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Protective and therapeutic effects of resveratrol on acetic acid-induced gastric ulcer

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Abstract
Sprague Dawley rats of both sexes were injected with either saline or RVT (10 mg/kg) either before or after acetic acid ulcer induction and decapitated 3, 5 or 10 days after ulcer. In the saline-treated ulcer groups, macroscopically evident ulcers were observed, while RVT-pretreated or RVT-treated groups had lower macroscopic ulcer scores. Likewise, the microscopic damage scores were lower for the RVT-administered groups. Gastric myeloperoxidase activity, malondialdehyde, collagen and tumour necrosis factor-alpha levels, as well as luminol- and lucigenin-enhanced chemiluminescence levels that were elevated in the saline-administered ulcer groups, were depressed with both RVT-pretreatment and RVT-treatment. Moreover, depleted glutathione levels in the ulcer groups were increased back to control levels by both pre- and post-treatments of RVT. Results demonstrate that resveratrol has both protective and therapeutic effects on oxidative gastric damage by suppressing pro-inflammatory cascades, including the activation of pro-inflammatory cytokines, accumulation of neutrophils and release of oxygen-derived free radicals.

Keywords: *Resveratrol, ulcer healing, oxidative stress*

Introduction

Despite continuous exposure to noxious factors, the gastric mucosa maintains structural integrity and function through a multifactorial and complex interaction between so-called protective and aggressive factors, including mucosal barrier, secretion of gastric acid and pepsin, gastroduodenal motility, *Helicobacter pylori* and use of nicotine and non-steroidal anti-inflammatory drugs [1]. When the barrier is broken by the aggressive offensive factors, the gastric mucosa allows a back diffusion of gastric acid into the mucosal layers, leading to mucosal damage. On the other hand, healing of gastric ulcer, which involves cell migration, proliferation and epithelial regeneration at the ulcer base, angiogenesis and matrix deposition [2], is largely coordinated by a variety of growth factors, transcription factors and cytokines [3,4]. Many studies were performed dealing with the mechanism of ulcer healing by using acetic acid ulcer model, which higly resembles human ulcers in terms of both pathological features and healing mechanisms. Therefore, this chronic ulcer model is frequently utilized to develop new anti-ulcer drugs that could potentially prevent ulcer relapse or enhance ulcer healing [5,6].

Resveratrol (RVT) is a potent member of the plant-derived chemicals known as polyphenols, which are synthesized by a wide diversity of plants, such as grapes, raspberries, mulberries, pistachios and peanuts, in response to stress, injury, ultraviolet irradiation and fungal infection as part of their defense mechanism [7]. Polyphenols have a variety

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of biological functions, including antioxidant, anti-inflammatory and anti-cancer effects [8–11]. We have previously reported that RVT treatment protects against cardiotoxicity, hepatic, pulmonary and renal injury in rats and mice by alleviating oxidative damage in the target tissues [12–19]. Accordingly, RVT was shown to protect gastric tissue against the oxidative stress in cholestatic rats [20]. On the other hand, RVT treatment was found to delay healing of gastric ulcer induced by ischemia–reperfusion [21] or acetic acid [22] via the specific inhibition of cyclooxygenase-1 (COX-1).

In the light of these findings, this study was designed to investigate whether resveratrol has protective and therapeutic effects on acetic acid-induced gastric ulcer by determining biochemical and histopathological parameters of oxidant tissue damage.

Materials and methods

All experimental protocols were approved by the Marmara University Animal Care and Use Committee. Both sexes of Wistar albino rats (200–250 g) were kept at a constant temperature ($22 \pm 1^{\circ}C$) with 12 h light and dark cycles and fed a standard rat chow.

Induction of acetic acid-induced gastric ulcer

Ulcers were induced using a model modified from that originally described by Okabe and Pfeiffer [23]. Before the surgery, rats were fasted overnight with free access to water and were anaesthetized (100 mg/kg ketamine and 0.75 mg/kg chlorpromazine; intraperitoneally). Briefly, a midline laparotomy was performed and the stomach was gently exteriorized. Half a millilitre of acetic acid (80%, vol/vol) was poured through the barrel of a 3-ml syringe placed on the serosal surface of the stomach in the corpus region (nearly an area of 60 mm^2) and allowed to remain in contact for 1 min. Then, the fluid was aspirated off carefully and the area that remained in contact with acid was gently rinsed with saline. It was previously demonstrated that ulcers, which develop in the gastric mucosa, become chronic within 2–3 days and heal completely within 2–3 weeks without perforation or penetration to the surrounding organs [24]. After the application of acetic acid, the rats were allowed to recover from anaesthesia and received normal pellets and the designated treatments for the following days. In the *pre-treatment* groups, rats were injected intraperitoneally with either saline ($n = 8$) or RVT (10 mg/kg; $n = 8$) for 10 days prior to ulcer induction and the treatments were continued for the following 3 days. In the *treatment* groups, RVT ($n = 16$) or saline ($n = 16$) was administered at the same dose for either 5 or 10 days starting on the day of ulcer induction. The rationale for selecting the dose of RVT depends upon our previous reports [12–19]. A sham control group ($n = 8$) underwent the surgical procedure of ulcer induction without the application of acetic acid.

