Syntehsis and gastroprotective activities of some zinc (II) complexes derived from (E)-2-(1-(2-(piperazin-1-yl)ethylimino)ethyl)phenol and (E)-4-(1-(2-(piperazin-1-yl)ethylimino)ethyl)benzene-1,3-diol Schiff bases against aspirin induced ulceration

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Abstract This study describes the protective effects of piperazine derived compounds against aspirin induced gastric injuries and evaluated the role of nitric oxide, inflammatory cytokines and serum level of aspartate aminotransaminases (AST), alanine aminotransaminases (ALT), high density lipoprotein (HDL) and malondialdehyde (MDA). The oral administration of the compounds at doses 30 and 60 mg/kg protected the gastric against the nectrotizing effects of aspirin. The level of nitric oxide (NO) was elevated in the group pretreated with the compounds. The results also showed that pre-treatment with piperazine compounds has led to the decrease in the amount of MDA and increased the activity of AST, ALT and HDL. In conclusion, pre-treatment with piperazine derived compounds; (E)-2-(1-(2-(piperazin-1-yl)ethylimino)ethyl)phenol (2HP), (E)-4-(1-(2-(piperazin-1-yl)ethylimino)ethyl) benzene-1,3-diol (DHP) and their zinc complexes has provided a significant protection to the gastric from damaging effects of aspirin.

1. Introduction

The therapeutic potencies of the non-steroidal anti-inflammatory drugs (NSAID) such as prophylaxis of cardiovascular events, conversion of fever and pains associated with common cold, arthritis and acute rheumatism have been reported (Bertagnolli, 2003; Monnier et al., 2005). Aspirin, one of the
famous NSAID has been frequently used as an analgesic to relieve minor aches, effective in the treatment of patients with aspirin exacerbated respiratory diseases (AERD) and many forms of cancer except for prostate cancer (Vane, 1971). Administration of aspirin in long-term at low doses to people at high risk of developing blood clots has been found to prevent heart attacks, strokes and blood clot formation (Patrono, 1994; Riddker et al., 1997). Apart from the health benefits attributed to aspirin, substantial gastric spachelus had been noticed in many studies. Some of the unwanted side effects of aspirin involved tinnitus, gastrointestinal and stomach bleedings particularly in high doses. The ability of aspirin to restrain the production of prostaglandin and thromboxanes was due to its non selective inhibition of the enzyme cylooxygenase by acting as acetylation agent and covalently binding to serine residue in the active site of enzyme (PTGS), or it buffers and transports proton (Smith and Willis, 1971). Moreover, the effects of aspirin involved inhibition of growth factor, infiltration of neutrophils and elevation of cytokines (Choi et al., 2010).

Recent study suggests that the cytokines such as tumor necrosis factor-alpha (TNF-a), interleukin-6 (IL-6) and interleukin-10 (IL-10) play important roles in the acute phase inflammation as well as in safeguarding and controlling the severity of gastric ulcers (Salga et al., 2011). Piperazine compounds have found substantial pharmaceutical applications such as anthelmintic for the treatment of worms like ascariasis, enterobiosis, and oxyriasis, acyl piperazine is used as an anti-inflammatory agent for the treatment of inflammatory disorders in human (Gauthier et al., 1996). Moreover, N-benzylpiperezines (BPZ) are also used as active ingredients in recreational pills commonly used to provide stimulant euphoric effect similar to that of methylenedioxyamphetamine (MDMA) ecstasy. BPZ predominantly affects dopamine transmission but the exact mechanism of action is still unknown (Johnstone et al., 2007). Zinc on the other hand, played an important role in cell-mediated immune functions and in children growth, it was also considered as a cofactor for metallo-enzymes, superoxide dismutase, (SOD) collagenase, alcohol dehydrogenase and spermatogenesis. It is required for the proper functioning of mucosal cells and it protects gastrointestinal diseases by acting as an antioxidant and anti-inflammatory agent. Furthermore, zinc deficiency leads to poor wound healing, loss of taste and smell and causes elevation of reactive oxygen species (ROS) (Tuoromaa, 1995). This study therefore, was aimed at synthesizing for the first time, the zinc complexes derived from (E)-2-(1 -(2-(piperazin-1-yl) ethylimi- 

