluated by assessing gastric juice volume and pH, ulcer index, curative index, and through morphological and histological examination of the stomach. Results: The study revealed a significant decrease ($p \leq 0.05$) in body weight percentage change in the curcumin-treated group compared to the positive control. Esomeprazole, chitosan, and the mixture showed no significant change ($p > 0.05$). Additionally, esomeprazole, curcumin, chitosan, and the mixture demonstrated a significant decrease ($p \leq 0.05$) in ulcer index and gastric juice volume, with a significant increase ($p \leq 0.05$) in pH compared to the positive control. Conclusion: In conclusion, esomeprazole, chitosan, and the mixture demonstrated notable protective and therapeutic effects by reducing ulcer index and gastric juice volume in ethanol-induced gastric ulcers in female rats.

Keywords. Gastric ulcer, Stomach, Curcumin, Esomeprazole, Chitosan, Female rats

Significance | Evaluate the healing effects of esomeprazole, curcumin, chitosan, and a curcumin-chitosan mixture on gastric injuries induced by ethanol in rats.

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Editor Mohammed Khadeer Ahamed Basheer And accepted by the Editorial Board Jan 21, 2024 (received for review Dec 27, 2023)

Introduction

Increasing intragastric pH and reducing daily gastric secretion output are two of the most effective ways to decrease gastric acid secretion. Proton pump inhibitors (PPIs) work by inhibiting the stomach H^+/K^+-ATPase enzyme, also known as the proton pump (Scott *et al.*, 2002; Miner *et al.*, 2003). Clinically available PPIs include pantoprazole, rabeprazole, lansoprazole, omeprazole, and esomeprazole. In addition to previous PPIs, another PPI was added called tenatoprazole has a 5-7 fold longer elimination half-life than previous PPIs (Al-Judaibi *et al.*, 2010).

Esomeprazole, the S-isomer of omeprazole (a racemic mixture of S-and R- optical isomers),is the first proton pump inhibitor to be developed as a single optical isomer, has a better pharmacokinetic profile and provides greater acid suppression than omeprazole (Lind *et al.*,2000).

Curcumin has a long history of administration in the traditional medicine of China, India, and Iran, and it has been used in

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Please cite this article.
Jenan Mahdi Jawad Al-kawaz, Fakhir Magtoof Al-Zubaidy, Hussein Jassim Obaid Al -Harbi, (2024). Therapeutic Effects of Esomeprazole, Curcumin, Chitosan, and Curcumin-Chitosan Mixture on Ethanol-Induced Gastric Ulcer in Female Rats, Journal of Angiotherapy, 8(1), 1-12, 9439

different folks to treat many diseases such as diabetes, liver diseases, rheumatoid diseases, atherosclerosis, infectious diseases, and cancers (Ammon,1991). The turmeric rhizome powder has been used in cookery, medicine, fabric dying, and cosmetics for centuries (Tilak *et al.*,2004). This important spice was introduced to the Western World in the 14th century (Aggarwal *et al.*, 2007), and up to now, it is still in use. In ancient Indian medicine, Ayurveda, a topical agent made of turmeric paste, was used to treat common ocular infections and inflammations. It has also been used in wound dressing in conditions such as bites, burns, and some other skin diseases (Thakur *et al.*, 1989), a curcumin poultice has been applied to the perineal area to improve the healing process of any birth canal lacerations, Powdered turmeric has been consumed with hot milk for cure of cough and related respiratory problems (Pandeya and Wives,2005) Roasted turmeric has also been administered as an anti-dysenteric agent. This ancient medicine has also been used to treat other digestive disorders, such as indigestion, dyspepsia, flatulence, and gastric and duodenal ulcers (Noorafshan and Ashkani-Esfahani,2013). It has also been used for Alleviating the hallucination states induced by some opioids and psychotropic drugs (Tilak *et al.*,2004).

Chitosan comprises a linear polysaccharide consisting of β-(1-4)-linked glucosamine and N-acetyl-d-glucosamine.It is prepared by alkaline deacetylation of chitin, which is present in the exoskeleton of marine crustaceans and insects and in the walls of most fungi and some algae (Ma *et al.*,2017). It is considered a natural most promising bio-polymer for future applications, which included the GRAS (Generally Recognized as Safe) category by the FDA, also characterized by its excellent biodegradability, biocompatibility, antimicrobial activity, non-toxicity, and economic advantages (Ahmed *et al.*,2014). It exhibits bacteriostatic or bactericidal effects against many microorganisms, so it possesses numerous technological and physiological properties useful in foods (Devlieghere *et al.*, 2004).

However, we aimed to evaluate the therapeutic effects of esomeprazole, curcumin, chitosan, and a curcumin-chitosan mixture on gastric ulcers induced by ethanol in rats.

