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**RESEARCH ARTICLE**

# EFFECT OF MELATONIN IN OXIDANT-INDUCED GASTRIC MUCOSAL LESIONS IN RAT

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**ABSTRACT**

**Background:** Oxidative stress is observed in gastric ulceras it contributes to the mucosal injury. Melatonin (Mel) has protective functions of the gastric mucosa. Theaim of this study is to examine the protective effect of Melon gastric mucosa in acute lesions induced by hydrogen peroxide ($H_2O_2$) induced gastric lesions in rat model of ethanol-induced gastric ulcer.

**Methodology:** This study was performed in male Sprague-Dawley rats (200–250 g) in 9 groups of equal size (n = 45): Intra-peritoneal (i.p.) injection of Mel (12.5, 25 and 50 mg/Kg) 30 min before $H_2O_2$treatment. Gastric ulcers were scored and ulcer index was calculated. Glutathione (GSH) and glutathione peroxidase (GSH-px) activity was measured using spectrophotometry. Gastric thiobarbituric acid reactive substance (TBARS) as an index of lipid peroxidation (LPO) was measured.

**Results:** Gastric mucosal level of reduced glutathione (GSH) and glutathione peroxidase (GSH-px) activity were significantlydecreased by $H_2O_2$ administration, while melatonin pretreatment significantly increased both TBARS was increased after $H_2O_2$ administration and this increase was inhibited by melatonin. Melatonin was more protective, when compared with ranitidine and omeprazole. Mel gastro-protective effect was reduced by indomethacin and completely blocked by diethylmaleate.

**Conclusions:** the results suggest that melatonin protects rat gastric mucosa against $H_2O_2$-induced damage possibly by scavenging reactive oxygen species via increases of GSH level, GSH-px activity and reduction of lipid peroxidation in the gastric mucosa. This suggests that Melmayprotect against alcohol induced peptic ulcer.



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## INTRODUCTION

Reactive oxygen species (ROS), e.g. superoxide anion ($O_2^{\cdot-}$), and hydrogen peroxide ($H_2O_2$) are physiologically produced in cells as metabolic by-products (Liochev *et al*., 1994; Landrick *et al*., 1997). Oxidative stress expressed as elevated level of ROS, is observed in acute physiological and pathological states that include but not limited to, sepsis, exposure to radiation, ischemia and, gastric ulcers. Excessive production of ROS due to acute and chronic inflammation of the gastrointestinal tract may contribute to the mucosal injury (Borg, 1993). Gastric (peptic) ulcer is a common disease that may result ofseveral etiologies. It is defined as a discontinuity in the gastric mucosa and penetration of the muscularis mucosa (Yeomans and Naesdal, 2008). Gastric ulceroftenresultsfroman imbalance between gastriclumen irritant factors (physical, chemical or psychological) and protective mechanisms in the duodenal mucosa i.e. mucus and bicarbonate secretions, as well as by prostaglandins, and dopamine (de Souza Almeida *et al*., 2011). This imbalance predisposes to chronic inflammation and disturbancein gastrin production (Dockray *et al*., 2001). The other known cause of ulcerative lesions in the gastro-duodenal mucosa is the hemorrhagic injury produced by exogenous compounds, mainly acetylsalicylic acid, non-steroidal anti-inflammatory drugs and high levels of ethanol. These compounds are known to affect the protective defense mechanisms of the mucus, leading to the formation of gastric mucosal lesions (Schurer-Maly *et al*., 1990; Laudanno *et al*., 1990). Acute administration of $H_2O_2$in rats produces gastric mucosal lesions and erosions similar to those occurring in gastric ulcer (reference needed). It is well known that $H_2O_2$ can be metabolized to the hydroxyl radical, which is one of the most potentially damaging free radicals (Bagchi *et al*., 1990). The pineal hormone, melatonin, is uniquely defined by its role as timehormone or the chemical expression of nighttime (Waly and Hallworth, 2015; Reiter, 1991; Hazlerigg, 2001). Ianas *et al*. (1991) first suggested that melatonin acted as a free radical scavenger and antioxidant. Melatonin is an efficient antioxidant and free radical scavenger. It differs from other classic antioxidants in that its production is inducible under moderate