2. Materials and methods

2.1. Materials

The chemicals 1-(2-hydroxyphenyl)ethanone, 1-(2,4-dihydroxyphenyl)ethanone, zinc(II) chloride hydrate, solvents and other reagents were of analytical grade and are purchased from Merck–Aldrich, Kuala Lumpur, Malaysia. Mass spectra were recorded on Maldi-TOF/TOF-ESI mass spectra. CHN analysis was carried out on Costech Elemental Combustion System CNHS-O elemental analyzer. NMR was performed on ECA-400 higher-performance FT-NMR Spectrophotometer. IR spectra were obtained from Perkin-Elmer FTIR Spectrophotometer. UV–visible spectroscopy was conducted on Perkin-Elmer-1650-UV–visible spectrophotometer; TGA was recorded on TGA-3000 thermo-analyzer.

2.2. Methods

2.2.1. Synthesis and characterizations of zinc (II) complexes

The Schiff bases 2HP and DHP were prepared according to the procedure reported (Saleh et al., 2011) in our previous work with some slight modifications. The zinc(II) complexes are synthesized according to the following general procedure.

A solution of the Schiff base 2HP (0.25 g, 1 mmol) in methanol (25 mL) was added to the equimolar solution of zinc(II) chloride hydrate (0.14 g, 1 mmol) in methanol (25 mL) and stirred at room temperature for 1 h. A light-yellow precipitate was formed. The solid product (Zn2HP) was filtered, washed with absolute ethanol and dried in a vacuum. The solid (Zn2HP) was then dissolved in ethanol-chloroform mixture (30:70) and after three days in the mother-liquor, it produces yellow needle-like crystals of very poor quality. Recrystallization was carried out in methanol-dichloromethane mixture but the nature and quality of the crystals still remained. It exhausted our effort to produce high quality crystals for X-ray diffraction. Same procedure was applied for the preparation of ZnDHP complex.

2.2.2. Analysis of the complex [Zn(2HP)Cl2]

Empirical formula: C14H19Cl2N3O2Zn. Molecular weight: 383.65 g/mol. Yield: (0.21 g, 54.8%). Anal. Cal. C, 43.83; H, 5.52; N, 10.95. Found: C, 42.13; H, 4.89; N, 10.52. IR (KBr, cm\(^{-1}\)): 3492 (NH); 2961 (C–H); 1597 (iminic); 1460 (arC–C); 1305 (phenolate); 538 (M–O); 493 (M–N). \(^{1}C\) NMR spectrum (DMSO-d6), \(\delta\) ppm: 6.02–7.56 (m, 2H, arH), 2.46 (d, 4H, 2CH2, aliphatic), 2.19 (t, 3H, ketoiminic), 2.31 (d, 4H, 2CH2, aliphatic), 6.02–6.56 (m, 2H, arH). \(^{1}C\) NMR spectrum (DMSO-d6), \(\delta\) ppm: 14.0 (CH3), 20.0 (CH2), 45.0 (DMSO-d6 + CH2), 116–172 aromatic (CH). UV–visible: 267 nm (17.157 mol\(^{-1}\)L cm\(^{-1}\), \(\pi\rightarrow \pi^*\)). m/z: 383.03 (molecular ion peak), 381.02, 385.02.

2.2.3. Analysis of the complex [Zn(DHP)Cl2]

Empirical formula; C14H23Cl2N3O2Zn. Molecular weight: 399.65 g/mol. Yield: (0.19 g, 47.6%). Anal. Cal. C, 42.07; H, 5.30; N, 10.95. Found: C, 40.75; H, 5.09; N, 9.85. IR (KBr, cm\(^{-1}\)): 3503 (NH); 2952 (C–H); 1594 (iminic); 1370 (arC–C); 1274 (phenolate); 537 (M–O); 459 (M–N). \(^{1}C\) NMR spectrum (DMSO-d6), \(\delta\) ppm: 2.08 (t, 3H, ketoiminic), 2.31 (d, 4H, 2CH2, aliphatic), 6.02–6.56 (m, 2H, arH). \(^{1}C\) NMR spectrum (DMSO-d6), \(\delta\) ppm: 14.0 (CH3), 19.5 (CH2), 40.0 (DMSO-d6 + CH2), 103–172 aromatic (CH). UV–visible: 260 nm (22037.9 mol\(^{-1}\)L cm\(^{-1}\), \(\pi\rightarrow \pi^*\)). m/z: 399.65 (molecular ion peak), 399.03, 397.03.

