INTRODUCTION

Peptic ulcer occurs when there is an imbalance between the damaging effects of gastric acid and pepsin, and the defense mechanisms, which protect the gastric and duodenal mucosa from these substances. The danger of epithelial arrosion and subsequent ulcer formation exists whenever the protective and reparative mechanisms are weakened and/or the chemical attack by the acid–pepsin mixture is too strong and persists for too long. The majority of gastric ulcers can be attributed to either H. pylori or NSAID-induced mucosal damage, particularly in the elderly. NSAIDs cause serious complications only in a small fraction of NSAID users. Hence, NSAID prophylaxis is advised for those older than 654, cases of cardiac disease5, those with previous history of ulcers or upper GI bleed4,5, and those using anticoagulants and corticosteroids4.

Recent studies suggest that NSAID-induced ulcers in at-risk patients can be prevented largely through co-administration of a proton pump inhibitor, such as Omeprazole that bind to the H+/K+-ATPase enzyme system (proton pump) of the parietal cell and suppress the secretion of hydrogen ions into the gastric lumen. This irreversibly inactivates the enzyme causing profound inhibition of acid secretion: a single 20 mg dose reduces gastric acid output by 90% over 24 h. Omeprazole is degraded at low pH and must be given in enteric-coated granules1. The coating is removed in the alkaline duodenum, and the prodrug, a weak base, is absorbed and transported to the parietal cell canaliculus. There, it is converted to the active form. Clinical studies have shown that PPIs reduce the risk of bleeding from an ulcer caused by aspirin and other NSAIDs7.

Folic acid (or folate), is a water-soluble vitamin8. Folic acid is used to prevent or cure deficiency of folate which is due either to a decreased supply or to an increased requirement. Deficiency of folic acid leads to a megaloblastic anaemia because it is necessary for the production of purines and pyrimidines, which are essential precursors of deoxyribonucleic acid (DNA)24. Folate modulates a number of disorders as a result of its anti-apoptotic and anti-oxidative properties9. The gastroprotective activity of folic acid supplementation at the basal requirement supplemental dose of 2 mg/kg diet against the lipid peroxidative activity of indomethacin was mentioned10.

MATERIALS AND METHODS

Animals: Fifty male albino rats of the Wistar strain weighing between 180-250 g were used for this study. The animals were separated randomly into ten cages of five rats each where they were kept for four weeks before the start of the experiment. Indomethacin was given to the rats by subcutaneous injection of 20 mg/kg i.p. daily for 7 days. The rats were separated into four groups with each containing 10 rats:

1. A control group
2. A group given folic acid (2 mg/kg, orally)
3. A group given Omeprazole (20 mg/rat, orally)
4. A group given folic acid and Omeprazole

Ulcers were induced by administration of indomethacin (20 mg/kg i.p.) for 7 days. The gastric ulcers in stomach's rat were examined histological and macroscopically. The ulcer Index, the protective Index, and the ulcer healing Index were calculated. Then the glycoproteines of stomach's mucus gel was measured by spectrophotometer. The combination-treated group (Folic acid and Omeprazole) gave significant increase in the amount of gastric mucosa as compared to Indomethacin group and in comparing to each drug alone the protective Index was increased while ulcer healing Index was decreased. It could be concluded that the combination-treated groups afford a good gastro-protective potential against the gastric ulceration induced by indomethacin better than each drug alone. The antioxidant effects of folic acid and omeprazole are involved and ameliorating the oxidative stress induced by indomethacin in the gastric mucosa.

Keywords: Ulcer, Omeprazole, Folic acid.
of the experiment. The animals were housed under standard conditions of temperature (23 ± 2°C), humidity (55 ± 15%) and 12 hour light (7.00 am - 7.00 pm). The cages were constantly cleaned in order to prevent the animals from contracting disease. They were fed with standard commercial rat pellets and allowed water ad libitum.