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oxidative stress. This special character of melatonin makes it anideal endogenous antioxidant to protect the body from stressful conditions (Tan *et al*., 2015). The gastrointestinal tract (GIT) is a major source of extra pineal melatonin (Sjoblom *et al*., 2001). Melatonin is probably synthesized in the enterochromaffin cells of the gastric mucosal lining (Reiter *et al*., 2003). In some animals, tissue concentrations of melatonin in the GIT exceed blood levels by 10-100 times. The digestive tract contributes significantly to melatonin concentrations in the peripheral blood, particularly during the day (Motilva *et al*., 2001; Bubenik, 2001). Melatonin seems to have a protective functions of the gastric mucosa and to also may regulate gastrointestinal motility (Reiter *et al*., 2003). In this study weinvestigated the protective effect of melatonin in $H_2O_2$-induced gastric lesions in rats. We hypothesized that melatoninexerts its gastro-protective effects viamodulation of gastric mucosal glutathione level, and GSH-px activity.

## MATERIALS AND METHODS

### Chemicals

Hydrogen peroxide was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Melatonin was purchased from Sigma (St. Louis, MO, USA). Ranitidine and omeprazole were a kind gift from GlaxoWellcome (Cairo, Egypt), and EIPICO (10 of Ramadan Egypt). Carboxymethyl cellulose (CMC). Glutathione, 5,5'-dithio-bis-(2-nitrobenzoic acid), L-cysteine, diethyl maleate (DEM), thiobarbituric acid (TBA), 1,1,3,3-tetraethoxypropane, were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

### Animals

Forty five Male Sprague-Dawley rats, weighing 200-250 g were used in this study. Rats were housed in wire-floored cages in a 12-h light/dark cycle for at least 5 days prior to treatment and fed standard laboratory chow (El-Nasr Co., Abu-Zaabal, Egypt) and tap water ad libitum. Room temperature was kept at 23 ± 2 $^0$C. Rats were deprived of food but allowed free access to water for 24 hours before the experiment-gastric hemorrhagic damage was induced by oral administrations of 10-30 % $H_2O_2$for 10 days[19]. In control groups, rats received an equivalent volume of respective administrative vehicles.

### Experimental protocol

Rats were randomly divided into the following groups:

Group I (control), treated with vehicle (saline, 0.3 ml).
Group II, treated with $H_2O_2$ (10 %, intra-gastric gavage daily for 10 days), melatonin (50 mg/kg) and indomethacin (5 mg/kg).
Group III, treated with $H_2O_2$ (20 %, intra-gastric gavage, through an oral gastric tube daily for 10 days), melatonin (50 mg/kg) and diethyl maleate (1ml/kg).
Group IV, treated with $H_2O_2$ (30 %, intra-gastric gavage daily for 10 days).
Group V, treated with $H_2O_2$ (30 %) and melatonin (12.5 mg/kg).
Group VI, treated with $H_2O_2$ (30 %) and melatonin (25 mg/kg).
Group VII, treated with $H_2O_2$ (30 %) and melatonin (50 mg/kg).
Group VIII, treated with $H_2O_2$ (30 %) and ranitidine (100 mg/kg).
Group IX, treated with $H_2O_2$ (30 %) and omeprazole (100 mg/kg).
Group II, treated with $H_2O_2$ (30 %), melatonin (50 mg/kg) and indomethacin (5 mg/kg).
Group III, treated with $H_2O_2$ (30 %), melatonin (50 mg/kg) and diethyl maleate (1ml/kg).

All test agents were dissolved in saline. Melatonin was dissolved in ethanol before being diluted with saline. The final concentration of ethanol in the melatonin solution was <1%. Ranitidine and omeprazole were dissolved in 0.5 % carboxymethyl cellulose. Melatonin was administered intraperitonealy (i.p.) 30 min before $H_2O_2$ instillation. Ranitidine or omeprazole was administered subcutaneously (s.c.) 60 min before $H_2O_2$ instillation. Indomethacin or diethyl maleate was administered 30 min before $H_2O_2$ instillation. At the end of any experiment, overnight fasting was done, and in the morning, animals were sacrificed by dislocation of the cervical vertebrate and the stomach was excised. Gastric contents were collected for estimation of pH, volume and total acidity. Stomachs were opened along the greater curvature and examined for gastric lesions. Gastric ulcers were scored and ulcer index was calculated by the method of Dasgupta *et al*. [20].

