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Protective Effects of Selenium and Vitamin E Combination on Experimental Colitis in Blood Plasma and Colon of Rats

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Abstract Ulcerative colitis increases oxidative damage accompanied by production of free oxygen radicals. Selenium (Se) and vitamin E are two natural antioxidants. The present study was undertaken to investigate the possible protective role of Se and vitamin E combination in experimental colitis induced by acetic acid (AA) in rats. This study was carried out on three groups, namely the first (control), the second (experimental colitis group, 2 ml 5% acetic acid), and the third groups (2 ml 5% acetic acid, vitamin E (100 mg/kg body weight (bw)) plus Se (0.2 mg/kg bw)). The activities of catalase (CAT), prolidase (PRS), myeloperoxidase (MPO), total antioxidant capacity (TAC), total oxidant status (TOS), oxidative stress index (OSI), total thiol (T-SH) were determined in plasma and colon samples. Macroscopic and microscopic damages in colon were increased by AA treatment ($p<0.01$ and $p<0.01$, respectively), whereas they were decreased by selenium and vitamin E treatment ($p<0.05$ and $p<0.01$, respectively). The activities of CAT and PRS in the plasma and colon were significantly affected ($p<0.05$ and $p<0.01$) by treatment of AA, Se, and vitamin E. MPO activity in colon was increased ($p<0.01$) by AA treatment and decreased ($p<0.05$) by Se and vitamin E administration. The values of TOS and OSI in plasma were increased ($p<0.5$) by AA. The TAC and T-SH in colon were decreased ($p<0.05$) by AA and increased

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($p<0.05$) by Se and vitamin E. Based upon these results, Se and vitamin E may play an important role in preventive indication of the oxidative damage associated by acetic acid caused inflammation.

Keywords Se · Vitamin E · Colitis · Acetic acid · Rats

Introduction

Experimental colitis induced by acetic acid in laboratory animals resembles to inflammatory bowel disease (IBD) in humans in terms of histopathological appearance. The colonic tissue is sensitive to acetic acid causing overproduction of oxidative mediators which have important roles in pathophysiology of colitis [1–4]. Different proinflammatory mediators including reactive oxygen species (ROS), hydrogen peroxide (H_2O_2), cytokines, accumulation of macrophages, and neutrophils in colonic mucosa may be responsible for IBD [4–7] and some skin disorders [8, 9]. In addition, ROS such as superoxide (O_2^-), the hydroxyl radical (OH^-), H_2O_2, hypochlorous acid (HOCl) and other free radicals in colitis may increase in colonic tissue. Free radicals have been implicated in etiopathogenesis of colitis [3, 4, 7, 10].

Se and vitamin E are two natural antioxidants which quench ROS [1, 3, 11–16]. Vitamin E may prevent the increase of free radicals (ROS) produced by oxidative damage of lipids and lipoproteins in cellular components and tissues [1, 11, 13, 16]. Se is also an essential element playing an important antioxidant role binding active site of glutathione peroxidase (GSH-Px). GSH-Px not only protects cells against damages that caused by free radicals but also permits regeneration of a cellular lipid molecule through reacylation [11]. ROS can attack double bonds in polyunsaturated fatty acids in cellular components and thus induce lipid peroxidation, which may result in more oxidative damage. Increasing attention has been focused on the role of ROS produced by activated neutrophils during IBD [1, 2, 4]. ROS molecules can highly attack cell components, causing further injury in colonic tissue [4, 8–10]. Several treatments have been used to protect the oxidative damage which caused by ROS in the colitis by administrations of some antioxidants [3, 10, 14, 17–19] in experimental animal models. The present study was designed to investigate the possible protective role of Se and vitamin E combination on the oxidative stress index, total oxidant status, antioxidant capacity, and enzyme activities in plasma and colonic tissue in experimental colitis induced by acetic acid in rats.

Materials and Methods

Animals and Treatments Thirty Wistar albino rats aged 2–2.5 months and weighing 150–200 g body weight were used in the study. During the study, animals were given water and fed ad libitum. Animal housing and experiments were carried out in accordance by the Guide for the Care and Use of Laboratory Animals and permit of ethical committee. Experimental animals were randomly divided into three equal groups ($n=10$). Animals were housed in cages at room temperature during the study. The first group (control) was intrarectally received only 2 mL physiologic saline (0.9% NaCl), the second group was given into rectum 2 mL 5% acetic acid and 2 mL physiologic saline was received by gastric gavage, and the third group was administrated 2 mL 5% acetic acid and vitamin E (100 mg/kg

body weight) plus Se (0.2 mg/kg body weight) were administered by gastric gavages. Acetic acid was purchased from Merck AG (Darmstadt, Germany). Se and vitamin E were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All treatments were applied three times in a week. After 24 h of the last dose being administered in 7th day, the blood samples were taken under ether sedation. The abdomens of all animals were opened by laparotomy, and their colon specimens were removed.

