Asian Journal of Surgery xxx (xxxx) xxx

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-asianjournalsurgery.com

ORIGINAL ARTICLE

# Potent therapeutic effects of ruscogenin on gastric ulcer established by acetic acid

Gulcin Ercan [a,*], Rumeysa Ilbar Tartar [a], Ali Solmaz [a], Osman Bilgin Gulcicek [a], Onur Olgac Karagulle [b], Serhat Meric [a], Huseyin Cayoren [a], Ramazan Kusaslan [a], Ahu Kemik [c], Damla Gokceoglu Kayali [d], Sule Cetinel [d], Atilla Celik [a]

[a] *Department of General Surgery, University of Health Science Bagcilar Training and Research Hospital, Istanbul, Turkey*
[b] *Department of General Surgery, Ergani State Hospital, Diyarbakır, Turkey*
[c] *Department of Biochemistry Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey*
[d] *Department of Histology and Embryology, Faculty of Medicine, Marmara University, Istanbul, Turkey*

Received 22 April 2019; received in revised form 13 June 2019; accepted 1 July 2019

**KEYWORDS**
Collagen;
Gastric ulcer;
Inflammation;
Ruscogenin;
Ultrastructure

**Summary** *Background/Objective:* The present study investigated the potent therapeutic effects of Ruscogenin, main steroid sapogenin of traditional Chinese plant called 'Ophiopogon japonicas', on chronic ulcer model established with acetic acid in rats.
*Methods:* 24 rats were attenuated to the sham (2 ml/kg/day isotonic solution), control (untreated ulcer) and treatment (3 ml/kg/day ruscogenin) groups. After treatment for 2 weeks, gastric tissues were collected and prepared for light microscopic (H&E), immunohistochemical (Collagen I, III and IV) and biochemical analysis [Epidermal growth factor (EGF), Prostaglandin E2 (PGE2), Tumor Necrosis Factor alpha (TNF-$\alpha$), Interleukin 6 and 8 (IL-6 and IL-8), Lipid Peroxidase (LPO), Myeloperoxidase (MPO), Glutathione (GSH) and Glutathione Peroxidase (GSH-Px)] and transmission electron microscopy (TEM).
*Results:* Macroscopic scoring showed that the ulceration area of ruscogenin-treated group decreased compared with control group. Immunohistochemical analysis revealed ruscogenin ameliorated and restored the levels of Collagen I and IV to the levels of sham group. Tissue levels of EGF and PGE2 enhanced significantly in untreated ulcer group while were higher in treated ulcer group than the control group. TNF-$\alpha$, IL-6, IL-8, LPO, MPO levels increased significantly in control group whereas decreased in treated rats after ruscogenin treatment. However, levels of GSH and GSH-Px increased significantly in treatment group. TEM showed chief cells and parietal cells of ulcer group having degenerated organelles while ruscogenin group had normal ultrastructure of cells.

* Corresponding author.
*E-mail address:* ghepgul@hotmail.com (G. Ercan).

https://doi.org/10.1016/j.asjsur.2019.07.001


Please cite this article as: Ercan G et al., Potent therapeutic effects of ruscogenin on gastric ulcer established by acetic acid, Asian Journal of Surgery, https://doi.org/10.1016/j.asjsur.2019.07.001

*Conclusion:* There are potent anti-inflammatory and anti-oxidant effects of ruscogenin on gastric ulcer and may be successfully used as a safe and therapeutic agent in treatment of peptic ulcer.


## 1. Introduction

As a type of peptic ulcers, gastric ulcer is a gastrointestinal disorder that is considered to be epidemic in modern age due to affecting 10% of the world population,[1] whereas the incidence in Turkey is not determined clearly yet.[2] Although the pathophysiology of the disease has not been described totally yet, it is accepted that an unbalance between the mucosal defense mechanisms (mucus and bicarbonate synthesis, epithelial barriers, continuous blood flow, etc.) and the destructive mechanisms/agents/inflammations [acid production, *Helicobacter pylori (H.pylori)* infection, non-steroidal anti-inflammatory (NSAI) drug usage, etc.] results in gastric ulcer.[3]

Ulcer cure is a dynamic process of elimination of mucosal damage by the epithelial and connective tissue cells, including various complex biological responses like cell proliferation, migration, regeneration, active angiogenesis and extracellular matrix (ECM) accumulation, controlled by several growth factors.[4] Ulceration induces the mucosal cells to express some factors such as epidermal growth factor (EGF), therefore these factors activate the epithelial cell migration and proliferation locally through autocrine and/or paracrine effects, assisting restoration and amelioration of the gastric ulcer.[5] The pharmaceuticals containing the agents which control these factors may help the clinical management of the peptic ulcers.

Ruscogenin [(1-beta, 3-beta, 25R)-Spirost-5-ene-1,3-diol], a main steroid sapogenin of a traditional Chinese plant called 'Ophiopogon japonicas', was firstly isolated from 'Ruscus aculeatus' and has been found to have notable anti-inflammatory and anti-thrombotic activities in different diseases.[6] Previous studies have revealed that the potent anti-inflammatory mechanism of ruscogenin is essentially related to the inhibition of nuclear factor kappa B (NF-kB) signal pathway and of the expression of intercellular adhesion molecule-1 (ICAM-1).[6,7] However, there are no reports about the therapeutic effects of ruscogenin on the chronic gastric ulcer and the underlying mechanisms remain unclear. Thus we aimed to evaluate the possible therapeutic effects of ruscogenin on acetic acid-induced chronic gastric ulcer by using macroscopic, histopathological, immunohistochemical, biochemical and ultrastructural methods.

## 2. Methods

### 2.1. Animals

The animal studies were performed after receiving approval of the Animal Care and Use Committee of The Bagcilar Training and Research Hospital of Health Science University approved (Protocol Number: 2016—25, Date of Approval: 22nd August 2016). The study complied with the ARRIVE guidelines and be carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

24 female Sprague—Dawley rats (250 ± 30 g, with ages 4—8 weeks), supplied by the Bagcilar Training and Research Hospital Animal Center (BADABEM), Istanbul, Turkey, were kept under the laboratory conditions of the same center, housed in a controlled room with 12-h light—dark cycles at 22 °C, and fed with standard pellet chow including 21% protein and daily fresh water. Exclusion criteria were a weight loss of more than 20%, irregular nourishment and less drinking water, and a significant decrease in response to stimuli during the experiments. All procedures were consistent with the standards recommended by European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS 123). In the study design, the rules of standard procedures concerned about pre-clinical animal studies and researches were followed.[8]

All rats were captured in separate cages including 8 rats in each one and were divided into three random groups (n = 8) as;

Group 1. Sham group received equal amount of isotonic solution (IS)

Group 2. Control (Ulcer) group received acetic acid, treated with 2 ml/kg/day dose of IS.

Group 3. Treatment group received acetic acid, treated with 3 mg/kg/day dose of ruscogenin.

### 2.2. Induction of ulcer

The rats were fasted 8 h before the operation of induction of the chronic gastric ulcer and caped and observed in metabolic cages 12 h before the operation. The sham group was cut through the abdomen and closed without any operation of ulcer induction. The control and treatment groups were operated by same surgeon to induce gastric ulcer and same operational steps were followed in each animal.

Under intraperiotenal 60 mg/kg Ketamine HCl (Ketalar®, Pfizer, Turkey) and 10 mg/kg Xylazine HCl (Rompun®, Bayer, Turkey) anesthesia as a general anesthesia method, 2 cm of anterior abdomen of rats were incised after shaving the region (Fig. 1A). Then the stomach was exteriorized by using clamp and forceps (Fig. 1B). Over the serosa of corpus-antrum region of anterior wall of stomach, 80% acetic acid was injected for 1 min by a readily prepared

Please cite this article as: Ercan G et al., Potent therapeutic effects of ruscogenin on gastric ulcer established by acetic acid, Asian Journal of Surgery, https://doi.org/10.1016/j.asjsur.2019.07.001

