Carvedilol can attenuate histamine-induced paw edema and formaldehyde-induced arthritis in rats without risk of gastric irritation

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A R T I C L E   I N F O

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A B S T R A C T

Background and aim: Rheumatoid arthritis treatment aims to control joint damage and any associated complications such as cardiovascular disease. Most anti-inflammatory drugs have a high tendency to cause gastrointestinal irritation. The present study is designed to investigate the anti-inflammatory effect of carvedilol and to study its effect on gastric mucosa.

Experimental approach: Lornoxicam (1.3 mg/kg) or carvedilol (10 mg/kg) was administrated orally 1 h before histamine injection into animals of a histamine-induced paw edema model and orally daily for 11 days into animals of a formaldehyde-induced arthritis model. Tumor necrosis factor-\(\alpha\) and prostaglandin \(E_2\) were measured in animals of the formaldehyde-induced arthritis model. The effect of lornoxicam and carvedilol on gastric mucosa was assessed in normal rats and after induction of cold stress ulcer.

Results: Carvedilol succeeded in reducing hind paw edema in both histamine-induced paw edema and formaldehyde-induced arthritis and in reducing the elevated level of tumor necrosis factor-\(\alpha\) and prostaglandin \(E_2\) nearly with near equal efficacy compared with lornoxicam. Carvedilol did not show any ulcerative effect on the gastric mucosa of normal rats, and its use was associated with an improvement of both the gross and histopathological pictures of gastric ulcers in animals of the cold stress ulcer model compared with lornoxicam treated rats.

Conclusion: The current findings support the use of carvedilol both in the management of inflammation as well as the prevention of cardiovascular complications in rheumatoid arthritis patients. The use of carvedilol was not associated with any gastrointestinal tract irritation.

1. Introduction

Rheumatoid arthritis (RA) is a systemic disease characterized by chronic inflammation of synovial joints, proliferation of synovial cells and infiltration of inflammatory cells leading to the destruction of cartilage and bone [20]. Activation of inflammatory cells such as neutrophils and macrophages results in the generation of reactive oxygen species (ROS) that cause damage of biomembranes [7].

Recruitment of leukocytes leads to the release of multiple cytokines such as TNF-\(\alpha\) and IL-6 into the inflamed joints. These cytokines contribute to the destruction of articular cartilage and subchondral bone via the activation of matrix metalloproteinases [34]. Another major class of inflammatory mediators is the eicosanoids which are comprised of prostaglandins and leukotrienes. Elevated serum levels of both prostaglandin \(E_2\) (PGE\(_2\)) and leukotriene B\(_4\) (LTB\(_4\)) have been reported to correlate with the severity of RA [28]. Cardiovascular complications have become the leading cause of morbidity in patients with RA. Ischemic heart disease and heart failure are common causes of death in RA [14].

Lornoxicam is a non-steroidal anti-inflammatory drug (NSAID). It has powerful analgesic and anti-inflammatory effects that can relieve symptoms of osteoarthritis, rheumatoid arthritis and ankylosing spondylitis [6].

The anti-inflammatory activity of lornoxicam is mediated through the inhibition of prostaglandin synthesis through the inhibition of cyclooxygenase (COX) enzymes [42]. Lornoxicam may produce gastrointestinal irritation and ulceration [21].

Carvedilol is a non-selective \(\beta\)-adrenergic antagonist that is used in the treatment of heart failure, ischemic heart disease and hypertension [37]. Carvedilol has been shown to protect against cisplatin-induced renal toxicities [44], endothelial dysfunction in streptozocin-induced diabetic rats [15] and hepatic injury [19]. These protective properties may contribute to its antioxidant and anti-inflammatory effects [13].

The aim of the present study was to assess the anti-inflammatory...
efficacy of carvedilol using two models of inflammation; histamine induced paw edema in rats and formaldehyde induced arthritis in rats, in comparison with lornoxicam; a standard drug for the treatment of arthritis. The levels of TNF-α and PGE₂ were measured in an attempt to deduce its possible anti-inflammatory mechanism of action. The gastric ulcerogenic effect of carvedilol on the gastric mucosa was also assessed, using the cold-stress ulcer test.

2. Materials and methods

2.1. Materials

The following were used: histamine, carvedilol and formaldehyde (SIGMA pharmaceutical company, Egypt), and lornoxicam (Xefo Nycomed Pharma, Oslo, Norway). All mentioned drugs/chemicals were dissolved in distilled water immediately before use.

2.2. Animals used

A total of 144 adult healthy male wistar rats - 180–200 g each - were included in the study. Animals were housed for at least 2 days before experiments under a 12 hourlight/dark cycle. Food and water were provided ad libitum. The study protocol was approved by the Institutional Review Board of the Faculty of Medicine, Cairo University and the animal experiments were carried out in accordance with the ethical guidelines of animal welfare.