2.3. Ulcerogenic study

2.3.1. Study design

2.3.1.1. Sprague Dawley. Healthy adult rats of 7–8 weeks weighing about 170–200 g were used throughout the study. The rats were divided into nine major groups of five rats each.
They were fed with a standard diet and tap water. Acute gastric lesions were induced by oral administration of absolute ethanol (5 ml/kg) to the rats, which were fasted for 24 h before the experiments, but had free access to water. This experiment was approved by the Ethics Committee for the care and use of laboratory animals organized by the University of Malaya, Kuala Lumpur, Malaysia.

The experimental rats were assigned to their respective groups as follows, Group I and II rats were given food and water for 14 days. Group III rats were treated with omeprazole (30 mg/kg) dissolved in carboxymethylcellulose (CMC) single dose, Group IV and V received the Schiff bases 2HP and DHP respectively at the dose of 30 mg/kg each, for 14 days. Rats in Groups VI and VII were given Zn2HP 30 and 60 mg/kg correspondingly for two weeks, while rats in Groups VIII and IX were orally administered with ZnDHP 30 and 60 mg/kg body weight respectively for 14 days. After two week treatments with or without the compounds, the animals were fasted for 24 h and on day 15, group III, received omeprazole. Groups IV–IX were given the compounds according to the fixed doses whereby Group I and II received vehicle. One hour after this treatment, animals in Group II–IX received aspirin (600 mg/kg) dissolved in CMC orally. The animals were sacrificed 8 h later by cervical decapitation under anesthetized xylazine and ketamine to obtain serum for biochemical analysis.

2.3.2. Determination of ulcerogenic effects
The rats were sacrificed under anesthesia (xylazine and ketamine, 5 ml/kg), the stomachs were excised and opened along the greater curvature. Lesion area was determined by measuring each lesion along its greatest diameter under a dissection microscope (1.8x). The area of each ulcer lesion was measured by counting the number of small squares (2 × 2 mm) covering the length and width of each ulcer band. The sum of the lesions’ affected areas for each stomach was calculated and used in the determination of ulcer area (UA mm²) and percent inhibition (I %) (Mahmood et al., 2010). The ulcer score was expressed as square millimeters.

2.3.3. Serum evaluations
Blood samples collected from the experimental rats were centrifuged at 4000 rpm for 10 min and analyzed for the changes in serum enzymes AST, ALT and HDL using Hitachi-auto analyzer at University Malaya Medical Center.

2.3.4. Cytokine evaluations
The level of pro-inflammatory (IL-6 and TNF-α) and anti-inflammatory IL-10 cytokines in the serum of experimental rats was assessed using ELISA kits for rats (Ray Biotech, Norcross GA, USA) according to the manufacturer’s instructions. Primary antibodies were first coated on the well plate and after washing, each well was blocked to remove the non-specific binding. 100 μL of sample or cytokine standards was added to each well and then followed with biotin conjugated secondary antibodies. To obtain a color reaction, streptavidin-HRP and the substrate solution were added. The absorbance was measured at 450 nm with an ELISA reader (TECAN, Manne-dorf, Switzerland). Standard curves were plotted on each assay plate using recombinant IL-6, IL-10, and TNF-α in serial dilution.

