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The effects of antioxidant combination on indomethacin-induced gastric mucosal injury in rats

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Abstract: The aim of this study is an investigation the protective effects of vitamin C (Vit C), vitamin E (Vit E), β-carotene, sodium selenate combination in indomethacin-induced gastric mucosal damage in rats. Rats were divided into 6 groups. Group I: Intact animals (control). Group II: Control animals receiving Vit C (100 mg/kg/day), Vit E (100 mg/kg/day), β-carotene (15 mg/kg/day) and sodium selenate (0.2 mg/kg/day) for 3 days. Group III: Animals receiving 25 mg/kg indomethacin. Group IV: Animals receiving Vit C, Vit E, β-carotene and sodium selenate (in same doses) for 3 days 2 h before the administration of indomethacin. Group V: Animals receiving ranitidine (150 mg/kg) for 3 days. Group VI: Animals receiving ranitidine for 3 days 2 h before to the administration of indomethacin (in same dose and time). The administration of indomethacin caused a decrease in the levels of glutathione, mucus, hexosamine and in the activities of glutathione-S-transferase, sodium-potassium ATPase, thromboplastic activity and an increase in the aspartate and alanine amino transferase, alkaline phosphatase, catalase, lactate dehydrogenase, myeloperoxidase activities and sialic acid, lipid peroxidation and protein carbonyl levels. Stomach caspase-8 immun$^+$ cell numbers showed a slight increase while caspase-9 immun$^+$ cell numbers reduced in indomethacin given group compared to control animals. Our results findings suggest that the combination of Vit C, Vit E, β-carotene, sodium selenate and ranitidine has a protective effect on indomethacin-induced gastric mucosal injury of rats.

Key words: Stomach; Antioxidant; Indomethacin; Oxidative stress; Apoptosis.

Introduction

Ulcers have been a big problem all around the world. It develops when the balance between normal defense and repair systems of stomach are weaken (1). The reason for the breaking of the balance is various agents like chemicals, stress, ischemia-reperfusion, nonsteroidal anti-inflammatory drugs (NSAIDs) especially indomethacin (2-4).

NSAIDs are used for their analgesic, anti-inflammatory and cardiovascular protective properties all around the world (5). Although they are preferred so much, their side effects cause undesirable results. The major adverse effects of NSAIDs occur in gastrointestinal system (6). Indomethacin, an indole acetic acid derivative NSAID, is commonly used for the treatment of rheumatoid arthritis, osteo- and gut arthritis, burst, tendinitis, traumatic synovitis and ankylosing spondylitis (7). Its usage is linked in a significant risk of hemorrhage and erosions of gastric and intestinal ulcers (8). However, gastric ulcer is the most known side effect of this drug. Indomethacin causes gastric mucosal damage by using many processes. It inhibits cyclooxygenase (COX) activity and reduces prostaglandin production (9). However, some reporters indicate reactive oxygen species (ROS) also play an important role in indomethacin induced gastric damages because drug has pro-oxidant activity (10).

ROS include superoxide, hydroxyl radicals, singlet oxygen, free radicals and peroxides (11). These species can attack to cells. If cells do not have enough defense systems, cell injuries might develop. In normal conditions, there is a balance between the generation of free radicals and antioxidant defense mechanism which include enzymatic and non-enzymatic antioxidant systems. The enzymatic and non-enzymatic antioxidant defences include superoxide dismutase, glutathione peroxidase, catalase, glutathione, glutathione reductase, α-tocopherol, ascorbic acid, β-carotene and vitamin A (12). These antioxidants probably exert their effects through their ability to scavenge reactive oxidants (13).