2.3. Experimental design

Rats were classified into three main groups in order to assess the effects of carvedilol versus lornoxicam on histamine-induced paw edema, formaldehyde-induced arthritis and cold stress induced ulcers.

2.3.1. Histamine-induced rat paw edema

Rats of this group were divided into 6 sub-groups (8 rats each):

Group I (untreated control group): Rats received 1 ml distilled water orally.

Group II (lornoxicam treated control group): Rats received lornoxicam orally at a dose of 1.3 mg/kg [39,48].

Group III (carvedilol treated control group): Rats received carvedilol orally at a dose of 10 mg/kg [5].

Group IV (histamine-induced rat paw edema group): Rats received 1 ml of distilled water 1 h before histamine injection. Animals of this group served as models of histamine-induced paw edema.

Group V (histamine-induced rat paw edema group treated with lornoxicam): Rats received lornoxicam as group II 1 h before histamine injection.

Group VI (histamine-induced rat paw edema group treated with carvedilol): Rats received carvedilol as group III 1 h before histamine injection.

Hind paw edema in the right foot of a rat was induced by subplantar injection of 0.1 ml of 1% freshly prepared histamine. The paw volume was measured initially and then at 1, 2 and 3 h after histamine injection by using a plethysmometer (Ugo Basil, Italy) [51].

Animals received a single oral dose of either of the two treatments 1 h before injection of histamine. The percentage of inhibition of inflammation was calculated using the following formula [50]:

\[ 1 - \left( \frac{V_t}{V_c} \right) \times 100 \]

where \( V_t \) represents the change in paw volume in the tested groups and \( V_c \) represents the change in paw volume in the model group.

2.3.2. Formaldehyde-induced arthritis in rats

Rats of this group were divided into 6 sub-groups (8 rats each):

Group I (untreated control group): Rats received 1 ml of distilled water daily orally for 11 days.

Group II (lornoxicam treated control group): Rats received lornoxicam orally at a dose of 1.3 mg/kg/day for 11 days [39,48].

Group III (carvedilol treated control group): Rats received carvedilol orally at a dose of 10 mg/kg/day for 11 days [5].

Group IV (arthritic group): Rats were injected with formaldehyde on day one and three of the experiment. Rats of this group were used as a model of formaldehyde-induced arthritis.

Group V (arthritic group treated with lornoxicam): Rats were injected with formaldehyde as in group IV and treated daily with lornoxicam as in group II for 11 days.

Group VI (arthritic group treated with carvedilol): Rats were injected with formaldehyde as in group IV and treated daily with carvedilol as in group III for 11 days.

Paw edema in the right foot of rats was induced by subplantar injection of 0.1 ml of 2% freshly prepared formaldehyde on day one and three of the experiment to model chronic arthritis. Formaldehyde was injected 30 min after drug dosing. One of the two treatments were given to the animals orally once per day for the duration of the experiment. Paw volume was measured initially and then on day 3, 5, 7, 9 and 11 using a plethysmometer (Ugo Basil, Italy) [32]. The percentage inhibition of inflammation was calculated using the following formula [50]:

\[ 1 - \left( \frac{V_t}{V_c} \right) \times 100 \]

where \( V_t \) represents the change in paw volume in the tested groups and \( V_c \) represents the change in paw volume in the model group.

On day 11, blood samples were collected from all animals of the formaldehyde-induced arthritis groups from rat tails and serum were used to measure TNF-α and PGE₂. TNF-α assays were performed using the quantitative sandwich enzyme immunoassay technique. The absorbance was read at 450 nm using the Biochrom Asys 96 microplate reader (UK). PGE₂ assays were performed using the forward sequential competitive binding technique. The absorbance was read at 450 nm using the Biochrom Asys 96 microplate reader (UK).

2.3.3. Assessment of ulcerogenic effect

Rats of this group were divided into 6 sub-groups (8 rats each):

Group I (untreated control group): Rats received 1 ml of distilled water daily orally for 11 days.

Group II (lornoxicam treated control group): Rats received lornoxicam orally at a dose of 1.3 mg/kg/day for 11 days [39,48].

Group III (carvedilol treated control group): Rats received carvedilol orally at a dose of 10 mg/kg/day for 11 days [5].

Group IV (cold stress ulcer group): Rats of this group were used as models of cases with gastric ulcer [38].

Group V (lornoxicam treated stress ulcer group): Rats received lornoxicam orally as in group II before the induction of cold stress ulcer.