2.3.5. Determination of Nitric Oxide (NO) level
The level of nitric oxide in the gastric tissue was evaluated as total nitrate/nitrite levels using Gries’s reagent (Lanza et al., 1980; Lopez-Belmonte et al., 1993). The gastric homogenate in 50 mM potassium phosphate buffer (pH 7.8) was centrifuged at 4000 rpm for 30 min at 4 °C. 50 μL of Gries’s reagent (0.1% N-(1-naphthyl) ethylenediamide dihydrochloride, 1% sulfanilamide in 5% phosphoric acid) was added to supernatant (50 μL) and mixed, then after 10 min the absorbance was measured at 540 nm. The standard curves were obtained by using sodium nitrite and expressed as micromoles nitrate/nitrite per gram of protein.

2.3.6. Evaluation of malondialdehyde (MDA)
The level of malondialdehyde was estimated using the thiobarbituric acid-reactive substance (as indicators of lipid peroxidation) assay according to the reported procedure (D’Argenio et al., 2008; Hung, 2004) with some modifications. A solution containing 26 mM thiobarbituric acid, 0.26 M hydrochloric acid, 15% trichloric acid and 0.02% butylated hydroxyl toluene was prepared and stocked. A portion of this solution (0.125 mL) was added to the stomach homogenates, heated at 96 °C for 15 min and centrifuged at 4000 rpm for 10 min. The supernatant was transferred to the 96-well plate, and the absorption was measured at 532 nm using tetramethoxypropane as standard.

2.3.7. Histological evaluations
Histological evaluations were performed to estimate the extent at which the ethanol damages the gastric mucosa. The pieces of tissue samples were prepared and placed in 10% buffered formalin for processing in a Paraffin Tissue Processing Machine. Sections of the gastric were made at a thickness of 5 μM and stained with hematoxylin and eosin for histological evaluations.

2.3.8. Statistical analysis
All values were reported as mean ± S.E.M. The significant differences between groups were assessed using one-way ANOVA. A value of p < 0.05 was considered significant.

3. Results and discussion
3.1. Synthesis and characterizations of zinc(II) complexes

In this study, a reflux condensation reaction was used to synthesize the complexes of zinc (II) chloride. Treatment of methanolic solution (25 mL) of the Schiff bases (E)-2-(1-(2-(piperazin-1-yl)ethylimino)ethyl) benzene-1,3-diol (DHP) with the solution of zinc(II) chloride in methanol (25 mL) resulted in the formation of complexes Zn2HP and ZnDHP, respectively. The compounds demonstrated MS, CHN, NMR, IR, UV–visible and TGA consistent with the targeted structure shown in Scheme 1. Compounds containing the imine group in their structure were reported to have E/Z geometrical isomers relative to –N==CH— double bond based on the solvent used (Demirbas et al., 2004). Examination of 1H NMR spectra of both complexes revealed definite chemical shift signals for –N==CH— which was shielded by the methyl...
group of the ketoimine and appeared once. This is in agreement with the report that higher percentage of imine-containing compounds in dimethyl sulfoxide-d$_6$ (DMSO-d$_6$) solution is present in the form of geometrical E isomer about A=N—CH double bond (Demirbas et al., 2004). The phenolic hydrogen observed at $\delta$ 7.6 and $\delta$ 10.95 in the spectra of the free Schiff bases 2HP and DHP had shifted to $\delta$ 8.01 and $\delta$ 7.16 in their zinc (II) complexes due to the chelate formation (Figs. 1–4). The characteristic IR bands of Zn (II) complexes derived from 2HP and DHP showed frequency bands at 1597 and 1594 cm$^{-1}$ for $\nu$(iminic) and at 3492 and 3503 cm$^{-1}$ for $\nu$(phenolate). These bands were observed at the frequencies of 1616 and 1610 cm$^{-1}$ for $\nu$(iminic) and 3394 and 3371 cm$^{-1}$ for $\nu$(phenolic), in the spectra of the free Schiff bases (Figs. 5–8). This is evidenced for the bonding of zinc with imine nitrogen and phenolate oxygen (Shama and Omara, 2001). The UV–visible of the complexes Zn2HP and ZnDHP recorded in DMSO solvent showed absorption at the band regions 267 and 260 nm which can be assigned to $\pi \rightarrow \pi^*$ electronic transition in the phenolic ring (Refat et al., 2008; Roncali, 1997). These
absorptions were blue shifted from 318 to 283 nm in the spectra of free Schiff bases and can be affordable to π → π* electronic transition originated from imine chromophore. Moreover, the TGA curves for the complexes were recorded from 50 to 900 °C at 20 °C intervals (Fig. 9) in order to determine the first decomposition temperature of the compounds. Both compounds exhibited two step decomposition with a clearly separated first step at 311 °C in Zn2HP curve. The observed mass loss corresponds to the departure of a molecule with a relative molar mass of about 71 g/mol which can presumably be attributed to the loss of one Cl2 molecule. Furthermore, the compound ZnDHP showed its first decomposition at 269 °C with a mass loss of 130.03 g. This corresponds to the departure of one hydroxyl group and ethyl piperazine molecules. Generally, the compounds showed high thermal stability, which is required for this study.
3.2. Anti-ulcerogenic activity

