The journal of Toxicology and pest control is one of the series issued twice by the Egyptian Academic Journal of Biological Sciences, and is devoted to publication of original papers related to the interaction between insects and their environment.

The goal of the journal is to advance the scientific understanding of mechanisms of toxicity. Emphasis will be placed on toxic effects observed at relevant exposures, which have direct impact on safety evaluation and risk assessment. The journal therefore welcomes papers on biology ranging from molecular and cell biology, biochemistry and physiology to ecology and environment, also systematics, microbiology, toxicology, hydrobiology, radiobiology and biotechnology.

www.eajbs.eg.net
Study of the Role of the Antioxidants; Silymarin and Vitamin C in Treatment of the Indomethacin-Induced Gastric Ulcer in Rats

Hassan Helaly A.A. Abu Rahma, Ali Abdelsalam Ahmed Attia and Walid Nasr El-dein Badawy Hammam

Pharmacology Department, Faculty of Medicine, Al-Azhar University (Assiut)

ARTICLE INFO

Article History
Received: 28/2/2017
Accepted: 1/4/2017

Key words:
Larvicidal
Antifeedant
Lantanacamara
Musca domestica.

ABSTRACT

Gastric ulcer represents a serious medical problem largely due to its frequency and high economic cost. Its incidence amounts up to 10% of the population in developed countries. Severe stress, Helicobacter pylori infection, ingestion of alcohol, aspirin and other NSAIDs are predisposing factors. Some studies have implicated the involvement of oxygen-derived free radicals in the pathogenesis of gastric ulcers and showed that antioxidants protect against gastro-duodenal injury. The present work was designed to evaluate the potential healing properties of the known antioxidants; silymarin and vitamin C as compared with omeprazole in the indomethacin-induced gastric ulcer in male albino rats. The present study showed that i.p administration of indomethacin (20m/kg) for two successive days produced several necro-hemorrhagic lesions in the gastric mucosa associated with decrease of the antioxidant parameters; Glutathione and Superoxide and increase of the gastric juice and total acid output. Treatment with silymarin (50 mg/kg/day), vitamin C (200 mg/kg/day) and omeprazole (3.6 mg/kg/day) for 5 days reduced the mean ulcer index, the oxidant parameter, malondialdehyde, the total gastric volume, the titratable acidity and total acid output and increased the antioxidant parameters; glutathione and superoxide dismutase.

In conclusion, silymarin and vitamin-c produced a significant healing effect in the indomethacin-induced gastric ulceration in male albino rats.

INTRODUCTION

Gastric ulcer represents a serious medical problem largely due to its frequency and high economic costs. Its incidence amounts up to 10% of the population in developed countries (Akimoto et al., 1998). Free oxygen radicals and lipid peroxidation play an important role in the pathogenesis of ischemia induced gastric mucosal injury by destruction of endothelial and epithelial cell components (Salim, 1992; Whittle, 2002 and Jimenez et al., 2004).

Antioxidants play a key role in these defense mechanisms. Many antioxidant agents; melatonin, Nigella sativa seed, Cucumismeloseeds and some vegetable oils have been reported to protect gastric mucosa against indomethacin ulcer (Rifat-uzz-Zaman et al., 2005). Silymarin has been used for centuries to treat liver, spleen and gall bladder disorders (Shaker et al., 2010). It is known to possess hepatoprotective, antioxidant (Morazzeni and Bombardelli, 1995), anticancer (Ziet et al., 1997) and antidiabetic (Maghrani et al., 2004) properties.
Vitamin C, also known as L-ascorbic acid, is a water-soluble vitamin that is naturally present in some foods, added to others, and available as a dietary supplement. Human, unlike most animals, are unable to synthesize vitamin C endogenously, so it is an essential dietary component (Li and Schellhorn, 2007). Due to its function as an antioxidant and its role in immune function, vitamin C has been promoted as a mean to help prevention and/or treatment of numerous health conditions as cancer (Carr and Frei, 1999; Li and Schellhorn, 2007), cardiovascular disease (Ye and Song, 2008; Willcox et al., 2008), age-related macular degeneration (AMD) (Van Leeuwen et al., 2005), and common cold (Douglas and Hemila, 2005).

Omeprazole is a selective and irreversible proton pump inhibitor. It suppresses stomach acid secretion by specific inhibition of the H+/K+-ATPase system found at the secretory surface of gastric parietal cells. Because this enzyme system is regarded as the acid (proton, or H+) pump within the gastric mucosa, omeprazole inhibits the final step of acid production. Omeprazole also inhibits both basal and stimulated acid secretion irrespective of the stimulus (Shin, et al., 2008). Omeprazole can be used in the treatment of gastroesophageal reflux disease (GERD), peptic ulcers, erosive esophagitis, and Zollinger-Ellison syndrome (Shamburek and Schubert 1993). Treatment of H. pylori infection can be completed by taking a triple therapy combination of omeprazole, amoxicillin, and clarithromycin for 7–14 days (Fuccio et al., 2007).

Several previous studies showed a potential protective effect of silymarin and vitamin C as compared with omeprazole against gastric ulcers and there is a little research information about their roles in ulcer healing, so the objective of the present study is to evaluate their possible role in gastric ulcer healing in the indomethacin induced gastric ulcer in male albino rats.

**MATERIALS AND METHODS**

**Materials**

**Apparatus:**
1. PH meter. (PYE UNICAM-Pye model 292 PH meter).
2. Centrifuge. VEB MLW Zentrifugenbavengelsdorf type T52.1.
3. Thermostatically controlled water bath adjusted at 37°C.

**Drugs and chemicals:**
1. **Indomethacin:** Indomethacin powder (Merck) was supplied in 50mg vials. It was dissolved in 10ml distilled water to make a concentration of 5 mg/ml.
2. **Silymarin powder** (Sigma Chemical Company) dissolved in distilled water.
3. **Vitamin C powder:** Vitamin C (Ascorbic acid) powder (Elnasr Pharmaceutical Company, Egypt) dissolved in distilled water.
4. **Omeprazole:** from (Sigma Chemical Company) dissolved in propylene glycol.
5. **Trichloroacetic acid** crystals (Merck) for preparing 6% trichloroacetic acid sol.
6. **Hydrochloric acid** (Merck) was used for preparing 0.1 HCL solution.
7. **Sodium Hydroxide** (Chemapole) crystals, was used for preparing 0.01 NaOH sols.
8. **Phenol Red** (Rhone-Poulenc) powder was used for preparing phenol red indicator.
9. **Diethyl ether** (Merck) was used for anesthesia.
10- Phosphate buffered saline, Hi-media- Lab. Pvt. Inc., USA.
11-SOD kit: Biodiagnostic, Egypt.
12-Lipid peroxide kit: Biodiagnostic, Egypt.
13-Glutathione reduced kit: Biodiagnostic, Egypt.
14- Saline, El-Nasr Pharmaceuticals chemical company, Egypt.

