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Original Article

Anti-ulcerative, anti-oxidative, and anti-inflammatory effects of rosuvastatin and isosorbide dinitrate on cysteamine-induced chronic duodenal ulcer in rats

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A B S T R A C T

Although many drugs are available for treatment of duodenal ulcer (DU), there is a growing concern in alternative therapies with reduced adverse effects in refractory patients. Oxidative stress, decreased generation and bioavailability of nitric oxide (NO), and various inflammatory pathways are widely implicated in the pathogenesis of DU. Statins display different gastroprotective effects through their antioxidant and anti-inflammatory roles with less well studied anti-ulcerative effects on DU diseases. Accordingly, this study was evaluated the possible anti-ulcerative effects of rosuvastatin and isosorbid dinitrate (ISDN) each alone and in combination versus omeprazole on cysteamine-induced chronic DU in rats. Cysteamine-HCl induced chronic DU models were done in 72 adult male albino Wistar rats. Rosuvastatin (20 mg/kg), ISDN (5 mg/kg), and omeprazole (20 mg/kg) were given orally. The anti-ulcerative activities were assessed through measuring the ulcer area, ulcer histopathological score, ulcer index, duodenal NO, tumor necrosis factors-α (TNF-α), interleukin (IL)-1β, malondialdehyde (MDA), and reduced glutathione (GSH) levels and duodenal superoxide dismutase (SOD) and catalase (CAT) activities. Rosuvastatin and/or ISDN significantly improved cysteamine-induced DU as evidenced by significant decreases in ulcer index, lipid peroxidation, TNF-α, and IL-1β levels, and increase in NO and GSH levels as well as increased antioxidant enzymes activities; SOD and CAT. Moreover, the combined treatment showed better results compared to rosuvastatin or omeprazole monotherapies. Finally, rosuvastatin and ISDN have anti-ulcerative, antioxidant and anti-inflammatory activities against cysteamine-induced DU in rats. Combined treatment is advantageous. Further studies for the combined-long term effects should be done especially in cases with co-morbidity.

Key Words: Cysteamine, Duodenal ulcers, Inflammatory cytokines, Isosorbide Dinitrate, Omeprazole, Oxidative stress, Rosuvastatin

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1. INTRODUCTION

Peptic ulcer diseases involve defects in the gastrointestinal mucosa that extend through the muscularis mucosae with implication of various aggressive and defensive factors such as acid-pepsin secretion, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents as, prostaglandins and epidermal growth factors (El-Moselhy et al., 2009; Adinortey et al., 2013).

The most common form of peptic ulcer is the duodenal ulcer (DU) in which several relapses to the development of complications with the potential for significant morbidity and mortality (Wang et al., 2011). During relapsing chronic ulcerations, various proinflammatory and inflammatory cytokines like tumor necrosis factors-α (TNF-α), interleukin (IL)-1β, nitric oxide (NO), reactive oxygen species (ROS), and hyperacidity are implicated in the pathogenesis of ulceration (Amita et al., 2012; GüCin et al., 2013).

NO is an endothelial derived relaxing factor that induced a reduction in the acid secretion. During peptic ulceration, there is a decrease in the expression of inducible NO synthase (iNOS), resulting in decreased generation and bioavailability of NO causing oxidative damage. In addition, inhibition of NO synthesis produces and intensifies acute gastric mucosal damage, suggesting that prolonged NO therapy may be necessary to restore the normal mucus layer, gastric mucosal blood flow and reduce myeloperoxidase activity (Ma et al., 2001).

Cysteamine hydrochloride has been found to be the most potent agent for inducing duodenal ulcer in animals, which resembles DU in man and it is now used

to study the antiulcer activity of drugs (Lahiri and Palit, 2012).

The goal of medical therapy for peptic ulcer disease is to relieve symptoms, heal craters, and prevent complications. Several traditional pharmaceutical drugs as histamine subtype 2-receptor antagonists, antacids, and proton-pump inhibitors have been employed in the management of peptic ulcers. These agents provoke many adverse effects especially with long-term usage recommendations for refractory cases, giving a growing interest in alternative therapies (Adinortey et al., 2013).

Statins are a group of drugs defined as inhibitors of 3-hydroxy-3 methylglutaryl-coenzyme A (HMG-CoA) reductase and have been recognized as the most effective therapeutic agents for reducing serum cholesterol levels (Tariq et al., 2007). Rosuvastatin is a fully synthetic HMG-CoA reductase inhibitor. In NSAIDs-induced gastric mucosal damage, rosuvastatin was associated with marked anti-oxidative, and anti-inflammatory gastroprotective effects, with induction of NO synthesis by iNOS ending in a significant increase in mucosal NO levels (Murrow et al., 2012; Özbakiş-Dengız et al., 2012; Samir et al., 2012).

The effects of statins on cysteamine-induced chronic DU diseases are in need to be explored including the highlights on oxidative, and inflammatory pathways. Moreover, prolonged NO administration in chronic DU is still a thought-provoking area. Therefore, the current study was designed to evaluate the possible anti-ulcerative, anti-oxidative, and anti-inflammatory effects of rosuvastatin and isosorbid dinitrate (ISDN, a NO donor) either alone or combined versus omeprazole as a gold standard in the management of peptic ulcer diseases on cysteamine-induced chronic DU in rats.

