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# Gastroprotective effects of telmisartan on experimentally-induced gastric ulcers in rats

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Telmisartan is an angiotensin II $T_1$ receptor blocker (ARB) with partial peroxisome proliferator-activated receptor gamma (PPARgamma) agonistic properties; two actions that are suggested to be efficacious for protecting against gastric ulcers. Hence, the aim of the present study was to evaluate the gastroprotective effect of telmisartan (1, 3, and 10 mg/kg) on indomethacin- and cold restraint stress (CRS)-induced gastric ulcer models in rats. Candesartan, another ARB with the lowest PPARgamma affinity, was used to justify the possible role of PPARgamma agonistic activity of telmisartan in gastroprotection. Ranitidine was used as a reference drug. Pre-treatment with telmisartan dose-dependently attenuated gastric ulcer indices induced by both models. The protective effect of telmisartan was accompanied by a significant rise in gastric mucosal nitric oxide (as nitrite/nitrate) with a concomitant fall in malondialdehyde concentrations as compared to the corresponding non-treated groups. Moreover, telmisartan significantly reduced free and total acid outputs in indomethacin-treated rats. On the other hand, telmisartan at the doses used did not alter gastric juice pH, peptic activity, mucin concentration or gastric mucosal prostaglandin $E_2$ content in both ulcer models. The telmisartan-treated rats exhibited greater protection from gastric ulceration than candesartan-treated animals. In conclusion, telmisartan, in a dose-dependent manner, protected rats' gastric mucosa from ulcerations possibly through its anti-oxidant action against oxidative stress induced by either indomethacin or CRS. Also, the greater gastroprotection afforded by telmisartan compared to candesartan could be partly ascribed to its PPARgamma-inducing property.

## 1. Introduction

Angiotensin II, a key peptide hormone in the renin-angiotensin-aldosterone system, induces oxidative stress and inflammation (Welch 2008) and constricts the gastric vasculature (Heinemann et al. 1999) through angiotensin II $T_1$ receptor stimulation. On the other hand, PPARgamma, a ligand-dependent nuclear receptor, has been reported to exert a variety of pleiotropic effects, including anti-oxidative and anti-inflammatory effects (Takano and Komuro 2009). Therefore, telmisartan that can concurrently block the angiotensin II $T_1$ receptor and activate PPARgamma (Benson et al. 2004) has the potential to be implicated in the protection against gastric ulcer. Alternatively, it has been reported that candesartan protected gastric mucosa from ulceration induced by either CRS (Bregonzio et al. 2003) or ischemia/reperfusion (Nakagiri et al. 2007) even as it was reported to have the lowest PPARgamma affinity among ARBs (Marshall et al. 2006). Consequently, telmisartan that has PPARgamma-activating properties is likely to be superior to one without this action. Then again, Sonnenberg (1988) reported that gastric ulcer coincided more frequently with cardiovascular diseases related to hypertension and both share a common etiologic factor, so, it may be beneficial to choose the optimal antihypertensive drug that will reduce such risk.

Indomethacin and CRS are frequently used and clinically relevant experimental models for induction of acute gastric ulcers. The choice of the indomethacin model was based on the fact that non-steroidal anti-inflammatory drugs are one of the most commonly used medicines throughout the world. However, they induce clinically significant ulceration in 17% of at-risk patients taking these drugs (Scheiman et al. 2006). On the other hand, the choice of CRS ulcer model was based on the fact that stress is a known inducer of acute gastric ulceration (Overmier and Murison 2000). Accordingly, the present study was designed to investigate the effects of telmisartan on gastric ulcers induced by indomethacin and CRS in rats. In addition, candesartan was used to support the promising role of PPARgamma agonistic activity of telmisartan against gastric ulceration. Gastric protection was evaluated by measuring the ulcer index, determination of gastric juice parameters (pH, free and total acid outputs, pepsin activity and mucin concentration), as well as determination of gastric mucosal concentrations of nitric oxide, malondialdehyde (as an indicator of lipid peroxidation), and prostaglandin $E_2$.

