CELL BIOCHEMISTRY & FUNCTION

CELL BIOCHEMISTRY AND FUNCTION
*Cell Biochem Funct* 2012; **30**: 303–308.
Published online 16 January 2012 in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/cbf.2802

# Effects of shock waves on oxidative stress and some trace element levels of rat liver and diaphragm muscles

İlhan Gecit[1]*, Servet Kavak[2], Ismail Meral[3], Mustafa Güneş[1], Necip Pirinççi[1], Fuat Sayır[4], Halit Demir[5] and Kadir Ceylan[1]

[1]*Faculty of Medicine, Department of Urology, Yuzuncu Yil University, Van, Turkey*
[2]*Faculty of Medicine, Department of Biophysics, Yuzuncu Yil University, Van, Turkey*
[3]*Faculty of Medicine, Department of Physiology, Yuzuncu Yil University, Van, Turkey*
[4]*Faculty of Medicine, Department of Thoracic Surgery, Yuzuncu Yil University, Van, Turkey*
[5]*Division of Biochemistry, Faculty of Science, Department of Chemistry, Yuzuncu Yil University, Van, Turkey*

This study was designed to investigate whether the short-term extracorporeal shockwave lithotripsy (ESWL) exposure to kidney produces an oxidative stress and a change in some trace element levels in liver and diaphragm muscles of rats. Twelve male Wistar albino rats were divided randomly into two groups, each consisting of six rats. The animals in the first group did not receive any treatment and served as control group. The right-side kidneys of animals in group 2 were treated with two-thousand 18 kV shock waves while anesthetized with 50 mg kg$^{-1}$ ketamine. The localization of the right kidney was achieved after contrast medium injection through a tail vein under fluoroscopy control. The animals were killed 72 h after the ESWL treatment, and liver and diaphragm muscles were harvested for the determination of tissue oxidative stress and trace element levels. Although the malondialdehyde level increased, superoxide dismutase and glutathione peroxidase enzyme activities decreased in the livers and diaphragm muscles of ESWL-treated rats. Although glutathione level increased in liver, it decreased in diaphragm muscles of ESWL-treated animals. Fe, Mg and Mn levels decreased, and Cu and Pb levels increased in the livers of ESWL-treated animals. Fe and Cu levels increased, and Mg, Pb, Mn and Zn levels decreased in the diaphragm muscles of ESWL-treated animals. It also causes a decrease or increase in many mineral levels in liver and diaphragm muscles, which is an undesirable condition for the normal physiological function of tissues. Copyright © 2012 John Wiley & Sons, Ltd.

KEY WORDS—ESWL treatment; antioxidants; trace elements; liver; diaphragm muscle; rat

## INTRODUCTION

Although extracorporeal shockwave lithotripsy (ESWL) is a non-invasive routine treatment modality for stones in the upper urinary tract, it is not completely free from side effects. Shock wave lithotripsy is thought to induce renal capillary disruption, and this causes relative ischaemia and hypoxia. Increased free radical formation and subsequent damage to the kidney can occur in the reperfusion period.[1–3] Munver *et al.* [4] introduced an innovative microdialysis system for *in vivo* sampling of interstitial fluids that can be analyzed for free radical-mediated lipid peroxidation products after ESWL treatment in the swine.

The mechanism of ESWL-induced cellular damage is still controversial. One of the mechanisms discussed for tissue damage is free radical formation during ESWL treatment.[5–8] In addition to the mechanical fragmentation of the calculus, each shock wave that is generated by thermal effects of 18 000–24 000 V of electrical energy at the second focus may result in some biochemical events, and the homolytic cleavage of molecules may take place leading to the formation of free radicals.[9,10] Because of their very high reactivities, free radicals can cause serious damage to the macromolecules in cells. Injuries to the adjacent organs have been reported in less than 1% of patients.[11] These include pulmonary contusion,[12–14] pancreatitis,[15] bile duct injury,[16] bowel perforation,[17,18] aortic aneurysm rupture,[19] retroperitoneal haemorrhage,[20] cardiac dysrhythmias and gastric erosions.[21] Respiratory muscle fatigue contributes to ventilatory pump failure. The cellular and the biochemical mechanisms underlying this phenomenon, however, remain unclear.[22] Massive hemoptysis and death from pulmonary damage due to vascular and alveolar rupture has been reported in laboratory animals after a single exposure of the thoracic region to ESWLs.[23] This potential of ESWLs to cause pulmonary damage poses a real problem during lithotripsy in children because of the close proximity of the kidneys to the lungs.[13]