Upon the completion of the treatments on the 3rd, 5th or 10th days of ulcer induction, the rats were decapitated. Immediately after decapitation, freshly excised stomachs were dissected out, cut along the greater curvature and the mucosae were rinsed with normal saline for the macroscopical analysis of haemorrhagic lesions in the glandular mucosa. The length (mm) of each lesion was measured (three petechia were counted as 1 mm), summed per stomach and expressed as ulcer index.

In order to evaluate the presence of oxidant injury, gastric tissue samples taken from the corpus region were stored at $-80^{\circ}C$ for the determination of tumour necrosis factor alpha (TNF-α), malondialdehyde (MDA), glutathione and collagen levels and myeloperoxidase (MPO) activity. Formation of reactive oxygen species (ROS) in the gastric samples was monitored by using a chemiluminescence (CL) technique with luminol and lucigenin probes. Additional tissue samples were placed in 10% formaldehyde for histological evaluation.

Measurement of tumour necrosis factor-alpha in gastric tissue

Tumour necrosis factor-alpha (TNF-α) was assayed in gastric tissue homogenate. TNF-α levels were quantified according to the manufacturer's instructions and guidelines using enzyme-linked immunosorbent assay (ELISA) kits specific for the previously mentioned rat cytokines (Biosource International, Nivelles, Belgium). These particular assay kits were selected because of their high degree of sensitivity, specificity, inter- and intra-assay precision and small amount of sample required to conduct the assay.

Malondialdehyde and glutathione assays

Stomach samples were homogenized with ice-cold 150 mM KCl for the determination of malondialdehyde (MDA) and glutathione (GSH) levels. The MDA levels were assayed for products of lipid peroxidation by monitoring thiobarbituric acid reactive substance formation as described previously [25]. Lipid peroxidation was expressed in terms of MDA equivalents using an extinction coefficient of $1.56 \times 10^5\ M^{-1}cm^{-1}$ and results are expressed as nmol MDA/g tissue. GSH measurements were performed using a modification of the Ellman procedure [26]. Briefly, after centrifugation at 3000 rev/min for 10 min, 0.5 ml of supernatant was added to 2 ml of 0.3 mol/l $Na_2HPO_4.2H_2O$ solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing.

GSH levels were calculated using an extinction coefficient of 1.36×10^4 $M^{-1}cm^{-1}$. Results are expressed in μmol GSH/g tissue.

Myeloperoxidase activity

Myeloperoxidase (MPO) is an enzyme that is found predominantly in the azurophilic granules of polymorphonuclear leukocytes (PMN). Tissue MPO activity is frequently utilized to estimate tissue PMN accumulation in inflamed tissues and correlates significantly with the number of PMN determined histochemically in tissues [27]. MPO activity was measured in tissues in a procedure similar to that documented by Hillegass et al. [28]. Stomach samples were homogenized in 50 mM potassium phosphate buffer (PB, pH 6.0) and centrifuged at 41 400 g (10 min); pellets were suspended in 50 mM PB containing 0.5% hexadecyltrimethylammonium bromide (HETAB). After three freeze and thaw cycles, with sonication between cycles, the samples were centrifuged at 41 400 g for 10 min. Aliquots (0.3 ml) were added to 2.3 ml of reaction mixture containing 50 mM PB, o-dianisidine and 20 mM H_2O_2 solution. One unit of enzyme activity was defined as the amount of MPO present that caused a change in absorbance measured at 460 nm for 3 min. MPO activity was expressed as U/g tissue.

Chemiluminescence (CL) assay

To assess the contribution of ROS in acetic acid-induced gastric damage, luminol and lucigenin chemiluminescences were measured as indicators of radical formation. Measurements were made at room temperature using a Junior LB 9509 luminometer (EG&G Berthold, Germany). Specimens were put into vials containing PBS-HEPES buffer (0.5 M PBS containing 20 mM HEPES, pH 7.2). ROS were quantitated after the addition of enhancers, lucigenin or luminol, for a final concentration of 0.2 mM. Luminol detects a group of reactive species, i.e..OH, H_2O_2, HOCl radicals, while lucigenin is selective for $O_2^{\cdot -}$ [29,30]. Counts were obtained at 1 min intervals and the results were given as the area under curve (AUC) for a counting period of 5 min. Counts was corrected for wet tissue weights and expressed as relative light units (rlu/mg tissue) [31].