Materials and Methods

Animals

The experimental animals utilized in this investigation were 30 adult female rats with an average weight of (190-200 gm) gathered from various sites within the province of Babylon. They were kept in specially designed rat cages with adequate cleanliness. The animals were given unlimited access to food and water. Before the trial began, the rats had around two weeks to adapt to the new environment.

The study was conducted following the ethical principles of the Declaration of Helsinki. A local ethics committee approved the study protocol according to document 7-17-7922 on November 3-2022 to get this approval.

Drug and Chemicals

Ajanta Pharma (India) manufactured the esomeprazole drug in this study. Esomeprazole has been obtained from the local pharmacy in Hilla-Iraq; each tablet contains 40 mg. The tablets were pink and oval. Determination of drug doses depended on the animal's body weight (Shin *et al.*,2010). Curcumin (98%) was purchased from Macklin Company (China). The Chitosan sample was obtained from Beijing company (china). All other chemicals were bought from local commercial suppliers.

Preparation of a Chitosan-curcumin mixture

Slowly adding the 150 mg of chitosan dissolved in 10 ml of 0.1M acetic acid to the 40 mg of curcumin, the mixture was then triturated to produce a uniformly yellow-colored liquid (Kaudkaew *et al.*,2021).

Study design

Animals were randomly divided into six study groups: two control groups and four treated groups (5 rats of each) as follows:

Group 1 (Distilled water-negative control group): All rats in this group received D.W. (2 ml/rat) by oral gavage during the experimental period.

Group 2 (Ethanol -positive control group): All rats in this group received ethanol (2ml/rat) by oral gavage in double dose after fasting for (19 hours) before administration of each ethanol dose.

Group 3 (Esomeprazole group): All rats in this group received ethanol (2ml/rat) by oral gavage in double dose after fasting for (19 hours) before administration of each ethanol dose. After one hour of the last dose, esomeprazole was administrated to the animals in a single oral daily dose of (40 mg/70kg) dissolved in 2 ml D.W. for 5 successive days.

Group 4 (Curcumin group): All rats in this group received ethanol (2ml/rat) by oral gavage in double dose after fasting for (19 hours) before administration of each ethanol dose. After one hour of the last dose, curcumin was administered to the animals in a single oral daily dose of (40 mg/kg) dissolved in 2 ml 0.06% (0.1 M) acetic acid for 5 successive days.

Group 5 (Chitosan group): All rats in this group received ethanol (2ml/rat) by oral gavage in double dose after fasting for (19 hours) before administration of each ethanol dose. After one hour of the last dose, chitosan was administered to the animals in a single oral daily dose of (150 mg/kg) dissolved in 2 ml 0.06% (0.1 M) acetic acid for 5 successive days.

Group 6 (Mixture group): All rats in this group received ethanol (2ml/rat) by oral gavage in double dose after fasting for (19 hours) before administration of each ethanol dose. After one hour of last

dose, mixture was administered to the animals in a single oral daily dose for 5 successive days.

Measurement of animal body weight

The body weight of the rats was measured on the day of commencement of treatment and the 30th day using an electronic weighing balance. The body weights of the rats were calculated using the following formula (Obasi *et al.*,2019):

$$\%\Delta \text{b.w.} = \frac{(b.w.AT) - (b.w.BT)}{b.w.BT} \times 100$$

Where:

%Δb.w.=Percentage change in body weight

b.w.AT=Body weight after treatment on day 30.

b.w.BT=Body weight before treatment on day 0

Measurement of stomach weight

The stomach weight of the animal was calculated after it was isolated and separated from the abdominal cavity by using electrical balance and estimated the relative weight according to the following equation (Mohammad et al., 2021):

$$\text{Relative weight of organ} = \frac{organ\ weight\ (gm)}{Final\ body\ weight(gm)} \times 10$$

Measurement of gastric ulcer index

The gastric ulcer index was estimated according to the method described by Mohammad et al. (2021). Each gastric cavity was scrutinized wholly, and the degree of ulceration was graded as follows: 0: no lesions (normal stomach), 0.5: hyperemia (red coloration),1: hemorrhagic spots,2: 1-5 small ulcers, 3: many smAll ulcers, 4: many small and large ulcers, 6: stomach full of ulcers with perforations

The protective index (PI) was calculated using the following formula:

$$PI = \frac{ulcer\ model - ulcer\ treated}{ulcer\ model} \times 100$$

Where:

PI: protective index

ulcer model: ulcer index of the ulcer control group.

Ulcer treated: ulcer index of treatment group.

Measurement of pH and volume of the gastric juice

The euthanized animal stomach was immediately dissected, and the gastric content was collected in sterilized tubes; the pH value of gastric juice was determined by pH paper and then centrifuged for 10 min at 3000 rpm to isolate the aqueous phase. The volume of centrifuged gastric juice was measured by a graduated cylinder and expressed as ml (Dokmeci *et al.*, 2005).