Obtaining Blood Samples The blood samples of all animals were taken by cardiac puncture in 12 h after the last application of acetic acid and Se and vitamin E. Whole blood was collected into the heparinized tubes (Beckon Dickinson Vacuntiner System Cedex France) and subsequently centrifuged at 1,500×*g* for 15 min. Plasma samples were stored at −80°C for further biochemical analysis.

Obtaining and Examination of the Colon Specimens At postmortem laparotomy, 6 cm of colon extending approximately from 2 cm above the anal margin was removed and split longitudinally. Colon specimens were divided into two portions. The first portion was stored at −80°C for further biochemical analysis. The other portion was used for histopathological examination. The macroscopic appearances of these colonic specimens scored on a scale adapted by Morris et al. [20] ranging from 0 to 4: 0—no macroscopic change; 1—mucosal erythema alone; 2—mild mucosal edema, slight bleeding, or small erosions; 3—moderate edema, bleeding, and ulcers; and 4—edema, severe ulceration, and tissue necrosis. Mean macroscopic scores were calculated according to four criteria above. Then, the colon specimens were fixed in formaldehyde (10%, *v*/*v*) and were embedded in paraffin for an ordinary histological examination and sectioned from 3 to 5 μ serial sections using a rotary microtome. The sections were stained by hematoxylin and eosin (H&E) for histological observations. The histological sections were evaluated according to the eight scales containing the vascular dilatation, edema, epithelial cell denudation, cellular mucin depletion, neutrophilic infiltration, eosinophilic infiltration, lymphocytic infiltration, and necrosis/ulceration, which have been described previously by Keshavarzian et al. [1]. The scores of above eight criteria were calculated on an ascending scale of 0 (normal), 1 (mild), 2 (moderate), 3 (severe), and 4 (most severe) for injuries in colonic tissue. The total score of eight criteria was measured as 32.

Biochemical Analysis Catalase (CAT) activity was assayed as described by Goth [21]. Serum T-SH was measured according to the method described by Kelly [22]. Total oxidant status (TOS) and total antioxidant capacity (TAC) were measured by using the methods described by Erel [23]. The percentage of TOS level to TAC level was regarded as the oxidative stress index (OSI) [24]. To perform the calculation, the unit of TAC (millimole Trolox equivalent per liter) was changed to micromole Trolox equivalent per liter, and the OSI was calculated as follows: OSI (arbitrary unit) = TOS [(micromole H_2O_2 equivalent per liter)/TAC (millimole Trolox equivalent per liter)×100]. Prolidase activities were measured by using the methods described by Myara et al. [25, 26]. The myeloperoxidase activity in the colonic specimens was analyzed by using the method described by Krawisz et al. [27].

Statistical Analysis Statistical analysis was carried out using the SPSS 11.5 statistical program (SPSS Inc., Chicago, IL, USA). For the analysis of macroscopic and microscopic scores and biochemical parameters, Kruskal–Wallis test and Mann–Whitney *U* tests were used for analysis of variance and post hoc test, respectively. The data were expressed as

means ± standard deviation (SD), and differences on the statistical analysis of results were considered to be significant at $p<0.05$.

Results

Macroscopic Findings The colon specimens taken from the control group usually demonstrated normal aspect. Only localized hyperemia was observed in one section. For the experimental colitis group, there were gross colonic damages characterized by marked mucosal thickening, edema, bleeding, linear ulceration in large areas, and tissue necrosis. Minimal mucosal erythema–edema, slight bleeding, or erosions were usually seen in Se and vitamin E group. Moderate edema, bleeding, and small ulcers occurred in some specimens. The scores of macroscopic changes were significantly increased ($p<0.01$) by intrarectal injection of acetic acid and decreased ($p<0.01$) by treatment of Se and vitamin E (Table 1).

