Gastro protective Effect of Pentagalloyl Glucose on Aspirin Induced Gastric Mucosal Ulcer in Comparison with Omeprazole, Famotidine and Melatonin

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Abstract
Phytochemical study on Harpullia pendula pericarp resulted in isolation of the five gallotannins 1,2,3,4,6-penta-O-galloyl-β-D-4Cl-glucopyranoside (1), 1,2,3,6-tetra-O-galloyl-β-D-4Cl-glucopyranoside (2), 1,3,6-tri-O-galloyl-β-D-4Cl-glucopyranoside (3), gallic acid (4), and methyl gallate (5) for the first time from the genus. Structures were established based on physical and spectroscopic data. The gastroprotective activity of pentagalloyl glucose (1) was evaluated in aspirin induced ulcer in rats, as compared to omeprazole, famotidine and melatonin. For assessment, pH of the gastric stomach juice as well as the ulcer number and severity were determined. In addition, the levels of prostaglandin, cyclooxygenase (COX-2), tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), glutathione (GSH) and malondialdehyde (MDA) were determined in gastric tissue homogenate. Results showed that pentagalloyl glucose induces increment of gastric juice pH, with decrement in number and severity of gastric mucosal ulcers and decreases the level of TNF-α and IL-1, as compared to the aspirin group. In addition, pentagalloyl glucose (100 and 200 mg/kg), famotidine and omeprazole normalized the elevated level of glutathione with decrease of malondialdehyde level, as compared to aspirin group. Furthermore, rats treated with either pentagalloyl glucose, famotidine, omeprazole or melatonin showed significant increase in prostaglandin level and significant decrease in gastric COX-2 level, as compared to aspirin group. Obtained results were supported by histopathological study.

Keywords
Antioxidant; aspirin; gastric mucosal damage; inflammatory cytokine; pentagalloyl glucose

INTRODUCTION
Peptic ulcer represents one of the most common disorders of the gastrointestinal tract, affecting about 5-10% of the people during their lives [1]. In addition, it is considered as one of the most important causes of morbidity and mortality during the 20th and 21st centuries [2]. Ulcers are responsible for upper gastrointestinal bleeding and major clinical problem resulting from the wide use of nonsteroidal anti-inflammatory drugs as aspirin, which is considered as one of the ulcer inducers [2,3]. Aspirin is a medication widely used for pain and inflammation treatment, in addition to its use in cardiovascular diseases due to its antiplatelet properties. The most common ulcers associated with peptic ulcer disease arise in the stomach and duodenum but the gastric ulcers are most frequently [4]. Gastric acid, cytokines, interleukin-1, tumor necrosis factor [5] represent major factors that play an important role in inducing mucosal damage [6,7]. Even the use of suitable gastrointestinal therapy may delay ulcers in high-risk patients, but incorrect diagnosis may lead to severe complications and mortality [8]. Many drugs are used for ulcer treatment, the most successful of which are intended for inhibiting gastric acid secretion. Famotidine and other H2-receptor antagonists were used for ulcer treatment and maintenance therapy, but by the time they became replaced by proton pump inhibitors as omeprazole, which is considered as a potent acid inhibitor that causes rapid healing of ulcers [2]. Aspirin can increase oxidation process and consequently elevate the hydrogen peroxide production, which leads to formation of the toxic oxidant HCl in presence of Cl- and H2O2, resulting in mucosal membrane lipid peroxidation and damage [9]. The antioxidant activity of melatonin under different biochemical conditions has been previously proven [10], which may explain its role in treatment of gastric ulcer. H. pendula Planch. ex F. Muell (Sapindaceae), - communally known as the tulipwood or tulip lancewood,
is a small to medium rainforest tree native to Australia [11] and widely cultivated as an ornamental shade tree. Previous studies on _H. pendula_ revealed that triterpene derivatives, quebrachitol and methyl p-coumarate were isolated from the bark and leaves [12,13]. In addition benzenacetic acid derivatives and flavonol glycosides were isolated from the leaves [14]. Furthermore, the cytoprotective activity of _H. pendula_ leaves was evaluated [15], as well as the molluscidicidal, miracidicidal and cercaricidal activities of the leaves and fruits were demonstrated [16]. Several reports on _in vitro_ and _in vivo_ studies of the natural polyphenolic compound 1,2,3,4,6-penta-O-galloyl-β-D-glucose (PGG) revealed that it exhibits many biological activities, which may be beneficial for avoidance of several human health problems as oxidative stress, mutagenicity, inflammation and ulcers [17].

The present study was designed to assess the gastroprotective effect of pentagalloyl glucose isolated from _H. pendula_ pericarp in aspirin induced mucosal damage in albino rats, as compared to the standard anti-ulcer drugs omeprazole and famotidine, and to the pineal hormone, melatonin.

