Original Article

Effects of caffeic acid phenethyl ester in the prevention of stress ulcer in rats

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Abstract

Background: Stress is one of the important factors responsible for the etiopathogenesis of many diseases. One of the diseases caused by stress is gastric ulcer. Gastric ulcer is called as stress ulcer when occurred because of stress. Purpose of this study was the investigation of caffeic acid phenethyl ester, an antioxidant and antiinflamatuar agent, effects on stress ulcer. **Materials and Methods:** Thirty Wistar Albino rats were used in this study. They were divided into three as control, stress and CAPE groups. "The cold-restraint method" was applied to induce stress ulcer to the stress and CAPE groups while controls did not. Stress and CAPE groups were given isotonic solution and 10 micromol/kg/day CAPE respectively once a week intraperitoneally three days before the stress application. Rats having stress were killed by cardiac puncture at the end of the experiment, and stomachs were removed and mean ulcer indices measured. Blood erythrocyte catalase (CAT) and nitric oxide (NO) as well as stomach tissue malondialdehyte (MDA) levels were analyzed. **Results:** At the end of study, it was seen that rats in CAPE group lost less weight than stress group. Severe hemorrhagic ulcers were observed in the stomach of all rats in stress group. Small ulcers like mucosal petechia were seen in CAPE group. It was determined that CAPE was 93.34% effective in preventing stress ulcer. Remarkable ulcerative lesions were observed microscopically in the stress group while there were minimal ulcerative lesions in CAPE group. CAT levels were found to be significantly higher in CAPE group than stress group ($p=0.001$) while lower than controls significantly ($p=0.043$). MDA levels were low in CAPE group compared to stress group ($p=0.143$) while high compared to controls ($p=0.009$). NO values of CAPE and stress groups were higher than those of controls ($p=0.000$). **Conclusion:** It was found that CAPE effectively prevented stress-dependent ulcer development in our study. The most important sign of this was the remarkably high inhibition percentage of CAPE. According to the findings obtained from this study CAPE prevented neutrouphil infiltration to gastric mucosa, and decreased mucosal damage considerably. With these results it was thought that CAPE probably prevented stress ulcer by blocking lipid peroxidation cascade triggered by free oxygen radicals.

Key Words: stress ulcer, caffeic acid phenethyl ester, cholestasis.

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Introduction

Helicobacter pylori (H pylori) is an exceptionally widespread infection and occurs One of the conditions caused by stress is gastric ulcer. If gastric ulcer develops due to stress, it is defined as stress ulcer. Among the main stress factors causing stress ulcer are; burns, trauma, sepsis, a serious surgery, renal insufficiency, vasculitis episodes and shock (1). Under conditions involving the above mentioned stress factors; mucosal surface erosion of the fundus and corpus of the stomach or deep lesions reaching down to muscularis mucosa can be observed.

Various studies have been conducted about the etiopathogenesis of stress ulcer. It was Hunter in 1772 who indicated for the first time that stress played an important role in ulcer development. In the following period, it was indicated that local ischemic areas in the stomach could form due to stress. In 1932, Cushing demonstrated that following brain surgery, acute stomach and duodenum ulcer developed in patients (2,3). In the following periods, it was indicated that there is a significant relation between stress ulcer and hypothalamic-pituitary-adrenal axis (4). Also, certain brain-intestine peptides or gastrointestinal neuropeptides were mentioned and they were indicated to play an important role in the development of stress ulcer (5). In recent studies, it was shown that mast cell degranulation and tissue damage triggered by free oxygen radicals play an important role in the development of stress ulcer (6-10). It was found that, during stress, nitric oxide release and vascular congestion develops due to neutrophile infiltration and that there would be increased oxygen radical formation with ischemic-reperfusion in mucosal veins. It was shown that this increased amount of radicals caused tissue damage in gastric mucosa and lipid peroxidation (11-13).

Caffeic acid phenethyl ester (CAPE) is an agent with antioxidant and anti-inflammatory properties and is found in propolis used by bees to protect their hives and reinforce their combs (14,15). In numerous studies, CAPE was shown to prevent tissue damage by sweeping away the free oxygen radicals and demonstrate antioxidant properties by blocking the reactive oxygen radicals in human neutrophiles and by inhibiting lipid peroxidation (15-18). In other studies, caffeic acid phenethyl ester was shown to have an anti-inflammatory effect by reducing proinflammatory cytokines (18-20).

In this study, we researched the role of caffeic acid phenethyl ester which has antioxidant and anti-inflammatory properties, in prevention of stress induced gastric ulcer.

Materials and Methods

In this study, 30 Wistar Albino breed rats reproduced in the laboratory and aged 4 to 5 months with weights between 178-241 grams were used. Rats were divided into 3 groups:

1. Group (Control group): 10 rats in this group comprised the control group.
2. Group (Stress group): 10 rats in this group were applied the stress ulcer model and were administered serum physiologic (SP) via intraperitoneal route during the 3 days prior to stress.
3. Group (CAPE group): 10 rats in this group were applied the stress ulcer model and were administered CAPE via intraperitoneal route during the 3 days prior to stress.

Stress Ulcer Model

In this study, "stress ulcer model with immobilization of rats in cold" was used (13,21). The stress group that was administered the stress ulcer model and CAPE group members were not fed with rat feed after 72 hours before the experiment and were only allowed to drink water. At the end of 3 days, the rats were administered ether anesthesia and were fixed on wooden plates of 30 x 50 cm using silk tapes attached to their four extremities (Figure - 1). After this, they were left in an ambient temperature of +4 ^{o}C for 4 hours.

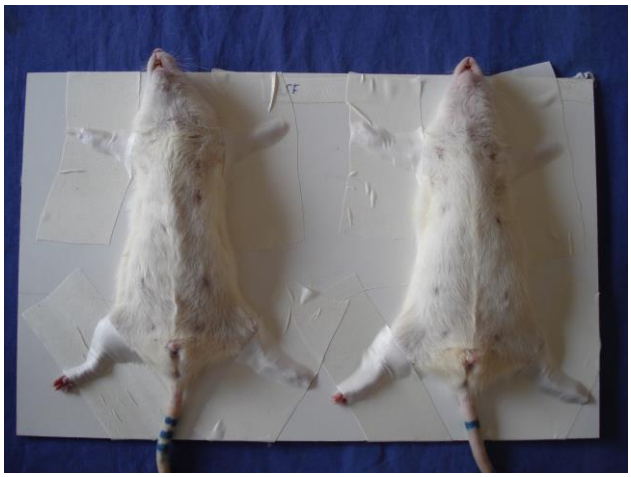

Figure 1. The rats were fixed on wooden plates

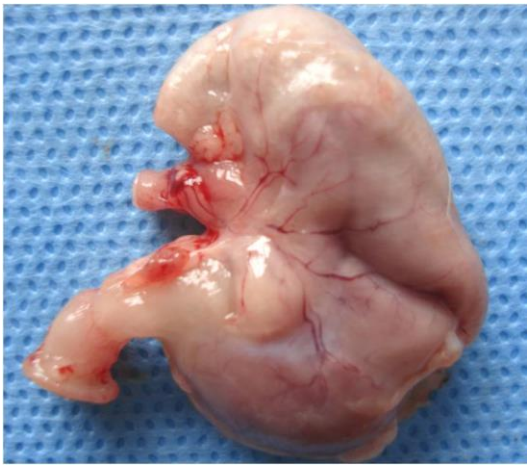

Figure 2. Their stomachs were removed by cutting the esophagogastric junction and pylorus

Pre-Stress Preventive Treatment

No agent or stress was applied to the rats in the control group. The rats in the stress group were administered a daily single dose of 0,4 cc SP from IP route and were applied stress at the end of the study. CAPE group was administered a daily single dose of 0,4 cc 10 micromol/kg/day CAPE from intraperitoneal route and were applied stress at the end of the study. CAPE preparation was obtained from Germany by Sigma Company (C 8221-1 g, SIGMA ALDRICH CHMIE, Gmbh p.o.1120, 89552 Steinheim / Germany 49-7239-970).