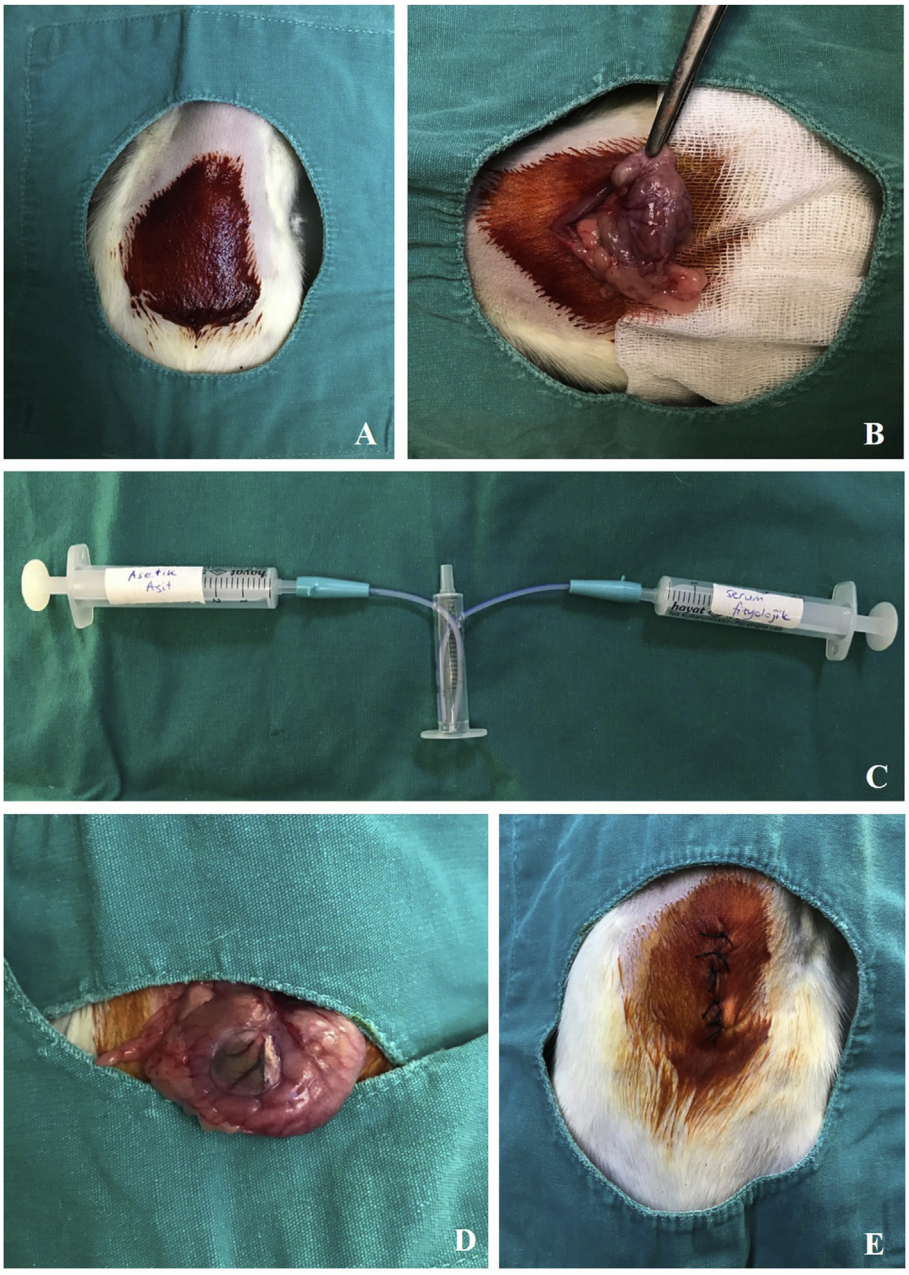


**Figure 1** Operational procedures on rat stomach to establish an ulcer model.

setup (Fig. 1C), avoiding the contact with surrounded tissues (Fig. 1D). Afterwards the fluid was aspirated off carefully and the area that remained in contact with acid was gently rinsed with IS. Then the stomach was located to the anatomical region, and the anterior wall of abdomen closed by continuous 3/0 silk suture (Fig. 1E). After operation, the rats were not limited by any oral regime.

Each of rats were left in the separate cages and checked for following 24 h after the operation. Daily one dose of 2 ml/kg/day IS was given to the sham and control groups, and daily 3 mg/kg/day dose of ruscogenin (H10000934, Abdi İbrahim Drug Industry and Trade Inc., Turkey) was given to the treatment group for 2 weeks via an oral gavage method. At the post-operative first day, one rat in the control group and 3 rats in the treatment group died, probably due to the anesthetic complications. There were no other criteria which led to exclude any other rat from the experimental procedures. There were no side effects like any inflammation on wounds or vomiting due to oral medication.

## 2.3. Macroscopic analysis

Upon the completion of 2 week-experiments, all rats were sacrificed by intra-cardiac puncture under general anesthesia and their stomach were dissected out, cut along the greater curvature and the ulcers were scored macroscopically in addition to measuring the ulcer region in

Please cite this article as: Ercan G et al., Potent therapeutic effects of ruscogenin on gastric ulcer established by acetic acid, Asian Journal of Surgery, https://doi.org/10.1016/j.asjsur.2019.07.001

millimeter. Then the ulcer regions of stomachs were sectioned into 1 $cm^2$ pieces for following analysis.

### 2.4. Histopathological analysis

The gastric tissues were fixed by immersion fixation in 10% neutral formalin solution for light microscopic analysis. Then they were dehydrated in increasing concentrations of alcohol, cleared in toluene and embedded in the paraffin blocks. Sections from the blocks in around 5 μM thickness were stained by hematoxylin and eosin (H&E) and examined for the characterization of histopathological changes under a photomicroscope (Olympus BX51, Tokyo, Japan) by an experienced histologist, who was unaware of the experimental groups. The sections were evaluated according to the criteria described in the literature before.[9] The shedding of surface epithelium; bleeding, focal necrosis and mucosal congestion; glandular cell degeneration, and inflammatory cell infiltration were given a histopathological score as 0: None, 1: Mild, 2: Moderate, 3: Severe.

### 2.5. Immunohistochemical analysis

In accordance with previously described method,[10] paraffin sections were stained immunohistochemically by using Streptavidin-Biotin-Peroxidase method with monoclonal and polyclonal antibodies tagged to indicate collagen I, collagen III and collagen IV (Anti-Collagen I antibody, orb322979; Anti-Collagen III antibody, orb10438; Anti-Collagen IV antibody, orb313870). Counter staining was performed by Mayer Hematoxylin and positively staining with relevant antigens were analyzed semi-quantitatively in terms of staining intensity (0: No staining, 1: weak reactivity, 2: moderate reactivity, 3: strong reactivity).[11]

### 2.6. Biochemical analysis

Gastric tissues were homogenized for 8 min at 20 000 rpm in 100 μl 0.02 M EDTA. Homogenates were centrifuged for 5 min at 5000 g and supernatants were collected. Enzyme-linked immunosorbent assay (ELISA) methods were applied according to the manufacturer's instructions without any modifications by using EGF ELISA Kit (E-EL-R0369, Elabscience, Houston, Texas), PGE2 ELISA Kit (201-11-0505, Sunred Biological Technology Co., Ltd, Shanghai, China), TNF-α ELISA Kit (E-EL-R001), IL-6 ELISA Kit (E-EL-R0015), IL-8 ELISA Kit (201-11-0138), LPO ELISA Kit (E-EL-R2481), MPO ELISA Kit (201-11-0575), GSH-Px ELISA Kit (201-11-5104), GSH ELISA Kit (201-11-5134). Optical densities were read on a plate reader set at 450 nm. The concentration of each parameter in the samples was calculated from the standard curve, multiplied by the dilution factor and was expressed as mean ± standard error mean (SEM).

### 2.7. Ultrastructural analysis

Gastric tissues of each rat in 1 $mm^3$ thickness was fixed in 2.5% glutaraldehyde in a 0.1-M phosphate buffer solution (PBS) (pH 7.2) at +4 °C for 12 h. The tissues were post-fixed in 2% $OsO_4$ prepared in PBS buffer, dehydrated with graded ethanol, and embedded in epon. Tissues were cut into ultra-thin 60-nm sections using an ultramicrotome (Leica R Ultracut) and the sections were positioned on copper grids (200 mesh), stained with uranyl acetate and lead citrate, and analyzed by TEM (Hitachi HT7800). Cellular regions were analyzed and photographed.

### 2.8. Statistical methods

All data are expressed as means ± SEM with all rats per group. Instat statistical package (GraphPad Software, San Diego, CA, USA) was used. Following the assurance of normal distribution of data, oneway analysis of variance (ANOVA) with the Tukey–Kramer post-hoc test was used for multiple comparison. Values of $p < 0.05$, $p < 0.01$ and $p < 0.001$ were regarded as significant.