**MATERIALS AND METHODS**

**Materials and reagents**

_Harpullia pendula_ Planck ex F. Muell (Sapindaceae) pericarps were obtained from Zoo Botanical Garden, Giza, Egypt, during August 2014. Plant authentication was done by Dr. Trease Labib, Consultant of Plant Taxonomy at the Ministry of Agriculture and former director of El Orman Botanical Garden, Giza, Egypt. A voucher specimen (H-21) was deposited at the Pharmacognosy and Medicinal Chemistry Department, Faculty of Pharmacy, Taibah University, Medina, Saudi Arabia. Polyamide S (Fluka Chemie AG, Switzerland), sephadex LH-20 (Sigma-Aldrich, Steinheim, Germany), cellulose (E. Merck, Darmstadt, Germany) were used for column chromatography. Whatman No.1 paper sheets were provided from Whatman Ltd., Maidstone, Kent, England. Ferric chloride, nitrous acid (NaNO₂/glacial AcOH) and potassium iodate were used for detection of the tannins. n-BuOH/HOAc/H₂O, 4:1:5 v/v/v, upper layer (S₁) and 15% acetic acid/H₂O (S₂) were used as solvent systems. Gastric mucosal cyclooxygenase (COX-2) and prostaglandin E₂ (PGE₂) were obtained fromCUSABIO Biotech Co. LTD, USA; Tumor necrosis factor (TNF-α) and Interleukin 1 (IL-1) were obtained from Immuno-Biological Laboratories, Inc. (IBL-America); malondialdehyde (MDA) and reduced glutathione (GSH) were obtained from Merck, Darmstadt, Germany and Sigma-Aldrich, MO, USA, respectively, while aspirin was obtained from Cid Company, Cairo, Egypt; Famotidine and melatonin were obtained from Amoun Company, Egypt and Omeprazole was obtained from Eipico Company, Egypt.

**Animals**

Adult Wister male albino rats (150-180 g) were obtained from the animal house, National Research Centre, Giza, Egypt. Animals were kept in controlled environmental breeding rooms and fed with standard laboratory food and water _ad libitum_. All animal experiments were performed according to the requirements of the National Research Center for experimental animal use, approved in January 2015 by the Ethics Committee of the National Research Centre, Egypt (registration number 13/022).

**Instrumentation and conditions**

NMR data were measured using JEOL GX-300 spectrometer (300 and 75 MHz for 1H and 13C NMR, respectively) and the results were expressed as δ ppm values relative to the internal reference TMS. Negative ESI-MS experiments were conducted with LCQ deca MS and LTQ-FT-MS spectrometers (Thermo Electron, Finnigan, Germany). A JASCO v-630 spectrophotometer was used for UV measurement of pure samples.

**Extraction and isolation**

Sample of _H. pendula_ pericarp (400 g) was dried in air and extracted by 80% aqueous EtOH under reflux (3 L x 3, 60 ºC, 4 h). The resulting extract was evaporated using reduced pressure to give a 60 g dry residue, which was desalted by dissolving in distilled water and precipitation with ethanol (1:10 v/v). The resulting filtrate was concentrated under reduced pressure to afford 40 g of a dry residue. The crude residue was suspended in water and fractionated through polyamide S column [250 g, 100 x 5 cm], using H₂O-MeOH mixtures (100:0-0:100% v/v) for elution. On the basis of comparative paper chromatography (Co-PC), UV-light and reactions towards different spraying reagents, the fractions were collected to give five major collective fractions (I-V). Fraction I was devoid of polyphenolic compounds. Fraction II (eluted with 20% aqueous MeOH; 15 g) was subjected to cellulose column using H₂O-MeOH mixtures (10-90%) giving major crude sample of compound 1, which was further purified using sephadex column eluted with H₂O-MeOH mixture (50%), to give chromatographically pure sample of compound 1 (12 mg). Fraction III (eluted with 30-50% aqueous MeOH; 3 g) was chromatographed on sephadex column and eluted with n-butanol saturated with water to yield crude sample of 2, which was further purified on sephadex column eluted with EtOH to give compound 2 (30 mg). Fraction IV (eluted with 60-70% aqueous MeOH; 2.3 g) was purified using successive sephadex columns with n-butanol/isopropanol/H₂O (4:1:5 v/v/v, upper layer) and EtOH as eluents, to afford compound 3 (23 mg). Fraction V (eluted with 80-100% aqueous MeOH; 3 g) was chromatographed on sephadex column eluted with n-butanol saturated with water to give two sub-fractions (i and ii). Both sub-fractions were individually purified using sephadex column eluted with EtOH, to give compounds 4 (25 mg) and 5 (20 mg). The
purity of all isolated compounds was traced using 2D-and Co-PC with S1 and S2 solvent systems.

1,2,3,4,6-penta-O-galloyl-β-D-β-D-β-D-β-D-β-D-c-gluco-pyranoside (1)