## 2. Investigations and results

### *2.1. Effects of various pretreatments on gastric ulcer score and juice analysis*

Ulcerative lesions were observed in both indomethacin- and CRS-treated rats. Pretreatment with telmisartan, in doses of 1, 3 and 10 mg/kg, significantly protected rats from gastric ulceration in both models. With the exception of the lowest dose of telmisartan (1 mg/kg) in indomethacin ulcer model, telmisartan was ineffective in altering gastric juice volume. On the other hand, in indomethacin-treated rats, the lowest dose of telmisartan (1 mg/kg) significantly increased gastric juice free and total acid outputs while the higher doses of telmisartan was accompanied by dose-dependent reduction in both parameters as compared to rats pretreated with the lowest dose. Nevertheless the highest dose of telmisartan (10 mg/kg) significantly reduced both parameters as compared to the indomethacin non-treated group. Moreover, in both ulcer models, candesartan significantly reduced gastric ulcer indices. In addition, in the indomethacin model, candesartan was not able to alter the volume of gastric secretion significantly, however, in CRS rats, candesartan significantly increased it, as compared to the corresponding non-treated groups. Only in indomethacin-treated rats, candesartan reduced free and total acid outputs. Ranitidine, in both models, significantly protected rats from gastric mucosal ulceration and significantly increased pH and mucin concentration with decreased free and total acid outputs (Fig. 1 and Table).

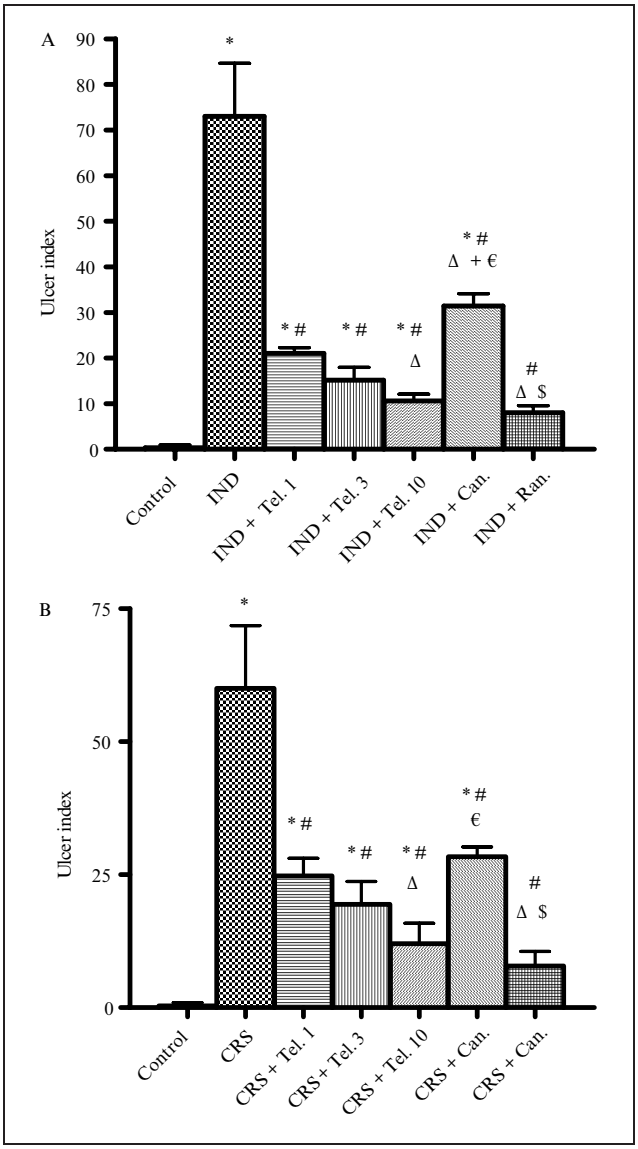


Fig. 1: Effects of telmisartan (Tel)-, candesartan (Can)-, and ranitidine (Ran)-pretreatment on ulcer index in (A) indomethacin (IND)- and (B) cold restraint stress (CRS)-induced gastric ulcer in rats. Values are means ± S.D. Tel. 1, 3, & 10 = 1, 3, & 10 mg/kg. * Significantly different from control group at $P < 0.05$; $^{\#,\Delta,+,€,\$}$ significantly different from the corresponding non-, telmisartan (1 mg)-, telmisartan (3 mg)-, telmisartan (10 mg)-, and candesartan-treated groups, respectively, at $P < 0.05$