The experimental animals pretreated with the compounds 2HP, DHP and their Zn(II) complexes for 14 days manifested good protection against the damaging effects of aspirin. This was substantiated by the ability of the compounds to safeguard the mucosal integrity and enhanced the production of nitric oxide synthase. The effective dose was chosen through

Figure 3  $^{13}$C NMR spectra for [Zn(2HP)Cl$_2$].

Figure 4  $^{13}$C NMR spectra for [Zn(DHP)Cl$_2$].
Figure 5  IR-spectra of 2HP.

Figure 6  IR spectra of DHP.
Figure 7  IR spectra for Zn-2HP.

Figure 8  IR spectra for Zn-DHP.
The oral pilot dose selection of aspirin at 400 mg/kg administered to the 12 and 24 h fasted rats. It was observed that this dose did not significantly induce ulcer even after 24 h of fasting. The dose was then adjusted to 600 mg/kg and administered to 12 and 24 h fasted rats. At this dose, few lesions and gastric bleeding were observed after 12 h whereby severe lesions appeared in the gastric of the rats fasted for 24 h. The parameters measured from the gastric content of the rats treated with aspirin after pretreatment with the Schiff bases 2HP, DHP and their zinc(II) complexes are presented in (Table 1). The ulcerated animals showed significant rise in acidic pH and low mucus content whereby the animals pretreated with the compounds exhibited considerable inhibition of high stomach acidity and increased mucus production dose dependently.

### 3.3. Gastric lesions

The compounds manifested protective activities against the aspirin gastric ulceration dose-dependently as observed macroscopically after 8 h of ulcer induction (Fig. 10). The Schiff bases 2HP and DHP had significantly inhibited the gastric injuries by 66.2% and 69.4%, respectively. However, this is below the percentage inhibition of 82.43% shown by the referenced drug (omeprazole). These inhibitory activities increase dose-dependently in the zinc complexes of the Schiff bases to 83.11% in Zn2HP and to 85.95% in ZnDHP at low doses. At high doses, a slight increase in the activity of the complexes to 83.90% in Zn2HP and 86.73% in ZnDHP was noticed. This result corresponds to the reported protective activities of the free zinc (Sivalingam et al., 2011) and the observed activities of the free Schiff bases in this study. The damage caused by aspirin was identified macroscopically as areas of mucosal hyperemia, leukocyte infiltrations and hemorrhagic lesions with edema covering the total glandular area of the stomach which was inferentially confirmed by the histological assessments of the gastric tissues.

### 3.4. Serum enzymes

The serum level of aspartate (AST), alanine aminotransaminases (ALT), high-density lipoprotein (HDL) and energy storage creatinine was assayed (Table 2). The liver enzymes AST and ALT rise significantly ($p < 0.01$) in rats that received aspirin. This indicates damage to the liver cells. However, the rats pretreated with the compounds showed no significant variations in the level of such enzymes when compared with the rats in the normal group. Also, the levels of creatinine and HDL in the rats treated with the compounds are comparable with those rats in the normal.