Vit C, a very important dietary antioxidant and a water soluble vitamin, has an ability of scavenging reactive oxygen species. Vit C has an ability to regenerate the real form of antioxidants, e.g. α-tocopherol, urate, β-carotene, when they become radical forms. Vit E is also an antioxidant which exists naturally in biological systems and this vitamin is concentrated many tissues, specially gastric and intestinal tissues. It is a major defense system itself due to scavenging of free radicals (14). β-carotene, which is responsible for the color of various fruits and vegetables, is also an anti-cancer agent as well as an inhibitor of inflammatory diseases (15). Selenium is an important essential trace element for many organisms (16). It plays a vital role in various biological and physiological functions including stabili-

zation of membrane structure, protection of macromolecules from oxidative attack, activation of some antioxidant enzymes (17). Ranitidine, one of the H_2 receptor antagonists, is widely used for treatment of prophylaxis and gastrointestinal system injuries (18).

Caspases are a family of endoproteases that play essential roles in inflammation and programmed cell death. These enzymes have been broadly classified with their major functions. Their known roles are in apoptosis (caspase-3, -6, -7, -8 and -9) and in inflammation (caspase-1, -4, -5, -11 and -12). Furthermore, the classification of caspases in apoptosis is divided into initiator caspases (caspase-8 and -9) or executioner caspases (caspase-3, -6 and -7) (19,20).

Although there have been many studies about gastric mucosal damage and prevention of this disease with antioxidants, there is not any study with Vit C, Vit E, β-carotene and selenium combination and ranitidine on indomethacin-induced gastric damage. Thus, our study will be the first study with quartet combination of these antioxidants and also ranitidine for the protection and healing of gastric injury induced by indomethacin.

Materials and Methods

Animals

The experimental procedures were performed according to the guidelines of the Local Ethic Committee of Animal Research (Istanbul University, HADYEK/21). In this study, 2.5-3 months aged male Sprague-Dawley rats were used. Their diet consisted of standart animal pellet food and tap water *ad libitum*.

Experimental design

A total of 48 rats were divided into 6 groups as follows. Group I: Intact animals (control, n=8). Group II: Control animals receiving Vit C (100 mg/kg/day), Vit E (100 mg/kg/day), β-carotene (15 mg/kg/day) and sodium selenate (0.2 mg/kg/day) for 3 days (n=8). Group III: Animals receiving 25 mg/kg indomethacin (n=8). Group IV: Animals receiving Vit C, Vit E, β-carotene and sodium selenate (in same doses) for 3 days 2 h before the administration of indomethacin (n=8). Group V: Animals receiving ranitidine (150 mg/kg) for 3 days (n=8). Group VI: Animals receiving ranitidine for 3 days 2 h before to the administration of indomethacin (in same dose and time) (n=8). The animals (Group III) were sacrificed at 6 hours after indomethacin administration. The animals (Group IV and VI) were sacrificed at 8 hours after antioxidants, ranitidine and indomethacin under anesthesia. The antioxidants, ranitidine and indomethacin were given to rats by gavage.

Animal model for gastric mucosal lesions

The gastric mucosal lesions were produced by a single oral dose of indomethacin 25 mg/kg after 18 h fasting (21).

Biochemical assays

Blood samples were withdrawn by syringe from hearts of the animals. Biochemical investigation was made in blood, serum and tissue samples. For the biochemical analyzes, the tissue samples of the stomach were washed with physiological saline and kept frozen until the day of the experiments. On the day of experiments, stomach tissue was homogenized in cold 0.9% NaCl with a glass homogenizer to make up 10% (w/v) homogenate. The homogenates were centrifuged. The supernatant fraction was removed for the biochemical analysis. Blood reduced glutathione (GSH) was determined according to the method of Beutler et al (22). Serum aspartate (AST) and alanine aminotransferase (ALT) activities were measured by the method of Reitman and Frankel (23). Serum alkaline phosphatase (ALP) activity was assessed by Two Point Method (24).

