Bull. Pharm. Sci., Assiut University, Vol. 47, Issue 2, 2024, pp. 1235-1249.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/

ANTIOXIDANT EFFECTS OF ESOMEPRAZOLE, CURCUMIN, CHITOSAN AND CURCUMIN-CHITOSAN MIXTURE ON GASTRIC ULCER IN FEMALE RATS : EXPERIMENTAL STUDY

Jenan Mahdi Jawad Al-kawaz [1*] , Hussein Jasim Obaid Al-Harbi[1], Fakir Magtoof Al-Zubaidy[2]

[1]*Department of Biology, College of Science, University of Babylon, Iraq*
[2]*College of Pharmacy, University of Babylon, Iraq*

Gastric ulcer (GU) is the most common health concern that occurs due to alcohol consumption, smoking and physiological stress. Ethanol-induced GU in animal model resembles the pathophysiology of human ulcer. The aim of this research was to examine the antioxidant properties of esomeprazole, curcumin, chitosan, and a mixture from curcumin and chitosan on gastric ulcers produced by ethanol in rats. The research included 60 animal, with an average weight between (179.60 - 180.30) gm. The rats were divided into a control group and five treated groups, each containing 10 rats. The treated groups received 0.1M acetic acid, esomeprazole, curcumin, chitosan, and curcumin – chitosan mixture. All groups were treated for a duration of 30 days. Following the conclusion of the experiment, the body was weighed both before to and subsequent to its completion. In order to develop stomach ulcers, a dose of 2 ml of absolute ethanol was administered orally to all groups, with the exception of the negative control group, after a 20-hour fasting interval. Subsequently, all the animals were euthanized 5 hours later. The study of gastric ulceration included a comparison of the amount and pH of gastric juice, ulcer index, protective index, and macroscopic inspection of the stomach. The research used gastric tissue homogenate to measure several parameters, including the quantity of MDA, the activity of SOD, and the concentration of NO. The findings of the research demonstrated reduction in the percentage change in body weight in the groups treated with curcumin, chitosan, and the combination, compared to the control groups. There was no significant difference ($p>0.05$) seen in the case of esomeprazole when compared to the control groups. Furthermore, the findings demonstrated reduction in the ulcer index and volume of gastric juice in the esomeprazole, curcumin, chitosan, and mixture groups compared to the positive control group. In the case of pH, there was rise. A substantial reduction was detected in the concentration of MDA in all treatment groups compared to the positive control group. The activity of SOD exhibited a significant increase ($p\leq0.05$). The analysis of gastric ulcers was conducted by comparing the histological examination of the stomach. However, the group treated with the mixture + ethanol did not exhibit any significant disruption in the epithelium of the mucosa.

***Key words**: Gastric ulcer, esomeprazole, curcumin, chitosan, rat*

INTRODUCTION

Oxidative stress refers to the harm caused to tissues due to an imbalance between the creation of reactive oxygen species (ROS) and the body's ability to counteract their damaging effects via antioxidant defense systems[1]. Oxidative stress has been implicated in the development of many disorders, including stomach ulcer[2]. The process of lipid peroxidation, which is facilitated by oxygen free radicals, is considered a significant factor in the damage to cell membranes[3].

Normal healthy cells include antioxidant enzymes, such as SOD, CAT, and GST, which operate as the first defense against oxidative damage. These enzymes break down O2 -• and H2O2 before they can combine and generate more reactive radicals[4]. Antioxidants have the ability to prevent lipid peroxidation and

Received : 27/5/2024 & Accepted : 27/7/2024

*Corresponding author: Jenan Mahdi Jawad Al-kawaz, E-mail: sci.jinan.mhadi@uobabylon.edu.iq

eliminate free radicals. Therefore, it is necessary to create medications that possess antioxidant properties and can effectively eliminate these harmful free radicals, resulting in positive benefits on stomach ulcers[5].

Curcumin is the principal component found in the herbal medicine and nutritional spice known as turmeric. Curcumin has a rich historical background in the traditional medical practices of China, India, and Iran. It has been used in many cultures to treat a wide range of disorders including diabetes, liver diseases, rheumatic diseases, atherosclerosis, infectious diseases, and malignancies[6]. Turmeric rhizome powder has been used for ages in several fields such as cuisine, medicinal, fabric dyeing, and cosmetics[7]. This significant spice was first brought to the Western World in the 14th century and continues to be used to this day [8]. In Ayurveda, an ancient Indian medicine, a paste made of turmeric has been traditionally used as a topical treatment for common eye infections and inflammations[9]. It has also been applied to wounds, such as bites, burns, and certain skin diseases, for dressing purposes. Additionally, a poultice containing curcumin, a compound found in turmeric, has been used on the perineal area to enhance the healing of lacerations in the birth canal[10]. According to Pandeya and Wives[11], powdered turmeric has been ingested with hot milk as a remedy for cough and associated respiratory issues. Additionally, roasted turmeric has been used as a remedy for dysentery. In addition to its historical usage, this traditional remedy has also been used to treat several gastrointestinal ailments, including indigestion, dyspepsia, flatulence, and gastric and duodenal ulcers[12]. Additionally, it has been used to mitigate the hallucinatory effects caused by some opioids and psychotropic medicines[13].

The aim of this research was to evaluate the antioxidant properties of esomeprazole, curcumin, chitosan, and a curcumin-chitosan mixture on experimentally produced stomach ulcer by ethanol in female rats.

MATERIALS AND METHODS

Experimental Animals

The experimental animals utilized in this investigation were 30 adult female rats with an average weight of 179.60 - 180.30 gm (5-weeks-of-age) that were 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 become used to the new environment.

Drug and Chemicals

Esomeprazole drug used in this study, which manufactured by Ajanta pharma company (India). Esomeprazole has been obtained from the local pharmacy in Hilla-Iraq, each tablet contains 40 mg. The tablets were pink, oval. Determination of drug doses was depended on the animals body weight [14]. Curcumin (98%) was purchased from Macklin company (China). Chitosan sample was obtained from Beijing company (China). All other chemicals were bought from Theera trading Co., LTD., Thailand.

Preparation of (Curcumin-Chitosan) 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[15].

Experimental design

The animals were separated into six groups: one group was a control, while the other five groups were treated (each consisting of 10 rats).

Control group

given distilled water 2 ml orally through stomach tube for 30 days , then subdivided into subgroup (1) sacrificed (n=5), and subgroup (2) sacrificed after 19 hours from fasting with access to water then 2 ml orally ethanol given for five hours (n=5).