Experimental Animals:
A total number of (80) male albino Westar rats weighing 150+30 gm were used. They were housed at ordinary room temperature, exposed to natural daily light-dark cycles, fed with standard laboratory diet pellets and given tap water.

II- Experimental protocol:
Forty eight hours before beginning of the experiments, animals were deprived of food to ensure complete gastric emptying (Cho and Ogle, 1979) but they were allowed free access to water (Martin et al., 1992). During fasting, rats were housed each in a separate cage with a wide raised mesh bottom to prevent coprophagy (Candido and Gutierrez-Cabano, 1994).

80 male albino Westar rats used in the present work were divided into two main groups:

Group-A: To investigate possible healing promoting effect of silymarin & vitamin C compared with omeprazole on indomethacin induced gastric ulcer. Rats are subdivided into 5 subgroups of 8 rats each as follows:

Group-A1: control group received orally distilled water.

Group-A2: Indomethacin-induced ulcer rats, given i.p with indomethacin 20 mg/kg once per day for consecutive 2 days for induction of gastric ulcer, then distilled water for 15 days (Guidobono et al., 1997; Alsaif, 2004; Jiang et al., 2009).

Group-A3: Silymarin treated rats (50 mg/kg orally once per day for 15 days) after induction of gastric ulcer by indomethacin (ShobhaandJamadar2012).

Group-A4: Vitamin C treated rats (200 mg/kg orally once per day for 15 days) after induction of gastric ulcer by indomethacin (Murat et al., 2008).

Group-A5: Omeprazole treated rats (3.6 mg/kg orally once per day orally for 15 days) after induction of gastric ulcer by indomethacin (Paget GE et al., 1994).

By the end of the treatment period for rats, blood samples were taken for determination of the following oxidants & antioxidants parameters: Malondialdehyde (MDA), Superoxide dismutase (SOD), Glutathione (GSH) activity and the animals were sacrificed by cervical dislocation, the abdomens were opened and the stomachs were exposed and opened from greater curvature, the ulcer indices were assayed and calculated and the gastric walls are prepared for the histopathological examination by using suitable dyes.

Group-B: To investigate treatment with silymarin & vitamin C compared with omeprazole on the volume and acidity of gastric secretion by pyloric ligation method. Rats were subdivided into 5 subgroups of 8 rats each as follows:

Group-B1: (control group) received orally distilled water.

Group-B2: Ulcer induced rats, given i.p with indomethacin 20 mg/kg once per day for consecutive 2 days for induction of gastric ulcer, then distilled water for 15 days.

Group-B3: Silymarin treated rats (50 mg/kg orally once per day for 15 days) after induction of gastric ulcer by indomethacin then pyloric ligation were done for 3 hours after 48 hours fasted rats.

Group-B4: Vitamin C treated rats (200 mg/kg orally once per day for 15 days) after induction of gastric ulcer by indomethacin then pyloric ligation were done for 3 hours after 48 hours fasted rats.

Group-B5: Omeprazole treated rats (3.6
mg/kg orally once per day for 15 days) after induction of gastric ulcer by indomethacin then pyloric ligation were done for 3 hours after 48 hours fasted rats.

Three hours after ligation of the duodenum of all rats of group B, the rats are sacrificed by cervical dislocation, the abdominal walls are opened and stomachs are exposed and gastric secretions are collected, then the collected gastric juice is analyzed for determination of the volume of the collected juice, titratable acidity and total acid output.

**The experimental procedures:**

**Administration of drugs:** Indomethacin was injected intraperitoneally (i.p.) to animals at a dose of 20 mg/ kg/day. Vitamin C is administered orally; vitamin C powder was dissolved in distilled water and was given in a dose of 200 mg / kg / day.

-Silymarin also administered orally, silymarin powder in 0.01% NaHCO3 was given in a dose of 3.6 mg / kg / day. Omeprazole also administered orally in propylene glycol and was given in a dose of 3.6 mg / kg / day. The drugs were administered by using a smooth stainless steel tube and connected to an ordinary 5 ml syringe. The tube was 5 cm long with a wide bore and a smooth beveled tip to avoid esophageal perforation. The tube was introduced into the esophagus before drug administration to ensure adequate drug delivery and to avoid regurgitation. Each individual animal was weighted before the start of therapy and was clearly marked by gentian violet to indicate its weight. The doses of drugs were accurately calculated according to the weight of each animal.

**Method of induction of gastric ulcer by indomethacin in male albino rats:**

Induction of indomethacin gastric ulcer were done according to a method of (Basso *et al*., 1983). The rats to be operated upon were kept fasting individually in separate cages with wide meshed wire bottom to prevent coprophagia for 48 hours before the experiment to insure emptying of the stomach.

One hour before the experiment, water was removed from the cages and rats were weighted and the doses of indomethacin, silymarin, vit C and omeprazole for each rat were determined according to experimental design. Indomethacin was injected intraperitoneally (i.p.) to the rats at a dose of 20 mg kg/b.w/day for two consecutive days which is well known to cause significant stomach ulcer in rats (Guidobono *et al*., 1997; Alsaif, 2004 and Jiang *et al*., 2009).

**Assessment of Indomethacin-induced Gastric Ulcers:**

Six hours after the second dose of indomethacin injection, rats were decapitated, abdomen was opened, and stomach removed. The stomach was opened along the greater curvature, rinsed with slowly running saline. The stomach was stretched by pins over a plate of cardboard and examined for ulcers. The ulcerated areas in each stomach appeared as streaks 1-2 mm wide. Their lengths were measured with a transparent millimeter scale with the help of a magnifying lens. Results of each group were calculated and expressed as ulcer index (UI) which indicates the mean length of ulcers in mm ± standard error “SE ”(Scepovic and Radmanovic, 1984).

The preventive index (PI) of the drug was calculated according to the formula used by (Yarno *et al*., 1976):

\[
PI = \frac{(UI \text{ indomethacin}) - (UI \text{ indomethacin + drug})}{UI \text{ indomethacin}} \times 100
\]
Curative ratio = (Control ulcer index – Test ulcer index) / (Control ulcer index) x 100 (Akhtar and Ahamed, 1995).

