The Possible Protective Effect of Ocimum Basilicum L. Aqueous Extract on Aspirin Induced Damage of Gastric Mucosa in Adult Male Albino Rats: Histological and Ultrastructural Study

Karima F. Abdelfadeel, Zeinab M. Alazouny

Departments of Histology and Cell Biology, Faculty of Medicine, Zagazig University, Egypt.

ABSTRACT

Background and Objectives: One of the new strategies to limit the gastric mucosal injury induced by aspirin is adding antioxidant. Therefore, our aim was to detect histological changes that may occur in fundic mucosa of adult male albino rats given aspirin and the protective role of Ocimum Basilicum administration.

Materials and Methods: Thirty adult male albino rats were classified into three groups: Group I (control group, which were subdivided into two equal subgroups, Group II (aspirin treated group, given aspirin in a dose of 12.5 mg/kg by orogastric tube for 4 weeks) and Group III (coadministration group, given aspirin in the same previous dose in concomitant with Basil in a dose of 200 mg/Kg b.w by orogastric tube).

Results: Fundic mucosa of aspirin treated group showed disorganized fundic glands, desquamation of surface epithelial cells, mononuclear cell infiltration and congested blood vessels. Many parietal cells exhibited small nuclei with increased amount of heterochromatin, dilated canaliculi containing few short microvilli and vacuole-like structures. Many eosinophils were seen in lamina propria. Many chief cells appeared with shrunken pyknotic nuclei and vacuolated cytoplasm. Statistically significant increase in the mean area percent of collagen fibers and decrease in the mean thickness of mucous film in PAS stained sections of fundic mucosa of aspirin treated group. These changes were reduced in the coadministration group.

Conclusion: Ocimum Basilicum aqueous extract has beneficial protective effects against the deleterious effects of aspirin on the fundic mucosa. Therefore, it may be a suitable nutritional supplement or therapeutic drug to protect against aspirin-induced gastric damage.

Key Words: Aspirin, fundic mucosa, ocimum basilicum, ultrastructure.

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin are widely used as anti-inflammatory and analgesic agents. Many people take an anti-inflammatory drug for arthritis, muscular pains, etc. Aspirin is also used by many people to protect against blood clots forming. However, these drugs cause gastric erosion as an important adverse effect by inhibiting prostaglandin biosynthesis. Sometimes they affect the mucous barrier of the stomach and allow acid to cause an ulcer. Therefore, effective strategies to protect the gastrointestinal mucosa are required.

There are enormous chemical agents available for the treatment of gastritis and gastric ulcer but proclaim serious side effects like H2 antagonists that are known to be the precipitating cause of impotence, headache, skin rash, arrhythmias whereas the use of proton pump inhibitors is a cause for atrophic gastritis. The use of antacids leads to stomach distention, belching, constipation and there is risk of ulcer perforation and other drugs like anticholinergics induce constipation, dry mouth, urinary retention, blurred vision, xerostomia and precipitation of glaucoma. In addition, the clinical evaluation of these drugs showed development of tolerance, incidences of relapse and side effects that make their efficacy arguable. It is the rationale for the development of new antiulcer drugs, which includes herbal drugs.

In traditional medicine, the plants and herbs are used for prevention and treatment of different gastrointestinal illnesses, including peptic ulcers without side effects since prehistoric times. Realizing the importance of plants in the discovery of new and safer therapeutic agents, screening of herbs for pharmacological activities and phytochemical constituents is one of the active fields of research round the world today.

Ocimum basilicum L. (Lamiaceae), commonly known as Sweet Basil, is part of a group of medicinal plants
widely used in cooking and known for its beneficial health properties. It belongs to the genus Ocimum of the family Lamiaceae. Ocimum (from Greek ozo for smell) is appropriate for the genus since its various species are known for their peculiar strong odours. Basilicum is the Latin translation of the Greek basilikon meaning king. Among more than 150 species of the genus Ocimum, basilicum (Basil) is the major essential oil crop which is cultivated commercially in many countries. It can be found in tropical Asia, Africa, Central America and South America[7,8].

Ocimum basilicum L., is an aromatic herb used extensively for its distinctive aroma and for food flavoring. The leaves can be used fresh or dried as a spice. Essential oils extracted from the fresh leaves and flowers can be used as food additives, and in pharmaceuticals and cosmetics[9]. Traditionally, Basil has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms, and kidney malfunctions[10].

There are many laboratory studies have shown various protective effects of Basil including chemopreventive activity, anti-inflammatory activity, bactericidal activity, a nervous system stimulant effect, modulator effect on glutathione and antioxidant enzymes, antidiarrheal effects, deduced risks of cancer, protective effects against hypercholesterolemia and age-related eye diseases, and blood-sugar lowering effect[11, 12, 13].

In modern medicine also, plants occupy a very significant place as raw material for some important drugs used as protective and therapeutic agents in many diseases. Also, use of plants for curing various diseases are not confined to the doctors only but is known to several households as well[14]. Therefore, the aim of this study was to investigate the possible protective effects of Ocimum Basilicum L. on the histological and ultrastructural damage of fundic mucosa induced by aspirin administration.