Recent studies have demonstrated that oxidative stress, an imbalance between oxidant flux and antioxidant defences where oxidants predominate, is associated with contractile

*Correspondence to: İlhan Gecit, Faculty of Medicine, Department of Urology, Yuzuncu Yil University, 65100 Van, Turkey.
E-mail: ilhan_gecit@hotmail.com

*Received 11 June 2011*
*Revised 24 November 2011*
*Accepted 16 December 2011*

dysfunction in adult muscle.[24] Moreover, investigators have shown that a major source of reactive oxygen species (ROS) in muscle is the mitochondria and that oxidant flux in muscle is directly related to the rate of mitochondrial oxygen consumption.[25] If the skeletal muscle production of ROS is enhanced by the maturational increase in mitochondrial capacity and not matched by an increase in antioxidant enzyme defence, then oxidative stress may result and contribute to an increased susceptibility to fatigue in the adult.

Trace elements serve essential metabolic functions in the liver and diaphragm muscles, and the liver plays a central role in the disposition of most trace elements. Much work is needed to identify the optimal measures of trace element adequacy and the functional consequences of deficiency states.[26]

By assuming that ESWL exposed to kidney also affects the adjacent organs in rats, this study was designed to investigate the effect of short-term ESWL exposure to kidney on oxidative stress and some trace element levels in liver and diaphragm muscles of rats.

## MATERIALS AND METHODS

### *Treatment of animals*

Twelve male Wistar albino rats, approximately 6 months of age, with an average body weight of 250 to 300 g were obtained from the Animal Laboratory of Yuzuncu Yil University. Rats were housed in specific cages. A 12:12-h light–dark cycle was maintained, and the rats were feed *ad libitum*. All animals received humane care according to the criteria outlined in the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and published by the National Institutes of Health.

### *Experimental design*

The animals were divided randomly into two groups, each consisting of six rats. The animals in the first group did not receive any treatment and served as control group. The right-side kidneys of animals in group 2 (ESWL treated) were treated with two-thousand 18-kV shock waves (Multimed 2001™ lithotripter; Elmed Co., Department of Urology, Faculty of Medicine, Yuzuncu Yil University, Van, Turkey) while anesthetized with 50 mg $kg^{-1}$ of ketamine. The localization of the right kidney was achieved after contrast medium injection through a tail vein under fluoroscopy control. The animals in both groups were killed 72 h after the ESWL treatment, and livers and diaphragm muscles were harvested for the determination of oxidant or antioxidant statues and trace element levels.

### *Biochemical analysis*

*Measurement of malondialdehyde level.* A tissue specimen of 50 mg was homogenized in 0.15 mol $l^{-1}$ of KCl. After the homogenate had been centrifuged at 1600*g*, the malondialdehyde (MDA) levels in tissue homogenate supernatant were determined by the thiobarbituric acid reaction according to Kavak *et al.* [27]. The principle of the method is based on measuring absorbance of the pink colour produced by the interaction of thiobarbituric acid with MDA at 530 nm. Values were expressed as milligrams per decilitre.

*Measurements of superoxide dismutase and glutathione peroxidase enzyme activities.* The tissues were homogenized in physiological saline (1 g in 5 ml) using a homogenizer (B. Braun Melsungen AG 853202, Germany) and then, centrifuged at 4000*g* for 20 min (Heraus Labofur 200, Germany). Glutathione peroxidase (GSH-Px) activity was measured by following changes in nicotinamide adenine dinucleotide phosphate absorbance at 340 nm,[28] by measuring decrease of $H_2O_2$ absorbance at 240 nm.[29] Superoxide dismutase (SOD) activity was measured by the method based on nitroblue tetrazolium (NBT) reduction rate. One unit for SOD activity was expressed as the enzyme protein amount causing 50 percent inhibition in NBT reduction rate.[30]

*Measurement of GSH level.* The GSH levels of tissues were measured at 412 nm using the method of Sedlak and Lindsay [31]. The samples were precipitated with 50% TCA and then centrifuged at 1000*g* force for 5 min. The reaction mixture contained 0.5 ml of supernatant, 2.0 ml of Tris-EDTA buffer (0.2 M, pH 8.9) and 0.1 ml of 0.01 M DTNB. The solution was kept at room temperature for 5 min and then read at 412 nm on the spectrophotometer.