## 3. Results

### 3.1. Macroscopic findings

The findings of macroscopic scoring for gastric tissues are given in Fig. 2. The sham group was not scored since any operation was not performed to induce ulcer. The mean area of gastric ulcer zone in the control group was 0.62 ± 0.06 while the mean of the treatment group reduced to 0.22 ± 0.06 in a statistically significant manner ($P < 0.05$).

### 3.2. Histopathological findings

The sham group had normal structure of gastric epithelium and glands (Fig. 3A) whereas the acetic acid induced ulcer group had dilations in both mucous neck cells and gland bodies and there were congestion in surface epithelial mucosa (Fig. 3B). On the other hand, this dilation in gastric glands and the congestion of mucosa was regressed in the treatment group (Fig. 3C).

According to the results of histopathological scoring, the scores for shedding of surface epithelium; bleeding, focal necrosis and mucosal congestion; glandular cell degeneration, and inflammatory cell infiltration in the control (ulcer)

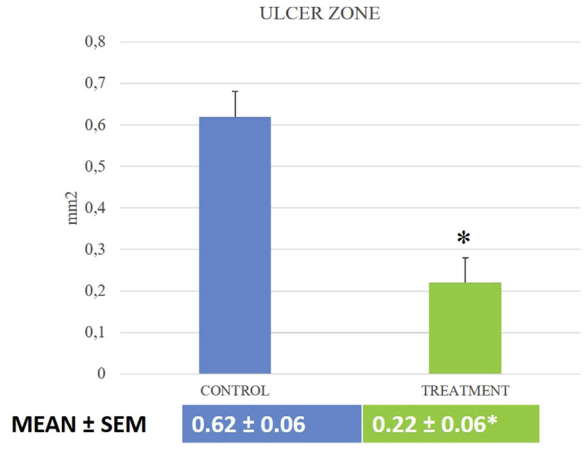


**Figure 2** A graphic for macroscopic ulcer score. *P = 0.012 (<0.05) vs control group.

Please cite this article as: Ercan G et al., Potent therapeutic effects of ruscogenin on gastric ulcer established by acetic acid, Asian Journal of Surgery, https://doi.org/10.1016/j.asjsur.2019.07.001

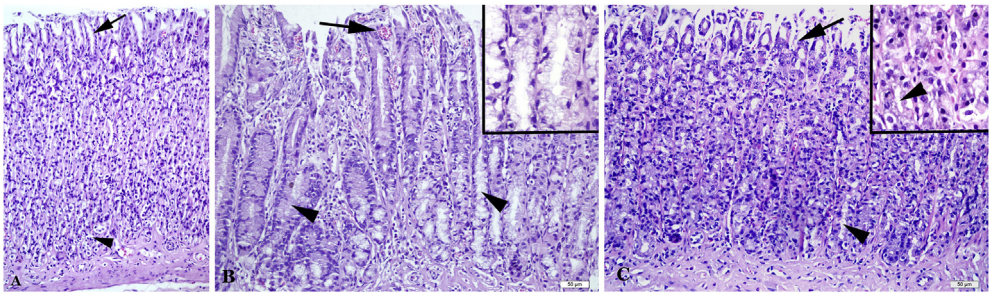

**Figure 3** **A:** Sham group, normal surface epithelium (ok) and gastric glands (arrow head), x20. **B:** Control (Ulcer) group, congestion of gastric epithelium (arrow) and highly dilation of gastric glands (arrowhead), x20; inlet figure: shedding of gastric cells, x40. **C:** Treatment group, recovered congestion of surface epithelium (arrow), x20; inlet figure: regenerated gastric epithelium (arrowhead), x40.

group increased significantly in comparison to the sham group ($P < 0.001$) while the scores were returned to the normal levels in the treatment group (Table 1).

### 3.3. Immunohistochemical findings

Collagen type I staining in the gastric tissues of sham group showed weak to moderate immunoreactivity specifically among the gastric glands and in the connective tissues (Fig. 4A) while the ulcer group showed an increase in immunoreactivites as moderate to strong (Fig. 4B) and post-treatment with ruscogenin led to a moderate immunoreactivity in the tissues (Fig. 4C). Comparing the statistical results of microscopic findings, the collagen type I immunoreactivity in the control group was significantly higher than the sham group ($P < 0.001$) and reactivity in the treatment group was significantly lower than the control group (Table 2).

Collagen type III staining in the sham group was observed slightly among the gastric glands and in the connective tissue (Fig. 4D). The control group showed moderate immunoreactivity in the glands (Fig. 4E) while the treatment group revealed generally moderate reactivities in the neck glands (Fig. 4F). Upon comparing the results, the collagen type III immunoreactivities in the control and treatment groups elevated compared to the sham group but there was no statistical significance in between groups (Table 2). The control and treatment groups also showed no significance among each other.

The gastric tissues of the sham group had strong collagen type IV immunopositivities in the basement membrane, especially among the gastric glands and in endothelium (Fig. 4G). However, the control group had lower (moderate) immunopositivity (Fig. 4H) with a statistical significance in comparison with the sham group ($p < 0.01$), and the treatment group had significantly stronger immunopositivity than the control rats (Fig. 4I) (Table 2).

### 3.4. Biochemical findings

The biochemical parameters and statistical comparisons of ELISA methods were introduced in Table 3. As compared to the gastric content of growth factors in the sham group, the EGF levels were dramatically increased in the groups with ulcer that received IS and ruscogenin ($P < 0.05$ and $P < 0.001$, respectively). The increment was approximately two times higher in the ulcer induced rats and 17 times higher in ulcer induced and treated rats, in comparison to the sham group (Table 3).

One of prostaglandins, PGE2 amount markedly increased in both of the ulcer groups compared with the sham group ($P < 0.05$ and $P < 0.01$, respectively). The control group showed approximately six times and the treatment group showed seven times more PGE2 than the sham group (Table 3).

The amount of one of inflammatory markers, TNF-$\alpha$ elevated only in the control group with a significantly difference compared to the sham group ($P < 0.001$) while the rats treated with ruscogenin had lower amount of TNF-$\alpha$ compared to the control group, measured the same amount as in the sham group (Table 3).

Other inflammatory marker, IL-6 amounts in the ulcer groups were greater than the sham group and the significance was higher in the control group ($P < 0.01$) than to the

**Table 1** Scores of histopathological findings of sham, control and treatment groups.

| Histopathological Parameters | Sham (n = 8) Mean ± SD | Control (n = 7) Mean ± SD | Treatment (n = 5) Mean ± SD |
|---|---|---|---|
| Shedding of surface epithelium | 0.25 ± 0.46 | 2.14 ± 0.38*** | 1.2 ± 0.45 |
| Bleeding, focal necrosis and mucosal congestion | 0.13 ± 0.35 | 1.71 ± 0.49*** | 0.80 ± 0.45 |
| Glandular cell degeneration | 0.13 ± 0.35 | 2.71 ± 0.49*** | 0.80 ± 0.45 |
| Inflammatory cell infiltration | 0.25 ± 0.46 | 2.14 ± 0.69*** | 1.00 ± 0.70 |

SD: Standard Deviation.
***$P < 0.001$ vs sham group.

Please cite this article as: Ercan G et al., Potent therapeutic effects of ruscogenin on gastric ulcer established by acetic acid, Asian Journal of Surgery, https://doi.org/10.1016/j.asjsur.2019.07.001