MATERIALS AND METHODS

2.1. Chemicals:

DEHP Aspirin (acetylsalicylic acid) in tablet form, each tablet contains 75 mg, was purchased from local pharmacy.

2.2. Plant collection:

Fresh leaves of Ocimum basilicum (Basil) were collected from botanical garden, Faculty of agriculture, Zagazig University in January 2019. The leaves were manually removed from the stem. Also, the brown old leaves were removed and the seeds separated manually. The particles were hand picked off in order to have clean leaves. The leaves were milled manually and stored at room temperature in a clean plastic bags for extraction process. The plant was identified as Ocimum basilicum l. (Basil) at Botany Department, Faculty of Science, Zagazig University, Egypt.

2.3. Preparation of aqueous extract of sweet basil leaves:

The extract was prepared at the Biology department, Faculty of Science, Zagazig University, Egypt. The aqueous extract was prepared as follows: The collected leaves were washed thoroughly using tap water and left to dry at room temperature for seven days. The dried leaves (200 g.) was milled and infused for 30 minutes in 1 liter sterile distilled water (100 °C), filtered with a 2 mm diameter mesh and the solution obtained was dried in oven under vacuum at 65 °C[15].

2.4. Animals:

Thirty two healthy adult Wistar male albino rats (4-6 months) weighing 200-250 gm were used in this study. The animals were obtained from the Animal House, Faculty of Medicine, Zagazig University, Zagazig, Egypt. They were fed standard balanced diet and allowed water ad-libitum. They were housed in hygienic cages in 12 h light/12 h dark cycle at room temperature. All the experimental procedures were performed according to the guidelines for animal research issued by the National Institute of Health and approved by Animal Ethics Committee, Zagazig University, Zagazig, Egypt.

2.5. Experimental Design:

At All experimental procedures were approved and carried out in accordance with the guidelines of the Institutional Animal Care and Use Committee accepted by Faculty of Medicine, Zagazig University, Zagazig, Egypt. After an acclimatization period of 1 week, rats were randomly divided into 3 equal groups;

Group I (Control group): included 12 rats, further subdivided into two equal subgroups (6 rats each):

Subgroup Ia (Negative control group): left without intervention.

Subgroup Ib: (Positive control group; Basil group): received Aqueous extract of Sweet Basil Leaves (200 mg/ Kg b.w) once daily by orogastric tube for 4 weeks[16].

Group II (Aspirin treated group): included 10 rats which were given aspirin in a dose of 12.5 mg/kg dissolved in sterile distilled water once daily by orogastric tube for 4 weeks[17].

Group III (Coadministration group): included 10 rats which were administered aspirin in a dose of 12.5 mg/ kg dissolved in 1 ml saline and aqueous extract of Basil Leaves (200 mg/Kg b.w) once daily by orogastric tube for 4 weeks[18].
2.6. General observations in rats

During the experimental period, clinical signs and general appearances, which included the amount of food and water consumed, were checked daily. Mortalities of the rats were recorded as it occurred.

2.7. Body weights:

The body weight of each animal was assessed before and at the end of experiment.

At the end of the experiment, the rats were fasted overnight. They were sacrificed with intraperitoneal injection of pentobarbitone sodium (60 mg/kg body weight)\(^{16}\), and their stomach were dissected out, rinsed and cut along the greater curvature. Specimens from the fundic region were prepared for light and transmission electron microscope (TEM) examination.

2.8. Histological study:

Specimens for light microscopic examination were fixed in 10% neutral formol saline, processed for paraffin block preparation, cut into 5 µm sections, and subjected to H&E\(^{19}\), Mallory trichrome stain for detection of collagen fibers. Staining with periodic acid Schiff (PAS) histochemical method was carried out for evaluation of the variations in mucosal glycoprotein production\(^{20}\).

2.9. Electron microscope study:

The 1 mm testis samples for electron microscope were immediately immersed in the 2.5% cocodylate buffered glutaraldehyde for 24 h at 4°C. Later on they were post fixed in 1.0% osmium tetroxide in 0.1 mol/l cacodylate buffer (pH 7.3) for 2 h at room temperature. Then samples were dehydrated in ascending graded ethanol. After immersion in propylene oxide (3 times for 10 min each), the samples were impregnated overnight in a mixture of propylene oxide and Epon-812 resin, then, finally, embedded in Epon-812 resin. Semithin sections (0.5 µm thick) were cut with an ultramicrotome (Leica Ultracut, Berlin, Germany) and stained with toluidine blue to select sites for the preparation of ultrathin sections of 80 nm thickness. Ultrathin sections were counterstained with 2% uranyl acetate and lead citrate\(^{21}\). The stained sections were examined and photographed by JEOL JEM 2100 electron microscope in Electron Microscope Research Laboratory of Faculty of Agriculture El Mansoura University.

