

## Effects of Shock Waves on Oxidative Stress, Antioxidant Enzyme and Element Levels in Kidney of Rats

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Received: 26 February 2011 / Accepted: 13 June 2011 /  
Published online: 24 June 2011  
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**Abstract** The aim of this study was to investigate the effect of extracorporeal shock wave lithotripsy (ESWL) on kidney oxidative stress and trace element levels of adult rats. Twelve male Wistar albino rats were divided equally into two groups. First group was used as control. The right-side kidneys of animals in second group were treated with 2,000 18-kV shock waves under anesthesia. Localization of the right kidney was achieved following contrast medium injection through a tail vein under flouroscopy control. The animals were sacrificed 72 h after the ESWL treatment, and the kidneys were taken. Malondialdehyde level was higher in the ESWL group than in the control. Reduced glutathione levels, superoxide dismutase, and glutathione peroxidase activities were lower in the ESWL group than those of the control. Fe, Cu, Pb, Mn, Cd, and Ni levels were lower in the ESWL group than in the control, although Mg level was higher in the ESWL group than in the control. In conclusion, the result of the present study indicated that ESWL treatment produced oxidative stress in the kidney and caused impairments on the antioxidant and trace element levels in the kidneys of rats.

**Keywords** ESWL treatment · Kidney · Oxidative stress · Antioxidant enzyme · Rat

### Abbreviations

ESWL Extracorporeal shock wave lithotripsy

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## Introduction

The mechanism of extracorporeal shock wave lithotripsy (ESWL)-induced cellular damage is still controversial. One of the mechanisms discussed for tissue damage is free radical formation during ESWL treatment [1–3]. 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 homolytic cleavage of molecules may take place leading to the formation of free radicals [4–6]. Because of their very high reactivities, free radicals can cause serious damage to the macromolecules in cells [7].

Free radical formation has been reported during ESWL treatment [1]. Serel et al. [1] adapted the Fricke ferrous sulfate radiation dosimeter to examine the chemical effects of high-energy shock waves, and significant free radical production was documented. Suhr et al. [3] presented intra- and extracellular *in vitro* measurements of free radicals and investigated cell viability after shock wave treatment. They demonstrated an elevated concentration of intracellular free radicals during such treatment in suspended cells *in vitro*. The source of reactive oxygen species (ROS) in the tissue are neutrophils recruited into the necrotic region, as well as metabolic transformation of hypoxanthine and xantine to uric acid. Subsequent reactions generate lipid peroxides as well as cytotoxic and oxidation products, among which is malondialdehyde (MDA) [8].

Many factors are involved in the formation of urolithiasis. The absence of crystallization inhibitors and the presence of crystallization promoters are critical to renal stone formation [9]. Various element concentrations are also found to be extremely variable in different categories of stones [10]. There are conflicting results regarding the role of Zn and Mg in urolithiasis. Zn is reported to act as a potent inhibitor of crystallization and is found to be abnormally low in the urine of stone formers [11]. In contrast, several studies reported that excretion of Zn increased in the urine of stone formers or no significant difference between healthy controls and stone formers [11, 12]. Moreover, early studies showed that dietary Mg deficiency causes experimental urolithiasis [12].

There are scarce reports on the effects of ESWL on antioxidant and element levels in patients and experimental animals with urolithiasis, and they are conflicting. The current study was designed to investigate the effect of short-term ESWL exposure on oxidative stress and element levels in the kidney of rats.

## Materials and Methods

### Animals

Twelve male Wistar albino rats, approximately 6 months of age, with an average body weight of 250–300 g, were obtained from the Animal Laboratory of Yuzuncu Yil University. Rats were housed in specific cages. A 12-h light/dark cycle was maintained, and the rats were fed *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 2,000 18-kV shock waves (Multimed 2001™ lithotripter–Elmed, located at the Department of Urology, School of Medicine, Yuzuncu Yil University, Van, Turkey. This lithotripter has an 80-nF capacitor, 135 mm focal distance, and a focal zone of about 22 mm in diameter and 7.5 mm in length) while anesthetized with 50 mg/kg ketamine. Localization of the right kidney was achieved following contrast medium injection through a tail vein under fluoroscopy control. The animals in both groups were sacrificed 72 h after the ESWL treatment, and the right kidneys were harvested for the determination of oxidant/antioxidant status and trace element levels.