The mucus content in gastric tissue was estimated as described by Corne et al (25). The gastric mucosal hexosamine content was used as an index of glycoprotein. Hexosamine concent was estimated by the method by Winzler (26) using acetyl acetone reagent and Ehrlich's reagent. Sialic acid levels were assayed by the method described by Lorentz et al (27). Tissue factor (TF) activity of stomach tissue was evaluated according to Quick's one-stage method using normal plasma (28). TF activity was expressed in seconds. Shorter clot formation time indicates increased TF activity.

The reduced GSH level in the stomach tissue was determined according to Beutler's method using Ellman's reagent (29). Lipid peroxidation in gastric tissue, as an index of malondialdehyde (MDA) production was assayed by the method of Ledwozyw et al (30). The protein content in supernatants was estimated by the method of Lowry using Bovine Serum Albumin (BSA) as a standart (31). Protein carbonyl content (PCC) level was assessed as described by Levine et al (32).

Catalase (CAT) activity was determined according to the method of Aebi (33). Glutathione -S- transferase (GST) activity was determined by the Habig and Jacoby (34). Lactate dehydrogenase (LDH) activity was measured by Bais and Philcox (35). The activity of Na^+-K^+-ATPase, a membrane-bound enzyme required for cellular transport, was measured by the method of Ridderstap and Bonting (36). Tissue associated myeloperoxidase (MPO) activity, an indicator of tissue neutrophil infiltration was determined in the stomach by the method described Wei and Frenkel method (37).

Immunohistochemical assays

For immunohistochemical studies, stomach tissues were fixed and embedded in paraffin. Embedding tissues in paraffin blocks cut into 5 μm sections. Deparaffinized sections heated in citrate buffer (0.01 mol / L, pH6.0) in a microwave oven for 25 min for antigen retrieval. Then slides were treated with 3% hydrogen peroxide (H_2O_2) solution in methanol for 15 min to inactive endogenous peroxidase. Immunoreactivities of antibodies were detected using a biotin-streptavidin-peroxidase-based Histostain Plus Broad Spectrum Kit (Invitrogen, 859043). The blocking solution in Histostain Plus Broad Spectrum Kit was used for caspase-3 and -9 slides for 30 min. The slides were incubated overnight at 4 °C with a primary polyclonal rabbit anti-rat antibody specific for caspase-9 (Neomarkers, RB-1205) at a dilution rate of 1:50 in an antibody diluent (Novex, 003118) and 48 h at 37 °C with a primary polyclonal rabbit anti-rat antibody specific for caspase-3 (Millipore, AB3623) at a dilution rate of 1:50 in an antibody diluent. The caspase-8 slides were blocked in 1 % BSA and 0.3% triton X-100 in Tris-

buffered saline (TBS) for 60 min and incubated overnight at 37 °C with anti-caspase-8 antibody (Neomarkers, RB-1200) at a 1:25 dilution rate in 1 % BSA. After three washes with PBS, the sections were incubated with a biotinylated secondary antibody for 15 min at room temperature and incubated with horseradish peroxidase (HRP)-labeled streptavidin. Antibody binding was visualized by using 3-amino-9-ethylcarbazole (AEC) and sections were counterstained in Mayer's hematoxylin for 40 seconds at room temperature and mounted in glycerin gelatin. For negative control, primary antibody was replaced with PBS. Tissue slides were examined using a Nikon Eclipse 80i light microscope equipped with a digital camera using NIS-Elements-D 3.1 microscope imaging software program. For the expressions of caspase-3, -8 and -9, ten randomly selected areas in each slides (n=5) were examined.

Statistical analyses

Biochemical results were evaluated using an unpaired t test and analysis of variance (ANOVA) using the NCSS computer package. The values were expressed as mean ± SD. Statistical calculations for immunohistochemical results were performed using SPPS software (version 21.0, SPSS, USA). Data are expressed as the mean ± standart error of the mean (SEM) for each group. The data were analyzed with One-Way ANOVA method followed by Tukey's post hoc test. The value of $P < 0.05$ was considered statistically significant.

