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Research Article

Effect of Ellagic Acid on Some Oxidative Stress Parameters and Cyclooxygenase-2 Reactivity in Mice with Experimental Gastric Injury

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1. Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) commonly used by people induces gastric injury. This study was designed to evaluate effects of ellagic acid as a natural compound on oxidative stress markers and immunohistochemical structure during experimental gastric damage. It was created five groups as follows. Group I was received standard pellet feed and drinking water. On the other hand, a single oral dose of 25 mg/kg indomethacin, 10 mg/kg ellagic acid, 25 mg/kg indomethacin plus 10 mg/kg ellagic acid and 20 mg/kg omeprazole were received to group II, III, IV and V, respectively. The reduced glutathione (GSH), nitric oxide (NO), malondialdehyde (MDA), total sialic acid (TSA) and ghrelin levels of samples taken after 6 hours from applications were analyzed by spectrophotometric methods. The cyclooxygenase 2 (COX-2) reactivity was analyzed by immunohistochemical staining method. The kidney NO and TSA levels of group II were found to be increased compared with group I, whereas these levels were lower in group IV compared with group II. The liver NO and MDA levels of group II were higher than in group IV. All blood ghrelin levels in group II were lower compared with other groups. It was revealed severe COX-2 immunoreactivity in stomach surface and foveola epithelium, parietal cells, macrophages and vascular endothelium near submucosa of group II, while this reactivity was less in group IV. It was concluded that ellagic acid significantly changed NO, TSA, MDA and ghrelin levels and stomach COX-2 activity of mice given indomethacin and ellagic acid prevented gastric injury.

2. Keywords: Gastric injury; ellagic acid; indomethacin; oxidative stress; cyclooxygenase.

3. Introduction

The gastric damage occurs in where the mucosal epithelium is unprotected against acid and pepsin. Symptoms such as bleeding in the stomach or duodenum, obstruction of food passage, and pain may be seen during gastric damage that occurs in vivo. In the formation of gastric damage, there is a role of impaired balance between aggressive and protective factors. Drugs (non-steroidal anti-inflammatory drugs, NSAIDs), alcohol use and stress are among the most aggressive factors leading to gastric and gastroduodenal damage [1].

The reactive oxygen species (ROS) such as peroxynitrite, hydroxyl and superoxide radicals lead directly or indirectly to tissue damage. Ellagic acid (2,3,7,8-tetrahydroxybenzopyrano [5,4,3-cde] benzopyran-5-10-dione) is an effective natural herbal phenolic compound with free radical scavenging and antioxidative effects in both in vivo and in vitro studies against ROS [2-4]. Application of ellagic acid (above 5 mg/kg) has been recorded to apparently prevent the ulcers in stress-induced gastric damage models [5]. It has been suggested that ROS and antioxidant defense systems play an important role in determining the pathogenesis of gastric damage using indomethacin-like drugs [6]. MDA level is used as a marker of lipid peroxidation severity, while ROS causes lipid peroxidation in the cell membrane [7]. Indomethacin which has many therapeutic activities and is evaluated as NSAIDs causes ulcerative lesions, especially in the gastrointestinal tract. Indomethacin causes gastric mucosal injury by increasing gastric acid secretion and affecting NO synthesis [8]. The L-arginine/NO pathway constitutes the major secondary defense system essential for gastric mucosa else than prostaglandins [9]. The NO is involved in modulation of gastric mucosal integrity and regulation of gastric acid secretion with ghrelin hormone which released mainly from the fundus of the stomach and endogenous prostaglandins [10,11].

In recent years, epidemiological studies on the gastrointestinal tract and the main effects of NSAIDs in animal experiments have been linked to cyclooxygenase (COX). The COXs that provide prostaglandin synthesis from arachidonic acid have two isoenzymes as constitutively (COX-1) and inducible (COX-2). The COX-1 enzyme is used in many organ and tissue in physiological protective functions such as platelet aggregation, mucosal protection and renal blood flow, whereas the COX-2 enzyme provides opposite functions [12,13].

Although the number of drugs used in the treatment of gastric damage or disorder is high nowadays, but it is not always possible to achieve the desired effect. Treatments are restricted sometimes due to the side effects of drugs, even when applied depending on the cause. The success of these treatment studies depend on a detailed understanding of the function of normal and variable conditions in many biomolecules synthesized from the stomach and other tissues. In this study on experimental gastric damaged mice, it was aimed to investigate the effect of ellagic acid on COX-2 reactivity with the levels of GSH, MDA, TSA, NO and ghrelin which are used in evaluating important metabolic pathways for oxidative stress.