Histopathological study and morphometry

The rat stomachs were removed from the abdominal cavity, fixed in 10% formalin, then dehydrated in ascending grades of alcohol, cleared in xylene, and embedded in paraffin. Serial sections of 5µm thick were cut and stained with hematoxylin and eosin (Bancroft *et al.*, 2013) for histopathological examination. Slides were examined and photographed using a digital microscope camera. Multiple measurements of the mucosa layer's thickness were done using Image J analysis software (NIH, USA). We took tissue samples from All animals in the study and measured 10 sections per tissue sample (Karasov *et al.*, 2004).

Statistical analysis

Statistical Package for Social Science (SPSS) version 23.0 (SPSS, Chicago, USA) was used to analyze the data. Data was given in the form of arithmetical mean values and standard error. One-way analysis of variance (ANOVA) was performed. The means were separated using the Duncan Multiple Test. The level of significance was accepted under($P \leq 0.05$).

Results

Mortality

The mortality rate in experimental groups is shown in Table (1). The highest mortality was observed in treated groups with ethanol (positive control group), esomeprazole, and chitosan (40%). The lowest mortality was found in the distilled water (negative control group) and in treated groups with curcumin and mixture (0%).

The therapeutic effect of esomeprazole, curcumin, Chitosan, and curcumin-chitosan mixture on body weight after 5 days of treatments in female rats

The results in Table (2) revealed that the percentage change in body weight in female rats significant increase ($P \leq 0.05$) in treated groups with esomeprazole drug (1.074±0.351), chitosan (1.171±-.335) and mixture (1.329±0.281) as compared withpositive control group (-2.026±0.345). While the results showed non-significant changes ($p>0.05$) in this groups as compared with the negative control group (2.055±0.488).

The results of this study showed non- significant change ($p>0.05$) in the percentage change of body weight for treated group curcumin (-1.702±0.350), as compared with the positive control group (-2.026±0.345), but there is significant decrease ($p \leq 0.05$) as compared with the negative control group (2.055±0.488).

A significant decrease($0 \leq 0.05$) was observedin the percentage change in body weight in the treated groupwith curcumin (-1.702±0.350) compared to treated groups with esomeprazole (1.074±0.351), chitosan (1.171±0.335) and mixture (1.329±0.281).

The present study showed that there is no significant change ($p>0.05$) in the percentage change of body weight in the treated group with chitosan (1.71±0.335) compared to treated groups with esomeprazole and mixture.

Therapeutic effect of esomeprazole , curcumin. Chitosan and curcumin-chitosan mixture on stomach weight after 5 days of treatment in female rats.

Results of this study showed that the relative weight of the stomach changed non-significantly (p>0.05) in all treated groups compared to control groups. The relative weight of the stomach in all the treated groups was found to show no significant change (p>0.05) when compared between them (Table 3).

The therapeutic effect of esomeprazole, curcumin, chitosan, and curcumin–chitosan mixture on gastric ulcer index after 5 days of treatment in female rats

The present study showed that there is a significant increase (p≤0.05) in the gastric ulcer index in the four treated groups, esomeprazole , curcumin , chitosan and mixture after 5 days from treatment (the 8th day after ulcer induction) as compared with the positive control group,but the ulcer index was found to show no significant change (p>0.05) as compared with negative control group, also no significant change was observed in the gastric ulcer index in the four treated groups as compared between them (Table 4).

The therapeutic effect of esomeprazole, curcumin, chitosan and curcumin–chitosan mixture on volume and pH of gastric juice after 5 days of treatment in female rats

The results of this study showed that the volume of the gastric juice not changed significantly (p>0.05) in treated groups with esomeprazole, curcumin, chitosan, and mixture after 5 days from treatment, as compared with the control groups, and also no significant change (p>0.05) was observed in the volume of the gastric juice in All the treated groups as compared between them (Table 5).

As shown in Table (6), non - significant change (p>0.05) was observed in pH of the gastric juice in treatment groups esomeprazole, curcumin, chitosan (5.333 ± 0.333, 5.2000 ± 0.2000, 5.000 ± 0.5774) respectively, as compared with control groups (5.400 ± 0.245 and 4.667 ± 0.333). While the pH of the gastric juice in the treated group with the mixture was observed to show a significant increase (p≤0.05)(5.8000 ± 0.2000) as compared with the positive control group (4.667 ± 0.333). The results revealed non-significant differences (p>0.05) in All treated groups compared to them.

Macroscopic examination of the stomach

Oral administration of two doses from absolute ethanol (2 ml/rat) for 8 days after the start of the experiment increased the mortality rate to 40% of the total rats (Table 1). Only a few and small hemorrhage lesions were observed in 2 rat of 3 rats (66.667%), and ulcer index and curation were (0.667±0.333 and 0%), respectively (Table 4) (Figure 1- 2).