2.10. Histo-morphometrical analysis:

The mean values of 10 non-overlapping fields (magnification power x 200) from 5 different rats in each group were taken using the image analyzer computer system Leica Qwin 500 (Cambridge, UK, Leica Microsystems Imaging Solutions Ltd). It was measured using the interactive measure menu\(^{22}\) in the image analyzing unit of the Pathology Department, Faculty of Dentistry, Cairo University, Egypt to evaluate the following parameters:

1) Thickness of mucous film in PAS-stained sections of fundic mucosa.

2) area percent of collagen fibers of the fundic mucosa in Mallory trichrome stained sections.

2.11. Statistical analysis:

All data were expressed as mean ± SD. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software, version 13.00 (Chicago, Illinois, USA). Statistical significance was determined by one-way analysis of variance for differences between the means of different groups. Further analysis was carried out using the post-hoc test to compare the parameters between the different groups with each other. Probability of P less than 0.05 was considered statistically significant.

RESULTS

3.1. General observations and Food intake:

Rats of aspirin-treated group showed reduced amount of food consumption compared to the control and the coadministration (aspirin+Basil) groups. However, the amount of food consumption was relatively normal in aspirin+Basil group. No mortalities were recorded.

3.2. Body Weight:

As a function of growth, body weight of experimental animals was monitored in comparison with the control group. Our data showed that the final body weight of aspirin treated group showed a significant decrease in body weight in comparison to the control group. The coadministration group showed a significant increase in body weight in comparison with the aspirin treated group which was non-significant with the control group. Results are presented in table (1).

3.3. Histological results:

Histological examination of subgroups 1a and 1b of the control group showed the same histological results. So, we used the negative control subgroup 1a as the control group. Haematoxylin and Eosin stained sections of stomach fundus of control albino rats showed the fundic mucosal layers; epithelium, lamina propria containing fundic glands and muscularis mucosa. Fundic glands were formed of isthmus, neck and base regions. They were long, straight, tubular and perpendicular to the surface occupying the whole thickness of the lamina propria and opened into the surface by narrow gastric pits. Muscularis externa was seen (Figure 1a). Contrary to the control, aspirin treated
group showed sloughing and detachment of the surface epithelium and loss of glandular cells. Notice, the wide gastric pits were seen. Mononuclear cell infiltration and congested blood vessels were observed in lamina propria and submucosa. Creeping of collagen fibers between the basal parts of the fundic glands and thick muscularis mucosa were noticed. Submucosal widening indicating edema was also noticed (Fig. 1b). Fundic glands of aspirin+Basil group (coadministration group) were regularly arranged and opened on the surface by narrow gastric pits. However, some congested blood vessels and few inflammatory cells were noticed in lamina propria (Fig. 1c).

Higher magnification of the upper part of the fundic glands of the control group revealed the surface mucous columnar cells with basal oval nuclei and the mucous neck cells. Parietal cells appeared as large cells with central pale nuclei and eosinophilic cytoplasm. Notice, the narrow gastric pits (Fig. 2a). However, higher magnification of the upper part of the fundic glands of aspirin treated group showed disorganized glands with loss of their normal architecture, some fundic glands were lined by cells with degenerated nuclei. Parietal cells had dark pyknotic nuclei and vacuolated cytoplasm. Some sections showed desquamation of the superficial epithelial cells. Notice the wide gastric pits (Fig. 2b & 2b'). On the other hand, fundic glands in the coadministration group were regularly arranged. They were lined by surface mucous cells and opened into the surface by relatively wide gastric pits. Many Parietal cells had rounded nuclei and eosinophilic cytoplasm. However, some parietal cells appeared with vacuolated cytoplasm (Fig. 2c).

Higher magnification of the lower part of the fundic glands of control group revealed the parietal cells with central rounded nuclei and eosinophilic cytoplasm. Chief cells appeared low columnar with basal rounded nuclei and basophilic cytoplasm. Muscularis mucosa was also noticed (Fig. 3a). Aspirin treated group showed dilated fundic glands. Some glands were lined by glandular cells with dark nuclei. Parietal cells had pyknotic nuclei and vacuolated cytoplasm. Chief cells appeared with pyknotic nuclei. Thick muscularis mucosa was seen. Notice mononuclear cell infiltration (Fig. 3b & 3b'). The coadministration group exhibited normal parietal cells and chief cells. However, some parietal cells appeared with vacuolated cytoplasm. Muscularis mucosa was also seen (Fig. 3c).

PAS-stained sections of the control group showed that there was a continuous PAS positive mucous film covering mucosal surface of the stomach extending to fill the gastric pits. (Fig. 4a). Examination of aspirin treated group showed absence of the PAS positive mucous film and weak reaction in the surface epithelium cells. (Fig. 4b). In the coadministration group, strong positive PAS-reaction was seen in the continuous mucous film over the surface epithelium extending to fill the gastric pits having the same pattern of distribution as those of the control group (Fig. 4c).