Results

Biochemical results

Blood GSH levels and serum AST, ALT and ALP activities of all groups are given in Table 1. According to the results, indomethacin treatment decreased blood GSH and increased serum ALP ($P < 0.0001$), AST and ALT activities ($P < 0.05$) in a significant manner when we compared to control group. While antioxidant treatment reversed GSH levels significantly ($P < 0.05$), this treatment reversed serum enzyme parameters insignificantly in indomethacin group. However, ranitidine treatment reversed blood GSH levels ($P < 0.0001$) and serum AST, ALT and ALP activities significantly as we compared to control group ($P < 0.05$ and $P < 0.005$ respectively).

Stomach mucus, hexosamine, sialic acid levels and TF activities of all groups are shown in Table 2. Administration of indomethacin decreased mucus and hexosamine levels significantly while sialic acid level and TF activity were found to be increased significantly ($P < 0.005$, $P < 0.0001$, $P < 0.05$ respectively). Antioxidant treatment reversed these effects in a significant manner ($P < 0.05$, $P < 0.005$) except in sialic acid levels. However ranitidine treatment reversed all the levels and activities in this table significantly in indomethacin group ($P < 0.0001$, and $P < 0.05$ respectively).

In Table 3, stomach GSH, LPO and PCC levels are shown. Administration of indomethacin decreased GSH levels ($P < 0.001$), and increased PCC levels significantly ($P < 0.0001$) while the level of increase in LPO levels was insignificant as we compared to control group. Antioxidant and ranitidine treatment to indomethacin group increased GSH levels in a significant manner ($P < 0.05$, $P < 0.005$ respectively). In addition, antioxidant and ranitidine treatments reversed LPO levels in indomethacin group insignificantly. However, antioxidant treatment decreased PCC levels significantly ($P < 0.05$) while ranitidine treatment reversed this level insignificantly in indomethacin group.

Stomach CAT, GST, LDH, Na^+/K^+-ATPase, MPO activities of all groups are given in Table 4. Accord-

Table 1. Blood GSH levels and serum AST, ALT, ALP activities of all groups.

Groups	GSH (mg %)*	AST (U/L)*	ALT (U/L)*	ALP (U/L)*
Control	45.08 ± 3.44	164.72 ± 3.24	79.37 ± 5.54	55.98 ± 5.34
Control+Antioxidant	48.87 ± 3.47	173.90 ± 1.68	80.66 ± 1.76	115.98 ± 3.55
Control+Ranitidine	53.66 ± 5.04	159.53 ± 4.91	68.90 ± 4.11	62.89 ± 3.98
Indomethacin	34.70 ± 3.00[a]	175.96 ± 4.08[d]	90.86 ± 5.50[d]	96.34 ± 10.85[a]
Indomethacin+Antioxidant	39.74 ± 3.80[b]	169.75 ± 0.35	81.00 ± 7.00	95.70 ± 7.04
Indomethacin+Ranitidine	56.58 ± 6.51[c]	166.44 ± 5.29[b]	75.80 ± 4.60[e]	61.62 ± 6.11[e]
P_{ANOVA}	0.0001	0.001	0.0001	0.0001

*Mean ± SD; [a]$P < 0.0001$ versus control group; [b]$P < 0.05$ versus indomethacin group; [c]$P < 0.0001$ versus indomethacin group; [d]$P < 0.05$ versus control group; [e]$P < 0.005$ versus indomethacin group.

Table 2. Stomach mucin, hexosamine, sialic acid contents and thromboplastic activities of all groups.

