Bitlis Eren University
Journal of Science and Technology

ISSN 2146-7706
2, (2012), 27-31

Research Article

The antioxidant effect of boric acid and CoQ10 on pulmonary fibrosis in bleomycin induced rats

Fatih Çağlar Çelikezen [a,*], Gökhan Oto [b], Hülya Özdemir [b], Ufuk Kömüroğlu [c], İbrahim Yörük [d], Halit Demir [d], Aslı Çilingir Yeltekin [d]

[a] *Bitlis Eren University, Faculty of Science and Arts, Department of Chemistry, 13000 Bitlis-TURKEY.*
[b] *Yuzuncu Yil University, Faculty of Medicine, Department of Pharmacology, 65080 Van-Turkey.*
[c] *Yuzuncu Yil University, Faculty of Medicine, Department of Biochemistry, 65080 Van-Turkey.*
[d] *Yuzuncu Yil University, Faculty of Science, Department of Chemistry, 65080 Van-Turkey.*
** Corresponding author: celikezen@hotmail.com*

Abstract

The purpose of this study is to investigate the antioxidant effects boric acid and CoQ10 has on pulmonary fibrosis in bleomycin induced rats. 32 wistar albino male rats were used in this study. The rats were categorised in four groups; a control group (only given normal saline), a bleomycin (BLM) group, a BLM + boric acid group, and a BLM + boric acid + CoQ10. The study period was adjusted to 30 days. Retinol, vitamin E, vitamin D, catalase, carbonic anhydrase, glucose, total protein, albumin, globulin, and total bilirubin levels were measured at the end of the study. There was a significant increase in the vitamin E levels of all groups ($p<0.05$). There was a statistically significant increase in the glucose level of all groups ($P<0.001$). There were significant decreases in albumin and total protein levels of all groups. While the significance level of the decrease in the albumin level was $p<0.001$, the significance level of the decrease in the total protein level was $p<0.05$. There was no significant difference in other biochemical parameters.

Keywords: Bleomycin, boric acid, CoQ10, pulmonary fibrosis, rat

1. Introduction

Pulmonary fibrosis (PF) is characterised by an altered cellular composition of the alveolar region with excessive deposition of collagen. However, its etiology is obscure. Lung inflammation is a major underlying component of a wide variety of pulmonary fibro proliferative disorders. In the last decades, it has been suggested that the main agent responsible for lung fibrosis are the reactive oxygen species (ROS), which are also produced under normal physiological conditions of the human body. Many possible treatment protocols for PF have been investigated, but none have succeeded in clinical trials (Taooka et al. 1997).

Bleomycin (BLM) is a mixture of closely related glycopeptide antibiotics isolated from Streptomyces verticillus. It has been used as an antitumor agent against head and neck cancer, squamous cell carcinomas, testicular cancer, and some lymphomas; however, its use is often limited by its toxicity, especially lung inflammation that can lead to fibrosis (Chen & Stubbe 2005).

Boron supplementation in animal and human nutrition may have important effects on various metabolic and physiological systems of an organisms. Some studies have demonstrated that boron has effects on the metabolism of minerals (Ca and P) (Meacham et al. 1994), vitamin D (Hunt 1996), energy substrates (triglycerides, glucose) (Eren et al. 2006), and reactive oxygen species (Turkuez et al. 2007).

Coenzyme Q10 (CoQ10), also known as ubiquinone 50, is a fat-soluble molecule produced in the majority of living cells. It plays a key role in the energy metabolism as an integral part of the electron transport system (Ernster & Dallner 1995). CoQ10 is also recognised as a powerful systemic radical scavenger (antioxidant) that blocks oxidative injuries to DNA, lipids, proteins, and other essential molecules, and is also capable of functioning synergistically with other antioxidants (Lass & Sohal 2000).

The aim of this study is to investigate the beneficial effects of boric acid and CoQ10 on pulmonary fibrosis.