Mallory trichrome stained sections of control group showed very thin collagen fibers in the lamina propria of the basal part of fundic mucosa (Fig. 5a). Increased collagen fibers in the lamina propria of fundic mucosa of aspirin treated group as compared to control group (Fig. 5b). Thin collagen fibers were detected in the lamina propria of the fundic mucosa of the coadministration group (Fig. 5c).

3.4. Electron microscopic results:

By electron microscopy, examination of the control group showed that the parietal cells had large central, regular and rounded nucleus, the cytoplasm contained numerous large mitochondria with dense matrix and often occupied nearly all of the cytoplasm, and there were characteristic intracellular canaliculi with microvilli projecting into their lumina (Fig. 6a). Many parietal cells in aspirin treated group exhibited small nuclei with increased amount of heterochromatin. Abnormally dilated canaliculi containing few short microvilli were seen. Light cytoplasmic areas resembling vacuole-like structures were displayed. Eosinophils were seen in lamina propria close to blood capillaries. They had bilobed nuclei and their cytoplasm was packed with specific granules containing an internal often electron-dense crystalline core surrounded by an electron-lucent matrix (Fig. 6b1&6b2). In the coadministration group, parietal cells appeared normal similar to the control group containing large central, regular and rounded nucleus, the cytoplasm contained numerous large mitochondria with dense matrix. However, some dilated intracellular canaliculi containing few short microvilli were seen (Fig. 6d).

The ultrastructural features of chief cells in the control group were those of protein secreting (zymogenic) cells in general; they appeared with basal, rounded nucleus with nucleolus, Their cytoplasm contained extensive RER, mitochondria, supranuclear Golgi apparatus and large number of membrane bound secretory vesicles (zymogenic granules) crowded in the apical cytoplasm (Fig. 7a). In aspirin treated group, many chief cells appeared with shrunken pyknotic nuclei. Their cytoplasm contained many vacuoles (Fig. 7b). In the coadministration group, most chief cells appeared with normal structure having basal, rounded nucleus with nucleolus. Their cytoplasm contained extensive RER, mitochondria, supranuclear Golgi apparatus and large number of zymogenic granules. However, few cells exhibited small vacuoles within their cytoplasm (Fig. 7c).

Enteroendocrine cells in the control group were seen resting with apical large euchromatic nuclei and small electron dense granules (Fig. 8a). In aspirin treated group, some enteroeendocrine cells had irregular nuclear envelope
In the coadministration group, enterendocrine cells appeared similar to that in the control group (Fig. 8c).

3.5. Histomorphometrical and statistical results:

Highly statistically significant decrease in the mean thickness of mucous film in PAS-stained sections of the fundic mucosa was detected in aspirin treated group as compared to the control group and the protective group. No statistically significant difference in the coadministration protective group as compared to the control group (Table 2).

Highly statistically significant increase in the mean area percent of collagen fibers of the fundic mucosa was detected in the aspirin treated group as compared to the control group and coadministration group. There was no statistically significant difference between the coadministration group and the control group (Table 3).

Table 1: Initial body weight (g) and Final body weight (g) in different studied groups using one-way ANOVA test:

<table>
<thead>
<tr>
<th></th>
<th>Control Group (G1)</th>
<th>Aspirin Group (G2)</th>
<th>Coadministration group (G3)</th>
<th>Fw</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>265.4±19.04</td>
<td>258.2±21</td>
<td>261.1±12.4</td>
<td>0.125</td>
<td>0.945**</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>352.6±17.96</td>
<td>321.3±22.1</td>
<td>252.2±31.21</td>
<td>3.214</td>
<td>0.032*</td>
</tr>
</tbody>
</table>

LSD for comparison between groups

<table>
<thead>
<tr>
<th></th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2</td>
<td>&lt;0.05*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>&gt;0.05**</td>
<td>&lt;0.05*</td>
<td></td>
</tr>
</tbody>
</table>

* Significant (p< 0.05)  
ns: non-significant (>0.05)

Table 2: Thickness of mucous film in in different studied groups using one-way ANOVA test.

<table>
<thead>
<tr>
<th></th>
<th>Mean ±SD</th>
<th>F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (G1)</td>
<td>67.3±10.6</td>
<td>53.6</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Aspirin group (G2)</td>
<td>14.4±6.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coadministration group (G3)</td>
<td>45.2±11.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD for comparison between groups

<table>
<thead>
<tr>
<th></th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2</td>
<td>&lt;0.001**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>&gt;0.05**</td>
<td>&lt;0.001**</td>
<td></td>
</tr>
</tbody>
</table>

*Significant (p<0.05) **Highly Significant (p<0.001)

Table 3: Area percent of collagen fibers in different studied groups using one-way ANOVA test

<table>
<thead>
<tr>
<th></th>
<th>Mean ±SD</th>
<th>F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (G1)</td>
<td>0.223±0.02</td>
<td>1398.99</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Aspirin group (G2)</td>
<td>0.786±0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coadministration group (G3)</td>
<td>0.232±0.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD for comparison between groups