Obtaining Tissue and Blood Samples

At the end of the study period, rats that were applied stress were sacrificed using cardiac puncture under ether anesthesia. Abdomens of the rats were opened with a rapid vertical middle line incision. Their stomachs were removed by cutting the esophagogastric junction and pylorus (Figure – 2). Blood obtained using puncture was divided into two separate tubes. Blood taken into heparin tubes were centrifuged at 3000 rpm while the blood taken into tubes containing biochemical gels were kept in an ambient temperature of -20°C.

Histopathological Assessment

Histopathological assessment was carried out at the laboratory of the Department of Pathology of School of Medicine at Pamukkale University by a pathology specialist who had no knowledge as to which group each material belonged to.

Evaluation of the Macroscopic Ulcer Index

The stomachs that were removed were opened along their *curvatura major*. Stomach content was cleaned by washing the stomachs in a utensil containing distilled water. Stomachs were affixed on foam platforms using needles. After cleaning the bloody mucus on the mucosa using a gauze bandage dampened with distilled water, stomachs were evaluated oculometrically. 5 pieces of petechia were accepted as 1 mm. The sizes of all lesions observed on the stomachs were summed up and divided into the number of rats in the group to calculate the average ulcer indexes (UI) of the groups (13). In order to evaluate CAPE's effectiveness in preventing ulcer, inhibition percentage was calculated using the formula below (13,21).

Inhibition percentage $=\{(UI_{stress} - UI_{cape}) / UI_{stress} \} \times 100$

Evaluation of Microscopic Gastric Mucosal Damage

Stomachs were fixed in 10% formol. 2 pieces of full fold tissue with a length of 1.5 cm and a width of 0.2 cm were followed up. After the tissue follow up, 2 sections of 5µ thickness each

were taken. The sections were stained with hematoxylin-eosine (H&E).

Using the sections obtained, all layers of the stomach were evaluated microscopically for signs of stress ulcer. Observation of any disruption of gastric mucosal integrity, epithelial cell chipping, fibrin accumulation, vascular congestion, erythrocyte extravasation or neutrophile infiltration was defined as stress ulcer (22,23). Also, the damage that occurred on the stomach mucosa of each rat in the groups was scored by using a percentage calculation as described by Tanaka et. Al. (24) (Table 1)

Table 1. Gastric mucosal damage and scores

Mucosal damage %	Score
≤ 9	0
10-39	+1
40-59	+2
60-79	+3
≥80	+4

Biochemical Assessments

Measurement of Malondialdehyde Levels in Gastric Tissue

It was decided to determine the amount of malondialdehyde (MDA) levels that resulted from lipid peroxidation. MDA forms a colored complex in the presence of TBA that occurs with absorbance measurement at 532 nanometers. Absorbance was measured using a Shimadzu UV-1601 spectrophotometer and the results were determined in terms of nmol/g wet tissue (25).

Measurement of Erythrocyte Catalase Activity

Blood samples in tubes with heparin were centrifuged for 5 minutes at 3000 rpm. For CAT measurement, erythrocytes were washed 3 times with cold SP. CAT activity was measured using Aebi's method (26). Results were recorded in terms of K/gr Hb.

Nitric Oxide Measurement

Because it has a very short half-life, NO rapidly transforms into nitrite and nitrate that have metabolites. Serum NO values were calculated in terms of μmol/L using the calibration curve prepared using the Griess method and the nitrate standards.

Statistical Analysis

Statistical analyses were conducted on all data and their descriptive properties were assessed. Averages and standard deviations of the data were calculated and were presented along with their numeric values. For statistical analysis, among the parametric tests, Kruskal-Wallis H and Mann-Whitney-U tests were used. Non-parametric assessments were not used in our study. For statistical tests, SPSS software was used (SPSS 10.0 for windows; Chicago, IL, USA). Regarding statistical significance, a "p" value less than 0,05 was considered to be an indicator of statistical significance.

Findings

Table 2 shows that compared to rats in the control group, rats in the CAPE group have lost more weight. It is noteworthy that the rats that were subjected to stress and were administered CAPE treatment lost more weight compared to those rats that were subjected to stress only.

Macroscopic Findings

Ulcer Index (UI)

At the end of the experiment, severe ulcerative lesions were observed in all of the rats in the stress group while only 8 of the rats in the CAPE group demonstrated mild lesions. While the lengths of the lesions in the stress group

ranged between 1 to 20 mm, those in the CAPE group ranged between 1 to 4 mm (Figure – 3,4). No macroscopic gastric lesion was observed in the control group (Figure - 5).

Figure 3. Macrocopic lesions was observed in the stress group

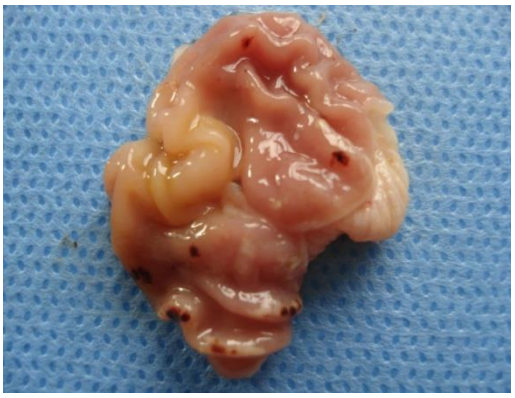

Figure 4. Punctate lesions was observed in the CAPE group

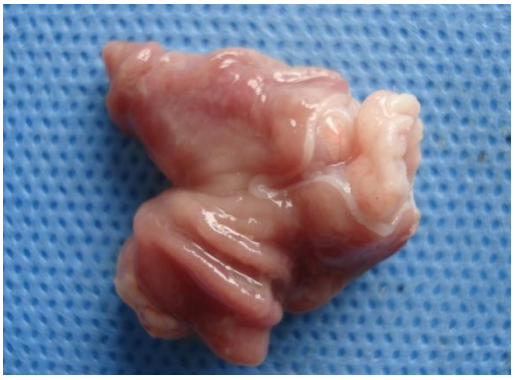

Figure 5. No gastric lesion was observed in the control group

Median UI's of the rats in the groups are shown in Table 2. While the average UI value in rats subjected to stress was 12.62 ± 3.78 mm, it was 0.84 ± 1.03 mm in rats in the CAPE group. Minimum UI value was found to be 6.8 mm in the rats in the stress group and the minimum UI value in the CAPE group was found to be 0.0 mm. Maximum values of the rats in these groups were found to be 19.4 and 3.2 mm respectively. Variance between average UI's in all groups (control, stress, CAPE) is highly statistically significant (p=0.000).

Inhibition Percentage

CAPE's capacity to inhibit stress ulcer was calculated using the inhibition percentage. It was found that CAPE had a 93.34% effectiveness in inhibiting ulcer development (Table 3).