2. Material and Method

2.1. Animals

3.5 month old wistar albino rats, weighing 180–200 g, were obtained from the animal laboratory at Yüzüncü Yıl University. All procedures involving animals were approved by the institutional ethics committee (30.06.2011-12). Rats were housed in specific cages. The rats had free access to water and food ad libitum.

2.2. Experimental protocol

32 male Wistar albino rats were used in this study. The planned study time was 30 days. The rats were divided into 4 groups. **Control group (n=8);** Sterile saline solution was given to these, administrated intraperitoneally. **BLM applied group (n=8);** Bleomycin (Nippon Kayaku, Tokyo, Japan) was dissolved in 250 µL of phosphate-buffered saline (PBS) solution and instilled into the animals at a dose of 7.5 mg/kg body weight intratracheally under

Received 16 January 2012
Accepted 23 May 2012
Available online June 2012

chloroform anaesthesia. The animals were shaken to facilitate distribution of bleomycin and saline (Ozyurt et al. 2004). **BLM+boric acid group (n=8);** in this group, boric acid was induced at a dose ratio of 10 mg/kg/body weight/day orally during 30 days, after BLM administration. **BLM+boric acid+CoQ_{10} (n=8);** CoQ_{10} adminstrated intraperytonnaly the ratio of 4 mg/kg/body weight/day in addition to BLM and boric acid. Animals were euthanized 30 days after the instillation. Blood samples were collected for analysis.

2.3. Determination of vitamin levels in the serum

Vitamin A, D, and E (retinol, tocopherol and vitamin D_3) levels were determined in accordance with the HPLC (high performance liquid chromatography, Agilent 1100, Germany) method (Zaspel & Csallany 1983; Reynolds & Judd 1984; Miller &Yang 1985).

2.4. Determination of catalase and carbonic anhydrase activity

A method described by (Aebi 1984) was used to conduct biochemical analysis of CAT activity in erythrocytes. The CA activity was assayed by hydrating CO_2. The hydration of CO_2 was measured using the method of Rickli and Wilbur-Anderson, with bromothymol blue as the indicator (Rickli et al. 1964).

2.5. Determination of the total protein, albumin, globulin, glucose, and total bilirubin levels in the serum

The total protein, albumin, globulin, glucose, and total bilirubin levels were determined by a Roche Modular PP auto analyser using commercial Roche Kits.

2.6. Statistical Analysis

A statistical package for the social sciences (SPSS 17) package programme was used to conduct statistical analysis together with the one way ANOVA test.

3. Result and Discussion

BLM is known to generate reactive oxygen metabolites, including superoxide and hydroxyl radicals. Generation of the ROS in the lung tissue results in DNA injury, lipid peroxidation, alteration in lung prostaglandin synthesis and degradation, and an increase in lung collagen synthesis. As a result of the injury, inflammation and cytokine dysregulation occur after BLM administration, fibroblasts are activated, and collagen production is stimulated, while collagen degradation is inhibited (Sleijfer 2001). In addition, several studies have demonstrated that bleomycin administration in rats decreases the antioxidative capacity, while increasing oxidative stress in the lung tissue (Mata et al. 2003).

There are three major enzymes that are in charge of reactive oxygen species; superoxide dismutase, glutatione peroxidase and catalase (Krinsky 1992). The main enzymatic defence is provided by albumin, uric acid, billuribin, sistein, glutatione, beta-caroten, dihydrolipoat, ubiquinone, ceruloplasmin, transfferine, zinc, manganase, selenium, vitamin A, vitamin C and vitamin E (Frei et al. 1988). One of the most important of these is albumin (Şahin 2006).