### *2.2. Effects of various pretreatments on gastric mucosal parameters*

There are significant reductions in nitrite/nitrate and prostaglandin $E_2$ and increase in malondialdehyde concentrations with both models. Telmisartan, 3 and 10 mg/kg, significantly increased nitrite/nitrate and reduced malondialdehyde concentrations as compared to the corresponding non-treated groups, while prostaglandin $E_2$ was not altered by telmisartan in the three tested doses. Alternatively, in both models, candesartan significantly decreased malondialdehyde and prostaglandin $E_2$ concentrations, while it did not alter gastric mucosal nitrite/nitrate concentration as compared to the corresponding non-treated groups. In both models, ranitidine significantly increased nitrite/nitrate and prostaglandin $E_2$ concentrations but reduced gastric mucosal malondialdehyde level as compared to the corresponding non-treated groups (Fig. 2A, B, and C).

## 3. Discussion

Among ARBs, telmisartan is characterized by the property of stimulating PPARgamma. Thus, the combined angiotensin II $T_1$ receptor blockade and selective PPARgamma activation with telmisartan could provide greater protection from gastric ulceration than other members of this family, e.g. candesartan, that target angiotensin II $T_1$ receptor alone. In the present study, telmisartan dose-dependently reduced the ulcer index in both ulcer models. These results are supported by findings of Nakagiri et al. (2007) who reported that ischemia/reperfusion-induced gastric mucosal lesions in rats were attenuated by pretreatment with ARBs; losartan, candesartan, or valsartan. In addition, infusion of saralasin, a specific ARB, decreased the ulcer index in CRS rats (Ender et al. 1993). The results obtained from gastric acid secretion in indomethacin model could be attributable to telmisartan's dose-dependent 1) reduction in gastric juice volume and 2) increase in nitric oxide which is known to inhibit gastric acid secretion (Kato et al. 1998). On the other hand, in the CRS ulcer model, telmisartan did not alter gastric acid secretion may be due to the unchanged gastric juice volume.

The increase in gastric mucosal level of nitric oxide, an important mediator of gastrointestinal mucosal defense, observed with rising telmisartan dose is in consistence with previous studies which explained that increase may be as a result of activation of PPARgamma signaling (Scalera et al. 2008), or angiotensin II $T_1$ receptor blockade and angiotensin II $T_2$ receptor stimulation (Unger and Stoppelhaar 2007). On the other hand, in accordance to the current study, previous studies denoted similar findings concerning the ability of telmisartan to decrease lipid peroxidation, assessed by malondialdehyde level (Iqbal et al. 2008; Takaya et al. 2006). This inhibitory effect of telmi-