<table>
<thead>
<tr>
<th></th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2</td>
<td>&lt;0.001**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>&gt;0.325**</td>
<td>&lt;0.001**</td>
<td></td>
</tr>
</tbody>
</table>

*Significant (p<0.05) **Highly Significant (p<0.001)
The Possible Protective Effect of Ocimum Basilicum L. Aqueous Extract. Abdelfadeel and Alazouny

Fig. 1: Photomicrographs of H&E stained sections of stomach fundus of:

The control group (1a): showing the fundic glands (arrows) in the lamina propria consists of [isthmus (l), neck (N) and base (B)]. They open into the surface by narrow gastric pits (arrow head). Thin muscularis mucosa (m) is also seen. (H&E×100, scale bar 50 µm)

Aspirin group (1b): showing sloughing and detachment of the surface epithelium cells (thick arrow) and wide gastric pits (arrow head). Inflammatory cells (curved arrow) and congested blood vessels (bv) are observed in lamina propria and submucosa. Creeping of collagen fibers (thin arrow) between the basal parts of the fundic glands and thick muscularis mucosa (m) are noticed. Submucosal widening is also noticed (asterix). (H&E×100, scale bar 50 µm)

The coadministration (1c): showing that the fundic glands are regularly arranged (arrows) and open on the surface by narrow gastric pits (arrow head). However, Thick muscularis mucosa (m), some congested blood vessels (bv) and few inflammatory cells (curved arrow) are seen in lamina propria. (H&E×100, scale bar 50 µm)
Fig. 2: Photomicrographs of H&E stained sections of higher magnification of the upper part of the fundic glands of:

The control group (2a): showing the surface mucous cells with basal oval nuclei (arrow) and the mucous neck cells (double arrow). Parietal cells have rounded nuclei and eosinophilic cytoplasm (thick arrow). Notice, the narrow gastric pits (arrow head). (H&E×400, scale bar 50 µm).

Aspirin group (2b & 2b'): showing disorganized fundic glands lined by cells with degenerated nuclei (curved arrow). Parietal cells have vacuolated cytoplasm and pyknotic nuclei (double arrow), desquamation of the surface epithelial cells (asterix) and wide gastric pits (arrow head). (H&E×400, scale bar 50 µm).

The coadministration (2c): showing regularly arranged fundic glands lined by surface mucous cells (arrow) and open into the surface by relatively wide gastric pits (arrow head). Many Parietal cells have rounded nuclei and eosinophilic cytoplasm (thick arrow). However, some parietal cells have vacuolated cytoplasm (double arrow). (H&E×400, scale bar 50 µm).
Fig. 3: Photomicrographs of H&E stained sections of higher magnification of the lower part of the fundic glands of:

The control group (3a): reveals the parietal cells with central rounded nuclei and eosinophilic cytoplasm (arrow). Chief cells appears low columnar with basal rounded nuclei and basophilic cytoplasm (arrow head). Musculais mucosa (m) is also noticed. (H&E×400, scale bar 50 µm)

Aspirin group (3b & 3b’): showing dilated fundic glands (curved arrow). Parietal cells have pyknotic nuclei and vacuolated cytoplasm (arrow). Chief cells appear with pyknotic nuclei (arrow head). Congested blood vessels (bv) and thick muscularis mucosa (m) are seen. Mononuclear cell infiltration is noticed (thin arrow). (H&E×400, scale bar 50 µm)

The coadministration (3c): showing that parietal cells (arrow) and chief cells (arrow head) are apparently normal. However, some parietal cells appear with vacuolated cytoplasm (double arrow). Musculais mucosa (m) is also seen. (H&E×400, scale bar 50 µm).
Fig. 4: Photomicrographs of PAS stained sections of:
The control group (4a): showing a continuous PAS positive mucous film (arrow) covering mucosal surface epithelium of the stomach extending to fill the gastric pits (arrow head). (H&E×400, scale bar 50 µm).
Aspirin group (4b): showing absence of the PAS positive mucous film and weak reaction in the surface epithelium cells (arrow head). (H&E×400, scale bar 50 µm).
The coadministration (4c): showing strong positive PAS reaction was seen in the continuous mucous film (arrow) over the surface epithelium extending to fill the gastric pits (arrow head). (H&E×400, scale bar 50 µm).
Fig. 5: Photomicrographs of Mallory trichrome stained sections of:
The control group (5a): showing very thin collagen fibers in the lamina propria of the basal part of fundic mucosa (arrow). (H&E×100, scale bar 50 µm).
Aspirin group (5b): showing increased collagen fibers in the lamina propria of fundic mucosa as compared to control group (arrow). (H&E×100, scale bar 50 µm).
The coadministration (5c): showing thin collagen fibers in the lamina propria of the fundic mucosa (arrow). (H&E×100, scale bar 50 µm).
Fig. 6: Electron micrographs of ultrathin sections of stomach fundus of the:
control group (Fig. (6a): showing a parietal cell (P) with large central, regular and rounded nucleus (N) with nucleolus (Nu), the cytoplasm contains numerous large mitochondria (M) with dense matrix and often occupy nearly all of the cytoplasm. Intracellular canaliculi (IC) with microvilli (mv) projecting into their lumina are seen. A Part of chief cell (C) can be seen. (TEM×4000, scale bar 5 µm)