*Measurements of mineral-heavy metal levels.* Exactly 2.0 ml of the mixture of $HNO_3/H_2O_2$ (2:1) was added to 0.7 g of the tissue samples. The mixture was placed into the water bath at 70 °C for 30 min and stirred occasionally. Then, 1.0 ml of the same acid mixture was added, and the mixture was transferred into a Teflon vessel bomb for the microwave oven. The bomb was closed, and the solution was placed inside the microwave oven. Radiation was applied for 3 min at 450 W. After the addition of 0.5 ml of the same acid mixture, radiation was repeated for 3 min. After cooling for 5 min, 2.0 ml of 0.1 mol $l^{-1}$ $HNO_3$ was added, and the solution was transferred into a Pyrex tube. After centrifugation, the clear solution was used for the determination of Cu, Zn, Mg, Mn, Pb, Cd, Ni and Fe.[32,33] They were measured by Atomic Absorption Spectrophotometer measurements using a UNICAM-929 spectrophotometer (Unicam Ltd, York Street, Cambridge, UK).

### *Statistical analysis*

The results were expressed as mean ± SD. The Kruskal–Wallis test (which is non-parametric) was used for the comparison of groups. When significant differences were observed ($P < 0.05$), Tukey multiple comparison test was used to determine the difference between groups. Statistical analyses were carried out using the SPSS® statistical software package (SPSS for Windows version 13.0, SPSS Inc., Chicago, IL, USA).

## RESULTS

Although MDA level increased significantly ($P < 0.05$), SOD and GSH-Px enzyme activities significantly decreased ($P < 0.05$) in the livers and diaphragm muscles of ESWL-treated rats (Figures 1–3). Although the GSH

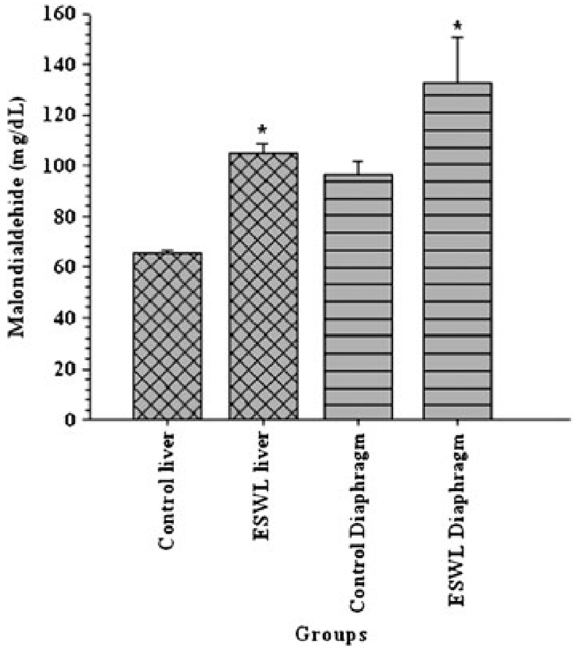


Figure 1. MDA levels in liver and diaphragms of control and ESWL-treated rats. *Significant difference ($P < 0.05$) between ESWL-treated and control groups

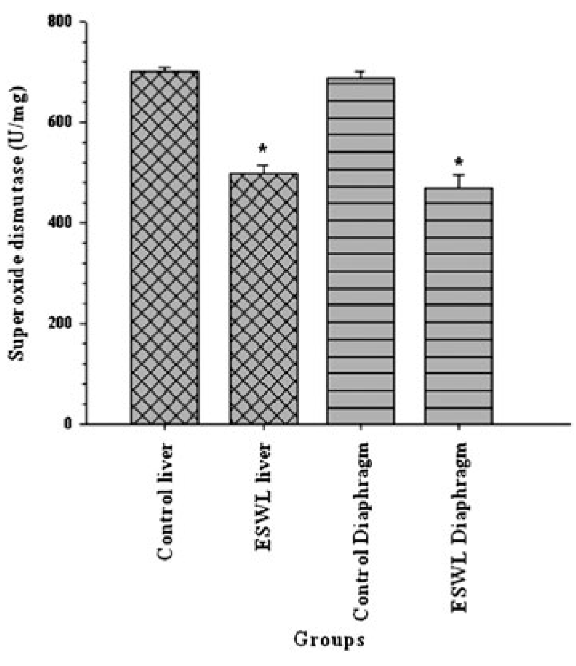


Figure 2. SOD levels in liver and diaphragms of control and ESWL-treated rats. *Significant difference ($P < 0.05$) between ESWL-treated and control groups