It was obtained as a yellowish brown amorphous powder: Rf 0.25 (S1), 0.31 (S2). It appeared as purple fluorescent spot under short UV-light (254). It gave indigo-red and deep blue colors after spraying with nitrous acid and FeCl3 reagents, respectively. UV (MeOH) λmax (nm): 221, and 272 (sh). Negative ESI-MS: m/z 939.0 [M-H]-, 787.0 [M-H-galloyl]-, 635.1 [M-H-3 galloyl]-, 331.0 [M-H-4 galloyl]-, 313.0 [monogalloylglucose-H2O]-, 169.0 [gallate]-. 1H NMR (300 MHz, (CH3)2CO-d6): galloyl moieties δH ppm 7.19, 7.13, 7.08, 7.03, 6.99 (each 2H, s, 5 x H-2′), glucose moiety: δH ppm 7.26, 7.22, 7.20 (each 2H, s, 4 x H-2′, galloyl moiety), 6.16 (1H, d, J = 9.9, 8.4 Hz, H-6 b), 4.07 (2H, m, H4/5). 13C NMR (75 MHz, CH3OH-d4): C ppm 165.80, 165.61, 165.37 (3 x C-7), 145.49, 145.34, 145.29, 145.25, 145.22 (5 x C-3/5), 139.07, 138.74, 138.69, 138.39 (5 x C-4), 120.74, 120.66, 119.97, 119.87, 119.86 (5 x C-1), 109.75, 109.65, 109.55, 109.50, 109.41 (5 x C-2/6); glucose unit: δC ppm 164.43 (5 x C-7), 145.49, 145.34, 145.29, 145.25, 145.22 (5 x C-3/5); 92.71 (C-1), 73.32 (C-5), 72.68 (C-3), 71.13 (C-2), 68.67 (C-4), 62.20 (C-6).

1,2,3,6-tetra-O-galloyl-β-D-β-D-β-D-β-D-c-gluco-pyranoside (2)

It was isolated as an off-white amorphous powder with Rf 0.34 (S1), 0.42 (S2). It appeared as purple fluorescent spot under short UV-light, which changed to indigo-red and deep blue colors after spraying with nitrous acid and FeCl3 reagents, respectively. UV (MeOH) λmax (nm): 222, and 270 (sh). Negative ESI-MS: m/z 787.0 [M-H]-, 635.1 [M-H-galloyl]-, 169.0 [gallate]-. 1H NMR (300 MHz, (CH3)2CO-d6): galloyl moieties δH ppm 7.17, 7.09, 7.08, 7.02 (each 2H, s, 5 x H-2′), glucose moiety: δH ppm 7.26, 7.22, 7.20 (each 2H, s, 4 x H-2′, galloyl moiety), 6.02 (1H, d, J = 9.9, 8.4 Hz, H-1), 5.50 (1H, t-like, J = 12.1, 3.9 Hz, H-6b), 4.43 (1H, dd, J = 12.9, 4.8 Hz, H-6a); 13C NMR (75 MHz, CH3OH-d4): galloyl moieties δC ppm 165.80, 165.61, 165.37 (3 x C-7), 145.49, 145.34, 145.29, 145.25, 145.22 (5 x C-3/5), 139.07, 138.74, 138.69, 138.39 (5 x C-4), 120.74, 120.66, 119.97, 119.87, 119.86 (5 x C-1), 109.75, 109.65, 109.55, 109.50, 109.41 (5 x C-2/6); glucose unit: δC ppm 164.43 (5 x C-7), 145.49, 145.34, 145.29, 145.25, 145.22 (5 x C-3/5); 92.71 (C-1), 73.32 (C-5), 72.68 (C-3), 71.13 (C-2), 68.67 (C-4), 62.20 (C-6).

1,3,6-tri-O-galloyl-β-D-β-D-β-D-c-glucopyranoside (3)

It was obtained as an off-white amorphous powder with Rf 0.25 (S1), 0.31 (S2). It appeared as shine violet fluorescent spot under short UV-light, which changed to pink and deep blue colors after spraying with nitric acid and FeCl3 reagents, respectively. UV (MeOH) λmax (nm): 220, and 271. Negative ESI-MS: m/z 1271.1 [M-H]-, 634.9 [M-H]-, 482.8 [M-galloyl]-, 169.0 [gallate]-. 1H NMR (300 MHz, (CH3)2CO-d6): δH ppm 7.26, 7.22, 7.20 (each 2H, s, 4 x H-2′, galloyl moiety), 6.02 (1H, d, J = 7.7 Hz, H-1), 5.50 (1H, t-like, J = 8.5 Hz, H-3), 4.61 (2H, m, H-6a/5), 4.12-3.90 (3H, m, H-6b, 2, 4). 13C NMR (75 MHz, CH3OH-d4): δC ppm 166.84, 166.77, 165.37 (3 x C-7), 145.53, 145.43, 145.46 (3 x C-3/5), 139.18, 138.63, 138.63 (3 x C-4), 121.42, 121.12, 120.28 (3 x C-1), 110.33, 110.17, 109.92 (3 x C-2/6), 95.23 (C-1), 78.32 (C-3), 75.30 (C-5), 72.03 (C-2), 69.04 (C-4), 63.72 (C-6).

Experimental design

Eight groups of rats, each of 6 were used for the study. The 1st group was used as normal control and was given orally 1 ml saline, while the 2nd served as aspirin control group and received orally 400 mg/kg of aspirin [18]. The 3rd-5th groups received orally pentagalloyl glucose (50, 100, 200 mg/kg), while the 6th-7th and 8th groups received orally 40 mg/kg famotidine [19], omeprazole (30 mg/kg) [20] and melatonin (10 mg/kg), respectively [21]. One hour later, the groups 3rd-8th were given aspirin orally (400 mg/kg). One hour later, rats were sacrificed and the stomachs were cut, opened through the greater curvature and their contents were collected, centrifuged at 3000 rpm for 10 min and the supernatants were then separated and used for estimation of gastric pH. In addition, the stomachs were washed with 5.0 ml of 0.9 % NaCl and the ulcers were counted to estimate their number and severity. Finally, portions of the gastric mucosal tissues were collected, frozen using liquid nitrogen and kept at –80 °C for determination of prostaglandin E2 (PGE2), cyclooxygenase (COX-2), tumor necrosis factor-alpha (TNF-α), interleukin 1 (IL-1), malondialdehyde (MDA) and reduced glutathione (GSH). The other portions of stomach tissue were used for histological study.