Vitamin A and its active metabolite credits are important factors in supporting epithelial differentiation and normal respiratory growth (Georgieff et al. 1991). Retinoids also have anti-fibrotic and anti-inflammatory properties (Tabata et al. 2006). Retinoic acid (RA) stimulates the cell proliferation (Nabeyrat et al. 1998) and affects the polyamine transport and synthesis in cultured type II pneumocytes. Polyamine maybe important during the lung cell repair process (Heger & Baybutt 1999). Tabata et al. (2006) investigated the preventive effect that All-trans-retinoic acid (ATRA) had on the progression of the lung fibrosis in irradiated and BLM-treated rats. They concluded that ATRA improved the fibrosis induced by BLM in the lung tissues of rats; their data may provide a basis to explore the clinical use of ATRA in order to prevent fibrosis induced by pathologic radiation of the lung implying pulmonary fibrosis. Mert et al. (2009) reported that there was a decrease in the retinyl ester level in the pulmonary fibrosis lung tissue, produced by giving bleomycin to rats. There was a decrease in the retinol level for all groups addressed in study ($p>0.05$) as seen in Table 1.

Table 1. The retinol, vitamin E and D levels in control, BLM, BLM+boric acid, BLM+boric acid+CoQ_{10} groups ($p<0.05$).

Groups	Retinol	Vitamin E	Vitamin D
Control	0.638 ± 0.021^a	1.424 ± 0.193^a	0.029 ± 0.001^a
BLM	0.568 ± 0.041^a	2.259 ± 0.308^b	0.030 ± 0.008^a
BLM+Boric Acid	0.554 ± 0.028^a	2.401 ± 0.240^b	0.026 ± 0.003^a
BLM+Boric Acid+CoQ_{10}	0.618 ± 0.056^a	2.146 ± 0.211^b	0.024 ± 0.004^a

BLM: Bleomycin

Vitamin E is a major antioxidant in biological systems acting as a powerful chain-breaking agent through the scavenging of peroxyl radicals (Shi et al. 1999). Vitamin E terminates the chain reaction of lipid peroxidation in membranes and lipoproteins. Thus, a number of studies have been carried out to determine the protective effects of vitamin E in different biological models of injury (Chen & Tappel 1995). Değer et al. (2007) proved that the pulmonary formation of fibriosis prevents vitamin E supplementation. Kato et al. (1990) noted that the concentration of vitamin E in the lung tissue increased significantly after intratrecheal administration of BLM. In this study, significant increase was determined in the vitamin E values of all the serum groups ($p<0.05$) as seen in Table 1.

The benefits of vitamin D were originally thought to be associated only with bone health based on increasing calcium and phosphorus absorption; several studies have extended the benefits to many noncalcemic effects. These effects include reduced risk of many forms of cancer (Garland 2006), bacterial infections (Bikle 2008), viral infections (Cannell 2006), autoimmune diseases (Munger et al., 2006), and cardiovascular disease (Wang et al. 2008). Mert et al. (2009) reported that there was a significant decrease in the vitamin D_3 level in the pulmonary fibrosis lung tissue, produced by giving rats bleomycin ($p<0.001$). For this study, while there was only an increase in the vitamin D levels of the BLM group, decreases were observed in all other groups ($p>0.05$) as shown in Table 1.

Carbonic anhydrase (CA) is present in nearly all organisms and catalyzes reversible hydration of CO_2 to HCO_3 and H^+ (Tashian et al. 1991). In addition to its role in the regulating of pH homeostasis, CA activity facilitates biosynthetic processes, which involve an early carboxylation step requiring bicarbonate. CA (IX) was first recognised as a novel tumor-associated antigen in

several human carcinomas (Liao et al. 1994). Simi et al. (2006) found that high levels of CA were found to be an independent prognostic feature in some cancer types. Ertekin et al. (2007) reported a significant increase in CA activity in lung fibrosis created using bleomycin in rats. Beydemir et al. (2002) reported that aminoglycoside antibiotics inhibited the activity of carbonic anhydrase in low concentrations in vitro, but activated it at high concentrations in vitro. In this study, there was a decrease in the CA activity of all groups ($p>0.05$) as seen in Table 2.