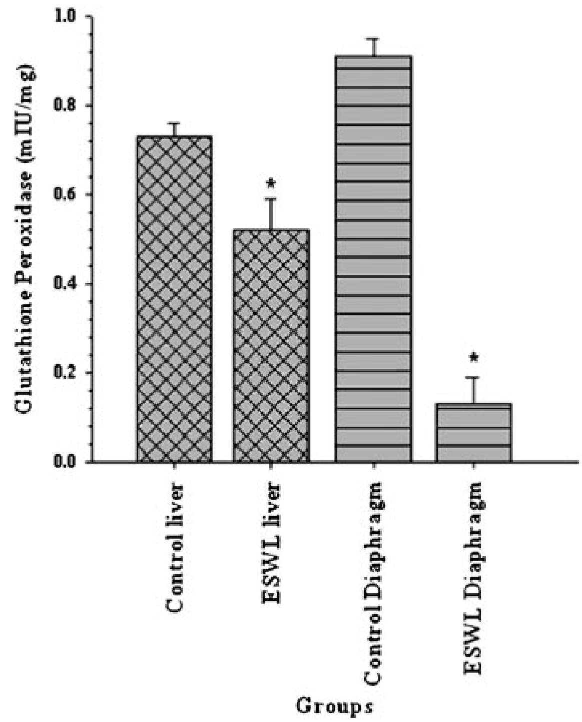


Figure 3. GSH-Px levels in liver and diaphragms of control and ESWL-treated rats. *Significant difference ($P < 0.05$) between ESWL-treated and control groups

level increased ($P < 0.05$) in the liver, it decreased ($P < 0.05$) in the diaphragm muscles of ESWL-treated animals (Figure 4).

Fe, Mg and Mn levels decreased ($P < 0.05$), and Cu and Pb levels increased ($P < 0.05$) in the livers of ESWL-treated animals (Table 1). Fe and Cu levels increased ($P < 0.05$), and Mg, Pb, Mn and Zn levels decreased ($P < 0.05$) in diaphragm muscles of ESWL-treated animals (Table 1).

## DISCUSSION

The aim of this study was to investigate the effect of short-term ESWL treatment of kidney on oxidative stress and some trace element levels in liver and diaphragm muscles of rat. It was found that the lipid peroxidation products increased in livers and diaphragm muscles after the ESWL treatment of kidney. This indicated the presence of increased tissue oxidative stress after ESWL treatment. In a previous study, it was demonstrated that ESWL treatment was responsible for the increased free radical production and impaired antioxidant defence potential.[34] In another experimental study, tissue antioxidant enzymes decreased, whereas free radical production and lipid peroxidation increased after exposure to shock wave energy.[35]

Free radicals are highly reactive compounds that are created in the body during normal metabolic functions or introduced from the environment. Free radicals are inherently unstable because they contain energy. To reduce their energy load, free radicals react with certain chemicals

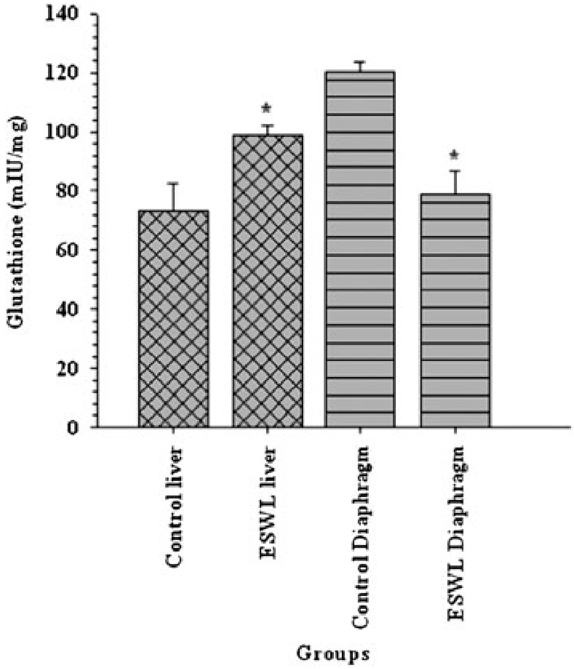


Figure 4. GSH levels in liver and diaphragms of control and ESWL-treated rats. *Significant difference ($P < 0.05$) between ESWL-treated and control groups

in the body and in the process interfere with the cells ability to function normally. Free radicals are believed to play a vital role in more than 60 different health conditions, including the ageing process, cancer and atherosclerosis. Fatty acyl side chains peroxidation in biological membranes by ROS and transition metal ions in a free radical chain reaction can be deleterious for membrane permeability as it can produce toxic compounds, such as MDA and acetaldehyde, which in turn produce abnormal adducts with biological substances, including DNA and RNA.[36,37]