**Histopathological examination:**
After gross examination of the opened stomach for ulceration, pieces of the stomach $1 \times \frac{1}{2} \text{ cm}$ were excised and fixed in 10% formalin solution. Paraffin sections were prepared and stained with hematoxylin, eosin and PAS stains for histopathological examination.

**Determination of glutathione reductase (mg/dL) activity:**
**Principle:** The method is based on the reduction of 5, 5' dithiobis2-nitrobenzoic acid (DTNB) with glutathione (GSH) to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 405 nm. Mix well, allow standing for 5 min. at R.T. centrifuge at 3000 rpm for 15 min. then taking the following aliquots:

<table>
<thead>
<tr>
<th>Reagents</th>
<th>BloodMI</th>
<th>Blank MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-</td>
<td>Trichloroacetic acid (TCA)</td>
<td>500 mmol/L</td>
</tr>
<tr>
<td>2-</td>
<td>Buffer</td>
<td>100 mmol/L</td>
</tr>
<tr>
<td>3-</td>
<td>DTNB</td>
<td>1 mmol/L</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Procedure</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 0.1</td>
<td>-</td>
</tr>
<tr>
<td>Distil. Water 0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Reagent 1 0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Supernatant 0.5 ml</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Reagent 2 1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Reagent 3 0.1 ml</td>
<td>0.1 ml</td>
</tr>
</tbody>
</table>

Mix well. Measure the absorbance after 5-10 min. at 405 nm of sample ($A_{\text{sample}}$) against the blank. Linearity up to 120 mg/dL (4 mmol/L).

**Calculation:**
GSH concentration = $A_{\text{sample}} \times \frac{66.66}{2.22} \text{ mg/dL} = A_{\text{sample}} \times 30 \text{ mmol/L}$

**Determination of superoxide dismutase (U/int/ml) activity:**
Superoxide dismutases (SODs) are metalloenzymes that catalyze the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide and thus form a crucial part of the cellular antioxidant defense mechanism. $2 \text{H}_2 \text{O}_2^- + 2\text{H}^+ + \text{SOD} \rightarrow \text{H}_2 \text{O}_2 + \text{O}_2$. Three types of SODs have been characterized according to their metal content: copper zinc (Cu/Zn), manganese (Mn), and iron (Fe). SOD is widely distributed in both plants and animals. It occurs in high concentrations in brain, liver, heart, erythrocytes, and kidney. In humans there are three forms of SOD: cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD. Extracellular SOD is found in the interstitial spaces of tissues and also extracellular, accounting for the majority of the SOD activity in plasma, lymph, and synovial fluid. The amount of SOD present in cellular and extracellular environments is crucial for the prevention of diseases linked to oxidative stress (Nishikimi et al., 1972).

**Principle:** This assay relies on the ability of the enzyme to inhibit the phenazinem-ethosulphate-mediated reduction of nitrobluetetrazolium dye.
Reagents:

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Phosphate Buffer pH 8.5</td>
<td>50 mmol/L</td>
</tr>
<tr>
<td>2</td>
<td>Nitroblue tetrazolium (NBT)</td>
<td>1 mmol/L</td>
</tr>
<tr>
<td>3</td>
<td>NADH</td>
<td>1 mmol/L</td>
</tr>
<tr>
<td>4</td>
<td>Phenazinemethosulphate (PMS)</td>
<td>0.1 mmol/L</td>
</tr>
</tbody>
</table>

Preparation of Solutions:
- Reagent 1, ready for use.
- Reagent 2, reconstitute in 5 ml d. Water. - Reagent 3, reconstitute in 5 ml buffer
- Reagent 4, reconstitute in 5 ml d. Water.

Procedure:
- R4 should be diluted 100 times immediately before use (0.1 ml + 9.9 ml dis. Water). Working reagent: mix R1 + R2 + R3 in ratio of (10+1+1 ml), immediately before use.

Control (Ml) | Sample (Ml)
---|---|
Buffer (R1) | 1.0 | 1.0 |
NBT (R2) | 0.1 | 0.1 |
Sample | - | 0.05 |
D. water | 0.05 | - |
Mix well initiate the reaction by addition of:

PMS (R4) | 0.01 | 0.01 |

Measure the increase in absorbance at 560 nm for 5 min for control ($\Delta A_{control}$) and for sample ($\Delta A_{sample}$) at 25°C.

Calculation:
Percent inhibition = $\left(\frac{\Delta A_{control} - \Delta A_{sample}}{\Delta A_{control}}\right) \times 100$

Where
$\Delta A_{control}$ = the change in absorbance at 560 nm over 5 min. Following the addition of PMS to the reaction mixture in the absence of sample.
$\Delta A_{sample}$ = the change in absorbance at 560 nm over 5 min. following the addition of PMS to the reaction mixture in the presence of sample.

C. Determination of Malondialdehyde (MDA):

Principle: The method based on the thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in acidic medium at temperature of 95°C for 30 min to form thiobarbituric acid reactive product the absorbance of resultant pink product can be measured at 534 nm.

Mix well, cover the test tube with glass bead, heat in boiling water bath for 30 min, cool, then add:

Reagents:

<table>
<thead>
<tr>
<th></th>
<th>Standard</th>
<th>10 nmol/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chromogen (Thiobarbituric acid detergent stabilizer)</td>
<td>25 mmol/L</td>
</tr>
</tbody>
</table>

Procedure:

<table>
<thead>
<tr>
<th></th>
<th>Sample (ml)</th>
<th>Standard (ml)</th>
<th>Blank (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>0.2</td>
<td>-</td>
</tr>
<tr>
<td>Chromogen</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Sample | - | - | 0.2 |
Mix well; read the absorbance of sample \((A_{\text{sample}})\) against the blank and standard against d. water at 534 nm color

**Calculation:**

\[
\text{Serum} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times 10 \text{nmol/ml}
\]

(B) **Methods of collection of gastric juice:** 64 male albinowister rats were used to study gastric secretion

**A- Preparation of the animal for collecting gastric secretions:**

Rats were prepared in the same way as in induction of ulceration. Pyloric ligation was made according to (Shay *et al.*, 1954) as follows: Under light ether anesthesia, hair of the abdominal skin was shaved, and a midline incision was made extending from the xiphoid process downwards for 2 cm. The duodenum was exposed and the pyloroduodenal junction was picked up gently by a curved probe. A pyloric ligation was made by silk suture, care being taken to avoid damage of blood vessels or traction on the stomach. The abdominal wound was then closed by suturing, cleaned thoroughly with saline, dried and covered with colloid solution. The anesthesia was discontinued and the rats were left to recover for 10 min.

