Biochemistry
Research Article – 48751

Sedat Bilgiç*, Elif Özerol, Mustafa Iraz, Nurhan Şahin, Kevser Tanbek, Ahmet Çığlı

Carbontetrachloride induced acute liver damage and protective effect of n-acetylcysteine on rats with regenerated and non-regenerated liver

Karaciğeri rejenere olan ve olmayan sıçanlarda, karbontetraklorürle indüklenen akut karaciğer hasarı ve n-asetilsisteinin koruyucu etkisi

DOI 10.1515/tjb-2016-0029
Received May 4, 2015; accepted February 3, 2016

Abstract: Objective: Our aim was to investigate 70% partial hepatectomy (PH) groups, compare with not subjected to PH groups after exposure to hepatotoxic agents for alterations in the protective effects of antioxidant agents and sensitivity of the liver. Accordingly, we aimed to investigate the toxicity of a hepatotoxic agent, carbon tetrachloride (CCl_4), and protective effects of an antioxidant, N-acetylcysteine (NAC), in experimental animal model.

Methods: 67 male Wistar Albino rats were divided into 2 main groups to total 9 subgroups: group 1, underwent PH; group 2, not subjected to PH. 0.5 ml/kg CCl_4 and 50 mg/kg NAC was given intraperitoneally (i.p.) to the groups. On postoperative day 9, 70% PH was performed according to the method of Higgins and Anderson. Finally, all rats were humanely killed.

Results: Catalase (CAT) and superoxide dismutase (SOD) activities were significantly lower in both groups when CCl_4 was administered. NAC treatment was found to significantly increase these parameters ($P<0.05$). Malondialdehyde (MDA) and protein carbonyl (PC) levels were significantly greater in both groups when CCl_4 was administered ($P<0.05$). NAC treatment was found to significantly reduce these parameters.

Conclusion: These results indicated that CCl_4 increased oxidation products, reduced liver enzymatic activity and reduced proliferation activity in both hepatectomised and nonhepatectomised liver. The liver injury of CCl_4 and the protective effect of NAC was similarly in both main groups. Consequently, making PH may not create a negative effect and an additional health problems in liver. Thus, these results can positively affect the decisions of the healthy liver donors.

Keywords: Partial hepatectomy, liver, NAC (N-acetylcysteine), CCl_4 (carbon tetrachloride)

Özet: Amaç: Bu çalışmada amacımız %70 parsiyel hepatektomi (PH) yapılan grupların, PH yapılmayan gruplara göre karbontetraklorür (CCl_4) gibi hepatotoksik ajanlara maruziyetleri sonrasında antioksidan ajanların koruyucu etkilerindeki değişiklikleri ve karaciğer duyarlılığını araştırmaktır. Buna uygun olarak, hepatotoksik bir ajan olan CCl_4'ün toksisitesinin ve bir antioksidan olan N-asetilsistein'in (NAC) koruyuculuğunun deneysel hayvan modeli ile araştırılması amaçlandı.

Metod: Altmış yedi adet Wistar albino tipi erkek sıçan PH yapılan ve yapılmayan olmak üzere iki ana grup altında dokuz alt gruba ayrıldı. Gruplara intraperitonel (i.p.) olarak 0.5 ml/kg CCl_4 ve 50 mg/kg NAC enjekte edildi.

***Corresponding author: Sedat Bilgiç:** Adıyaman University, Department of Biochemistry, Adıyaman, Turkey, e-mail: sbilgic@adiyaman.edu.tr
Elif Özerol: İnönü University, Department of Biochemistry, Malatya, Turkey, e-mail: eozerol@gmail.com
Mustafa Iraz: İnönü University, Department of Pharmacology, Malatya, Turkey, e-mail: mustafairaz@yahoo.com
Nurhan Şahin: İnönü University, Department of Pathology, Malatya, Turkey, e-mail: sahin.nurhan@gmail.com
Kevser Tanbek: İnönü University, Department of Biochemistry, Malatya, Turkey, e-mail: ktanbek@hotmail.com
Ahmet Çığlı: İnönü University, Department of Biochemistry, Malatya, Turkey, e-mail: ahmet.cigli@inonu.edu.tr

Postoperatif dokuzuncu günde, Higgins and Anderson metoduna uygun olarak %70 PH gerçekleştirildi. Deneyin sonunda bütün sıçanlar usulünce kesildi.