Microscopic Findings

All stomach sections stained with H&E were assessed histopathologically. No obvious histopathological change was observed in the stomachs of the rats in the control group. In the stomachs of the rats in the CAPE group, mild erosive changes were observed in certain areas of the stomach. These lesions were limited to the mucosa layer of the stomach. In the CAPE group, mild mucosal vascular congestion was observed. On the other hand, in the rats of the stress group, it was seen that gastric mucosal integrity was disrupted and epithelial cells were chipped. Mucosal vascular congestions were quite obvious. It was seen that these congested veins had erythrocyte extravasation and that their destructive lesions extended down to sub-mucosa. Again, on the sub-mucosa layers of certain rats in the stress group, areas with fibrin accumulation were observed (Figure 6-8).

Table 2. Median UI's of the rats in the groups*

	Control	Stress	CAPE
UI in rats (mm)	0.0	12.0	0.0
	0.0	11.0	0.2
	0.0	14.6	1.8
	0.0	10.2	0.0
	0.0	8.6	3.2
	0.0	6.8	1.2
	0.0	13.2	0.2
	0.0	19.4	0.2
	0.0	13.4	0.4
	0.0	17.0	1.2
Median UI (mm)	0.00	12.62 ± 3.78	0.84 ± 1.03
Minimum – maximum value	0.0 – 0.0	6.8 – 19.4	0.0 – 3.2

**p=0.000 (in all groups)*

Table 3. Inhibiton percentage and effect of CAPE*

Groups	Median UI (mm)	Inhibiton percentage (%)
Control	0.0	--
Stress	12.62 ± 3.78	--
CAPE	0.84 ± 1.03	93.34

**p=0.000 (in all groups)*

Assessment of Mucosal Damage

Gastric mucosal damage scores and average mucosal damages of the groups are given in Table 4. No mucosal loss was observed in the control group. Average mucosal damage in the CAPE group was calculated as 0.6 ± 0.5.

Average mucosal damage in the stress group, on the other hand, was found to be 2,8 ± 0.7. Mucosal damage in the CAPE group was found to be decreased compared to the stress group in a highly statistically significant manner (p=0.000).

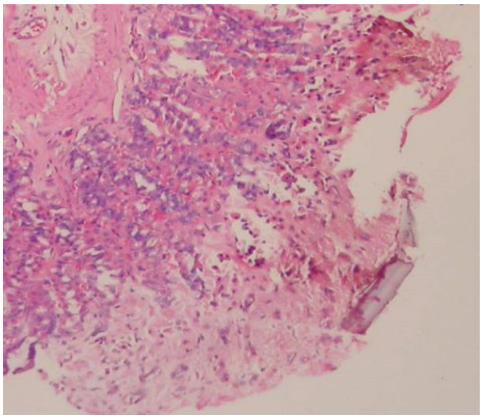

Figure 6. Stress group. H&E X 100

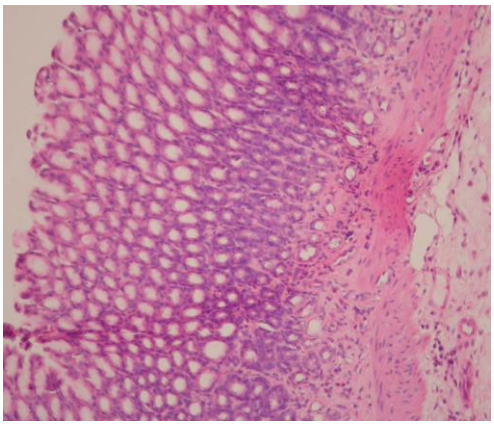

Figure 7. CAPE group. H&E X 100

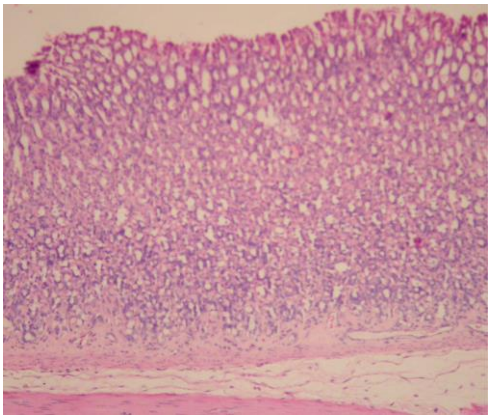

Figure 8. Control group. H&E X 100

Biochemical Findings

Tissue Malondialdehyde Assessment

Average wet tissue MDA levels in the control, stress and CAPE groups were found to be 75.17 ± 6.14, 147.09 ± 21.02, 107.42 ± 10.56 nmol/g respectively. A comparison of the control and stress groups showed that MDA value in the stress group is high in a highly statistically significant manner (p=0.000), and the comparison of the control and CAPE group revealed that MDA values in the CAPE group are high in a statistically significant manner (p=0.009). Comparing the stress and CAPE groups, it was seen that although the MDA values were less in the CAPE group compared to those in the stress group, difference between the groups was not statistically significant (p=0.143)

Erythrocyte Catalase Assessment

Average CAT values in the control, stress and CAPE groups were found to be 25.16 ± 2.16, 12.05 ± 1.20, 20.01 ± 1.42 K/gr Hb respectively. A comparison of the control and stress group showed that CAT value in the stress group was low in a statistically significant manner (p=0.001). Comparing the control and CAPE groups, it was seen that CAT value in the CAPE group was low in a statistically significant manner (p=0.043). And comparing the stress and CAPE groups, it was seen that the CAT value in the stress group was low in a statistically significant manner (p=0.001).

Nitric Oxide Assessment

Average NO values in the control, stress and CAPE groups were 4.51 ± 0.26, 9.12 ± 0.63, 8.96 ± 0.93 μmol/L respectively. Total nitrite + nitrate values in the control group were found to be lower than those in the CAPE and stress groups in a highly statistically significant manner (p=0.000). Comparing the stress and CAPE groups it was seen that despite the lower total nitrite + nitrate values in the CAPE group, there was not statistically significant difference between the two groups (p=0.481).

Table 4. Gastric mucosal damage scores and average mucosal damages of the groups

Rats	Control	Stress	CAPE
1	0	+++	+
2	0	+++	+
3	0	+++	0
4	0	++	0
5	0	+++	0
6	0	++	+
7	0	++++	+
8	0	++	0
9	0	++	+
10	0	++++	+
Median mucosal damage (score)	0	2.8 ± 0.7	0.6 ± 0.5*

* *p=0.000 According to the stress group*

Discussion

Until today, numerous studies have been conducted on the ethiopathogenesis of stress ulcer. It was Hunter in 1772 who indicated for the first time that stress played an important role in ulcer development. In 1853 Virchow argued that local ischemias played a role in the development of ulcerative lesions. In 1913 von Bergman, and in 1932 Cushing drew attention to neuropsychic factors by demonstrating that acute stomach and duodenum ulcer developed in patients following brain surgery (27). In 1936, Selye et al. indicated that there was a significant relation between stress ulcer and hypothalamus-hypophysis axis (28). In the following period, various brain-intestine peptides or gastrointestinal neuropeptides were mentioned and were emphasized as having an important role in the development of stress ulcer (2). In one study, it was shown that somatostatine, a neuropeptide with inhibitor effects inhibited acid secretion, increased mucus formation and increased PG synthesis. Also, it was argued that various substances that inhibit gastric acid secretion exerted their effects by increasing somatostatine secretion (3). It was indicated that leptin which is another neuropeptide, is secreted from stomach with the effect of cholecyst and resulted in defense against certain agents. It was argued that this effect may be due to CCK_B receptors, vagal activity and sensory nerves and that the resulting hyperemic lesions were possibly due to NO (2). It was determined that bombesine which is another neuropeptide, inhibits gastric acid secretion when injected into cerebrospinal fluid. It was also demonstrated that bomesine plays a role in gastric mucosal defense by increasing endogenous gastrine release and that it prevented this effect by iNOS inhibition (5).