**B- Collection and analysis of gastric secretions:**

Indomethacin, silymarin, vit C, omeprazole are given for each rat. The titratable acidity was calculated by multiplying the volume of gastric juice (in ml) by the titratable acidity value (in mEq/L) divided by 1000 (Brodie, 1966, Brodie & Hook, 1971 and Okabe *et al.*, 1975). It was expressed as mEq/3 hours.

\[
\text{Acidity Value in mEq/L} = \left( \frac{\text{Volume of gastric juice in ml}}{\text{titratable acidity in meq/L}} \right) \times 1000
\]
Statistical analysis: Statistical analysis of the difference between groups was performed by using student t test. The data were presented in the form of mean ± standard error A value of P< 0.05 were used as the limit for statistical significance.

RESULTS

Induction of gastric ulcer by indomethacin (20mg/kg) in male albino rats:
Macroscopically: The mucosa of the corpus showed ulcers spread all over. The ulcers were present in linear streaks as blood clots on a necrotic base. The mucosa in between ulcers appeared normal or hyperemic. The ulcer index (mm) showed significant increase (P <0.001) in indomethacin treated rats (6.6 ± 0.11) as compared to their control vehicle rats (0.00± 0.00) as shown in Table (1) and Figs. (1&2).

Microscopically: Histopathological examination revealed that there were cone shaped ulcer with mucosal loss and hemorrhage. The base of ulcer showed granulation tissue. The submucosal layer and serosa showed inflammatory reactions as shown in Figs. (3&4).

Effect of treatment with silymarin & vitamin C compared with omeprazole on indomethacin-induced gastric ulceration in male albino rats.

Effect of treatment with silymarin:
Macroscopically: The mucosa of the corpus showed moderate hyperemia shown in Fig. (5). The ulcer index (mm) showed significant decrease (P <0.05) in silymarin (50 mg/kg) treated rats (5.31± 0.13) as compared to their indomethacin treated rats (6.6 ± 0.11). The curative index was 18% as shown in Table (1). Approximated by healing with narrowing of ulcer gapes with decreasing of the inflammatory reaction in submucosa, musculosa, serosa and decreasing of granulation tissue as shown in Fig. (5).

Microscopically: Histopathological examination revealed that edges of healthy mucosa approximated by healing with narrowing of ulcer gaps with decreasing of the inflammatory reaction in submucosa, musculosa, serosa and decreasing of granulation tissue as shown in Fig. (6).

Effect of treatment with vit C (200 mg/kg):
Macroscopically: The mucosa of the corpus showed mild hyperemia as shown in Fig. (7), the ulcer index (mm) showed significant decrease (P <0.01) in vit C (200 mg/kg) treated rats (4.01± 0.12) as compared to their indomethacin treated rats (6.6 ± 0.11). The curative index was 38.2% as shown in Table (1).

Table 1: Effect of treatment with silymarin & vitamin C compared with omeprazole on indomethacin-induced gastric ulceration in male albino rats.

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Group-1 Control Vehicle</th>
<th>Group-2 Indomethacin (20mg/kg)</th>
<th>Group-3 Indomethacin treated silymarin (50 mg/kg)</th>
<th>Group-4 Indomethacin treated vit c (200 mg/kg)</th>
<th>Group-5 Indomethacin treated omeprazole (3.6mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer index (mm)</td>
<td>0.0±0.00</td>
<td>6.6 ± 0.11 P &lt; 0.001***</td>
<td>5.31± 0.13 P &lt; 0.05*</td>
<td>4.01± 0.12 P &lt; 0.01**</td>
<td>0.96±0.19 P &lt; 0.001***</td>
</tr>
<tr>
<td>Preventive index(%)</td>
<td>18%</td>
<td>38.2%</td>
<td>85.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Curative index(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Values given are mean of ulcer indices ± S.E.
- Group B2 were compared to group B1 (control vehicle).
- Groups, B3, B4 and B5 were compared to group B2 (Indomethacin Treated).

Microscopically: Histopathological examination revealed that the ulcer gap is completely disappeared by healing. There are minimal inflammatory responses as shown in Figs. (3&4).

- Values given are mean of ulcer indices ± S.E.
- Group B2 were compared to group B1 (control vehicle).
- Groups, B3, B4 and B5 were compared to group B2 (Indomethacin Treated).
reactions. The vasculatures of submucous layer are within normal with regression of granulation tissue. There is minimal inflammatory reactions in submucosa, muscularosa and serosa as shown in Fig. (8).

**Effect of treatment with omeprazole:**

**Macroscopically:** The mucosa of the corpus was free of hyperemia as shown in fig (9). The ulcer index (mm) showed significant decrease ($P < 0.001$) in omeprazole (3.6 mg/kg) pretreated rats ($0.96 ± 0.19$) as compared to their indomethacin treated rats ($6.6 ± 0.11$). The preventive index was 85.2% as shown in Table (1).

**Microscopically:** Histopathological examination revealed that the mucosa appeared as normal but showed minimal inflammatory reactions in mucosa, submucosa, muscularosa and serosa as shown in Fig. (10).

Table 2: Effect of treatment with silymarin (50mg/kg) & vitamin C (200mg/kg) compared with indomethacin (3.6mg/kg) on indomethacin-induced changes in oxidants and antioxidants parameters:

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Tested Parameters</th>
<th>Group-1 Control Vehicle</th>
<th>Group-2 Indomethacin (20mg/kg)</th>
<th>Group-3 Indomethacin treated silymarin (50 mg/kg)</th>
<th>Group-4 Indomethacin treated vit c (200 mg/kg)</th>
<th>Group-5 Indomethacin treated omeprazole (3.6mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione (mg/dl)</td>
<td></td>
<td>5.53±0.24</td>
<td>2.8±0.16 $P &lt; 0.001^{***}$</td>
<td>3.1±0.23 $P &lt; 0.05^{*}$</td>
<td>3.2±0.33 $P &lt; 0.05^{*}$</td>
<td>5.08±0.24 $P &lt; 0.001^{***}$</td>
</tr>
<tr>
<td>Superoxide dismutase (U/ml)</td>
<td></td>
<td>117±0.89</td>
<td>70.5±0.85 $P &lt; 0.001^{***}$</td>
<td>75.2±0.83 $P &lt; 0.05^{*}$</td>
<td>78.6±0.28 $P &lt; 0.05^{*}$</td>
<td>90.48±0.36 $P &lt; 0.001^{***}$</td>
</tr>
<tr>
<td>Malondialdehyde (nmol/ml)</td>
<td></td>
<td>43.8±0.7</td>
<td>80.5±1.1 $P &lt; 0.001^{***}$</td>
<td>64.2±0.43 $P &lt; 0.01^{**}$</td>
<td>59.2±0.17 $P &lt; 0.001^{***}$</td>
<td>56.1±0.26 $P &lt; 0.001^{***}$</td>
</tr>
</tbody>
</table>

- Values represents mean of oxidants & antioxidants ± S.E.
- Group -2 were compared to group -1 (control vehicle).
- Groups,3,4 and 5 were compared to group 2 (Indomethacin Treated).