4. Materıals and Methods

All experimental and medium conditions were conducted in accordance with the “Guide for Care and Use of Laboratory Animals”, published by the National Institutes of Health and the ethical guidelines of the International Association for the Study of Pain. The study was performed in Kafkas University Research and Aplication Center after the acceptance of the local ethic committee of Kafkas University (Registration number: KAU HADYEK 2010/34). A total of 35 Swiss albino-type male mice (12-15 weeks old, 29-34 g bw) were used in the study. The mice were divided into five groups each consisting of seven animals. They were kept in stainless-steel cages (26/15/50 cm) with standard mouse feed and water for one month of adaptation period before experimental applications. The medium conditions of animals had a temperature of 22 ± 1°C and an humidity of 50 ± 5 % with 12 h light/dark cycle.

4.1. Experimental Design

The group I (control as a healthy group) was kept under non-treatment standard maintenance conditions. The model of experimental gastric damage was prepared according to the procedure that was reported by Guidobono et al. [14]. The treatments were done after animals were starved one day. It was administered a

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single oral dose of 25 mg/kg indomethacin (Cas: 53-86-1, Sigma), 10 mg/kg ellagic acid (Cas: 476-66-4, Sigma), 10 mg/kg ellagic acid plus 25 mg/kg indomethacin after 5 minutes and 20 mg/kg omeprazole (Nobel Pharmaceutical Industry and Trade Inc., Istanbul) to groups II, III, IV and V, respectively.

4.2. Preparation of Samples

After six hours from experimental aplications, the blood samples from the mice were taken as intracardial during ether anesthesia and systemic necropsy was performed followed by euthanasia using cervical dislocation method and then sections from liver, kidney and stomach tissues were taken for biochemical and immunohistochemical analysis. Tissue samples were 10 times diluted (pH 7.4) through homogenizator (Wiggen Hauser) on ice using 0.1 M phosphate buffer solution. After homogenates were centrifuged at 15000 g for 10 minutes at 4°C, the obtained supernatants were kept in deep freeze (-50°C) until the working day for biochemical analysis. Stomach tissue samples were fixed in 10 % buffered formaldehyde solution. The paraffin blocks were prepared with routine operations of the fixed tissues. It was taken 5 µ sections from paraffin blocks and stained with hematoxylin. All layers of the stomach were evaluated microscopically for the stress ulceration.

4.3. Biochemical Analysis

The nitric oxide levels were determined through absorbance values at 540 nm for total levels of nitrate and nitrite values in the samples according to spectrophotometric (UV-1201, Shimadzu, Japan) method reported by Miranda et al. [15]. MDA levels were analyzed by the method of Yoshioka et al. [16]. The principle of experiment is based on the fact that MDA and thiobarbituric acid form a pinkish complex in acidic and hot medium. The absorbance at 535 nm of this colored complex is directly proportional to MDA concentration. MDA calibration curve was prepared using 1,1,3,3-tetraethoxypropane. GSH levels were analyzed by the method of Beutler et al. [17]. The principle of experiment is based on measurement of optical density at 412 nm wavelength of yellow complex formed by 5,5'-2-dithiobis nitrobenzoic acid in clear liquid obtained by precipitating of the proteins with non-sulfhydryl (-SH) group in sample. The ghrelin levels were measured colorimetrically (Epoch™ Microplate Spectrophotometer, BioTek) using an enzyme immunoassay (EIA) method and an in vitro quantitative kit (Cat no: EIA-GHR-1; RayBio) after obtaining all blood samples to tubes containing aprotinin. TSA levels were measured using a spectrophotometer by the method of Sydow [18] in that all bound sialic acid was separated by perchloric acid in plasma and tissue homogenates, and then the supernatants were boiled by Erlich reagent, and finally the product was read at 525 nm. N-acetylneuraminic acid (NANA, N-acetylneuraminic acid from *Escherichia coli*, A2388, Sigma) was used for the TSA calibration curve.

4.4. Immunohistochemical Analysis

Immunostaining was performed with avidin-biotin-peroxidase method using a commercial kit specific for primary antibodies (Abcam, Cat No: ab21704) to determine COX-2 immunoreactivity in gastric damage. Sections taken from paraffin blocks prepared from stomach tissue were transferred onto 3 poly-L-lysine coated slides and left to dry overnight. Sections were deparaffinized, then washed 3 times for 5 minutes in phosphate buffer solution (PBS). In order to elicit antigenic receptors, they were incubated with 0.001 % trypsin for 30 minutes at 37°C. Sections washed with PBS were incubated for 20 minutes in 3 % solution of hydrogen peroxide to prevent endogenous peroxide activity in the tissues. Sections were incubated at room temperature for 30 minutes with non-immun goat serum (Zymed Laboratories Inc, Cat No: 85-9043A) to prevent non-specific staining. Then sections were incubated with Rabbit polyclonal anti-COX-2 (Abcam, Cat No: ab21704) diluted 1/200 in PBS for 1 hour at 37°C. At the end of this period, the sections washed three times for 5 minutes in PBS were treated with biotinylated secondary antibody (Zymed Laboratories Inc, Cat No: 85-9043B) for 30 minutes. Then sections were incubated with peroxidase-conjugated streptavidin (Zymed Laboratories Inc, Cat No: 85-9043C) for 30 minutes, washed 3 times with PBS. Following washing again 3 times with PBS for 5 minutes each, a solution of 3.3'-diaminobenzidine tetrahydrocholoride-H_2O_2 (Lab Vision, Cat No: TA-125) was applied onto the sections as a color substrate. When the color change started to occur, the reaction was stopped with distilled water. At the last stage, the sections were stained with Mayer Haematoxylin for 20 seconds and then washed in tap water for 5 minutes. The stained