Measurement of collagen content

Gastric tissue samples were cut with a razor blade, immediately fixed in 10% formalin (in 0.1 M phosphate buffer, pH 7.2), embedded in paraffin and ~15 μm thick sections were obtained. Evaluation of collagen content was performed according to the method published by Lopez de Leon and Rojkind [32] based on the selective binding of the dyes Sirius Red and Fast Green to collagen and non-collagenous components, respectively. Both dyes were eluted readily and simultaneously using 0.1 N NaOH-methanol (1:1, v/v). Finally, the absorbances at 540 and 605 nm were used to determine the amount of collagen and protein, respectively.

Histopathological analysis

For light microscopic investigations, tissue specimens that were fixed in normal 10% buffered formalin for 48 h were dehydrated in alcohol series, cleared in toluene and embedded in paraffin. Paraffin sections (5 μm) were stained with hematoxylin and eosin (H&E) and examined for the characterization of histopathological changes under a photomicroscope (Olympus BH 2, Tokyo, Japan) by an experienced histologist, who was unaware of the experimental groups.

Gastric injury was assessed semi-quantitatively and the scores were given according to the following criteria: 0: Regular morphology; 1: Vacuolization in superficial cells and picnotic nucleus; 2: Moderate degeneration in superficial cells and mild in glandular cells; 3: Moderate degeneration in glandular cells, capillary congestion; and 4: Severe degeneration in glandular cells.

For scanning electron microscopic (SEM) examination, tissue samples were fixed and dehydrated in alcohol series, put into amyl acetate series, dried with liquid CO_2 under pressure with critical point drier (Bio-Rad E 3000, Hertfordshire, UK), covered with gold particles (Bio-Rad SC502, Hertfordshire, UK) and examined under Jeol JSM SEM (Tokyo, Japan).

Statistics

Statistical analysis was carried out using GraphPad Prism 3.0 (GraphPad Software, San Diego, CA). All data were expressed as means ± SEM. Groups of data were compared with an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Values of $p < 0.05$ were regarded as significant.

Results

Serosal application of acetic acid to the saline-treated animals resulted in extensive gastric lesions on the 3rd, 5th and 10th days of ulcer induction, where the ulcer index was found to be significantly high as compared to sham-operated control rats without any lesion ($p < 0.001$). On the contrary, systemic administration of RVT before or after ulcer induction reduced the ulcer index significantly at all time points (Figure 1A). Similarly, the high damage scores in the gastric tissues of saline-treated groups in both pretreated and treated groups were also found to be significantly less in RVT-treated ulcer groups (Figure 1B).

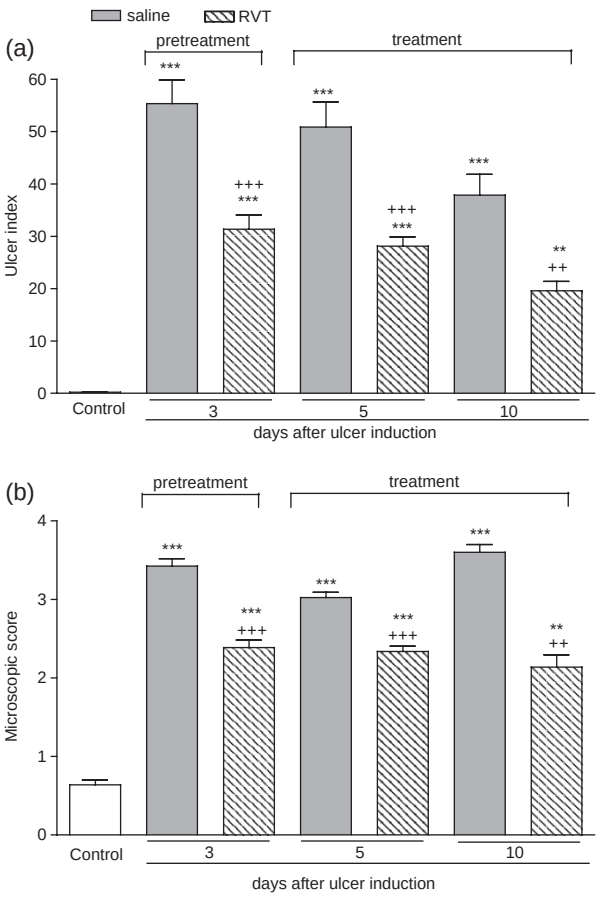

Figure 1. (a) Ulcer index and (b) Microscopic score in the control and saline- or resveratrol (RVT)-treated ulcer groups. $^{**}p<0.01$, $^{***}p<0.001$ vs saline-treated control group; $^{++}p<0.01$, $^{+++}p<0.001$ vs saline-treated ulcer group. For each group $n=8$.