**Effect of treatment with vitamin C, 200mg/kg (Table2):** The value of the glutathione (mg/dl) showed significant increase ($P < 0.05$) in vitamin C (200mg/kg) treated rats ($3.2±0.33$) as compared to their indomethacin treated rats ($2.8 ± 0.16$). The value of the superoxide dismutase (U/ml) showed significant increase ($P < 0.05$) in vitamin C (200mg/kg) treated rats ($78.6 ± 0.28$) as compared to their indomethacin treated rats ($70.5 ± 0.85$). The Value of the malondialdehyde (nmol/ml) showed significant decrease ($P <0.001$) in vitamin C (200mg/kg) treated rats ($59.2 ± 0.17$) as compared to their indomethacin treated rats ($80.5 ± 1.1$).

**Effect of treatment with omeprazole, 3.6 mg/kg (Table 2):** The value of the glutathione (mg/dl) showed significant increase ($P < 0.001$) in omeprazole (3.6 mg/kg) treated rats ($5.08± 0.24$) as compared to their indomethacin treated rats ($2.8 ± 0.16$).
The value of the superoxide dismutase (U/ml) showed significant increase (P <0.001) in omeprazole (3.6 mg/kg) treated rats (90.48 ± 0.36) as compared to their indomethacin treated rats(70.5± 0.85).

The Value of the malondialdehyde (nmol/ml) showed significant decrease (P <0.001) in omeprazole (3.6 mg/kg) treated rats (56.1 ± 0.26) as compared to their indomethacin treated rats (80.5± 1.1).

**Effect of indomethacin (20mg/kg) on gastric secretions in pyloric ligated rats.**

Table (3):

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Tested Parameters</th>
<th>Group-1 Control Vehicle</th>
<th>Group-2 Indomethacin Treated (20mg/kg)</th>
<th>Group-3 Indomethacin treated silymarin (50 mg/kg)</th>
<th>Group-4 Indomethacin treated vitC (200 mg/kg)</th>
<th>Group-5 Omeprazole (3.6mg/kg) Treated Indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of juice (ml)</td>
<td>2.2± 0.06</td>
<td>3.7 ± 0.04</td>
<td>3.68±0.06</td>
<td>3.5±0.07</td>
<td>2.9± 0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &lt; 0.001**</td>
<td>P &gt;0.05 #</td>
<td>P &gt;0.05 #</td>
<td>P &lt;0.01**</td>
<td></td>
</tr>
<tr>
<td>Titratable Acidity(mEq/L)</td>
<td>73.3 ± 0.4</td>
<td>85.4 ± 0. 3</td>
<td>69.1 ± 0.36</td>
<td>60.3 ± 0.36</td>
<td>50.1 ± 0.53</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &lt; 0.01***</td>
<td>P &lt;0.05*</td>
<td>P &lt; 0.05*</td>
<td>P &lt; 0.01**</td>
<td></td>
</tr>
<tr>
<td>Total acid output (mEq/3hours)</td>
<td>0.160±0.004</td>
<td>0.305± 0.001</td>
<td>0.247±0.01</td>
<td>0.191 ± 0.008</td>
<td>0.137± 0.005</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &lt; 0.001***</td>
<td>P &lt;0.05*</td>
<td>P &lt;0.01**</td>
<td>P &lt;0.001***</td>
<td></td>
</tr>
</tbody>
</table>

- Values represents mean of gastric secretions ± S.E.
- Group -2 were compared to group-1 (control vehicle).
- Groups-3, 4 and 5 were compared to group 2 (Indomethacin Treated).

**Effect of treatment with silymarin (50mg/kg) & vitamin C (200mg/kg) compared with omeprazole(3.6mg/kg) on indomethacin induced changes in gastric secretion in male albino rats:**

**Effect of treatment with silymarin 50 mg/kg(Table 3):**

The volume of the gastric juice (ml) showed a non-significant decrease (P >0.05) in treated rats (3.7 ± 0.04). The titratable acidity (mEq/L) showed significant decrease(P <0.05) in silymarin (50 mg/kg) treated rats (69.1 ± 0.36) as compared to their indomethacin treated rats (85.4 ± 0.3).- The total acid output (mEq/3hours) showed significant decrease(P<0.05) in silymarin (50 mg/kg) treated rats (0.247 ± 0.01) as compared to their indomethacin treated rats(0.305± 0.01).

**Effect of treatment with vitamin-C 200 mg/kg(Table3):**

The volume of the gastric juice (ml) showed non-significant decrease (P >0.05) in vit c (200 mg/kg) treated rats (3.5 ± 0.07) as compared to their indomethacin treated rats (3.7 ± 0.04).- The titratable acidity (mEq/L) showed significant decrease (P < 0.05) in vit c (200 mg/kg) treated rats (60.3 ± 0.36) as compared to their indomethacin treated rats (85.4 ± 0.3).- The total acid output (mEq/3hours) showed significant decrease (P < 0.01) in vit c (200 mg/kg) treated rats (0.191 ± 0.008) as compared to their indomethacin treated rats(0.305± 0.01).
Effect of treatment with omeprazole 3.6 mg/kg (Table 3):

The volume of the gastric juice (ml) showed significant decrease (P < 0.01) in omeprazole (3.6 mg/kg) treated rats (2.9 ± 0.06) as compared to their indomethacin treated rats (3.7 ± 0.04). The titratable acidity (mEq/L) showed significant decrease (P < 0.01) in omeprazole (3.6 mg/kg) treated rats (50.1 ± 0.53) as compared to their indomethacin treated rats (85.4 ± 0.3).

The total acid output (mEq/3 hours) showed significant decrease (P < 0.001) in omeprazole (3.6 mg/kg) treated rats (0.137 ± 0.005) as compared to their indomethacin treated rats (0.305 ± 0.01).