Table 2. The catalase (CAT) and CA levels in control, BLM, BLM + boric acid, BLM + boric acid+ CoQ_{10} groups ($P>0.05$).

Groups	CAT	CA
Control	11.4679±4.3345[a]	0.8044±0.2374[a]
BLM	20.9605±5.2859[a]	0.6119±0.1553[a]
BLM + Boric Acid	11.5679±3.3105[a]	0.6733±0.1307[a]
BLM + Boric Acid+ CoQ_{10}	14.7444±2.4574[a]	0.6977±0.1258[a]

BLM: Bleomycin, CA: Carbonic anhydrase, CAT: Catalase

Catalase (CAT) is essential to protect aerobic organisms from the toxic effects of H_2O_2. Catalase was also utilised in an enzymatic oxidation reaction to depress the deactivation of the relevant enzyme and/or the side reaction due to H_2O_2 that was produced (Yoshimoto et al. 2005). Özyurt et al. (2004) reported the significant decrease in CAT and SOD activities in lung tissue in bleomycine-induced lung fibrosis in rats. Yen et al. (2005) identified the markedly lower MnSOD and CAT activities in VA13 cells, which were observed after the after bleomycin treatment. In this study, there was an increase in the catalase level of all groups ($P>0.05$) as seen in Table 2.

Garcia-Gonzalez et al. (1991) suggested that boron deficiency increased the concentration of superoxide dismutase (SOD), catalase (CAT), and peroxidase in Anabaena PCC 7119 heterocysts. In addition, Turkez et al. (2007) observed that at low doses (15 mg/L) boron compounds increased both SOD and CAT activities in the erythrocytes when compared to the control group, while at high doses (500 mg/L) these decreased both SOD and CAT activities in erythrocytes. In our study, boric acid may have been the reason behind the increase in CAT levels, and the decrease in CA activity. No studies were found in literature that addressed the relationship between CA and boric acid. We believe this is the only study that addresses the same relationship.

Responses to oxidative stress can be affected by several factors in the cellular environment such as glucose. Glucose deprivation results in a loss of substrate for ATP production via glycolysis. Oxidative phosphorylation would then be the alternative pathway to produce energy. Evidence suggests that glucose deprivation causes metabolic oxidative stress imposed by the generation of superoxide and hydrogen peroxide during mitochondrial respiration (Blackburn et al. 1999). It was seen that the glucose metabolism was directly related to cellular sensitivity to hydrogen peroxide (Averill-Bates & Przybytkowski 1994).

Baynes set forth the relationship between diabetes and oxidative stress, and reported that the increase in lipid peroxidation caused hyperglycemia (Baynes 1991). Giri et al. (1985) reported bleomycin-treated animals were hyperglycemic in comparison to nutritionally comparable pair-fed animals, and had plasma glucose levels similar to those of control-fed animals. There was a significant increase in the glucose level of all groups ($p<0.001$) as seen in Table 3.

Albumin has several important physiological and pharmacological functions. In general, albumin represents the major and predominant antioxidant in the plasma, a body compartment known to be exposed to continuous oxidative stress. A large proportion of the total serum antioxidant properties can be attributed to albumin. Previous works indicate that more than 70% of the free radical-trapping activity of the serum was due to human serum albumin (HSA) as assayed using the free radical-induced hemolysis test (Bourdon & Blache 2001). Albumin is a more effective antioxidant than globulin. The reason behind the effectiveness of albumin is its structure (Roche et al. 2008). Albumin has the capacity to catch hydroxyl radicals (Wang et al. 2008).

Globulin levels increased and albumin levels decreased according to the laboratory measurements conducted on patients diagnosed and being treated for multiple myeloma (bone marrow cancer) (Türe et al. 2002). There were decreases in the albumin levels, and increases in the gamma globulin levels of some patients suffering from autoimmune diseases (Parlak et al. 1999).