Aspirin group (6b1): showing a parietal cell having small nucleus (N) with increased amount of heterochromatin (arrow). Light cytoplasmic areas resembling vacuole-like structures (V) are seen. (TEM×4000, scale bar 5 µm)

Aspirin group (6b2): showing a parietal cell (P) with dilated intracellular canaliculi (IC) containing few short microvilli (mv). An eosinophil (E) is seen in lamina propria close to blood capillaries (bc). They had bilobed nuclei (N) and their cytoplasm is packed with specific granules (G) containing an internal electron-dense crystalline core surrounded by an electron-lucent matrix. (TEM×3000, scale bar 10 µm)

The coadministration (Fig. 6c): showing a parietal cell similar to the control group having large central, regular and rounded nucleus (N). The cytoplasm contains numerous large mitochondria (M) with dense matrix. However, some dilated intracellular canaliculi (IC) containing few short microvilli (mv) are seen. Chief cells (C) are seen. (TEM×5200, scale bar 10 µm).
Fig. 7: Electron micrographs of ultrathin sections of stomach fundus of the:
control group (Fig. 7a): showing chief cells (C) with basal, rounded nucleus (N) with nucleolus (Nu). Their cytoplasm contains extensive RER (RE) mitochondria (M), supranuclear Golgi apparatus (GA), large number of membrane bound secretory vesicles (zymogenic granules) (arrow) crowded in the apical cytoplasm. (TEM×6200, scale bar 5 µm).
Aspirin group (7b): showing chief cells (C) with shrunken pyknotic nuclei (N). Their cytoplasm contains many vacuoles (V). A parietal cell (P) is seen. (TEM×5600, scale bar 5 µm).
The coadministration (7c): showing chief cells (C) with normal structure having basal, rounded nucleus (N) with nucleolus (Nu). Their cytoplasm contains extensive RER (RE), mitochondria (M), supranuclear Golgi apparatus (GA) and large number of zymogenic granules (arrow). However, small vacuoles (V) within their cytoplasm can be seen. (TEM×6400, scale bar 5 µm).
Fig. 8: Electron micrographs of ultrathin sections of stomach fundus of the:
control group (Fig. (8a)): showing an enteroendocrine cell (EN) with apical large euchromatic nucleus (N) and small electron dense granules (g). A part of chief cell (C) is seen. (TEM×8400, scale bar 5 µm).
Aspirin group (8b): showing an enteroendocrine cell (EN) and a parietal cell (P) resting on the basement membrane (bm). A blood capillary (bc) is seen in the lamina propria. The nucleus (N) of enteroendocrine cell is surrounded by irregular nuclear envelope (arrow) and its cytoplasm contains small electron dense granules (g). (TEM×7000, scale bar 5 µm).
The coadministration (8c): showing an enteroendocrine cell (EN) similar to that in the control group. It has a large euchromatic nucleus (N) and small electron dense granules (g). (TEM×10800, scale bar 5 µm).
DISCUSSION

The Aspirin is one of the most commonly used non-steroidal anti-inflammatory drugs (NSAIDs). It is widely used as anti-inflammatory, analgesic drug and in prevention of cardiovascular events. Despite putting efforts in improving the safety, efficacy, and potency of NSAIDs, adverse effects such as gastrointestinal irritation, renal and hepatic toxicity, interference with hemostasis, and reproductive problems still persist[21].

The gastrointestinal tract is the most common site for side effects of aspirin. The major serious side-effects to its use include damage of gastrointestinal mucosa, aggravation of stress ulcersations, and exacerbation of preexisting gastric ulcerations. These could be due to its topical irritating effect, activation of neutrophils, increased reactive oxygen species (ROS), fall in the microcirculation and the reduction in mucosal generation of prostaglandin E2 (PGE2) which is essential for homeostatic functions including maintenance of the mucosal integrity and mucosal blood flow[24].

Several studies have shown that a person exposed to aspirin has three to four times the risk of upper gastrointestinal bleeding, perforation or both than a non aspirin user. These side effects are the cause of major limitations of its clinical application. One of the new strategies to limit the gastric mucosal injury induced by aspirin was adding antioxidant[29].

Food consumption and energy expenditure are the determinant for weight gain or loss[29]. Previous studies have shown that non-steroidal anti-inflammatory drugs (NSAIDs) decreased nutrient digestion and absorption through their direct effect on the gastric and intestinal mucosal cells[29]. However, the direct effect of NSAIDs on gastric and intestinal mucosal cells is attributed to the inhibitory effects of NSAIDs on constitutive cyclooxygenase in the gastrointestinal cells[29]. Therefore, the significant decrease in food consumption and body weight change that were detected in aspirin treated group can be attributed to the anorexia that was occurred during aspirin treatment or inhibition of prostaglandins synthesis in the stomach. An increase in food consumption and body weight were observed in the coadministration group (aspirin+Basil).