Histopathological Findings For the experimental colitis group, hemorrhage, edema, massive mucosal and submucosal inflammatory infiltration, glandular destruction, and ulcerous areas were observed in all sections. Neutrophilic infiltration was observed predominantly in the mucosa, submucosa, and occasionally in muscularis propria. Eosinophilic and lymphocytic cell infiltrations were observed around the ulcerous areas. The values of above histopathological scores for the experimental colitis group were significantly higher ($p<0.001$) than for the control group. The score values of colonic injury for Se–vitamin E group were significantly lower ($p<0.01$) than for the experimental colitis group (Table 1). Mean histopathological score in experimental colitis group was 25.12 of the inflammatory score of 32, which was previously described by Keshavarzian et al. [1]. Histological slides from acetic acid and Se–vitamin E groups were shown in Figs. 1 and 2.

Biochemical Findings The levels of TAC, TOS, OSI, T-SH, PRS, CAT activities in plasma are given in Table 3. CAT and PRS activities in plasma were higher ($p<0.05$–0.01) in experimental colitis group than in control, and CAT activity was lower ($p<0.01$) in Se–vitamin E group than in experimental colitis group. While the TAC levels were lower in experimental colitis group than in control, its values were higher ($p<0.05$) in Se–vitamin E group than in experimental colitis group. The values of TOS and OSI were statistically higher ($p<0.05$) in experimental colitis group than in control (Table 2).

Table 1 Macroscopic and Microscopic Scores for Colons of Study Groups

Scores/groups	Macroscopic and microscopic scores of colonic injury		
	Control	Acetic acid	Acetic acid + vitamin E–Se
Mean macroscopic scores	0.1±0.01	3.20±0.13	1.40±0.51**
Minimum–maximum scores	0–1	2–4	1–3
Mean microscopic scores	1.87±0.51	25.12±1.24*	13.87±1.41**
Minimum–maximum scores	1–4	18–29	7–19

Data are presented in mean±SD

*$p<0.001$ (statistically significant compared to control group); **$p<0.001$ (statistically significant compared to control and acetic acid groups)

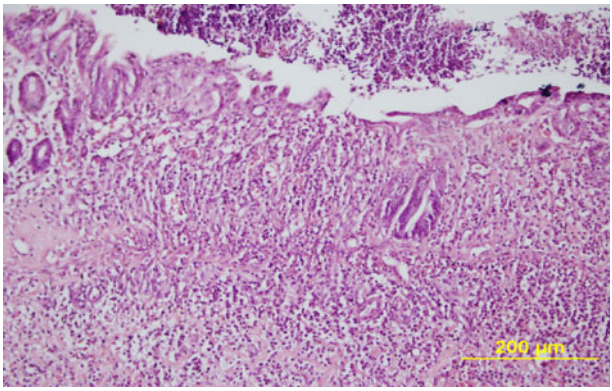

Fig. 1 Histological appearance of an acetic acid-induced lesion. Cellular debris in the luminal surface, luminal epithelial denudation, glandular destructions and some scattered glands, mucosal and submucosal ulceration, and predominantly neutrophilic infiltration are seen. These microscopical features are described an ulcerous lesion in colonic mucosa and submucosa (inflammatory score=25; H&E stain; original magnification, ×200)

The values of CAT, PRS, MPO, TAC, T-SH, TOS, and OSI in colonic tissue are given in Table 3. We determined that CAT activity was higher in the experimental colitis group than in the controls. While the PRS activity, TAC, and T-SH levels in colonic tissue were lower ($p<0.01$, respectively), the values of TOS and OSI were higher ($p<0.05$) in experimental colitis group than in controls. While the TAC was higher ($p<0.01$), OSI was lower ($p<0.01$) in Se–vitamin E group than in experimental colitis group. MPO activity was higher ($p<0.01$) in experimental colitis group than in control, but it was lower ($p<0.05$) in Se and vitamin E group than in experimental colitis group (Table 3).

Discussion

Several factors such as overproduction of proinflammatory mediators including reactive oxygen mediators, cytokines, arachidonate metabolites, and neutrophil infiltration have been implicated in the pathogenesis of colitis [10, 14, 28]. The reactive oxygen species in colonic mucosa have been described in colorectal specimens of patients with IBD. There is an increasing attention on the role of free radicals in the pathogenesis of colitis [14, 29]. Therefore, it has been proposed that increase in ROSs in IBD may be an important factor for etiopathogenesis of colitis [3, 7, 30]. Inflamed colon is exposed to oxidative stress produced by infiltrating macrophages and neutrophils within lamina propria of colon [3].