Hernnäs et al. (1992) reported that albumin in bronchoalveolar lavage fluid (BALF) reached a peak level, 20 times more than the control values, after 3 days, and then rapidly decreased. In another study, Jordana et al. (1988) reported that the concentration of albumin in the BALF began to increase 48 hours after bleomycin, becoming 10 times greater than control values by day 7 (150 +/- 38 versus 16 +/- 3 micrograms/ml), and returned to the control values by day 28.

In this study, there was a significant decrease in the albumin level of all groups ($p<0.001$). However, there was an increase in globulin levels ($p>0.05$) as shown in Table 3. Liu et al. (2007) reported that there was a significant decrease in the total protein level of the group when they applied alpha lipoic acid to bleomycin-induced lung pulmonary fibrosis. In this study, there was a significant decrease in the total protein level ($p<0.05$) as seen in Table 3.

Reports selected from the last two decades on the antioxidant role of bilirubin (Ihara et al. 1998), Stocker and co-workers (1994), in particular, highlight the important role of bilirubin as a natural antioxidant, at least in vitro. There is also evidence that bilirubin can be

Table 3. The glucose, T. protein, albumin, globulin and T.billuribin levels in control, BLM, BLM+boric acid, BLM+boric acid+CoQ_{10} groups ($p>0.05$, $p<0.05$, $p<0.001$).

Groups	Glucose	T. Protein	Albumin	Globulin	T.Billuribin
Control	149.4286±1.5562[a]	7.4029±0.1629	4.5614±0.0606[b]	2.8286±0.2408	0.0971±0.0129[a,b]
BLM	160.5714±2.2768[b]	7.0529±0.1747	3.9243±0.0633[a]	3.0000±0.2204	0.0743±0.0149[a]
BLM + Boric Acid	192.4286±2.6445[d]	6.9857±0.1327	4.1014±0.1283[a]	3.0857±0.1993	0.1286±0.0163[b]
BLM + Boric Acid+ CoQ_{10}	172.2857±2.9416[c]	7.3986±0.1606	4.4443±0.0676[b]	3.0714±0.1358	0.1371±0.0180[b]

BLM: bleomycin, CA: carbonic anhydrase, CAT: catalase

protect cells (Dudnik et al. 2001) against lipid peroxidation, and contribute to the antioxidant capacity of the jaundiced new born infants (Bélanger et al. 1997).

There are no studies available in literature regarding the relationship between BLM-induced pulmonary fibrosis and bilirubin. We believe our study is the first. In our study, while there were significant decreases in the bilirubin levels of the BLM group, there were significant increases in the bilirubin levels of all the other groups ($p<0.05$) as seen in Table 3.

Results concluded that boric acid and CoQ_{10} had an antioxidant effect against bleomycin-induced pulmonary fibrosis.

References

Aebi H (1984). Catalase in vitro. Method Enzymol 105, 121-126.

Averill-Bates D, Przybytkowski E (1994). The role of glucose in cellular defenses against cytotoxicity of hydrogen peroxide in Chinese hamster ovary cells. Arch Biochem Biophys 312, 52–58.

Baynes JW (1991). Role of oxidative stress in development of complications in diabetes. Diabet 40, 405-412.

Bélanger S, Lavorie JC, Chessex P (1997). Influence of bilirubin on the antioxidant capacity of plasma in newborn infants. Biol Neonate 71, 233–238.

Beydemir S, Çiftçi M, Küfrevioğlu OI, Büyükokuroğlu ME (2002). Effects of gentamicin sulfate on enzyme activities of carbonic anhydrase from human erythrocytes in vitro and from rat erythrocytes in vivo. Biol Pharm Bull 25, 966-969.

Bikle DD (2008). Vitamin D and the immune system: role in protection against bacterial infection. Curr Opin Nephrol Hy 17, 348–352.