The stomach obtained from esomeprazole 40mg, curcumin 40mg/kg, chitosan 150mg/kg, and mixture (curcumin40mg/kg-chitosan 150mg/kg) treated groups showed normal external morphology and intact gastric mucosa without hemorrhage, with ulcer index and curation (0.00±0.00 and 100%) respectively, as distilled water negative control group (Table 4) (Figure 1- 3,4,5,6).

Microscopic examination of the stomach

The histological sections from the stomach of the rat in the ethanol-treated group showed normal epithelial lining of gastric mucosa, and there were no significant pathological changes as in the negative control group (Figure 2A), While there were histological sections of another rat in the same group showed hemorrhagic mucosal erosions with little necrosis of gastric mucosa (Figure 2B)

The histological sections of the stomach of rats in the esomeprazole 40mg/kg, curcumin 150mg/kg, chitosan 150mg/kg, and mixture (curcumin 40mg/kg-chitosan 150mg/kg) treated groups showed no clear lesion and disruption of mucosa (Figure 2 - 3,4,5,6) as in the negative control group (Figure 2).

Morphometric study

Mucosa thickness of the stomach

As shown in Table (7), a significant decrease (p≤0.05) was observed in the mucosa thickness of the stomach in the ethanol group (positive control group) (60.0214μm±2.16835) as compared with the distilled water group (negative control group) (68.0178μm±2.27421).

The thickness of mucosa in the esomeprazole, curcumin, and mixture groups (70.2747μm±1.19469, 67.5267μm±2.24844 and 69.4963μm±2.11946), respectively was found to show no significant change (p>0.05) as compared with the distilled water group (68.0178μm ± 2.27421), but there was a significant increase (p≤0.05) as compared with ethanol group (60.0214μm ± 2.16835).

The mucosa thickness of the stomach in the chitosan group (64.5199μm±0.91793) was observed to show no significant change (p>0.05) as compared with the distilled water group (negative control group)(68.0178μm±2.27421) and ethanol group (positive control group) (60.0214μm±2.16835).

The mucosa thickness in all the treatment groups was found to show no significant change (p>0.05) when compared between them.

Number and depth of the GUs

As shown in Table (8), a significant increase (p≤0.05) was observed in the number and depth of the GUs in the ethanol group (positive control group) (0.6273±0.3151 and 11.4513μm±5.8348), respectively as compared with the distilled water group (negative control group) (0.00±0.0000 and 0.00μm±0.0000).

The present study results showed that All treatment groups showed a significant decrease (p≤0.05) in the number and depth of the GUs when compared with the ethanol group (positive control

Table 1. Mortality in experimental groups of female rats after 5 days of treatment with esomeprazole, curcumin, chitosan and mixture

Groups	Number of rats before treatment (n)	Mortality (n)	Mortality (%)
Distilled water (Negative control)	5	0	0
Ethanol (Positive control)	5	2	40
esomeprazole 40mg	5	2	40
Curcumin 40 mg/kg	5	0	0
Chitosan 150mg/kg	5	2	40
Mixture (curcumin40mg/kg-chitosan 50mg/kg)	5	0	0

Table 2. Therapeutic effect of esomeprazole, curcumin, chitosan and curcumin-chitosan mixture on Percentage change in body weight after 5 days of treatments in female rats.

Groups	Initial body weight (gm) Mean±S.E	Final body weight (gm) Mean±S.E	Percentage change in body weight % Mean±S.E.
Distilled water (Negative control group)	195.600±2.839* a	199.600±2.713 * a	2.055±0.488* b
Ethanol (Positive control group)	193.400±8.091* a	188.667±13.421 ** a	-2.026±0.345** a
Esomeprazole 40 mg	190.200±7.559* a	192.000±10.583** a	1.074±0.351** b
Curcumin 40 mg/kg	200.200±2.354* a	196.800±2.577* a	-1.702±0.350* a
Chitosan 150 mg/kg	201.600±2.542* a	201.667±2.729** a	1.171±0.335** b
Mixture (curcumin 40 mg/kg - chitosan150 mg/kg)	198.600±3.855* a	201.200±3.426* a	1.329±0.281* b

Different letteres in same column indicated significant (p≤0.05) among groups. *n=5 , **n=3

Table 3. Therapeutic effect of esomeprazole, curcumin, chitosan, and curcumin-chitosan mixture on stomach weight after 5 days of treatments in female rats

Groups	Relative weight of stomach % Mean±S.E.
Distilled water (Negative control group)	1.046±0.072* a
Ethanol (Positive control group)	0.940±0.1499** a
Esomeprazole 40mg	1.005±0.194** a
Curcumin 40mg/kg	1.162±0.074* a
Chitosan 150mg/kg	1.011±0.358** a
Mixture(curcumin 40 mg/kg + chitosan 150mg/kg)	1.022±0.005* a

