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What is the most effective antioxidant against ulcers? An experimental study

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Abstract

Background: *The aim of this study was to evaluate the effects of selenium, gingko biloba, melatonin and vitamin E, which are antioxidants in the prevention of ulcerative lesions occurring in rats exposed to immobility and cold stress by using comparative histochemical and statistical methods.*

Material and Methods: *6 groups were created with 42 Wistar albino rats; control, ulcer (performed by immobilization and cold stress), selenium (0.5 ml/kg), Gingko biloba (300 mg/kg), melatonin (60 mg/kg), vitamin E (60 mg/kg). Gastric tissues were taken and examined by histochemical methods. Statistical methods were used to determined gastric pit and glands leight.*

Results: *In the ulcer group; expansive degenerative changes was seen in gastric mucosa and lots of different types necrotic areas were seen. In the vitamin E administered group, similar findings with the control group were observed except the superficial mucosal epithelium spilth in the located area and moderate dilatation in gastric glands. With the alcian blue dying to observe the mucus compared with other groups, the most evident increase in mucus was found in this group.*

Conclusions: *In the histochemical and statistical evaluations we considered that prevention of the formation the gastric lesions in case of stress, vitamin E was the most effective agent.*

Keywords: *Gastric ulcer, Gingko biloba, selenium, melatonin, vitamin E, rat.*

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Introduction

The stomach is one of the most affected organs by stress, and various biological and psychosocial conditions have been reported to increase the formation of ulcer[1]. Experimental animal studies have demonstrated that physiological stress rapidly decreases the intra-mucosal blood flow resulting in mucosal ischemia[2], and the direct relationship between the severity of ischemia, mucosal ATP destruction and the severity of mucosal injury[3]. The formation of ulcers in the gastrointestinal system occurs due to the interaction with non-steroidal anti-inflammatory drugs such as indomethacin and aspirin, ethanol, hydrochloric acid, and due to the effects of stress, free radicals, bile acids, and intrinsic factors such as protease[4]. Apart from these, many stress models such as exercise, fasting, cold and immobility increase the formation of free radicals and these radicals induce lipid peroxidation[5].

In rats, cold and immobility stress cause ulcerations in the gastric mucosa, and several pathological factors such as the increase in gastric secretion, inhibition of gastric mucosal prostaglandin synthesis, decrease in the mucosal blood flow and damage of the gastric mucosal barrier play roles in the formation of these ulcerative lesions[6].

Several studies have reported the effects of free radicals in the etiology of gastric ulcer. The effect of antioxidants that prevent the formation of free radicals or removing these free radicals have been presented in stress independent and stress dependent gastric ulcer models, in addition to various stress models[7,8,9]. The exact changes during the formation of ulcers have not yet been clarified; however, various hypotheses have been put forth from time to time. Increased motility, severe vagal activity[10], degranulation of mast cells, decrease of mucosal blood flow and decreased prostaglandin levels are thought to play roles in ulcer formation as a result of stress[6]. In stress induced mucosal damage, it is generally accepted that gastric ulcers are formed due to the imbalance of progressive and regressive mucosal factors.

Based on these data, the aim of this study was to evaluate the effects of selenium, *Gingko biloba* (Gbe), melatonin and vitamin E, which are antioxidants in the prevention of ulcerative lesions occurring in rats exposed to immobility and cold stress by using comparative histochemical and statistical methods.

Materials And Methods

Animals and Experimental Protocols

In this study, 42 Wistar rats weighing between 250 gr and 300 gr were used. The rats were kept at 21°C to 23°C and under 12 hour light:12 hour dark lighting schedule in the Laboratory Animal Research Center. This study was approved by the Animal Experimentation Ethics Committee of University of Dicle (18.06.2009/16). After 7 days of acclimation to the environment, the rats were divided into six groups. The experimental animals were fasted for 24 hours before the procedure except the control group and they were allowed access to drinking water only. Metal mesh was placed under the cages for the test animals to avoid caprophagy. For inactivity and cold stress, modified shape of the model which was used by Güneli E, et al[11] was implemented.