Acetic acid group

given 2 ml of 0.1M (0.06%) acetic acid orally through stomach tube for 30 days , then subdivided into subgroup (1) sacrificed (n=5), and subgroup (2) sacrificed after 19 hours from fasting with access to water then 2 ml orally ethanol given for five hours (n=5).

Esomeprazole treated group

given esomeprazole (3.54 mg/kg) orally through stomach tube for 30 days, then

subdivided into subgroup (1) sacrificed (n=5), and subgroup (2) sacrificed after 19 hours from fasting with access to water then 2 ml orally ethanol given for five hours (n=5).

Curcumin treated group

given curcumin (40 mg/kg) orally through stomach tube for 30 days , then subdivided into subgroup(1)sacrificed (n=5), and subgroup (2) sacrificed after 19 hours from fasting with access to water then 2 ml orally ethanol given for five hours (n=5).

Chitosan treated group

given Chitosan (150 mg/kg) orally through stomach tube for 30 days, then subdivided into subgroup (1) sacrificed (n=5), and subgroup (2) sacrificed after 19 hours from fasting with access to water then 2 ml orally ethanol given for five hours (n=5).

Curcumin-Chitosan mixture treated group

given Curcumin-Chitosan combination (containing 40 mg/kg of curcumin and 150 mg/kg of chitosan) orally through stomach tube for 30 days, then subdivided into subgroup (1) sacrificed (n=5), and subgroup (2) sacrificed after 19 hours from fasting with access to water then 2 ml orally ethanol given for five hours (n=5).

Measurement of body and stomach weight

The final rats body weight was determined using the method outlined by Obasi *et a*l.,[16]. Stomach weight of rat was calculated after isolated and separated from abdominal cavity by using electrical balance and estimated the relative weight according to the equation described by Al-kawaz & Jawad,[17].

Measurement of gastric ulcer index

Gastric ulcer index was estimated according to the method described by Mohammad *et al*.,[18]

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, 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 [19].

Measurement of oxidative markers and NO in the gastric tissue

Gastric tissues were weighed and homogenized in phosphate buffer (pH 7.4) then centrifuged at 3.000 rpm for 10 minutes and supernatant was used for biochemical analysis of Malondialdehyde (MDA) concentration[20], Superoxide dismutase (SOD) activity[21] and Nitric oxide (NO) concentration [22].

Histological study

The stomach of the rats were removed from abdominal cavity and fixed in 10% formalin and then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Serial sections of 5µm thick were cut and stained with haematoxylin and eosin[23].

Statistical analysis

Statistical Package for Social Science (SPSS) version 23.0 (SPSS,Chicago,USA) was used for statistical analysis of 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 Duncan Multiple Test.The level of significance was accepted under($P \geq 0.05$) [17].

RESULTS AND DISCUSSION

Results

Protective effect on body weight

The results of the current study showed that the percentage change in body weight decreased significantly ($p \leq 0.0.5$) in treated groups with esomeprazole, curcumin, chitosan and mixture as compared with the control and 0.1M acetic acid groups. On the other hand, the percentage change in body weight decreased significantly ($p \leq 0.05$) in the treated group with chitosan and mixture when compared to esomeprazole and curcumin groups **(Table 1)**.

Protective effect on stomach weight

The results in **Table (2)** revealed that the relative weight of the stomach in female rats changed in treated groups with esomeprazole, curcumin, chitosan and mixture (curcumin - chitosan), as compared to control groups. The

results showed a significant increase ($p \leq 0.05$) in groups treated with curcumin, curcumin + ethanol, chitosan + ethanol, and mixture + ethanol as compared with the distilled water group. While the results of this study showed non-significant change ($p > 0.05$) in groups treated with ethanol group, 0.1 M acetic acid + ethanol, esomeprazole, esomeprazole + ethanol, chitosan and mixture as compared with the distilled water group.

Table 1: Protective effect on Percentage change in body weight of treated female rats for 30 days.

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 (control)	179.60 ±0.909 a	199.70±1.342 cd	11.192±0.498 d
0.1 M Acetic acid	179.80±1.245 a	201.20±1.218 d	11.913±0.375 d
Esomeprazole	179.60±1.147 a	197.000±1.174 c	9.694±0.295 c
Curcumin	179.10±0.888 a	191.70±1.044 b	7.043±0.496 b
Chitosan	179.80±0.892 a	182.80±0.952 a	1.688±0.679 a
mixture	180.30±1.350 a	184.300±0.684 a	2.248±0.4669 a

* Different letters in same column indicated significant ($p \leq 0.05$) among groups.
* n=10 for each group.

Table 2: Protective effect on stomach weight in treated female rats for 30 days.

Groups	Realtive weight of stomach % Mean±S.E.
Distilled water (Negative control group)	0.782±0.234 a
Ethanol (Positive control group)	0.838±0.265 ab
0.1 M acetic acid	0.969±0.595 abc
0.1M acetic acid + Ethanol	1.1895±0.483 c
Esomeprazole 3.54mg/kg	0.932±0.113 abc
Esomeprazole 3.54mg/kg + Ethanol	0.945±0.696 abc
Curcumin 40mg/kg	1.118±0.188 bc
Curcumin 40mg/kg + Ethanol	1.199±0.117 c
Chitosan 150mg/kg	0.799±0.228 a
Chitosan 150mg/kg + Ethanol	1.0902±0.106 bc
mixture (curcumin 40 mg/kg -chitosan 150 mg/kg)	0.847±0.361 ab
mixture (curcumin 40 mg/kg + chitosan 150mg/kg)+ Ethanol	1.165±0.777 c

* Different letters indicated significant($p \leq 0.05$) among groups.
* n=5 for each group.

Protective effect on gastric ulcer index

The results of this study showed that gastric ulcer index increased significantly ($p \leq 0.05$) in groups treated with ethanol and 0.1 M acetic acid + ethanol compared to the groups treated with distilled water and 0.1 M acetic acid. While the group treated with 0.1 M acetic acid showed non–significant changes ($p > 0.05$) when compared to the group treated with distilled water **(Table 3)**.

Protective effect on volume of gastric juice

As shown in **Table (3)**, a significant increase ($P \leq 0.05$) was observed in the volume of the gastric juice in the groups treated with the ethanol and 0.1 M acetic acid + ethanol groups, compared to the treated groups with distilled water and 0.1 M acetic acid, but no significant change ($p > 0.05$) was observed in the volume of the gastric juice in groups treated with 0.1 M acetic acid, esomeprazole, curcumin, chitosan and mixture, compared to distilled water group.