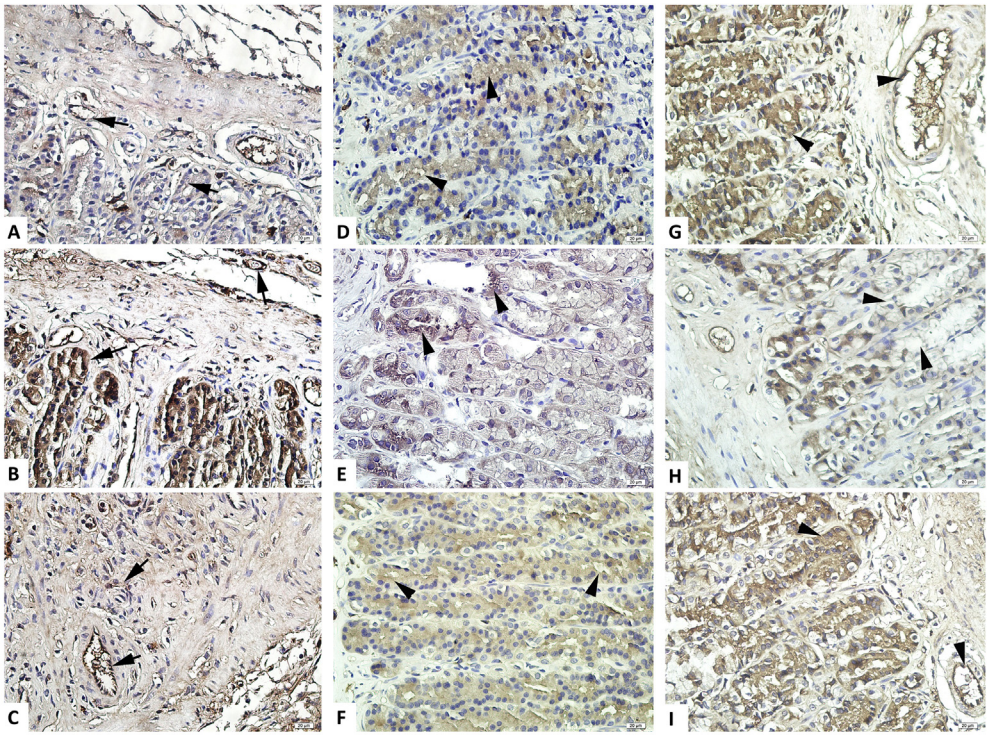


**Figure 4** Immunohistochemical staining for Collagen I (A, B and C), Collagen III (D, E and F) and Collagen IV (G, H and I). **A:** Sham group with mild-medium staining in gastric glands and endothelia (arrows), **B:** Control group with medium—high staining in glands and endothelia (arrows), **C:** Treatment group with medium staining (arrows), x40. **D:** Sham group with mild staining in gastric glands (arrowheads), **E:** Control group with medium staining in gastric glands (arrowheads), **F:** Treatment group with regularly distributed medium staining in glands (arrowheads), x40. **G:** Sham group with high staining in gastric glands and endothelium (arrowhead, **H:** Control group with decreased staining in mucous neck cells (arrowheads), **I:** Treatment group with high staining both I endothelium and glands (arrowheads), x40.

treatment group ($P < 0.05$). The last inflammatory marker IL-8 amount in the gastric tissue highly elevated in the control group as compared to the sham group with a marked statistical significance ($P < 0.001$) but the treated rats preserved the IL-8 amount in the levels of sham tissues (Table 3).

Measuring the LPO amount in the gastric tissue, one of the enzymes enrolled in lipid metabolism, only the control group showed a significant elevation in comparison to the sham group ($P < 0.001$). Ruscogenin treatment completely prevented ulcer-induced elevation in gastric LPO levels (Table 3).

Ulcerogenesis caused significant increase in gastric GSH levels as compared to the sham group ($P < 0.05$) while the ulcer group administered with ruscogenin had dramatically higher increased levels of GSH ($P < 0.001$), approximately nine times higher than the sham group (Table 3). In accordance with this result, the amount of an antioxidant enzyme GSH-Px was also increased significantly in the ulcer groups compared with the sham group ($P < 0.05$ and $P < 0.001$, respectively). In addition, GSH-Px amount in ulcer-induced rats treated with ruscogenin was nine times higher than the amount of sham rats but untreated ulcer-induced rats had lower increase compared to the sham group (Table 3).

Myeloperoxidase enzyme (MPO) activity, which accepted as an indicator of oxidative stress, was significantly higher in the gastric tissues of ulcer groups untreated and treated with ruscogenin in comparison to the sham group ($P < 0.001$ and $P < 0.05$, respectively). The elevation in untreated

**Table 2** Immunohistochemical scores of Collagen I, III and IV reactivities of sham, control and treatment groups.

| | Sham (n = 8) Mean ± SD | Control (n = 7) Mean ± SD | Treatment (n = 5) Mean ± SD |
|---|---|---|---|
| Collagen I | 0.88 ± 0.35 | 2.86 ± 0.38*** | 1.60 ± 0.55 |
| Collagen III | 0.88 ± 0.35 | 1.57 ± 0.79 | 1.60 ± 0.55 |
| Collagen IV | 2.88 ± 0.35 | 1.71 ± 0.76** | 2.60 ± 0.55 |

SD: Standard Deviation.
**$P < 0.01$ and ***$P < 0.001$ vs sham group.

Please cite this article as: Ercan G et al., Potent therapeutic effects of ruscogenin on gastric ulcer established by acetic acid, Asian Journal of Surgery, https://doi.org/10.1016/j.asjsur.2019.07.001

**Table 3** Comparison of biochemical parameters of sham, control and treatment groups.

| | | Sham (n = 8) Mean ± SD | Control (n = 7) Mean ± SD | Treatment (n = 5) Mean ± SD |
|---|---|---|---|---|
| Growth Factor | EGF (pg/ml) | 35.23 ± 8.63 | 75.94 ± 9.71* | 609.0 ± 66.56*** |
| Prostaglandin | PGE2 (ng/ml) | 1.55 ± 0.46 | 6.79 ± 1.14* | 7.88 ± 0.87** |
| Inflammation | TNF-α (pg/ml) | 102.48 ± 12.39 | 1170.0 ± 120.42*** | 174.53 ± 59.80 |
| | IL-6 | 129.2 ± 16.82 | 199.38 ± 11.48** | 156.94 ± 29.60* |
| | IL-8 | 4.50 ± 0.85 | 84.20 ± 6.69*** | 7.13 ± 1.43 |
| Lipid Metabolism | LPO | 4.81 ± 0.37 | 15.74 ± 0.33*** | 10.33 ± 0.85 |
| Oxidative Stress | MPO | 5.84 ± 1.60 | 40.86 ± 3.95*** | 11.04 ± 2.74* |
| Antioxidant Metabolism | GSH | 21.50 ± 6.12 | 34.13 ± 4.97* | 195.2 ± 74.65*** |
| | GSH-Px | 11.5 ± 1.60 | 26.0 ± 6.85* | 78.2 ± 10.08*** |

SD: Standard Deviation.
EGF: Epidermal growth factor, PGE2: Prostaglandin E2, TNF-α: Tumor necrosis factor α, IL-6: Interleukin 6, IL-8: Interleukin 8, MPO: Myeloperoxidase, LPO: Lipid Peroxidase, GSH: Glutathione, GSH-Px: Glutathione Peroxidase.
*P < 0.05, **P < 0.01 ve ***P < 0.001 vs sham group.

ulcer group was observed as four times higher than the elevation in treated ulcer group (Table 3).

### 3.5. Ultrastructural findings

TEM micrographs of chief cells in sham group showed a number of zymogenic secretory granules located in the apical cytoplasm, intact mitochondria, heterochromatic oval nuclei, evenly distributed rough endoplasmic reticulum (RER) and membrane-limited lipid droplets in normal ultrastructure (Fig. 5A). However, the chief cells of untreated ulcer group had a few number of zymogenic secretory granules, disrupted mitochondria, heterochromatic but invaginated degenerated nuclei and RER with dilated cisterna (Fig. 5B). The chief cells of ruscogenin treated group had also a few number of zymogenic secretory granules located in basal cytoplasm, but intact mitochondria, heterochromatic oval nuclei, evenly distributed RER, a juxtanuclear vacuole and membrane-limited lipid droplets (Fig. 5C). The parietal cells of sham group had normal heterochromatic nuclei, evenly distributed mitochondria and regular intracellular and intercellular canaliculi (Fig. 5D) whereas the cells of untreated ulcer group showed peculiarly invaginated heterochromatic nuclei, reduced number and size of mitochondria and irregular, dilated intracellular and intercellular canaliculi (Fig. 5E). Ruscogenin treatment ameliorated the ulcer related degenerations as the parietal cells of treated group revealed normal heterochromatic nuclei, evenly distributed mitochondria and regular intracellular and intercellular canaliculi (Fig. 5F).