### 3.5. Cytokine assessments

The serum levels of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-$\alpha$), interleukin-6 (IL-6) and anti-inflammatory cytokine like interleukin-10 (IL-10) were evaluated (Table 3). The rats pretreated with CMC alone

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### Table 1

<table>
<thead>
<tr>
<th>Animals</th>
<th>Pretreatment (5 ml/kg)</th>
<th>Gastric pH</th>
<th>Mucus wt (g)</th>
<th>UA (mm)</th>
<th>% I</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal (CMC)</td>
<td>3.54 ± 1.6</td>
<td>2.92 ± 1.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>Aspirin (600 mg/kg)</td>
<td>0.98 ± 1.2</td>
<td>1.39 ± 1.8</td>
<td>872.2 ± 9.2</td>
<td>–</td>
</tr>
<tr>
<td>III</td>
<td>Omeprazole (30 mg/kg)</td>
<td>3.46 ± 1.1</td>
<td>3.08 ± 1.4</td>
<td>153.2 ± 7.9</td>
<td>82.43</td>
</tr>
<tr>
<td>IV</td>
<td>Ligand 2HP (30 mg/kg)</td>
<td>2.32 ± 2.3</td>
<td>2.97 ± 1.2</td>
<td>294.6 ± 2.4</td>
<td>66.22</td>
</tr>
<tr>
<td>V</td>
<td>Ligand DHP (30 mg/kg)</td>
<td>2.45 ± 1.9</td>
<td>2.98 ± 1.7</td>
<td>267.4 ± 2.2</td>
<td>69.34</td>
</tr>
<tr>
<td>VI</td>
<td>Zn-2HP (30 mg/kg)</td>
<td>3.79 ± 1.4</td>
<td>3.69 ± 2.2</td>
<td>147.3 ± 4.2</td>
<td>83.11</td>
</tr>
<tr>
<td>VII</td>
<td>Zn-2HP (60 mg/kg)</td>
<td>4.62 ± 1.3</td>
<td>4.31 ± 2.1</td>
<td>140.4 ± 3.8</td>
<td>83.90</td>
</tr>
<tr>
<td>VIII</td>
<td>ZnDHP (30 mg/kg)</td>
<td>4.64 ± 1.5</td>
<td>3.82 ± 1.6</td>
<td>122.5 ± 4.2</td>
<td>85.95</td>
</tr>
<tr>
<td>IX</td>
<td>ZnDHP (60 mg/kg)</td>
<td>5.82 ± 1.5</td>
<td>4.89 ± 1.3</td>
<td>115.7 ± 5.1</td>
<td>86.73</td>
</tr>
</tbody>
</table>

Ulcer area (UA) = (mean ± S.E.M), % I = percent inhibition.
showed a significant increase in the level of pro-inflammatory cytokines IL-6 and TNF-α and a decrease in the amount of anti-inflammatory cytokine IL-10 whereby the rats pretreated with the compounds showed high level of anti-inflammatory cytokine IL-10.

3.6. Assessment of nitric oxide level

The animals pretreated with the compounds prior to aspirin induced ulceration showed an increased level of nitric oxide synthase (NO) ($p < 0.05$) (Table 3). This signifies an enhance-