## Biochemical Analysis

### *Measurement of MDA Level*

A tissue specimen of 50 mg was homogenized in a solution (0.15 mol/l KCl). After the homogenate had been centrifuged at  $1,600\times g$ , the MDA levels in tissue homogenate supernatant were determined by the thiobarbituric acid (TBA) reaction according to Placer et al. [13]. The principle of the method is based on measuring absorbance of the pink color produced by the interaction of TBA with MDA at 530 nm. Values were expressed as milligrams per deciliter.

### *Superoxide Dismutase and Glutathione Peroxidase Assays*

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  $4,000\times g$  for 20 min (Heraus Labofur 200, Germany). Glutathione peroxidase (GSH-Px) activity was measured by following changes in NADPH absorbance at 340 nm [14] and by measuring decrease of  $H_2O_2$  absorbance at 240 nm [11]. Superoxide dismutase (SOD) activity was measured by the method based on nitroblue tetrazolium (NBT) reduction rate. One unit of SOD activity was expressed as the enzyme protein amount causing 50% inhibition in NBT reduction rate [15].

### *Measurement of CAT Level*

Biochemical analysis of erythrocyte catalase (CAT) activity was performed with a method described by Aebi [16]. Briefly, the supernatant (0.1 ml) was added to a quartz cuvette containing 2.95 ml of 19 mmol/l  $H_2O_2$  solution prepared in potassium phosphate buffer (0.05 M, pH 7.00). The change in absorbance was monitored at 240 nm for 5 min using a spectrophotometer (Shimadzu UV-1201, Japan).

### *Measurement of Reduced Glutathione Level*

The glutathione (GSH) levels of tissues were measured at 412 nm using the method of Sedlak and Lindsay [17]. The samples were precipitated with 50% TCA and then centrifuged at  $1,000\times g$  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 Levels

Exactly 2.0 mL of the mixture of  $\text{HNO}_3/\text{H}_2\text{O}_2$  (2:1) was added to 0.7 g of the tissue samples. The mixture was placed into the water bath at  $70^\circ\text{C}$  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 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  $\text{HNO}_3$  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 [18]. They were measured by atomic absorption spectrophotometer measurements using a UNICAM-929 spectrophotometer (Unicam Ltd, York Street, Cambridge, UK).

### Statistical Analysis

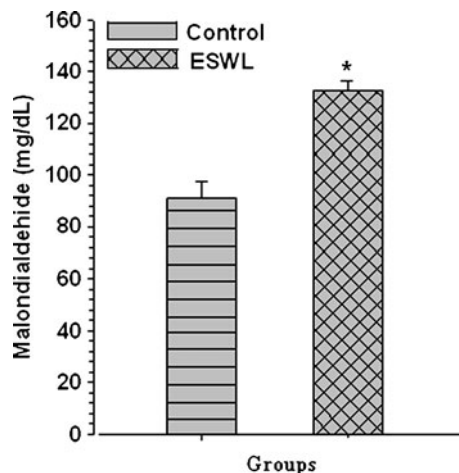
The results were expressed as mean  $\pm$  standard deviation (SD). Kruskal–Wallis test was used for the comparison of groups. When significant differences were observed ( $p < 0.05$ ), Tukey multiple comparison test was used to determine the difference between groups. Statistical analyses were carried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

### Results

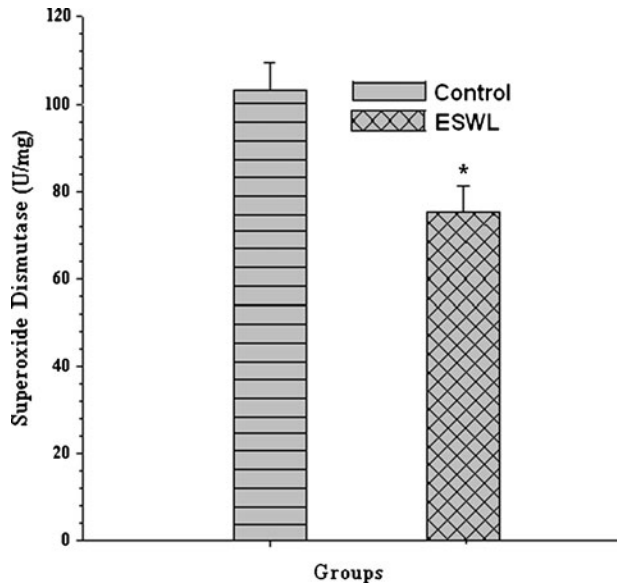
MDA levels in both groups are shown in Fig. 1. Mean MDA levels in control and ESWL groups were 92 and 128 mg/dL, respectively. MDA level was significantly ( $p < 0.05$ ) higher in the ESWL group than in the control. Hence, ESWL exposure induced oxidative stress.