Groups	Mucus (µg/g tissue)*	Hexosamine (µg glucosamine/ mg protein)*	Sialic Acid (µmol/g protein)*	Thromboplastic Activity (sec)*
Control	491.62 ± 44.79	2.59 ± 0.62	70.00 ± 2.82	78.33 ± 15.71
Control+Antioxidant	579.62 ± 57.44	1.51 ± 0.66	93.18 ± 34.55	86.00 ± 6.57
Control+Ranitidine	563.14 ± 132.42	3.46 ± 2.85	99.48 ± 8.29	69.8 ± 7.30
Indomethacin	263.46 ± 24.17[a]	0.70 ± 0.42[d]	207.4 ± 77.6	50.2 ± 12.35[f]
Indomethacin+Antioxidant	377.55 ± 48.79[b]	3.42 ± 2.06[e]	172.00 ± 45.25	76.58 ± 10.24[e]
Indomethacin+Ranitidine	419.83 ± 43.35[c]	2.98 ± 1.00[c]	92.4 ± 48.36[b]	69.75 ± 6.22[b]
P_{ANOVA}	0.0001	0.010	0.004	0.0001

*Mean ± SD; [a]$P < 0.005$ versus control group; [b]$P < 0.05$ versus indomethacin group; [c]$P < 0.0001$ versus indomethacin group; [d]$P < 0.0001$ versus control group; [e]$P < 0.005$ versus indomethacin group; [f]$P < 0.05$ versus control group.

Table 3. Stomach GSH, LPO and PCC levels of all groups.

Groups	GSH (nmol GSH/ mg protein)*	LPO (nmol MDA/ mg protein)*	PCC (nmol carbonyl/ mg protein)*
Control	17.99 ± 1.19	0.66 ± 0.37	10.16 ± 0.97
Control+Antioxidant	20.02 ± 4.70	0.58 ± 0.34	20.14 ± 0.91
Control+Ranitidine	12.89 ± 6.30	1.49 ± 1.10	12.53 ± 2.21
Indomethacin	7.08 ± 2.09[a]	0.97 ± 0.22	23.89 ± 3.10[d]
Indomethacin+Antioxidant	17.53 ± 5.74[b]	0.88 ± 0.43	14.21 ± 2.26[b]
Indomethacin+Ranitidine	12.88 ± 1.19[c]	0.80 ± 0.52	20.26 ± 4.24
P_{ANOVA}	0.029	0.268	0.0001

*Mean ± SD; [a]$P < 0.001$ versus control group, [b]$P < 0.05$ versus indomethacin group, [c]$P < 0.005$ versus indomethacin group,[d]$P < 0.0001$ versus control group.

Table 4. Stomach CAT, GST, LDH, Na^+/K^+-ATPase and MPO activities of all groups.

Groups	CAT (U/mg protein)*	GST (U/g protein)*	LDH (U/g protein)*	Na^+/K^+-ATPase (µmol Pi /mg proteinxh)*	MPO (mU/ g tissue)*
Control	3.56 ± 2.46	2.63 ± 0.69	5.87 ± 1.77	99.35 ± 8.54	0.706 ± 0.175
Control+Antioxidant	3.83 ± 2.00	1.63 ± 0.45	7.82 ± 1.60	113.00 ± 47.81	0.833 ± 0.715
Control+Ranitidine	5.39 ± 1.64	0.30 ± 0.08	3.54 ± 0.65	165.43 ± 26.79	0.848 ± 0.62
Indomethacin	5.87 ± 1.84[a]	1.50 ± 0.80[a]	14.34 ± 2.43[c]	65.46 ± 8.46[a]	3.954 ± 1.537[a]
Indomethacin+Antioxidant	2.96 ± 1.63[b]	0.67 ± 0.10	6.35 ± 1.86[d]	125.42 ± 22.95[d]	1.362 ± 0.801[b]
Indomethacin+Ranitidine	4.48 ± 2.37	0.29 ± 0.02[b]	5.81 ± 0.68	74.19 ± 20.41	1.021 ± 0.456[c]
P_{ANOVA}	0.103	0.0001	0.0001	0.013	0.0001

*Mean ± SD; [a]$P < 0.05$ versus control group, [b]$P < 0.05$ versus indomethacin group, [c]$P < 0.0001$ versus control, [d]$P < 0.0001$ versus indomethacin group, [e]$P < 0.005$ versus indomethacin group.