2. MATERIALS AND METHODS

2.1. Drugs and chemicals

Rosuvastatin was purchased as a white powder from AstraZeneca Company, Egypt, while ISDN was purchased as a white powder from Minapharm Company, Egypt. Omeprazole was purchased as commercial capsules (Losec®, 20 mg), AstraZeneca Company, Egypt. These capsules were evacuated and the inside enteric coated white pellets were used. Cysteamine-HCI and all other chemicals and solvents were of analytical grades and were obtained from commercial sources.

2.2. Animals

Seventy-two adult male albino Wistar rats, weighing 180-200 g were obtained from Egyptian Organization for Biological Products and Vaccines. The animal chow diet and water were provided ad libitum. Rats were maintained on normal light-dark schedule and temperature 25 ± 3 °C throughout the experiment and left one week for acclimatization. The experimental protocol was approved by the institutional animal care and use committee at Suez Canal University, which is following the National Institutes of Health Guide for the care and use of laboratory animals (Maryland, USA).

2.3. Experimental protocol

2.3.1. Induction of chronic DU in rats

Rats were fasted for 24 hours prior to the experiments. The day of cysteamine-HCl administration was defined as day (0). Chronic DU model was induced through administration of two intragastric doses of cysteamine-HCl (450 mg/kg dissolved in distilled water) through rubber stomach tube at an interval of 4 hours on day (0) (Elberry,

2013), then the rats were accessed freely to drinking water containing 0.2% cysteamine-HCI from day (1) and continued for day (25) (Szabo et al., 1979).

2.3.2. Study groups

Animals were allocated randomly into nine groups, each comprising of eight rats (n=8), group 1: control-untreated group, normal rats without induction of DU and did not receive any medications; group 2: rosuvastatin-control group, rats without DU received two intragastric doses of distilled water through rubber stomach tube at an interval of 4 hours on day (0), then on day (10) received rosuvastatin by oral gavage at a dose of 20 mg/kg dissolved in 0.9% saline daily for 14 days (Özbakiş-Dengız et al., 2012); group 3: ISDN-control group, rats without DU received two intragastric doses of distilled water through rubber stomach tube at an interval of 4 hours on day (0), then on day (10) received ISDN by oral gavage at a dose of 5 mg/kg dissolved in 0.9% saline, three times daily for 14 days (Qui et al., 2004); group 4: rosuvastatin & ISDN-control group, rats without DU received two intragastric doses of distilled water through rubber stomach tube at an interval of 4 hours on day (0), then on day (10) received both rosuvastatin and ISDN as previously mentioned doses; group 5: cysteamine-untreated group, rats with chronic DU as described; group 6: cysteamine & omeprazole-treated group, rats with chronic DU that received freshly prepared omeprazole suspension (4 mg/ 2ml distilled water) at an intragastric dose of 20 mg/kg/d (Elberry, 2013) for 14 days started on day (10) of cysteamine-HCl administration and continued for 14 days. The animals

fasted for food two hours before and one hour after omeprazole administration, which was followed immediately by administration of 1ml distilled water to ensure washing of the gavage tube from any remaining drug particles. group 7: cysteamine & rosuvastatin-treated group, rats with chronic DU that received rosuvastatin started on day (10) of cysteamine-HCl administration and continued for 14 days; group 8: cysteamine & ISDN-treated group, rats with chronic DU that received ISDN started on day (10) of cysteamine-HCl administration and continued for 14 days; group 9: cysteamine, rosuvastatin & ISDN-treated group, rats with chronic DU that received rosuvastatin and ISDN started on day (10) of cysteamine-HCl administration and continued for 14 days.

2.4. Duodenal samples collection and processing

Body weight of each animal was measured before administration of any drug. Rats were sacrificed on the day (25) of the experiment by cervical dislocation (Lee et al., 1987a). The duodena (5 cm in length) were removed after clamping at the esophagus and duodenum. Each duodenum was incised along its antimesenteric side and rinsed with cold saline. Duodenal tissue samples were weighed and from each sample, 0.5 g was homogenized and the homogenates were centrifuged for 15 min at 17.000 rpm. The supernatants were collected and kept frozen at -80°C for subsequent biochemical studies. The rest of duodenal samples were fixed in 10% neutral buffered formaldehyde for 24 hours for further histopathological assessment.

2.5. Determination of duodenal oxidative stress and antioxidant markers

The oxidative stress parameter including malondialdehyde (MDA) (Preuss et al., 1998) and antioxidant markers including superoxide dismutase (SOD) and catalase (CAT) activities (Marklund, 1992; Mueller et al., 1997) as well as reduced glutathione (GSH) (Beutler et al., 1963) levels were measured according to manufacturer instructions using a UV-visible spectrophotometer (UV-1601PC, Shimadzu, Japan).

2.6. Determination of duodenal NO level

Duodenal NO levels were measured by using the Griess reaction. Briefly, samples were diluted with distilled water and deproteinized by adding 1/20th volume of zinc sulfate (300 g/L) to give a final concentration of 15 g/L. After centrifugation at 10.000 g for 5 mm at room temperature, 100 L of supernatant was applied to a microtiter plate well, followed by 100 mL of Griess reagent (1 g/L sulfanilamide, 25 g/L phosphoric acid, and 0.1 g/L N-1-naphthyl ethylenediamine). After 10 mm of color development at room temperature, the absorbance was measured on a microplate reader (Moshage et al., 1995).