Any operation is not applied to the rats in control group. In the ulcer group, before the occurrence of stress ulcer, 0.5 ml / 100 gr of saline was administered through the gastric catheter. One hour later, the rats were placed in sacs made of dense gap wires. Immobilization was provided and they were kept at room temperature for 16 hours. After exposure to +4 °C for 4 hours rats were sacrificed under anesthesia and the gastric tissues were excised (11). 60 mg/kg melatonin was administrated intraperitoneally instead of saline in the melatonin and ulcer group, 300 mg/kg *Ginkgo biloba* was administered through the gastric catheter in the *Ginkgo biloba* and ulcer group instead of saline, 0.46 mg/ml selenium was administered through the gastric catheter in the selenium and ulcer group instead of saline and 60 mg/kg was administered through the gastric catheter in the vitamin E and ulcer group. All other applications were the same as ulcer group. After sacrification, stomachs were removed completely by made the abdominal incision.

Histochemical Method

The obtained gastric samples were processed by routine light microscopic methods and paraffin blocks were prepared. From these paraffin blocks, 4 μm thickness cross sections were obtained using the microtome (Leica RM 2265). For evaluation of the microscopic changes, these sections were stained with hematoxylene-eosin & Masson trichrome, and for observation of the mucus accumulation in the glands, they were specifically stained with alcian blue (12). The obtained preparations were then evaluated with the light microscope (Olympus BH2). The photolight microscope (Leica DM 4000) and QWin 3 program were used for the measurements.

Statistical Method

The results were expressed as the number of observations (n), mean ± standard deviation, median and min-max values. The results of the homogenity (Levene's Test) and normality test (Shapiro Wilk) were used to decide the statistical methods to compare the study groups. Normally distributed and with homogeneous variances groups were compared two groups by Student's t test and compared three or more groups by Analysis of Variance. According to those tests results parametric test assumptions were not available for some variables, so the comparisons of two independent groups were performed by Mann-Whitney U test and three independent groups were performed by Kruskal Wallis test. Multiple comparison tests, the adjusted Bonferroni test was used. All statistical analyses were performed with the SPSS software (SPSS Ver. 17.0; SPSS Inc., Chicago IL, USA). P value of < 0.05 was considered statistically significant.

Results

Histochemical Results

In the rat gastric sections of the control group, all the layers were observed to have normal histological structure (Fig. 1).

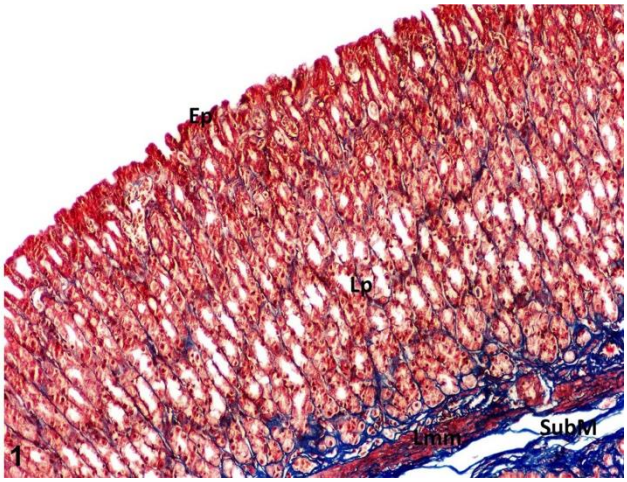


Figure 1: Control group. Rat gastric mucosa**. Ep:** Mucosal epithelium, **Lp:** Lamina propria, **Lmm:** Muscularis mucosa, **SubM:** Submucosa. (Masson's trichrome ×40)

In the ulcer group, typical acute gastric ulcer lesions were observed in the light microscopic evaluations. In some sections, the mucosal damage was seen to have reached the deeper layers, mucus accumulation in the mucosal area was observed to be decreased, and in some areas, deep ulcer craters were observed even reaching the lamina muscularis mucosa. In some sections, there were focal necrotic areas with dominance of necrotic cells. Capillary hemorrhage and congestion, together with dilatation were observed in the gastric pits and glands. Furthermore, lymphatic infiltration was evident throughout the mucosa. In this group, similar to the mucosa, lymphatic infiltration, edema and congestion of the vessels were determined in the evaluations performed in the submucosa (Fig. 2 A-D).

In the group in which selenium had been administered before ulcer formation, while the superficial gastric epithelium was seen to have been partially protected, edema and lymphatic infiltration were observed in the lamina propria. Compared to the ulcer group, despite the absence of deep ulcer craters and focal necrotic areas, there was visualization of tissue loss in the lamina propria, enlargement of the gastric pits, dilatation of the glands and diffuse capillary hemorrhage. Furthermore, mucus accumulation in the superficial glands was evident. Similar to the mucosa, there were observed that lymphatic infiltration and edema in the submucosa (Fig. 3 A, B).