### Ulcer index

After removing the stomach under light ether anesthesia, it was washed with cold normal saline and mucosa was exposed by cutting along the greater curvature. The stomach was laid flat on a Petri dish inverted over ice and examined for the severity of gastric lesions with help of a magnifying glass. The grade of gastric lesions was scored according to the following scale: 0 = no pathology; 1 = a small ulcer (1 to 2 mm); 2 = a medium ulcer (3 to 4 mm); 4 = a large ulcer (5 to 6 mm); 8 = a larger ulcer (> 6 mm) (Dasgupta *et al*., 1969). The sum of the total severity scores in each group of rats divided by the number of animals was expressed as the mean ulcer index.

### Assay of lipid peroxidation (LPO)

LPO was determined by measuring malondialdehyde (MDA) content in tissue homogenate according to Mihara and Uchiyama (Mihara and Uchiyama, 1978). Protein was quantified by the Lowry protein assay using bovine serum albumin as standard (Lowry *et al*., 1951).

### Assay of GSH level and GSH-px activity

GSH level was determined according to the method of Adams *et al*. (1983) and expressed as nmol/mg protein. GSH-px activity was spectrophotometrically measured by the method of Paglia and Valentaine (1967) and expressed as units (U)/mg protein.

### Statistical Analysis

The GRAPHPAD (ISI Software, Philadelphia, PA, USA) computer program was used to conduct regression analysis and to plot collected data. Data were expressed as means ± SEM. Assessment of the results was performed using one-way ANOVA procedure followed by Tukey-Kramer multiple comparison tests using Software GRAPHPAD INSTAT

(Version 2). The 0.05 level of probability was used as the criterion for significance.

## RESULTS

$H_2O_2$ showed dose-dependent induction of gastric ulcer compared to control (Fig. 1a). Intra-gastric administrations of 30 % $H_2O_2$ induced hemorrhagic lesions in gastric fundus. On gross examination these lesions were characterized by multiple hemorrhagic red bands of different sizes along with longitudinal axis of the glandular stomach. Pretreated animals with melatonin at 12.5-50 mg/kg prevented the gastric lesions in a dose-dependent manner, when given 30 min before $H_2O_2$ (30%) administration. The protective action of melatonin was significant at all the doses used (12.5–50 mg/kg) (Fig. 1b).

Additionally $H_2O_2$ intoxication induced a significant decrease of gastric GSH level and GSH-px activityin a concentration-dependent manner. Melatonin at 12.5-50 mg/kg, 30 min prior to $H_2O_2$ instillation inhibitedthe decrease of gastric GSH level and GSH-px activity, dose dependently (Table 1). Measuring the effects of various concentrations of $H_2O_2$ (10-30 %) on lipid peroxidation indicated by TBARS generation revealed a concentration-dependent increase of TBARS production in gastric tissues. Pretreatment by melatonin at 12.5 and 50 mg/kg prior to $H_2O_2$ instillation induced significant decreased in the TBARS production in a dose-dependent manner (Table 1). Consequently 30% $H_2O_2$ and melatonin (50mg/kg) were selected as the concentrations of choice and used throughout the remainder of this study. Effect of antacid drugs on hemorrhagic mucosal lesions-induced by intra-gastric

**Table 1. Effects of intra-gastric administration of different concentrations of $H_2O_2$ and the protective effect of altered doses of melatonin on total GSH, GSH-px activity, and lipid peroxidation levels in rat stomach**

| Groups | Lipid peroxidation (nmol/mg protein) | GSH-px activity (U/mg protein) | GSH (nmol/mg protein) |
|---|---|---|---|
| Control (saline) | 2.9± 0.02 | 0.501± 0.001 | 50.1±0.09 |
| $H_2O_2$ (10 %) | 5.6 ± 0.1 * | 0.412 ± 0.003 * | 40.2 ± 2.4 * |
| $H_2O_2$ (20 %) | 7.2 ± 0.2 * | 0.343 ± 0.002 * | 30.9 ± 1.7 * |
| $H_2O_2$ (30 %) | 8.9± 0.4* | 0.299± 0.001 * | 19.9± 1.4* |
| $H_2O_2$ (30 %) + 12.5 mg/kg MT | 5.6± 0.4*,# | 0.380± 0.003 *,# | 33.1± 1.1 *,# |
| $H_2O_2$ (30 %) + 25 mg/kg MT | 4.7± 0.2*,# | 0.399± 0.002 *,# | 39.7± 1.4 *,# |
| $H_2O_2$ (30 %) + 50 mg/kg MT | 3.4± 0.1# | 0.462± 0.001 # | 45.1± 2.3 # |

Data expressed as mean ± SEM of six determinations.
GSH-px: glutathione peroxidase; MT: melatonin
Melatonin (12.5-50 mg/kg) were injected i.p. 60 min before the $H_2O_2$ instillation.
*: significantly different from control group at $p < 0.05$.
#: significantly different from $H_2O_2$ (30 %)-intoxicated animals at $p < 0.05$.