Bulgular: Katalaz (CAT) ve süperoksit dismutaz (SOD) enzim aktiviteleri hem PH yapılan hem de yapılmayan gruplarda CCl_4 uygulandığında anlamlı olarak azaldığı gözlendi. NAC uygulandığında ise bu parametrelerin anlamlı olarak arttığı görüldü ($P<0.05$). CCl_4 uygulandığında her iki grupta doku malondialdehit (MDA) ve protein karbonil (PC) düzeylerinin anlamlı olarak yükseldiği tespit edildi ($P<0.05$). NAC'ın bu parametreleri anlamlı olarak düşürdüğü görüldü.

Sonuç: Bu sonuçlar, CCl_4'ün PH yapılan/yapılmayan karaciğerde oksidan maddeleri arttırdığını, karaciğer enzim aktivitelerini ve proliferasyonunu azaltığını göstermektedir. Ayrıca CCl_4'ün karaciğer hasarının ve NAC'ın koruyucu etkisinin her iki ana grupta da benzer şekilde olduğu görülmektedir. Sonuç olarak PH yapılması karaciğer donörlerinde olumsuz yönde bir etki ve ek bir sağlık sorunu oluşturmayabilir. Dolayısıyla bu sonuçlar sağlıklı karaciğer donörlerinin karar vermelerini olumlu etkileyebilir.

Anahtar Kelimeler: (PH) parsiyel hepatektomi, karaciğer, NAC (N-Asetilsistein), CCl_4 (karbontetraklorür),

Introduction

The liver is the only organ that exhibits a tremendous potential to regenerate after injury or surgical resection in the mammalian body. The recovery of patients after resection of tumors or donation for a living-related liver transplantation depends on the regenerative capacity of the remnant liver [1]. Most commonly, regeneration of the liver is studied by performing a surgical procedure which removes 2/3 of the liver mass in rodents, a technique known as 2/3 PH [2].

Loss of liver mass can be induced by administering hepatotoxic chemicals [2]. CCl_4 is a lipid-soluble potent hepatotoxic that is widely used as an animal model of acute hepatocellular necrosis [3]. The administration of CCl_4 significantly increases the release of hepatic enzymes, increases destruction of cytochrome P-450, increases lipid peroxidation products, and elicits an inflammatory response [4]. While, after PH, the liver restores its mass and function within days, CCl_4 toxicity leads to an irreversible impairment of liver function [5]. Treatment with antioxidants may modulate the toxic effects of CCl_4 on the liver. NAC has antioxidant, anticytotoxic and antiapoptotic properties and may therefore be useful in counteracting damaging events of CCl_4-induced hepatitis [6]. This drug has a diversity of applications, largely because of the chemical properties of the thiol moiety present in its structure. The ability of the reduced thiol moiety to sweep reactive oxygen species is well-established by NAC [7].

Partial liver grafts from living donors are usually transplanted to patients in liver transplantation. Therefore, in the present study our aim was to investigate alterations and sensitivities of the regenerated liver of healthy liver donors. Therefore regenerated liver, underwent 70% PH, compare with not regenerated liver, not subjected to PH, after exposure to hepatotoxic agents for alterations in the protective effects of antioxidant agents and sensitivity of the liver. Accordingly, we aimed to investigate the toxicity of a hepatotoxic agent, CCl_4, and protective effects of an antioxidant, NAC, in the regenerated liver and not regenerated liver in experimental animal model.

Materials and Methods

Chemicals

Carbon tetrachloride (CCl_4), 5,5'-dithiobis (2-nitrobenzoic acid; Ellman's reagent), thiobarbituric acid (TBA), trichloroacetic acid (TCA), hypochlorous acid (HOCl), and 2,4-dinitrophenylhydrazine (DNPH) were from Sigma Aldich GmbH, Germany. NAC (Fluimucil, 10% solution) was purchased from Zambon Group (Italy). Ki-67 mouse monoclonal antibody (MMI) was purchased from Leica (United Kingdom). All other chemicals and solvents were of the highest grade commercially available.