Protective effect on pH of gastric juice

As shown in **Table (3),** a significant decrease ($p \leq 0.05$) was observed in the pH of gastric juice in treated groups with ethanol and 0.1 M acetic acid + ethanol, compared to the treated groups with distilled water and 0.1 M acetic acid. But no significant change ($p > 0.05$) was observed in the pH of gastric juice in groups treated with 0.1 M acetic acid, esomeprazole, curcumin, chitosan and mixture compared to the distilled water group.

Table 3: Protective effect on ulcer index, protective index, volume and pH of gastric juice in treated female rats for 30 days.

Groups	Ulcer index	Protective index %	Volume of gastric juice (ml)	PH of gastric juice
Distilled water (Negative control group)	0.00±0.00 a	100	1.30±0.20 ab	5.40±0.25 ab
Ethanol (Positive control group)	4.80±0.49 b	0.00	3.80±0.26 e	3.00±0.32 c
0.1 M acetic acid	0.00±0.00 a	100	1.60±0.19 abc	5.00±0.32 ab
0.1M acetic acid + Ethanol	3.00±0.45 c	37.5	3.60±0.25 e	3.60±0.25 c
Esomeprazole 3.54mg/kg	0.00±0.00 a	100	1.00±0.16 a	5.60±0.5 a
Esomeprazole 3.54mg/kg + Ethanol	1.70±0.30 d	64.58	2.00±0.16 bcd	5.20±0.37 ab
Curcumin40mg/kg	0.00±0.00 a	100	1.10±0.19 a	5.20±0.37 ab
Curcumin 40mg/kg + Ethanol	2.60±0.51 cd	45.83	2.30±0.37 cd	4.60±0.51 b
Chitosan 150mg/kg	0.00±0.00 a	100	1.10±0.19 a	5.40±0.25 ab
Chitosan 150mg/kg + Ethanol	1.80±0.37 d	62.5	2.50±0.27 d	5.00±0.32 ab
mixture (curcumin 40mg/kg - chitosan 150mg/kg)	0.00±0.00 a	100	1.00±0.27 a	5.80±0.20 a
mixture (curcumin 40mg/kg - chitosan 150mg/kg) + Ethanol	1.70±0.30 d	64.58	1.90±0.33 bcd	5.20±0.37 ab

***** Different letters indicated significant ($p \leq 0.05$) among groups.
*n=5 for each group.

Protective effect on MDA concentration in gastric tissue

The concentration of MDA in treated groups with ethanol and 0.1 M acetic acid + ethanol was significantly increased ($p \leq 0.05$) compared to the values observed in groups treated with distilled water and 0.1 M acetic acid as shown in **Fig. (1)**.

Protective effect on SOD activity in gastric tissue

The results of current study showed that SOD activity significant decrease ($p \leq 0.05$) in treated group with ethanol (positive control group) compared to normal control group, While in treated group with 0.1M acetic acid +ethanol showed non-significant differences ($p > 0.05$) as compared to treated group with 0.1 M acetic acid **(Fig. 2)**.

Protective effect on NO concentration in gastric tissue

As shown in **Fig. (3-C)**, a significant decrease ($p \leq 0.05$) was observed in the concentration of NO in the gastric tissue in the treated group with ethanol as compared to normal control group, but no significant change ($p > 0.05$) was observed in the concentration of NO in gastric tissue of the treated group with 0.1M acetic acid + ethanol as compared to the treated group with 0.1M acetic acid.

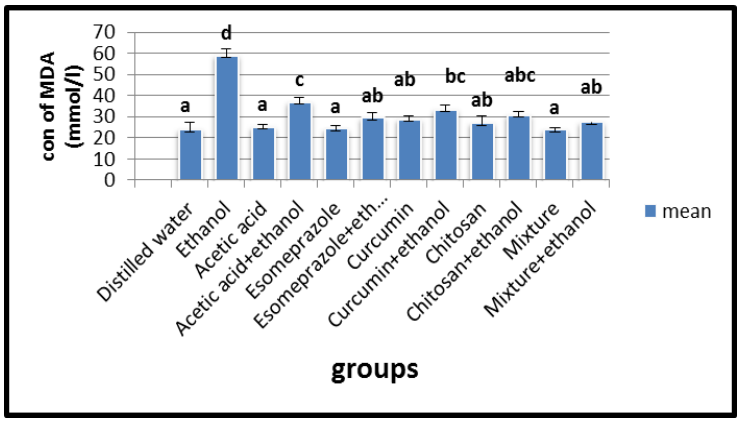

Fig. 1: Protective effect of esomeprazole, curcumin, chitosan and curcumin-chitosan mixture on MDA concentration in gastric tissue of experimental rats.

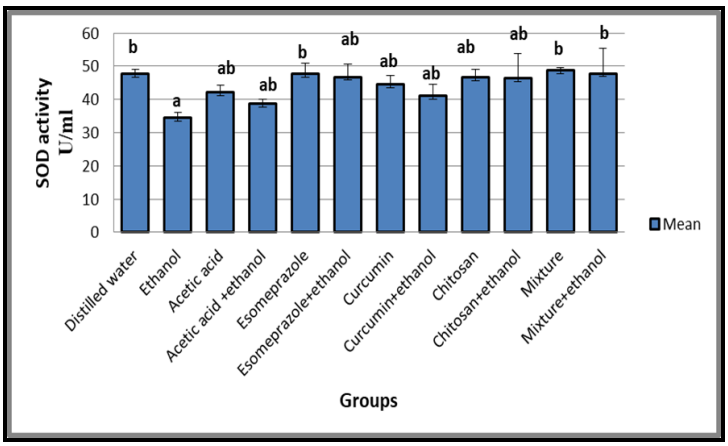

Fig. 2: Protective effect of esomeprazole, curcumin, chitosan and curcumin-chitosan mixture on SOD activity in gastric tissue of experimental rats.

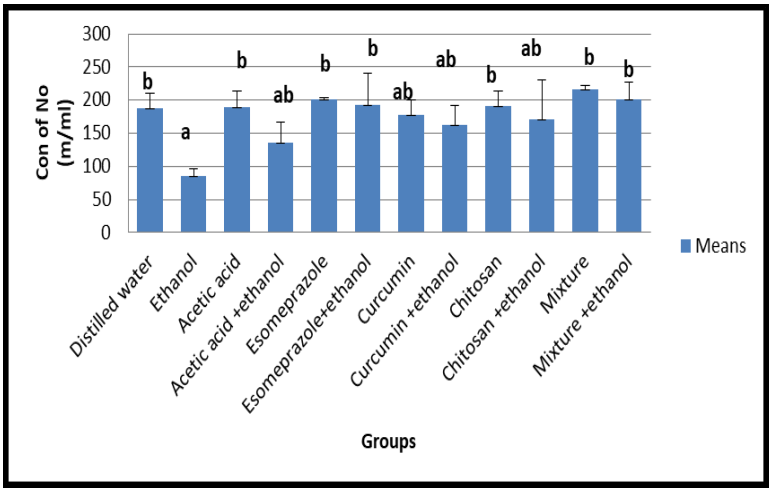

Fig. 3: Protective effect of esomeprazole, curcumin, chitosan and curcumin-chitosan mixture on NO concentration in gastric tissue of experimental rats.