As compared to gastric content of TNF-α in the sham-operated control rats, the TNF-α levels were markedly increased in the groups with ulcer that received saline as pretreatment or treatment ($p<0.05$–0.001; Figure 2A). In the groups that were treated with RVT either before or after the induction of ulcer, the increments in gastric TNF-α levels were significantly inhibited ($p<0.01$–0.001). ROS generation as measured by CL revealed that gastric luminol and lucigenin CL levels in the saline-treated ulcer groups were increased significantly on the 5th and 10th days of ulcer ($p<0.05$–0.001), while the elevation was much greater on the 3rd day of ulcer ($p<0.001$) (Figure 2B). RVT, however, abolished the elevations in CL values at both the earlier and later phases of ulcer ($p<0.05$–0.01).

The gastric tissue MDA content measured in the control group was significantly elevated in the saline-treated rats when the gastric tissues were obtained 3, 5 or 10 days after ulcer surgery ($p<0.05$–0.01; Figure 3A). On the other hand, RVT treatment completely prevented ulcer-induced elevation in gastric MDA levels ($p<0.05$–0.001). In accordance with that, ulcerogenesis caused significant decreases in gastric GSH levels at all time points ($p<0.05$–0.001) as compared to control group (Figure 3B), while in the ulcer groups administered with RVT, either as a treatment or a pretreatment, gastric GSH contents were found to be preserved ($p<0.001$) and not different from that of the control group.

MPO activity, which is accepted as an indicator of neutrophil infiltration to the inflamed tissue, was significantly higher in the gastric tissues of saline-treated ulcer groups as compared to the sham-operated group ($p<0.001$) (Figure 3C). In the RVT-treated rats observed at both the earlier and later phases of ulcer, MPO activities were significantly depressed to levels that were not different from that of the sham group ($p<0.05$–0.001).

Tissue collagen content that was measured as a free radical-induced fibrosis marker was elevated in the gastric tissues of saline-treated ulcer groups on the 5th and 10th days of ulcer induction as compared with the control group ($p<0.001$), while in the ulcer group observed on the 3rd day of induction, collagen level was not significantly different than the control group (Figure 3D). On the other hand, RVT treatment significantly reduced the collagen content of both groups with ulcer ($p<0.001$).

Microscopic evaluation of the control group at light microscopic or SEM level (Figure 4, right and left column-A) revealed a regular glandular epithelium with normal gastric pits and glandular cells. In the saline-pretreated ulcer group, severe inflammation with dilations in fundic glandular cells and accumulation of leukocytes were observed (Figure 4, right column-B), while the superficial morphology was observed as desquamation of cells along with nuded and degenerated lamina propria (Figure 4, left column-B). In the RVT-pretreated ulcer group, dilations of fundic glandular cells were reduced and limited, but the neck cell rows showed moderate dilations (Figure 4, right column-C). SEM analysis in this RVT-pretreatment group showed that the lamina propria was regenerated with the renewal of superficial cells (Figure 4, left column-C). The saline-treated ulcer group observed on the 5th day of ulcer induction demonstrated a high density of leukocytes dominantly in the lamina propria with dilations in fundic glands (Figure 4, right column-D), whereas in the RVT-treated match of this group dilations were reduced and the density of leukocytes was diminished (Figure 4, right column-E). Similarly, SEM evaluation in the saline-treated ulcer group on the 5th day revealed desquamation of superficial cells with some erythrocytes (Figure 4, left column-D), while in the RVT-treated corresponding ulcer group the desquamation was reduced and superficial mucus layer was settled in some areas (Figure 4, left column-E). The saline-treated ulcer group examined on the 10th day of ulcer induction demonstrated severe degeneration of fundic glands, which was accompanied by