Experimental animals

The animals involved in this study were produced, maintained and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals prepared by the Inonu University, Animal Ethical Committee. All experiments were performed on male Wistar rats (each weighing 200–250 g at the time of the experiment). Rats were housed in polycarbonate cages with wire lids and given the standard laboratory chow and water. The housing room was maintained at 24°C with 42±5% relative humidity and had a 12-12-h light-dark cycle (lights on 06:00–18:00 h). Body temperature was maintained around 37±5°C throughout the surgical procedure.

Induction of hepatotoxicity by CCl_4

Oral LD_{50} values for the CCl_4 ranging from 1000 to 12800 mg/kg of body weight were reported in mice and rats [8]. For CCl_4, the dose was administered on the basis of the LD_{50} dose as determined by Lewis [9]. Previous studies indicated that animals were given a single dose of 0.5 ml/kg, i.p. CCl_4 [10]. The i.p. LD_{50} of NAC was reported in the literature as 1205 mg/kg body weight in rats. [11]. In our study, animals were administered a single dose of 0.5 ml/kg, i.p. CCl_4 and 6 doses of 50 mg/kg, i.p. NAC as reported in the literature.

Experimental protocol

67 male Wistar Albino rats (12 weeks old) weighing around 200–250 g were used. Rats were divided into 2 main groups to total 9 subgroups: group 1, underwent PH; group 2, not subjected to PH. Both main groups were assigned into four subgroups respectively: subgroup 1, control; subgroup 2, CCl_4 (0.5 ml/kg, i.p.); subgroup 3, NAC+CCl_4 (50 mg/kg, i.p. NAC and 0.5 ml/kg, i.p. CCl_4); subgroup 4, NAC (50 mg/kg, i.p.). Besides to the groups that underwent a 70% PH, a sham control group was added. In subgroups, which underwent PH were designated 8 tried numbers, in sham control and subgroups which were not subjected to PH were designated 7 tried numbers. 0.5 ml/kg CCl_4 and 50 mg/kg NAC was given i.p. to the groups which underwent PH in the 8th day of operation and to the groups which were not subjected to PH 24 hours before the tissues were taken off. Treated subgroups were administered a total of 6 doses of NAC (50 mg/kg, i.p.), 3 hrs before CCl_4 and 1, 3, 6, 12, 18 hrs after CCl_4 administration. On the other hand, the same volume of serum physiologic was given to the control groups. Treatment to the whole groups was carried out synchronously. As for PH, approximately 70% of the rat liver was surgically removed under general anesthesia. On postoperative day 9, all rats were killed ethical and then serum and liver tissues were removed.

Partial hepatectomy

70% PH was performed according to the method described by Higgins and Anderson [2]. On postoperative day 9, all rats were killed and then serum and liver tissues were removed. A sample of removed liver tissue was kept in 10% formalin for histopathological analysis. Livers were rapidly frozen and stored at -70°C.

Histopathological examination

For all histopathological examinations, liver tissues were fixed overnight in 10% formaldehyde solution (dissolved in phosphate buffer, pH 7.4), and then the incubation solution was altered successively from 70% alcohol to 100% alcohol. The incubating solution was further changed from 100% alcohol to xylene, and the tissues were then embedded in paraffin wax, sectioned in 5 μm slices and stained with H&E.

Ki-67 immunostaining

Immunostaining for Ki-67, a marker for cell proliferation, was performed to evaluate the proliferation of hepatocytes. It was determined as previously described [12]. Slides were examined by a single pathologist unaware of the group identity. The mean of hepatocytes labeled with the anti-Ki67 mouse monoclonal antibody (MMI) over 400 high-power fields was measured in the nine experimental groups.