Macroscopic and Microscopic examination of the stomach

Ethanol group (positive control group)

Oral administration of 2ml ethanol produced swelling in the stomach, and multiple lesions were seen in gastric mucosa, affecting mostly the glandular portion and running parallel to the long axis of the stomach, as shown in **Fig**. (**4-2**). The histological sections of the stomach of rats in this group showed hemorrhagic mucosal erosions with severe necrosis of gastric mucosa **(Fig. 5-B).**

0.1 M acetic acid - treated group

The stomach obtained from the group treated with 0.1M acetic acid alone for 30 days showed external and internal normal morphology **(Fig. 4-3)** as the distilled water negative control group **(Fig. 4-1)**. The histological sections from the stomach of rats showed normal epithelial lining of gastric mucosa, and there were no significant pathological changes **(Fig. 5-C)**, as in the negative control group **(Fig. 3-A)**.

0.1 M a cetic acid + ethanol - treated group

The stomach obtained from the group treated with 0.1M acetic acid + ethanol showed less degree of swelling and ulceration than that occurred in the ethanol group with a protective index of 37.5% **(Fig. 4-4).** The histological sections of the stomach showed hemorrhagic mucosal erosions with necrosis of gastric mucosa but less than the positive control group **(Fig. 5-D)**.

Esomeprazole 3.54 mg/kg-treated group

The stomach obtained from group treated with esomeprazole alone for 30 days showed external and internal normal morphology as distilled water negative control group **(Fig. 4-5)**. The histological sections from the stomach showed normal epithelial lining of gastric mucosa, and there were no significant pathological changes **(Fig. 5-E),** as in the negative control group.

Esomeprazole 3.54 mg/kg + ethanol-treated group

The stomach obtained from the group treated with esomeprazole + ethanol showed slight swelling in the external morphology, and small lesions affectting the gastric mucosa compared to positive control groups with a protective index of 64.58% **(Fig. 4-6).** The histological sections of the stomach of rats in the esomeprazole + ethanol-treated group showed hemorrhagic mucosal erosions with necrosis of gastric mucosa but less than the positive control group **(Fig. 5-F)**.

Curcumin 40 mg/kg-treated group

The stomach obtained from group treated with curcumin alone for 30 days showed external and internal normal morphology **(Fig. 4-7)** as distilled water group. The histological sections showed normal epithelial lining of gastric mucosa, and there were no significant pathological changes as in the negative control group **(Fig. 5-G)**.