sections were covered with entellan and examined using light microscopy.

4.5. Statistical Analysis

Statistical analysis of data obtained from the experimental and control groups was performed using the statistical package program (SPSS 20.0 for Windows). One-way analysis of variance (ANOVA) was used to determine whether there were significant differences between the groups. Tukey test was used multiple comparison analysis and Spierman test was used for the correlation analysis. Results were expressed as mean ± standard deviation (X ± SD).

5. Results

5.1. Biochemical Findings

It was determined that levels of kidney NO and TSA were increased in group II given indomethacin compared to the control group (group I), and decreased in group IV with indomethacin plus ellagic acid (P< 0.01 and P< 0.05, respectively). Plasma NO levels were found to be increased in the groups (group III and V) given ellagic acid and omeprazole compared to the group II (P<0 .05). The levels of liver NO and MDA increased in group II when decreased in group IV compared with group I (P< 0.01). All blood ghrelin levels were found to be lower in the group II than in the other groups (P< 0.05). The levels of NO, TSA, MDA, GSH and ghrelin in group I and experimental gastric injured mice have showed in (**Table 1**). Correlation coefficient test revealed a positive correlation between the levels of the plasma NO and all blood ghrelin (P< 0.05), liver NO and MDA (P< 0.01), liver TSA levels with kidney MDA and GSH (P< 0.01). Correlation coefficients among levels of NO, TSA, MDA, GSH and ghrelin have showed in (**Table 2**).

5.2. Ulceration Score

The ulceration and stomach damage caused by oral indomethacin application were quantitatively determined, and there was no ulcereration in group I. The ulceration scores were significantly lower in group III and IV compared with group II (P< 0.001). The ulceration score is shown in (**Table 3**).

Table 1. The levels of NO, TSA, MDA, GSH, and ghrelin in control and experimental gastric injured mice

		Group I	Group II	Group III	Group IV	Group V	P
Plasma	NO (µM)	20.54 ± 4.80^{a}	18.78 ± 7.1^{b}	28.71 ± 9.3^{ab}	27.79± 0.39^{ab}	28.39 ± 7.54^{c}	<0.05
	TSA (mg/dL)	73.15 ± 4.41	76.01 ± 8.61	71.86 ± 8.63	73.43 ± 8.52	66.43 ± 5.29	>0.05
	MDA (µmol/L)	5.38 ± 1.08^{a}	8.06 ± 1.85^{b}	6.23 ± 1.84^{a}	6.73 ± 1.82^{a}	5.92 ± 1.65^{a}	<0.01
All Blood	GSH (mg/dL)	8.58 ± 2.02	8.13 ± 2.41	10.95 ± 2.54	8.92 ± 4.81	9.04 ± 1.98	<0.01
	Ghrelin (pg/mL)	12.69±3.56^{ab}	6.79±2.92^{b}	13.61±4.31^{a}	8.92±2.32^{ab}	13.48±5.09^{a}	<0.05
Kidney	NO (µM)	6.54 ± 1.38^{a}	11.98 ± 2.32 ^{c}	6.95 ± 1.74^{ab}	9.19 ± 2.30^{bc}	8.42 ± 1.92^{ab}	<0.01
	TSA (mg/dL)	6.78 ± 0.91^{ab}	7.47 ± 0.88^{a}	5.95 ± 1.16^{ab}	5.42 ± 1.02^{b}	6.12 ± 0.91^{ab}	<0.05
	MDA (µmol/L)	2.07 ± 0.65	2.63 ± 1.02	2.21 ± 0.67	1.51 ± 0.26	2.38 ± 0.71	>0.05
	GSH (mg/dL)	10.48 ± 5.21	7.47 ± 2.25	8.48 ± 2.31	8.95 ± 2.49	9.48 ± 2.98	>0.05
Liver	NO (µM)	10.84 ± 1.88^{ab}	11.97 ± 3.29^{b}	7.37 ± 2.08^{a}	10.52± 2.89^{ab}	13.78 ± 2.55^{b}	<0.01
	TSA (mg/dL)	11.90 ± 2.32	12.88 ± 2.71	11.29 ± 1.51	10.04 ± 0.67	11.37 ± 1.92	>0.05
	MDA (µmol/L)	3.93 ± 0.81^{a}	4.31 ± 0.82^{b}	3.22 ± 0.43^{c}	4.07 ± 0.54^{ab}	4.02 ± 0.78^{a}	<0.01
	GSH (mg/dL)	7.01 ± 1.75	5,72 ± 2.33	6.43 ± 2.05	6.32 ± 2.52	6.85 ± 2.68	>0.05