2.7. Determination of duodenal TNF-α and IL-1β levels

Total TNF-α and IL-1β contents were measured in duodenal homogenates by an automated ELISA reader (Metertech, M960) and according to the manufacturer's instructions.

2.8. Assessment of duodenal mucosa

The fixed duodenal samples were examined under 5-fold binocular magnification to assess ulcerative lesions. The number of ulcers, ulcer surface area, ulcer score, and ulcer index was determined. For histopathologic assessment, the formalin-fixed specimens were embedded in paraffin, sectioned (5 microns) and stained with Hematoxylin and Eosin (H & E) (Elberry, 2013).

2.8.1. Evaluation of ulcer score

Ulcer intensities were scored, by histopathological examination, using 4- point scale, 0, no lesion; 1, superficial mucosal erosion; 2, deep ulcer or transmural ulcer (necrosis); 3, Perforated or penetrated ulcer (into the pancreas or liver) (Elberry, 2013; Rao et al., 2004).

2.8.2. Evaluation of ulcer surface area

The ulcer surface area was estimated using 3M® scaled surgical transport tapes fixed on a light, transparent sheet. The tape transport was divided into cells each 1 mm2 in the area; the number of cells was counted and the ulcer area was thus measured for each duodenum (Elberry, 2013; Minaiyan et al., 2009).

2.8.3. Evaluation of ulcer index

The ulcer index was calculated by the following equation:

$$UI = UN + US + UA \times 0.1$$

Where: UI = ulcer index. UN= ulcer number. US= ulcer score. UA= ulcer surface area for each duodenum (Elberry, 2013; Szabo et al., 1979).

2.9. Statistical analysis

Results were collected and expressed as the mean ± standard deviation (SD). Results were analyzed using The Statistical Package for the Social Sciences, version 20 (SPSS Software, SPSS Inc., Chicago, USA). One-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc test was used to test the significance of the difference between quantitative variables. The p-value < 0.05 was considered to be statistically significant.

3. RESULTS

3.1. Effects of rosuvastatin and ISDN on oxidative stress and antioxidant markers

3.1.1. Effects on cysteamine-induced alterations in duodenal MDA Levels

Normal rats administered rosuvastatin and ISDN showed a statistical decrease in mean duodenal MDA level compared to the control-untreated group ($p < 0.05$). In cysteamine-induced chronic peptic ulcer model, the mean duodenal MDA level displayed a statistically significant elevation compared to the control-untreated group ($p < 0.05$). All cysteamine-treated groups showed a significant reduction in the elevated mean MDA levels ($p < 0.05$), from which the cysteamine group treated with combined therapy showed the most significant decrease compared to the monotherapy with omeprazole or rosuvastatin ($p < 0.05$; Figure 1).

3.1.2. Effects on cysteamine-induced alterations in duodenal SOD and CAT activities and GSH Levels.

In normal rats, administration of rosuvastatin and ISDN revealed a statistical increase in means SOD activities compared to means obtained from the control-untreated group ($p < 0.05$). Regarding means CAT activities, normal rats administered rosuvastatin alone or combined with ISDN showed significant elevations compared to the control-untreated group ($p < 0.05$), while GSH levels were increased significantly in control rats received ISDN alone or combined compared to the control-untreated group ($p < 0.05$; Figure 2).

Untreated-cysteamine challenged animals, revealed a significant reduction in means duodenal SOD, and CAT activities and GSH levels compared to the control- untreated group ($p < 0.05$). Administration of rosuvastatin and/or ISDN elevated these reductions significantly ($p < 0.05$). The statistical highest elevations were attained by the combined regimens compared to the omeprazole- or rosuvastatin-treated groups ($p < 0.05$; Figure 2). Regarding means duodenal GSH levels in rats with chronic duodenal ulcers, administration of either ISDN individually or simultaneously with rosuvastatin showed marked significant elevations when compared to the omeprazole- or rosuvastatin-treated groups ($p < 0.05$; Figure 2C).

3.2. Effects of rosuvastatin and ISDN on cysteamine-induced alterations in duodenal NO Levels

Regarding the control groups, means duodenal NO levels after administration of rosuvastatin and/or ISDN showed statistically significant elevations in comparison with the control-untreated group ($p < 0.05$). After the cysteamine-chronic challenging, there was a significant decrease in mean duodenal NO levels compared to the control-untreated animals ($p < 0.05$). Administration of omeprazole showed a significant increase in NO level compared to cysteamine untreated group ($p < 0.05$). Treatment with rosuvastatin and ISDN each alone and in a combined manner significantly elevated the marked reductions in means duodenal NO levels approached in the cysteamine-untreated group ($p < 0.05$). ISDN alone or combined treated groups showed significant elevations in NO level as compared to omeprazole or rosuvastatin-treated group and control-untreated group ($p <0.05$; Table 1).