Determination of gastric juice pH

Gastric juice pH was measured using pH meter after diluting 1 ml of gastric content supernatant with 10 ml distilled water.

Assessment of ulcer number and severity

The gastric erosions on the surfaces of the stomachs were examined macroscopically for mucosal necrotic lesions, red streaks and red erosions. The total lesion number was counted and the severity of lesions based on the following scores: 0 = No ulcer, 1 = Lesion size < 1 to 2 mm, 2 = Lesion of size 1 to 2 mm, 4 = Lesion of size 3 to 4 mm. A total lesion score for each rat was calculated by multiplying the total number of lesions by the respective severity score. Results were expressed as the severity of lesions/rat [22].

Determination of gastric mucosal prostaglandin E2 (PGE2)

Gastric mucosal PGE2 was determined in the gastric tissue by homogenization of 1g with 5 ml of phosphate buffer (0.1 M, pH 7.4) containing EDTA (1 mM) and indomethacin (10 μM), then centrifuging the lyate at 16,000 rpm for 15 min at 2-4 °C. PGE2 concentrations were measured using PGE2 ELISA kits.

Determination of gastric mucosal cyclooxygenase (COX-2)
Gastric mucosal of cyclooxygenase (COX-2) activity was measured according to Hemler and Lands, 1976 [23].

**Determination of tumor necrosis factor-alpha (TNF-α) and interleukin 1 (IL-1)**

TNF-α stomach contents were estimated using rat specific immunoassay kit and represented as μg/g wet tissue.

**Determination of gastric reduced glutathione (GSH)**

Stomach homogenate GSH content was measured using Ellman’s reagent (5,5-dithiobis-2-nitrobenzoic acid) [24] and expressed as mg/g wet tissue.

**Determination of gastric malondialdehyde (MDA)**

Malondialdehyde level in the tissue homogenate was determined using the thiobarbituric acid reactive substances (TBARS) assay [25] and represented as nmol/g wet tissue.

**Histopathological study**

Gastric tissues were embedded in formalin solution (10%, 24 h), followed by paraffin and were cut into sections. The sections were examined by light microscopy after staining with hematoxylin and eosin stain (H&E).

**Statistical analysis**

All data were represented as the mean ± S.E. Results were statistically evaluated using One way ANOVA, followed by Tukey test for multiple comparisons. Data was considered as statistically significant at P < 0.05. Statistical analysis of ulcer scores was done by the proper post-hoc test by SPSS 11.0 J (SPSS Japan, Tokyo). Data is represented as mean ± SE; *statistically significant from the normal control group (saline treated): P < 0.05 statistical analysis using one way ANOVA followed by Tukey test for multiple comparisons.

**RESULTS**

Investigation of the 80% aqueous ethanol extract of *H. pendula* pericarp using two dimensional PC, revealed the presence of a mixture of tannins on the basis of their appearance in UV-light and their reaction with different spray reagents. The extract was fractionated and purified using polyamide, cellulose and sephadex LH-20 columns. This resulted in the isolation of five tannins for the first time from genus *Harpullia*. Structure elucidation of the compounds (Fig. 1) was based on their chemical and spectral data (UV, 1H, 13C NMR and ESI-MS), in addition to comparison with previously published data [26-28]. Compound 1 exhibited chromatographic behavior for gallotannins (Rf-values, fluorescence under UV-light and changes with FeCl₃ and nitrous acid spray reagents). The UV spectrum showed absorption peaks at λ_max 270, characteristic for gallotannins. Complete acid hydrolysis of 1 revealed the presence of gallic acid and glucose in organic and aqueous phases, respectively (comparative PC with authentic samples). Negative ESI-MS spectrum demonstrated a molecular ion peak at m/z 939.0 [M-H]⁻, with other ion fragments for consecutive loss of four galloyl moieties. In the aromatic region, the 1H NMR spectrum showed five singlet signals, each for two equivalent protons at δ ppm 7.19, 7.13, 7.08, 7.03 and 6.99, assignable for H-2/6 of five galloyl moieties. Moreover the presence of a downfield doublet signal at 6.34 (J = 8.4 Hz) for H-1, together with splitting pattern, δ₆ and J-values of the other sugar protons gave an evidence that the sugar moiety of (1) is β-D-C₆ glucopyranose. Examination of the 13C NMR spectrum revealed the presence of five repeated characteristic 13C resonances, each assigned for the five signals of the five galloyl moieties in the aromatic region. In addition, the stereo-structure of the glucose moiety was confirmed as full esterified β-D-C₆ glucopyranose, based on the downfield shift of the six sugar signals [27,28]. Accordingly, compound (1) was established as 1,2,3,4,6-penta-O-galloyl-β-D-C₆ glucopyranoside.