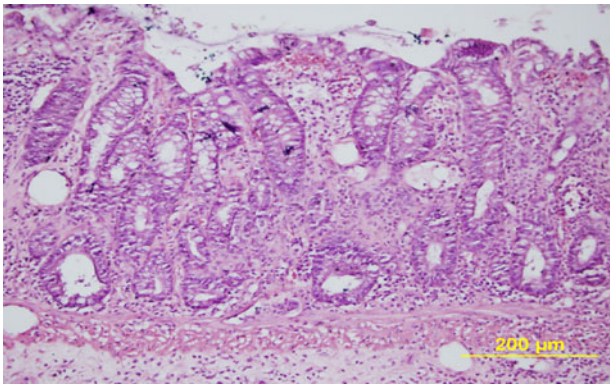

Fig. 2 Histological appearance of an acetic acid + vitamin E plus Se-treated lesion. Cellular mucin depletion, focal luminal epithelial denudation, vascular dilatation, edema, and moderate degree neutrophilic infiltration are seen. However, colonic glands in the lamina propria appear to be better protected compared with the acetic acid group (inflammatory score=14; H&E stain; original magnification, ×200)

Table 2 The Activities of CAT and PRS and the Values of Total SH, TAC, TOS, and OSI in Plasma of all Groups

	Control	Acetic acid	Acetic acid + vitamin E–Se
Catalase (kU/L)	51.31±6.90	55.31±8.751**	50.79±9.11****
Prolidase (kU/L)	0.46±0.14	0.49±0.15*	0.47±0.13
Total SH (mmol/L)	0.41±0.07	0.43±0.07	0.43±0.07
TAC (mmol Trolox eqv/L)	0.91±0.07	0.81±0.09*	0.86±0.10***
TOS (μmol H_2O_2 eqv/L)	16.09±2.94	17.75±2.63*	17.80±0.74
OSI (arbitrary unit)	18.39±0.66	20.84±0.63**	20.45±0.66

Data are presented in mean±SD

*$p<0.05$ (statistically significant compared to control group); **$p<0.01$ (statistically significant compared to control group); ***$p<0.05$ (statistically significant compared to acetic acid group); ****p<0.01 (statistically significant compared to acetic acid group)

The respiratory burst by neutrophils and macrophages is characterized by marked changes in oxygen metabolism that results in increased production of superoxide and H_2O_2 radicals [31]. Excessive unneutralized H_2O_2 diffuses from any intracellular location to the extracellular space where can be converted to the highly destructive hydroxyl radical via a metal catalyzed Haber–Weiss reaction causing significant oxidative damage in colonic epithelial membranes [4]. The tissue damage produced by neutrophils and macrophages has been attributed to their ability to release ROS, nitrogen metabolites, cytotoxic proteins, lytic enzymes, and cytokines as well as their disrupting effects on epithelial integrity [6, 14, 32].

Acetic acid is one of the important agents for experimental colitis which causes inflamed colon in laboratory animals. Colitis induced by acetic acid is also known to produce excess ROS and antioxidants that reduce these oxygen radicals [3, 4, 10, 14]. ROS cause reversible or irreversible damage to biological molecules including DNA, lipids, proteins, carbohydrates, or any nearby molecule causing a cascade of chain reactions resulting in cellular damage [1, 2, 4, 33]; hence, the biomolecules may lose their physiological functions in the tissues. Damage of these cellular components by free radicals may play an important role in various disorders of skin [8, 9], colon [1–8, 10, 17], brain [33], and liver [15, 16, 34]. In

Table 3 The Activities of CAT, PRS, MPO, and the Values of Total SH, TAC, TOS, and OSI for Colonic Tissue of all Groups

	Control	Acetic acid	Acetic acid + vitamin E–Se
Catalase (kU/g protein)	0.21±0.01	1.15±0.14**	1.12±0.13
Prolidase (IU/g protein)	10.89±1.46	5.02±0.14**	4.97±0.15
Total SH (mmol/g protein)	18.14±2.26	7.53±0.33**	7.70±0.16
TAC (mmol Trolox eqv/g protein)	25.00±0.90	21.14±1.00**	24.67±2.40****
TOS (μmol H_2O_2 eqv/g protein)	394.30±87.70	498.30±20.40**	495.60±21.00
OSI (arbitrary unit)	16.05±3.12	23.68±1.84**	20.01±1.76****
MPO (IU/g protein)	4.91±0.74	6.77±0.93**	5.92±1.07***