Different letters indicated significant(p≤0.05) among groups. *n=5,**n=3

Table 4. Therapeutic effect of esomeprazole, curcumin, chitosan and curcumin-chitosan mixture on gastric ulcer index after 5 days of treatments in female rats. Different letters indicated significant($p \leq 0.05$) among groups. *n=5,**n=3

Groups	Ulcerated rats %	Ulcer index Mean±S.E.	Curation %
Distilled water (Negative control group)	0	0.000±0.00* a	100
Ethanol (Positive control group)	66.667	0.667±0.333** b	0
Esomeprazole 40mg	0	0.000±0.000** a	100
Curcumin 40mg/kg	0	0.000±0.000* a	100
Chitosan 150mg/kg	0	0.00±0.00** a	100
Mixture(curcumin 40mg/kg -chitosan 150mg/kg)	0	0.00±0.00* a	100

Table 5. Therapeutic effect of esomeprazole, curcumin, chitosan and curcumin-chitosan mixture on volume of gastric juice after 5 days of treatments in female rats. Different letters indicated significant($p \leq 0.05$) among groups. *n=5,**n=3

Groups	Volume of gastric juice (ml) Mean±S.E.
Distilled water (Negative control group)	1.300±0.200* a
Ethanol (Positive control group)	1.8333±0.333** a
Esomeprazole 40 mg	1.333±0.441** a
Curcumin 40mg/kg	1.800±0.2000* a
Chitosan 150mg/kg	1.500±0.2887** a
Mixture(curcumin 40mg/kg -chitosan 150mg/kg)	1.100±0.100* a

Table 6. Therapeutic effect of esomeprazole , curcumin , chitosan and curcumin-chitosan mixture on pH of gastric juice after 5 days of treatments in female rats. Different letters indicated significant($p \leq 0.05$) among groups. *n=5,**n=3

Groups	pH of gastric juice, Mean ± S.E.
Distilled water (Negative control group)	5.400±0.245* ab
Ethanol (Positive control group)	4.667±0.333** a
esomeprazole 40 mg	5.333±0.333** ab
Curcumin 40mg/kg	5.2000±0.2000* ab
Chitosan 150mg/kg	5.000±0.5774** ab
Mixture(curcumin 40mg/kg -chitosan 150mg/kg)	5.8000±0.2000* b

Table 7. Therapeutic effect of esomeprazole , curcumin , chitosan and curcumin-chitosan mixture on mucosa thickness after 5 days of treatments in female rats. Different letters indicated significant($p \leq 0.05$) among groups. *n=5,**n=3

Groups	Mucosa thickness (µm), Mean±S.E.
Distilled water (Negative control group)	68.0178±2.27421* b
Ethanol (Positive control group)	60.0214±2.16835** a
Esomeprazole 40mg	70.2747±1.19469** b
Curcumin 40mg/kg	67.5267±2.24844* b
Chitosan 150mg/kg	64.5199±0.91793** ab
Mixture (curcumin 40 mg/kg + chitosan 150mg/kg)	69.4963±2.11946* b

Table 8. Therapeutic effect of esomeprazole , curcumin , chitosan and curcumin-chitosan mixture on number and depth of gastric ulcer after 5 days of treatments in female rats. Different letters indicated significant($p \leq 0.05$) among groups. *n=5,**n=3

Groups	Number of gastric ulcer Mean±S.E.	Depth of gastric ulcer (µm), Mean±S.E.
Distilled water (Negative control group)	0.00±0.0000* a	0.00±0.00* a
Ethanol (Positive control group)	0.6273±0.3151** b	11.4513±5.8348** b
Esomeprazole 40mg	0.00±0.0000** a	0.00±0.00** a
Curcumin 40mg/kg	0.00±0.0000* a	0.00±0.00* a
Chitosan 150mg/kg	0.00±0.0000** a	0.00±0.00** a
Mixture (curcumin 40mg /kg - chitosan 150mg/kg)	0.00±0.0000* a	0.00±0.00* a