GSH levels, SOD, and GSH-Px activities are shown in Figs. 2, 3, and 4. They were significantly ( $p < 0.05$ ) lower in the ESWL group than those of the controls.

**Fig. 1** MDA levels in the kidneys of control and ESWL-treated rats (mean  $\pm$  SD and  $n = 6$ ). \* $p < 0.05$  and versus control



**Fig. 2** SOD activity in the kidneys of control and ESWL-treated rats (mean $\pm$ SD and  $n=6$ ). \* $p<0.05$  and versus control

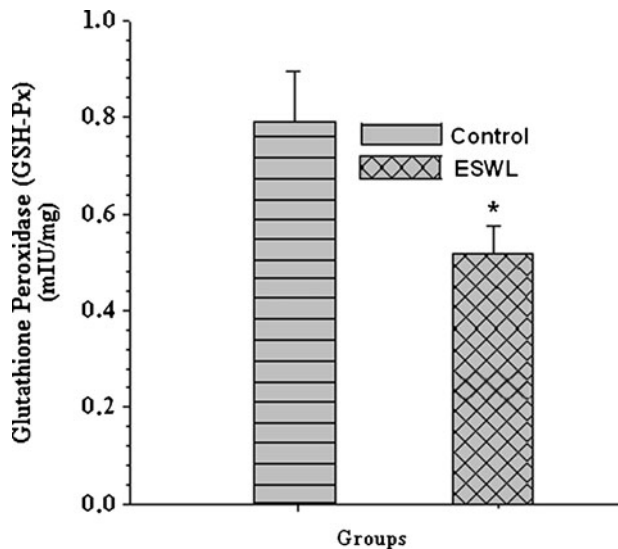


Tissue levels of Fe, Mg, Cu, Pb, Mn, Zn, Cd, and Ni of the control and ESWL groups are shown in Table 1. Fe, Cu, Pb, Mn, Cd, and Ni levels were significantly ( $p<0.05$ ) lower in the ESWL group than those of controls, although Mg levels were significantly ( $p<0.05$ ) higher in the ESWL group than in the control.

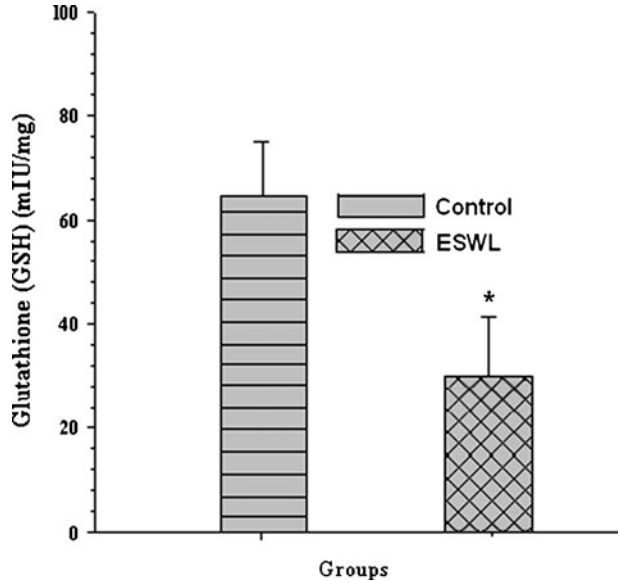
## Discussion

This study was designed to investigate the effect of short-term ESWL exposure to kidney on oxidative stress and some trace element levels in the kidneys of rats. It was found that

**Fig. 3** GSH-Px activity in the kidneys of control and ESWL-treated rats (mean $\pm$ SD and  $n=6$ ). \* $p<0.05$  and versus control



**Fig. 4** GSH levels in the kidneys of control and ESWL-treated rats (mean±SD and  $n=6$ ). \* $p<0.05$  and versus control



the lipid peroxidation levels increased in the kidney after the ESWL exposure, although GSH, SOD, and GSH-Px values decreased by ESWL exposure. This result indicated that ESWL treatment caused an increase in the free radical production and produced oxidative stress in the kidney.