ing to the results, we observed significant increases in LDH ($P < 0.0001$), CAT, MPO activities and significant decreases in GST and Na^+/K^+-ATPase in indomethacin group as we compared to control group ($P < 0.05$). Administration of antioxidant reversed all enzyme activities significantly ($P < 0.05$, $P < 0.0001$) except GST activity in indomethacin group. Ranitidine treatment also reversed GST and MPO activities in a significant manner ($P < 0.05$, $P < 0.005$ respectively) while the reversing effect on CAT, LDH and Na^+/K^+-ATPase activities were insignificant.

Immunohistochemical results

Determination of caspase-3, -8 and -9 expressions in stomach

We have not observed any change in the number of caspase-3 immun$^+$ cells among groups. However, the number of caspase-8 showed a slight increase in indomethacin given group compared to control animals. In stomach of indomethacin + ranitidine treated rats, the number of caspase-8 immun$^+$ cells was the same with indomethacin given rats. The mean of caspase-8 immun$^+$ cells was showed very little reduction in indomethacin + Vit C + Vit E + β-carotene + sodium selenate group when compared to indomethacin group. Compared to the control rats, the number of caspase-9 immun$^+$ cells in indomethacin group was insignificantly reduced, whereas in indomethacin + antioxidant and indomethacin + ranitidine groups were observed an increase (Fig. 1).

Discussion

All around the world, many patients use more than one drug at the same time (38). So the effects of combined drugs have recently become an important issue. NSAIDs are widely used for the treatment of pain, in-

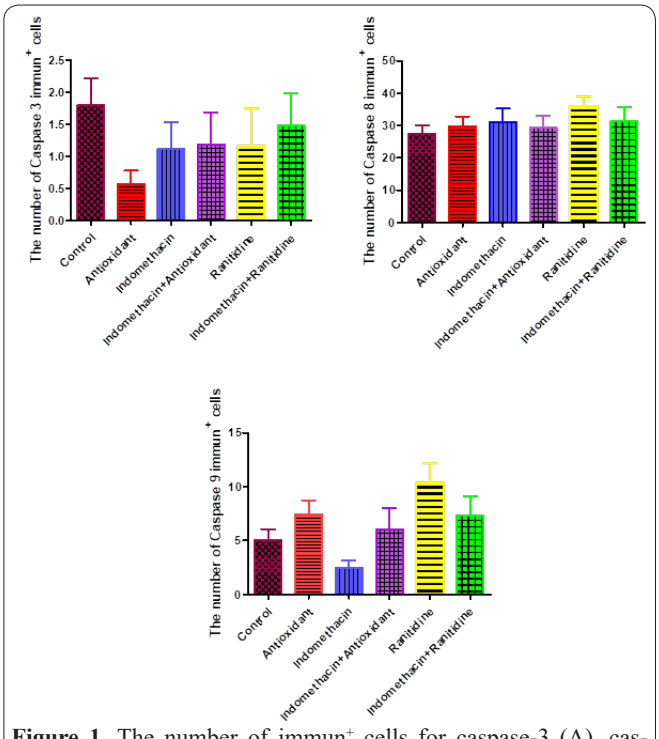

Figure 1. The number of immun$^+$ cells for caspase-3 (A), caspase-8 (B) and caspase-9 (C) in stomach of all groups. Graphical results were reported as mean ± SEM.

flammation, rheumatic disorders, and osteoarthritis (39-40). However, dyspepsia, peptic ulceration, hemorrhage and even death are well known side effects of NSAIDs (41). So finding a solution for preventing these effects becomes crucial.

GSH is a major low molecular weight scavenger of free radicals in cytoplasm (42). In our study, blood GSH levels were significantly decreased probably due to its consumption during oxidative stress which is produced by the presence of indomethacin. Administration of antioxidants and ranitidine increased the content of reduced glutathione levels in the blood. This increase provides a protection against ROS.