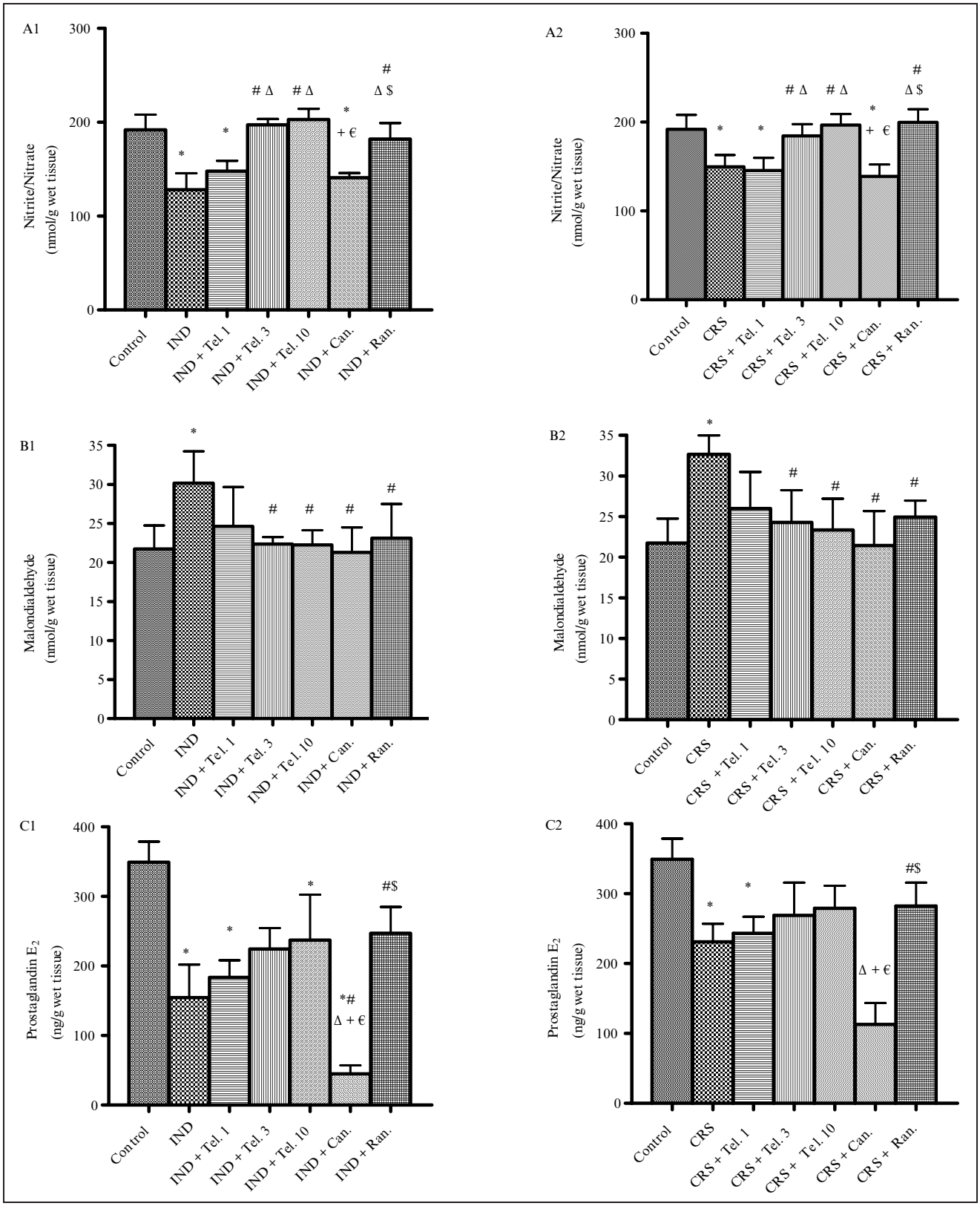


Fig. 2: Effects of telmisartan (Tel)-, candesartan (Can)-, and ranitidine (Ran)-pretreatment on gastric mucosal (A) nitrite/nitrate, (B) malondialdehyde, and (C) prostaglandin $E_2$ concentrations in (1) indomethacin (IND)- and (2) cold restraint stress (CRS)-induced gastric ulcer in rats. Values are means ± S.D. Tel. 1, 3, & 10 = 1, 3, & 10 mg/kg. * Significantly different from control group at $P < 0.05$; $^{\#,\Delta,+,€,\$}$ significantly different from the corresponding non-, telmisartan (1 mg)-, telmisartan (3 mg)-, telmisartan (10 mg)-, and candesartan-treated groups, respectively, at $P < 0.05$

sartan on lipid peroxidation was explained by decrease in superoxide production and increase in reduced glutathione content. Therefore, in this study, the well-recognized antioxidant property of nitric oxide (Martín et al. 2001) could partly be involved in the observed decrease in lipid peroxidation.

Prostaglandin $E_2$ is a well-established mediator in gastric mucosal defense and repair (Martin and Wallace 2006).