**Table 2. Effect of melatonin and antacid drugs on volume, pH and total acidity of gastric juice in rats intoxicated with $H_2O_2$ (30 %)**

| Groups | Total acidity(µEq/h) | Volume (ml) | pH |
|---|---|---|---|
| Control (saline) | 124± 11 | 6.9± 0.31 | 2.22± 0.09 |
| $H_2O_2$ (30 %) | 121± 13 | 6.2± 0.24 | 2.13± 0.18 |
| $H_2O_2$ + melatonin | 126± 14 | 7.3± 0.22 | 3.83± 0.21*,# |
| $H_2O_2$ + ranitidine | 24±3*,# | 5.5± 0.12*,# | 5.26± 0.17*,# |
| $H_2O_2$ + omeprazole | 19±7*,# | 4.9± 0.21*,# | 5.08± 0.19*,# |

Data expressed as mean ± SEM of six determinations.
Ranitidine or omeprazole (100 mg/kg) were injected i.p. 60 min before the $H_2O_2$ instillation.
*: significantly different from control group at $p < 0.05$.
#: significantly different from $H_2O_2$ (30 %)-intoxicated animals at $p < 0.05$.

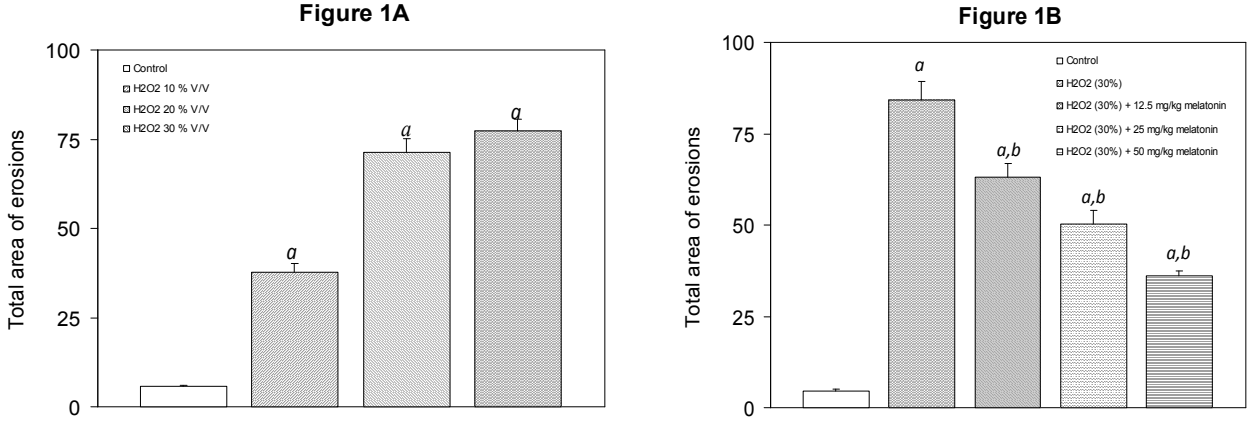


**Figure 1. Effect of various concentrations of $H_2O_2$ (A) and melatonin (B) on gastric mucosal lesions in rat stomach**

Each value is expressed as mean ± SEM of six determination. Rats were received a different concentrations of $H_2O_2$ (10, 20 and 30 %) by gavage (1 ml/100 g) daily for consecutive 10 days. Rats were treated with various concentrations of melatonin (12.5, 25, 50 mg/kg i.p) 30 min prior to $H_2O_2$ administration.
a: significantly different from control group at $p < 0.05$. b: significantly different from $H_2O_2$ intoxicated animals at $p < 0.05$.