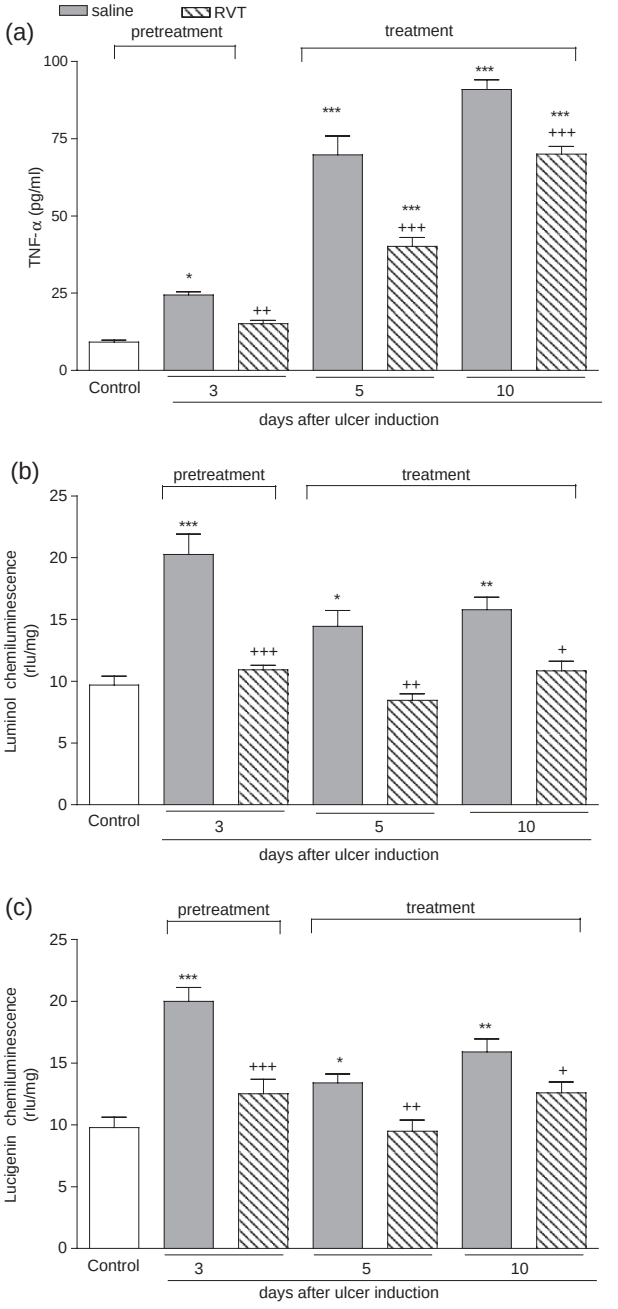

Figure 2. (a) Tumour necrosis factor-alpha (TNF-α), (b) Luminol and (c) Lucigenin chemiluminescence levels in the control and saline or resveratrol (RVT)-treated ulcer groups. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs saline-treated control group; $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ vs saline-treated ulcer group. For each group $n=8$.

accumulation of leukocytes and oedema in the lamina propria (Figure 4, right column-F), but 10 days of RVT treatment following ulcer induction caused regeneration of gastric tissue with reductions in glandular dilations and density of leukocytes, whereas the oedema in lamina propria was decreased but still present (Figure 4, right column-G). Accordingly, SEM observation of the saline-treated ulcer group treated for 10 days revealed that the desquamation of superficial cells was severe (Figure 4, left column-F), while in the corresponding RVT group a prominent regeneration was observed with the settlement of superficial structures (Figure 4, left column-G) in most of the regions.

Discussion

When the gastric mucosa is exposed to noxious agents, the extent of gastric damage depends upon the balance between the factors promoting this damage and those supporting the natural defense mechanisms. In the current study, all the parameters indicating the presence of oxidative injury in the gastric mucosa were markedly reversed by RVT treatment, while the chronic ulcer healing following acetic acid application was facilitated, suggesting that RVT has potent anti-inflammatory, antioxidant and healing-supporting effects on the injured stomach. Increased TNF-α content of the injured stomach, the enhanced release of toxic oxygen metabolites, determined by luminol- and lucigenin-enhanced CL data, as well as the recruitment of neutrophils, shown by MPO activity, were depressed when the rats were treated with RVT either before or after ulcer induction. The extent of injury was also significantly reduced by RVT treatment as assessed by macroscopic and microscopic scores, decreased collagen content and reduced MDA content. In concomitant with these findings, gastric GSH levels, which were depleted in rats possessing ulcers, were preserved when the animals were treated with RVT. In summary, the results indicate that RVT pretreatment followed by a 3-day treatment, as well as 5- or 10-day treatments after ulcer induction facilitated gastric healing by similar mechanisms, through the enhancement of anti-oxidant defense of gastric mucosa.

Since reactive oxygen species (ROS), primarily superoxide anions, hydroxyl radicals and lipid peroxides have been shown to play an important role in the pathogenesis of gastric mucosal damage [33], radical-scavenging antioxidants are found to be useful by protecting the gastric mucosa from oxidative damage or by accelerating the healing of gastric ulcers [34]. As efficient antioxidants, as well as stimulators for prostaglandin synthesis and angiogenesis, many phenolics are known to show anti-ulcerogenic activities [35]. The strong antioxidant property of RVT, the naturally occurring phytoalexin, was shown to yield a variety of cardioprotective, neuroprotective and chemopreventive results [36–38]. However, RVT treatment was found to delay healing of gastric ulcer induced by ischemia–reperfusion or acetic acid [21,22], while RVT treatment was shown to support antioxidant defenses and to reduce oxidative gastric damage in cholestatic rats [39]. Similar to that observed in cholestatic rats, we have