Blackburn RV, Spitz DR, Liu X, Galoforo SS, Sim JE, Ridnour LA, Chen JC, Davis BH, Corry PM, Lee YJ (1999). Metabolic oxidative stress activates signal transduction and gene expression during glucose deprivation in human tumor cells. Free Radical Bio Med 26, 419–430.

Bourdon E, Blache D (2001). The importance of proteins in defense against oxidation. Antioxid Redox Sign 3, 293–311.

Cannell JJ, Vieth R, Umhau JC, Holick MF, Grant WB, Madronich S, Garland CF, Giovannucci E (2006). Epidemic influenza and vitamin D. Epidemiol Infect 134, 1129–1140.

Chen H, Tappel AL (1995). Protection by vitamin E, selenium, trolox C, ascorbic acid palmitate, acetylcysteine, coenzyme Q, coenzyme Q10, beta carotene, canthaxantine, and (+)-catechin against oxidative damage to rat blood and tissues in vivo. Free Radical Bio Med 18, 949–953.

Chen J, Stubbe J (2005). Bleomycins: towards better therapeutics. Nat Rev Cancer 5,102–112.

Değer Y, Yur F, Ertekin A, Mert N, Dede S, Mert H (2007). Protective effect of alpha-tocopherol on oxidative stress in experimental pulmonary fibrosis in rats. Cell Biochem Funct 25, 633-637.

Dudnik LB, Tsyupko N, Khrenov AV, Alessenko AV (2001). Effect of bilirubin on lipid peroxidation, sphingomyelinase activity, and apoptosis induced by sphingosine and UV irrdatiation. Biochem 66, 1019–1027.

Eren M, Kocaoğlu-Güçlü B, Uyanık F, Karabulut N (2006). The effects of dietary boron supplementation on performance, carcass composition and serum lipids in Japanese quails. J Anim Vet Adv 5, 5–8.

Ernster L, Dallner G (1995). Biochemical, physiological and medical aspects of ubiquinone function. Biochem Biophys Acta 1271, 95-204.

Ertekin A, Değer Y, Mert H, Mert N, Yur F, Dede S, Demir H (2007). Investigation of the effects of α-tocopherol on the levels of Fe, Cu, Zn, Mn and Carbonic Anhydrase in rats with bleomycin-induced pulmonary fibrosis. Biol Trace Elem Res 116, 289-300.

Frei B, Stocker R, Ames BN (1988). Antioxidant defenses and lipid peroxidation in human blood plasma. P Natl A Sci 85, 9748-9752.

Garcia-Gonzalez M, Mateo P, Bonilla I (1991). Boron requirement for envelope structure and function in Anabaena PCC 7119 heterocysts. J Exp Bot 42, 925–929.

Garland CF, Garland FC, Gorham ED, Lipkin M, Newmark H, Mohr SB, Holick MF (2006). The role of vitamin D in cancer prevention. Am J Public Health 96, 252–261.

Georgieff MK, Radmer WJ, Sowell AL, Yeager PR, Blaner WS, Gunter EW, Johnson DE (1991). The effect of glucocoticoids on serum, liver and lung vitamin A and retinyl ester concentration. J Pediatr Gastr Nutr 13, 376-382.

Giri NS, Nakashima JM, Curry DL (1985). Effects of intratracheal administration of bleomycin or saline in pair-fed and control-fed hamsters on daily food intake and on plasma levels of glucose, cortisol, and insulin, and lung levels of calmodulin, calcium, and collagen. Exp Mol Pathol 42, 206-219.

Heger RJ, Baybutt RC (1999). Regulation of polyamine synthesis and transport by retinoic acid and epidermal growth factor in cultured type II pneumocytes. J Nutr Biochem 10, 518-524.

Hernnäs J, Nettelbladt O, Bjermer L, Särnstrand B, Malmström A, Hällgren R (1992). Alveolar accumulation of fibronectin and hyaluronan precedes bleomycin-induced pulmonary fibrosis in the rat. Eur Respir J 4, 404-410.