Group VI (carvedilol treated stress ulcer group): Rats received carvedilol orally as in group III before the induction of cold stress ulcers.

2.3.4. Induction of cold-stress ulcer

Rats were made to fast for 48 h before the induction of ulcers to allow for complete gastric emptying. Free access to water was allowed. Each animal was enclosed in a small gridded metallic cage to limit movement. Rats were kept for 2–3 h at a temperature of 4 °C [38].

2.3.5. Gross examination of stomach

Animals were sacrificed at the end of the experiment, using diethyl ether. Their stomachs were excised, opened along the greater curvature, washed with saline and fixed on a platform, photographed and examined with a 6 × magnifying lens. All the ulcerative lesions were accurately measured. Each hemorrhagic lesion was measured along its greatest length. The individual lengths were summed to obtain a total lesion length in each animal and expressed in millimeters. Five pectechiae were taken as equivalent to a 1 mm ulcer [46].

Sections of the stomach were stained with hematoxylin and eosin.
(H & E) stains and examined microscopically. The results were recorded and tabulated according to the following grading system [40]:

Grade 0: normal gastric mucosal cells appearing intact with normal shape and location.

Grade 1 ulcer: erosion of the superficial epithelial layer.

Grade 2 ulcer: erosion of the superficial epithelial layer and less than one third the thickness of the mucosa.

Grade 3 ulcer: erosion or necrosis of more than of the mucosal surface.

2.4. Statistical methodology

Data were statistically described in terms of mean ± standard deviation (± SD). Comparison between the study groups was done using the Kruskal Wallis test with post hoc multiple two-group comparisons. P values < 0.05 were considered statistically significant. All statistical calculations were done using SPSS (Statistical Package for the Social Sciences; SPSS Inc., Chicago, IL, USA, release 15 for Microsoft Windows, 2006).

3. Results

3.1. Histamine-induced rat paw edema

Treatment of normal rats with either lornoxicam (group II) or with carvedilol (group III) did not result in any significant change in mean hind paw volumes compared with rats of the untreated control group (group I) (p > 0.05) (Table 1).

Rats of histamine-induced paw edema group (group IV) showed significant rise in mean hind paw volumes after 1, 2 and 3 h of subplantar injection of histamine compared with the mean paw volume of rats of the untreated control group (group I) (p < 0.05) (Table 1).

Rats pretreated with oral lornoxicam (group V) showed significant decrease in mean paw volumes after 1, 2 and 3 h of subplantar injection of histamine compared with the histamine-induced rat paw edema group (group IV) with 58.9%, 59.2% and 65.6% percent inhibition rates respectively (p < 0.05) (Table 1).

Rats pretreated with oral carvedilol (group VI) showed significant decrease in mean paw volumes after 1, 2 and 3 h of subplantar injection of histamine compared with the histamine-induced rat paw edema group (group IV) with 48%, 50% and 60.8% percent inhibition rates respectively (p < 0.05). There was no significant difference between mean paw edema volumes between groups V and VI (p > 0.05) (Table 1).

3.2. Formaldehyde-induced arthritis

Treatment of normal rats with both lornoxicam (group II) and with carvedilol (group III) did not result in any significance difference in the mean volumes of hind paws compared with rats of the untreated control group (group I) on days 3, 5, 7, 9 and 12 (p > 0.05) (Table 2).

Subplantar injection of formaldehyde on the first and third day of the experiment resulted in significant increase in mean paw volumes in group IV on days 3, 5, 7, 9 and 12 compared with mean paw volumes of rats of the untreated control group (group I) (p < 0.05) (Table 2).

Lornoxicam treated arthritic rats (group V) showed significant decrease in mean hind paw volumes on days 3, 5, 7, 9 and 11 of the experiment compared with untreated arthritic group (group IV) (p < 0.05). Percent inhibition rates of paw volumes were 62.6%, 63.2%, 66.4%, 68.4% and 68.9%, respectively (Table 2).

Carvedilol treated arthritic rats (group VI) showed significant improvement in mean hind paw volumes on days 3, 5, 7, 9 and 11 of the experiment compared with untreated arthritic group (group IV) (p < 0.05). Percent inhibition rates of paw volumes were 56.5%, 59.2%, 65.6%, 70.9% and 72.2%, respectively (Table 2). There was insignificant improvement of paw volumes on days 9 and 11 in the group of carvedilol treated rats compared with that of lornoxicam treated rats (p > 0.05) (Table 2).

3.3. Serum level of tumor necrosis factor-α (TNF-α)

Treatment of normal rats with either lornoxicam (group II) or with carvedilol (group III) did not show any significant change in mean serum levels of TNF-α compared with the untreated control rats (group I) (p > 0.05) (Table 3).