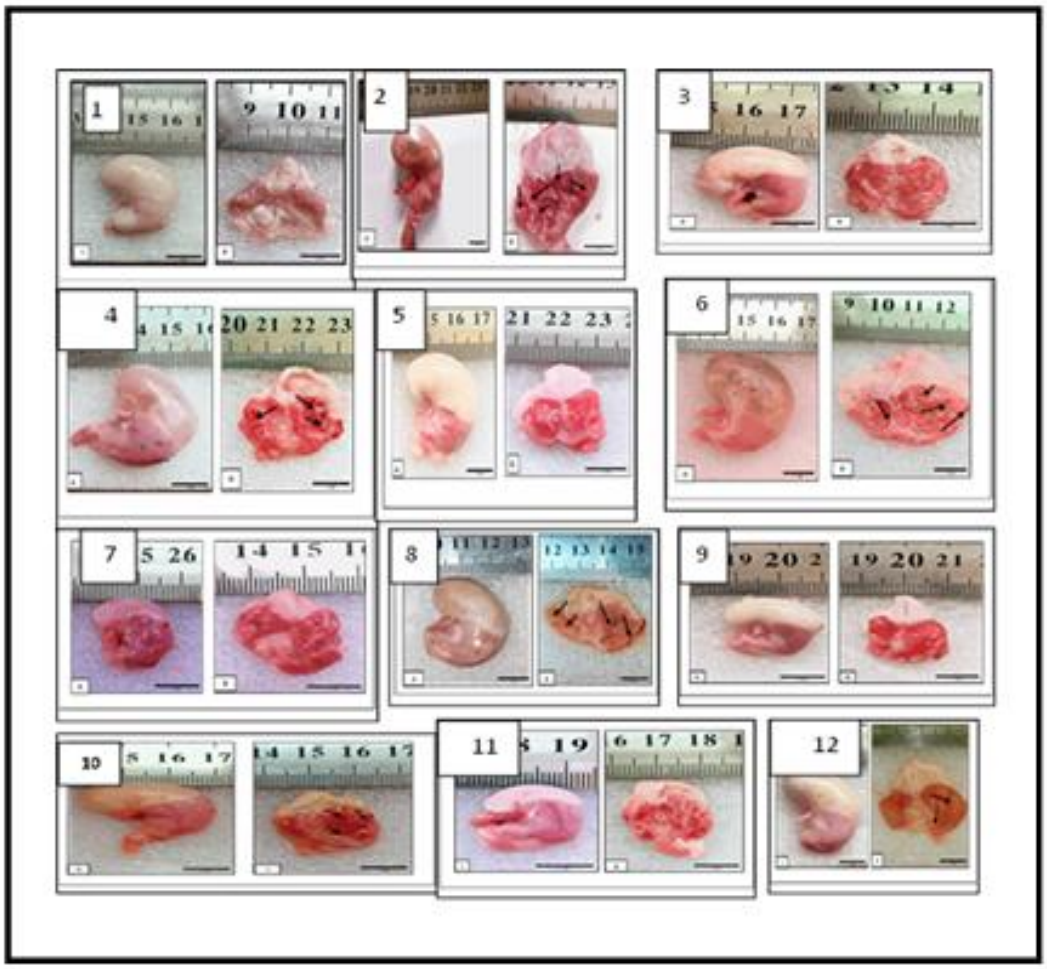

Fig. 4: Stomach of the treated female rats for 30 days:(1):distilled water group (negative control group).A:Normal external morphology. B:Internal morphology showing intact gastric mucosa.(2): ethanol treated group (positive control group). A: Swelling external morphology .B: Internal morphology showing the multiple lesions in gastric mucosa (black arrows).(3) :0.1 acetic acid treated group. A: Normal external morphology .B: Normal internal morphology.(4): 0.1 M acetic acid + ethanol treated group. A: External morphology showing slight swelling. B:Internal morphology showing the mild lesions in gastric mucosa compared to the lesions in distilled water + ethanol (ulcer control group) .(5):esomeprazole treated group. A: Normal external morphology .B: Normal internal morphology.(6):esomeprazole + ethanol treated group. A: External morphology showing slight swelling . B:Internal morphology showing the small lesions (black arrows) in gastric mucosa compared to positive control groups . (7):curcumin treated group. A: Normal external morphology. B: Normal internal morphology. (8):curcumin + ethanol treated group. A: External morphology showing slight swelling. B: Internal morphology showing small lesions in gastric mucosa (black arrows) compared to positive control group. (9):chitosan treated group. A:Normal external morphology B: Normal internal morphology.(10):chitosan + ethanol treated group. A: External morphology showing slight swelling. B: Internal morphology showing small lesions in gastric mucosa (black arrows) compared to positive control groups (11: mixture (curcumin - chitosan) treated group. A:Normal external morphology. B:Normal internal morphology.(12):mixture (curcumin-chitosan) + ethanol treated group. A:External morphology showed slight swelling. B:Internal morphology showed small lesions (black arrows) in gastric mucosa as compared with positive control groups

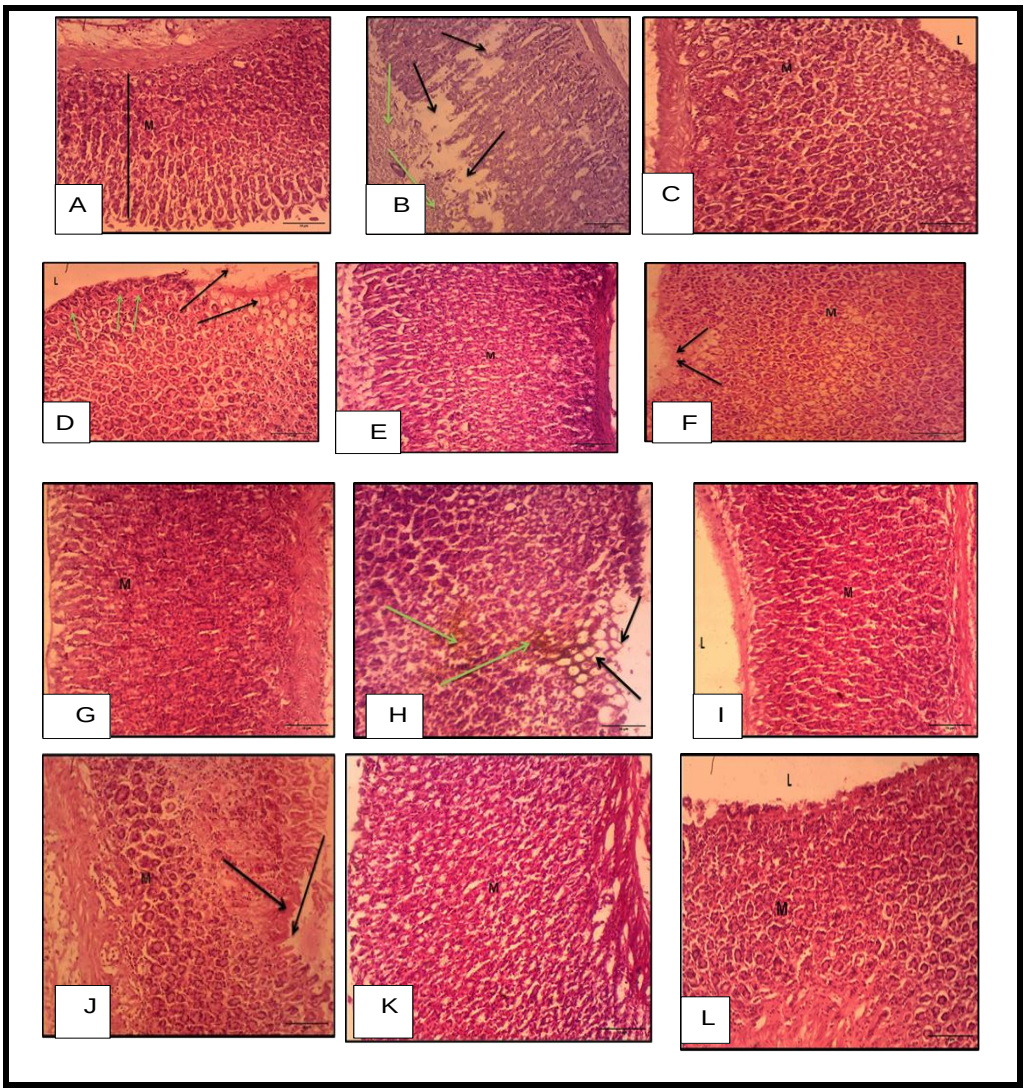

Fig. 5: Cross section in the stomach of the treated female rats for 30 days: (A): distilled water group (negative control group) showing intact gastric mucosa M (H&E, 100X).(B): ethanol group (Positive control group) showing hemorrhagic mucosal erosions (green arrow) with severe necrosis in the gastric mucosa (black arrow) (H&E, 100X).(C) :0.1M acetic acid group showing intact gastric mucosa (M), Lumen (L) (H&E, 100X).(D): 0.1M acetic acid + ethanol group showing hemorrhagic mucosal erosions (green arrow) with necrosis of gastric mucosa (black arrow), lumen (L) (H&E, 100X).(E): esomeprazole -treated group showing intact gastric mucosa (M) (H&E, 100X). (F): esomeprazole + ethanol-treated group showing necrosis (black arrows) of the gastric mucosa (M) (H&E, 100X). (G): curcumin -treated group showing intact gastric mucosa (M) (H&E, 100X). (H): curcumin + ethanol - treated group showing mucosal ulceration (black arrows) and hemorrhage in the epithelial cells (green arrows) (H&E, 100X).(I): chitosan treated group showing intact gastric mucosa (M), Lumen (L) (H&E, 100X).(J): chitosan + ethanol group showing mucosal ulceration (black arrows), Mucosa (M) (H&E, 100X).(K): mixture treated group showing intact gastric mucosa (M) (H&E, 100X).(L): mixture + ethanol-treated group showing no clear lesion and disruption in the gastric mucosa (M), Lumen (L) (H&E, 100X).

Curcumin 40 mg/ kg + ethanol- treated group

The stomach obtained from the group treated with curcumin + ethanol showed slight swelling in the external morphology with small lesions affecting the gastric mucosa compared with positive control groups but more severe than the lesion observed in esomeprazole + ethanol treated group with a protective index of 45.83 % **(Fig. 4-8)** The histological sections of

the stomach of rats in the curcumin + ethanol-treated group showed hemorrhagic mucosal erosions with necrosis of gastric mucosa but less than the positive control group **(Fig. 5-H)**.