Hunt CD (1996). Biochemical effects of physiological amounts of dietary boron. J Trace Elem Exp Med 9, 185–213.

Ihara H, Aoki Y, Hashizume N, Aoki T, Yoshida M, Osawa S (1998). Comparison of antioxidant activity of bilirubin species in vitro. Clin Chem Enzym Commun 8, 31–36.

Jordana M, Dolovich M, Irving LB, Tomioka M, Befus D, Gauldie J, Newhouse MT (1988). Solute movement across the alveolar-capillary membrane after intratracheally administered bleomycin in rats. Am Rev Respir Dis 138, 96-100.

Kato S, Kudo Y, Takahashi H, Osanai K, Yakuwa N, Nakamura H, Sato S, Takahashi K (1990). Effects of vitamin E deficiency on bleomycin induced pulmonary fibrosis in hamsters. Kok To Jun 38, 445-450.

Krinsky NI (1992). Mechanism of action of biological antioxidants. Proc Soc Exp Biol Med 200, 248-254.

Lass A, Sohal RS (2000). Effect of coenzyme Q10 and alpha-tocopherol content of mitochondria on the production of superoxide anion radicals. FASEB J 14, 87–94.

Liao SY, Brewer C, Závada J, Pastorek J, Pastoreková S, Manetta A (1994). Identification of the MN antigen as a diagnostic biomarker of cervical intraepithelial squamous and glandular neoplasia and cervical carcinomas. Am J Pathol 145, 598–609.

Liu R, Ahmed KM, Nantajit D, Rosenthal FS, Hai C X, Li JJ (2007). Therapeutic effects of alpha-lipoic acid on bleomycin-induced pulmonary fibrosis in rats. Int J Mol Med 19, 865-73.

Mata M, Ruiz A, Cerda M, Martinez-Losa M, Cortijo J, Santangelo F, Serrano-Molar A, Llombart-Bosch A, Morcillo EJ (2003). Oral N-acetylcysteine reducesbleomycin-induced lung damage and mucin Muc5ac expression in rats. Eur Respir J 22, 900–905.

Meacham SL, Taper LJ, Volpe SL (1994). Effects of boron supplementation on bone mineral density and dietary, blood, and urinary calcium, phosphorus, magnesium, and boron in female athletes. Environ Health Persp 102, 79–82.

Mert H, Yörük I, Ertekin A, Dede S, Değer Y, Yur F, Mert N (2009). Vitamin levels in lung tissue of rats with bleomycin induced pulmonary fibrosis. J Nutr Sci Vitaminol 55,186-190.

Miller KW, Yang CS (1985). An isocratic high-performance liquid chromatography method for the simultaneous analysis of plasma retinol, α-tocopherol and various carotenoids. Anal Biochem 145, 21-26.

Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A (2006). Serum 25- hydroxyvitamin D levels and risk of multiple sclerosis. JAMA 296, 2832–2838.

Nabeyrat E, Besnard V, Corroyer S, Cazals V, Clement A (1998). Retinoic acid-induced proliferation of lung alveolar epithelial cells: relation with the IGF system. Am J Physiol 275, 71-79.

Neužil J, Stocker R (1994). Free and albumin-bound bilirubin are efficient coantioxidants for alpha-tocopherol, inhibiting plasma and low density lipoprotein lipid peroxidation. J Biol Chem 24, 16712–16719.

Özyurt H, Söğüt S, Yıldırım Z, Kart L, Iraz M, Armut F, Temel I, Özen S, Uzun A, Akyol O (2004). Inhibitory effect of caffeic acid phenethyl ester on bleomycine-induced lung fibrosis in rats. Clin Chim Acta 339, 65–75.

Parlak E, Oğuz P, Şaşmaz N, Şahin T, Koşar Y, Kovalı E (1999). Coeliac disease, autoimmune thyroid disease and primary biliary cirrhosis: Case report. Turkish J Gastroenterol 10, 167-170.