3.3. Effects of rosuvastatin and ISDN on cysteamine-induced alterations in duodenal TNF-α and IL-1β levels

In the cysteamine-untreated group, the mean duodenal TNF-α and IL-1β levels showed statistically significant increases compared to the control-untreated group ($p < 0.05$). All cysteamine-treated groups showed significant reductions in the elevated means duodenal TNF-α and IL-1β levels when compared to the cysteamine-untreated group ($p < 0.05$), from which the cysteamine group treated with combined therapy showed the most significant reductions compared to the monotherapy with omeprazole or rosuvastatin ($p < 0.05$). Of notice, monotherapy with rosuvastatin induced a significant decrease in TNF-α and IL-1β levels compared to omeprazole group ($p < 0.05$; Table 1).

3.4. Effects of rosuvastatin and ISDN on cysteamine-induced chronic alterations in ulcer score, ulcer surface area, and ulcer index

The DU scores, ulcer surface areas, and ulcer index were measured in each animal group after induction of chronic duodenal ulcer model with cysteamine. Animal groups treated with omeprazole, or rosuvastatin and/or ISDN showed significant reductions in the three mentioned parameters compared to the cysteamine-untreated group ($p < 0.05$). The combination therapy resulted in the greatest significant reductions in ulcer score, ulcer surface area, and ulcer index compared to omeprazole-treated group ($p < 0.05$; Table 2).

3.5. Effects of rosuvastatin and ISDN on cysteamine-induced alterations in duodenal histopathological pattern

As shown in Figure 3, duodena from the control untreated group showed normal intact mucosa thrown into folds with regularly arranged mucosal glands. The mucosa was lined by columnar epithelium with regular

nuclei and lamina propria formed of loose fibrovascular tissue, Figure 3A. All the previous findings were found in the rosuvastatin-control group, Figure 3B, ISDN-control group, Figure 3C and rosuvastatin and ISDN-control groups, Figure 3D.

Chronic cysteamine administration revealed total loss of duodenal mucosal lining and glands with sloughing and ulceration formed of intense inflammatory infiltrate formed of few polymorphonuclear leukocytes, plasma cells, and lymphocytes, surrounded by marked full wall thickness fibrosis, Figure 3E. Obvious reversions in the chronic cysteamine-induced histopathological changes were noticed after administration of omeprazole, rosuvastatin and/or ISDN. In omeprazole- or rosuvastatin-treated animals, duodenal mucosa were showing superficial ulcerations of the surface epithelium with loss of mucosal folds and shed epithelial cells with chronic inflammatory infiltrates and the underlying stroma showed regularly arranged mucosal glands, Figure 3F and G. Rats treated with ISDN showed ulceration of duodenal mucosa with shed epithelial cells and few mucosal glands with most of the thickness replaced by chronic inflammatory infiltrate and few regenerating cells showing enlarged nuclei, Figure 3H. Rosuvastatin and ISDN co-administration revealed by mucosa with intact surface epithelium folds, mucosal glands, loose fibrovascular stroma, and very few chronic inflammatory cells, with no ulceration or erosion, and few regenerating epithelial cells showing enlarged nuclei, Figure 3I**.**

Table 1: Effects of rosuvastatin, ISDN, and omeprazole on cysteamine-induced chronic alterations in duodenal NO, TNF-α and IL-1β levels.

Study Groups (n= 8 rats/group)	Duodenal NO levels (µmol/gm)	Duodenal TNF-α levels (pg/ml)	Duodenal IL-1β levels (pg/ml)
Group (1)	3.96 ± 0.48	32.20 ± 4.98	76.54 ± 6.71
Group (2)	5.98 ± 0.57*	30.71 ± 2.46	75.68 ± 5.11
Group (3)	10.20 ± 1.34*	31.27 ± 4.58	77.40 ± 6.31
Group (4)	11.74 ± 1.14*	30.57 ± 3.41	75.38 ± 4.57
Group (5)	0.80 ± 0.10*	114.81 ± 12.48*	204.17 ± 17.31*
Group (6)	3.86 ± 0.95 $^{\#}$	83.64 ± 11.23$^{*, \#}$	125.34 ± 15.68$^{*, \#}$
Group (7)	3.14 ± 0.69$^{*, \#}$	68.50 ± 8.9$^{*, \#, @}$	102.64 ± 11.1$^{*, \#, @}$
Group (8)	8.73 ± 1.51$^{*, \#, @, \$}$	71.20 ± 10.5$^{*, \#}$	123.27 ± 13.6$^{*, \#, \$}$
Group (9)	12.11 ± 1.93$^{*, \#, @, \$}$	42.20 ± 6.25 $^{\#, @, \$}$	81.43 ± 8.68 $^{\#, @, \$}$

Results were expressed as mean ± SD, and analyzed using one-way ANOVA followed by Bonferroni's post-hoc test, n=8. Group (1): Control-untreated group, group (2): rosuvastatin -control group, group (3): ISDN-control group, group (4): rosuvastatin and ISDN-control group, group (5): cysteamine-untreated group, group (6): omeprazole-treated group, group (7): rosuvastatin-treated group, group (8): ISDN-treated group, group (9): rosuvastatin & ISDN-treated group. ISDN, Isosorbide-dinitrate. *p<0.05 compared to control-untreated group, #p<0.05 compared to cysteamine-untreated group, @p<0.05 compared to omeprazole-treated group, $p<0.05 compared to rosuvastatin-treated group.

Table 2: Effects of rosuvastatin, ISDN, and omeprazole on cysteamine-induced chronic alterations in ulcer score, ulcer surface area and ulcer index.