**Table 2** Effects of the compounds on serum biomarkers.

<table>
<thead>
<tr>
<th>Animals</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
<th>HDL</th>
<th>Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Normal (CMC)</td>
<td>68.47 ± 3.2</td>
<td>194.6 ± 5.2</td>
<td>1.93 ± 0.2</td>
<td>71.70 ± 4.1</td>
</tr>
<tr>
<td>II Aspirin (600 mg/kg)</td>
<td>88.42 ± 7.5</td>
<td>269.2 ± 8.1</td>
<td>0.04 ± 1.4</td>
<td>11.40 ± 1.3</td>
</tr>
<tr>
<td>III Omeprazole (30 mg/kg)</td>
<td>66.62 ± 3.1</td>
<td>178.8 ± 3.1</td>
<td>1.42 ± 0.4</td>
<td>57.70 ± 6.1</td>
</tr>
<tr>
<td>IV 2HP (30 mg/kg)</td>
<td>62.11 ± 2.3</td>
<td>165.2 ± 6.1</td>
<td>1.12 ± 0.9</td>
<td>42.20 ± 1.3</td>
</tr>
<tr>
<td>V DHP (30 mg/kg)</td>
<td>64.31 ± 4.3</td>
<td>167.4 ± 5.1</td>
<td>1.22 ± 0.4</td>
<td>49.40 ± 1.2</td>
</tr>
<tr>
<td>VI Zn2HP (30 mg/kg)</td>
<td>69.64 ± 5.3</td>
<td>182.3 ± 5.7</td>
<td>1.47 ± 0.6</td>
<td>59.20 ± 9.6</td>
</tr>
<tr>
<td>VII Zn2HP (60 mg/kg)</td>
<td>71.22 ± 3.2</td>
<td>188.3 ± 3.1</td>
<td>2.12 ± 0.9</td>
<td>64.30 ± 2.1</td>
</tr>
<tr>
<td>VIII ZnDHP (30 mg/kg)</td>
<td>70.46 ± 4.1</td>
<td>190.1 ± 2.1</td>
<td>2.20 ± 2.3</td>
<td>69.31 ± 1.8</td>
</tr>
<tr>
<td>IX ZnDHP (60 mg/kg)</td>
<td>72.32 ± 6.1</td>
<td>195.4 ± 1.6</td>
<td>2.62 ± 2.2</td>
<td>72.24 ± 1.5</td>
</tr>
</tbody>
</table>

**Figure 10** Gross gastric lesion: (A) Normal rats, (B) Aspirin induced ulcerated rats, 600 mg/KG, (C) Omeprazole (30 mg/kg, 82.43%), (D) 2HP (30 mg/kg, 66.22%), (E) DHP (30 mg/kg, 69.34%), (F) Zn-2HP (30 mg/kg, 83.11%), (G) Zn-2HP (60 mg/kg, 83.90%), (H) ZnDHP (30 mg/kg, 85.95%), and (I) ZnDHP (60 mg/kg, 86.73%).
ment in the gastric defense by the compounds against the attack of the ulcer model (Hutcheson et al., 1990). In contrast, the rats that received aspirin alone showed an acute decrease in NO. This displayed the damaging effects of aspirin in weakening the gastric mucosal barrier (Cheung and Porterfield, 1979; Fiorucci et al., 2004; Whittle et al., 1990).

**Table 3** Effects of the compounds on MDA, NO, TNF-α, IL-6 and IL-10 in ulcerated rats.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Treatments</th>
<th>MDA (μmol/g tissue)</th>
<th>NO (μmol)</th>
<th>TNF-α (pg/mg)</th>
<th>IL-6 (pg/mg)</th>
<th>IL-10 (pg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal (CMC)</td>
<td>13.21 ± 2.10</td>
<td>12.92 ± 1.20</td>
<td>0</td>
<td>0</td>
<td>245 ± 5.2</td>
</tr>
<tr>
<td>II</td>
<td>Aspirin (600 mg/kg)</td>
<td>28.11 ± 1.40</td>
<td>3.32 ± 0.06</td>
<td>386.0 ± 2.3</td>
<td>67.0 ± 2.1</td>
<td>72.0 ± 6.2</td>
</tr>
<tr>
<td>III</td>
<td>Omeprazole (30 g/kg)</td>
<td>14.21 ± 1.20</td>
<td>9.55 ± 0.12</td>
<td>112.0 ± 2.4</td>
<td>10.4 ± 1.5</td>
<td>264 ± 4.5</td>
</tr>
<tr>
<td>IV</td>
<td>2HP (30 mg/kg)</td>
<td>13.12 ± 1.10</td>
<td>9.13 ± 0.19</td>
<td>75.0 ± 2.1</td>
<td>15.30 ± 1.8</td>
<td>236 ± 3.3</td>
</tr>
<tr>
<td>V</td>
<td>DHP (30 mg/kg)</td>
<td>13.21 ± 0.30</td>
<td>9.42 ± 0.65</td>
<td>81.50 ± 1.1</td>
<td>14.60 ± 1.6</td>
<td>245 ± 2.1</td>
</tr>
<tr>
<td>VI</td>
<td>Zn2HP (30 mg/kg)</td>
<td>14.46 ± 1.23</td>
<td>9.65 ± 1.20</td>
<td>123.2 ± 2.2</td>
<td>12.67 ± 1.1</td>
<td>253 ± 5.2</td>
</tr>
<tr>
<td>VII</td>
<td>ZnHP (60 mg/kg)</td>
<td>14.51 ± 1.31</td>
<td>10.32 ± 1.13</td>
<td>129.8 ± 1.5</td>
<td>10.22 ± 1.4</td>
<td>278 ± 6.5</td>
</tr>
<tr>
<td>VIII</td>
<td>ZnDHP (30 mg/kg)</td>
<td>14.87 ± 1.92</td>
<td>10.88 ± 3.20</td>
<td>133.2 ± 1.7</td>
<td>10.15 ± 1.7</td>
<td>283 ± 4.2</td>
</tr>
<tr>
<td>IX</td>
<td>ZnDHP (60 mg/kg)</td>
<td>14.89 ± 2.10</td>
<td>11.92 ± 2.10</td>
<td>138.6 ± 1.5</td>
<td>8.21 ± 1.3</td>
<td>295 ± 5.1</td>
</tr>
</tbody>
</table>