**Experimental Design**

Table 1: Effect of folic acid, omeprazole and combination between them on ulcer number, ulcer acuity, ulcer index, and preventive index in indomethacin- (INDO-; 20 mg/kg, i.p.) induced gastric injury.

<table>
<thead>
<tr>
<th>Number of animals</th>
<th>Ulcer number</th>
<th>ulcer acuity</th>
<th>percentage of animals with ulcers / 10</th>
<th>Ulcer index</th>
<th>preventive index</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONT</td>
<td>10</td>
<td>0.4 ± 0.266</td>
<td>0.2±0.133</td>
<td>2</td>
<td>2.6</td>
</tr>
<tr>
<td>INDO</td>
<td>9</td>
<td>9.22±1.128**</td>
<td>3.77±0.547**</td>
<td>10</td>
<td>22.99</td>
</tr>
<tr>
<td>FOLIC</td>
<td>10</td>
<td>8.6±1.147</td>
<td>3.4±0.4</td>
<td>10</td>
<td>22</td>
</tr>
<tr>
<td>OMEPRAZOLE</td>
<td>7</td>
<td>6.57±0.841</td>
<td>2±0.488*</td>
<td>10</td>
<td>18.57</td>
</tr>
<tr>
<td>OMEPRAZOLE + FOLIC</td>
<td>8</td>
<td>3.125±0.895*</td>
<td>1.25±0.313*</td>
<td>7.5</td>
<td>11.875</td>
</tr>
</tbody>
</table>

*Parametric data were expressed as mean ± S.E.M. P < 0.05. As compared to control (CONT) (**). As compared to INDO (*).
Grouping: The animals were divided into five groups of ten rats each.

- **Group One (NORMAL):** Animals were treated with normal saline for seven days. They were called the control group.
- **Group Two (INDO):** Animals were treated with normal saline for seven days before indomethacin (CSPC Ouyi pharmaceutical.co) administration (20 mg/kg).
- **Group Three (FOLIC):** Animals were treated with 2 mg/kg of folic acid (Ibn Sina laboratories) for seven days before indomethacin administration (20 mg/kg).
- **Group Four (OMEPRAZOLE):** Animals were treated with omeprazole (ASIA Pharmaceutical Industries) for seven days (20 mg/rat) then indomethacin was administrated (20 mg/kg). This group served as the positive control group.
- **Group Five (FOLIC + OMEPRAZOLE):** Animals were treated with omeprazole (10 mg/rat) + folic acid (2 mg/kg) for seven days then indomethacin was administrated (20 mg/kg).

The route of administration for folic acid was oral. Folic acid was dissolved in normal saline to give a suspension. Omeprazole pellets were administrated by feeding tube without dissolving to reach the duodenum in this form and protect them from degradation at low pH in the stomach.

Operative procedure: After seven days of treatment with the appropriate drug for each group, animals were sacrificed as follow: The rat was anesthetized and a midline incision was made and the stomach is removed, then the stomach was opened along the greater curvature, stretched moderately by pinning on a cork board, and then the gastric mucosa was examined by naked eye and magnifying lens to:
- count the number of lesions.
- determine the ulcer acuity for each group. Gastric lesions were scored according to the following system:
  - 0: no lesion;
  - 1: <5 lesions, all <2 mm;
  - 2: <5 lesions, at least one lesion >2 mm;
  - 3: 5-10 lesions, all <2 mm;
  - 4: 5-10 lesions, at least one lesion >2 mm;
  - 5: >10 lesions, all <2 mm;
  - 6: >10 lesions, at least one lesion >2 mm.