X ± Sd$^{a, b, c}$: The changes between the means with different letters in the same line is important. Gruop I: Control, Gruop II: Indomethacin, Gruop III: Ellagic acid, Gruop IV: Indomethacin plus ellagic acid, Gruop V: Omeprazole.

Table 2. Correlation coefficients among levels of NO, TSA, MDA, GSH, and ghrelin in control and experimental gastric injured mice

		Group I	Group II	Group III	Group IV	Group V	P
Plasma	NO (µM)	20.54 ± 4.80^{a}	18.78 ± 7.1^{b}	28.71 ± 9.3^{ab}	27.79± 0.39^{ab}	28.39 ± 7.54^{c}	<0.05
	TSA (mg/dL)	73.15 ± 4.41	76.01 ± 8.61	71.86 ± 8.63	73.43 ± 8.52	66.43 ± 5.29	>0.05
	MDA (µmol/L)	5.38 ± 1.08^{a}	8.06 ± 1.85^{b}	6.23 ± 1.84^{a}	6.73 ± 1.82^{a}	5.92 ± 1.65^{a}	<0.01
All Blood	GSH (mg/dL)	8.58 ± 2.02	8.13 ± 2.41	10.95 ± 2.54	8.92 ± 4.81	9.04 ± 1.98	<0.01
	Ghrelin (pg/mL)	12.69±3.56^{ab}	6.79±2.92^{b}	13.61±4.31^{a}	8.92±2.32^{ab}	13.48±5.09^{a}	<0.05
Kidney	NO (µM)	6.54 ± 1.38^{a}	11.98 ± 2.32 ^{c}	6.95 ± 1.74^{ab}	9.19 ± 2.30^{bc}	8.42 ± 1.92^{ab}	<0.01
	TSA (mg/dL)	6.78 ± 0.91^{ab}	7.47 ± 0.88^{a}	5.95 ± 1.16^{ab}	5.42 ± 1.02^{b}	6.12 ± 0.91^{ab}	<0.05
	MDA (µmol/L)	2.07 ± 0.65	2.63 ± 1.02	2.21 ± 0.67	1.51 ± 0.26	2.38 ± 0.71	>0.05
	GSH (mg/dL)	10.48 ± 5.21	7.47 ± 2.25	8.48 ± 2.31	8.95 ± 2.49	9.48 ± 2.98	>0.05
Liver	NO (µM)	10.84 ± 1.88^{ab}	11.97 ± 3.29^{b}	7.37 ± 2.08^{a}	10.52± 2.89^{ab}	13.78 ± 2.55^{b}	<0.01
	TSA (mg/dL)	11.90 ± 2.32	12.88 ± 2.71	11.29 ± 1.51	10.04 ± 0.67	11.37 ± 1.92	>0.05
	MDA (µmol/L)	3.93 ± 0.81^{a}	4.31 ± 0.82^{b}	3.22 ± 0.43^{c}	4.07 ± 0.54^{ab}	4.02 ± 0.78^{a}	<0.01
	GSH (mg/dL)	7.01 ± 1.75	5,72 ± 2.33	6.43 ± 2.05	6.32 ± 2.52	6.85 ± 2.68	>0.05

r*: Correlation is significant at the 0.05 level (2-tailed) r**: Correlation is significant at the 0.01 level (2-tailed)

Table 3. Histopathological grading scores for ulseration of control and experiment mice

Groups (n=7)	(%) Ulceration
Control or omeprazole	0 ± 0^{a}
Ellagic acid	0.28 ± 0.15^{a}
Indomethacin	2.89 ± 0.61^{c}
Indomethacin plus ellagic acid	0.76 ± 0.29^{b}

X ± Sd$^{a, b, c}$: The changes between the means with different letters in the same line is important (P< 0.001).