In the group in which Gbe had been administered before the ulcer, mucosal erosion and local desquamation in the superficial epithelium were observed. There were dilatation in the gastric glands and a decrease in the mucus accumulation. In addition to diffuse capillary hemorrhage in the lamina propria, there were seen diffuse lymphatic infiltration and edema (Fig. 4).

In the group in which melatonin had been administered before the ulcer, it was observed in some areas that the integrity of the superficial epithelium was impaired. Compared to the ulcer group, dilatation in the gastric pits and glands was observed to be decreased and the mucus accumulation was observed to be increased. In this group, lymphatic infiltration was seen to be decreased significantly compared to the ulcer group. When compared to the control group, the vascular congestion was observed to persist (Fig. 5).

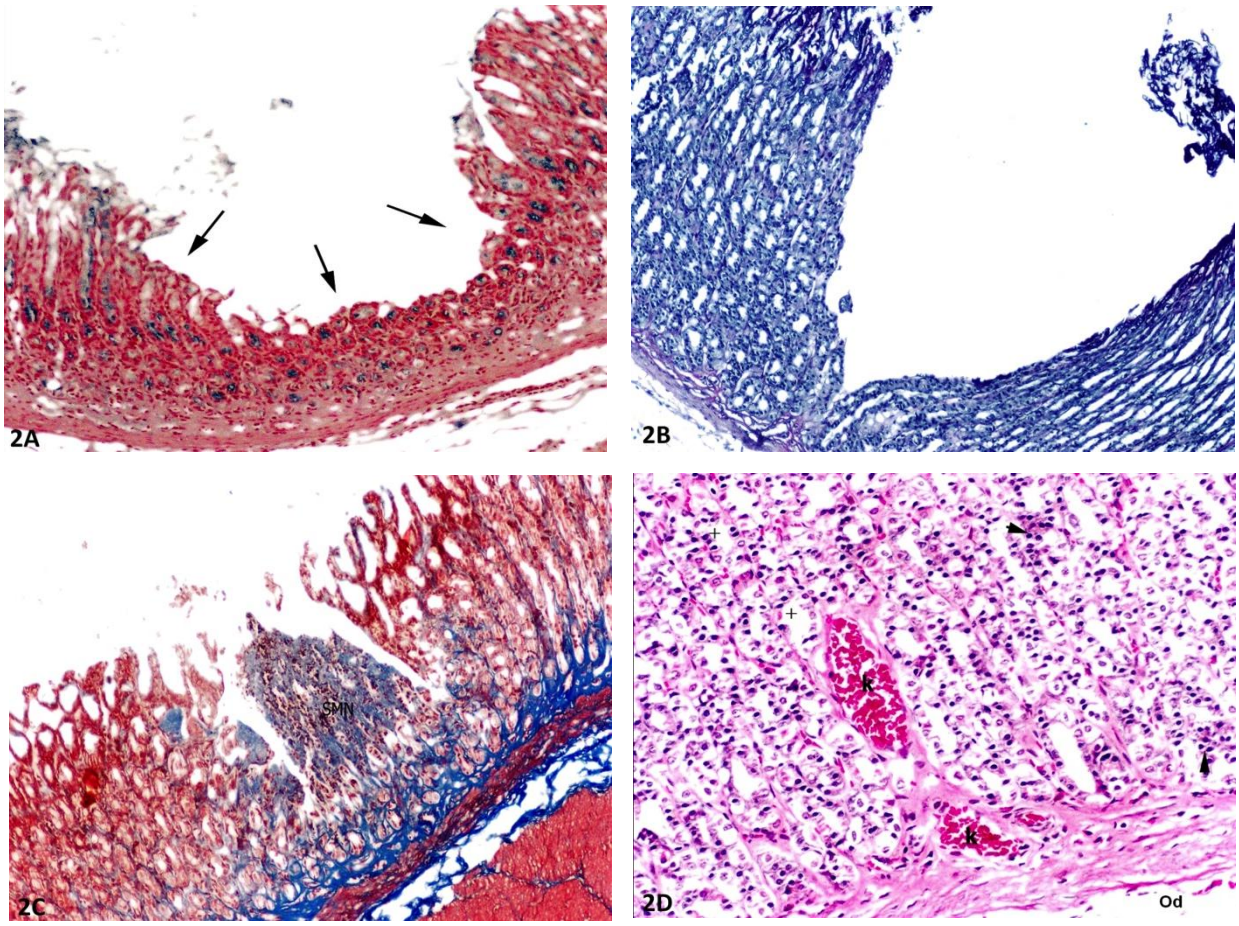


Figure 2: Ulcer group. **(A)** Partial loss of mucosa and reduction in the accumulation of mucus in section of rat gastric, **(↑):** A great loss of mucus that including the gastric lamina muscularis mucosa, typical appearance of ulcer. **(B)** Appearance of a funnel-shaped ulcer crater that is down to the lamina muscularis mucosa of the stomach