Data are presented in mean±SD

*$p<0.05$ (statistically significant compared to control group); **$p<0.01$ (statistically significant compared to control group); ***$p<0.05$ (statistically significant compared to acetic acid group); ****$p<0.01$ (statistically significant compared to acetic acid group)

this study, macroscopic and microscopic damages were significantly increased ($p<0.01$) by intrarectal injection of acetic acid and decreased ($p<0.05$ and $p<0.01$, respectively) in group treated by Se–vitamin E (Table 1). These results are in agreement with the findings of the earlier studies showing lipid peroxidation decrease in bowel disorders [1, 3, 7, 14, 19, 35]. Thus, the reactive oxygen radicals may defeat the antioxidant defense system controlling the production of ROS during activities of normal cellular metabolism. Indeed, it has been reported that ROSs were held responsible for colonic toxicity of acetic acid and caused oxidative damage in unsaturated lipids in some cellular components of colon [3, 10].

The values of TOS in plasma was significantly increased ($p<0.05$) in the experimental colitis group. These results are consistent with the observations in some studies indicating lipid peroxidation decrease in bowel disorders [14, 35]. The values for TAC were calculated significantly lower ($p<0.05$) in the plasma of the experimental colitis group than in control and Se–vitamin E groups (Table 2). The plasma OSI was significantly increased ($p<0.01$) by acetic acid injection but not decreased ($p>0.05$) by administration of Se and vitamin E. These findings are similar to the results of the studies demonstrating the decrease of TAC values in experimental colitis [2, 18].

Some thiol-containing compounds such as cysteine, methionine, taurine, glutathione, and lipoic acid are considered as effective antioxidants. The reduced form of thiol-containing compound is known as its strongest antioxidative activity. The number of sulfur atoms in thiol-containing compounds determines partially the activity of glutathione-related antioxidant enzymes [12, 36]. The difference among plasma total thiol values of control, experimental colitis, and Se–vitamin E groups were not seen significant, but the thiol values in colonic tissue decreased ($p<0.05$) by acetic acid injection (Tables 2 and 3). The decrease of thiol values may be attributed to the oxidation of protein thiols such as on GAPDH, or depletion of low molecular weight thiols for instance glutathione. Our present findings are in accordance by the results of Blackburn et al. [18].

Prolidase, a highly specific peptidase, may play an important role in proline conservation [37]. Serum prolidase activity was correlated by increased oxidative stress. Moreover, it has been reported that plasma prolidase activity might be useful in evaluating bowel disorders and fibrotic processes in chronic liver disease in human [35]. In our study, while the prolidase activity in colonic tissue was statistically decreased ($p<0.05$), the values in plasma significantly increased ($p<0.05$) by intrarectal injection of acetic acid and decreased ($p<0.05$) by administration of Se–vitamin E (Tables 2 and 3). These results are similar to the findings of the some other studies [35, 37]. MPO activity has been widely used to detect the neutrophil infiltration in mucosal inflammation of bowel, and a reduction in this enzyme activity can be seen as an indicator for the anti-inflammatory activity of a given compound. In our study, MPO activity was significantly increased ($p<0.01$) by the intrarectal injection of acetic acid and decreased ($p<0.05$) by administration of Se and vitamin E (Table 3). These results are in accordance with the results of the studies showing MPO activity increase in experimental colitis [7, 18, 38].

We determined that CAT activities were higher in the experimental colitis group both in serum and in colonic tissue than the values of the controls. In addition, serum CAT activity was decreased ($p<0.05$) by administration of Se–vitamin E combination (Tables 2 and 3). These observations are in agreement with results of the investigations indicating the activity of CAT affected by acetic acid induced colitis [3, 34]. CAT, an essential peroxisomal hydrogen peroxide-consuming enzyme, also decreased the oxidative effects of H_2O_2, superoxide dismutase, and GSH-Px that may play an important role as a system scavenging oxidative radicals. These enzymes are defined as the antioxidant defense enzymes against ROS [4, 11, 24, 30]. Thus, catalase and superoxide dismutase prevent the harmful effects of

free radicals and reduce the reactive metabolites produced by effects of acetic acid. Several treatments in experimental models have been used to protect the free radical injury in the bowel diseases by administrations of antioxidants and other chemical substances [3, 7, 13].