Chitosan 150 mg/kg-treated group

The stomach obtained from the group treated with chitosan alone for 30 days showed external and internal normal morphology **(Fig. 4-9)** as the distilled water negative control group. The histological sections from the stomach of rats this group showed normal epithelial lining of gastric mucosa, and there were no significant pathological changes as in the negative control group **(Fig. 5-I)**.

Chitosan 150 mg/kg + ethanol- treated group

The stomach obtained from the group treated with chitosan + ethanol showed slight swelling in external morphology, and small lesions affected the gastric mucosa compared with positive control groups but less severe than the lesion observed in curcumin + ethanol treated group and more than that occur in esomeprazole + ethanol treated group with protective index 62.5 % **(Fig. 4-10)** . The histological sections of the stomach of rats in the chitosan + ethanol-treated group showed hemorrhagic mucosal erosions with necrosis of gastric mucosa but less than the positive control group **(Fig. 5-J)**.

mixture-treated group

The stomach obtained from the group treated with the mixture alone for 30 days showed external and internal normal morphology **(Fig. 4-11)** as the distilled water group. The histological sections from the stomach of rats this group showed normal epithelial lining of gastric mucosa, and there were no significant pathological changes as in the negative control group **(Fig. 5-K).**

mixture + ethanol - treated group

The stomach obtained from the group treated with the mixture + ethanol showed slight swelling, and small lesions affected the gastric mucosa compared with positive control groups (ulcer groups) but less severe than lesion that observed in (esomeprazole + ethanol, curcumin + ethanol and chitosan + ethanol) treated groups, with protective index 64.58 % **(Fig. 4-12)** The histological sections of the stomach of rats in this group showed no clear lesion and disruption of mucosa **(Fig. 5-L).**

Discussion

The ingestion of ethanol leads to the development of gastric lesions by infiltrating and dissolving the stomach lining via its proteolytic and hydrolytic properties. Additionally, it damages endothelial cells due to the impaired blood flow[24]. The present study showed that oral administration of 2 ml of pure ethanol per rat over a period of 5 hours led to the formation of gastric ulcers. The groups that were administered pretreatment with esomeprazole, curcumin, chitosan, and the combination of protective index exhibited a statistically significant decrease ($p \leq 0.05$) in the stomach ulcer index. The decreases in the ulcer control group were 64.58%, 45.83%, 62.5%, and 64.58%, respectively. The observed gastroprotective effects of esomeprazole, curcumin, chitosan, and their combination on the stomach mucosa may be related to their respective qualities. This discovery is consistent with the findings presented by Xie *et al*.[25], who provided evidence that esomeprazole decreases the ulcer index in rats. The effect of esomeprazole is closely correlated with its mechanism of action, which entails binding to the H+/K+ ATPase enzyme system located in the parietal cells. This binding efficiently prevents the escape of hydrogen ions into the stomach lumen [26].

Bahijri *et al*.,[27] demonstrated that chitosan's capacity to cling to the gastric mucosa serves as a protective barrier, limiting damage by creating a physical barrier between the wounded mucosa and the stomach's natural environment. Moreover, it hinders the proliferation of bacteria at the location of the wound. In addition, it impedes the enzymatic activities involved in the synthesis of the microorganism's cell wall at the site of damage [28].

The research indicates that the antiulcer effect of the combination of curcumin and chitosan is likely attributed to their potent combination, which possesses various advantageous properties including antioxidant, anti-inflammatory, gastroprotective, and ulcer healing effects[13].

The findings in **Table (3)** clearly showed a significant increase ($p \leq 0.05$) in the amount of gastric juice in the ethanol group. The rise in this phenomenon may be ascribed to the direct influence of ethanol on the stomach mucosa. Ethanol is well acknowledged for its capacity to cause stomach injuries via many mechanisms, including dehydration, which compromises the integrity of mucosal cell barriers, and cytotoxicity. The substance's cytotoxicity induces the recruitment of leukocytes that secrete reactive oxygen species (ROS) and inflammatory cytokines. These variables have the ability to cause cell death. Interestingly, NF-kB plays a substantial role in the link between these adverse events[1 & 30].

The findings revealed a substantial decrease ($P \leq 0.05$) in the acidity level of stomach fluid in rats exposed to ethanol as compared to animals in the normal control group. The possible reason for this activity might be the stimulation of the vagus nerve, which triggers the release of gastrin, resulting in an elevation in the production of stomach acid [23]. Gastric acid hypersecretion plays a crucial role in the onset of gastric ulcer disease [28]. The reflux of acid into the mucosa may immediately lead to increased vascular permeability and cause severe damage to the basement membrane of both epithelial and mucosal cells in the stomach wall. This might hinder the natural healing processes in the damaged mucosa and initiate the advancement of apoptosis to inner layers of the mucosa[29]. The experimental studies consistently shown that ethanol has the capacity to decrease stomach pH levels[1].

In esomeprazole, curcumin, chitosan and mixture-pre-treated groups, pH of gastric juice levels returned near normal value as a result to daily oral administration of esomeprazole, curcumin, chitosan and mixture for 30 days, this result reflects the hyposecretion of the gastric acid which indicates protective effect of esomeprazole, curcumin, chitosan and mixture on pH of gastric juice. These alteration in acidity is also seen in microscopic study of stomach which indicates protective effect of these materials on stomach mucosa.

Multiple studies have shown that the action of antioxidant substances in scavenging free radicals may effectively prevent ethanol-induced gastric ulcers[3]. Administration of esomeprazole (3.54 mg/kg), curcumin (40mg/kg), chitosan (150mg/kg), and a mixture from curcumin (40mg/kg) and chitosan (150mg/kg) as pretreatment resulted in a reduction in the concentration of MDA in the gastric tissue. The findings we have align with several publications that have shown curcumin's effectiveness as a powerful scavenger of free radicals in different tissues, such as the heart [32], liver [34], kidney [4], and pancreas[23]. The gastroprotective action of curcumin in rat stomach may be attributed to its ability to reduce the concentration of MDA, a prominent marker of lipid peroxidation. This effect is achieved by the elimination of free radicals and the reduction of oxidative stress. The authors of the study suggested that the ability of curcumin to remove free radicals may be linked to the existence of both phenolic OH and CH2 groups in the □-diketone moiety of its chemical structure [32]. Hence, it seems that curcumin's ability to protect against ethanol-induced gastric ulcers may be attributed, at least in part, to its strong antioxidant properties and its capacity to eliminate free radicals.