Compounds 2-5, were identified as 1,2,3,6-tetra-O-galloyl-β-D-C₆ glucopyranoside (2), 1,3,6-tri-O-galloyl-β-D-C₆ glucopyranoside (3), gallic acid (4) and methyl gallate (5), by comparison of their UV, ESI-MS and H and 13C NMR data with those of reported data [27,28].

Administration of aspirin to rats induced significant reduction in gastric juice pH in comparison to normal control. In addition, groups treated with 50, 100 or 200 mg/kg b.wt. pentagalloyl glucose showed neutralization of gastric juice pH (pH: 7.17-7.40), while famotidine, omeprazole and melatonin induced elevation of gastric juice pH to 6.70, 6.39 and 6.5, respectively (Table 1). Therefore, pentagalloyl glucose treatment was more efficient in neutralization of rat’s gastric pH.

**Table 1: Determination of pH of gastric stomach juice in rats treated orally with pentagalloyl glucose, famotidine, omeprazole, melatonin on gastric mucosal injury induced by aspirin (N=6).**

<table>
<thead>
<tr>
<th>Treated groups</th>
<th>Dose (mg/kg)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>1ml</td>
<td>7.40 ± 0.55</td>
</tr>
<tr>
<td>Aspirin</td>
<td>400</td>
<td>5.70 ± 0.48*</td>
</tr>
<tr>
<td>Pentagalloyl glucose</td>
<td>50</td>
<td>7.17 ± 0.37</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>7.40 ± 0.64</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>7.20 ± 0.27</td>
</tr>
<tr>
<td>Famotidine</td>
<td>40</td>
<td>6.70 ± 0.45</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>30</td>
<td>6.39 ± 4.78</td>
</tr>
<tr>
<td>Melatonin</td>
<td>10</td>
<td>6.50 ± 0.27</td>
</tr>
</tbody>
</table>

Data is represented as mean ± SE; *statistically significant from the normal control group (saline treated): P < 0.05 statistical analysis using one way ANOVA followed by Tukey test for multiple comparisons.
Fig. 1: Structures of compounds 1-5 isolated from *Harpullia pendula* pericarp

1: R=H
2: R=CH₃

4: R=H
5: R=CH₃
Table 2: Determination of prostaglandin E₂ and COX-2 level in stomach tissues content in rats treated with pentagalloyl glucose, famotidine, omeprazole and melatonin on gastric mucosal injury induced by aspirin (N=6).

<table>
<thead>
<tr>
<th>Treated groups</th>
<th>Dose (mg/kg)</th>
<th>PGE2 µg/g tissue</th>
<th>1</th>
<th>2</th>
<th>COX-2 µm/g tissue</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1ml</td>
<td>27.93±1.244**</td>
<td>---</td>
<td>---</td>
<td>+287.92</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Aspirin</td>
<td>400</td>
<td>7.200±0.209*</td>
<td>74.22</td>
<td>---</td>
<td>4.180±0.169*</td>
<td>378.81</td>
<td>---</td>
</tr>
<tr>
<td>Pentagalloyl glucose</td>
<td>50</td>
<td>12.20±1.290*</td>
<td>56.32</td>
<td>69.44</td>
<td>3.390±0.195**</td>
<td>288.32</td>
<td>18.89</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>16.35±1.666**</td>
<td>41.46</td>
<td>127.08</td>
<td>2.440±0.195**</td>
<td>179.49</td>
<td>41.62</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>21.81±1.231**</td>
<td>21.91</td>
<td>202.92</td>
<td>1.803±0.118**</td>
<td>106.53</td>
<td>56.87</td>
</tr>
<tr>
<td>Famotidine</td>
<td>40</td>
<td>17.93±0.523*</td>
<td>35.80</td>
<td>149.03</td>
<td>2.057±0.049*</td>
<td>135.62</td>
<td>50.79</td>
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<tr>
<td>Omeprazole</td>
<td>30</td>
<td>19.95±1.420*</td>
<td>28.57</td>
<td>177.08</td>
<td>2.220±0.214*</td>
<td>154.29</td>
<td>46.89</td>
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<tr>
<td>Melatonin</td>
<td>10</td>
<td>11.70±1.029*</td>
<td>58.11</td>
<td>62.5</td>
<td>1.550±0.152 @</td>
<td>77.55</td>
<td>62.92</td>
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Statistical analysis was carried out using repeated measures one way ANOVA, followed by Tukey test for multiple comparisons; *statistically significant from the normal group: P< 0.05; **Statistically significant from the aspirin control group: P< 0.05; 1= Percent of change was calculated as regard normal control group; 2= Percent of change was calculated as regard aspirin control group.