Biochemical determination

Liver was washed, finely minced, and submerged in ice-cold 0.2 mmol/L Tris-HCl (pH 7.4) and mechanically homogenized (Ultra Turrax T 25 basic; IKA, Wilmington, NC, United States) at 16000 g for 2 min at 4–8°C. In order to evaluate the prooxidant-antioxidant balance, we determined the free radicals production by measuring of lipid peroxidation, PC, glutathione (GSH) levels, and activity of SOD, CAT, and glutathione peroxidase (GSH-Px).

Determination of CAT, SOD, and GSH-Px activity

CAT activity was determined according to Aebi's method [13]. The principle of the assay is based on the determination of the rate constant (s^{-1}, k) or the H_2O_2 decomposition rate at 240 nm.

SOD activity was determined according to the method of Sun et al. [14] with a slight modification [15]. The principle of the method is based on the inhibition of NBT reduction by the xanthine-xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the sample after 1.0 ml ethanol/chloroform mixture (5/3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as

Table 1: Effects of NAC on biochemical parameters of liver in CCl_4 intoxicated rats (p<0.05).

Design of treatment		SOD (units/g protein)	CAT (k/g protein)	GSH-Px (units/g protein)
PH(+)control	(n=8)	0.138±0.012[c,d,e,f,h,i]	1.703±0.184[b,c,e,f,h]	1.864±0.202[d]
PH(+)CCl_4	(n=8)	0.108±0.010[c,d,e, f,h,i]	1.030±0.164[a,c,d,e,f,g,h,i]	1.951±0.101[d]
PH(+)NAC+CCl_4	(n=8)	0.199±0.012[a,b,g]	2.516±0.276[a,b,d,g]	2.261±0.103[g]
PH(+)NAC	(n=8)	0.189±0.007[a,b,g]	1.940±0.163[b,c]	2.491±0.183[a,b,g]
Sham control	(n=7)	0.213±0.027[a,b,g]	2.415±0.149[a,b,g]	2.138±0.187
PH(-)control	(n=7)	0.194±0.010[a,b,g]	2.379±0.259[a,b,g]	2.311±0.247[g]
PH(-)CCl_4	(n=7)	0.115±0.006[c,d,e,f,h,i]	1.756±0.164[b,c,e,f,h]	1.766±0.085[c,d,f]
PH(-)NAC+CCl_4	(n=7)	0.185±0.015[a,b,g]	2.377±0.210[a,b,g]	2.053±0.241
PH(-)NAC	(n=7)	0.201±0.011[a,b,g]	2.250±0.137[b]	2.193±0.085

NAC: N-Acetylcysteine; CCl_4: Carbontetrachloride; GSH-Px; Glutathione peroxidase; SOD: Superoxide dismutase; CAT: Catalase. Values are mean±SEM. [a]*P*<0.05 vs PH (+) control group; [b]*P*<0.05 vs PH (+) CCl_4 group; [c]*P*<0.05 vs PH (+) NAC+CCl_4 group; [d]*P*<0.05 vs PH (+) NAC group; [e]*P*<0.05 vs sham control group; [f]*P*<0.05 vs PH (-) control group; [g]*P*<0.05 vs PH (-) CCl_4 group; [h]*P*<0.05 vs PH (-) NAC+CCl_4 group; [i]*P*<0.05 vs PH (-) NAC group.

the enzyme amount causing 50% inhibition in the NBT reduction rate.

GSH-Px activity was measured by the method of Paglia and Valentine [16]. The enzymatic reaction in the tube, which is containing following items: NADPH, reduced glutathione (GSH), sodium azide, and glutathione reductase, which was initiated by addition of H_2O_2 and the change in absorbance at 340 nm was monitored by a spectrophotometer.

Detection of liver MDA, and GSH levels

The formation of thiobarbituric acid reactive substances (TBARS) during an acid-heating reaction was measured as an index of oxidative stress as previously described [17]. Briefly, the samples were mixed with 1 ml of trichloroacetic acid 10% and 1 ml of thiobarbituric acid 0.67% (Sigma Chemical) and then heated in a boiling water bath for 15 mins. Malondialdehyde equivalents were determined by the absorbance at 535 nm using 1,1,3,3-tetramethoxypropane (Sigma Chemical) as an external standard. Results were expressed as malondialdehyde equivalents per milligram of protein (Lowry assay).