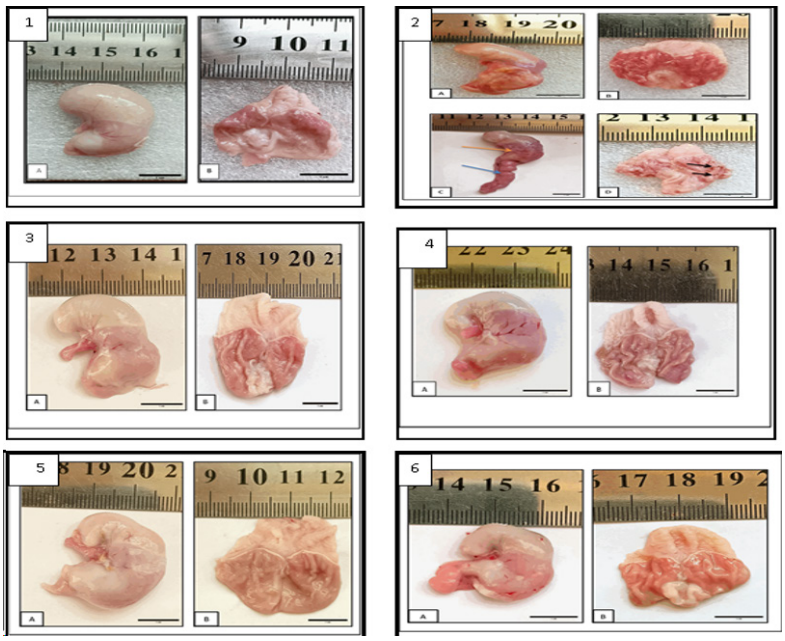

Figure 1. Morphological examination of the stomach after 5 days of treatment in female rats. (1): stomach in distilled water group (negative control group). A: Normal external morphology. B: Internal morphology shows intact gastric mucosa. (2): stomach in ethanol-treated group (Positive control group) after 8 days from treatment. A: Normal external morphology. B: Normal internal morphology. C: Normal external morphology showing the stomach (orange arrow), and duodenum(blue arrow). D: Internal morphology showing few and small hemorrhage lesions in the gastric mucosa(black arrows).(3): Stomach in esomeprazole 40mg treated group after 5 days from treatment A: Normal external morphology. B: Normal internal morphology.(4): Stomach of curcumin 40mg treated group after 5 days from treatment A: Normal external morphology. B: Normal internal morphology.(5): Stomach of chitosan 150mg/kg treated group after 5 days from treatment A: Normal external morphology. B: Normal internal morphology.(6): Stomach of mixture (curcumin 40mg/kg - chitosan 150mg/kg) treated group after 5 days from treatment A: Normal external morphology. B: Normal internal morphology.

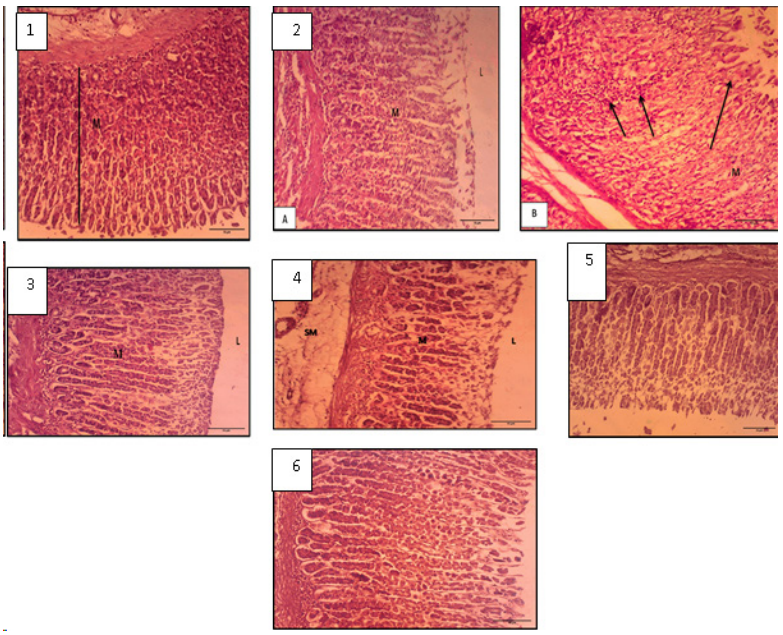

Figure 2. Histological examination of the stomach after 5 days of the treatment in the female rats . (1): Cross sections in the stomach of rat in distilled water group (negative control group) showing no clear lesions M: Mucosa (H&E,100X), (2): Cross sections in the stomach of rat in ethanol treated group (positive control group) after 5 days from last dose showing (A): no clear lesions, (B): mucosal ulceration (black arrows) M: Mucosa, L: Lumen (H&E,100X), (3): Cross section in stomach of rat in esomeprazole 40mg- treated group showing intact gastric mucosa (M), lumen (L) (H&E,100x), (4): Cross section in stomach of rat in curcumin 40mg/kg- treated group showing intact gastric mucosa (M), lumen (L), submucosa (SM) (H&E,100x).(5): Cross section in the stomach of rat in chitosan 40mg/kg treated group showing intact gastric mucosa (M), lumen (L) (H&E,100x),(6): Cross section in stomach of rat in the mixture-treated group showing intact gastric mucosa (M), Lumen (L)(H&E,100x).

group), but no significant change ($p>0.05$) as compared with the distilled water group (negative control group).