Study Groups (n= 8 rats/group)	Ulcer score	Ulcer surface area (mm2)	Ulcer index
Group (5)	3.32 ± 0.15	49.81 ± 3.89	5.68 ± 0.68
Group (6)	1.53 ± 0.38*	11.52 ± 4.61*	1.32 ± 0.28*
Group (7)	1.67 ± 0.23*	14.10 ± 1.29*	1.61 ± 0.19*
Group (8)	2.13 ± 0.10$^{*, @, \#}$	18.23 ± 1.18$^{*, @, \#}$	2.12 ± 0.25$^{*, @, \#}$
Group (9)	0.17 ± 0.05$^{*, @, \#}$	9.12 ± 1.08$^{*, @, \#}$	1.01 ± 0.12$^{*, \#}$

Results were expressed as mean ± SD, and analyzed using one-way ANOVA followed by Bonferroni's post-hoc test, n=8. Group (5): cysteamine-untreated group, group (6): omeprazole-treated group, group (7): rosuvastatin-treated group, group (8): ISDN-treated group, group (9): rosuvastatin & ISDN-treated group. ISDN, Isosorbide-dinitrate. * p<0.05 compared to cysteamine-untreated group, @p<0.05 compared to omeprazole-treated group, #p<0.05 compared to rosuvastatin-treated group.

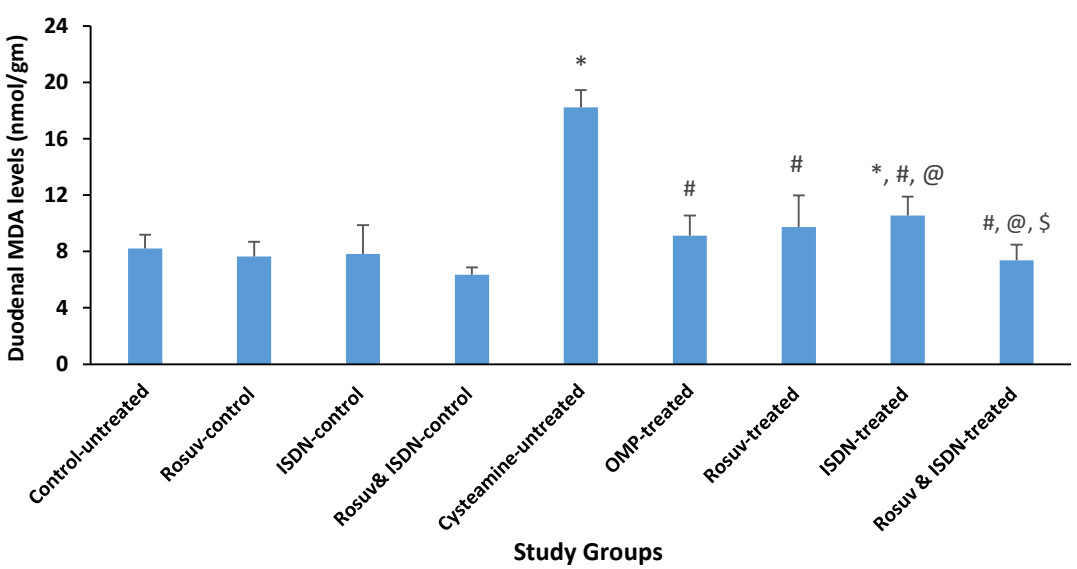

Figure (1): Malondialdehyde (MDA) level in control and cysteamine groups of the chronic peptic ulcer study. Group (1): Control-untreated group, group (2): rosuvastatin -control group, group (3): ISDN-control group, group (4): rosuvastatin and ISDN-control group, group (5): cysteamine-untreated group, group (6): omeprazole-treated group, group (7): rosuvastatin-treated group, group (8): ISDN-treated group, group (9): rosuvastatin & ISDN-treated group. ISDN: Isosorbide-dinitrate. Results were expressed as mean ± SD and analyzed using one-way ANOVA followed by Bonferroni's post-hoc test, n=8. *p<0.05 compared to control-untreated group, $^{\#}$p<0.05 compared to cysteamine-untreated group, @p<0.05 compared to omeprazole-treated group, $^{\$}$p<0.05 compared to rosuvastatin-treated group.