DISCUSSION

Gastric ulcer represents a serious medical problem largely due to its frequency and high economic costs. Its incidence amounts up to 10% of the population in developed countries (Akimoto et al., 1998). Some studies have implicated the involvement of oxygen-derived free radicals in the pathogenesis of gastric ulcers and showed that antioxidants protect against gastro-duodenal injury (Yoshikawa et al., 1990; Naito et al., 1995). It is thought that free oxygen radicals and lipid peroxidation play a role in the pathogenesis of ischemia induced gastric mucosal injury by destruction of endothelial and epithelial cell components (Salim, 1992; Whittle, 2002; Jimenez et al., 2004). Some investigators suggest that superoxide and active O₂ particles lead to contractions in vascular smooth muscles by either a direct effect or interaction with nitric oxide. Ischemia induced by these contractions leads to gastro-duodenal necrosis and injury. In severe ischemia, xanthine dehydrogenase turns into xanthine oxidase, which is the first documented biological source of superoxide radicals and is considered to be an important source of oxygen radicals (Salim, 1992). In the present work, we investigated the effect of treatment with silymarin (50 mg/kg), vitamin C (200 mg/kg) and omeprazole (3.6 mg/kg) on the indomethacin induced gastric ulcer. The present data showed that intraperitoneal injection of indomethacin showed that the mucosa of the corpus contain ulcers spread all over. The ulcers were present in linear streaks as blood clots on a necrotic base. The mucosa in between ulcers appeared normal or hyperemic macroscopically. Also microscopic examination revealed that there were cone shaped ulcer with mucosal lose and hemorrhage, the base of ulcer show granulation tissue, submucosal layer and serosa showed inflammatory reactions. The present results are in agreement with the results obtained by (Takeuchi et al., 1989, Abd El-Kader et al., 2011, Nygard et al., 1994; Anthony, et al., 1996; Whittle, 2002, Scarpignato and Hunt, 2010). Cheung and Ashley, 1987, claimed that gastric mucosal ischemia is the primary factor that leads to ulceration. Ueki et al., (1988, claimed that stimulated gastric hypermotility is an important factor in the pathogenesis of indomethacin induced gastric mucosal lesions in rats. The present data showed...
that, Intraperitoneal injection of indomethacin lead to decreased gastric reduced glutathione (GSH) level, superoxide dismutase (SOD) level and increase Malondialdehyde (MDA) level. These results are in agreement with the studies of Dengiz et al., 2007 and Bilici et al., 2009 where they found lower concentrations of GSH in indomethacin ulcerated gastric tissues. Basivireddy et al., (2003) reported that decreased SOD activity and release of ROS produce gastric damage in indomethacin ulcerated gastric tissues, and Demircan et al., 2005; Bayir et al., 2006 and Dengiz et al., 2007 reported that MDA levels in stomach tissue increase in indomethacin-induced gastric damage. This decrease in glutathione (GSH) level because of its consumption in removal of oxygen radicals. Our results were further supported by the data obtained from Morsy and Fouad, 2008 who reported depletion in glutathione contents in gastric tissues of indomethacin-treated rats. The free radicals produced from infiltration of neutrophils induce lipid peroxidation and changes in membrane lipid composition that further aggravates gastric damage Sandhya and Varalakshmi, 1997. This view is supported by increased lipid peroxidation products in stomach tissues of indomethacin-treated rats Tamura et al., 2001. Consistent with this finding, we found elevated lipid peroxide levels in the indomethacin-treated group associated with necrosis of gastric mucosa and hypertrophy in muscularis. The scavenging of superoxide radicals is achieved through superoxide dismutase enzyme, which catalyzes the dismutation of superoxide to H$_2$O$_2$. The reduction in superoxide dismutase activity after indomethacin treatment has been previously recorded Motawiet et al., 2007 suggesting that oxidative stress is one of the causes of indomethacin-induced gastropathy. The present data showed that intraperitoneal injection of indomethacin leads to increased the mean gastric juice volume, mean titratable acidity, mean total acid output. These results are in agreement with the studies done by Feldman and Colturi (1984); Rifat-uz-Zaman et al., (2005); Ajeigbe et al., 2008 were observed that indomethacin increased the gastric juice volume, mean titratable acidity and total acid output. On the other hand, these results are in contrast with the findings of Nicoloff, (1967) who found that indomethacin induced a decrease in gastric acid output. Another group, however, found that indomethacin at the ulcerogenic dose had no effect on gastric acid output (Ueki et al., 1988 and Takeuchi et al., 1989). These gastric changes have been reported due to its lipid peroxidation/apoptosis activity (Smith and Marnett, 1991). Also due to the inhibitory effects of indomethacin on prostaglandin production (Yokotani et al., 1996). The present data showed that treatment with silymarin (50mg/kg), vit C (200mg/kg) and Omeprazole (3.6mg/kg) significantly reduced the ulcer index in Indomethacin induced gastric ulcer in rats. These results are in agreement with the study done by Nishikawa et al., (2005). They reported that pretreatment with vitamin C prevents gastric ulceration in stressed rats and this was explained by its antioxidant effect. Furthermore, the present data are in agreement with the previous study recorded by Murat et al., (2008). They investigated the gastro protective and antioxidant properties of ascorbic acid on indomethacin-induced gastric injury in rats. Furthermore, the present data are in agreement with the previous study recorded by S. Samini et al., (2013). They investigated antiulcer effects of coenzyme Q10 and vitamin C on indomethacin induced gastric ulcer in rats. The present data are in agreement with the study done with Shobha and Jamadar, (2012). They found that silymarin has significant antiulcer
activity. It perhaps acts by decreasing hydrochloric acid output and increasing buffering power. The present data are in agreement with the study of Samaret al. (2013) who found a preventive effect of omeprazole against indomethacin-induced gastric ulcer in rats. Vitamin C is the most important water-soluble biological antioxidant, it can scavenge the reactive oxygen species (ROS) e.g. hydrogen peroxide (H2O2) and the superoxide radical, which shown to play a critical role in the gastric ulceration process (Ozdil S., et al. 2004). Also, ascorbic acid attenuates the deleterious effect of indomethacin on ulcer healing due to its anti-oxidant activity by mechanism involving preservation of gastric microcirculation, attenuation of lipid peroxidation and release of proinflammatory cytokines (Konturek PC, et al., 2006). Administration of both indomethacin and vitamin C produced less gastric mucosal damage due to the increase in expression and activity of hemeoxygenase-1 (HO-1). HO-1 plays an important role in gastric protection against indomethacin, by making cells more resistant to apoptotic death (Zhu GH, et al., 2000). The possible mechanism is that silymarin stimulates DNA-dependent RNA polymerase, leading to increased protein synthesis and thus promoting healing and reparative processes as explained by Alarcon de la lastra et al., (1992). A recent study showed antioxidant and antiapoptotic role of omeprazole to block gastric ulcer through scavenging of endogenous hydroxyl radical associated lipid peroxidation and protein oxidation, indicating that its antioxidant role plays a major part in preventing oxidative damage. Omeprazole prevents loss of membrane permeability and dysfunction of the cellular proteins, leading to survival of the functionally active cells. Moreover, it offers an antiapoptotic effect by blocking DNA fragmentation during ulceration (Biswas K, et al., 2003). The present data showed that treatment with silymarin (50mg/kg), vit C (200mg/kg) and Omeprazole (3.6mg/kg) significantly increase gastric reduced glutathione (GSH) level, superoxidedismutase (SOD) level and decrease Malondialdehyde (MDA) in indomethacin induced gastric ulcer in rats. The present data are in agreement with the study done by Murat et al., (2008) where they found administration of indomethacin caused a significant decrease in the levels of superoxide dismutase, glutathione peroxidase, glutathione S -transferase and glutathione, and an increase in the lipid peroxidation level the administration of ascorbic acid reversed the trend. The present data are in agreement with the study done by Shobha and Jamadar, (2012) where they found that cytoprotective action of silymarin could be by prevention of peroxidative processes by increasing superoxide dismutase (SOD) and glutathione levels, increases the endogenous levels of antioxidants. The present data are in agreement with the study done by Goksel et al. (2001) where they found that acetyls-alicylic acid treatment decreased significantly the gastric GSH levels, and pretreatment with omeprazole, famotidine, or melatonin increased it significantly. The present data are in agreement with the study done by Nermin and Anaam, (2014) where they found that both omeprazole and L-carnitine significantly increased gastric total antioxidant capacity and SOD activity. Glutathione is an important intracellular antioxidant that also plays a role in the detoxification and elimination of potential carcinogens and toxins. Studies in animals have found that glutathione synthesis and tissue glutathione levels are significantly lower in aged animals than in younger animals, leading to decreased ability of aged animals to respond to oxidative stress or toxin exposure (Hagen et al., 2000). Superoxide dismutase plays an important role in eliminating gastric
damage by partially preventing oxidative damage and it destroys the highly reactive radical O^·-G_2 by converting it into the less reactive H_2O_2 that can be destroyed by CAT. Furthermore, the relation between SOD activity and prostaglandin synthesis may be a possible mechanism to explain indomethacin-induced ulcer (El-Missiry et al., 2001). The possible mechanism might be silymarin being lipid soluble diffuses into the biological membrane, as acute stress depletes glutathione levels, pretreatment with silymarin replenishes glutathione and superoxide dismutase (SOD) levels. The peroxyl and alkoxyl radicals generated during oxidative stress are scavenged thus preventing peroxidation mediated injury. Thus it can be proposed that silymarin protects gastric mucosa against stress injury by its antioxidant and scavenging activity (Alarcon de la lastra C., et al., 1992). The present data showed that treatment with silymarin (50mg/kg), vitamin C (200mg/kg) and Omeprazole (3.6mg/kg) significantly decrease the mean acid concentration and total acid output. The present data are in agreement with the study done by Nitin Mahurkar et al. (2014) who found that the parameters like free acidity, total acidity, volume of gastric juice, pH, and ulcer index have shown significant reduction in their values when given vitamin C. The present data are in agreement with the study done by Shobha and Jamadar, (2012) who found that silymarin significantly reduced free and total acidity, gastric juice volume, total acid output, and combined acid content. The possible mechanism of the antiulcer effect of silymarin might be its ability to decrease the HCl secreted by gastric glands in pylorus-ligated rats. The possible mechanism of the antiulcer effect of vitamin C may be the antioxidant activity which neutralizes all the free radicals, nitrates, nitrites that cause cellular damage. An oral supplement of vitamin C is sufficient to maintain the gastric blood flow, intragastric vitamin C levels, antioxidant enzyme activities, which is impaired due to peptic ulcer disease. Further it translates HO-1 mRNA into active protein, which then may exert gastro protection by its antioxidant and vasodilator properties. The present data are in agreement with the study done by Osamu Yamamoto et al., (1984) who found that omeprazole has a potent and long-lasting activity on gastric secretion in rats and the possible mechanism of antisecretory activity of omeprazole might be by inhibiting gastric secretion in humans and experimental animals through a specific inhibition of the proton pump in parietal cells. In conclusion, treatment with silymarin, vitamin C and omeprazole reduced the mean ulcer index, the oxidant parameter; malondialdehyde, the total gastric volume, titratable acidity and total acid output and increase of antioxidants parameters; glutathione and superoxide dismutase and produced a significant healing effect in the indomethacin-induced gastric ulceration in male albino rats.