mucosa. **(C)** Segmental appearance of mucosal necrosis **(SMN)** that is characterized by necrotic cells in rat gastric mucosa. **(D)** Dilatation of the stomach glands in rat gastric mucosa **(+)**, lymphatic infiltration **(►)**, vascular congestion **(k)**, edema in submucosa **(Od)**. (A. Alcian blue ×10, B. Alcian blue ×40, C. Masson's trichrome ×10, D. H-E ×10).
roup. Rat gastric mucosa**. Ep:** Mucosal epithelium, **Lp:** Lamina propria, **Lmm:** Muscularis mucosa, **SubM:** Submucosa. (Masson's trichrome ×40)

In the sections of the group in which vitamin E had been administered before the ulcer, apart from the local mucosal epithelial loss and moderate dilatation, similar findings to the control group were observed. Compared with the other groups, the most evident increase in mucus was found in this group after having applied alcian blue staining to observe the mucus (Fig. 6).

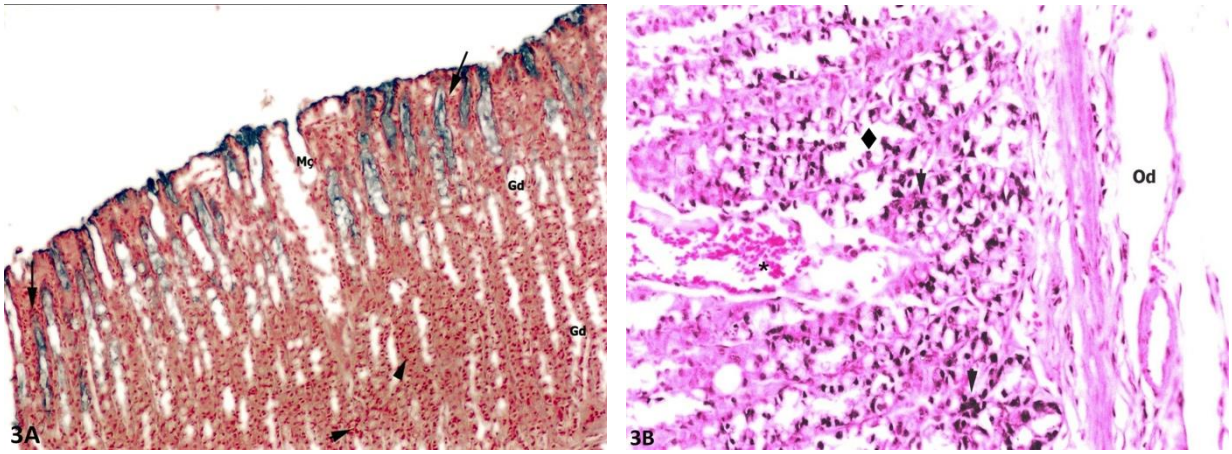


Figure 3: Selenium + Ulcer group. **(A)** Dilatation, lymphatic infiltration **(▶)**, the accumulation of mucus **(↑)** are seen in gastric pits **(Mç)** and glands **(Gd)**. **(B)** Lymphatic infiltration **(▶)**, loss of lamina propria **(♦)**, capillary hemorrhage **(*)**, edema in submucosa **(Od)**. (A. Alcian blue ×10, B. H-E ×100).