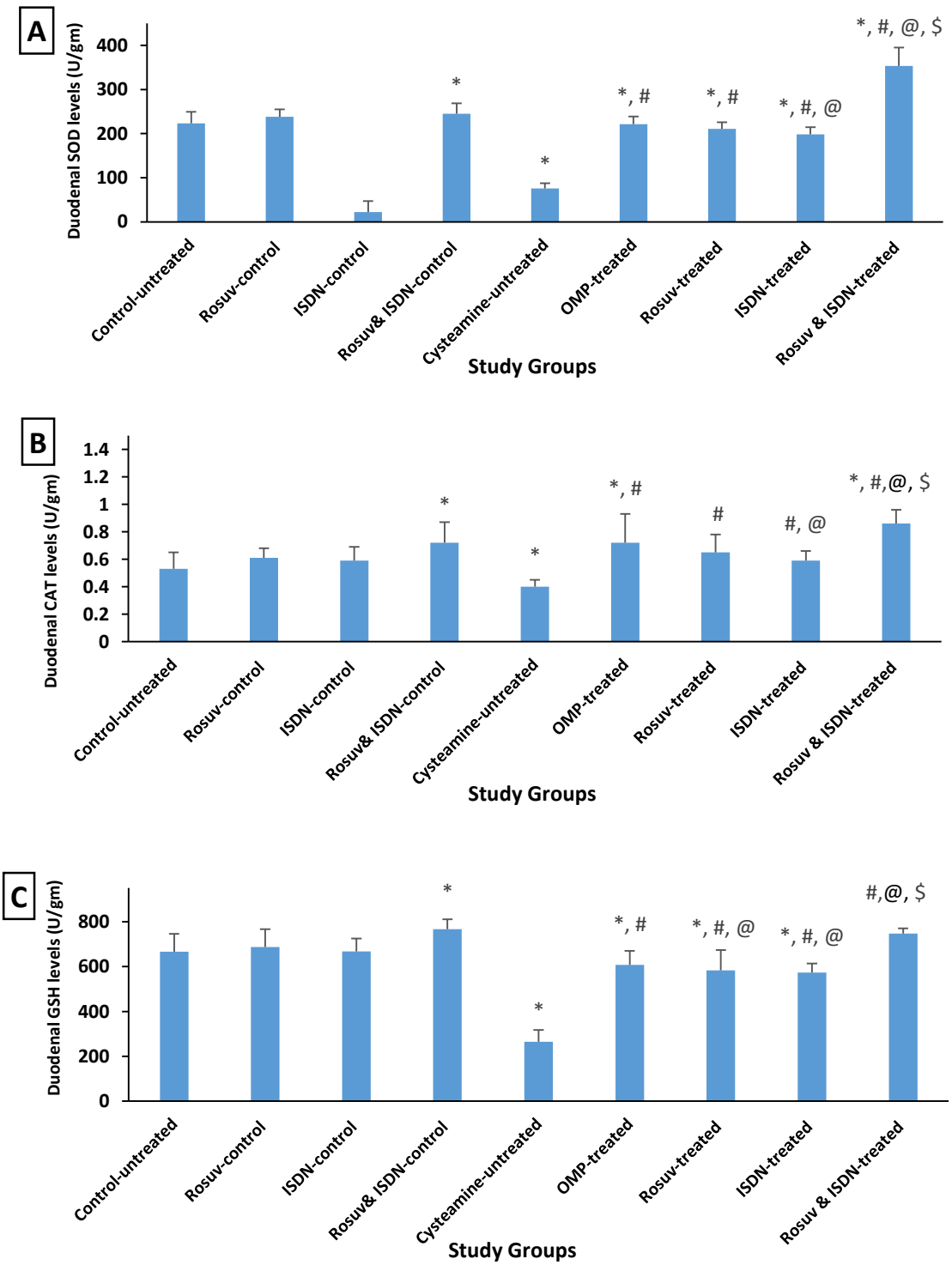

Figure (2): oxidative stress markers; superoxide dismutase (SOD) (A) and catalase (CAT) (B) activities as well as reduced glutathione (GSH) (C) levels in control and cysteamine groups of the chronic peptic ulcer model. Group (1): Control-untreated group, group (2): rosuvastatin -control group, group (3): ISDN-control group, group (4): rosuvastatin and ISDN-control group, group (5): cysteamine-untreated group, group (6): omeprazole-treated group, group (7): rosuvastatin-treated group, group (8): ISDN-treated group, group (9): rosuvastatin & ISDN-treated group. ISDN: Isosorbide-dinitrate. Results were expressed as mean ± SD and analyzed using one-way ANOVA followed by Bonferroni's post-hoc test, n=8. *p<0.05 compared to control-untreated group, #p<0.05 compared to cysteamine-untreated group, @p<0.05 compared to omeprazole-treated group, $p<0.05 compared to rosuvastatin-treated group.