Rats of the formaldehyde-induced arthritis group (group IV) had significant elevations in mean serum levels of TNF-α compared with the rats of the untreated control group (group I) (p < 0.05) (Table 3).

Rats with formaldehyde-induced arthritis treated with lornoxicam (group V) and carvedilol (group VI) had a significant reduction in mean serum levels of TNF-α compared with untreated rats with induced arthritis (group IV) (p < 0.05) (Table 3).

Treatment of rat with induced arthritis using carvedilol (group VI) resulted in a more significant reduction in the elevated serum level of TNF-α on day 11 of the experiment compared with similar rats treated with lornoxicam (group V) (p < 0.05) (Table 3).

3.4. Serum level of prostaglandin-E2 (PGE2)

Treatment of normal rats with lornoxicam (group II) and with carvedilol (group III) did not result in any significant change in the mean serum levels of PGE2 compared with rats of the untreated control group (group I) (p > 0.05) (Table 4).

The mean serum levels of PGE2 showed significant elevation in rats of the formaldehyde-induced arthritis group (group IV) compared with the rats of the untreated control group (group I) on day 11 of the experiment (p < 0.05) (Table 4).

Treatment of the rats with formaldehyde-induced arthritis with lornoxicam (group V) and carvedilol (group VI) resulted in significant reductions in mean serum levels of PGE2 on day 11 of the experiment as compared with untreated rats with rats with formaldehyde-induced arthritis (group IV) (p < 0.05) (Table 4).

The reduction in mean serum levels of PGE2 was nearly equal in rats with formaldehyde-induced arthritis treated with carvedilol (group VI) and those treated with lornoxicam (group V) (Table 4).

Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean hind paw volumes in ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1h</td>
</tr>
<tr>
<td>Group I (Untreated control group)</td>
<td>0.29 ± 0.03</td>
</tr>
<tr>
<td>Group II (Lornoxicam treated control group)</td>
<td>0.31 ± 0.02</td>
</tr>
<tr>
<td>Group III (Carvedilol treated control group)</td>
<td>0.3 ± 0.02</td>
</tr>
<tr>
<td>Group IV (Histamine-induced paw edema group)</td>
<td>1.58 ± 0.08a</td>
</tr>
<tr>
<td>Group V (Histamine-induced paw edema group treated with lornoxicam)</td>
<td>0.65 ± 0.04b, b</td>
</tr>
<tr>
<td>Group VI (Histamine-induced paw edema group treated with carvedilol)</td>
<td>0.82 ± 0.02b, b</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for 8 animals in each group.

a Statistically significant compared to untreated control group (group I) (p < 0.05).

b Statistically significant compared to arthritic group (group IV) (p < 0.05).
Gross examination of stomachs of rats of the untreated control group (group I) revealed normal gastric mucosae. Gastric mucosae of most rats treated with lornoxicam (group II) showed ulcers of 1 mm and petechiae with significant increases in ulcer scores compared with rats of the untreated control group (group I) \((p < 0.05)\). Rats treated with carvedilol (group III) showed normal gastric mucosae with no evidence of erosions or hemorrhagic lesions (Table 5, Fig. 1).

Stomachs of rats with cold stress induced ulcers (group IV) showed small linear hemorrhagic lesions and mucosal erosions with a significant rise in the mean ulcer score compared with rats of the untreated control group (group I) \((p < 0.05)\) (Table 5, Fig. 1).

Treating rats with lornoxicam for 11 days before induction of cold stress induced ulcers (group V) resulted in significant deterioration of mean ulcer scores compared with the stress ulcer group \((p < 0.05)\) (group IV). On the other hand, rats treated with carvedilol for 11 days before induction of cold stress induced ulcers (group VI) showed significant improvement of mean ulcer scores compared with the stress ulcer group (group IV) and lornoxicam treated stress ulcer group (group V) \((p < 0.05)\) (Table 5, Fig. 1).

Gastric microscopic examination of animals of the untreated control group (group I) showed normal mucosae with normal glands and mucus volume. No evidence of erosions was seen (grade 0). Gastric mucosae of rats treated with lornoxicam (group II) showed a picture of erosive gastritis with grade I erosion in 75% of cases and normal mucosae (grade 0) in 25% of cases while all rats treated with carvedilol (group III) showed normal intact mucosae (grade 0) (Figs. 2 & 3).