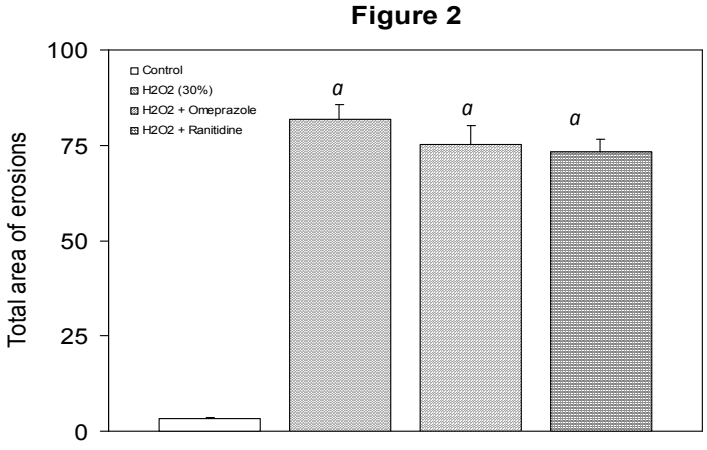


**Figure 2. Effect of ranitidine and omeprazole on gastric mucosal lesions induced by intra-gastric administration of $H_2O_2$**

Each value is expressed as mean ± SEM of six determination. Ranitidine and omeprazole were given 60 minutes before $H_2O_2$ administration, at 100 mg/kg i.p. a: significantly different from control group at $p < 0.05$. b: significantly different from $H_2O_2$ intoxicated animals at $p < 0.05$.

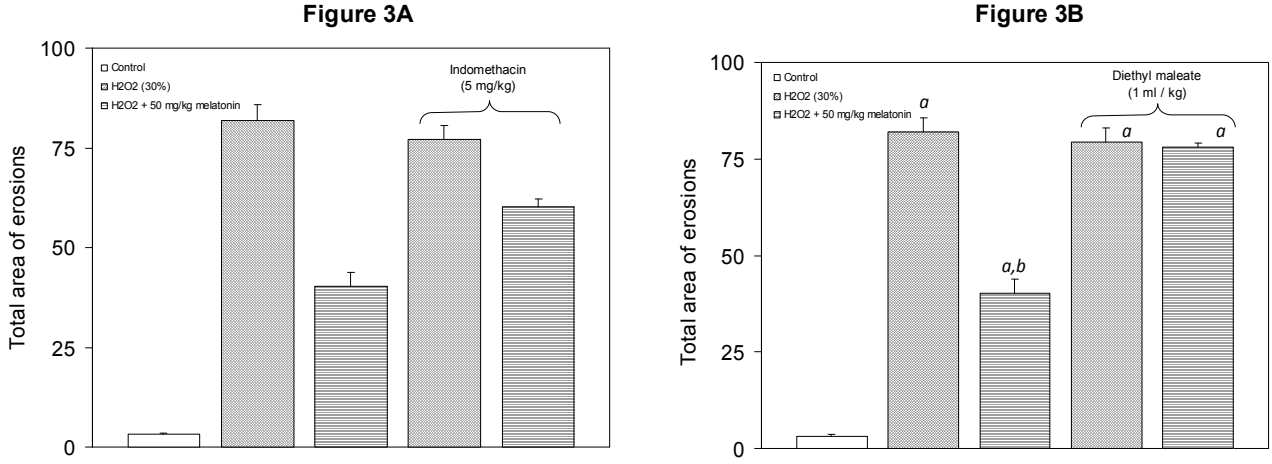


**Figure 3. Effect of pretreatment with indomethacin (A) and diethyl maleate (B) on the protective effect of melatonin against $H_2O_2$-induced gastric mucosal lesions**

Each value is expressed as mean ± SEM of six determination. Indomethacin (5 mg/kg) and diethyl maleate (1 ml/kg) were given s.c. 30 min prior to $H_2O_2$ instillation. a: significantly different from control group at $p < 0.05$. b: significantly different from $H_2O_2$ intoxicated animals at $p < 0.05$.