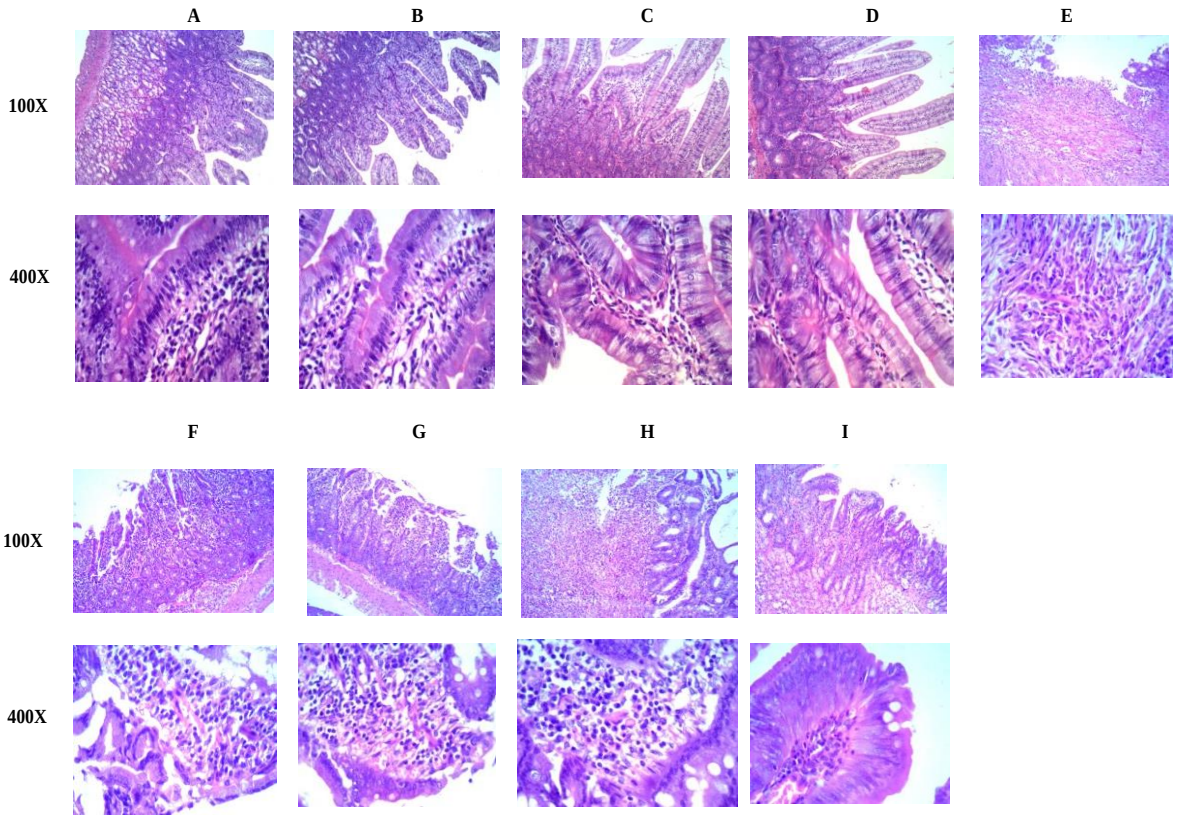

Figure (3): Representative duodenal tissues photomicrographs for different study groups stained with (H&E). Original magnification, 100X, and 400X. Group (1): control-untreated group (A), group (2): rosuvastatin -control group (B), group (3): ISDN-control group (C), group (4): rosuvastatin and ISDN-control group (D), group (5): cysteamine-untreated group (E), group (6): omeprazole-treated group (F), group (7): rosuvastatin-treated group (G), group (8): ISDN-treated group (H), group (9): rosuvastatin & ISDN-treated group (I). ISDN: Isosorbide-dinitrate. Total loss of duodenal mucosal lining and glands ending with ulceration (green arrows) with intensive inflammatory infiltrates (black arrows) surrounded by a fibrotic full-thickened wall (red arrows) were observed after cysteamine challenging. The reversals of these ulcerative/inflammatory changes were obvious after omeprazole, or rosuvastatin and/or ISDN administration with the advantageous effects of the combined regimens.

4. DISCUSSION

In the present study, continuous oral administration of rosuvastatin and/or ISDN versus omeprazole, as a gold standard therapeutic approach, for 14 days were evaluated regarding the anti-ulcerative, anti-oxidative, and anti-inflammatory effects on cysteamine-induced chronic DU in rats.

In the current study, the chronic cysteamine model of duodenal ulceration in rats was evident by an increase in the ulcer index and duodenal MDA, TNF-α, and IL-1β levels with a significant decrease in duodenal CAT and SOD activities and GSH, and NO levels.

The oxidative, inflammatory, and ulcerogenic effects of cysteamine could be explained by its cytotoxic effects through generation of reactive oxygen species that with the presence of ferric ions are forming the cysteamine free radicals with development of oxidative stress (Adinortey et al., 2013; Elberry, 2013), and induction of cytotoxic inflammatory reactions including many proinflammatory cytokines as TNF-α, IL-1β, and IL-6, in addition to reduction in mucosal NO levels with affection of the mucosal blood flow (Amita et al., 2012; Choi et al., 2012; Lu et al., 2014).

The present findings of higher TNF-α and IL-1β after cysteamine challenging were similar to the previous evidences which concluded that in peptic ulcers, the inflammatory cytokines TNF-α and IL-1β have potential roles in impairment of the healing of chronic peptic ulcers and aggravates the acute lesions (Konturek et al., 2010). Locally, the higher TNF-α can augment the tissue inflammation ending in mucosal ischemia and hypoxia with decreased gastric mucosal blood flow with the critical development of consecutive cascades of inflammatory, oxidative stress, and apoptotic reactions (Gao et al., 2014).

As a mediator of acute and chronic relapsing inflammation, IL-1β displays a vital role in the generating and disseminating the systemic inflammatory responses with other pro-inflammatory cytokines release as TNF-α and IL-6 (Choi et al., 2012). Furthermore, the endogenous oxidative stress and generation of oxidative species could stimulate and activate endogenous activation of IL-1β, which explains its importance in the pathogenesis of peptic ulcer diseases (Dinarello, 2011; Warzecha et al., 2012).

The present oxidative, inflammatory, and ulcerogenic effects of cysteamine were supported histopathologically through obvious large full wall thickness fibrotic areas with excessive inflammatory reactions in lamina propria in the duodenal tissues. These outcomes were in consistency with the comparable findings reported by Tham et al. (2001). Moreover, the secretagogues effects on gastric acid and inhibition of the proximal duodenal Brunner's glands-released mucus are done by cysteamine, alongside with the reduction in dopamine levels, the bioavailability of somatostatin, and gastric motility, and emptying (Adinortey et al., 2013; Khomenko et al., 2012).