Microscopic examination of gastric mucosae of rats subjected to cold stress (group IV) revealed a picture of erosive gastritis with grade I erosion in 62.5% of cases and grade II erosions in 37.5% of cases. Congestion and edema were noticed in all cases (Figs. 2 & 3). Gastric

### Table 2
Mean paw volumes (mean ± SD) in formaldehyde-induced arthritis groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Paw volume in ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 3</td>
</tr>
<tr>
<td>Group I (Untreated control group)</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td>Group II (Lornoxicam treated control group)</td>
<td>0.25 ± 0.03</td>
</tr>
<tr>
<td>Group III (Carvedilol treated control group)</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>Group IV (Arthritic group)</td>
<td>1.15 ± 0.05(^a)</td>
</tr>
<tr>
<td>Group V (Arthritic group treated with lornoxicam)</td>
<td>0.43 ± 0.02(^a)</td>
</tr>
<tr>
<td>Group VI (Arthritic group treated with carvedilol)</td>
<td>0.50 ± 0.06(^a,b)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for 8 animals in each group.

\(^a\) Statistically significant compared to treated control group (group I) \((p < 0.05)\).

\(^b\) Statistically significant compared to arthritic group \((p < 0.05)\).

### Table 3
Mean serum levels (mean ± SD) of tumor necrosis factor-α (TNF-α) in formaldehyde-induced arthritis groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>TNF-α on day 12 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Untreated control group)</td>
<td>12.2 ± 0.13</td>
</tr>
<tr>
<td>Group II (Lornoxicam treated control group)</td>
<td>12 ± 0.22</td>
</tr>
<tr>
<td>Group III (Carvedilol treated control group)</td>
<td>12.8 ± 0.3</td>
</tr>
<tr>
<td>Group IV (Arthritic group)</td>
<td>50.5 ± 0.38(^b)</td>
</tr>
<tr>
<td>Group V (Arthritic group treated with lornoxicam)</td>
<td>22.8 ± 0.32(^ab)</td>
</tr>
<tr>
<td>Group VI (Arthritic group treated with carvedilol)</td>
<td>18.6 ± 0.24(^abc)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for 8 animals in each group.

\(^a\) Statistically significant compared to untreated control group (group I) \((p < 0.05)\).

\(^b\) Statistically significant compared to arthritic group \((p < 0.05)\).

### Table 4
Mean serum levels (mean ± SD) of prostaglandin-E\(_2\) (PGE\(_2\)) in formaldehyde-induced arthritis groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>PGE(_2) on day 12 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Untreated control group)</td>
<td>210.3 ± 4.1</td>
</tr>
<tr>
<td>Group II (Lornoxicam treated control group)</td>
<td>214.6 ± 5.4</td>
</tr>
<tr>
<td>Group III (Carvedilol treated control group)</td>
<td>212.8 ± 3.3</td>
</tr>
<tr>
<td>Group IV (Arthritic group)</td>
<td>870.6 ± 8.38(^a)</td>
</tr>
<tr>
<td>Group V (Arthritic group treated with lornoxicam)</td>
<td>560.8 ± 8.32(^b)</td>
</tr>
<tr>
<td>Group VI (Arthritic group treated with carvedilol)</td>
<td>544.6 ± 7.24(^b)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for 8 animals in each group.

\(^a\) Statistically significant compared to untreated control group (group I) \((p < 0.05)\).

\(^b\) Statistically significant compared to arthritic group \((p < 0.05)\).

### 3.5. Assessment of ulcerogenic effect

Gross examination of stomachs of rats of the untreated control group (group I) revealed normal gastric mucosae. Gastric mucosae of most rats treated with lornoxicam (group II) showed ulcers of 1 mm and petechiae with significant increases in ulcer scores compared with rats of the untreated control group (group I) \((p < 0.05)\). Rats treated with carvedilol (group III) showed normal gastric mucosae with no evidence of erosions or hemorrhagic lesions (Table 5, Fig. 1).

Stomachs of rats with cold stress induced ulcers (group IV) showed small linear hemorrhagic lesions and mucosal erosions with a significant rise in the mean ulcer score compared with rats of the untreated control group (group I) \((p < 0.05)\) (Table 5, Fig. 1).

Treating rats with lornoxicam for 11 days before induction of cold stress induced ulcers (group V) resulted in significant deterioration of mean ulcer scores compared with the stress ulcer group \((p < 0.05)\) (group IV). On the other hand, rats treated with carvedilol for 11 days before induction of cold stress induced ulcers (group VI) showed significant improvement of mean ulcer scores compared with the stress ulcer group (group IV) and lornoxicam treated stress ulcer group (group V) \((p < 0.05)\) (Table 5, Fig. 1).

Gastric microscopic examination of animals of the untreated control group (group I) showed normal mucosae with normal glands and mucus volume. No evidence of erosions was seen (grade 0). Gastric mucosae of rats treated with lornoxicam (group II) showed a picture of erosive gastritis with grade I erosion in 75% of cases and normal mucosae (grade 0) in 25% of cases while all rats treated with carvedilol (group III) showed normal intact mucosae (grade 0) (Figs. 2 & 3).