The number and depth of the GUs in All treatment groups were found to show no significant change ($p>0.05$) as compared between them.

Discussion

Alcohol-related disease is a major cause of mortality, disability, and social disruption worldwide (Palmese *et al*., 2023).

Ethanol is known to have a variety of effects on immune function, including decreased lymphocyte responsiveness to mitogen, decreased neutrophil chemotactic and phagocytic functions, and alteration of cytokine production by lymphocytes and macrophages (Yu & Chaudry, 2009; Castro *et al*., 2013).

In addition, it is well known that ethanol produces a wide range of effects in the body due to its action on the central nervous system (CNS) or peripheral organs (Tabakoff *et al*.,1996). In humans, ethanol tends to lower body temperature, an effect that depends on different factors such as the dose, clothing or physical activity (Kalant & Le, 1984). Generally, ethanol administered by the peripheral route or intracerebroventricularly (*icv*) induces a rapid body temperature in laboratory animals, monitored at normal ambient or lower temperatures (Mizinga *et al*.,1995). Ethanol affects CNS function by several different mechanisms, such as the induction of primary perturbations of neural membranes, reduction of calcium influx through voltage-sensitive calcium channels (Wang *et al*.,1991), alteration in the release of the neurotransmitters dopamine (Morikawa & Morrisett, 2010), acetylcholine (Aistrup *et al*.,1999), norepinephrine (Shefner & Tabakoff,1985), serotonin (Tabakoff *et al*.,1977), and alteration in neurotransmission mediated by inhibitory and excitatory amino acids (Tabakoff & Hoffman, 1991). Hypothermia induced by ethanol has been related, at least partially, to neurotransmission mediated by monoamines (French & Weiner, 1991) and to cellular Ca^{2+} (Paez & Myers, 1989), and may be modulated by other substances such as PGs (Morato *et al*.,1986).

On the other hand, ethanol is toxic to vital organs, producing harmful effects on resistant tissues, such as bones. Therefore, alcohol consumption is prejudicial to bone tissue integrity, and hence, it can make it difficult bone repair after traumatic injuries (Garcia *et al*., 2015).

Also, ethanol induces several deleterious metabolic changes in the liver. Its excessive use for a long time leads to the development of steatosis, alcoholic hepatitis, and cirrhosis, resulting in weight and volume changes (Kumar *et al*., 2003; Rehman *et al*., 2011). At least 80% of heavy drinkers had been reported to develop steatosis, 10-35% alcoholic hepatitis, and approximately 10% liver cirrhosis (Saravanan & Nalini, 2007). Recent studies in animal models suggest that liver injury in chronic alcoholics is due to oxidative stress that leads to fibrosis, impaired liver functions, and increased apoptosis (Ronis *et al*., 2004). Hence, the death and loss of rats in therapeutic experiments after the administration of ethanol in ulcer, esomeprazole, and chitosan groups may be attributed to its effects, as we mentioned previously.

The present study showed that the ethanol-induced GU-treated group significantly decreased the whole body weight compared with the negative control group. This result was accepted for the ethanol group because it is well-known that ethanol causes decreased food intake, which leads to weight loss (Aguiar *et al*., 2004).

This result is consistent with another study that showed decreased body weight in ethanol rats (Piano *et al*., 2001).

Results showed a significant decrease ($p\leq0.05$) in percentage change of body weight in curcumin rats compared to normal control rats. This result agrees with the results obtained by Koboziev *et al.* (2020), who revealed that mice treated with curcumin decreased body weight, which may be explained by the modified serum lipid profile in mice and insulin sensitivity, as mentioned previously in the protective experiment.

In addition, the results showed non-significant differences in percentage change of body weight in esomeprazole, chitosan, and mixture groups after treated for 5 days as compared with the normal group. This may indicate that neither esomeprazole, chitosan, or mixture adversely affected body weight.

The data shown in Table (4-10) demonstrated a significant increase ($p\leq0.05$) in the relative weight of the stomach in ulcer rats treated with two doses of ethanol, and these results are consistent with Rahman *et al.* (2020) who revealed that ulcer animals treated with ethanol showed an increase in stomach weight and stomach coefficient which may be explained by hemorrhage, edema, necrosis, and inflammation.

In All treatment study groups in this experiment, the relative weight of the stomach was not significantly changed after treatment for 5 days with esomeprazole, curcumin, chitosan, and mixture as compared with control groups. This enhancement may reflect the ability of these materials to repair the physiological and histological defect that causes this organ weight change, as seen in these results.

Table (4-11) shows that the ulcer index of the stomach in esomeprazole, curcumin, chitosan, and mixture groups decreased significantly (0.000 ± 0.000) as compared to the positive control group (0.667 ± 0.333). As conclusion, treatment of ethanol rats with these materials for 5 days showed significant enhancement in ulcer index and could repair the defect that caused the change in the stomach, which reflected in enhancement of their histological structure as confirmed by macroscopic and microscopic examination.