5.3. Immunohistochemical Findings

The COX-2 immunostaining was lightly observed in the mouse stomach in the control group (Figure I). COX-2 immunostaining on more magnified views was found to be intracytoplasmic in macrophages with a small number of foveola epithelial cells. Similar immunostaining was also observed in the group III and V given alone ellagic acid and omeprazole (Figure III). In the stomach of mice in the indomethacin-treated group II was noted a markedly increase for COX-2 immunostaining. In this group, intense and severe COX-2 immunoreactivity was detected in the stomach mucosa, foveola epithelium and parietal cells, macrophages and vascular endothelium near to submucosa, mostly cytoplasmic (Figure II). In group IV was observed moderate immunoreactivity in COX-2 immunostaining compared to other groups (Figure IV), although there was a significant decrease compared to group II. It was noted that COX-2 immunostaining in group IV was lightly in vascular endothelial cells, macrophages, foveola epithelium near the mucosal surface.

6. Discussion and Conclusion

The effects of plant-derived phenolic compounds on human health are being clarified day by day. Experimental studies demonstrate that these constructs have anti-inflammatory, antioxidant, anticarcinogenic and antiatherosclerotic effects [19,20]. Ellagic acid as a phenolic acid derivate with strong antioxidant property is intensive in pomegranate and all grape juice [21]. Ellagic acid is used in many modifying reactions such as methylation, methoxylation and glycosylation in organisms [4,22]. It has been suggested that the protective effects of ellagic acid against gastric damage have been achieved through the antioxidant defense system components [20,23]. In experimental oxidative stress-induced rats with N^{ω}-Nitro-L-arginine methyl ester hydrochloride (L-NAME) were reported that the ellagic acid was increased superoxide production in the vascular tissue, plasma MDA levels as a marker of lipid peroxidation and reactive oxygen species and decreased expression of $p47^{phox}$ subunit of NADPH oxidase responsible for hypertension. It has also been claimed that ellagic acid in rats causes these effects when the NO ratio restores according to the optimum living conditions [24]. The findings of present study support previous studies on oxidative stress. Oxidative stress markers were used to assess the protective effect of ellagic acid

in blood, liver and kidneys of mice treated indomethacin from NSAIDs to induce gastric damage. Omeprazole is a benzimidazole derivative drug that strongly blocks gastric acid secretion and is given to the positive control group for comparison of the gastroprotective effect of ellagic acid. When the groups were compared for NO, TSA, MDA, GSH and ghrelin levels, it was found that significant changes were observed especially in terms of plasma NO and MDA, liver MDA and kidney TSA levels.

It has been reported that oxidative stress is important due to increased reactive oxygen species in the pathogenesis of indomethacin-induced gastric damage and proton pump inhibitors may be effective in reducing oxidative stress in the gastric mucosa [25,26]. In the study for this reason, the levels of oxidant and antioxidant parameters were analyzed in order to evaluate the gastroprotective effect of the ellagic acid which was claimed to be a strong antioxidant and potential proton effector. It was detected that levels of kidney NO and TSA were increased in mice given alone indomethacin compared to the control group, whereas these levels in the indomethacin plus ellagic acid treated group were close to the control group. It was determined that liver NO and MDA levels in mice alone indomethacin treated were increased compared to the control group, but these levels decreased in group ellagic acid plus indomethacin treated (Table 1). These findings suggest that ellagic acid may be a gastroprotective agent by showing similar effects to omeprazole on renal NO and TSA levels with liver NO and MDA levels.

Sialic acids (SA) as derivatives of N-acetyl neuraminic acid are important in cellular acceptance and adhesion events in macromolecule and receptor components as terminal carbohydrate residues of the oligosaccharide side chain of glycoproteins, polysaccharides, and mucoproteins [27]. It is noted that SA concentration has increased in many health disorders such as cardiovascular [28], cancer [29], chronic glomerulonephritis and diseases related to renal disorders [30]. Sialic acids have been reported to help prevent oxidative stress by functioning primarily to remove oxygen from the vascular system [31,32]. It was reported that oxygen production in the vascular system increased when the SAs were removed in neutrophils [31]. It has also been reported that SA may be evaluated as a marker of peroxidation severity as a product of membrane lipid peroxidation degradation [33]. In this study, it was thought that kidney TSA levels were increased in alone indomethacin treated mice compared to the control group. Decrease of these levels in group given indomethazine plus ellagic acid might be due to the antioxidative action of ellagic acid in the kidney. This opinion is supports by lower MDA levels in the ellagic acid-treated group compared to other groups (**Table 1**).