Microscopic examination of gastric mucosae of rats subjected to cold stress (group IV) revealed a picture of erosive gastritis with grade I erosion in 62.5% of cases and grade II erosions in 37.5% of cases. Congestion and edema were noticed in all cases (Figs. 2 & 3).

### Table 5
Mean gastric ulcer scores (mean ± SD) in different studied groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ulcer scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Untreated control group)</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Group II (Lornoxicam treated control group)</td>
<td>1.5 ± 0.32(^a)</td>
</tr>
<tr>
<td>Group III (Carvedilol treated control group)</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Group IV (Stress ulcer group)</td>
<td>5.33 ± 0.72(^a)</td>
</tr>
<tr>
<td>Group V (Lornoxicam treated stress ulcer group)</td>
<td>7.00 ± 0.62(^ab)</td>
</tr>
<tr>
<td>Group VI (Carvedilol treated stress ulcer group)</td>
<td>3.82 ± 0.61(^ab)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for 8 animals in each group.

\(^a\) Statistically significant compared to untreated control group (group I) \((p < 0.05)\).

\(^b\) Statistically significant compared to stress ulcer group (group II) \((p < 0.05)\).
mucosae of rats treated by lornoxicam before the induction of cold
stress (group V) revealed a picture of erosive gastritis with grade I
erosion in 37.5% of cases and grade II erosions in 62.5% of cases.
Parietal cell destruction was seen in most cases. Hemorrhage was
seen in a few cases (Figs. 2 & 3).

Microscopic examination of gastric mucosae of rats treated with
carvedilol before the induction of cold stress (group VI) revealed a
picture of erosive gastritis with grade I erosion in 75% of the cases and
grade II erosions in 25% of the cases with improvement in congestion.
No parietal cell destruction or hemorrhage was detected (Figs. 2 & 3).

4. Discussion

Despite continuous improvement in RA treatment, the mortality gap
between patients with this disease and the general population has not
been closing [18]. Oxidative stress may share in the pathogenesis of RA
where excessive generation of ROS by activated neutrophils and mac-
rophages cause damage to joints [41]. Chronic inflammation can pro-
 mote endothelial cell activation and vascular dysfunction, which would
lead to decreased blood vessel compliance, atheroma formation and
vascular complications associated with RA [26].

The present study aimed to detect the possible anti-inflammatory
effects of carvedilol in two models of inflammation; histamine-induced
paw edema and formaldehyde-induced arthritis and to evaluate the
possible underlying mechanisms and the gastric ulcerogenic effects of
such treatments.

In the present study, subplantar injection of histamine in rats caused
significant increase in the mean hind paw volumes after 1, 2 and 3 h
compared with mean paw volumes of rats of the untreated control
group.

Inflammation likely consists of three stages; an increase in vascular
permeability; leukocyte migration; and proliferation of connective
tissue. Swelling is the first stage in the inflammatory process. Although
the second stage of inflammation is not dependent on the first stage, the
therapeutic effects of agents acting on the second stage may be influ-
enced by the first stage. Histamine evokes the release of neuropeptides
and prostaglandins from endothelial cells leading to hyperalgesia and
other pro-inflammatory effects [43].

In the present study, subplantar injection of formaldehyde on day
one and three of the experiment resulted in significant elevations of
mean paw volumes on days 3, 5, 7, 9 and 11 compared with mean paw
volumes of rats of the untreated control group, which was associated
with significant increases in TNF-α and PGE_2 levels in the sera of rats
with induced arthritis compared with rats of the untreated control
group.

Studying the effect of drugs on formaldehyde-induced joint edema is
one of the most suitable methods to evaluate the anti-arthritic activity
of such drugs. Inflammation induced by formaldehyde injection is
possibly mediated through the release of histamine, serotonin and
prostaglandins at the site of injection. These mediators stimulate local
pain receptors and nerve terminals causing hypersensitivity at the area
of injury [31].
Tumor necrosis factor-α (TNF-α) is a pleiotropic cytokine which is involved in many aspects of inflammation relevant to the pathogenesis of RA, including leukocyte stimulation through the activation of endothelial cells, upregulating E-selectin and VCAM-1, and the release of several chemokines and osteoclast and chondrocyte activation factors, promoting articular destruction and probably attendant cardiovascular comorbidity [8].