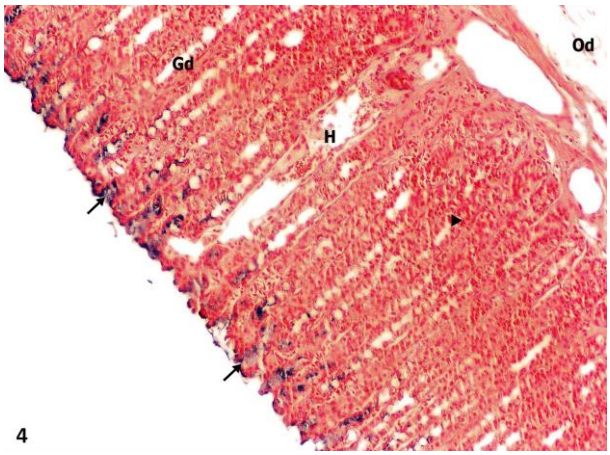


Figure 4: Gbe+Ulcer group. Dilatation and hemorrhage in gastric glands and also edema in submucosa. **Gd:** Dilatation in gastric glands, **H:** Hemorrhage, **Od:** Edema, lymphatic infiltration **(▶)**, decrease of mucus **(↑)**. (Alcian blue ×10).

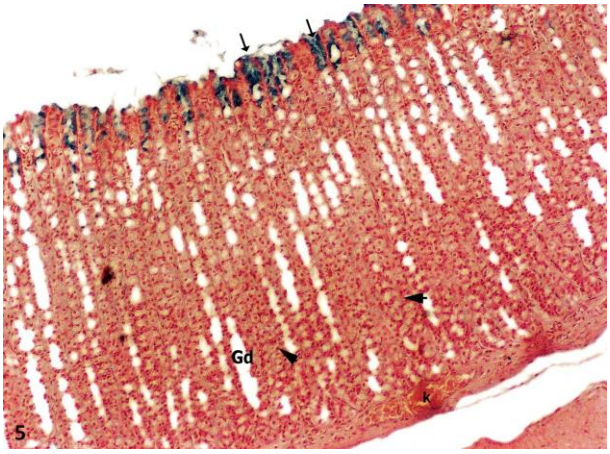


Figure 5: Melatonin + Ulcer group. Decreased dilatation of gastric glands **(Gd)** and lymphatic infiltration **(▶)**, increase in the accumulation of mucus **(↑)**, slight congestion of blood vessels **(k)**. (Alcian blue ×10).

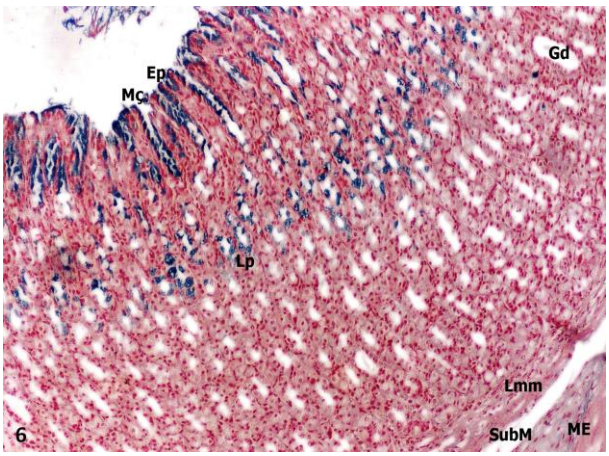
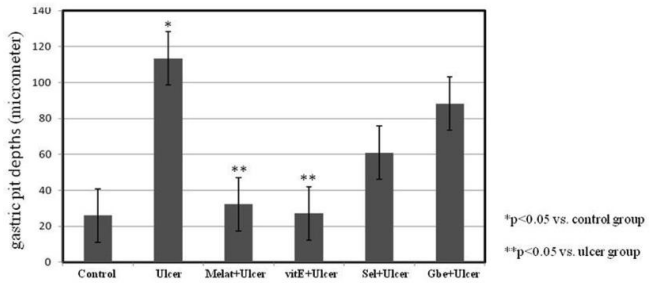


Figure 6: Vitamin E + Ulcer group. Similar appearance to the control group except slight dilatation in gastric pits and glands. **Ep:** Mucosal epithelium, **Lp:** Lamina propria, **Lmm:** Muscularis mucosa, **SubM:** Submucosa, **ME:** Muscularis externa, **Gd:** Dilatation of gastric glands, **Mç:** Gastric pits.