Our results regarding body weight gain agreed with Rehman et al., 2018[29] who reported that Basil improved the feed consumption of broiler chickens in heat stress condition. The previous authors mentioned that constituents of basil, like linalool, estragole and eugenol are antimicrobials, which cause sterilization of gastrointestinal tract improving feed utilization.

The findings of the present study clearly revealed that subchronic administration (for 4 weeks) of aspirin to albino rats caused histopathological changes in gastric mucosa compared to the control group manifested by disorganized glands with loss of their normal architecture and wide gastric pits. Sloughing and detachment of the surface epithelial cells. Also, some fundic glands were lined by cells with dark and degenerated nuclei. Similar findings were detected by[30] who explained the cause of these alterations by decreasing antioxidant enzymes with aspirin administration. On the other hand, others[31] found that the aspirin administration was increasing the volume of gastric juice secretion and total acidity leading to sloughing and ulceration of the mucosa. Some researchers[32] mentioned that the administration of aspirin for 2 weeks induced widening of gastric pits and desquamation of mucosal cells, moreover the prolonged period of aspirin administration for 4 weeks resulted in more extensive lesions.

Yousif, 2002[33] stated that Prostaglandins play an important role in protecting the gastric mucosa and enhancing the perception of pain. Inhibition of prostaglandin synthesis by aspirin is the major established mechanism by which NSAIDs render the gastric mucosa vulnerable to mucosal injury. Prostaglandins also stimulate mucus and bicarbonate secretion, decrease acid secretion and cause vasodilatation, thereby increasing elimination of acid that has diffused into the submucosa. High proportion of NSAIDs associated ulcers are silent as depression of prostaglandin synthesis resulting in diminishing pain perception.

Aspirin induces the reactive oxygen metabolites in animal models, which may contribute to mucosal injury. Therefore, Oxygen-derived free radicals play a key role in the mechanism of aspirin-induced acute gastric mucosal lesions[34].

In the current work, aspirin treated group revealed also dilated fundic glands and gastric pits, creeping of collagen fibers between the basal parts of the fundic glands, mononuclear cell infiltration and thick muscularis mucosa. These findings were in agreement with Wang et al., 2011[35] who mentioned that inflammation and neutrophil infiltration are also important in the pathogenesis of the gastric damage induced by NSAIDs. According to Mahmoud et al., 2015[36], the induced inflammation by aspirin is accompanied by increased tumour necrosis factor alpha (TNFα) which is a proinflammatory cytokine, that been shown to be a crucial mediator of aspirin-induced gastric mucosal injury.

In addition, other researchers mentioned that cellular infiltration is a constant feature in any inflammatory process, which in turn gives rise to generation of reactive oxygen species (ROS) which play an important role in the
The dilatation of the gastric pits and upper part of fundic gland of aspirin treated group of this study could be due to excessive production of mucous secretion by mucous neck cells, an attempt by the body to safe guard against more damage to the surface coat of the gland, or, it might be related to inhibition of prostaglandin I2 production, which is considered as a potent anti-secretory agent.[38]

Presence of congested blood vessels in the lamina propria of gastric mucosa was explained by Ibrahim et al., 2018[39] who mentioned that NSAIDs exert direct toxic effect of on the wall of small blood vessels leading to ischemia followed by vasodilatation and extravasations of blood from their luminae. The same authors added that another factor might be implicated is the destructive effect of the drug on the mucosal barrier and intercellular integrity exposing the capillaries and venules to the harmful effects of hydrochloric acid and gastric secretions.

Interestingly, aspirin administration resulted in absence of PAS positive mucous layer leaving raw unprotected surface of gastric mucosa. Wallace, 2008[40] explained these findings by the direct cytotoxic effect of aspirin while, Kwiecien et al., 2012[41] added that the absence of gastric mucus protective layer was due to the accumulation of oxygen free radicals. Perini et al., 2004[42] mentioned that the non-steroidal anti-inflammatory drugs (NSAIDs) cause suppression of cyclo-oxygenase enzyme-1 (COX-1) and inhibition of prostaglandin synthesis which resulting in loss of mucous layer from the gastric mucosa. Seleem et al., 2010[43] had another explanation that aspirin might lead to decrease in the number of mucous cells within gastric mucosa.

Decreased mucous secretion allows diffusion of peptic and hydrogen ions from the lumen into the mucosa stimulating further acid and peptic secretion, decreasing mucosal blood flow and decreasing gastric motility. The acid also damages connective tissue and submucosal capillaries leading to mucosal haemorrhage[43].

In the present study, our results revealed increased amount of collagen fibers in the lamina propria of fundic mucosa of aspirin treated group as compared to control group. This result could be explained by Damiano et al., 1999[44] who reported that disruption of the normal microenvironment of the fibroblasts may be the crucial event for fibroblast activation, cell division and collagen deposition. Fibroblast proliferation and collagen deposition were found to occur when epithelial cells were severely damaged and there was a delay in the epithelial repair process.