## 4. Discussion

Even the gastric mucosa is continuously exposed to detrimental factors, mucosal barrier is able to protect its structural integrity and functions through multifactorial and complex interactions and protective mechanisms including the gastric acid and pepsin release, mucosal blood flow and gastroduodenal motility.[12] Secondary components of the defense system are prostaglandins (PGs) and nitric oxide (NO) which protect the gastric microcirculation by stimulating the synthesis of mucus and bicarbonate.[13,14] Oxidative stress (OS) is shown to disturb this natural defense system via reducing the adherent mucus layer, resulting in direct increase in sensitivity against the mechanical powers by producing the hydroxyl radicals, as well as indirect exacerbation of inflammatory response by activating redox-sensitive transcription factors.[15] Moreover, gastric inflammation around the ulcer region induce the migration of macrophages and polymorphonuclear cells, leading to an increase in release of pro-inflammatory cytokines and mediators from these cells. Among these mediators, tumor necrosis factor (TNF-α) and interleukin 6 and 8 (IL-6 and IL-8) are known to increase especially in *H. pylori* positive ulcer patients.[16] To investigate these mediators and to examine their effects in the healing process of peptic ulcers, four types of experimental chronic ulcer models, named acetic acid ulcer models, have been developed.[17] Animal models are best experimental choices to screen anti-ulcer drugs, and evaluate the adverse effects of various anti-inflammatory drugs on the gastrointestinal mucosa. The model easily and reliably produces round, deep ulcers in the stomach and duodenum, allowing acetic acid ulcer production in mice, rats, Mongolian gerbils, guinea pigs, cats, dogs, miniature pigs, and monkeys. These ulcer models highly resemble human ulcers in terms of both pathological features and healing process. One of the characteristic features of acetic acid ulcers in rats is the spontaneous relapse of healed ulcers >100 d after ulceration, an endoscopically confirmed phenomenon. However, ulcers induced in other animals spontaneously healed and did not relapse, which is in distinct contrast to ulcers induced in rats. Anti-secretory drugs (e.g. omeprazole), prostaglandin analogs, mucosal defense agents (e.g. sucralfate), and various growth factors all significantly enhance healing of acetic acid ulcers in rats.[17–21] Therefore, acetic acid ulcer rat models are quite useful for various studies related to peptic ulcers. By given the reasons above, this study investigated the therapeutic effects of Ruscogenin, a major steroidal sapogenin of Radix Ophiopogon japonicas, in a chronic gastric ulcer model in rats.

Please cite this article as: Ercan G et al., Potent therapeutic effects of ruscogenin on gastric ulcer established by acetic acid, Asian Journal of Surgery, https://doi.org/10.1016/j.asjsur.2019.07.001

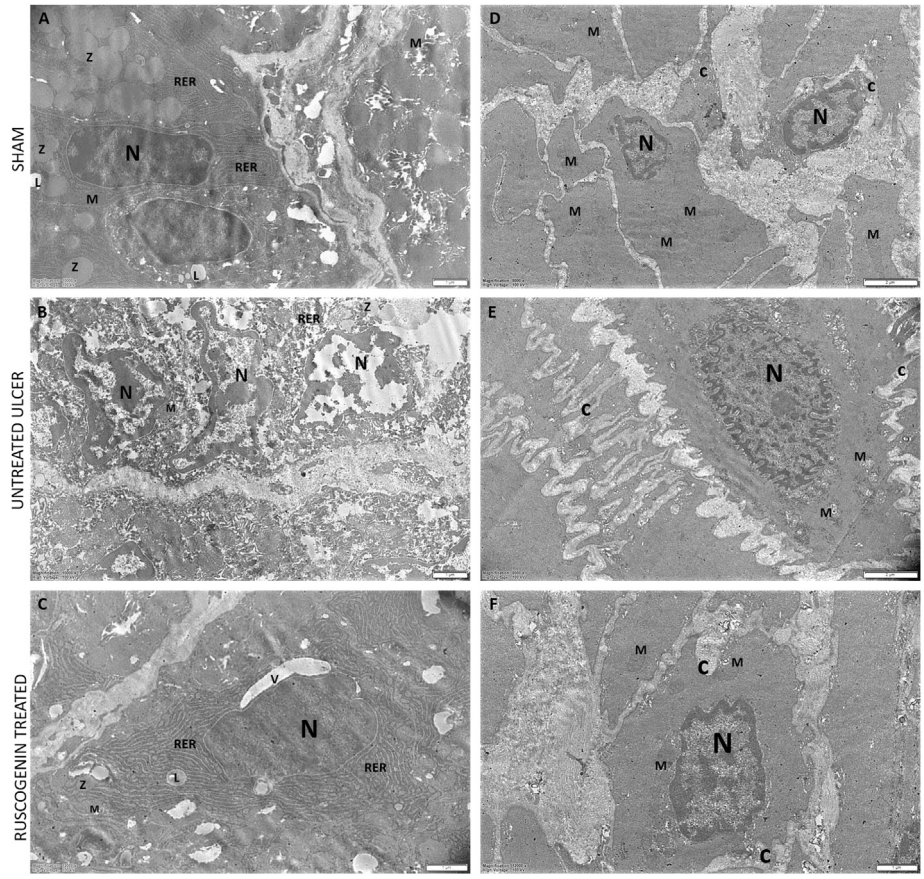


**Figure 5** Transmission electron micrograph of chief cells (A, B, C) and parietal cells (D, E, F). **A:** The chief cells of sham group with a number of zymogenic secretory granules (Z) located in apical cytoplasm, intact mitochondria (M), heterochromatic oval nuclei (N), evenly distributed rough endoplasmic reticulum (RER) and membrane-limited lipid droplets (L). **B:** Chief cells of untreated ulcer group with rare zymogenic secretory granules (Z), disrupted mitochondria (M), heterochromatic but invaginated degenerated nuclei (N), dilated rough endoplasmic reticulum (RER). **C:** Chief cells of ruscogenin group with a low number of zymogenic secretory granules (Z) located in basal cytoplasm, intact mitochondria (M), heterochromatic oval nuclei (N), evenly distributed rough endoplasmic reticulum (RER), a juxtanuclear vacuole (L) and membrane-limited lipid droplets (L). **D:** The parietal cells of sham group with heterochromatic nuclei (N), evenly distributed mitochondria (M) and regular intracellular and intercellular canaliculi (C). **E:** Parietal cells of untreated ulcer group with peculiarly invaginated heterochromatic nuclei (N), reduced smaller mitochondria (M) and irregular, dilated intracellular and intercellular canaliculi (C). **F:** The parietal cells of treated group with normal heterochromatic nuclei (N), evenly distributed mitochondria (M) and regular intracellular and intercellular canaliculi (C).

Natural polyphenols have been reported to play various beneficial roles in gastrointestinal track. Among these roles, antispasmodic, anti-colitic, anti-secretory, antidiarrheal, anti-ulcerative and anti-oxidant properties have been determined until now.[18] Additionally the therapeutic effects of several traditional and supplementary drugs on peptic ulcers are related to their polyphenol contents.[20] The extracts of *R.aculeatus* plant, colloquially known as butcher's broom have been subject to many studies in terms of their pharmacological features. Two main active materials of this plant, ruscogenin and neoruscogenin recently rise to prominence by their anti-inflammatory and anti-oxidant characteristics, as well as their vasoconstrictive and venotonic features. In Europe, Ruscogenin has been largely used in the treatment of chronic venous insufficiency, varicose veins, hemorrhoids, etc.[21] In Turkey, the fluid of boiled roots of *R.aculeatus* in conventional medicine has been used as a diuretic and for the treatment of urinary system disorders and also against eczema.[22] In Turkey, 5 taxa of Ruscus L. are registered; *R. aculeatus* L. var. *aculeatus*, *R. aculeatus* L. var. *angustifolius Boiss.*, *R. hypoglossum* L., *R. colchicus Yeo* and R. *hypophyllum* L.[23] Steroidal saponins (ruscogenin and neoruscogenin as aglycone and their glycosides) are determined to be the main active ingredients responsible from its pharmacological effects.[24] However, these possible effects of ruscogenin have not been investigated before, as a therapeutic agent in the treatment of peptic ulcer until the present report. To accomplish this goal, we examined a chronic ulcer model established by acetic acid induction by a light microscopic, immunohistochemical, biochemical analysis and transmission electron microscopy examination. Since there has not been any research about the effects of ruscogenin in acetic acid induced gastric ulcer, we examined the macroscopic findings to demonstrate the underlying mechanisms of its therapeutic actions, accomplished by

Please cite this article as: Ercan G et al., Potent therapeutic effects of ruscogenin on gastric ulcer established by acetic acid, Asian Journal of Surgery, https://doi.org/10.1016/j.asjsur.2019.07.001

improvements in histopathology, biochemistry, and electron microscopy.