Proton pump inhibitors are a broad and important therapeutic strategy for peptic ulcer diseases. Omeprazole is the prototype of this important drug group, which reduces the gastric acid secretion with gastric mucosal protective actions (Bedekovic et al., 2003; Gao et al., 2014; Watanabe et al., 2002).

In the present study, administration of daily oral omeprazole ameliorated the ulcerative, inflammatory and oxidative effects-induced by cysteamine. Previously, Gao et al. (2014) reported that omeprazole prevents the gastric mucosal peptic injury through enhancing the release of NO after influencing the parietal cells proton pump activity with enhanced gastric mucosal blood flow and decreasing TNF-α levels. Furthermore, the inhibition of ulcer recurrence by omeprazole administration was caused by the decrease in duodenal IL-1β beside inhibition of the expression of adhesion molecules and many inflammatory cytokines (Watanabe et al., 2001a, 2001b).

Rosuvastatin has been recognized as the most effective therapeutic agent for reducing serum cholesterol level and interestingly it exhibits other effects unrelated to its lipid lowering effects, among which are antioxidant and anti-inflammatory actions, which is considered as a new therapeutic target for inflammation, atherosclerosis, diabetes and peptic ulcer (Lee et al., 1987; Maheshwari et al., 2015; Virdis et al., 2009).

The present study showed that rats had received rosuvastatin, and ISDN orally for 14 days either separately or combined had a significant anti-ulcerative effect on cysteamine-induced DU. They effectively reduced ulcer score, ulcer surface area, ulcer index and histopathological changes. Likewise, they successfully improved duodenal SOD, GSH, CAT and NO levels while they lowered the higher duodenal MDA, TNF-α, and IL-1β.

Previous studies showed that the gastroprotective effects of rosuvastatin were mediated by scavenging the free radicals, increasing mucosal NO synthesis by iNOS and prostaglandin-E2 levels and increasing gastric juice mucin production, with a reduction in gastric levels of TNF-α (Heeba et al., 2009). Additionally, rosuvastatin possessed a stronger reducing power due to the fluorophenyl moiety of its structure leading to more powerful antioxidant effects. Rosuvastatin has also a great inhibitory effect on myeloperoxidase, which increases NO consumption and affects its bioavailability at the site of inflammation (Feng et al., 2015; Samir et al., 2012).

In the chronic and recurrent peptic ulcers, many inflammatory, and apoptotic pathways are involved in which the recruited inflammatory cells including neutrophils and macrophages with their released mediators as TNF-α, and IL-1β are considered the key players (Watanabe et al., 2001). Furtherly, Özbakiş-Dengız et al., (2012) stated that low dose of rosuvastatin (20 mg/kg) could decrease the mononuclear leucocytes and neutrophil infiltrations with a final anti-inflammatory effect. This previous evidence could be an additional explanatory rationale on how rosuvastatin could decrease the present cysteamine-induced higher levels of duodenal TNF-α and IL-1β, and support the improvement in the present cysteamine-induced histopathological changes after rosuvastatin administration.

An earlier study contradicted our current findings and showed that use of rosuvastatin did not confirm gastroprotection and could provoke hyperemia and pro-ulcerogenic effects especially at the higher doses of 40 mg/kg/day, where the indomethacin-induced gastric injury model in rats was performed (Özbakiş-Dengız et al., 2012). These incomparable findings may be related to the diversity in the present experimental protocol versus the previous study, such as the durations of drug administration (14 days versus once before indomethacin challenging), the used doses (20 mg/kg versus 40 mg/kg), the use of different experimental animal models (cysteamine versus indomethacin).

Regarding the role of NO, many studies attempted to explain the mechanisms of NO in cell protection, where NO plays a vasodilatory role, and in a maintenance of the mucosal blood flow through reduction of leukocyte-endothelial adherence. In addition, this protection is accompanied by the participation of reactive oxygen species and a fall in

oxidative stress parameters, namely decrease of MDA and increase of SOD activity (Feng et al., 2015; Ma et al., 2001; Vera-Arzave et al., 2012). Furtherly, NO donors were shown to enhance cyclo-oxygenase activity with protective prostaglandin-E2 production (Calatayud et al., 2001). Additionally, Szlachcic et al. (2013) have reported that NO is an important regulator of the gastric acid, mucus and bicarbonate secretions that has essential protective roles against virulent, and corrosive agents.

This previous evidence was reinforced the present biochemical results of ISDN administration and supported the histopathological results of the significant reductions in ulcer scores and ulcer indexes indicating healing of the cysteamine-induced duodenal ulcers. Additionally, the present simultaneous use of ISDN with rosuvastatin enhanced the anti-oxidative, anti-inflammatory, and anti-ulcerative effects of each other.

5. CONCLUSION

The present study provided evidence that rosuvastatin and ISDN each alone and concomitantly caused substantial therapeutic effects in chronic cysteamine-induced duodenal peptic ulcer as omeprazole, through their anti-oxidative and anti-inflammatory abilities alongside their permissive effects on duodenal NO levels. Further recommendations for appropriate studies and clinical trials in patients with co-morbidities as ischemic heart diseases, hypercholesterolemia, and peptic ulcer diseases whom on nitrates and statins to evaluate the effects of these drugs when combined with the other approved therapeutic regimens for peptic ulcers.

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