Electron microscopic examination in the current experiment confirmed the light microscopic results. Some degenerative changes of the parietal, cheif cells and enteroendocrine cells were detected in aspirin treated group. These results agreed with Yehia et al., 2014[45]. These changes were explained by[45] who mentioned that aspirin intake induces generation of reactive oxygen species (ROS) resulting in oxidative damage in cellular membrane and cell lysis. Another explanation was offered by[45] who attributed these changes to increased mitochondrial permeability, mitochondrial failure and translocation of intramitochondrial protein (apoptosis-inducing factor).

In the current study, many eosinophils were seen in lamina propria of aspirin treated group. Eosinophils are known to produce tissue damage and are associated with some fibrozing disorders. It is possible that they may play a role in the pathogenesis of collagenous disorders of the gastrointestinal tract, although their activation could also be a byproduct of a non-specific inflammatory response[46].

Basil is traditionally used worldwide as a medicinal herb for prevention and treatment of numerous diseases. The leaves and flowering parts are used as antispasmodic, aromatic, carminative, and digestive remedies, and to treat abdominal cramps, gastro-enteritis, fever, poor digestion, nausea, migraines, insomnia, depression and dysentery. They have been applied externally to treat acne, insect stings, snake bites, and skin infections[47].

Hussain et al., 2008[48] mentioned that the chemical composition of Basil is affected by the four seasons namely, summer, autumn, winter and spring. The previous authors found that the content of the essential oils is distributed unevenly among seasons. The highest amount of the oil in the O. basilicum is found during winter (0.8%) which decreases significantly (p<0.05) in summer to 0.5%. Therefore, in our study, the plant was collected in winter.

Our results revealed that concomitant use of Ocimum Basilicum (Basil) aqueous extract with aspirin drug actually protected the gastric mucosa from injurious effects of aspirin. The fundic glands were regularly arranged and opened on the surface by narrow gastric pits. The cells lining fundic glands showed nearly normal appearance. However, few parietal and chief cells exhibited vacuolated cytoplasm and pyknotic nuclei. This was in agreement with others[48] who reported that Basil has important antioxidant and anti-inflammatory effects. Its antioxidant and anti-inflammatory properties have numerous beneficial effects on the organism, for example, as aprotector against endothelial lesions and cancer, preventing the occurrence of diseases that cause the highest death rates worldwide.

Histopathological results of the present study also revealed protection of gastric mucosa and inhibition of mononuclear cell infiltration of gastric wall in rats administered Basil extract and aspirin. Activation and infiltration of neutrophils appear to be involved in the initial processes of formation of the gastric lesion. Similarly,
Abdulla et al., 2010(49) demonstrated that the reduction of cellular infiltration into ulcerated gastric tissue promotes the prevention of gastric ulcers in rats. Wasman et al., 2010(49) showed that oral administration of plant extract before ethanol administration significantly decreased neutrophil infiltration of gastric mucosa.

Prostaglandin E2 (PGE2) plays an important role in the regulation of gastric mucus secretion. PGE2 has protective effects against various gastric injury models(50). It has been shown that prostaglandins influence virtually every component of the mucosal defense: stimulating mucus and bicarbonate secretion, maintaining mucosal blood flow, enhancing the resistance of epithelial cells to injury induced by cytotoxins, and inhibiting leukocyte recruitment(51). Mahmood et al., 2007(52) suggested that the gastroprotective effect of Basil is mediated partially by PGE2 as direct measurement of its mucosal level confirmed that its biosynthesis was significantly enhanced by the plant extract.

The beneficial effects of Basil are attributed to its active components. Its antioxidant properties can be attributed to rosmarinic acid, one of the esters of caffeic acid(53). The betulinic, oleanolic, ursolic, 3-epi-maslinic, alphitolic and euscaphic acids isolated from Basil exhibited hepatoprotective effects in rats(54). Ursolic acid has anti-inflammatory, antirheumatic, antiviral, antioxidant and antitumor properties(55).

CONCLUSION

In conclusion, our study provided evidence that Ocimum Basilicum aqueous extract has conferred protective effects on aspirin induced histological and ultrastructural damage of gastric mucosa. Based on the findings of this study, Basil may be used as a nutritional supplement or therapeutic drug to protect against aspirin-induced gastric ulcers, a common problem resulting from the use of aspirin.

FUNDING

This research was personally funded.

CONFLICT OF INTEREST

There are no conflicts of interest.

ETHICAL APPROVAL

The study was carried out according to the guidelines of the ethical committee of the faculty of medicine, Zagazig University.

ACKNOWLEDGMENTS

The authors acknowledge Botany and Biology Departments, Faculty of Science, Zagazig University, Egypt for providing help in plant identification and extract preparation.

AUTHOR CONTRIBUTION

Both authors contribute equally in performing the experiment and in writing and revising the research.

REFERENCES