In the following periods, studies were conducted to research the physiopathology between stress ulcer and mast cell degranulation. In one study, it was demonstrated that leukotrienes that are revealed by mast cell degranulation delayed the amelioration of stress ulcer (29). It was also indicated that, with leukotrien antagonists, stress development is prevented. In an experimental study conducted by Kalia et al. (30), it was indicated that cholinergic system is activated and this activation aggravated mast cells. In another study, it was indicated that serotonin

that occurs as a result of mast cell degranulation caused vasoconstriction in arterioles and that histamine caused vasodilatation in venules (31). It was demonstrated that as a result of these events, gastric mucosal micro hemorrhages developed due to erythrocyte diapedesis. In the studies conducted by Ohta et al (32,33), it was found that gastric bleeding decreased with mast cell degranulation and that as a result, reactive oxygen radicals were released. It was mentioned that, the damage that occurred in micro-vascular endothelium as a result of reactive oxygen radicals caused mucosal lesions. In numerous studies, the effects of mast cell degranulators in preventing stress ulcer were analyzed. In these studies, various agents such as ketotyphen, zinc compounds, sodium chromoglikate, phosphatidylcholine and vasoactive intestinal peptide were used and were shown to be sufficiently effective in preventing stress ulcer (34-36). It was indicated that these agents prevented the tissue damage that resulted from mast cell degranulation and particularly mucosal bleeding.

It is indicated that inflammatory process plays an important role in the etiology of stress ulcer. In an experimental study, it was demonstrated that neutrophiles that become activated with xhantine–xhantine oxidase system during the development of acute gastric lesions, play a role in the inflammatory process (37). In two studies conducted separately by Brzozowski and Watanabe, it was demonstrated that proinflammatory cytokines IL-1 β and TNF-α increase in the stomach tissue due to stress (38,39). Brzozowski et. Al, determined that in the treatment group that was administered omeprazole and ranitidine which have anti-inflammatory effects as well, IL-1 β increase in the stomach tissue was less than those in other groups. On the other hand, Watanabe et. Al. emphasized the importance of TNF-α and particularly IL-1 β in the recurrence of ulcer. In an experimental study by Handa et al. (40), changes observed in gastric epithelial cells as a result of stress were analyzed. It was seen that in the gastric tissue, TNF-α increased the level of CINC-1 which is a neutrophile cemoattractant. It was also shown that reactive oxygen radicals and NF-kB are activated as a result of TNF-α stimulation. In another study, it was shown that congestion due to neutrophile infiltration and microvascular ischemia developed in the area damaged due to stress. And as a result of the reperfusion conducted following ischemia, it was seen that SOR development was increased (41). In an experimental stress ulcer study conducted by Jia et al (42), NF-kB was found to demonstrate biphasic activation. It was seen that NF-kB reached peak levels in the 45^{th} and 360^{th} minutes of the study. With Western blot analysis, NF-kB was shown to be activated as a result of IkappaBalpha and IkappaBbeta degradation. Also, it was found that as a result of rapid and permanent activation of NF-kB; TNF-α, IL-1 β, CINC-1 and ICAM-1, mRNA increased in the 15^{th} and 30^{th} minutes. And 30 and 90 minutes after stress, it was seen that iNOS mRNA gene expression increased and this increase lasted until the end of stress. As a result, it was found that NF-kB activation played an important role in stress ulcer by increasing proinflammatory gene over expression in the gastric mucosa. In another study, it was indicated that there might be a pathway through which NF-kB and AP-1 activation is triggered by SOR and this was defined as the SOR/NF-kB pathway (43). In the same study, SOR/NF-kB pathway was shown to increase TNF-α, IL-1 and CINC-1and that AP-1, allowed for the transcription of genes such as C/EBP or Stat3 genes.

It is indicated that particularly free radical damage can have a major effect on stress ulcer formation. In related studies, free radicals were shown to have an important role in the development of mucosal damage and bleeding erosions due to stress (7,10). Free radicals may affect all components of the cell but lipids, proteins and nucleic acids are the main targets. The most prevalent of these toxic substances are superoxide anion and hydroxyl radical. Production of superoxide anion forms the starting phase of the event. After that, superoxide anion is converted into hydrogen peroxide via SOD. Hydrogen peroxide is broken down with glutathione peroxidase enzyme. Hydroxyl radical is produced via

mainly two reactions. The first one involves direct reduction of hydrogen peroxide via superoxide radical and it is catalyzed by iron (Haber-Weiss reaction). The other is hydroxyl radical production as a result of the reaction between iron and hydrogen peroxide (Fenton reaction). Hydroxyl radical has a longer half-life than superoxide radical and is more toxic. These radicals result in damage by causing hyaluronic acid which is one of the basic components of epithelial membrane to break down and by setting lysosomal enzymes free (8,44,45). In a study regarding the effects of free radicals on the development of stress ulcer, it was found that particularly the hydroxyl radical proliferate in stomach tissue (10). It was found that hydroxyl radicals depolymerize polysaccharides by inactivating the enzymes after interacting with proteins. It was also indicated that nucleotide synthesis is inhibited and fatty acids enter into peroxidation as a result of interaction with free radicals and that membrane structure gets disrupted as a result of these reactions (8).

It is stated that stomach micro-circulation as well displays significant changes during mucosal damage due to stress. It is indicated that particularly the protective mechanism in the mucosa get damaged after ischemia. And also SOR develops as a result of the reperfusion that develops following ischemia. Guth (46) who conducted a pathophysiological study about the issue, indicated that during the early periods of stress, blood flow decreased and this resulted in a cell protective effect by decreasing gastric acid secretion. He also stated that in the later stages, however, ischemia due to vasoconstriction developed which resulted in gastric mucosal damage. In an experimental stress ulcer study conducted by Khadzhiev et al.(31), stomach tissues of rats were analyzed and it was indicated that perivascular edema, erythrocyte, diapedeses and micro-hemorrhage developed as a result of arteriolar spasm and venous congestion. It was seen that a decrease in blood flow limited oxygen use and ATP production. It is known that energy change may disrupt ion gradients in the membrane and this resulted in a disruption in the distribution of Ca^{+2} ion. Increased cytosolic Ca^{+2} concentration activates a protease that causes the xanthine form to be transformed into XO. On the other hand, with decreased intracellular ATP, AMP increase would be observed. And AMP, by being converted into adenosine, inosine and hypoxantine, forms a resource for free radical production (37,47).

Reviewing the above mentioned studies regarding stress ulcer etiopathogenesis, one can say that an ischemia-reperfusion process is experienced due to oxidative stress in general and that the resulting free radicals disrupts the gastric mucosal integrity. One may also argue that mast cell degranulation and NF-kB activation plays a role in this process and that they realize this mainly by increasing the release of proinflammatory cytokines. As a result, it is mainly SOR and neutrophile infiltration that is responsible from the etiopathogenesis of stress ulcer.