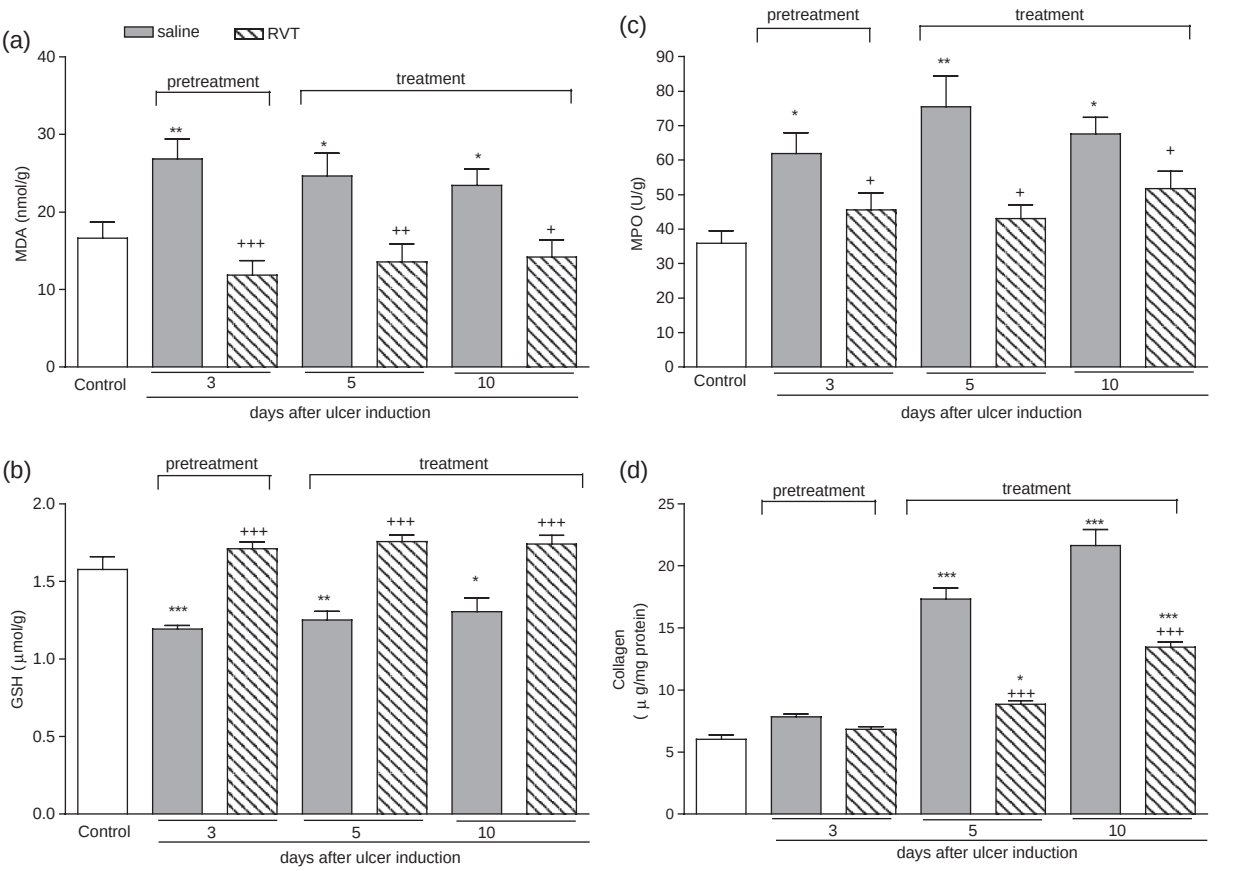

Figure 3. (a) Malondialdehyde (MDA) level, (b) glutathione (GSH) level, (c) myeloperoxidase (MPO) activity and (d) collagen content in the control and saline or resveratrol (RVT)-treated ulcer groups. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs saline-treated control group; $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ vs saline-treated ulcer group. For each group $n=8$.

also reported the therapeutic effects of RVT on ischemia-reperfusion- [14,15] or drug-induced injuries of several organs [13,16–19], as well as its inhibitory effects on oxidative damage due to sepsis [12]. In accordance with these reports, the current findings also show the potent preventive and therapeutic effects of RVT on chronic gastric ulcerogenesis. In the studies which have shown that RVT prolongs ulcer healing, the treatments were applied intragastrically [21,22], which may be the reason for the lack of its facilitatory effect on the healing of gastric ulcer as observed in the present study. This delaying effect of RVT was attributed to its inhibitory effect on COX-1. On the other hand, along with COX-1, COX-2 and nitric oxide synthase (NOS) are also important enzymes involved in ulcer healing, but the interactions between them have not been clearly defined yet. Non-selective inhibition of NOS delays gastric ulcer healing, decreases COX-2 expression and COX activity in the ulcer tissues, suggesting that COX-2 and eNOS promote ulcer healing [40]. Since RVT enhances the expression and activity of eNOS [41], our results suggest that the anti-ulcer effect of RVT may be mediated by the increased expression of eNOS, which was previously shown to enhance COX-2 expression [40].