As a result of our findings, the ruscogenin treatment macroscopically reduced the ulceration in gastric tissues in comparison to the rats with untreated ulcer. Our histopathological analysis indicated that the untreated ulcer group had exfoliation on surface epithelium, hemorrhage, focal necrosis and mucosal congestion, degeneration of glandular cells and inflammatory cell infiltration with significantly higher levels of degenerations compared to the sham group; however, treatment with ruscogenin dramatically ameliorated these pathologic features. Immunohistochemical findings revealed that ruscogenin elevated the collagen type IV but depressed collagen type I content. Biochemical analysis demonstrated the enhanced antioxidant and anti-inflammatory properties of ruscogenin, as well as the suppression of oxidative stress and induction of EGF and PGE2 biosynthesis by ruscogenin. Lastly, the ultrastructure of gastric mucosa was ameliorated and restored by the ruscogenin treatment.

Acute oral and parenteral toxicities of ruscogenin have been reported to be low in mice and rats, and long term oral application of high doses were found to be well-tolerated in rats.[25] Rudofsky reported a 10% rate of decrease in venous capacities in healthy individuals in 2 h following an oral introduction of Ruscus hydroalcolic extracts. The patients with chronic venous insufficient gave a constant venous tonus after treated with Ruscus extracts and improved the venous outflow compared with the placebo applied patients.[26] A study evaluated the contributory factors to the effects of R.aculeatus revealed that ruscogenin was not effective on the hyaluronidase activity but had a distinctive anti-elastase activity,[27] as well as anti-edematous effects.[28] Another trial showed that Ruscus extract was able to inhibit the endothelial activation in hypoxia induced cells, principally a similar condition to venous blood stasis. This effect was shown with a depression in ATP content and phospholipase A2 activation, as well as an elevation in neutrophil adherence. Therefore, this may explain some therapeutic efficacy of ruscogenin in chronic venous insufficiency.[29,30] In the present study, ruscogenin is considered to exert its potent efficacy in gastric ulcer via anti-inflammatory actions through reducing TNF-$\alpha$, IL-6 and IL,8, anti-oxidant actions through enhancing GSH and GSH-Px activities, in addition to depression in oxidative stress levels and suppressive effects on lipid metabolism.

Related to the pathogenesis of peptic ulcer, several molecular mechanisms have been determined recently, especially by in vivo studies indicating intracellular and molecular pharmacological action mechanisms of many drugs, nutritional supplements or other agents. Since the recovery period of ulcer needs a series of well-coordinated complex processes, it should be finely controlled by several growth factors and prostaglandins, and tissue healing includes main cellular functions of tissue restoration and angiogenesis.[20] The improvements in the cellular defense, re-epithelization, neovascularization and angiogenesis steps of healing process controlled by enhanced prostaglandins, tissue growth factors and immune complexes, and reduced anti-angiogenic factors play prominent roles in the anti-ulcer potentials of herbal extracts. Therefore, we investigated the EGF and PGE2 levels biochemically in a chronic ulcer model and found that the ruscogenin treatment induced their biosynthesis markedly in gastric tissues of ulcer group, in consistent with the notion that the enhancement in PGE2 is a protective mechanism against gastric mucosal damage.[31,32] Kang et al. investigated the effects of ethyl acetate fraction of a herb on experimental gastric ulcer models and its mechanisms of action in gastric ulcer healing. They found that the rats treated with EtOH/HCl showed a tendency to increase mucosal PGE2 levels.[14] We also observed that acetic acid induced an increase in mucosal PGE2 levels. By contrast, Huang et al. reported that ruscogenin at the 0.3, 1, and 3 mg/kg doses did not exert any remarkable effects on PGE2 content in peritoneal fluid of peritonitis induced mice.[6] This is an expected discrepancy that ulcer and peritonitis may have different physiological action mechanisms in the body, therefore, mucosal and peritoneal levels of PGE2 may differ in these different disorders. Thus, the promontory effect of ruscogenin on mucosal PGE2 levels should not be ignored in gastric ulcers.

EGF, a polypeptide growth factor, exerts a wide variety of biological effects including the promotion of proliferation, and is essential for gastric ulcer repair and healing. Within 3 days after ulcer formation, cells lining the gastric glands in the ulcer margin undergo dedifferentiation, express EGF and its receptor, and actively proliferate. EGF in turn locally stimulates cell proliferation, migration and hence ulcer healing.[5] We observed a robust effect of ruscogenin on mucosal EGF levels in acetic acid induced gastric tissues, therefore, it may be concluded that ruscogenin may accelerate the steps of ulcer healing controlled by EGF.

Anti-inflammatory effect of the crude steroidal saponin from the rhizomes of *Ruscus aculeatus* L. (Ruscaceae) were investigated in two rat models of acute inflammation and a dose-dependent effect of R. aculeatus was found, even with a superiority over a reference drug, diclofenac.[21] Ruscogenin has also been found to exert significant anti-inflammatory and anti-thrombotic activities in related diseases. A study suggested that ruscogenin remarkably inhibited adhesion of leukocytes to a human umbilical vein endothelial cell line (ECV304) injured by TNF-alpha in a dose-dependent manner.[33] Another study by Huang et al., the in vivo effects of ruscogenin on leukocyte migration and celiac PGE2 level induced by zymosan A were studied in mice.[6] The results showed that ruscogenin significantly suppressed zymosan A-evoked peritoneal total leukocyte migration in mice in a dose-dependent manner, while it had no obvious effect on PGE2 content in peritoneal exudate. Ruscogenin also inhibited TNF-$\alpha$-induced over expression of ICAM-1 both at the mRNA and protein levels and suppressed NF-kB activation considerably. Since the main cause of gastric ulcers is acute or chronic inflammation, we expected and observed an inhibitory effect of ruscogenin on TNF-$\alpha$, IL-6 and IL-8 levels in ulcer-induced gastric tissues, therefore one of possible molecular mechanism of ruscogenin is supposed to be anti-inflammatory effect through TNF-$\alpha$, IL-6 and IL-8. Another mechanism may be an inductive effect of ruscogenin on PGE2 levels since ruscogenin-treated rats had higher levels of PGE2 in the gastric tissues.[6] Cao and his colleagues investigated the

Please cite this article as: Ercan G et al., Potent therapeutic effects of ruscogenin on gastric ulcer established by acetic acid, Asian Journal of Surgery, https://doi.org/10.1016/j.asjsur.2019.07.001

protective effect of ruscogenin after ischemic stroke, and showed that it could inhibit IL-1β and Caspase-1, thus decreasing inflammation in vivo and in vitro. Furthermore, ruscogenin might inhibit mitogen activated protein kinase (MAPK) pathway therefore protecting cerebral cells.[34]

By far, an accumulating body of evidence suggests that, among a broad reach of natural molecules, dietary polyphenols with multiple biological mechanisms of action play a pivotal part in the management of gastric ulcers.[35] The dietary polyphenols are currently known to possess a protective and therapeutic potential in peptic ulcer mediated by up-regulating tissue growth factors and prostaglandins; enhancing endothelial nitric oxide synthase derived NO; suppressing oxidative mucosal damage; amplifying antioxidant performance, antacid, and anti-secretory activity; increasing endogenous mucosal defensive agents; and blocking gastroduodenal inflammation and ulceration.[35] Activated neutrophils are one of reactive oxygen species (ROS) sources in gastric mucosal damage. Accumulated active neutrophils lead to release of MPO in the tissue, resulting in the production of hypochlorous acid from hydrogen peroxide ($H_2O_2$) and chloride ions. Then hypochlorous acid causes oxidation of sulfides, and disruption of cytochrome and proteins. Elevated MPO activity is a marker for increase of neutrophil accumulation in the tissue that is an inflammation in the gastric mucosa.[16] Thus, we assessed the level of MPO in gastric tissues of ruscogenin treated rats. Ruscogenin successfully reduced MPO levels induced by ulcer while revealed a boost effect on the levels of indicators of antioxidant metabolism, GSH and GSH-Px. It was readily apparent that ruscogenin exerts its anti-oxidant effects through eliminating the oxidative stress and promoting anti-oxidant metabolism in the chronic ulcers.

Application of various antioxidant agents presents many protective or therapeutic effects in peptic ulcers.[35] Some of them were reported to have protective effects in gastric lumen via altering antioxidant enzyme levels such as GSH and GSH-Px. Moreover, they are known to inhibit lipid peroxidation and to support the integrity of cellular membrane. For example, prevention of GSH depletion and protein oxidation are among curcumin's anti-oxidative stress mechanisms in peptic ulcer. Pretreatment with curcumin can alleviate gastric lesions through amelioration of oxidative damage, scavenging ROS, suppressing thiol depletion and lipid peroxidation, and protecting gastric mucosal peroxidase against drug-associated inactivation resulting in inhibiting the accumulation of endogenous $H_2O_2$ and its OH derivative.[36] Therefore, we tried to elucidate the potent effects of ruscogenin in gastric ulcer via LPO activity as well as MPO activity. Ruscogenin treatment apparently reduced the enzyme levels in gastric tissues, resulting in an alleviation of inflammation, thereby suggesting a conducive effect on lipid metabolism in the chronic ulcer model.