Most living organisms possess enzymatic and non-enzymatic defence systems against excess production of ROS. However, different external factors such as those induced by ESWL, decrease the capability of such protective systems resulting in disturbances of the redox equilibrium that is established in healthy conditions. In the present study, it was found that SOD and GSH-Px enzyme activities decreased in the liver and diaphragm muscles of ESWL-treated rats. SOD, an enzyme, is found in the cytosol and mitochondria of cells. It is responsible for decreasing superoxide levels. GSH-Px is in the mitochondria and cytosol and reduces organic hydroperoxides and hydrogen peroxide in reaction that involves GSH. GSH is not only a cofactor for GSH S-transferase but also serves as a reductant for GSH-Px, an enzyme involved in natural protection by free radicals, in addition to SOD and catalase.[38,39] Decreased tissue SOD and GSH-Px enzyme activities in this study suggested that ESWL treatment produced damage of the cell membrane, leading to an increase in MDA level, and also damage to cell components such as mitochondria, which contains SOD and GSH-Px.[40]

GSH is one of the most important water-soluble antioxidants because of its action not only as a scavenger but also as an indispensable factor in proper catalytic action of some antioxidative enzymes.[41] In the present study, although the GSH level increased in liver, it decreased in diaphragm muscles of ESWL-treated animals. Decreased diaphragm muscle GSH content might be due to the export of the oxidized form of the damaged tissue. It could be explained by the fact that oxidized GSH, produced by oxidant stress, was being exported from the tissue at a faster rate than the production of new GSH.[42] Because the glucose-6-phosphate dehydrogenase (G-6-PD) catalyzes the first step of the pentose phosphate pathway, which provides the nicotinamide adenine dinucleotide phosphate necessary for the conversion of oxidized GSH (GSSG) to GSH, decreased tissue GSH level was probably due to the decreased G-6-PD activity that caused the diminished production of the GSH.

In biological systems, metals are mostly bound to proteins, forming metalloproteins. Many of the metals in metalloproteins are part of enzymatic systems, have structural and storage functions or use the protein to be transported to their target site in the organism. In humans, Mn, Fe, Cu, Zn and Se accomplish decisive functions to maintain human health. Deficiency in any of these trace elements leads to undesirable pathological conditions that can be prevented or reversed by adequate supplementation.[43] In sufficiently nourished persons, supplementation should be carefully controlled, given the toxic effects ascribed to trace elements when present

Table 1. Some mineral concentrations in the liver and diaphragm muscles of control and ESWL-treated rats ($n = 6$)

| Minerals ($\mu g\ dl^{-1}$) | Control | | ESWL treated | |
|---|---|---|---|---|
| | Liver | Diaphragm | Liver | Diaphragm |
| Fe | 4.43 ± 1.8 | 1.7 ± 0.3 | 2.80 ± 1.4* | 2.34 ± 0.89* |
| Mg | 11.8 ± 0.12 | 13.7 ± 2.8 | 7.72 ± 1.97* | 8.75 ± 2.75* |
| Cu | 0.08 ± 0.01 | 0.08 ± 0.04 | 0.13 ± 0.02* | 0.20 ± 0.01* |
| Pb | 0.11 ± 0.01 | 0.16 ± 0.04 | 0.23 ± 0.03* | 0.09 ± 0.01* |
| Mn | 0.02 ± 0.001 | 0.04 ± 0.03 | 0.012 ± 0.002* | 0.012 ± 0.001* |
| Zn | 0.51 ± 0.17 | 0.84 ± 0.38 | 0.57 ± 0.08 | 0.34 ± 0.07* |
| Cd | 0.02 ± 0.003 | 0.04 ± 0.008 | 0.02 ± 0.003 | 0.05 ± 0.003 |
| Ni | 0.22 ± 0.05 | 0.35 ± 0.03 | 0.3 ± 0.06 | 0.25 ± 0.01 |

The results are expressed as mean ± SD.
*Significant difference ($P < 0.05$) between ESWL-treated and control groups.

in quantities exceeding those required for accomplishing their biological functions.[43]