Se, an essential cofactor of GSH-Px, may play an important antioxidant role binding active site of glutathione peroxidase. GSH-Px not only detoxifies organic peroxides and H_2O_2 but also permits the regeneration of cellular lipid molecules through reacylation in the cellular membrane [11–14]. Vitamin E, the most effective chain-breaking lipid soluble antioxidant likely to quench oxidants, may prevent the increase of free radicals produced by oxidative damage of lipids and lipoproteins in progress of IBD. Moreover, vitamin E is an antioxidant that protect phagocytic cells and surrounding tissues from oxidative attack by free radicals produced by the respiratory burst of neutrophils and macrophages [31]. Thus, Se and vitamin E may prevent the certain types of the oxidative damages produced by infiltrating macrophages and neutrophils within inflamed colon. These considerations are in agreement with opinions of the authors who investigate prophylactic effects of antioxidants against oxidative damage in blood [13, 34], liver [39], and colon tissues [14, 40].

In conclusion, our findings show a beneficial effect of Se and vitamin E on the oxidative damage produced during the process of inflammatory response of experimental colitis. These antioxidants may be used for protective purposes in animals exposed to acetic acid, and prophylactic treatment could be taken by acting colonic mucosa for experimental colitis. However, there is a need for more detailed studies in order to assess possible relationships between colitis induced by acetic acid and antioxidants.

References

1. Keshavarzian A, Morgan G, Sedghi S et al (1990) Role of reactive oxygen metabolites in experimental colitis. Gut 31:786–790
2. Loguercıo C, Dargenıo G, Delle Cave M et al (1996) Direct evidence of oxidative damage in acute and chronic phases of experimental colitis in rats. Dig Dis Sci 41:1204–1211
3. Millar AD, Rampton DS, Chander CL et al (1996) Evaluating the antioxidant potential of new treatments for inflammatory bowel disease using a rat model of colitis. Gut 39:407–415
4. Pravda J (2005) Radical induction theory of ulcerative colitis. World J Gastroenterol 11:2371–2384
5. Sartor RB (1997) Pathogenesis and immune mechanisms of chronic inflammatory bowel diseases. Am J Gastroenterol 92:5S–11S
6. Fiocchi C (1998) Inflammatory bowel disease: aetiology and pathogenesis. Gastroenterology 115:182–205
7. Camuesco D, Ivez JG, Nieto A et al (2005) Dietary olive oil supplemented with fish oil, rich in EPA and DHA (n-3) polyunsaturated fatty acids, attenuates colonic inflammation in rats with DSS-induced colitis. J Nutr 135:687–694
8. Kokçam I, Nazıroglu M (2002) Effects of vitamin E supplementation on blood antioxidants levels in patients with Behcet's disease. Clin Biochem 35:633–639
9. Naziroğlu M, Kökçam I, Şimşek H et al (2003) Lipid peroxidation and antioxidants in plasma and red blood cells from patients with pemphigus vulgaris. J Basic Clin Physiol Pharmacol 14(1):31–42
10. Keshavarzian A, Sedghi S, Kanofsky J et al (1992) Excessive production of reactive oxygen metabolites by inflamed colon: analysis by chemiluminescence probe. Gastroenterology 103:177–185
11. McPherson A (1994) Selenium, vitamin E and biological oxidation. In: Cole DJ, Garnsworthy PJ (eds) Recent advances in animal nutrition. Butterworth and Heinemann's, Oxford, pp 3–30
12. Chen HJ, Wu SB, Chang CM (2003) Biological and dietary antioxidants protect against DNA nitration induced by reaction of hypochlorous acid with nitrite. Arch Biochem Biophys 415:109–116
13. Karakilcik AZ, Hayat A, Zerin M et al (2003) Effects of intraperitoneally injected selenium and vitamin E in rats anesthetized with halothane. J Trace Elem Med Biol 17:33–38
14. Ademoglu E, Erbıl Y, Tam B et al (2004) Vitamin E and selenium have beneficial effects on rinitrobenzenesulfonic acid-induced experimental colitis. Dig Dis Sci 49:102–108