Serum AST, ALT and ALP activities are indicators of hepatic injury as well as extra-hepatic tissue injuries (43). We have got elevated activities of these three enzymes in indomethacin group. This may suggest extra-hepatic injuries like indomethacin induced damages can elevate these enzyme activities. On the other hand, administration of antioxidants and ranitidine reversed these activities by using their protective effect.

Gastric mucus is an important factor for protection of gastric mucosa and consists of a viscous, elastic, adherent and transport gel formed by 95% water and 5% glycoproteins. Mucus is capable of acting as an antioxidant agent and can be damaged by oxygen free radicals (42-44). When cells are damaged by extracellular oxygen radicals, intracellular mucus might be released into the gastric tissue and thus prevention is provided for a possible additional damage by free radicals (45). In the present study, a significant reduction in the levels of mucus was observed in indomethacin group as compared to control rats. Administration of antioxidant and ranitidine significantly increased the amount of mucus produced by rat mucosa in indomethacin group. This enhancement supported by antioxidant and ranitidine may prevent the action of acid and pepsin on the stomach mucous epithelium (46).

Gastric mucosal hexosamine derived from macromolecular glycoprotein is a precursor in the biosynthesis of gastric mucin. A decreased level of hexosamine means quantitative reduction in mucus synthesis. This situation also indicates deterioration of mucosal enzyme activity involved in glycoprotein biosynthesis and glucose uptake during gastric hexosamine formation (47). In the present study, antioxidant and ranitidine prevented a marked decrease in gastric mucosal hexosamine levels, an index of gastric mucus.

Sialic acid is a terminal component of the non-reducing end of the carbohydrate chains of glycoproteins and glycolipids. They are suggested to be major participants in many biological functions (48). In this study, we found increased levels of sialic acid in indomethacin treated group. However, antioxidant combination and ranitidine pretreatment diminished this level and showed their protective effects.

TF is an important coagulation factor that initiates extrinsic blood coagulation with factor VII. It is a cell membrane component and its activity has been measured by prothrombin time test. Shortened clot formation time indicates increased TF activity. Increased TF activity was observed in the stomach tissue of indomethacin group according to control group. TF activity can be easily changed by alterations in membrane composition, heating, change in pH level or lipid peroxidation. Indomethacin is a source for lipid peroxidation process (49). The increased activity of TF in indomethacin group may be associated with this property. Administration of antioxidant and ranitidine reversed this activity in indomethacin group by using their protective effect about free radical scavenging.

ROS plays an important role in the pathogenesis of mucosal damage caused by indomethacin (50). Superoxides are one of the part of ROS and produced by peroxidases in the tissues can damage membranes and cause ulcer in the stomach tissues by increasing LPO (51). Preventive antioxidant such as GSH is the first line of defence against ROS. Based on the present results, indomethacin caused the reduction of GSH levels and an increase in LPO levels in the stomach tissues of rats. Antioxidant combination and ranitidine reversed these levels. In addition to antioxidant properties of vitamins and sodium selenate, ranitidine has also a protective effect on stomach tissue due to its antioxidant capacity as reported by Ahmadi et al (52).

The stomach ulceration is associated with oxidative damage to proteins, lipids and the thiol-dependent antioxidant defense systems (53). PCC is the most general indicator and used marker of protein oxidation (54). In the lights of this information, we found that indomethacin caused a considerable increase in the PCC as we compared to control group. Vit C, Vit E, β-carotene, sodium selenate and also ranitidine were found to attenuate indomethacin induced effects, probably due to scavenging activity of free radicals.