REFERENCES


Shaker, E., Mahmoud, H., and Mnaa, S. (2010): Silymarin, the antioxidant component and Silybummarianum
extracts prevents liver damage. Food and Chemical Toxicology. Biological Research Association, 48(3):803-806.


Fig. 1: Naked eye appearance of gastric mucosa in control vehicle rats.

Fig. 2: Naked-eye appearance of the effect of 20 mg/kg indomethacin I.P on gastric mucosa.

Fig. 3: Photomicrograph of rat gastric mucosa of control vehicle rats.

Fig. 4: Photomicrograph of rat gastric mucosa given 20mg/kg indomethacin.

Fig. 5: Naked eye appearance of the effect of treatment with 50 mg/kg silymarin on indomethacin-induced gastric ulcer in male albino rats.
Study of the role of the antioxidants silymarin and vitamin C in of the indomethacin-induced

Fig. 6: Photomicrograph of rat gastric mucosa treated with 50 mg/kg silymarin on indomethacin induced gastric ulcer in male albino rats.

Fig. 7: Naked eye appearance of the effect of treatment with 200 mg/kg vit C on indomethacin induced gastric ulcer in male albino rats.

Fig. 8: Photomicrograph of rat gastric mucosa treated with 200 mg/kg vit C on indomethacin induced gastric ulcer in male albino rats.

Fig. 9: Naked eye appearance of the effect of treatment with omeprazole (3.6 mg/kg) on indomethacin induced gastric ulcer in male albino rats.