The results observed that daily intake of mixture of curcumin (40mg/kg) and chitosan (150mg/kg) for 30 day showed no significant differences in MDA concentration and SOD activity in the gastric tissue of mixture + ethanol group compared to negative control group. This result is due to antioxidant activities of the curcumin and chitosan compounds which may act separately or synergistically to reduce or prevent generation of free radicals. Similar observations were also obtained by Kuadkaew *et al.*[15], who reported that mixture comprising of curcumin and chitosan to have an antioxidant effect.

The experiment included analyzing the morphological structure and tissue composition of rats used as positive controls. The findings indicated the existence of many lesions arranged in a parallel way along the long axis of the stomach, which resulted in ulceration of the stomach mucosa. One potential explanation might be that the administration of pure ethanol at a concentration of 100% induces oxidative stress, resulting in a decrease in antioxidant levels and an increase in the generation of reactive oxygen species (ROS)[35].

Conclusion

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

Ethical approval

The research was done in compliance with the ethical guidelines derived from the Declaration of Helsinki. The research protocol underwent assessment and received clearance from a local ethics commission on November 3, 2022, under document number 7-17-7922.

Acknowledgements

We would like to extend our profound appreciation to the Biology Department of the Science College at Babylon University for their provision of essential resources during this research endeavor.

REFERENCES

1. M. Raish , M. Shahid, Y. Jardan, M.A. Ansari, *et al.,* ″Gastroprotective effect of sinapic acid on thanol-induced gastric ulcers in rats: Involvement of Nrf2/HO-1 and NF-kB signaling and antiaptotic role″, ***Front Pharmacol***, 12,1-15(2021).
2. A.S. Al-Rashdi, S.M. Salama, S.S. Al-Kiyumi, M. A. Abdulla, A.H. Hadi, and S.I. Abdelwahab, ″Mechanisms of gastroprotective effects of ethanolic leaf extract of *Jasminum sambac* against HCL/ethanol induced gastric mucosal injury in rats″, ***Evid Based Complement Alternat Medm,*** 76, 1-15(2012).
3. S. Arulkumaran, V. R. Ramprasath, P. Shanthi and P. Sachdanandam, ″Alteration of DMBA-induced oxidative stress by additive action of a modified indigenous preparation-kalpaamruthaa″, ***Chem Biol Interact***, 167, 99-106 (2007).
4. S. Damiano, E. Andretta, C. Longobardi, F. Prisco, O. Paciello, C. Squillacioti, N. Mirabella, S. Florio and R. Ciarcia,″Effects of curcumin on the renal toxicity induced by orchratoxin A in rats″, ***Antioxidants***, 9 (332), 1-13(2020).
5. D. Dokmeci, M. Akpolat, N. Aydogdu, L. Doganay and F. N. Turan,″L-carnitine inhibits ethanol-induced gastric mucosal injury in rats″, ***Pharmacol Rep***,57,481-488 (2005).
6. H. Ammon and M.A. Wahl, ″Pharmacology of Curcuma longa″ , ***Planta Med,***57:1-7 (1991).
7. J. Tilak, M. Banerjee, H. Mohan and T.P.A. Devasagayam, "Antioxidant availability of turmeric in relation to its medicinal and culinary uses″, ***Phytother Res***, 18(10), 798-804 (2004).
8. B. B. Aggarwal, C. Sundaram, N. Malani and H. Ichikawa, ″Curcuminn: The Indian solid gold″, ***Adv Exp Med Biol***, 95, 1-75 (2007).
9. M. Awasthi, S. Singh, V.P. Pandey, U.N. Dwivedi, ″Curcumin: Structure-activity relationship towards its role as a versatile multi-targeted therapeutics. Mini Rev″, ***Org Chem,*** (14), 311–332 (2017).
10. P.G. Bradford,″Curcumin and obesity″, ***Biofactors,*** 39(1),78-87 (2013).
11. N.K. Pandeya and T. Wives,″Modern Miracles —Turmeric as Traditional Medicine in India″, ***Trees for Life J***, 1,3 (2005).
12. 12. A. Noorafshan. and S. Ashkani-Esfahani, ″ A Review of Therapeutic Effects of Curcumin″, ***Current Pharmaceutical Design***, 19, 2032-2046 (2013).
13. J. Tilak, M. Banerjee, H. Mohan, and T.P.A. Devasagayam, ″Antioxidant availability of turmeric in relation to its medicinal and culinary uses″, ***Phototherapy Research***, 18(10), 798-804 (2004).
14. J.W. Shin, I.C. Soel and C.G. Son, "Interpretation of Animal Dose and Human Equivalent Dose for Drug Development", ***J Korean Med,*** 31(3), 1-7(2010).
15. S. Kuadkaew, S. Ungphaiboon, N. Phdoongsombut, S. Kaewsuwan and S.