In parallel to this, in numerous studies regarding prevention of stress ulcer, agents with anti-inflammatory and antioxidant effect were researched. For preventing stomach mucosa damage that results from indomethacin, Santucci et al (48) used pentoxifylline which is a methylxantine derivative and exerts an anti-inflammatory effect. They found that, in the treatment group, this agent prevented neutrophile migration and mucosa damage by increasing TNF-α level. In a study conducted by Bregonzio et al. (49), treatment group was administered candeartan which is an angiotensin II AT_1 receptor and it was found that it prevented gastric mucosal damage due to stress in a statistically significant manner. It was indicated that the AT_1 receptor antagonist exerted this influence by decreasing TNF-α and ICAM-1 expression and thus prevented neutrophile infiltration. In an experimental ulcer study by Salim (50), allopurinol and dimethyl sulphoxide were used as antioxidant agents. Allopurinol, by inhibiting the XO enzyme which plays a role in superoxide radical, and dimethyl sulphoxide, by sweeping away the hydroxyl radicals from the environment, exert antioxidant effect. In this study, antioxidant agents were found to be decreasing ulcer formation. In an experimental stress ulcer study,

Güzel et al. (51) found that Vitamin E strengthens gastric mucosal barrier. Vitamin E prevented the formation of stress ulcer by sweeping away free radicals after inhibiting lipid peroxidation. In a study by Al-Moutary et. al (52), rats in the treatment group were given antioxidant selenium and Vitamin E and these agents were found to be decreasing ulcer development in a statistically significant manner compared to the control group. In an experimental stress ulcer study conducted by Bülbüller et al (24), treatment group was administered pentoxifylline and L-tryptophan and stress ulcer was found to be decreased. It was shown that L-tryptophan which is an essential amino acid protected gastric mucosa against SOR. In another study, the substance called ginko bilboa which is extracted from a tea species grown in China, prevented ulcer by exerting antioxidant and cytoprotective effects (53). In studies by Ohta et al., agents named ebselen, selenium-organic compounds and oren-gedoku-to were used and these agents were found to be preventing acute gastric mucosal lesions to a great extent (36,54). In another study, lansoprozale which is a benzimidazole derivative exerted antioxidant effects by increasing the amount of mucus sulfhydryl compounds. And as a result, it was found to exert preventive effect against acute gastric mucosal lesions by increasing gastric blood flow related due to this increase in the amount of mucus sulfhydryl compounds (55). While numerous studies have researched the potential of various agents with antioxidant and anti-inflammatory effects for preventing stress ulcer, no study has been conducted to research the effect of CAPE in preventing the formation of stress ulcer.

In numerous experimental studies CAPE was shown to have strong antioxidant effects. (18,56-58). CAPE exerts its antioxidant effect by inhibiting the XO enzyme and by binding the dose dependent free radicals (17,59).

XO enzyme is an oxidant enzyme that plays a role in lipid peroxidation and is one of the major free radical sources (17,37). Particularly during ischemia, hypoxantine forms from ATP and then hypoxantine is reduced to xanthine. On the other hand, xanthine which is synthesized in large amounts during ischemia, is converted into uric acid via a reaction whereby XO is catalyzed following provision of oxygen into the environment via reperfusion. During this reaction, superoxide radical is produced and this radical is converted into hydroxyl anion that damages radical tissues.

CAPE which carries two hydroxyl groups with antioxidant properties in its structure, acts as a SOR collector. Numerous studies have been conducted to research this free radical collecting property of CAPE. Yılmaz et al. researched SOD and CAT activities in diabetic rat liver developed by streptozisine. It was found that SOD and CAT decreased in the group treated with CAPE. They argued that due to its SOR sweeping effect, CAPE prevented the increase in SOD and CAT activities (60). In an experimental study conducted by Koltuksuz et al., CAPE's effect in preventing small intestine ischemia-reperfusion damage was researched. It was stated that CAPE prevented SOR formation and prevented the tissue from damage by inhibiting the leukocyte infiltration (14). In a study where kidney ischemia-reperfusion damage was created, CAPE was shown to suppress ischemia-reperfusion damage more effectively than Vitamin E and prevent lipid peroxidation by decreasing SOR formation (17,61). In our study, CAT value in the CAPE group was found to be higher than that in the stress group in a statistically significant manner ($p=0.001$), and less than that in the control group in a statistically significant manner ($p=0.043$). Here, CAPE may have exerted an influence similar to that of Vitamin E by inhibiting lipid radicals or by blocking the OH radical formation phase chain after fenton reaction and may have increased the erythrocyte CAT level. Also, CAPE may have prevented extreme increase in erythrocyte activity with its SOR blocking effect.

In the literature, there are different results for antioxidant enzyme levels in experimental models where stomach mucosa damage and stomach outer tissue damage is formed. There are studies indicating decreased or increased (62-64) SOD, GSH-Px and CAT enzyme levels

in rats in which gastritis was caused. In this study, CAT and GSH-Px enzyme activities in the erythrocytes of humans who were given aspirin at a dose of 10 mg/kg/day for 30 days, were observed to be increased. Again in the same study, analysis of the heart tissues of the guinae pigs that were given aspirin at a dose of 440 mg/kg/day revealed decreased SOD and unchanged GSH-Px. CAT enzyme level increased but it was not statistically significant (65). In an experimental study where gastritis was caused using indomethacin, CAT enzyme activity was found to be high (64). In an experimental gastric ulcer study conducted by Fesharaki et al, it was shown that activity of antioxidant enzymes such as SOD and GSH-Px in gastric tissue decreased and that mucosal damage and hemorrhage due to these changes developed (62). In summary, CAT activity can bring different results with the same experimental models in different studies. In our study, CAT level was found to have decreased in rats in which stress ulcer was caused via immobilization in cold. CAT, which is a natural antioxidant is in fact, expected to increase in the presence of oxidative stress. However in certain situations where stress response is extreme, it is known that antioxidants may not reach sufficient levels. CAT demonstrates activity in situations where peroxide concentration increases extremely. On the other hand, in situations where hydrogen peroxide level is low, other enzymes such as GSH-Px take action. In our study, in the group that was given CAPE treatment, CAT values were observed to be lower compared to those in the control group ($p=0.043$). Also, erythrocyte CAT activity was found to be higher in the CAPE group than in the stress group ($p=0.001$).

MDA is the final product of lipid peroxidation and is used as an indicator of tissue damage. It is stated that the tissue damage that develops in stress ulcer forms as a result of lipid peroxidation. In an experimental study conducted by Yoshikawa et al., it was shown that, as a result of the activation of XO which is one of the SOR sources, the MDA value that results from the peroxidation of polyunsaturated fatty acids located inside the cell membrane increases significantly in the gastric tissue (44,66). In an experimental stress ulcer study, lipid peroxidation was assessed using tissue MDA values (13). MDA values were found to be lower in a statistically significant manner in the group that was given phosphatidyl choline treatment compared to those in the stress group. In a study conducted by Kwiecien et. al (67), MDA values in the stomach were found to have been increased due to reactive oxygen radicals. In our study, when the control and stress groups were compared, MDA values in the stress group were found to be high in a statistically significant manner ($p=0.000$). A comparison of the stress and CAPE groups showed that MDA value was lower in the CAPE group than in the stress group ($p=0.143$). With these findings in our study, lipid peroxidation played an important role in formation of stress ulcer and CAPE may have exerted an effect by inhibiting lipid peroxidation.