Reynolds SL, Judd HJ (1984). Rapid procedure for the determination of vitamins a and d in fortified skimmed milk powder using high-performance liquid chromatography. Analyst 109, 489-492.

Rickli EE, Ghazanfar SAS, Gibbons BH, Edsall JT (1964). Carbonicanhydrase from human erytrocytes. Preparation and properties of two enzymes. J Bio Chem 239, 1065-1078.

Roche M, Rondeau P, Singh NR, Tarnus E, Bourdon E (2008). The antioxidant properties of serum albumin. FEBS Lett 582,1783–1787.

Shi H, Noguchi N, Niki E (1999). Comparative study on Dynamics of antioxidative action of tocopheryl hydroquinone, ubiquinol, and tocopherol against lipid peroxidation. Free Radical Bio Med 27, 334–346.

Simi L, Venturini G, Malentacchi F, Gelmini S, Andreani M, Janni A, Pastorekova S, Supuran CT, Pazzagli M, Orlando C (2006). Qua ntitative analysis of carbonic anhydrase IX mRNA in human non-small cell lung cancer. Lung Cancer 52, 59–66.

Sleijfer S (2001). Bleomycin-induced pneumonitis. Chest 120, 617–624.

Şahin T (2006). Postoperatif erken dönemde cerrahi travma, oksidatif stres ve serum albümini arasındaki ilişki. Sağlık Bilimleri Enstitüsü, Uzmanlık Tezi, Gazi Üniversitesi Tıp Fakültesi, 50 s.

Tabata B, Kadokawa Y, Tabata R, Takahashi M, Okoshi K, Sakai Y, Mishima M, Kubo H (2006). All-trans-retinoic acid prevents radiation or bleomycin-induced pulmonary fibrosis. Am J Resp Crit Care 174, 1352-1360.

Taooka Y, Maeda A, Hiyama K, Ishioka S, Yamakido M, (1997). Effects of neutrophils elastase inhibitor on bleomycin-induced pulmonary fibrosis in mice. Am J Respir Crit Care Med, 156, 260–265.

Tashian RE, Hewett-Emmet D, Venta PJ (1991). In diversity and evolution in the carbonic anhydrase gene family, F. Botrè, G. Gros, B.T. Storey, eds., VCH Verlagsgesellschaft, Weinheim.

Turkez H, Geyikoğlu F, Tatar A, Keles S, Özkanç A (2007). Effects of some boron compounds on peripheral human blood. Z Naturforsch 62, 889–896.

Türe ÖT, Salepçi T, Sargın H, Koç Y, Yayla A (2002). Multiple myelom tanısı ile takip ve tedavisi düzenlenen olguların klinik ve patolojik durumlarının retrospektif analizi. Kartal Eğt Arşt Hast Tıp Derg 13, 83-86.

Wang JZ, Zhang H, Zhang M, Yao WT, Mao XY, Ren FZ (2008). Antioxidant activity of hydrolysates and peptide fractions of porcine plasma albumin and globulin. J Food Biochem 32, 693–707.

Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, Benjamin EJ, D'Agostino RB, Wolf M, Vasan RS (2008). Vitamin D deficiency and risk of cardiovascular disease. Circul 117, 503–511.

Yen HC, Chang HM, Fan-Yi Chen HM, Li SH (2005). Levels of reactive oxygen species and primary antioxidant enzymes in WI38 versus transformed WI38 cells following bleomcyin treatment. Free Radical Bio Med 38, 950–959.

Yoshimoto M, Wang S, Fukunaga K, Fournier D, Walde P, Kuboi R, Nakao K (2005). Novel immobilized liposomal glucose oxidase system using the channel protein OmpF and catalase. Biotechnol Bioeng 90, 231–238.

Zaspel BJ, Csallany S (1983). Determination of alpha-tocopherol in tissues and plasma by high-performance liquid chromatography. Anal Biochem 130, 146-150.