Each lesion was considered as an ulcer to calculate the ulcer index (UI) as described by Robert 15:

\[
UI = \frac{a + b + c}{10}
\]

- **a**: percentage of animals with ulcers
- **b**: average of acuity score
- **c**: average of number of ulcers per group animals

Then the preventive index (P.I.) of a given drug was calculated by the equation of Hano et al 16.

\[
P.I. = \frac{U.I. \text{ of IND group} - U.I. \text{ of pretreated group}}{X 100}
\]

**Histopathological techniques:** Each stomach was fixed with 10% formalin solution for 24 hours. After fixation, five specimens were taken from each stomach. Then sections were stained with conventional EH stain; at least four sections were prepared from each animal. The stained sections were examined under light microscope and the relevant data were registered.
The severity and the degree of mucosal damage were assessed according to modified Sedny scale, so that the following grades were obtained: Grade (0): no mucosal lesions; Grade (1): mucosal edema, congestion, and neutrophils infiltration; Grade (2): surface mucosal erosion. Grade (3): ≤ 2 Gastric ulcers. Grade (4): > 2 Gastric ulcers.

Mucin Content Determination: The gastric mucin was determined according to the method described by Winzler17. Briefly, to diluted samples orcinol (1.6%) and sulphuric acid (60%) were added, vortexed, and boiled for 10min. Mixtures were cooled in ice-cold water to stop the reaction and the absorbance was measured at 425 nm.

**STATISTICAL ANALYSIS**

All obtained values were expressed as mean + standard error of mean (SEM). By using Mann-Whitney test the proportion of histopathological changes and ulcer acuity in various groups of animals were compared. While the significance of differences between means of ulcers number and mucin content were calculated using the student’s t-test. P-values < 0.05 were considered significant. All analysis utilized Prizm4.

**RESULTS**

Macroscopic study: The macroscopic findings of the opened stomach are shown in Fig. 1. Numerous, tiny, pin-point petechial hemorrhages (figure 3) and large erosions (figure 2) were observed in the indomethacin administered group (INDO, Group 2). Folic acid alone (FOLIC, Group...
Table 3: Effect of folic acid, omeprazole as well as their combination on mucin secretion in indomethacin-induced (INDO; 20 mg/kg, i.p.) gastric injury. Values are means ± SEM; comparisons were carried out using Student t-test, P < 0.05. As compared to control (CONT)(**); INDO (*).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of animals</th>
<th>Glycoprotein concentration in mucin SEM ± X</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL</td>
<td>10</td>
<td>8.309 ± 0.346</td>
</tr>
<tr>
<td>INDO</td>
<td>9</td>
<td>2.031 ± 0.183**</td>
</tr>
<tr>
<td>FOLIC</td>
<td>10</td>
<td>2.536 ± 0.243</td>
</tr>
<tr>
<td>OMEPRAZOLE</td>
<td>7</td>
<td>3.655 ± 0.426*</td>
</tr>
<tr>
<td>OMEPRAZOLE + FOLIC</td>
<td>8</td>
<td>±0.558 *</td>
</tr>
</tbody>
</table>

Values given as mean ± SEM. P < 0.05. As compared to control (CONT) (**), INDO (*).

DISCUSSION
Experimental studies have demonstrated that nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin are capable of producing injury to gastrointestinal mucosa in experimental animals and humans18. In the present study, injecting indomethacin intraperitoneally (20 mg/kg) induced raise in ulcer acuity, number of ulcers and ulcer index, and decrease mucin content (75%) as compared to control group. Research works on the effect of indomethacin on the gastric mucosa are in agreement with our results. In a previous study, indomethacin decrease gastric mucin concentration by 55.7% and increase ulcer index12. Histological observation showed comparatively extensive damage to the gastric mucosa, and oedema and leucocytes infiltration of the submucosal layer in most of the animals in indomethacin group. There was a statistically significant difference on gastric erosion score between indomethacin group and control group (p <0.05).