RVT possesses antioxidant and anti-inflammatory activities, as evidenced by its ability to inhibit superoxide generation in stimulated neutrophils [42] and macrophages [43], as well as its ability to inhibit the production of different ROS in resting or activated platelets [44]. In addition, RVT inhibits lipid peroxidation mainly by scavenging lipid peroxyl radicals within the membrane [45]. Although it is difficult to quantitate ROS because of their reactive nature and short lives, CL method used in the present study is a simple and reproducible technique [46], using two CL probes with different selectivities. Lucigenin is particularly sensitive to superoxide radical, whereas luminol detects H_2O_2, OH^-, hypochlorite, peroxynitrite, and lipid peroxyl radicals [30]. The current luminol- and lucigenin-enhanced CL data confirm that gastric ulcer induced by acetic acid involves toxic oxygen metabolites and RVT acts as an antioxidant agent to reduce the generation of ROS. The results also showed that RVT treatment significantly inhibited MDA production, implying a reduction in lipid peroxidation and cellular injury that protected the stomach against oxidative damage.

GSH and other thiol-containing proteins constitute the primary line of cellular defense against oxidative damage [47]. In an environment where there is

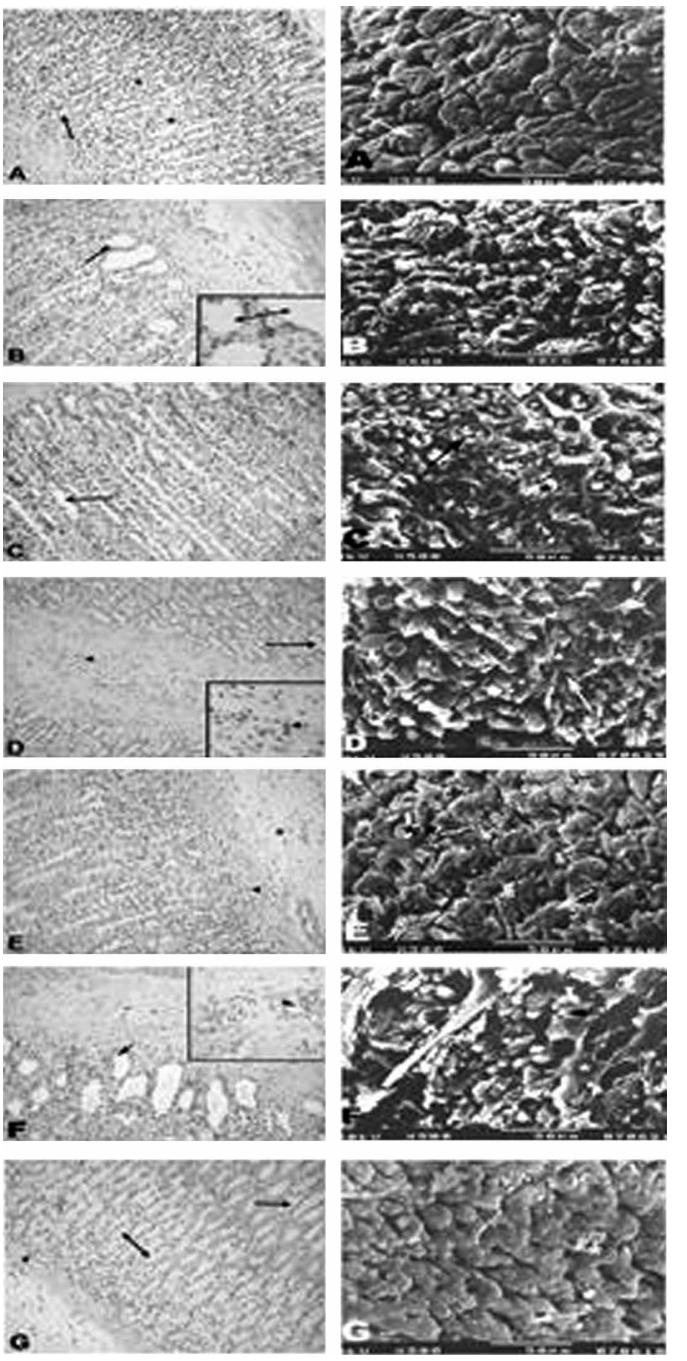

Figure 4. Light microscopic photographs (left column) illustrating the histological appearances of gastric tissues in different experimental groups: (A) *Control group*: Regular structure of neck (*) and fundic glands (arrow), (B) *Saline-preatreated ulcer group*: Dilation of fundic glands (arrow and inset-double-side arrow), note the increased density of leukocytes, (C) *RVT-preatreated ulcer group*: Mild dilation in both fundic glands and neck region (arrow), note the mild congestion throughout the neck region, (D) *Saline-treated ulcer group* (5^{th} day): Severe inflammation in lamina propria (arrowheads), dilation and degeneration of fundic glands (arrow), (E) *RVT-treated ulcer group* (5^{th} day): Reduced inflammation (arrowhead) and mild oedema (*) in lamina propria, note the decreased dilation of fundic glands, (F) *Saline-treated ulcer group* (10^{th} day): Severe inflammation with high density of leukocytes (arrowhead), dilation of fundic glands (arrow) and oedema in lamina propria note the congestion, (G) *RVT-treated ulcer group* (10^{th} day): Prominent reduction of dilation in fundic glands (double-side arrow) and inflammation. (arrowhead) persistance of oedema and dilation of neck-region (arrow). ×200, insets ×400. Hematoxylen-eosin. SEM photomicrographs (right column) illustrating the superficial morphology of gastric tissues in different experimental groups: (A) Control group: superficial cells (arrow) alignining regularly, (B) Saline-preatreated ulcer group: superficial cellular loss (arrow) with dense mucus, (C) RVT-preatreated ulcer group: the clear amelioration in superficial cellular layer with cells and lamina propria (arrow) note the mucus (*), (D) Saline-treated ulcer group (5^{th} day): cellular loss in superficial layer with nude lamina propria (arrow) note the erytrocytes (arrowhead) and mucus, (E) RVT-treated ulcer group (5^{th} day): prominent regeneration of superficial layer with cellular alignment (arrow) note the settlement of mucus (**), (F) Saline-treated ulcer group (10^{th} day): severe desquamation in superficial cells and lamina propria (arrow) and excess mucus (*), (G) RVT-treated ulcer group (10^{th} day): renewal of superficial cells and lamina propria with regular alignment (arrow) note the presence of some degenerated regions (*). ×500.