Groups with different superscript alphabets are statistically significant.

![Figure 11](image-url) **Figure 11** Histological view of gastric mucosa: (A) Normal (CMC), (B) Aspirin induced ulcerated rats, 600 mg/kg, (C) Omeprazole (30 mg/kg, 82.43%), (D) 2HP (30 mg/kg, 66.22%), (E) DHP (30 mg/kg, 69.34%), (F) Zn-2HP (30 mg/kg, 83.11%), (G) ZnHP (60 mg/kg, 83.90%), (H) ZnDH (30 mg/kg, 85.95%), and (I) ZnDH (60 mg/kg, 86.73%).

3.7. Level of MDA in tissue homogenate

The level of lipoperoxidation in the gastric of rats that are pretreated with CMC alone (5% in distilled water) was high (28.11 ± 1.40 μmol of MDA/mg of tissue) when compared with the observed lipoperoxidation (13.21 ± 2.1 μmol of
MDA/mg of tissue) in the normal rats. Furthermore, the stomachs of rats treated with 30 mg/kg of 2HP and DHP showed a decreased level of MDA/mg in the tissue as 14.76 ± 1.23 and 14.57 ± 1.57 μmol respectively. This is bit high (p < 0.001) than the value of 13.65 ± 1.31 and 13.89 ± 2.70 μmol observed at the high dose of 60 mg/kg correspondingly. This clearly indicates the ability of the compounds to reduce the level of lipoperoxidation significantly (p < 0.001) at both doses (Table 3).

3.8. Histological assessments

Histological evaluations of the gastric wall showed no significant changes between the rats pretreated with the compounds and the rats in the normal groups (Fig. 10 A). The ulcerated rats showed mucosal hyperemia and hemorrhagic lesions with edema covering the total glandular area of the stomach (Fig. 10B) which is a proof of acute ulceration (Choi et al., 2010; Kraft et al., 1963; Lo et al., 1988). Pretreatment with omeprazole and the compounds (30 and 60 mg/kg body weight orally for 14 days) significantly reduced the number of gastric lesions (p < 0.05) (Fig. 10C–I) when compared with the ulcer group (Fig. 10B). Furthermore, the expansion of gastric epithelial cells, damage of mucosal design and predisposition of exfoliation of gastric pits observed with ulcerated rats were insignificant in the rats pre-treated with omeprazole (Fig. 11C) and the compounds (Fig. 11D–I). Therefore, overall histological scores revealed the protective efficacies of the compounds against aspirin induced ulceration by increasing the tendency of restoration of the mucosa, reduced size of ulcer crater and glandular organization.

4. Conclusion

These findings suggest the protective efficacies of zinc complexes derived from the Schiff bases 2HP and DHP in protecting the gastric mucosal cells against aspirin induced ulcers. However, further investigations need to be conducted on these novel compounds to exactly determine their chemotherapeutic mechanism of action.

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References