NO is an oxidant from the free radical group. Peroxynitrite forms from the reaction between NO and superoxide (44). Increased NO stimulates inflammation and tissue damage. Also, by entering into a reaction with intracellular gluthanione, it decreases gluthanione levels and causes sensitivity towards oxidative stress in the cell. As a result, in addition to its own cytotoxic effect, it increases cytotoxicity by reacting with free radicals. With these effects, NO is one of the important factors in the pathogenesis of the gastric mucosal lesion in ulcer caused by stress. iNOS and cNOS enzymes play a role in NO synthesis. In a study, NO produced by iNOS was indicated to have cytotoxic effects on gastric mucosa and that NO produced by cNOS was shown to be cytoprotective (68). Within the first 6 hours of stress ulcer, a dramatic increase in the gastric mucosal iNOS activity and a decrease in the cNOS activity was determined. It was stated that, with the decrease in the cNOS activity, NO made a significant contribution to the formation of gastric mucosal lesions due to an increase in iNOS activity. In another study, bombes was shown to play a role in gastric mucosal defense by increasing endogenous gastrin release and to prevent this effect by iNOS inhibition (5). On the other hand, even though Calatayud et al. argue that, in the

experimental stress ulcer model, formation of acute gastric mucosal lesions are prevented by increased NO release, no definite consensus about this view was reached (69).

CAPE exerts antioxidant effect by decreasing the NO level by means of blocking NOS (iNOS) enzyme which has a catabolic effect (70). In our study, in the stress group and CAPE group, NO value was found to be higher than that in the control group in a highly statistically significant manner (p=0.000). Comparison of the stress group and the CAPE group showed no statistically significant difference between the two groups despite the lower NO value in the CAPE group (p=0.481). The significant increase in the NO value in the stress group indicates that an ischemia reperfusion process is experienced in the stomach during stress. Serum level of NO with vasodilator effect may be increasing in order to compensate for decreased gastric blood flow. Thus it might be thought that NO which an oxidant enzyme damages the mucosa and contributes to the vascular congestion observed during the development of ulcerative lesions. And in our study, while mucosal congestions that are microscopically obvious in the stomachs of the rats in the stress group were observed, these congestions were at minimal level in the CAPE group. Also, in our study, unexpectedly NO with oxidant properties was found to be higher in the CAPE group in a statistically significant manner (p=0.000). The reason for this finding may be that CAPE exerts its antioxidant effect primarily not via iNOS but via inhibiting XO and its SOR collecting effect.

CAPE is a strong NF-kB inhibitor and it is a transcription factor that has a very important role in the arrangement of immune and inflammatory events and in cell life (71). Cytokines activate neurotransmitters and SOR activates NF-kB. NF-kB triggers the expression of cytokines, proteases, adhesion molecules and other inflammatory mediators (25). On the cyclooxygenase path, on the other hand, CAPE supersedes the enzyme activity of cyclooxygenase -1 (COX-1) and cyclooxygenase-2 (COX-2) and inhibits the activation of gene expression of COX-2. With its strong chemotactic potential, SOR stimulates the formation and release of various inflammatory mediators such as IL 1, IL 6 and TNF-α. These mediators cause neutrophile infiltration in the tissue. CAPE, by inhibiting NF-kB in a potent and specific manner, prevents neutrophile accumulation and release of systemic inflammatory mediators (30,62). And in our study, CAPE was shown to prevent neutrophile infiltration during stress. It may have exerted this influence via the NF-kB pathway.

As a result, in our study, based on our findings, it was determined that CAPE effectively prevents stress induced ulcer formation with an inhibition percentage of 93.34%. We believe that CAPE exerts its stress ulcer preventive effect via its antioxidant and anti-inflammatory properties. It was determined that in the CAPE group, histopathologically, the mucosal damage was lower than that in the stress group in a statistically significant manner (p<0.009). Again as a result of microscopic assessment, while no neutrophile infiltration was observed in the CAPE group and the control group, areas of serious neutrophile infiltration were observed in the stress group. Current findings demonstrate the anti-inflammatory effect of CAPE. It was also seen that activity of CAT, an antioxidant enzyme, was higher in the CAPE group compared to the stress group and lower compared to the control group. At this point, we believe that CAPE, with its antioxidant effect, may have increased CAT and may have prevented an extreme increase in CAT with its SOR keeping effect. Also in our study, CAPE was found to have decreased MDA values. During lipid peroxidation, lipid radicals are transformed into cytotoxic products, the most important being MDA. Based on our study, one can believe that the effect of CAPE on lipid peroxidation chain is one of the most important factors preventing the development of stress ulcer.

References

1. Spirt MJ. Stress-Related Mucosal Disease: Risk Factors and Prophylactic Therapy. Clinical Therapheutics 2004; 26: 197-213.

2. Büyükçoşkun Nİ. Stres ülseri ve nöropeptitler. Uludağ Üniversitesi Tıp Fakültesi Dergisi 2002; 28: 109-113.

3. Jenkins SA, Taylor BA, Nott DM, Ellenbogen S, Haggie J, Shields R. Management of massive upper gastrointestinal haemorrhage from multiple sites of peptic ulceration with somatostatin and octreotide a report of few case. Gut 1992; 33: 404-407.

4. Konturek SJ, Brzozowski T, Piastucki I, Dembinski A, Radecki T, Dembinska-Kiec A, Zmuda A et al. Role of mucosal prostaglandins and DNA synthesis in gastric cytoprotection by luminal epidermal growth factor. Gut 1981; 22: 927-932.

5. Castaneda AA, Kim YS, Chang LK, Cui Y, Mercer DW. Nitric oxide synthase inhibition negates bombesin-induced gastroprotection. Surgery 2000; 128: 422-428.

6. Thayer WR, Toffler AH, Chapo G. İnhibitin of restraint ulcers in the rat by pridoxine deficiency. Yale Journal of Biology and Medicine 1965; 38: 257-264.

7. Hung CR and Hsu DS. Roles of Histamine Receptors and Oxyradicals in Aggravation of Acid-Induced Gastric Haemmorhagic Ulcers in Endotoxaemic Rats. Inflammopharmacology 1998; 6: 339-355.

8. Çavdar C, Sifil A, Çamsarı T. Reaktif oksijen partikülleri ve antioksidan savunma. Türk Nefroloji Diyaliz ve Transplantasyon Dergisi 1997; 3-4: 92-95.

9. Ishıı M, Shımızu S, Nawata S, Kıuchı Y and Yamamoto T. Involvement of reactive oxygen species and nitric oxide in gastric ıschemia-reperfusion ınjury in rats. Digestive Diseases and Sciences 2000; 45: 93-98.

10. Das D, Bandyopadhyay D, Bhattacharjee M, Banerjee RK. Hydroxyl radical is the major causutive factor in stress-induced gastric ulceration. Free Radic Biol Med 1997; 23: 8-18.

11. Herken H, Uz E, Özyurt H, Söğüt S, Virit O, Akyol Ö. Evidence that the activities of erythrocyte free radical scavenging enzymes and the products of lipid peroxidation are increased in different forms of schizophrenia. Molecular Psychiatry 2001; 6: 66-73.

12. Lata H, Ahuja GK, Narang APS. Effect of immobilitation stress on lipid peroxidation and lipid profile in rabbits. İndian Journal of Clinical Biochemistry 2004; 19: 1-4.