The molecular basis for the gastrointestinal toxicity of NSAIDs is widely believed to their inhibitory activity against cyclooxygenase, which causes them to block the production of prostaglandins18. The physiological function of mucosal prostaglandins is cytoprotective, by inhibiting acid secretion, promoting the secretion of mucus and strengthening resistance of the mucosal barrier to back-diffusion of acid from the gastric lumen into the submucosal tissues where it causes damage1. NSAIDs significantly decreased the mucosal prosta-glandin E2 (PGE2) concentration. Characteristically, the damage was observed along the long axis of the stomach and consisted mostly of hemorrhagic lesions, with a few non-hemorrhagic lesions22. Suppression of prostaglandin synthesis is associated with reduction of gastric mucosal blood flow, disturbance of microcirculation, decrease in mucus secretion, lipid peroxidation, and neutrophil activation, which are involved in the pathogenesis of gastrointestinal mucosal disorders18. All NSAIDs, except aspirin, increased gastric motility at ulcerogenic doses, leading to the development of gastric lesions. Gastric hypermotility causes microvascular disturbances, especially at specific sites on mucosal folding, leading to various events including neutrophil-endothelial interaction. In addition, indomethacin caused oxyradical production and lipid peroxidation in the gastric mucosa, probably resulting from the ischemic-reperfusion changes due to rhythmic hypercontraction of the stomach that is mediated by a vagal-cholinergic mechanism22. Stress, nonsteroidal anti-inflammatory drugs, and H. pylori cause mucosal damage through a number of mechanisms, of which some reactive oxygen species (ROS) such as O2•- and OH• are now considered to be one of the major causative factors for mucosal lesions through oxidative damage. Lipid peroxidation, an important parameter for...
OH•-induced oxidative damage of membrane, is increased in gastric lesions caused by ethanol, indomethacin, and water immersion stress. Increased lipid peroxidation, increased protein oxidation, and decreased glutathione level are also evident in restraint cold stress-induced gastric lesions as a result of oxidative damage caused by the significant generation of OH•. Recent studies also indicate that programmed cell death or apoptosis plays a significant role in gastric ulceration. Gastric mucosal lesions caused by stress, indomethacin, ethanol, and H. pylori are also due to increased cell death by apoptosis. The gastroprotective activity of folic acid supplementation at the basal requirement supplemental dose of 2 mg/kg diet against the lipid peroxidative activity of indomethacin was mentioned. Folate, an important factor in the de novo synthesis of purines, and thymidine, Deoxyribonucleic acid (DNA) stability, and apoptosis, is able to attenuate the development of gastric ulcer. Increase the superoxide dismutase and mucus concentration observed in the folic acid pre-treated group suggests gastroprotective activity of folic acid, because they are scavengers which mop up and resist free radicals predisposing the stomach to inflammation. Moreover, this is underscored by a decrease in the MDA concentration observed in this group of animals. This could mean that folate inhibits the lipid peroxidative activity of indomethacin. Folic acid (2 mg/kg) was used in previous studies for 21 days. It showed a gastroprotective activity according to its antioxidative and antisecretory properties. The combination between omeprazole and folic acid was able to attenuate gastric ulceration induced by indomethacin. Ulcer index was low in combination group compared to the indomethacin-induced ulcer group. The combination between omeprazole and folic acid was able to attenuate gastric ulceration induced by indomethacin. Ulcer index was low in combination group compared to the indomethacin-induced ulcer group. Pretreatment of Omeprazole and folic acid 1 hour before administration of indomethacin resulted in a decrease in mean gastric erosion score.

In addition to the gastroprotective effect of omeprazole through inhibiting gastric acid secretion, 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. As a result, the effect of co-administration of folic acid and omeprazole in preventing indomethacin-induced gastric ulcer can be explained through their antioxidant role. So they are able to attenuate oxidative stress induced after indomethacin administration. Indomethacin produces an increase in formation of ROS that catalyze lipid peroxidative and gastric epithelial damage, while folic acid and omeprazole play antioxidant and antisecretory roles, scavenge ROS and reduce their harmful effects thus as a result prevent gastric ulcer formation.

CONCLUSIONS
The combination between folic acid and omeprazole have preventive effect against indomethacin-induced gastric ulcer in rats. Using folic acid (2 mg/kg) for seven days is ineffective in preventing indomethacin-induced gastric ulcer in rats.

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