It was reported that ghrelin had directly and indirectly effects on activating different protective mechanisms in providing gastric mucosal integrity in animals exposed to various harmful substances, and NO was important for gastroprotective and hyperemic effects of ghrelin [34]. It has been suggested that nitric oxide synthase (NOS) inhibition totally reverses the gastroprotective effect of ghrelin against experimental ulcers and ghrelin promotes luminal NO concentration via NOS in gastric stress conditions [34,36]. In the present study, it was determined that all ghrelin and plasma NO levels in mice increased significantly indomethacin and ellagic acid combination compared with mice applied indomethacin alone. Considered in this regard, ellagic acid may be useful on levels of ghrelin which are important functions in controlling food intake. Indomethacin has been reported to reduce NOS activity [36]. Ellagic acid has antioxidative effects such as inhibition of nitrate reactions and peroxynitrite-induced radicals and lipid peroxidation in vivo and in vitro [3,37]. In the present study, it was determined that mouse liver and kidney tissue NO levels significantly increased in indomethacin group compared to the other groups, and these levels were reduced by ellagic acid treatment to a rate close to the control group (**Table 1**). According to this findings, it can be argued that ellagic acid may contribute to prevention of NO-induced peroxynitrite formation.

In a study applied on rats, it was recorded that more than 2/3 of the stomach mucosa occured peelings and large openings after 4 hours from application of orally 30 mg/kg indomethacin followed pyloric ligation [38]. In a similar study, it was reported that severe hemorrhagic lesions occurred at the level of closing the entire gland region of rat stomach after six hours from administration of orally 25 mg/kg indomethacin [39]. It has been reported that protective additives such as sodium alginate or L-carnitine were effective in improving the

lesions [38,39]. In the present study, the stomach tissues of mice treated orally 25 mg/kg indomethacin showed erosive and ulcerative changes due to the deterioration of gastric mucosal integrity and to be poured of epithelial cells after six hours. It was observed mucosal hyperemia and bleedings with necrosis of mucosal surface and foveola epithelium in tissue samples with severe lesions. The ellagic acid treatment was an important protective to reduce the ulceration and lesions caused by indomethacin administration (**Table 3**). These protective effects of the ellagic acid are probably caused by inhibition of the reactive substances.

It was reported that indomethacin has a high risk of gastrointestinal toxicity in COX-2 non-specific NSAID drugs and its derivatives rather than indomethacin inhibit COX-2 production [40]. Indomethacin is used experimentally in gastric damage-inducing studies because it inhibits the metabolic pathway of cyclooxygenase-prostaglandin (COX-PG) in the gastric mucosal defense system and has inflammatory effects in the gastrointestinal tract [8,34,41]. In this study, it was detected intense and severe COX-2 immunoreactivity in mice with gastric mucosal injury (**Figure II**). In the mice given ellagic acid plus indomethacin, it was found that the COX-2 immunoreactivity was markedly decreased compared with alone indomethacin-treated group (**Figure IV**). Accordingly, it is conceivable that ellagic acid may have important functions in suppression of the COX-2 reactivity. In addition, the increases in TSA levels were parallel to this while the COX-2 reactivity increased during gastric injury. The increased TSA levels may also reduce immunohistochemical staining due to the intense sialic acids in the cellular region where the COX-2 antibody will react [42].

As a result, it was concluded that mice with indomethacin application could show protective effects by reducing oxidative stress of ellagic acid on changes in NO, TSA, MDA and ghrelin levels in plasma, kidney and liver tissues with COX 2 reactivity of stomach. It is also needed further investigations related with contribute rate of indomethacin and ellagic acid to the production and elimination of free radicals in the organism.

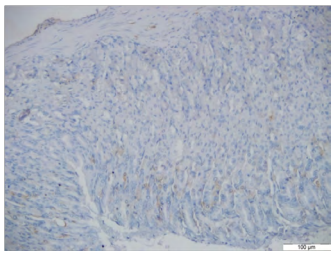

Figure I. COX-2 immunostaining of mouse stomach in control group

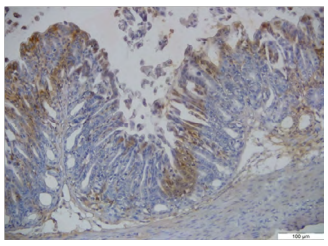

Figure II. Severe COX-2 immunostaining in indomethacin-treated group (white arrowheads)

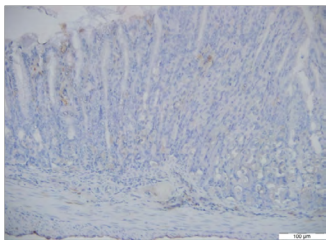

Figure III. COX-2 immunostaining of mouse stomach in ellagic acid group (white arrowhead)

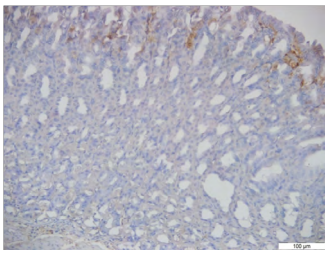

Figure IV. COX-2 immunostaining in ellagic acid plus indomethacin treated group (white arrowheads)

7. Acknowledgement

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References

1. Belaiche J, Burette A, De MV, Louis E, Huybrechts M, Deltenre M. Observational survey of NSAID-related upper gastro-intestinal adverse events in Belgium. Acta Gastroenterol Belg. 2002; 65:65-73.