CAT is a highly reactive enzyme that reacts with H_2O_2 to form water and molecular oxygen. Experimental studies show that CAT activity increases in the presence of indomethacin-induced stomach damage (38,55). In the present study, the activity of CAT was found to be increased in indomethacin administrated rats as compared with the stomach tissues of healthy rat. This increase may be due to an elevated amount of mucosal H_2O_2 and OH^- level occurred by inhibition of peroxidase (56). However, administration of antioxidant combination and ranitidine possess reducing effect against increased CAT activity.

Na^+/K^+-ATPase is a membrane-dependent enzyme that enables the transport of sodium and potassium across the membrane against a concentration gradient by hydrolysis of ATP and maintenance of intracellular electrolyte homeostatis (57). Na^+/K^+-ATPase requires phospholipids for its activity and is very susceptible to structural changes due to lipid peroxidation. For reason, assessment of this enzyme activity can be used as an index for oxidant-induced tissue injury and lipid peroxidation. In this study, we found lowered Na^+/K^+-ATPase activity in indomethacin group in comparison with the control group. The decreased activity of membrane-bound ATPase may be due to the inactivation of phosphates by free radicals formed by indomethacin. In contrast to this situation, pretreatment either antioxidant combination or ranitidine significantly maintained the activities of membrane bound Na^+/K^+-ATPase by the counteraction of indomethacin-induced free radicals by the radical scavenging property of antioxidant activities.

The MPO enzyme is present in neutrophils and acts in the presence of superoxide anion and chloride anion

(Cl-) to form the hypochloric acid. In the present study, we demonstrated that MPO activity was increased in the gastric mucosa after indomethacin administration. The increase in this enzyme activity may be associated with increases in the levels of neutrophil infiltration and H_2O_2 in those gastric damage tissues with indomethacin administration. Antioxidants and ranitidine decreased MPO activity. This effect of antioxidants and ranitidine may be related their gastroprotective ability about either antioxidant activity or suppressive effect of acid secretion. In parallel to our study, Van Zyl et al.(58) have reported ranitidine and other H_2 antagonists in clinical use have potent inhibitory effect on MPO catalyzed reactions.

Indomethacin has some side effects especially on the gastrointestinal tract such as gastric mucosal erosions. In addition, it is the main cyclooxygenase-2 (COX-2) inhibitors. COX-2 is known playing roles in inflammation, carcinogenesis and apoptosis (59). In the study of Wallace et al. (60) on animals, inhibition of both COX-1 and COX-2 must occur for gastric injury. Caspase-8 has been associated with both activation of apoptosis and suppression of necrosis (19,61). In our study, we observed that indomethacin non-significantly enhanced caspse-8 expression in contrast to both caspase-3 and -9 expressions. It can be thought that indomethacin may induce the increased caspase-8 for suppression of increased necrosis in gastric injury. In addition, the decrease in caspase-3 and -9 expressions may show that there is a reduction of apoptotic cell numbers. It can be originated from inhibition of COX-1 and COX-2. While caspase-8 expression was not change in indomethacin induced gastric injury with treated ranitidine, it showed a slight decrease with Vit C + Vit E + β-carotene + sodium selenate. These treatments also increased caspase-9 in indomethacin induced gastric injury. The rising in caspase-9 can induce apoptosis and, it may prevent carcinogenesis. The studies indicated that COX-2 expression was upregulated in gastric, colon, pancreas cancers (62-64). The apoptotic markers (caspase-3 and -9) may be decreased due to indomethacin induced COX-2 inhibition.

In conclusion, Vit C, Vit E, β-carotene and sodium selenate can be united for protective effects on indomethacin induced gastric mucosal damage by decreasing the negative effects of free radicals. In addition to this, ranitidine has an effect like suppressing acid secretion and also an antioxidant activity. Based on these properties, ranitidine can be used to prevent indomethacin induced stomach injury. According to our present findings, ranitidine, and combination of Vit C, Vit E, β-carotene, sodium selenate may cause apoptosis in indomethacin induced gastric injury. Thus, pretreatment either antioxidant combination or ranitidine may prevent carcinogenesis.

Conflict of interest

None.

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