Fig. 10: Photomicrograph of rat gastric mucosa treated with 20 mg/kg indomethacin then (3.6 mg/kg) omeprazole.
دراسة دور مضادات الأكسدة (السيليلامين وفيمتامين ج) في علاج فرة المعدة الحميدة في ذكور الجرادان البيضاء

حسن هلالى على ابرر حمهم – عبد السلام أحمد عطية – وليد نصر الدين بدو همام

قسم الفارماكولوجي كلية الطب – جامعة الأزهر (السويس)

تعتبر فرة المعدة من المشاكل الصحية ذات الخطورة، وذلك يترتب على تسمم جلودة ونفاذ أو انسداد الألتهابات والمصابات مثل الإسهال والإسهاليني، فعندما يعاني الإنسان أو الحيوان في حالات فرة المعدة، يؤدي ذلك إلى نقص في شحنة الأحماض الهيدروكلورية، حيث يعاني الجرادان البيضاء النشط من فرة المعدة ويدفعه إلى تأثير هذه الدراسة.

باستخدام إنجاز دراسة تأثير عقار الإوميبازول على علاج فرة المعدة الأوميبازول في الجرادان البيضاء، ويتم استخدام الفيمتامين ج، كما يتم استخدام مضادات الأكسدة (السيليلامين) في العلاج.

تُستعمل هذه الفئات في فترات معينة من الحياة، لعلاج فرة المعدة الحميدة في ذكور الجرادان البيضاء.

وفي هذه الدراسة تم استخدام جرادان التحدي البضاء الذكور (الاستغلال الذئبي) لاستخدام الفيمتامين ج. حصلت دراسة تأثير الفيمتامين ج في فرزة المعدة الحميدة. وقد تم استخدام مضادات الأكسدة (السيليلامين) في الجرادان البيضاء.

باستخدام إنجاز دراسة تأثير الفيمتامين ج في علاج فرة المعدة الأوميبازول في الجرادان البيضاء، يتم استخدام مضادات الأكسدة (السيليلامين) في العلاج.

تُستعمل هذه الفئات في فترات معينة من الحياة، لعلاج فرة المعدة الحميدة في ذكور الجرادان البيضاء.

باستخدام إنجاز دراسة تأثير الفيمتامين ج في علاج فرة المعدة الأوميبازول في الجرادان البيضاء، يتم استخدام مضادات الأكسدة (السيليلامين) في العلاج.

تُستعمل هذه الفئات في فترات معينة من الحياة، لعلاج فرة المعدة الحميدة في ذكور الجرادان البيضاء.

باستخدام إنجاز دراسة تأثير الفيمتامين ج في علاج فرة المعدة الأوميبازول في الجرادان البيضاء، يتم استخدام مضادات الأكسدة (السيليلامين) في العلاج.

تُستعمل هذه الفئات في فترات معينة من الحياة، لعلاج فرة المعدة الحميدة في ذكور الجرادان البيضاء.

باستخدام إنجاز دراسة تأثير الفيمتامين ج في علاج فرة المعدة الأوميبازول في الجرادان البيضاء، يتم استخدام مضادات الأكسدة (السيليلامين) في العلاج.

تُستعمل هذه الفئات في فترات معينة من الحياة، لعلاج فرة المعدة الحميدة في ذكور الجرادان البيضاء.

باستخدام إنجاز دراسة تأثير الفيمتامين ج في علاج فرة المعدة الأوميبازول في الجرادان البيضاء، يتم استخدام مضادات الأكسدة (السيليلامين) في العلاج.

تُستعمل هذه الفئات في فترات معينة من الحياة، لعلاج فرة المعدة الحميدة في ذكور الجرادان البيضاء.

باستخدام إنجاز دراسة تأثير الفيمتامين ج في علاج فرة المعدة الأوميبازول في الجرادان البيضاء، يتم استخدام مضادات الأكسدة (السيليلامين) في العلاج.

تُستعمل هذه الفئات في فترات معينة من الحياة، لعلاج فرة المعدة الحميدة في ذكور الجرادان البيضاء.

باستخدام إنجاز دراسة تأثير الفيمتامين ج في علاج فرة المعدة الأوميبازول في الجرادان البيضاء، يتم استخدام مضادات الأكسدة (السيليلامين) في العلاج.

تُستعمل هذه الفئات في فترات معينة من الحياة، لعلاج فرة المعدة الحميدة في ذكور الجرادان البيضاء.

باستخدام إنجاز دراسة تأثير الفيمتامين ج في علاج فرة المعدة الأوميبازول في الجرادان البيضاء، يتم استخدام مضادات الأكسدة (السيليلامين) في العلاج.

تُستعمل هذه الفئات في فترات معينة من الحياة، لعلاج فرة المعدة الحميدة في ذكور الجرادان البيضاء.

باستخدام إنجاز دراسة تأثير الفيمتامين ج في علاج فرة المعدة الأوميبازول في الجرادان البيضاء، يتم استخدام مضادات الأكسدة (السيليلامين) في العلاج.

تُستعمل هذه الفئات في فترات معينة من الحياة، لعلاج فرة المعدة الحميدة في ذكور الجرادان البيضاء.

باستخدام إنجاز دراسة تأثير الفيمتامين ج في علاج فرة المعدة الأوميبازول في الجرادان البيضاء، يتم استخدام مضادات الأكسدة (السيليلامين) في العلاج.

تُستعمل هذه الفئات في فترات معينة من الحياة، لعلاج فرة المعدة الحميدة في ذكور الجرادان البيضاء.

باستخدام إنجاز دراسة تأثير الفيمتامين ج في علاج فرة المعدة الأوميبازول في الجرادان البيضاء، يتم استخدام مضادات الأكسدة (السيليلامين) في العلاج.

تُستعمل هذه الفئات في فترات معينة من الحياة، لعلاج فرة المعدة الحميدة في ذكور الجرادان البيضاء.

باستخدام إنجاز دراسة تأثير الفيمتامين ج في علاج فرة المعدة الأوميبازول في الجرادان البيضاء، يتم استخدام مضادات الأكسدة (السيليلامين) في العلاج.

تُستعمل هذه الفئات في فترات معينة من الحياة، لعلاج فرة المعدة الحميدة في ذكور الجرادان البيضاء.

باستخدام إنجاز دراسة تأثير الفيمتامين ج في علاج فرة المعدة الأوميبازول في الجرادان البيضاء، يتم استخدام مضادات الأكسدة (السيليلامين) في العلاج.

تُستعمل هذه الفئات في فترات معينة من الحياة، لعلاج فرة المعدة الحميدة في ذكور الجرادان البيضاء.

باستخدام إنجاز دراسة تأثير الفيمتامين ج في علاج فرة المعدة الأوميبازول في الجرادان البيضاء، يتم استخدام مضادات الأكسدة (السيليلامين) في العلاج.

تُستعمل هذه الفئات في فترات معينة من الحياة، لعلاج فرة المعدة الحميدة في ذكور الجرادان البيضاء.