Table 3: Determination TNF-α and interleukin 1 (IL-1) level in stomach tissues in rats treated with aspirin, pentagalloyl glucose, famotidine, omeprazole, melatonin on gastric mucosal injury induced by aspirin (N=6)

<table>
<thead>
<tr>
<th>Treated groups</th>
<th>Dose (mg/kg)</th>
<th>TNF-α pg/g tissue</th>
<th>1</th>
<th>2</th>
<th>IL-1 pg/g tissue</th>
<th>1</th>
<th>2</th>
</tr>
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<tbody>
<tr>
<td>Normal</td>
<td>1ml</td>
<td>12.33 ± 0.49@</td>
<td>---</td>
<td>---</td>
<td>77.45</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Aspirin</td>
<td>400</td>
<td>54.68 ± 2.59 *</td>
<td>343.47</td>
<td>---</td>
<td>56.43 ± 2.39 *</td>
<td>315.84</td>
<td>---</td>
</tr>
<tr>
<td>Pentagalloyl glucose</td>
<td>50</td>
<td>26.63 ± 3.12**</td>
<td>115.98</td>
<td>51.29</td>
<td>33.03 ± 2.22*</td>
<td>143.4</td>
<td>41.47</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>20.51 ± 1.18@</td>
<td>66.34</td>
<td>62.49</td>
<td>32.45 ± 2.87*</td>
<td>139.13</td>
<td>42.49</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>18.30 ± 1.04@</td>
<td>48.42</td>
<td>66.53</td>
<td>20.87 ± 2.41</td>
<td>53.79</td>
<td>63</td>
</tr>
<tr>
<td>Famotidine</td>
<td>40</td>
<td>24.23 ± 1.03*</td>
<td>96.51</td>
<td>55.69</td>
<td>26.40 ± 2.65*</td>
<td>94.55</td>
<td>53.20</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>30</td>
<td>27.47 ± 2.06**</td>
<td>122.79</td>
<td>49.76</td>
<td>36.13 ± 1.26*</td>
<td>166.25</td>
<td>35.97</td>
</tr>
<tr>
<td>Melatonin</td>
<td>10</td>
<td>15.28 ± 0.81@</td>
<td>23.93</td>
<td>72.11</td>
<td>18.03 ± 1.20</td>
<td>32.87</td>
<td>68</td>
</tr>
</tbody>
</table>

Statistical analysis was carried out using repeated measures one way ANOVA, followed by Tukey test for multiple comparisons; *statistically significant from the normal group: P< 0.05; @Statistically significant from the aspirin control group: P< 0.05; 1= Percent of change was calculated as regard normal control group; 2= Percent of change was calculated as regard aspirin control group.

Table 4: Determination gastric tissue level of GSH and MDA content in rats treated orally with pentagalloyl glucose, famotidine, omeprazole and melatonin on gastric mucosal injury induced by aspirin in rats (N=6).

<table>
<thead>
<tr>
<th>Treated groups</th>
<th>Dose (mg/kg)</th>
<th>GSH µM/g</th>
<th>1</th>
<th>2</th>
<th>MDA nM/mg</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>1ml</td>
<td>3.794±0.032**</td>
<td>---</td>
<td>---</td>
<td>259.28</td>
<td>194.5±16.60*</td>
<td>---</td>
</tr>
<tr>
<td>Aspirin</td>
<td>400</td>
<td>1.056±0.012*</td>
<td>72.17</td>
<td>---</td>
<td>362.5±26.62*</td>
<td>86.38</td>
<td>---</td>
</tr>
<tr>
<td>Pentagalloyl glucose</td>
<td>50</td>
<td>2.938±0.210*</td>
<td>22.56</td>
<td>178.22</td>
<td>265.4±15.65</td>
<td>36.45</td>
<td>26.79</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>3.410±0.241*</td>
<td>10.12</td>
<td>222.92</td>
<td>264.6±24.15</td>
<td>36</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>3.560±0.24*</td>
<td>6.17</td>
<td>208.71</td>
<td>194.3±16.68*</td>
<td>0.10</td>
<td>46.4</td>
</tr>
<tr>
<td>Famotidine</td>
<td>40</td>
<td>3.932±0.304*</td>
<td>3.64</td>
<td>272.35</td>
<td>227.4±14.52*</td>
<td>16.92</td>
<td>37.27</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>30</td>
<td>3.170±0.254*</td>
<td>16.45</td>
<td>200.19</td>
<td>253.3±21.45</td>
<td>30.23</td>
<td>30.12</td>
</tr>
<tr>
<td>Melatonin</td>
<td>10</td>
<td>2.330±0.168*</td>
<td>38.59</td>
<td>120.64</td>
<td>211.7±14.64*</td>
<td>8.84</td>
<td>41.6</td>
</tr>
</tbody>
</table>

Statistical analysis was carried out using repeated measures one way ANOVA, followed by Tukey test for multiple comparisons; *statistically significant from the normal group: P< 0.05; **Statistically significant from the aspirin control group: P< 0.05; 1= Percent of change was calculated as regard normal control group; 2= Percent of change was calculated as regard Aspirin control group.

Aspirin treated rats exhibited significant increase in number and severity of gastric ulcers, by 4.25 and 7.75, respectively. Pentagalloyl glucose treated groups showed significant decrease in number of gastric ulcers, by 81.10, 90.58 and 90.58% and severity by 87.09, 92.25 and 94.83%, for 50, 100 and 200 mg/kg, respectively, in comparison with aspirin control group. Moreover, treatment with famotidine and melatonin induced significant decrease in gastric ulcer number by 94.11 and 88.23% and severity by 96.77 and 90.32%, respectively, while omeprazole treated group showed no ulcers (Fig. 2,3). Rats given aspirin showed significant decrease in the PGE2 level in gastric tissue content by 74.22%, as
compared with normal control group. The oral administration of pentagalloyl glucose (50, 100 and 200 mg/kg b.wt), famotidine, omeprazole and melatonin induced significant decrease in PGE2 levels by 56.32, 41.46, 21.91, 35.80, 28.57 and 58.11%, respectively as compared with control group. On the other hand, by comparing with aspirin control group, pentagalloyl glucose (50, 100 and 200 mg/kg b.wt), famotidine, omeprazole and melatonin induced significant increase in PGE2 level, by 69.44, 127.08, 202.92, 149.03, 177.08 and 62.5%, respectively (Table 2).