Prostaglandin E2 (PGE2) is the major PG generated by chondrocytes and synovial fibroblasts. Its synthesis is enhanced by proinflammatory cytokines [35]. PGE2 can regulate the function of immune cell as macrophages, T and B lymphocytes and cytokine expression profile of dendritic cells and T cell differentiation [61].

Elevated levels of inflammatory mediators were detected by Agha and Mansour [2] in a rat model of arthritis and by Kaur and Sultana [28] in an adjuvant-induced arthritis model in mice. Honda et al. [22] showed prostaglandin-mediate development of paw swelling associated with collagen-induced arthritis. Likewise, studies of carrageenan induced paw edema and pleurisy revealed the elevation of EP2 in the inflammatory exudates [64].

In the present study, rats treated with lornoxicam 1 h before subplantar injection of histamine showed significant reductions in mean paw volumes compared to untreated rats. Rats treated with lornoxicam showed significant improvement in mean paw volumes in the formaldehyde-induced arthritis model compared with untreated rats.

Fig. 2. Histopathological sections of rat gastric mucosa (H & E X 200).
A: Normal gastric mucosa in group I (untreated control group).
B: Erosion of the superficial epithelial layer and less than one third the thickness of the mucosa (Grade 2) in group II (lornoxicam treated control group).
C: Normal gastric mucosa in group III (carvedilol treated control).
D: Erosion of the superficial epithelial layer and less than one third the thickness of the mucosa (Grade 2) in group IV (cold stress ulcer group).
E: Erosion of the superficial epithelial layer and less than one third the thickness of the mucosa (Grade 2) with appearance of focal hemorrhage in group V (lornoxicam treated stress ulcer group).
F: Gastric mucosal erosion (Grade 1) in group VI (carvedilol treated stress ulcer group).

Fig. 3. Percentage of histopathological grades of gastric mucosa in different studied groups.
-Data was measured as a percentage of occurrence of a histopathological grade with respect to the total number of animals within the same group (each group contained 8 rats). Group I: untreated control group, Group II: lornoxicam treated control group, Group III carvedilol treated control group, Group IV: cold stress ulcer group, Group V: lornoxicam treated stress ulcer group, Group VI: carvedilol treated stress ulcer group.
-Grade 0: normal gastric mucosal cells appeared intact with normal shape and location.
-Grade 1 ulcer: erosion of the superficial epithelial layer.
-Grade 2 ulcer: erosion of the superficial epithelial layer and less than one third the thickness of the mucosa.
Treatment of this group with lornoxicam led to significant reductions in mean serum levels of TNF-α and PGE_2 compared with untreated rats.

The anti-inflammatory effect of lornoxicam on carrageenan induced paw edema model in rats was recorded by Buritova and Besson [9] and Tyagi and Kori [56].

Lornoxicam can inhibit polymorphonuclear (PMN) leukocyte migration [54], superoxide release from human PMN leukocytes and nitric oxide release from macrophages [12].

In the present study, rats treated with carvedilol one hour before subplantar injection of histamine showed a significant reduction in mean paw volume compared to untreated rats. This improvement is nearly equal to that detected in rats treated with the standard anti-inflammatory lornoxicam.

Rats treated with carvedilol showed significant improvement in mean paw volumes in the formaldehyde-induced arthritis model compared with untreated rats with insignificant improvement compared with rats treated with lornoxicam on days 9 and 11 of the experiment. Treatment of carvedilol in this group lead to significant reductions in mean serum levels of TNF-α compared with rats treated with lornoxicam and an insignificant reduction in mean serum levels of PGE_2 compared with rats treated with lornoxicam.

The results of the present study are in agreement with Yue-Chun et al. [62] who showed the ability of carvedilol to inhibit leucocyte migration, oxidative stress and release of inflammatory mediators such as TNF-α and eicosanoids such as PGE_2 in a murine model of viral myocarditis. Li et al. [33] and Wang et al. [59] demonstrated that carvedilol can attenuate myocardial lesions and cellular infiltration in mice with acute viral myocarditis.

Chen and Hong [11] showed that carvedilol is able to reduce the symptoms of conjunctivitis in rat models. Also, Amirshahrorkhi and Khalili [4] demonstrated the anti-inflammatory properties of carvedilol in the inhibition of paraquat-induced lung injury.

Arab and El-Sawalhi [5] detected the ability of carvedilol to decrease cytokines and eicosanoids in joint exudates and sera of rats in adjuvant-induced arthritis and subcutaneous air pouch models. Sunitha et al. [52] showed that carvedilol can improve carrageenan induced rat paw edema.