**Table: Effects of telmisartan (Tel)-, candesartan (Can)-, and ranitidine (Ran)-pretreatment on various gastric juice parameters in indomethacin (IND)- and cold restraint stress (CRS)-induced gastric ulcer in rats**

| Groups | Gastric juice | | | | | |
|---|---|---|---|---|---|---|
| | pH | volume (ml/3 h) | free acid output (μeq/3 h) | total acid output (μeq/3 h) | pepsin activity (μg/ml tyrosine) | mucin conc. (mg% hexose) |
| Control | 4.0 ± 0.2 | 0.8 ± 0.2 | 6.9 ± 1.1 | 12 ± 2.9 | 142 ± 36 | 93 ± 16 |
| IND | | | | | | |
| Non-treated | 3.1 ± 0.3[a] | 1.3 ± 0.3[a] | 40 ± 14[a] | 51 ± 14[a] | 214 ± 30[a] | 57 ± 9.8[a] |
| Tel (1 mg) | 3.0 ± 0.2[a] | 2.2 ± 0.2[ab] | 64 ± 20[ab] | 74 ± 24[ab] | 173 ± 21 | 62 ± 11[a] |
| Tel (3 mg) | 3.1 ± 0.4[a] | 1.1 ± 0.2[ac] | 34 ± 8.0[ac] | 45 ± 9.0[ac] | 201 ± 44 | 87 ± 22 |
| Tel (10 mg) | 3.4 ± 0.7 | 0.9 ± 0.1[c] | 13 ± 5.2[bcd] | 19 ± 4.9[bcd] | 160 ± 44 | 81 ± 33 |
| Can (2 mg) | 3.2 ± 0.4[a] | 1.0 ± 0.2[c] | 20 ± 7.3[bc] | 28 ± 9.5[bc] | 229 ± 22[ae] | 64 ± 19[a] |
| Ran (50 mg) | 4.9 ± 0.2[abcdef] | 0.7 ± 0.1[bcd] | 2.0 ± 0.9[bcd] | 7.2 ± 0.7[bcd] | 204 ± 20[a] | 108 ± 2[b] |
| CRS | | | | | | |
| Non-treated | 2.8 ± 0.2[a] | 0.7 ± 0.2 | 32 ± 2.1[a] | 43 ± 2.1[a] | 249 ± 84[a] | 67 ± 15[a] |
| Tel (1 mg) | 2.8 ± 0.9[a] | 1.2 ± 0.38 | 49 ± 3.1[a] | 64 ± 5.6[a] | 258 ± 42[a] | 66 ± 12[a] |
| Tel (3 mg) | 2.8 ± 0.3[a] | 1.2 ± 0.36[a] | 48 ± 6.7[a] | 60 ± 13[a] | 211 ± 32 | 65 ± 13[a] |
| Tel (10 mg) | 3.1 ± 0.3[a] | 1.0 ± 0.1 | 45 ± 13[a] | 57 ± 16[a] | 198 ± 26 | 74 ± 13 |
| Can (2 mg) | 2.8 ± 0.2[a] | 1.4 ± 0.4[ab] | 45 ± 8.8[a] | 62 ± 14[a] | 224 ± 37 | 74 ± 15 |
| Ran (50 mg) | 4.3 ± 0.7[acdef] | 0.8 ± 0.2[f] | 17 ± 1.0[bcdef] | 22 ± 1.4[bcdef] | 212 ± 45 | 94 ± 22[b] |

Values are presented as means ± S.D.; n = 6 rats. conc. = concentration. All doses are per kg. [a] Significantly different from control group at $P < 0.05$; [b,c,d,e,f] significantly different from the corresponding non-, telmisartan (1 mg)-, telmisartan (3 mg)-, telmisartan (10 mg)-, and candesartan-treated groups, respectively, at $P < 0.05$

Previous studies reported reduction in angiotensin II-induced prostaglandin $E_2$ generation by ARBs (Beltrán et al. 2009; Cipollone et al. 2006). However, telmisartan at the used doses, in both ulcer models, did not alter gastric mucosal prostaglandin $E_2$ level. It seems that the angiotensin II $T_1$ receptor antagonistic activity of telmisartan which possibly tends to reduce prostaglandin $E_2$ level has antagonized the presumed nitric oxide-induced prostaglandin $E_2$ elevation, as there is a considerable correlation between nitric oxide and prostaglandins, including prostaglandin $E_2$, biosynthetic pathways (Mollace et al. 2005), and the net result was the trivial alteration in the prostaglandin $E_2$ level.