2. Solon S, Lopes L, de Sousa Jr PT, Schmeda-Hirschmann G. Free radical scavenging activity of Lafoensia pacari. J Ethnopharmacol. 2000; 72:173-8.

3. Ippoushi K, Takeuchi A, Azuma K. Prevention of peroxynitrite-induced oxidation and nitration reactions by ellagic acid. Food

Chem. 2009; 112:185-8.

4. Dolatshahi M, Farbood Y, Sarkaki A, Mansouri SMT, Khodadadi A. Ellagic acid improves hyperalgesia and cognitive deficiency in 6-hydroxidopamine induced rat model of Parkinson's disease. Iran J Basic Med Sci. 2015; 18(1):3846.

5. Murakami S, Isobe Y, Kijima H, Nagai H, Muramatu M, Otomo S. Inhibition of gastric H+, K+-ATPase and acid secretion by ellagic acid. Planta Med. 1991; 57:305-8.

6. Miura T, Muraoka S, Fujimoto Y. Lipid peroxidation induced by indomethacin with horseradish peroxidase and hydrogen peroxide: involvement of indomethacin radicals. Biochem Pharm. 2002; 63:2069-74.

7. Dehpour AR, Mani AR, Amanlou M, Nahavandi A, Amanpour S, Bahadori M. Naloxone is protective against indomethacin-induced gastric damage in cholestatic rats. J Gastroenterol. 1999; 34:178-81.

8. Konturek SJ, Brzozowski T, Majka J, Pytko-Polonczyk J, Stachura J. Inhibition of nitric oxide synthase delays healing of chronic gastric ulcers. Eur J Pharmacol. 1993; 239:215-7.

9. Khattab MM, Gad MZ, Abdallah D. Protective role of nitric oxide in indomethacin-induced gastric ulceration by a mechanism independent of gastric acid secretion. Pharmacol Res. 2001; 43:463-7.

10. Kojima M, Kangawa K. Ghrelin: structure and function. Physiol Rev. 2005; 85:495-522.

11. Takeuchi K, Sugamoto S, Yamamoto H, Kawauchi S, Tashima K. Interactive roles of endogenous prostaglandin and nitric oxide in regulation of acid secretion by damaged rat stomachs. Aliment Pharmacol Ther. 2000; 14:125-34.

12. Yoshimura R, Matsuyama M, Kawahito Y, Tsuchida K, Kuratsukuri K, Takemoto Y, et al. Study of cyclooxygenase-2 in renal cell carcinoma. Int J Mol Med. 2004; 13:229-33.

13. Güçer H, Şahan E, İğdem AA, Tetikkurt ÜS, Erdoğan N. Cox-2 Expression and microvessel density in clear cell type renal cell carcinoma. Turk Patoloji Derg. 2009; 25:13-19.

14. Guidobono F, Pagani F, Ticozzi C, Sibilia V, Pecile A, Netti C. Protection by amylin of gastric erosions induced by indomethacin or ethanol in rats. Br J Pharmacol. 1997; 120:581-6.

15. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide. 2001; 5:62-71.

16. Yoshioka T, Kawada K, Shimada T, Mori M. Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. Am J Obstet Gynecol. 1979; 135:372-6.

17. Beutler E. Red cell metabolism: a manual of biochemical methods. Grune & Stratton, Newyork 1976; 49(3): 310-311.

Sydow G. A simplified quick method for determination of sialic acid in serum. Biomed Biochim Acta. 1985; 44:1721-3.

18. Vattem DA, Ghaedian R, Shetty K. Enhancing health benefits of berries through phenolic antioxidant enrichment: focus on cranberry. Asia Pac J Clin Nutr. 2005; 14:120-30.

19. Selim MM, ElShal EB, Elwahab AHA. Effect of ellagic acid on gastric mucosa of experimentally induced gastric ulcer: Histological and immunohistochemical study. EJPMR. 2016; 3:658-667.

20. Farbood Y, Rashno M, Ghaderi S, Khoshnam SE, Sarkaki A, Rashidi K, et al. Ellagic acid protects against diabetes-associated behavioral deficits in rats: Possible involved mechanisms. Life Sci. 2019; 225:8-19.

21. Mullen W, Yokota T, Lean ME, Crozier A. Analysis of ellagitannins and conjugates of ellagic acid and quercetin in raspberry fruits by LC–MSn. Phytochemistry. 2003; 64:617-24.

22. Iino T, Tashima K, Umeda M, Ogawa Y, Takeeda M, Takata K, et al. Effect of ellagic acid on gastric damage induced in ischemic rat stomachs following ammonia or reperfusion. Life Sci. 2002; 70:1139-50.