Rats treated orally with 400 mg/kg aspirin exhibited significant increase in COX-2 activity by 378.81, as compared with control group, as well as pentagalloyl glucose (50, 100 and 200 mg/kg b.wt), famotidine, omeprazole and melatonin induced significant increase in COX-2 activity by 288.32, 179.49, 106.53, 135.62, 154.29 and 77.55%, respectively as compared with normal control group. In addition, pentagalloyl glucose (50, 100 and 200 mg/kg b.wt), famotidine, omeprazole and melatonin induced significant decrease in COX-2 activity by 18.89, 41.62, 56.87, 49.76 and 62.92%, respectively in comparison to aspirin group (Table 2).

Aspirin treated rats, showed significant increase in gastric tissue TNF-α level by 343.47%, as compared with normal control group. Pentagalloyl glucose (50 mg/kg), famotidine and omeprazole treatment induced significant increase in gastric tissue TNF-α level by 115.98, 96.51 and 122.79%, respectively as compared with control, while treatment with pentagalloyl glucose (50, 100 and 200 mg/kg), famotidine, omeprazole and melatonin showed decrease of TNF-α level in stomach tissues by 51.29, 62.49, 66.53, 55.69, 49.76 and 72.11%, respectively in comparison with aspirin control group (Table 3).

Aspirin treatment exhibited significant increase in gastric tissue interleukin-1 by 315.84%, in comparison to control group. Oral administration of pentagalloyl glucose (50, 100 mg/kg), famotidine and omeprazole induced significant decrease in gastric tissue interleukin-1 levels, by 41.47, 42.49, 53.20 and 35.97%, respectively in comparison with aspirin control group (Table 3).

As regards gastric tissue GSH level, aspirin administration induced significant decrease by 72.17%, while pentagalloyl glucose (100 and 200 mg/kg), famotidine and omeprazole were able to normalize gastric tissue GHS levels.

Moreover, aspirin increased the gastric tissue MDA level by 86.38%, as compared to control group, while rats receiving pentagalloyl glucose, famotidine, omeprazole and melatonin showed significant decrease in gastric tissue MDA levels (Table 4).

Furthermore, the higher dose of pentagalloyl glucose (200 mg/kg) and melatonin (10 mg/kg) normalized the gastric tissue GHS levels (Table 4).
Histopathology results
Photomicrograph section of stomach gastric mucosa of control rats showed normal intact gastric mucosa (Fig. 4), while gastric mucosa of aspirin treated rats showed degeneration, necrosis, desquamation of the gastric mucosal epithelial linings, congestion of the sub-mucosal blood vessels with marked edema and mild degree of inflammatory cell infiltration, dilatation of the lower proprial blood vessels and marked sub-mucosal edema with mild inflammatory cell infiltration (Fig. 5A-C).

Stomach gastric mucosa of rats treated with pentagalloyl glucose (50 mg/kg) showed marked vacuolar degeneration of the epithelial cells (Fig. 6A). The stomach of rats treated with 100 mg/kg showed intact mucosal epithelial linings (Fig. 6B), while 200 mg/kg treatment showed moderate degree of degenerative changes of the mucosal cells with sub-mucosal congestion and edema (Fig. 6C).

A photomicrograph section of stomach gastric mucosa of rats treated with famotidine showed nearly normal mucosa, with slight congestion of the lower proprial blood vessels (Fig. 7A,B).

Omeprazole treated rats, showed intact mucosal epithelial lining with mild degree of sub-mucosal congestion and inflammatory cell infiltration (Fig. 8), while stomach section of rat’s gastric mucosa treated with melatonin (10 mg/kg) revealed a normal intact mucosa with mild congestion of the lower mucosa (Fig. 9).
Fig. 6 (A): A photomicrograph section of stomach gastric mucosa of rats pretreated with pentagalloyl glucose group (50 mg/kg), showing marked vacuolar degeneration of the epithelial cells. (H&E x 400).

Fig. 6 (B): A photomicrograph section of stomach gastric mucosa of rats pretreated with pentagalloyl glucose group (100 mg/kg), showing intact mucosal epithelial linings (H&E x 200).

Fig. 6 (C): A photomicrograph section of stomach gastric mucosa of rats pretreated with pentagalloyl glucose (200 mg/kg) showing moderate degree of degenerative changes of the mucosal cells with sub-mucosal congestion and edema (H&E x 200).

Fig. 7 (A): A photomicrograph section of stomach gastric mucosa of rat pretreated with famotidine group (40 mg/kg), showing nearly normal mucosa with slight congestion of the lower proprial blood vessels (H&E x 200).