In the present study, Fe, Mg and Mn levels decreased, and Cu and Pb levels increased in the liver of ESWL-treated rats. Fe and Cu levels increased, and Mg, Pb, Mn and Zn levels increased in the diaphragm muscles of ESWL-treated animals. Excess or deficiency of these minerals is not desirable and may cause disturbance in organ functions. Although Fe is an essential nutritional mineral for all life forms, it is known that excess in Fe and Fe deficiency also leads to oxidative DNA damage.[44] Fe chelators can deplete Fe or cause oxidative stress in the tumour because of redox perturbations in its environment. Cu is an essential element that plays a role in the production of haemoglobin, myelin, collagen and melanin.[45] Recent evidence also suggests that adequate intake of Cu is necessary for normal immune function.[46] Cu deficiency affects various physiological functions that may be important in immunological defence to pathogenic challenge.[47] Pb increases oxidative stress, affects endothelial function, promotes inflammation, down-regulates nitric oxide production and induces renal dysfunction.[48] Mn is essential for normal physiologic functioning in humans and animals.[49] It also plays a role in the free radical scavenging activity of SOD. Cd is a dangerous occupational and environmental toxin. It accumulates in the human organism mainly in liver and kidneys.[50] Cd is a ubiquitous toxic heavy metal and unlike organic compounds, it is not biodegradable and has a very long biological half-life.[51] On the basis of data in animals and humans, Cd in blood soon after exposure is present in plasma, whereas it later becomes bound to red blood cells.[52] Mg is the fourth most abundant cation in the body and plays a pivotal role as an enzyme cofactor in biosynthesis of proteins and mineral administration. Its metabolism is connected with the bone, and it is indispensable to osteogenesis and mineralization of bones.[53] Subacute Mg deficiency has caused lymphopoietic neoplasms in young rats.[54]

## CONCLUSIONS

The short-term ESWL treatment of kidney may increase lipid peroxidation and decrease the antioxidant enzyme activity in the adjacent tissues such as liver and diaphragm muscles. It also causes a decrease or increase in many mineral levels in liver and diaphragm muscles, which is an undesirable condition for the normal physiological function of tissues. However, more studies are needed to verify and clarify the roles of the oxidative stress, antioxidant enzyme activities and trace element levels in the pathogenesis of ESWL.

## CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.


## ACKNOWLEDGEMENT

The authors thank Dr Sıdık Keskin for his help in the statistical evaluation of our data.


## REFERENCES


1. Aksoy Y, Malkoc İ, Atmaca AF, Aksoy H, Altinkaynak K, Akcay F. The effects of extracorporeal shock wave lithotripsy on antioxidant enzymes in erythrocytes. *Cell Biochem Funct* 2006; **24**: 467–469.
2. Evan AP, Willis LR, Connors B, Reed G, McAteer JA, Lingeman JE. Shock wave lithotripsy-induced renal injury. *Am J Kidney Dis* 1991; **17**: 445–450.
3. Gunasekaran S, Donovan JM, Chvapil M, Drach GW. Effects of extracorporeal shock wave lithotripsy on the structure and function of rabbit kidney. *J Urol* 1989; **141**: 1250–1254.
4. Munver R, Delvecchio FC, Kuo RL, Brown SA, Zhong P, Preminger GM. In vivo assessment of free radical activity during shock wave lithotripsy using a microdialysis system: the renoprotective action of allopurinol. *J Urol* 2002; **167**: 327–334.
5. Serel TA, Ozguner F, Soyupek S. Prevention of shock wave-induced renal oxidative stress by melatonin: an experimental study. *Urol Res* 2004; **32**: 69–71.
6. Morgan TR, Laudone PV, Heston WD, Zeitz L, Fair WR. Free radical production by high energy shock waves comparison with ionising irradiation. *J Urol* 1988; **139**: 186.
7. Suhr D, Brummer F, Hulcer DF. Cavitation-generated free radicals during shock wave exposure: investigations with cell-free solutions and suspended cells. *Ultrasound Med Biol* 1991; **17**: 761.
8. Sokolov DL, Bailey MR, Crum LA, Blomgren PM, Connors BA, Evan AP. Prefocal alignment improves Stone comminution in shockwave lithotripsy. *J Endourol* 2002; **16**: 709.
9. Kırkali Z, Kırkali G, Tahiri Y. The effect of extracorporeal electromagnetic shock waves on renal proximal tubular function. *Int Urol Nephrol* 1994; **26**: 255.
10. Crum LA. Cavitation microjets as a contributory mechanism for renal calculus disintegration in ESWL. *J Urol* 1988; **140**: 1587.
11. Ilnyckyj A, Hosking DH, Pettigrew NM, Bernstein CN. Extra-corporeal shock wave lithotripsy causing colonic injury. *Dig Dis Sci* 1999; **44**: 2485–2487.
12. Tiede JM, Lumpkin EN, Wass CT, Long TR. Hemoptysis following extracorporeal shock wave lithotripsy: a case of lithotripsy-induced pulmonary contusion in a pediatric patient. *J Clin Anesth* 2003; **15**: 530–533.
13. Eroglu M, Cimentepe E, Demirag F, Unsal E, Unsal A. The eVects of shock waves on lung tissue in acute period: an in vivo study. *Urol Res* 2007; **35**: 155–160.
14. Malhotra V, Rosen RJ, Slepian RL. Life-threatening hypoxemia after lithotripsy in an adult due to shock-wave-induced pulmonary contusion. *Anesthesiology* 1991; **75**: 529–531.
15. Hassan I, Zietlow SP. Acute pancreatitis after extracorporeal shock wave lithotripsy for a renal calculus. *Urology* 2002; **60**: 1111–1113.
16. Hung SY, Chen HM, Jan YY, Chen MF. Common bile duct and pancreatic injury after extracorporeal shock wave lithotripsy for renal stone. *Hepatogastroenterology* 2000; **47**: 1162–1163.
17. Geh JL, Curley P, MayWeld MP, Price JJ. Small bowel perforation after extracorporeal shock wave lithotripsy. *Br J Urol* 1997; **79**: 648–649.
18. Castillon I, Frievro O, Gonzales-Enguita C, Vela-Navarrete R. Colonic perforation after extracorporeal shock wave lithotripsy. *BJU Int* 1999; **83**: 720–721.
19. Gugulakis A, Matsagas MI, Liapis CD, Vasdekis SN, Sechas MN. Rupture of the abdominal aorta following extracorporeal shock-wave lithotripsy. *Eur J Surg* 1998; **164**: 233–235.
20. Bex A, Goepel M, MollhoV S. Extensive retroperitoneal hematoma following extracorporeal shock wave lithotripsy with second generation lithotripter. *Urol Int* 1992; **48**: 111–114.