23. Berkban T, Boonprom P, Bunbupha S, Welbat JU, Kukongviriyapan U, Kukongviriyapan V, et al. Ellagic acid prevents L-NAME-induced hypertension via restoration of eNOS and p47phox expression in rats. Nutrients. 2015; 7:5265-80.

24. Dursun H, Albayrak F, Bilici M, Koc F, Alp HH, Candar T, et al. Gastroprotective and antioxidant effects of opipramol on indomethacin-induced ulcers in rats. Yakugaku Zasshi. 2009; 129:861-9.

25. Bandyopadhyay D, Biswas K, Bandyopadhyay U, Reiter RJ, Banerjee RK. Melatonin protects against stress-induced gastric lesions by scavenging the hydroxyl radical. J Pineal Res. 2000; 29:143-51.

26. Schauer R. Chemistry, metabolism, and biological functions of sialic acids. Advances in carbohydrate chemistry and biochemistry. Adv Carbohydr Chem Biochem. 1982; 131-234.

27. Serdar Z, Yeşilbursa D, Dirican M, Sarandöl E, Serdar A. Sialic acid and oxidizability of lipid and proteins and antioxidant status in patients with coronary artery disease. Cell Biochem Funct. 2007; 25:655-64.

28. Kökoğlu E, Sönmez H, Uslu E, Uslu I. Sialic acid levels in various types of cancer. Cancer Biochem Biophys. 1992;13:57-64.

29. Sillanaukee P, Ponnio M, Jaaskelainen IP. Occurrence of sialic acids in healthy humans and different disorders. Eur J Clin Invest. 1999; 29:413-25.

30. Henricks PA, Van Erne-van der Tol ME, Verhoef J. Partial removal of sialic acid enhances phagocytosis and the generation of superoxide and chemiluminescence by polymorphonuclear leukocytes. J Immunol. 1982; 129:745-50.

31. Kumagai R, Lu X, Kassab GS. Role of glycocalyx in flow-induced production of nitric oxide and reactive oxygen species. Free Radic Biol Med. 2009; 47:600-607.

32. Yapar K, Kart A, Karapehlivan M, Citil M. Dose-dependent effects of L-carnitine on blood sialic acid, mda and gsh concentrations in BALB/c mice. Acta Vet. 2007; 57:321-7.

33. Brzozowski T, Konturek PC, Konturek SJ, Kwiecień S, Drozdowicz D, Bielanski W, et al. Exogenous and endogenous ghrelin in gastroprotection against stress-induced gastric damage. Regul Pept. 2004; 120:39-51.

34. Sibilia V, Torsello A, Pagani F, Rapetti D, Lattuada N, Locatelli V, et al. Effects of hexarelin against acid-independent and acid-dependent ulcerogens in the rat. Peptides. 2004; 25:2163-70.

35. Rao CV, Reddy BS, Steele VE, Wang CX, Liu X, Ouyang N, et al. Nitric oxide–releasing aspirin and indomethacin are potent inhibitors against colon cancer in azoxymethane-treated rats: effects on molecular targets. Mol Cancer Ther. 2006; 5:1530-8.

36. Devipriya N, Sudheer AR, Menon VP. Dose-response effect of ellagic acid on circulatory antioxidants and lipids during alcohol-induced toxicity in experimental rats. Fundam Clin Pharmacol. 2007; 21:621-30.

37. El-Awdan SA, Zaki HF, Salam OMA, El-Iraqy WI, Kenawy SA. Impact of the dopaminergic system on mucosal integrity in indomethacin-induced gastric ulcers in rats: possible modulation by ranitidine or L-carnitine. Pharmacologia 2013; 4(1): 22-23.

38. Yamamoto A, Itoh T, Nasu R, Nishida R. Sodium alginate ameliorates indomethacin-induced gastrointestinal mucosal injury via inhibiting translocation in rats. World J Gastroenterol. 2014; 20:2641-52.

39. Gökşen US, Kelekçi NG. A new avenue in anti-inflammatory therapy: Dual inhibitors of cyclooxygenase and 5-lipoxygenase. Hacettepe Univ Eczacilik Fak Derg. 2010; 1:81-118.

40. Naito Y, Yoshikawa T, Matsuyama K, Nishimura S, Yagi M, Kondo M. Effects of free radical scavengers on indomethacin-induced aggravation of gastric ulcer in rats. Dig Dis Sci. 1995; 40:2019-2021.

41. Sproviero D, Julien S, Burford B, Taylor-Papadimitriou J, Burchell JM. Cyclooxygenase-2 enzyme induces the expression of the α-2, 3-sialyltransferase-3 (ST3Gal-I) in breast cancer. J Biol Chem. 2012; 287: 44490-7.