The current study included candesartan to address the effectiveness of angiotensin II $T_1$ receptor antagonists into perspective and leave a space for exploring PPARgamma agonism of telmisartan and its role in gastroprotection. Telmisartan provided better gastroprotection than candesartan; its highest dose produced comparable effects to that of ranitidine. The results showed that telmisartan, in all tested doses, significantly reduced gastric lesions as compared to candesartan. The likely mechanism for this greater protection could be attributed to its PPARgamma-mediated action as telmisartan was reported to have the strongest PPARgamma affinity among ARBs (Marshall et al. 2006). The mechanisms underlying telmisartan's gastroprotective effect involves increase in nitric oxide level and reduction of lipid peroxidation aided by resistance to reduction in prostaglandin $E_2$. The differences in gastroprotection between telmisartan and candesartan could be relevant when choosing a therapy for hypertensive patients at high risk of developing gastric ulceration, but, the applicability of these data to the clinical situations has to be verified.

## 4. Experimental

### *4.1. Animals*

Male Wistar rats weighing 180–200 g were used after acclimatization for a period of 1 week to animal house conditions and had free access to food and water. The experiments were conducted according to the ethical standards approved by Institutional Animal Ethics Committee guidelines for animal care and use.

### *4.2. Chemicals*

Telmisartan and indomethacin were purchased from Sigma Chemical Co., USA. Candesartan and ranitidine were kind gifts from AstraZeneca, and GlaxoSmithKline, respectively, Egypt. All other chemicals were of analytical grade and were obtained from commercial sources.

### *4.3. Induction of gastric ulceration*

Rats were deprived of food for 24 h prior to the experiment in mesh-bottomed cages to minimize coprophagia but allowed free access to water except for the last hour before the experiment. Pyloric ligation was carried out to enable collection of the gastric juice. Immediately after pyloric ligation, acute gastric ulcers were induced either by i.p. administration of indomethacin (30 mg $kg^{-1}$, suspended in 1% Tween 80) (Khattab et al. 2001) or rats were restrained, and maintained at 4 °C for 2 h (Senay and Levine 1967). All experiments were performed during the same time of the day to avoid diurnal variations of putative regulators of gastric functions.

### *4.4. Experimental procedures*

Animals were divided into three main groups; the first group contained control non-ulcer rats, the second group contained indomethacin-induced ulcer rats, and the third group contained CRS-induced ulcer rats. The second and third groups were further subdivided into six subgroups; non-, telmisartan (1 mg/kg)-, telmisartan (3 mg/kg)-, telmisartan (10 mg/kg)-, candesartan (2 mg/kg)-, and ranitidine (50 mg/kg)-treated, in a single oral dose 1 h before ulcers' induction, subgroups. The animals were killed 3 h after indomethacin administration or 2 h after CRS and their gastric juice was collected, and gastric mucosa was scrapped for gastric injury evaluation after assessment of the gastric ulcer index.

### *4.5. Assessment of gastric mucosal lesions*

Gastric mucosal lesions were expressed in terms of the ulcer index according to the method of Till et al. (1988).

### *4.6. Analysis of gastric juice*

The gastric juice was drained, centrifuged at 600 × g and the volume of the supernatant was measured. The supernatant was then assayed for the pH (Moore 1968), pepsin activity (Sanyal et al. 1971), and mucin concentration (Winzler 1955). Free and total acid outputs, a well-established method of studying gastric acid secretion, were calculated (Feldman 1998) by multiplying gastric juice volume by the measured (Hara et al. 1991) free and total acid concentrations, respectively.

### *4.7. Biochemical analysis of gastric mucosa*

Nitric oxide content was determined as total nitrite/nitrate, the stable degradation products of nitric oxide (Sastry et al. 2002). Malondialdehyde level was measured by the method of Buege and Aust (1978). Prostaglandin $E_2$ assay was performed with prostaglandin $E_2$ enzyme immunoassay kit (R&D Systems, Inc., MN, USA) according to supplier's instructions.

### *4.8. Statistical analysis*

The data are expressed as means ± S.D. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey-Kramar post analysis test for multiple comparisons with $P < 0.05$ being considered as statistically significant.

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