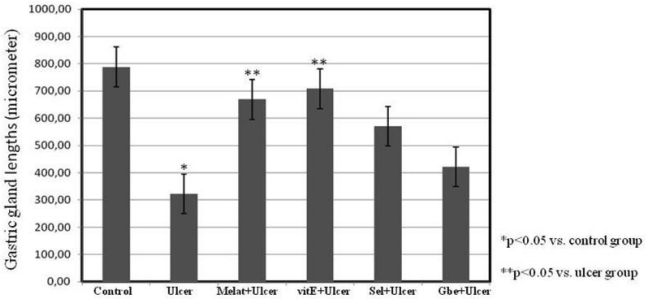
Statistical Result

On statistical evaluation of the measurements made for the gastric pits, a statistically significant deepening in the gastric pits was determined in the ulcer group ($P < 0.05$). In the Gbe and the selenium administered ulcer groups, there was a difference with the non treated ulcer group, but this difference was not statistically significant ($P > 0.05$). While the melatonin and the vitamin E administered ulcer groups were statistically significantly different to the non treated ulcer group with regard to the depth of the gastric pits ($P < 0.05$), no statistically significant difference was observed between the control group and the treated ulcer group ($P > 0.05$) (Graphic 1).

On statistical evaluation of the measurements made for the gastric glands, the shortening in the lengths of gastric glands was found to be statistically significant compared to the control, and the melatonin, vitamin E and the selenium administered groups ($P < 0.05$). There was no statistically significant difference with regard to the gland lengths between the control, melatonin and vitamin E administered ulcer groups ($P > 0.05$) (Graphic 2).



Graphic 1: *Statistical determination of gastric pits in all groups*



Graphic 2: *Statistical determination of gastric gland lengths in all groups*

Discussion

Stress induced ulcers in humans are common in the fundus and the corpus of the stomach, and are multiple, asymptomatic, superficial mucosal lesions, which are commonly localized in areas where there is functional oxyntic glandular mucosa[13]. Collected under a general group named stress induced ulcer, acute mucosal lesions of the gastrointestinal system can appear following various systemic diseases such as trauma, central nervous system diseases, burns, shock, sepsis and surgical interventions[14].

Free oxygen radicals have an important role in tissue damage originating from the stress factor. During stress, the level of free oxygen radicals in the circulation increases, and it has been demonstrated that in this way, they reduce the circulating blood volume and increase the vascular permeability, which leads to mucosal damage[15]. Increase in gastric secretion, decreases in gastric mucosal blood flow, prostaglandin synthesis, bicarbonate synthesis, and mucus production, and

impairment of the gastric mucosal barrier are considered as the pathological mechanisms that are responsible for gastric lesions due to stress[16].

In their study, Das et al.[17] found diffuse hemorrhagic ulcer areas in the gastric glands in 80% of rats they had exposed to cold administration for two hours. Depending on the effect of the administered stress, they drew attention to the lesions extending from the superficial epithelium through the submucosa. Furthermore, they determined a decrease in the cell number and alterations in the organizations together with the changes in the area extending through the mucosa to the submucosa. Depending on the cold and immobility stress that we administered, while partial mucosal loss and a decrease in the mucus accumulation were observed in some of the sections, typical ulcerative lesions were observed in some other sections. This segmental necrosis, characterized with an atypical appearance and the cell structures advancing towards necrosis clearly reveal the mucosa. Consequently, the shortening of the length of the mucosal glands was also found to be statistically significant ($P < 0.05$). With the effect of stress, hemorrhage in the mucosal vessels, lymphatic cell infiltrations in the connective tissues, and vascular congestion and edema in the submucosa, have been accepted as changes that support ulcerations.

In a study conducted on the protective effect of selenium on gastric lesions in the gastric mucosa of the rat ischemia and reperfusion model, Mobarok Ali at al.[18] reported that the gastric lesions and the damage occurring after ischemia could be prevented with the antioxidant selenium, dose dependently. However, in their rat model in which they created gastric mucosal damage with ethanol, Parmar et al.[19] suggested that sodium selenite caused a very significant decrease in gastric lesions.

Unlike the studies of Mobarok Ali and Parmar, in our study in which the ulcer group was compared to the sodium selenium administered ulcer group, despite the partial protection of the superficial epithelium, the dilatation in the gastric glands, the presence of focal bleeding, the edema in the submucosa and the observation of lymphatic cell infiltration, demonstrate that selenium provides total protection against gastric ulcerations. The statistical analysis of the measurements in gastric pits and glands support our microscopic findings. It was observed that sodium selenite did not provide a total protection against mucosal damage due to stress; however, it was seen to provide partial protection on the superficial epithelium against mucosal lesions.