The anti-inflammatory effects of carvedilol may be due to its ability to reduce excessive oxidative stress and preserve intracellular mitochondrial function by inhibition of low-density lipoprotein oxidation by macrophages through scavenging of ROS [47] and the formation of reactive hydroxyl radicals [36]. The powerful antioxidant activity of carvedilol has been demonstrated by Kumar and Dogra [30] who concluded that carvedilol is approximately 10 times more potent than vitamin E as an antioxidant.

Carvedilol probably decreases levels of inflammatory cytokines through downregulation of their mRNA expression [63]. This could be due to inactivation of NF-kB mediated expression of proinflammatory cytokines. Carvedilol has been reported to inhibit the protein expression of NF-kB during inflammation in vivo [19] as well as in vitro [60].

Carvedilol inhibits NF-kB migration to the nucleus and expression of genes by prevention of inhibitor of kB (IkBα) degradation and dissociation of the IkBα inhibitory protein from NF-kB. Also, carvedilol may block NF-kB-dependent reporter gene transcription, which results in in the inhibition of T cell activation [10].

Carvedilol's effects may also be due to it mediating an increase IL-10 levels. IL-10 is a potent anti-inflammatory molecule that has been shown to inhibit the production of TNF-α and IL-1 and to suppress the activation of NF-kB. IL-10 reduces macrophage production of nitric oxide and reactive oxygen intermediates, and also reduces the expression of adhesion molecules and chemokines [16].

The observed decrease of PGE_2 with carvedilol usage may be also due to inhibition of TNF-α, since TNF-α increases the overexpression of COX-2 in inflamed joint tissues [35].

The present study was extended to evaluate the safety of carvedilol treatment on the gastric mucosa. Normal rats treated with lornoxicam for 11 days showed significant increase in gastric ulcer scores compared with rats in the untreated control group, while normal rats treated with carvedilol for 11 days did not show any evidence of gastric irritation or ulceration. Rats treated by carvedilol for 11 days before induction of cold stress ulcers showed significant improvement detected on both gross and histopathological examinations compared with untreated rats with cold stress induced ulcers as well as those treated with lornoxicam for 11 days. The latter's ulcer scores had even significantly deteriorated.

The results of the present study matched those of Aabakken et al. [1] regarding the ulcerogenic effects of lornoxicam treatment in healthy volunteers. Lornoxicam can damage gastric mucosa via; an irritant effect on the epithelium; the impairment of the barrier properties of the mucosa; suppression of gastric prostaglandin synthesis; reduction of gastric mucosal blood flow; and interference with the repair of superficial injury [57]. The slow rate of dissolution of lornoxicam may enhance its gastric irritant effect. The presence of undissolved particles of the drug in contact with the gastric mucosa leads to high local concentration of the drug which leads to local irritation and ulceration of the mucosa [27].

Mechanisms of the protection of the mucosa induced by carvedilol may be due to its ability to bind free radicals and inhibit lipid peroxidation. There is considerable evidence concerning the participation of reactive oxygen species in the etiology and pathophysiology of the gastric ulcer disease [29]. Carvedilol can prevent lipid peroxidation [23].

Tunez et al. [55] confirmed the cytoprotective effect of carvedilol in cell culture. Ronsein et al. [45] described the cytoprotective effect of carvedilol against free radicals in rat liver. Al-Rejaie [3] demonstrated that carvedilol has the ability to decrease gastric acid secretion and to protect against ethanol induced ulceration in rats - both effect of which can be explained by the inhibitory effect of carvedilol on lipid peroxidation.

Carvedilol may enhance the secretion of PGE_2 in the gastric mucosa. Kaan et al. [25] reported that propranolol can increase prostaglandin E_2 levels in gastric mucosa which may explain its anti-ulcer effects. Prostaglandin E_2 protects the gastric mucosa by promoting increased secretion of the protective mucus that lines the gastrointestinal tract. Increased mucus secretion can prevent gastric ulceration by: decreasing stomach wall friction during peristalsis and acting as a barrier preventing back diffusion of hydrogen ions [49]; protecting the underlying epithelium against acid [17] and pepsin [24]; and facilitating the repair of damaged gastric epithelium [53].

In conclusion, carvedilol may have a promising role in treatment of patients with rheumatoid arthritis. Carvedilol, by virtue of its anti-arthritic properties, can delay the progression of rheumatoid arthritis as well as lower the risk of cardiovascular disorders. The anti-arthritis effects of carvedilol may be due to the suppression of pro-inflammatory cytokines (such as TNF-α) and eicosanoids (such as PGE_2). Use of carvedilol showed an advantage over the use of nonsteroidal anti-inflammatory drugs, having no risk of inducing gastric ulceration.

Further studies are needed to detect the safety of the long-term usage of carvedilol in patients with rheumatoid arthritis and any drug interactions between carvedilol and other ant-arthritic drugs.

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References