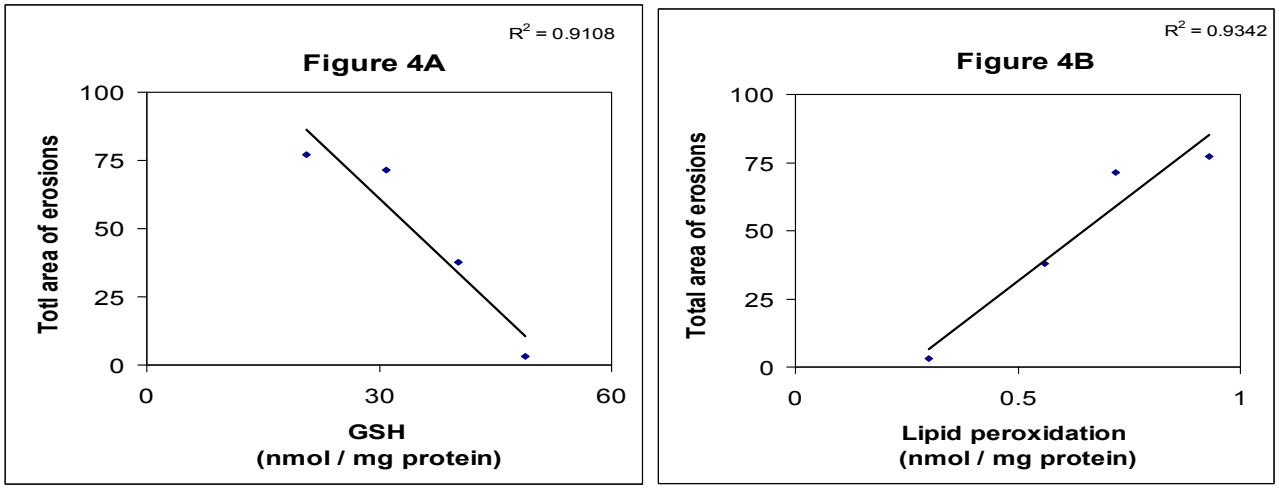


**Figure 4. The linearity of the correlation between gastric lesions induced by different concentration of $H_2O_2$, and total GSH (A) or lipid peroxidation (B)**

Gastric lesions were reflected by total areas of erosions, as described in materials and methods.

administration of 30 % $H_2O_2$ in the rat stomach, was investigated by injection of ranitidine and omeprazole intraperitonealy 60 min before the induction of the damage. Both drugs did not prevent $H_2O_2$-induced lesion formation (Fig. 2). Table 2 showed the volume, pH and total acidity of the gastric content of the animals belonging to the control, $H_2O_2$ (30%), melatonin (50 mg/kg), and antacids treated groups. The volume was decreased significantly with antacid treatment, while the pH value was increased significantly with melatonin and antacid treatment groups. Ranitidine or omeprazole (antacids) significantly reduced the total acidity from 126 ± 11 to 23 ± 4 and 21 ± 5 μEq/h respectively, when compared to the control group. Hemorrhagic mucosal lesions induced by 30% $H_2O_2$ were not aggravated by pretreatment with indomethacin (5 mg/kg, s.c.) or diethyl maleate (1 ml/kg, s.c.), 30 min before $H_2O_2$ instillation (Fig.3a and Fig. 3b), The protective effect of melatonin was partially reversed by pretreatment with indomethacin, while it was almost completely inhibited by diethyl maleate (Fig. 3a and Fig.3b). The linearity of the relationship between ulcer index and GSH or lipid peroxidation was evaluated. The correlation coefficients (r value) for both linearity curves were 0.91 and 0.93 respectively (Fig. 4a and Fig. 4b).