Matrix metalloproteinase (MMPs) are a group of endopeptidases which selectively degrade constituents of the ECM. MMPs possess dynamic function in remodeling the ECM and regulation of matrix proteins like collagens. Some antioxidant and anti-inflammatory agents possess gastroprotective and healing properties through varying of MMP expressions in gastric tissue causing re-epithelialization and remodeling of endothelial tissue.[35,37] Furthermore, angiogenesis and collagenization within gastric tissues are among the main molecular mechanisms of these agents, but these kinds of effects of ruscogenin has not been shown in gastric ulcer. Thereby, we provided inside into the modulatory effects of ruscogenin on the structure of ECM by showing the immunoreactivities of collagen type I, type III and type IV. The most common collagen in ECM, collagen type I has roles in cell attachment, growth, differentiation, migration and tissue morphogenesis. Only one study showing the effect of ruscogenin on collagen content evaluated the impact of ruscogenin on the adhesion of lymphocytes on ECM, and reported that ruscogenin inhibits their adhesion on type I collagen as well as on fibronectin and laminin.[38] In the present study, the content of Collagen type I elevated in ulcer group while ruscogenin treatment lowered the amount to the sham levels. Collagen type III, along with type I is one of essential components of the interstitial matrix. Synthesized from fibroblasts, it plays a major role in inflammation associated pathologies, such as liver damage, renal fibrosis, hernia or vascular diseases.[39] Especially used as an indicator for fibrosis in our study, the amount of type III collagen increased in gastric ulcer regardless of ruscogenin treatment, in a non-significant manner.

Type IV collagen is the main component of basal membrane, providing many attachment points in the epithelium and establishing the backbone of basal membrane. This collagen has a potential for signaling which is pivotal for various physiological and pathological functions.[39] In the present study, reduced content of collagen type IV in chronic ulcer induced group due to disorganization of epithelial integrity was restored to the normal levels in ruscogenin treated ulcer group. Hence, in compliance with the literature, it could be speculated that some therapeutic effects of ruscogenin on the chronic gastric ulcer are attributed to the modulation in collagen content.

In last years, although several compounds have been offered as candidates to alleviate gastrointestinal diseases according to the mucosal lesion index, mucous production, the levels of inflammatory markers and prostaglandins,[40] no studies have described the ultrastructural outcomes of acetic acid induced gastritis, as well as ultrastructural effects of ruscogenin on gastric cells. Scanning and transmission electron microscopy usually employed to evaluate mucosal surface cells affected by ulcer, which reveals a flattened or swollen mucosal epithelium and irregularly gastric pits in animal models.[41,42] The gastric mucosal epithelium in chronic gastritis presents the swelling, vacuolar degeneration, ribosome dissociation, dilatation of the RER and Golgi's apparatus together with mitochondrial swelling in chief cells.[43] Kengkoom et al. demonstrated fatty degeneration in submucosal layer and vacuolated degeneration in muscular layer in an ethanol-induced gastritis rats.[42] They also presented that omeprazole, a basic medication on the World Health Organization's list of essential medicine, improved cellular architecture in the stomach, recovered the gastric cells. However, at ultrastructural level, some defects on RER and mitochondria were still observed. They also confirmed that ethanol-induced gastritis caused RER alterations in chief cells indicated by a number of large, dilated, and fragmented RERs while omeprazole preserves their integrity in relation to its

Please cite this article as: Ercan G et al., Potent therapeutic effects of ruscogenin on gastric ulcer established by acetic acid, Asian Journal of Surgery, https://doi.org/10.1016/j.asjsur.2019.07.001

anti-oxidative and anti-inflammatory effects.[42] Similarly, in the present study, we showed acetic acid induced gastritis resulting in devastating damage on ultrastructure of chief and parietal cells while ruscogenin almost completely ameliorated these effects and preserved the ultrastructure, suggesting a correlation between cellular integrity and anti-inflammatory effects.

As a limitation in the present study, we lost one rat from the control group and three rats from the treatment group, hence, the number of samples in whole groups was not homogenous. It may be confused that this limited number may cause a quite reserved and doubtful conclusion. However, all aspects of the experimental procedures reveals the effectiveness of ruscogenin in the gastric ulcer and thus, the valuable information added to the literature by this study is incontrovertible.

The present findings have demonstrated that the healing effects of ruscogenin in acetic acid-induced gastric ulcer may be due to inhibiting the oxidative stress, promoting the antioxidant mechanisms and the inhibition of lipid peroxidation by maintaining a balance in collagen content of ECM and ultrastructure. Notably, this nominates the ruscogenin as a highly promising supplementary agent to be considered in the treatment of gastric ulcer for a qualified ulcer healing. Utilizing advanced molecular biology techniques, including the gene therapy, it may be possible to more precisely analyze the mechanisms underlying ulcer healing by ruscogenin. With the use of other ulcer models in other animals and lastly human beings, the ruscogenin could be potentially a new anti-ulcer drug that enhances ulcer healing, as well as prevents ulcer relapse.

## Author contributions statement

Conception and design of study was established by GE, RIT, AS, OBG, OOK. Animal studies were performed by GE, RIT, OBG, OOK, AS, SM, HC, RK. Acquisition of data was done by GE, RIT, AK, DG, SC. Analysis and/or interpretation of data were performed by GE, RIT, OBG, SC. GE, RIT, OBG, OOK, DG, SC drafted the manuscript and GE, RIT, RK, AC revised the manuscript critically for important intellectual content. AC supervised the findings of this work. All authors discussed the results and contributed and approved the final manuscript.

## Conflicts of interest

None declared.

## Acknowledgements

The present project was supported by Research and Development Expenses Account under Circulating Capital Budget of University of Health Science Bagcilar Training and Research Hospital, Istanbul, Turkey (Project No: 2016/12).

Authors thank to Assoc. Prof. Ilknur Dag from Central Research Laboratory Application and Research Center of Eskisehir Osmangazi University, Turkey, for the analysis by transmission electron microscopy.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.asjsur.2019.07.001.

## References

1. Prabhu V, Shivani A. An overview of history, pathogenesis and treatment of perforated peptic ulcer disease with evaluation of prognostic scoring in adults. *Ann Med Health Sci Res*. 2014; 4(1):22–29. https://doi.org/10.4103/2141-9248.126604.
2. Zakaria ZA, Abdul Hisam EE, Rofiee MS, et al. In vivo antiulcer activity of the aqueous extract of Bauhinia purpurea leaf. *J Ethnopharmacol*. 2011 Sep 2;137(2):1047–1054.
3. Prachland VK, Klingensmith ME. Stomach. In: Doherty BM, Lowney JK, Mason JE, Reznik SI, Smith MA, eds. *The Washington Manual of Surgery*. 3rd ed. Philadelphia: Lippincott William & Wilkins; 2002:224–226.
4. Wallace JL. Nonsteroidal anti-inflammatory drugs and the gastrointestinal tract. Mechanisms of protection and healing: current knowledge and future research. *Am J Med*. 2001; 110(1A):19S–23S.
5. Tarnawski AS, Ahluwalia A. Molecular mechanisms of epithelial regeneration and neovascularization during healing of gastric and esophageal ulcers. *Curr Med Chem*. 2012;19(1): 16–27.
6. Huang YL, Kou JL, Ma L. Possible mechanism of the anti-inflammatory activity of ruscogenin: role of intercellular adhesion molecule-1 and nuclear factor-kappa B. *J Pharmacol Sci*. 2008 Oct;108(2):198–205.
7. Guan T, Liu Q, Qian Y. Ruscogenin reduces cerebral ischemic injury via NF-κB-mediated inflammatory pathway in the mouse model of experimental stroke. *Eur J Pharmacol*. 2013 Aug 15; 714(1–3):303–311.
8. de Vries RBM, Hooijmans CR, Langendam MW, et al. A protocol format for the preparation, registration and publication of systematic reviews of animal intervention studies. *Evid Based Preclin Med*. 2015;2(1):1–9.
9. Ozveri ES, Bozkurt A, Haklar G, et al. Estrogens ameliorate remote organ inflammation induced by burn injury in rats. *Inflamm Res*. 2001 Dec;50(12):585–591.
10. Ertürküner SP, Yaprak Saraç E, Göçmez SS, et al. Anti-inflammatory and ultrastructural effects of Turkish propolis in a rat model of endotoxin-induced uveitis. *Folia Histochem Cytobiol*. 2016;54(1):49–57.
11. Seckin I, Uzunalan M, Pekpak M, et al. Experimentally induced puromycine aminonucleoside nephrosis (PAN) in rats: evaluation of angiogenic protein platelet-derived endothelial cell growth factor (PD-ECGF) expression in glomeruli. *J Biomed Sci*. 2012 Feb 16;19:24.
12. Eastwood GL. Is smoking still important in the pathogenesis of peptic ulcer disease? *J Clin Gastroenterol*. 1997;25(Suppl 1): S1–S7.
13. Wallace JL, Granger DN. The cellular and molecular basis of gastric mucosal defense. *FASEB*. 1996;10(7):731–740.
14. Kang JW, Yun N, Han HJ, Kim JY, Kim JY, Lee SM. Protective effect of flos lonicerae against experimental gastric ulcers in rats: mechanisms of antioxidant and anti-inflammatory action. *Evid Based Complement Alternat Med*. 2014;2014:596920.
15. Gloire G, Legrand-Poels S, Piette J. NF-κB activation by reactive oxygen species: fifteen years later. *Biochem Pharmacol*. 2006;72(11):1493–1505.
16. Haghazali M, Molaei M, Mashayekhi R, et al. Proinflammatory cytokines and thrombomodulin in patients with peptic ulcer disease and gastric cancer, infected with Helicobacter pylori. *Indian J Pathol Microbiol*. 2011 Jan-Mar;54(1):103–106.