Although ESWL is a noninvasive 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 ischemia and hypoxia. Increased free radical formation and subsequent damage to the kidney can occur in the reperfusion period [18–21]. Munver et al. [22] 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 model. In the current study, MDA levels were higher in the ESWL group than in the control. These results supported the view that enhanced oxidative stress is involved in the etiology of kidney pathophysiology in ESWL treatment.

GSH-Px is the main enzyme of the enzymatic antioxidant defense system responsible for protection against the increase in ROS production [23]. Hydrogen peroxide formed by the catalytic reaction of SOD is both a reactive form of oxygen and a normal cellular

**Table 1** Some mineral and heavy metal concentrations in the kidneys of control and ESWL-treated rats (mean±SD and  $n=6$ )

	Control	ESWL
Fe ( $\mu\text{g}/\text{dL}$ )	1.70±0.2	1.30±0.17*
Mg ( $\mu\text{g}/\text{dL}$ )	4.50±1.12	8.30±2.24*
Cu ( $\mu\text{g}/\text{dL}$ )	0.20±0.04	0.12±0.02*
Pb ( $\mu\text{g}/\text{dL}$ )	0.20±0.02	0.09±0.07*
Mn ( $\mu\text{g}/\text{dL}$ )	0.03±0.001	0.014±0.002*
Zn ( $\mu\text{g}/\text{dL}$ )	0.46±0.17	0.41±0.16
Cd ( $\mu\text{g}/\text{dL}$ )	0.032±0.006	0.011±0.006*
Ni ( $\mu\text{g}/\text{dL}$ )	0.34±0.03	0.23±0.06*

\* $p<0.05$  and versus control

metabolite, and it is further detoxified by GSH-Px and catalase [7]. The kidney is a relatively abundant focus of both free radical-mediated injury by virtue of enhanced endogenous rates of production of ROS and impairment of tissue free radical defense mechanisms. In addition, the cysteine derivative, GSH, is synthesized in erythrocytes, and glutathione disulfide is transported outside the cell to maintain a high GSH/oxide glutathione ratio [23]. Disulfide exchange reactions occur between protein thiols and low-molecular-weight disulfide. In the current study, we observed that GSH, SOD, and GSH-Px values were lower in the ESWL group than in the control. The decrease in kidney GSH, SOD, and GSH-Px values in rats during ESWL treatment has been attributed to the generation of free radicals and lipid peroxidation. Similarly, Sheng et al. [19] reported that renal SOD activity decreased and MDA concentration increased in patients with renal urolithiasis treated with ESWL. Aksoy et al. [21] investigated the effects of ESWL on SOD, catalase (CAT), and MDA values in erythrocytes of patients undergoing ESWL. They observed that MDA levels were higher in the ESWL group than in the control, although SOD and CAT activities were lower in the ESWL group than in the control. SOD, CAT, and MDA results of current study were supported by results of Sheng et al. [19] and Aksoy et al. [21].

Although Fe is an essential nutritional mineral for all life forms, it is known that excess Fe and Fe deficiency also lead to oxidative DNA damage [7]. Fe chelators can deplete Fe or cause oxidative stress in the tumor due to redox perturbations in its environment. Cu is an essential element that plays a role in the production of hemoglobin, myelin, collagen, and melanin [24]. Cu deficiency affects various physiological functions that may be important in immunological defense to pathogenic challenge [25, 26]. Cu has also function as cofactor of SOD and CAT in antioxidant redox system [26, 27]. Pb increases oxidative stress, affects endothelial function, promotes inflammation, downregulates nitric oxide production, and induces renal dysfunction [28]. Mn is essential for normal physiologic functioning in humans and animals. It also plays as a cofactor in the antioxidant SOD enzyme. In the present study, Fe, Cu, Pb, Mn, Cd, and Ni levels decreased, although Mg level increased in ESWL-treated kidneys. This result indicated that ESWL treatment caused a decrease in the element levels in the kidney. The decrease in the antioxidant element levels in the kidney of rats during ESWL treatment has been attributed to the generation of free radicals and lipid peroxidation.

## Conclusions

We observed that short-term ESWL treatment caused an increase in the lipid peroxidation production and a decrease in the antioxidant enzyme activity and element levels in the kidneys of ESWL-treated rats. It was concluded that ESWL treatment caused an increase in the free radical production and decrease in antioxidant enzyme activity. However, more studies are needed to verify and clarify the stress between antioxidant status, and trace element and heavy metal levels in the pathogenesis of ESWL.

**Acknowledgments** We thank Dr. Sidik Keskin for his help in statistical evaluation of our data.

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