21. Newmark JR, Wong MYC, Lingeman JE. Complications. In *Smith's Textbook of Endourology*, Smith AD (ed.)(ed.). Quality Medical Publishing: St. Louis, 1996; 681–693.
22. Watchko JF, Mayock DE, Standaert TA, Woodrum DE. The ventilatory pump: neonatal and developmental issues. *Adv Pediatr* 1991; **38**: 109–134.
23. Chaussy CG. *Extracorporeal shock wave lithotripsy: new aspects in the treatment of kidney stone disease*. Karger: Munich, 1982; 24–35.
24. Anzueto A, Supinski GS, Levine SM, Jenkinson SG. Mechanisms of disease: are oxygen-derived free radicals involved in diaphragmatic dysfunction? *Am J Respir Crit Care Med* 1994; **149**: 1048–1052.
25. Boveris A, Chance B. The mitochondrial generation of hydrogen peroxide. *Biochem J* 1973; **134**: 707–716.
26. Versieck J. Trace elements in human body fluids and tissues. *Crit Rev Clin Lab Sci* 1985; **22**: 97–184.
27. Kavak S, Ayaz L, Emre M, Inal T, Tamer L, Günay I. The effects of rosiglitazone on oxidative stress and lipid profile in left ventricular muscles of diabetic rats. *Cell Biochem Funct* 2008; **26**(4): 478–485.
28. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967; **70**: 158–169.
29. Aebi H. Catalase. In *Methods of enzymatic analysis*, Bergmayer HU (ed.)(eds). Academic Press Inc: New York, 1974; 673–677.
30. Durak İ, Canbolat O, Kavutcu M, Oztürk HS, Yurtarslanı Z. Activities of total, cytoplasmic, and mitochondrial superoxide dismutase enzymes in sera and pleural fluids from patients with lung cancer. *J Clin Lab Anal* 1996; **10**: 17–20.
31. Sedlak J, Lindsay RHC. Estimation of total, protein bound and non-protein sulfhydryl groups in tissue with Ellmann's reagent. *Anal Biochem* 1968; **25**: 192–205.
32. Bush VJ, Moyer TP, Batts KP, Parisi JE. Essential and toxic element concentrations in fresh and formalin-fixed human autopsy tissues. *Clin Chem* 1995; **41**: 284–294.
33. Yaman M, Akdeniz I. Sensitivity enhancement in flame atomic absorption spectrometry for determination of copper in human thyroid tissues. *Anal Sci* 2004; **20**: 1363–1366.
34. Biri H, Ozturk HS, Buyukkocak S, *et al*. Antioxidant defense potential of rabbit renal tissues after ESWL: protective effects of antioxidant vitamins. *Nephron* 1998; **79**: 181–185.
35. Sarica K, Kosar A, Yaman O, *et al*. Evaluation of ischemia after ESWL: detection of free oxygen radical scavenger enzymes in renal parenchyma subjected to high-energy shock waves. *Urol Int* 1996; **57**: 221–223.
36. Wei YH, Lee HC. Oxidative Stress, Mitochondrial DNA Mutation And Impairment Of Antioxidant Enzymes In Aging. *Exp Biol Med* 2002; **227**: 671–682.
37. Abdulah W, Hamedan AL, Lalualit M, Anfenan K. Antioxidant Activity of Leek towards Free Radical Resulting from Consumption of Carbonated Meat in Rats. *Life Sci J* 2002; **8**(1): 2011.