13. Demirbilek S, Gürses I, Sezgin N, Karaman A, Gürbüz N. Protective effect of polyunsaturated Phosphatidylcholine pretreatment on stress ulcer formation in rats. J Pediatr Surg. 2004; 39: 57-62.

14. Koltuksuz U, Ozen S, Uz E. Caffeic acid phenethyl ester prevents intestinal reperfusion injury in rats. J Pediatr Surg 1999; 34: 1458-62.

15. Koltuksuz U, Irmak MK, Karaman A. Testicular nitric oxide levels after unilateral testicular torsion/detorsion in rats pretreated with caffeic acid phenethyl ester. Urol Res 2000; 28: 360-63.

16. Sud'ina GF, Mirzoeva OK, Pushkareva MA, Korshunova GA, Sumbatyan V, Varfolomeev SD. Caffeic acid phenethyl ester as a lipoxygenase inhibitor with antioxidant properties. FEBS Lett 1993; 329: 21-24.

17. Irmak MK, Fadillioglu E, Sogut S, Erdogan H, Gulec M, Ozer M, Yagmurca M, Gozukara ME. Effects of caffeic acid phenethyl ester and alpha-tocopherol on reperfusion injury in rat brain. Cell Biochem Funct 2003; 21: 283-89.

18. Rossi A, Longo R, Russo A, Borrelli F, Sautebin L. The role of the phenethyl ester

of caffeic acid (CAPE) in the inhibition of rat lung cyclooxygenase activity by propolis. Fitoterapia 2002; 73: 30-37.

19. Chen MF, Keng PC, Lin PY, Yang CT, Liao SK, Chen WC. Caffeic acid phenethyl ester decreases acute pneumonitis after irradiation in vitro and in vivo. BMC Cancer 2005; 158: 1-9.

20. Singh J, Khan M, Singh I. Caffeic acid phenethyl ester induces adrenoleukodystrophy (Abcd2) gene in human X-ALD fibroblasts and inhibits the proinflammatory response in Abcd1/2 silenced mouse primary astrocytes. Biochim Biophys Acta. 2013 Apr;1831(4): 747-58.

21. Yeşilada E, Gürbüz I, Ergun E. Effect of Cistus Iaurifolius L. Flowes on gastric duodenal lesions. J Ethnopharmocol 1997; 55: 201-211.

22. Ribeiro-Rama AC, Figueiredo IV, Veiga F, Castel-Branco MM, Cabrita AM, Caramona MM. Evaluation of gastric toxicity of indomethacin acid, salt form and complexed forms with hydroxypropyl-beta-cyclodextrin on Wistar rats: histopathologic analysis. Fundam Clin Pharmacol. 2009 Dec;23(6):747-55.

23. Noffsinger AE, Stemmermann GN, Lantz PE. Gastrointestinal Pathology An Atlas and Text. Philadelphia: Lippincott Williams & Wilkins, 1999.

24. Bülbüller N, Akkuş A, İlhan Y.S, Baysal F, Özercan İ, Aygen E, Kırkıl C. L-Triptofan ve pentoksifilinin stres ülseri üzerine etkisi. Ulus Trav Der 2003; 9: 90-95.

25. Tüközkan N, Erdamar H, Seven I. Measurement of Total Malondialdehyde in Plasma and Tissues by High-Performance Liquid Chromatography and Thiobarbituric Acis Assay. Fırat Tıp Dergisi 2006; 11: 88-92.

26. Aebi H, Catalase. In: Bergmeyer HU editors. Methods of Enzymatic Analysis. New York: Academic Press, 1974.

27. Yabana T, Yachi A. Stress induced vascular damage and ulcer. Digestive Disease and Sciences. 1988; 33: 751-761.

28. Szabo S. Understanding biologic stres for study design and interpretation of results. Digestive Disease and Sciences 1985; 30: 28-31.

29. Ogle CW and Cho CH. The protective mechanism of FPL55712 against stress-induced gastric ulceration in rats. Agents and Actions 1989; 26: 3-4.

30. Kalia N, Bardhan KD. Mast cells and gastric mucosal damage: yet another mechanism of injury?. J Gastroenterol Hepatol 1998; 13: 1081-83.

31. Khadzhiev OC, Lupal'tsov VI, Simonenkov AP, Klimenko NA, Tatarko SV. Microcirculatory disturbances in gastric mucosa during ulcer disease and effects of serotonin on their dynamics. Bull Exp Biol Med 2000; 130: 843-45.

32. Ohta Y, Kobayashi T, Ishiguro I. Role of endogenous serotonin and histamine in the pathogenesis of gastric mucosal lesions in unanaesthetised rats with a single treatment of compound 48/80, a mast cell degranulator. Pharmacol Res 1999; 39: 261-67.

33. Ohta Y, Kobayashi T, Inui K, Yoshino J, Nakazawa S. Protective effect of teprenone against acute gastric mucosal lesions induced by compound 48/80, a mast cell degranulator, in rats. J Pharmacol Sci 2003; 93: 337-46.

34. Kiraly A, Suto G, Tam B, Hermann V, Mozsik G. Vagus-mediated activation of mucosal mast cells in the stomach: effect of ketotifen on gastric mucosal lesion formation and acid secretion induced by a high dose of intracisternal TRH analogue. J Physiol Paris 2000; 94: 131-134.

35. Tunçel N, Erkasap N, Şahintürk V, Doğrukol D, Tunçel M. The Protective Effect of Vasoactive Intestinal Peptide (VIP)

on Stres-Induced Gastric Ulceration in Rats. Ann N Y Accad 1998; 309-323.

36. Ohta Y, Kobayashi T, Inui K, Yoshino J, Nakazawa S. Protective effect of ebselen, a seleno-organic compound, against the progression of acute gastric mucosal lesions induced by compound 48/80, a mast cell degranulator, in rats. Jpn J Pharmacol 2002; 90: 295-303.

37. Ohta Y and Nishida K. Protective effect of coadministered superoxide dismutase and catalase against stres-induced gastric mucosal lesions. Clinical and Experimental Pharmacology and Physiology 2003; 30: 545-550.

38. Brzozowski T, Konturek PC, Konturek SJ, Drozdowicz D, Kwiecien S, Pajdo R, Bielanski W et al. Role of gastric acid secretion in progression of acute gastric erosions induced by ischemia-reperfusion into gastric ulcers. Eur J Pharmacol 2000; 398: 147-58.

39. Watanabe T, Higuchi K, Tanigawa T, Tominaga K, Fujiwara Y and Arakawa T. Mechanisms of peptic ulcer recurrence: role of inflammation. Inflammopharmacology 2002; 10: 291-302.

40. Handa O, Naito Y, Takagi T, Shimozawa M, Kokura S, Yoshida N, Matsui H et al. Tumor necrosis factor-alpha-induced cytokine-induced neutrophil chemoattractant-1 (CINC-1) production by rat gastric epithelial cells: role of reactive oxygen species and nuclear factor-kappaB. J Pharmacol Exp Ther 2004; 309: 670-76.

41. Nishida K, Ohta Y, Ishiguro I. Contribution of NO synthases to neutrophil infiltration in the gastric mucosal lesions in rats with water immersion restraint stress. FEBS letters 1998; 425: 243-248.