15. Ozardalı İ, Bitiren M, Karakılcik Z et al (2004) Effects of selenium on histopathological and enzymatic changes in experimental liver injury of rats. Exp Toxicol Pathol 56:59–64
16. Karakılcık Z, Zerin M, Arslan O et al (2004) Effects of dietary vitamins C and E on liver enzymes and biochemical parameters of rabbits exposed to aflatoxin-B_1. Vet Hum Tox 46:190–192
17. Yoshikawa T, Takahashi S, Kondo M (1992) Possible role of free radicals in chronic inflammation in the gut. EXS 62:353–358
18. Blackburn AC, Doe WF, Buffinton GD (1997) Colonic antioxidant status in dextran sulfate-induced colitis in mice. Infl Bowel Dis 3:198–203
19. Choudhary S, Keshavarzıan A, Yong S et al (2001) Novel antioxidants zolimid and AEOL11201 ameliorate colitis in rats. Dig Dis Sci 46:2222–2230
20. Morris GP, Beck PL, Herridge MS et al (1989) Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology 96:795–803
21. Goth L (1991) A simple method for determination of serum catalase activity and revision of reference range. Clin Chim Acta 196:143–152
22. Kelly G (2003) The interaction of cigarette smoking and antioxidants. Part III: ascorbic acid. Altern Med Rev 8:43–54
23. Erel O (2005) A new automated colorimetric method for measuring total oxidant status. Clin Biochem 38:1103–1111
24. Aycicek A, Ipek A (2008) Maternal active or passive smoking causes oxidative stress in cord blood. Eur J Pediatr 167:81–85
25. Myara I, Charpentier C, Lemonnier A (1982) Optimal conditions for prolidase assay by proline colorimetric determination: application to iminodipeptiduria. Clin Chim Acta 125:193–205
26. Myara I, Myara A, Mangeot M et al (1984) Plasma prolidase activity: a possible index of collagen catabolism in chronic liver disease. Clin Chem 30:211–215
27. Krawisz JE, Sharon P, Stenson WF (1984) Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity: assessment of inflammation in rat and hamster models. Gastroenterology 87:1344–1350
28. Nieto N, Torres MI, Fernandez MI et al (2000) Experimental ulcerative colitis impairs antioxidant defense system in rat intestine. Dig Dis Sci 45:1820–1827
29. Lih-Brody L, Powell SR, Collier KP et al (1996) Increased oxidative stress and decreased antioxidant defenses in mucosa of inflammatory bowel disease. Dig Dis Sci 41:2078–2086
30. Mansour MA, Nagi MN, El-Katip AS et al (2002) Effect of tymoquinone on antiioxidant enzyme activities lipid peroxidation and DT-diaphorase in different tissues of mice: a possible mechanism of action. Cell Biochem Funct 20:143–151
31. Hogan JS, Smıth KL, Weıss WP et al (1990) Relationships among vitamin E, selenium and bovine blood neutrophils. J Dairy Sci 73:2372–2378
32. Grisham MB, Yamada T (1992) Neutrophils, nitrogen oxides, and inflammatory bowel disease. Ann NY Acad Sci 664:103–115
33. Nazıroglu M (2009) Role of selenium on calcium signaling and oxidative stress-induced molecular pathways in epilepsy. Neurochem Res. doi:10.1007/s11064-009-0015-8
34. Gumral N, Naziroglu M, Koyu A et al (2009) Effects of selenium and L-carnitine on oxidative stress in blood of rat induced by 2.45-GHz radiation from wireless devices. Biol Trace Elem Res. doi:10.1007/s12011-009-8372-3
35. Aslan M, Nazligul Y, Horoz M et al (2007) Serum prolidase activity and oxidative status in Helicobacter pylori infection. Clin Biochem 40:37–40
36. Komarnisky LA, Christopherson RJ, Basu TK (2003) Sulfur: its clinical and toxicologic aspects. Nutrition 19:54–61
37. Namiduru ES, Özdemir Y, Kutlar I et al (2001) A study of prolidase in mothers, their newborn babies and in non-pregnant controls. Arch Gynecol Obstet 265:73–75
38. Hajj Hussein IA, Tohme R, Barada K et al (2008) Inflammatory bowel disease in rats: bacterial and chemical interaction. World J Gastroenterol 14:4028–4039
39. Ding M, Potter JJ, Liu X et al (2009) Selenium supplementation decreases hepatic fibrosis in mice after chronic carbon tetrachloride administration. Biol Trace Elem Res. doi:10.1007/s12011-009-8414-x
40. Reifen R, Nissenkorn A, Matas Z et al (2004) 5-ASA and lycopene decrease the oxidative stress and inflammation induced by iron in rats with colitis. J Gastroenterol 39:514–519