oxidative stress, GSH is consumed by glutathione peroxidase enzyme during H_2O_2 elimination and the intracellular GSH content is quickly depleted. The current findings demonstrate that depletion of gastric GSH, which is one of the major factors that permit lipid peroxidation and subsequent gastric damage, was also prevented by RVT. Since administration of RVT as a treatment or pretreatment prevented the gastric GSH depletion, it appears that the protective effect of RVT involves the maintenance of the intracellular antioxidant pools. Similarly, RVT treatment was shown to significantly decrease mitochondrial lipid peroxidation and exhibit a significant increase in mitochondrial GSH content of the neurones in the ischemic brain [48]. Moreover, reports have demonstrated that RVT directly increases sirtuin 1 (SIRT1) activity, a NAD(+) (oxidized form of nicotinamide adenine dinucleotide)-dependent histone deacetylase and provides cellular protection against cerebral ischemia via SIRT1 activation [49].

The MPO activity, a marker of neutrophil aggregation at the site of inflammation, frequently increases in ulcerated condition and reduces with the healing process [50]. In consonance with this, in the present study it is also found that acetic acid-induced ulceration leads to increased MPO activity in the gastric tissue, suggesting an inappropriate recruitment of leukocytes into the inflamed gastric tissue, while RVT-enhanced healing of ulcer involves, in part, the inhibition of neutrophil recruitment. Among several cytokines, the proinflammatory TNF-α secreted by activated macrophages plays a crucial role in regulating immune response and in promoting the release of other pro-inflammatory mediators [51] and has a largely irreplaceable role in leukocyte movement within the inflamed tissues [52]. The current findings demonstrate that the potent antioxidant RVT suppressed the neutrophil recruitment and its major regulator TNF-α in the gastric tissue, which appear to be responsible for the amelioration of oxidative injury in the stomach. Furthermore, RVT inhibits the inflammatory response by suppressing prostaglandin biosynthesis [53] and by down-regulating the gene expression of intercellular adhesion molecule-1 (ICAM-1) and NF-k-B, the latter taken as an indirect biomarker of oxidative stress [54]. The NF-k-B signalling pathway can be evoked by oxidative stress and is in turn inhibited by RVT [55].

The results of the present study show that collagen content of gastric tissue was elevated by the progression of ulcer in the 5^{th} or 10^{th} days of ulcer, but no change in gastric collagen level was observed in the early phase of ulcerogenesis. It was documented that RVT pretreatment inhibits growth paths stimulated by angiotensin-II, epidermal growth factor or transforming growth factor-β, which are essential in proliferation and differentiation in cardiac fibroblasts and myofibroblast [56], suggesting that resveratrol is potentially anti-fibrotic. Parallel to these studies, the current data show that RVT depressed the collagen content in the gastric tissue. Similarly, researchers have found that RVT attenuates stricture formation following oesophageal inflammation [57] and inhibits cell growth in activated hepatic stellate cells [58]. Moreover, research on the synthesis and secretion of human type I collagen at the protein level has shown that RVT can inhibit collagen synthesis effectively in a dose-dependent manner, suggesting its use for anti-fibrosis treatment purposes [59].

In conclusion, the present findings have demonstrated that the gastroprotective effects of resveratrol in acetic acid-induced oxidative damage may be due to an augmentation of the endogenous antioxidants and the inhibition of lipid peroxidation by maintaining a balance in oxidant–antioxidant status and inhibiting neutrophil infiltration, nominating resveratrol as a highly promising supplementary agent to be considered in the treatment of gastric ulcer for a qualified ulcer healing.

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