We can link these results with other studies, the role of *Bryophyllum pinnatum* leaf extract in healing acetic acid-induced chronic GU model in rats (Araugo *et al*., 2021) while Kuadkaew *et al*.(2021) observed the biological effects of the curcumin-chitosan mixture in treating indomethacin-induced acute GU and indicated that a mixture possessed the potent multi-target antioxidant, anti-inflammatory, gastroprotective and ulcer healing promoting actions attributed to curcumin and chitosan containing in the mixture. Also, Ibrahim *et al*. evaluated in 2019 the curative effects of curcumin on GU induced by piroxicam in albino rats by inhibiting lipid peroxidation and TNF-α alongside with activating of antioxidants enzymes like SOD, CAT and GPX.

All study groups showed non-significant differences in the volume of gastric juice, which was still within the normal range compared to the normal control group. This result is in agreement with Ibrahim *et al*. (2019), who revealed that the piroxicam-induced ulcer model in rats showed non-significant changes in the gastric juice volume.

The data in Table (4-13) demonstrated a significantly higher pH of gastric juice in ethanol animals after daily oral administration of (curcumin-chitosan) mixture for 5 days than the positive control animals, leading to complete suppression of histological changes in the gastric mucosa. But, the effect of the two compounds in the mixture was more than that with either of the compounds alone. This effect of the mixture may be because containing antioxidant compounds that reduce ROS formation in the stomach induced by ethanol and enhance the defense antioxidant mechanism against ROS production in GU. The result of the significant increase in pH of gastric juice in a mixture of animals is consistent with the previous study (Kuadkaew *et al*., 2021).

This study's macroscopic observation in ethanol control showed few small hemorrhage lesions with ulcer index (0.667±0.333) and curation (0%). A probable explanation may be related to oxidative stress resulting from ethanol decreasing the antioxidant levels and increasing ROS (Bhattacharyya *et al*., 2023).

The results observed that daily intake of esomeprazole, curcumin, chitosan, and mixture for 5 days to ethanol rats showed no significant differences in the means of ulcer index (0.000±0.000) compared to negative control (0.000±0.000) and the curation was (100)% as we mentioned earlier which were confirmed by microscopical examination.

The present study reveals the efficacy of esomeprazole (40mg/70kg), curcumin (40 mg/kg), chitosan (150mg/kg), and (curcumin 40mg/kg-chitosan 150 mg/kg) mixture in healing ethanol-induced GU model occurs after 5 days from treatment, which may be attributed to its antioxidant effects. Previous study confirms that the action of lupeol stearate (1 mg/kg) and omeprazole (20mg/kg) in healing acetic acid-induced GU model occurs after 7 days from treatment, whereas ranitidine (100 mg/kg) after ten days (Somensi *et al*., 2022).

In the experimental model of GU, it was observed that pentoxifylline administration for 14 days or the first 7 days, but not the last 7 days, of the 14-day treatment period, accelerated healing of aceticacid–induced GU in rats by reduced TNF-α concentration and myeloperoxidase MPO activity in ulcer tissue (Shimizu *et al*., 2000).

Based on our morphometric measurements, the changes in the thickness of gastric mucosa included a significant decrease in ulcer control group compared to the normal control with a non-significant change in esomeprazole, curcumin, chitosan and (curcumin-chitosan) mixture. This may mean that the ethanol may affect mucosa thickness when given to normal animals as reflected in histological structure in the stomach which is similar to the results obtained by Hajrezaie *et al*. (2015), but when esomeprazole, curcumin, chitosan and (curcumin-chitosan) mixture were given to ulcer rats, they could increase the thickness causing non-significant differences in treated groups for 5 days as compared to normal control.

Also, the morphometric study revealed an increase in the number and depth of GU in ethanol control rats. This increase may be related to the effects of ethanol as we mentioned previously that, ethanol exposure produced gastric lesions by penetrating and digesting the gastric wAll due to its proteolytic and hydrolytic action as well as by endothelial cell damage due to reduction in blood circulation (Adinortey *et al*., 2013; Rahman *et al*., 2020).

Conclusion

The mixture of curcumin (40 mg) and chitosan (150 mg) was more effective in the treatment of ethanol-induced acute gastric ulcers in rats than esomeprazole (PPI), a common antiulcer drug. It was discovered that a curcumin-chitosan mixture has antioxidant effects. Curcumin and chitosan in the mixture may be responsible for these therapeutical properties.

Author contribution

J.M.J.A., F.M.A., H.J.O.A. conceived and designed the analysis and collecting the data, performed the analysis and wrote the Paper.

Acknowledgment

The author would like to thank the University of Babylon, College of Science.

Competing financial interests

The authors have no conflict of interest.

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