38. Kanno T, Sato EE, Muranaka S, Fujita H, Fujiwara T, Utsumi T, Inoue M. Oxidative Stress Underlies The Mechanism For Ca(2+)-Induced Permeability Transition Of Mitochondria. *Free Radical Res* 2004; **38**(1): 27–35.
39. Linder MC. Other trace elements and the liver. *Semin Liver Dis* 1984; **4**: 264–276.
40. Fridovich I. Superoxide dismutases. *Ann Rev Biochem* 1995; **64**: 97–112.
41. Briviba K, Sies H. Non enzymatic antioxidant defense system. In *Natural Antioxidants in Human Health and Disease*, Frei B (ed.)(ed.). Acad Press: San Diego, 1994; 107–128.
42. Riley PA. Free radicals in biology: oxidative stress and the effects of ionizing radiation. *Int J Radiat Biol* 1994; **1**: 27–33.
43. Cesar G. Fraga * Relevance, essentiality and toxicity of trace elements in human health. *Mol Aspect Med* 2005; **26**: 235–244.
44. Ames BN. DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. *Mutat Res* 2001; **475**: 7–20.
45. Aggett PJ. An overview of the metabolism of copper. *Eur J Med Res* 1999; **4**: 214–216.
46. Ertekin A, Değer Y, Mert H, Mert N, Yur F, Dede S, Demir H. An investigation of the effects of alpha-tocopherol on the levels Fe, Cu, Zn, Mn and carbonic anhydrase in rats with bleomycin-induced pulmonary fibrosis. *Biol Trace Elem Res* 2006; **114**: 1–12.
47. Stabel JR, Spears JW, Brown TT. Effect of copper deficiency on tissue, blood characteristics and immune function of calves challenged with infectious bovine rhinotracheitis virus and Pasteurella hemolytica. *J Anim Sci* 1993; **71**: 1247–1255.
48. Lustberg M, Silbergeld E. Blood lead levels and mortality. *Arch Intern Med* 2002; **162**: 2443–2449.
49. Bureau I, Anderson RA, Arnaud J, Raysiguier Y, Favier AE, Roussel AM. Trace mineral status in post menopausal women: impact of hormonal replacement therapy. *J Trace Elem Med Biol* 2002; **16**: 9–13.
50. Kowalczyk E, Kopff A, Fijalkowski P, Kopff M, Niedworok J, Blaszczyk J, Kêdziora J, Tyoelerowicz P. Effect of anthocyanins on selected biochemical parameters in rats exposed to cadmium. *Acta Bio Pol* 2003; **50**(2): 543–548.
51. Messner B, Knoflach M, Seubert A, Ritsch A, Pfaller K, Henderson B, Shen YH, Zeller I, Willeit J, Laufer G, Wick G, Kiechl S, Bernhard D. Cadmium Is a Novel and Independent Risk Factor for Early Atherosclerosis Mechanisms and In Vivo Relevance. *Arterioscler Thromb Vasc Biol* 2009; **29**: 1392.
52. Nordberg M. *Environ Res* 1978; **15**: 381–404.
53. Rahnama M, Marcınıak A. Influence of Estrogen Deficiency on The Level of magnesium In Rat Mandible and Teeth. *Bull Vet Inst Pulawy* 2002; **46**: 267–271.
54. Ilıcan G. Serum copper and magnesium levels in leukemia and malignant lymphoma. *Lancet* 1971; **6**: 1036.


 