In a study in which they investigated the protective effect of Gbe against gastric mucosal damage in their gastric ulcer model created with ethanol in rats, Shetty et al.[20] suggested that the gastric mucosal ulceration created in the ethanol group was protected with Gbe and there was only presence of tiny losses on the mucosal superficial epithelium.

In another study conducted for the protective effect of vitamin E and Gbe on mucosal damage[21], the researchers expressed that the mucosal protective effects of vitamin E and Gbe on the mucosal lesions occurring due to stress on gastric mucosa were to a higher extent in the Vitamin E group than the Gbe group, and that the gastric mucosa was protected in the Gbe group.

In our study, it was determined that Gbe did not provide a total protection in the prevention of gastric mucosal lesions occurring due to stress, and that there were partial detachments in the epithelium, hemorrhage in the mucosa, dilatation in the gastric glands and edema in the submucosa, in addition to lymphatic cell infiltration in the lamina propria. Our statistical findings also supported these findings. Our study was seen to be parallel to the study of Chen et al.[22], in which they evaluated the protective effect of Gbe on gastric ulcer induced by ethanol. In this study, it was put

forth that beside the mucosal lesion due to gastric mucosal ulcer, the mucus accumulation was also decreased. The researchers emphasized that the decrease in mucus accumulation was an important finding of ulcer formation, and demonstrated that Gbe administration following ulcer formation significantly prevented the mucus accumulation. According to our study, it could not be demonstrated that Gbe provides a total protection against gastric mucosal damage due to cold and immobility stress.

Bandyopadhyay et al.[23] reported that one of the best anti-oxidants that protected against gastric ulceration was melatonin. In a study, depending on the protective effect of melatonin[7], it was suggested that melatonin exerted an important protective effect on indomethacin induced gastric damage. In their study titled "the role of melatonin in the injury of gastrointestinal mucosa due to water floating test", Ercan et al.[24] determined mucous cell degeneration, dilatation in gastric glands, vascular congestion and lymphatic cell infiltration in the gastric mucosa of stress group. In the melatonin administered group, they observed moderate vascular congestion, decrease in gastric gland dilatation, and regular epithelium together with gastric pits.

In our study, the melatonin dose was administered as 60 mg/kg and as the protectiveness against ulcer was evaluated, mucosal integrity was seen to be impaired in some areas, there was presence of partial desquamation of the superficial epithelium, decreased dilatation of the glands, decrease in the lymphatic cell infiltration and persistence of vascular congestion. Mucus accumulation was observed clearly. With these findings, the protective effect of melatonin in different stress models was demonstrated. On statistical evaluation of the measurements of gastric pits and glands, consistent values with the microscopic findings close to that of controls were obtained.

Krinsky[25] suggested that vitamin E alone or combined with selenium, decreased the basal acid secretion and mucus loss due to cold stress, and thereby protected the gastric mucosa.

In our study, the appearance of superficial epithelium of the gastric mucosa close to that of the control group, increase in the diffuse mucus accumulation and mild dilatation in the glands, denoted that vitamin E protects the mucosa significantly. On the other hand, in a study conducted for gastric ulcer, Armari et al.[26] determined that vitamin E did not provide a protective effect in stress created by 18 hours of immobility after ulcer occurrence in the gastric mucosa of rats.

In our study, immobility was maintained for 4 hours, and in rats that were exposed to cold stress for 4 hours, since there was a higher degree of mucosal injury in the ulcer group, we determined that the gastric mucosal damage was very small in the vitamin E administered group and that the observed mucosal structure was close to that of the control group. In another study in the ulcer model created by immobility and cold, the protective effect of vitamin E depending on the dose (1, 5 ,25 mg/kg) on the gastric mucosal injury was evaluated, and mucus accumulation and partial protection of the mucosal structure were determined in the 5 mg/kg dose-administered group[27]. In our study, protection of vitamin E (60 mg/kg) on gastric mucosal injury created by stress was observed in the cross sections. In the statistical evaluations of the gastric pits and the glands, the ulcer group treated with vitamin E provided the closest results to that of the controls.

Conclusion

In conclusion, the results of the present study suggest that, all the administered antioxidants were found to be effective on prevention of gastric lesions created by cold and immobility stress, and compared to the control group a significant difference was found histochemically and

statistically. Melatonin has an efficiency close to that of vitamin E in preventing stress induced gastric lesions. The most effective agent in preventing the formation of stress induced gastric lesions was observed to be vitamin E.

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