Fig. 7 (B): Higher magnification of a photomicrograph section of stomach gastric mucosa of rat pretreated with famotidine group (40 mg/kg), showing intact gastric epithelial lining (H&E x 400).

Fig. 8: A photomicrograph section of stomach gastric mucosa of rats pretreated with omeprazole group (30 mg/kg), showing intact mucosal epithelial lining with mild degree of sub-mucosal congestion and inflammatory cell infiltration (H&E x 200).
Fig. 9: A photomicrograph section of stomach gastric mucosa of rats pretreated with melatonin (10 mg/kg), showing nearly normal intact mucosa with mild congestion of the lower mucosa (H&E x 200).

**DISCUSSION**

Conventional antiulcer drugs as omeprazole and famotidine are widely used because they possess a protective effect against gastric mucosal injury, which may be induced by different agents and stress conditions; in addition they cause improvement in gastric damage produced by aspirin in humans and animals [29]. This effect may be attributed to their ability to suppress the gastric acid secretion and to their cytoprotective and antioxidant activities. The present research was performed to evaluate the effect and mechanism of the gallotannin pentagalloyl glucose on gastric injury induced by aspirin, in comparison to famotidine, omeprazole and melatonin. Previous studies reported that aspirin is able to reduce gastric juice pH and that low doses of aspirin can induce ulceration of the gastric mucosa under experimental conditions and in humans [6,30,31]. Results of the present study revealed that the main cause of ulcer formation in aspirin-treated rats is the lowering in pH of gastric secretions, with the high acidity resulting in appearance of multiple, elongated, reddish bands of hemorrhagic erosions in rats' gastric mucosa. Oral administration of pentagalloyl glucose, famotidine, omeprazole and melatonin in this work caused neutralization of gastric juice acidity, as compared with control group, so all treatments caused significant decrease in ulcer formation.

Prostaglandins (PGs) of E and I series are produced throughout the gastrointestinal tract, especially in the gastric and duodenal mucosa, and are released into the gut lumen in response to vagal and hormonal stimulation. Endogenous PGs may be involved in the maintenance of mucosal integrity, control of mucosal blood flow and protection against harmful agents. In peptic ulcer patients, the gastric mucosa tends to produce smaller amounts of PGs of E and I series and exhibits a reduced ratio of PG to thromboxane generation, suggesting that the deficiency of protective PG may play a role in the pathogenesis of peptic ulcer [32]. Prostaglandins are lipid autacoids derived from arachidonic acid, and are involved in sustaining homeostatic functions and mediating pathogenic mechanisms, including the inflammatory response. PGs are generated from arachidonate by the action of cyclooxygenase (COX) enzymes, while their biosynthesis is blocked by the effect of nonsteroidal anti-inflammatory drugs (NSAIDs), including those selective for inhibition of COX-2. Despite the clinical efficacy of NSAIDs, prostaglandins may function in the promotion and resolution of inflammation [33-35]. In addition, they have been shown to inhibit gastric secretion, stimulate bicarbonate secretion, as well as inhibition of mast cell activation, and leukocyte and platelet adhesion to the vascular endothelium [36]. Therefore, they can promote ulcer healing through inhibition of gastric acid secretion.

In results of the current study, rats treated with aspirin showed marked decrease in gastric PGE2 level, while rats treated with either pentagalloyl glucose, famotidine, omeprazole and melatonin showed elevation in the gastric PGE2 level. In addition, aspirin treatment caused increase in COX-2 gastric level, which was decreased by oral administration of a high dose of pentagalloyl glucose (200 mg/kg) and melatonin (10 mg/kg). Aspirin induced gastric mucosal inflammation results in the increase of gastric mucosal TNF level [37,38], which in turn improves neutrophil-derived superoxide generation and stimulates IL-1 production, resulting in the neutrophil accumulation [39]. Moreover, current study results revealed that rats treated with aspirin showed increase in TNF-α and IL-1 levels, which was significantly decreased by administration of all doses of pentagalloyl glucose, as well as with famotidine, omeprazole and melatonin. This is important evidence, which supports the claim that reactive oxygen species (ROS) are represented in gastric injury caused by aspirin exposure [40]. Furthermore, the current work proved that aspirin administration enhances ROS activity, as indicated by elevated serum MAD and reduced GSH levels. Such increased activity of ROS often leads to mucosal damage with the subsequent destruction of epithelial basement membrane. As regard lipid peroxidation, changes in the glutathione peroxidase activity was considered as gastric injury index [35,41]. The antulcer activity of the pineal hormone melatonin and pentagalloyl glucose may be attributed to their powerful scavenging activity for both hydroxyl and peroxyl radicals [17,31,42,43]. Moreover, recent studies stated that omeprazole and famotidine possess antioxidant activity [10].
CONCLUSIONS
The current study clarified the pharmacological mechanisms of antiulcer activity of pentagalloyl glucose against aspirin induced ulcer in rats. It has cytoprotective, anti-secretory and healing properties, possibly due to stimulation of PGE2 by the up regulation of COX-2. Moreover, it modulates the defensive factors by improvement of gastric cytoprotection, decrease of oxidative stress and inflammatory cytokine (TNF-α and IL-1), in addition to its antioxidant activity.

CONFLICT OF INTEREST
All authors approved the final manuscript and they declare no conflict of interest.

REFERENCES