## DISCUSSION

We investigated the gastro-protective activity of Melatonin in experimental oxidative stress rat model of gastric injury as well as its mechanism of action. Our results show that intra-gastric administration of 30 % $H_2O_2$ induced gastric mucosal hemorrhagic lesions in rats while pretreatment with Mel dose dependently inhibited this effect. In addition pretreatment with Mel at 12.5-50 mg/kg inhibited the $H_2O_2$ induced decrease of gastric GSH level and GSH-px activity, dose dependently. These results suggest that the gastro-protective effects of melatonin may be attributed to its antioxidant function. Our study also showed that melatonin administration at 12.5-50 mg/Kg, i.p. reduced acute gastric mucosal lesion formations after $H_2O_2$ exposure. Our findings are in line with those of Kato *et al*. (2001) who showed that administration of melatonin significantly inhibit the induction of acute gastric mucosal lesions by stress or ischemia-reperfusion in rats. Dasgupta *et al*., 1969 reported that melatonin has a potent activity to scavenge the hydroxyl radical, which is thought to be one of the most toxic free radicals. These results suggest that hydroxyl radical generated from $H_2O_2$ via Fenton reaction in the gastric mucosa might be related to these lesions and that melatonin scavenging of hydroxyl radical may play some roles in its protective effect. Mel antioxidant protective effect observed hereby is similar to its gastro-protective effect observed in ethanol and aspirin (Sener-Muratoglu *et al*., 2001; Ko and Cho, 1995; El-Sokkary *et al*., 1999; Antunes *et al*., 1999) Since our results suggest that $H_2O_2$ is capable of inducing oxidative stress as indicated by gastric GSH depletion and decreased in the GSH-px activity (Table 1) we tested the hypothesis that Mel gastro-protective effect is via modulation of GSH / GSH-px system. Indeed, Mel reversed the negative effects of $H_2O_2$ on GSH / GSH-px system significantly (Table 1). Among the endogenous detoxifying systems, GSH-px plays a critical role in protection against oxidants and products of oxidative stress. Previous studies showed that intracellular GSH was mainly responsible for protecting against gastric mucosal injury induced by ethanol (Mutoh *et al*., 1990). At high concentrations of $H_2O_2$ (30 %), the levels of GSH decreased and the activity of GSH-px decreased indicating the presence of an oxidative insult. This was in expected considering the known role of GSH in intracellular metabolism and its protective role in gastric tissues particularly when challenged with chemicals (Hirota *et al*., 1989). In addition, the gastric tissues are highly dependent on GSH that is required for their normal function, where inhibiting the synthesis of GSH leads to marked cellular degradation (Martensson *et al*., 1990). Oxygenation of proteins by $H_2O_2$ and $H_2O_2$-metabolites might be related to the decrease of GSH-px (Cho *et al*., 1991). Furthermore, the development of gastric mucosal injury may occur when the generation of ROS exceeds the ability of free radical scavenging enzyme glutathione peroxidase (a scavenging enzyme of $H_2O_2$) to handle these radicals.

The present study results also show that the increase in TBARS (index of LPO) in the gastric mucosa 30 min after $H_2O_2$ instillation was significantly inhibited by pretreatment with melatonin (12.5-50 mg/kg). Melatonin significantly reduces the lipo-peroxidation levels (Saad *et al*., 2013) and excessive LPO can cause increased GSH consumption (Younes and Siegers, 1981). Together with our above-mentioned data of the decrease in gastric mucosal GSH these results suggest that Mel exerts its gastro-protective effect via enhancement of GSH/GSH-px and inhibition of LPO. Pretreatment with diethyl maleate (DEM), which is a depressor of GSH, completely blocked the protective effect of melatonin. This result confirms that Mel exerts its gastro-protective via GSH modulation. Mel gastro-protective effects were diminished with the pretreatment of non-steroidal anti-inflammatory drug, indomethacin. This data suggests that the elevation of gastric prostaglandins might explain the protective effect of melatonin. It was previously reported that i.p. administration of melatonin stimulated prostaglandin synthesis in the rat stomach (Mihara and Uchiyama, 1978). Furthermore, 16, 16-dimethyl $PGE_2$ was previously reported to protect gastric mucosa against $H_2O_2$-induced injury (Dockray *et al*., 2001). On the other hand, ranitidine and omeprazole were not able to protect the gastric mucosa from $H_2O_2$-induced lesions (Fig. 2). The selected antacid drugs were able to significantly decrease the total acidity of the rat gastric content, when compared to the control group (Table 2). There are conflicting results in the literature regarding the antacid drugs in necrotizing agent-induced gastric mucosal damage and protection. Tarnawski *et al*. (1985) reported that neither ranitidine nor cimetidine was able to protect the gastric mucosa from ethanol-induced lesions. On the contrary, Sener-Muratoglu *et al*. (2001) reported that both omeprazole and famotidine have a protective effect against gastric mucosal damage induced by acetylsalicylic acid and other non-steroidal anti-inflammatory drugs. These findings suggest that inhibition of acid secretion cannot protect the gastric mucosa from $H_2O_2$-induced lesions.

### Conclusion

In conclusion, our data suggest that melatonin is a putative gastro-protective agent in gastric ulcer. Melatonin possibly exerts its gastro-protective by reducing the oxidative stress in gastric mucosa via enhancement of GSH/GSH-px and inhibition of LPO in addition to stimulation of prostaglandin secretion. Further studies are required to evaluate its possible clinical use in the management of gastric ulcer.

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**Conflict of interest**

There is no conflict of interest.

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