17. Okabe S, Amagase K. An overview of acetic acid ulcer models-the history and state of the art of peptic ulcer research. *Biol Pharm Bull*. 2005 Aug;28(8):1321—1341.
18. Farzaei MH, Rahimi R, Abdollahi M. The role of dietary polyphenols in the management of inflammatory bowel disease. *Curr Pharmaceut Biotechnol*. 2015;16:196—210.
19. Kuwayama H, Matsuo Y, Eastwood GL. Effects of sucralfate, lansoprazole, and cimetidine on the delayed healing by hydrocortisone sodium phosphate of chronic gastric ulcers in the rat. *Am J Med*. 1991 Aug 8;91(2A):15S—19S.
20. Farzaei MH, Abbasabadi Z, Shams-Ardekani MR, Abdollahi M, Rahimi R. A comprehensive review of plants and their active constituents with wound healing activity in traditional Iranian medicine. *Wounds*. 2014;26:197—206.
21. Balica G, Vostinaru O, Tamas M, Crisan G, Mogosan C. Anti-inflammatory effect of the crude steroidal saponin from the rhizomes of Ruscus aculeatus L. (Ruscaceae) in two rat models of acute inflammation. *J Food Agric Environ*. 2013;11: 106—108.
22. Guvenc A, Satir E, Coskun M. Determination of ruscogenin in Turkish Ruscus L. species by UPLC. *Chromatographia*. 2007;66: S141—S145.
23. Davis PH. *Flora of Turkey and the East Aegean Islands*. Edinburgh: University Press; 1984.
24. Ozer G, Guzelmeric E, Sezgin G, et al. Comparative determination of ruscogenins content in Butcher's Broom rhizome samples gathered from the populations grown in different soil conditions in the Marmara Region and attempts for pilot field cultivation of rhizomes. *J Chem Metrol*. 2018;12(1):79—88.
25. Capra C. Pharmacology and toxicology of some components of Ruscus aculeatus. *Fitoterapia*. 1972;43:99—113.
26. Rudofsky G. Improving venous tone and capillary sealing. Effect of a combination of Ruscus extract and hesperidine methyl chalcone in healthy probands in heat stress. *Fortschr Med*. 1989;107(52):55—58.
27. Facino RM, Carini M, Stefani R, Aldini G, Saibene L. Anti-elastase and anti-hyaluronidase activities of saponins and sapogenins from Hedera helix, Aesculus hippocastanum, and Ruscus aculeatus: factors contributing to their efficacy in the treatment of venous insufficiency. *Arch Pharm (Weinheim)*. 1995;328:720—724.
28. Cluzan RV, Alliot F, Ghabboun S, Pascot M. Treatment of secondary lymphedema of the upper limb with CYCLO 3 FORT. *Lymphology*. 1996;29:29—35.
29. Bouaziz N, Michiels C, Janssens D, et al. Effect of Ruscus extract and hesperidin methylchalcone on hypoxia-induced activation of endothelial cells. *Int Angiol*. 1999;18:306—312.
30. Hadzifejzovic N, Kukic-Markovic J, Petrovic S, et al. Bioactivity of the extracts and compounds of Ruscus aculeatus L. and Ruscus hypoglossum L. *Ind Crops Prod*. 2013;49:407—411.
31. Júnior FE, de Oliveira DR, Boligon AA, et al. Protective effects of Croton campestris A.Protective effects of Croton campestris A. St-Hill in different ulcer models in rodents: evidence for the involvement of nitric oxide and prostaglandins. *J Ethnopharmacol*. 2014 Apr 28;153(2):469—477.
32. Nordin N, Salama SM, Golbabapour S, et al. Anti-ulcerogenic effect of methanolic extracts from Enicosanthellum pulchrum (King) Heusden against ethanol-induced acute gastric lesion in animal models. *PLoS One*. 2014 Nov 7;9(11), e111925.
33. Ma L, Kou JP, Huang Y, Yu BY. Effect of ruscogenin in on adhesion of HL-60 cells to ECV304 cells. *Chin Pharmacol Bull*. 2006;22:706—709.
34. Cao GN, Jiang Y, Hu Y, et al. Ruscogenin attenuates cerebral ischemia-induced blood-brain barrier dysfunction by suppressing TXNIP/NLRP3 inflammasome activation and the MAPK pathway. *Int J Mol Sci*. 2016;17:E1418.
35. Farzaei MH, Abdollahi M, Rahimi R. Role of dietary polyphenols in the management of peptic ulcer. *WJG*. 2015;21(21):6499—6517.
36. Vecchi Brumatti L, Marcuzzi A, Tricarico PM, Zanin V, Girardelli M, Bianco AM. Curcumin and inflammatory bowel disease: potential and limits of innovative treatments. *Molecules*. 2014;19:21127—21153.
37. Sharma AV, Ganguly K, Paul S, Maulik N, Swarnakar S. Curcumin heals indomethacin-induced gastric ulceration by stimulation of angiogenesis and restitution of collagen fibers via VEGF and MMP-2 mediated signaling. *Antioxidants Redox Signal*. 2012;16: 351—362.
38. Liu J, Chen T, Yu B, Xu Q. Ruscogenin glycoside (Lm-3) isolated from Liriope muscari inhibits lymphocyte adhesion to extracellular matrix. *J Pharm Pharmacol*. 2002 Jul;54(7):959—965.
39. Nielsen MJ, Karsdal MA. Chapter 3 - type III collagen. In: Karsdal MA, ed. *Biochemistry of Collagens, Laminins and Elastin*. London UK: Academic Press; 2016:21—30.
40. Wang QS, Zhu XN, Jiang HL, Wang GF, Cui YL. Protective effects of alginate-chitosan microspheres loaded with alkaloids from Coptis chinensis Franch and Evodia rutaecarpa (Juss.) Benth. (Zuojin Pill) against ethanol-induced acute gastric mucosal injury in rats. *Drug Des Dev Ther*. 2015;9:6151—6165.
41. Cho KR, Kwon KY, Chang ES. Ultrastructural study of alcohol-induced gastric mucosal change of rat. *Korean J Pathol*. 1993;27:362—370.
42. Kengkoom K, Tirawanchai NN, Angkhasirisap W, Ampawong S. Omeprazole preserves the RER in chief cells and enhances re-epithelialization of parietal cells with SOD and AQP-4 up-regulation in ethanol-induced gastritis rats. *Exp Ther Med*. 2017 Dec;14(6):5871—5880.
43. Zhang ZL, Bu JK, Zhao JX. Ultrastructural observation of the gastric mucosa in chronic gastritis patients treated by traditional Chinese medicine. *World J Gastroenterol*. 1997;3: 185—188.

Please cite this article as: Ercan G et al., Potent therapeutic effects of ruscogenin on gastric ulcer established by acetic acid, Asian Journal of Surgery, https://doi.org/10.1016/j.asjsur.2019.07.001