42. Jia YT, Wei D, Chen XL, Xia ZF. Activation of nuclear factor-kappaB signaling pathway in rat gastric mucosa during stress ulceration. Zhonghua Shao Shang Za Zhi 2006; 22: 351-54.

43. Jia YT, Wei W, Ma B, Xu Y, Liu W-J, Wang Y, Lv K-Y et al. Activation of p38 MAPK by Reactive Oxygen Species Is Essential in a Rat Model of Stress-Induced Gastric Mucosal Injury. The Journal of Immunology 2007; 179: 7808–7819.

44. Akkuş İ. Serbest radikaller ve fizyopatolojik etkileri. Konya: Mimoza yayınları, 1995.

45. Onat T, Emerk K, Sözmen E.Y. İnsan biyokimyası, Ankara: Palme yayınları, 2002

46. Guth PH. Current concepts in gastric microcirculatory pathophysiology. Yale J Biol Med 1992; 65: 677-88.

47. Glavin G, Szabo S. Experimental gastric mucosal injury: laboratory models reveal mechanisms of pathogenesis and new. The FASEB Journal 1992; 6: 825-831.

48. Santucci L, Fiorucci S, Giansanti M, Brunori PM, Di Matteo FM, Morelli A. Pentoxifylline prevents indomethacin induced acute gastric mucosal damage in rats: role of tumour necrosis factor alpha. Gut 1994; 35: 909-15.

49. Bregonzio C, Armando I, Ando H, Jezova M, Baiardi G, Saavedra JM. Anti-inflamatuar effect of angiotensin II AT_1 receptor antagonism prevent stress-induced gastric injury. AJP. Gastrointest Liver Physiol 2003; 285: 414-423.

50. Salim AS. The Relationship between Helicobacter Pylori and Oxygen-Derived Free Radicals in the Mechanism of Duodenal Ulceration. Internal Medicine 1993; 32: 359-364.

51. Güzel C, Kurt D, Şermet A. The effects of vitamin E on gastric ulcers and gastric mucosal barrier in stress induced rats. Tr. J of Medical Sciences 1998; 28: 19-21.

52. Al-Moutairy AR, Tariq M. Effect of vitamin E and selenium on hypothermic restraint

stress and chemically-induced ulcers Digestive Diseases and Sciences 1996; 41: 1165-1171.

53. Jane C. J. Chao, Hung HC, Chen SH, Fang CL. Effects of Ginkgo biloba extract on cytoprotective factors in rats with duodenal ulcer. World J Gastroenterol 2004; 10: 560-566.

54. Ohta Y, Kobayashi T, Nishida K, Nagata M, Ishiguro I. Therapeutic effect of Oren-gedoku-to extract on stress-induced acute gastric mucosal lesions in rats. Phytother Res 1999; 13: 588-92.

55. Natale G, Lazzeri G, Lubrano V, Colucci R, Vassalle C, Fornai M, Blandizzi C et al. Mechanisms of gastroprotection by lansoprazole pretreatment against experimentally induced injury in rats: role of mucosal oxidative damage and sulfhydryl compounds. Toxicol Appl Pharmacol 2004; 195: 62-72.

56. Russo A, Longo R, Vanella A. Atioxidant activity of propolis: role of caffeic acid phenethyl ester and galangin. Fitoterapia 2002; 73: 21-29.

57. İlhan A, Koltuksuz U, Ozen S, Uz E, Ciralik H, Akyol Ö. The effects of caffeic acid phenethyl ester (CAPE) on spinal cord ischemia / reperfusion injury in rabbits. Eur J Cardiothorac Surg 1999; 16: 458-463.

58. Özyurt B, Güleç M, Özyurt H, Ekici F, Atış Ö, Akbaş A. The effect of antioxidant caffeic acid phenethyl ester (CAPE) on some enzyme activities in cisplatin-induced neurotoxicity in rats. Eur J Gen Med 2006; 3: 167-172.

59. Fadıllıoglu E, Erdogan H, Söğüt S, Kuku İ. The effects of caffeic acid phenethyl ester on DNA-turnover rates and nitric oxide level in Doxorubicin-induced myocardial injury. T Klin Tıp Bilimleri 2003; 23: 366-370.

60. Yılmaz HR, Uz E, Yücel N, Altuntas I, Ozcelik N. Protective effect of caffeic acid phenethyl ester (CAPE) on lipid peroxidation and antioxidant enzymes in diabetic rat liver. Biochem Mol Toxicol 2004; 18: 234-238.

61. Irmak MK, Koltuksuz U, Kutlu NO, Yagmurca M, Ozyurt H, Karaman A, Akyol O. The effect of caffeic acid phenethyl ester on ischemia-reperfusion injury in comparison with α-tocopherol in rat kidneys. Urol Res 2001; 29: 190-193.

62. Fesharaki M, Nasimi A, Mokhtari S, Mokhtari R, Moradian R, Amirpoor N. Reactive oxygen metabolites and anti-oxidative defenses in aspirin-induced gastric damage in rats: Gastroprotection by Vitamin E. Pathophysiology 2006; 13: 237-243.

63. Bulbuloglu E, Inanc F, Bakarıs S, Kantarceken B, Cetinkaya A, Caglar R, Ilhami TK et al. Association of adenosine deaminase, superoxide dismutase and catalase activities with Helicobacter pylori. Digestive Diseases and Sciences 2005; 50: 2296-99.

64. Odabasoglu F, Cakir A, Suleyman H, Aslan A, Bayir Y, Halici M, Kazaz C. Gastroprotective and antioxidant effects of usnic acid on indomethacin-induced gastric ulcer in rats. Journal of Ethnopharm 2006; 103: 59-65.

65. Durak I, Karaayvaz M, Cimen MYB, Avcı A, Cimen OB, Buyukkocak S, Ozturk HS. Aspirin impairs antioxidant system and causes peroxidation in human erythrocytes and guinea pig myocardial tissue. Human and Experimental Toxicology 2001; 20: 34-37.

66. Yoshikawa T, Minamiyama Y, Ichikawa H, Takahashi S, Naito Y, Kondo M. Role of lipid peroxidation and antioxidants in gastric mucosal injury induced by the hypoxanthine-xanthine oxidase system in rats. Free Radic Biol Med 1997; 23: 243-50.

67. Kwiecien S, Brozozowski T, Konturek PC, Pawlik MW, Pawlik WW, Kwiecien W,

Konturek SJ. The role of reactive oxygen species and capsaicin-sensitive sensory nerves in the pathomechanisms of gastric ulcers induced by stress. Journal of Physiology and Pharmacology 2003; 54: 423-437.

68. Nishida K, Ohta Y, Ishiguro I. Role of gastric mucosal constitutive and inducible nitric oxide synthases in the development of stres-induced gastric mucosal lesions in rats. Biochem Biophys Res Commun 1998; 236: 275-9

69. Calatayud S, Barrachina D, Esplugues JV. Nitric oxide: relation to integrity, injury, and healing of the gastric mucosa. Microsc Res Tech 2001; 53: 325-35.

70. Hosnuter M, Gurel A, Babuccu O, Armutcu F, Kargı E, Isikdemir A. The effect of CAPE on lipid peroxidation and and nitric oxide levels in the plasma of rats following thermal injury. Burns 2004; 30: 121-5.

71. Çam H. Sıçanlarda Aspirin ile uyarılan gastritin önlenmesinde kafeik asit fenetil ester'in etkinliğinin araştırılması. Isparta Süleyman Demirel Üniv. 2007.