Mahattanadul, ″Efficacy of a chitosan-curcumin mixture in treating indomethacin-induced acute gastric ulcer in rats″, ***Curr Pharm Biotechnol***, 22(14),1919-1931 (2021).
16. E. Obasi, K. Iheanacho, N.C. Nwachukwu and P.C. Chikezie, ″ Evluation of body weight, serum glucose level and oxidative stress parameters of diabetic rats administration phenolic aqueous leaf extract of Vitex doniana″, ***Arthritis Res Ther***, 6(9), 3359-3367 (2019).
17. J.M. Al-kawaz, and N.M. Jawad, ″Morphological and histological study of large intestine in adult local rabbits subjected to starvation″, ***Int J Health Sci***,6(S3),2387-2402 (2022).
18. R. Mohammad, M. Shahid, A.B.Jardan and M.A.Ansari, ″Gastroprotective effect of sinapic acid on thanol-induced gastric ulcers in rats: Involvement of Nrf2/HO-1 and NF-kB signaling and antiaptotic role″, ***Front Pharmacol***, 12, 1-15(2021).
19. S.J. Gilni and M.N. Bin-Jumah, ″Protective effect of fustin against ethanol-activated gastric ulcer via down regulation of biochemical parameters in rats″, ***ACS Omega***,7(27), 23245-23254 (2022).
20. J.A. Buege and S.D. Aust, ″Microsomal lipid peroxidation″, ***Methods Enzymol***, 52, 302-310 (1978).
21. S. Marklund and G. Marklund, ″Involvement of the Superoxide Anion Radical in the Autooxidation of Pyrogallol and a Convenient Assay for Superoxide Dismutase″, ***Eur J Biochem***, 47(3), 467-474 (1974).
22. L.C. Green, D. A.Wagner and J. Glogowski, ″Analysis of nitrate nitrite and [15 N] nitrate in biological fluids″, ***Anal Biochem***, 126(1), 131–138 (1982).
23. J. D. Bancroft, C. Layton and S. K. Suvarna, Bancrofts, theory and practice of histological technique, Seventh edition. Livingstone. Elsevier Limited, (2013).
24. Z. Rahman, D. K. Dwivedi and G.B. Jena,″Ethanol-induced gastric ulcer in rats and intervention of tert-butylhydroquinone: Involvement of Nrf2/HO-I signaling pathway″, ***HET,*** 39(4), 547-562 (2020).
25. W. Xie, X. Huang, R. Chen, R. Chen, T. Li, W. Wu and Z. Huang, ″Esomeprazole alleviates the damage to stress ulcer in rats through not only its antisecretory effect but its antioxidant effect by inactivating the p 38 MAPK and NF-kB signaling pathways", ***Drug Des Devel Ther***, 13, 2969-2984 (2019).
26. P. J. Miner, P.O. Katz, Y. Chen, and M. Sostek, ″Gastric acid control with esomeprazole, lansoprazole, omeprazole, pantoprazole, and rabeprazole: a five-way crossover study″, ***Am J Gastroenterol***, 98(12), 2616–20 (2003).
27. S. M. Bahijri, L. Alsheikh, G. Ajabnoor and A. Borai ,″Effect of Supplementation With Chitosan on Weight, Cardiometabolic, and Other Risk Indices in WistarRats Fed Normal and High-Fat/High-Cholesterol Diets Ad Libitum″, ***Nutr Metab Insights***, 10, 1–8 (2017).
28. G. B. Glavin and S. Szabo, ″Experimental gastric mucosal injury: laboratory models reveal mechanisms of pathogenesis and new therapeutic strategies″, ***FASEB J,*** 6(3), 825-831 (2018).
29. J.H. Kim, S.K. Choi, S. Y. Choi, H.K. Kim and H.I. Chang,″Suppressive effect of astaxanthin isolated from the Xanthophyllomyces dendrorhous mutant on ethanol-induced gastric mucosal injury in rats″, ***Biosci Biotechnol Biochem***, 69(7),1300-1305 (2005).
30. J. L. Wallace, ″Recent advances in gastric ulcer therapeutics″, ***Curr Opin Pharmacol,*** 5(6), 573-577 (2005).
31. J.K. Ko, C.H. Cho, and C.W. Ogle, ″The vagus nerve and its non-cholinergic mechanism in the modulation of ethanol induced gastric mucosal damage in rats″, ***J Pharm Pharmacol,*** 46(1), 29-31 (2016).
32. S. A. Hussein, M. E. Azab and S.M. Abdelwahed, ″Biochemical effect of

curcumin on experimentally induced myocardial injury in rats", ***BVMJ,*** 35(1), 375-387 (2018).

33. R. Mohammad, M. Shahid, A.B. Jardan and M.A. Ansari, "Gastroprotective effect of sinapic acid on thanol-induced gastric ulcers in rats: Involvement of Nrf2/HO-1 and NF-kB signaling and antiaptotic role", ***Front Pharmacol***, 12,1-15 (2024).
34. K. Iurii, S. Shane, G. Xiaoxia, M. Parvin, Z.M. Masoud, Y. Mohammad, M. Hanna, J.H. Yava and M. Naima, "Effects of Curcumin in a Mouse Model of Very High Fat Diet-Induced Obesity", ***Biomolecules***, 10(10), 1368 (2020).
35. F. Ahmadi, Z. Oveisi, S.M. Samani and Z. Amoozgar, "Chitosan based hydrogels: characteristics and pharmaceutical applications", ***Res Pharm Sci***, 10(1), 1-16 (2015).

Bull. Pharm. Sci., Assiut University, Vol. 47, Issue 2, 2024, pp. 1235-1249.

نشرة العلوم الصيدليـــــة
جامعة أسيوط

BULL. PHARM. SCI. Assiut Univ.

التأثيرات المضادة للأكسدة للإيزومبيرازول والكركمين والكيتوسان ومزيج من الكركمين والكيتوسان على القرحة المعدية في إناث الجرذان : دراسة تجريبية

جنان مهدي جواد الكواز [١]* – حسين جاسم عبيد الحربي [١] – فاخر مكطوف الزبيدي [٢]

[١] قسم علوم الحياة ، كلية العلوم ، جامعة بابل، العراق

[٢] كلية الصيدلة ، جامعة بابل ، العراق

مرض القرحة المعدية الناتج عن التدخين والاجهاد وتناول الكحول هو من أكثر المشاكل الصحية انتشارًا في الوقت الحاضر لذلك هدفت الدراسة الحالية الى الكشف عن التأثيرات المضادة للأكسدة لعقار الايزومبيرازول (٣.٥٤ ملغم/كغم) ، الكركمين (٤٠ ملغم / كغم) ، الكيتوسان (١٥٠ ملغم / كغم) ومزيج من الكركمين (٤٠ ملغم/ كغم) والكيتوسان (١٥٠ ملغم / كغم) على قرحة المعدة التي سببها الإيثانول في إناث الجرذان : شملت الدراسة الحالية ٦٠ جرذ تراوحت اوزانها بين (١٧٩.٦٠-١٨٠.٣٠ غم) قسمت إلى مجموعة سيطرة وخمس مجاميع معاملة، كل مجموعة احتوت على ١٠ جرذان . تم وزن الجسم قبل وبعد اكتمال التجربة. بعد فترة صيام استمرت ١٩ ساعة ، تم إعطاء الإيثانول المطلق (٢ مل / جرذ) عن طريق الفم لجميع المجاميع ، باستثناء مجموعة السيطرة السالبة لغرض استحثاث قرحة في المعدة ، بعد ٥ ساعات تم قتل جميع الحيوانات . من خلال مقارنة كمية ودرجة الحموضة لعصير المعدة ، مؤشر القرحة ، مؤشر الحماية ، والفحص العياني والنسجي للمعدة ، تمت متابعة تطور قرحة المعدة. اظهرت النتائج انخفاض النسبة المئوية لوزن الجسم بشكل ملحوظ في المجاميع المعاملة بالكركمين والكيتوسان والمزيج مقارنة بمجاميع السيطرة. بينما في حالة عقار الايزومبيرازول لا يوجد تغير معنوي. بالإضافة إلى ذلك ، أظهر مؤشر القرحة وحجم العصير المعدي في المجاميع المعاملة بعقار الايزومبيرازول ، والكركمين ، والكيتوسان ، والمزيج انخفاضا معنويا ($p\leq0.05$) مقارنة بمجموعة السيطرة الموجبة.