GSH content was determined as described by Ellman [18]. Briefly, liver homogenates (500 µl) were precipitated by adding 500 µl 4% sulfosalicylic acid and centrifugation at 2,858 x g for 10 min. The supernatant (900 µl) was mixed with 100 µl 0.004% 5.5'dithiobis-2-nitrobenzoic acid (DTNB). After standing for 10 min at room temperature, the absorbance of chromophoric products (2-nitro-5-thiobenzoic acid) was determined at 412 nm using a spectrophotometry. GSH content was calculated using a standard curve.

Methods for detection of PC groups

The oxidative damage to proteins was assessed by the determination of carbonyl groups based on the reaction with dinitrophenylhidrazine as previously described [19]. Briefly, proteins were precipitated by the addition of 20% trichloroacetic acid and redissolved in dinitrophenylhidrazine, and the absorbance was read at 370 nm. Protein was determined by the method of Lowry et al [20].

Statistical analysis

The distribution of the groups was analyzed by the Shapiro Wilk test. Except GSH, all groups showed a normal distribution, so parametric statistical methods were used to analyze the data. Non-parametric tests were used for GSH. One Way ANOVA, LSD and Kruskal-Wallis were used for the differences between groups. Mann-Whitney *U* test was employed in comparison of data between groups. All values are given as mean and standard error of mean (SEM). Significance was determined at P<0.05.

Results

Hepatic antioxidant enzyme activities

We measured activities of CAT, SOD, GSH-Px in the liver and results are shown in Table 1.

SOD activity decreased significantly in the PH (-) CCl_4 group (0.115±0.006, p=0.001) when compared with its control group. The decrease in SOD activity was signifi-

Table 2: Effects of NAC on biochemical parameters of liver in CCl_4 intoxicated rats (p<0.05).

Design of treatment		MDA (nmol/g wet weight)	GSH (µmol/g wet weight)	PC (nmol/mg protein)
PH(+)control	(n=8)	89.085±9.233^{c,d,e,f,h,i}	1.197±0.062^{h,i}	0.625±0.046^{b,c,e,f,g,i}
PH(+)CCl_4	(n=8)	99.281±5.607^{c,d,e,f,h,i}	1.201±0.038^{h,i}	0.836±0.088^{a,c,d,e,f,h,i}
PH(+)NAC+CCl_4	(n=8)	61.210±2.816^{a,b,g}	1.494±0.237	0.398±0.060^{a,b,g}
PH(+)NAC	(n=8)	54.427±5.585^{a,b,g}	1.214±0.026^{h,i}	0.494±0.046^{b,g}
Sham control	(n=7)	53.322±3.670^{a,b,g}	1.228±0.041^{h,i}	0.296±0.028^{a,b,g}
PH(-)control	(n=7)	63.334±5.937^{a,b,g}	1.256±0.046^{i}	0.347±0.035^{a,b,g}
PH(-)CCl_4	(n=7)	94.531±3.752^{c,d,e,f,h,i}	1.384±0.099	0.846±0.165^{a,c,d,e,f,h,i}
PH(-)NAC+CCl_4	(n=7)	53.018±4.547^{a,b,g}	1.600±0.133^{a,b,d,e}	0.480±0.062^{b,g}
PH(-)NAC	(n=7)	62.864±7.222^{a,b,g}	1.450±0.062^{a,b,d,e,f}	0.377±0.059^{a,b,g}

NAC: N-Acetylcysteine; CCl_4: Carbontetrachloride; MDA: Malondialdehyde; GSH; Glutathione; PC; protein carbonyl. Values are mean±SEM. $^{a}P<0.05$ vs PH (+) control group; $^{b}P<0.05$ vs PH (+) CCl_4 group; $^{c}P<0.05$ vs PH (+) NAC+CCl_4 group; $^{d}P<0.05$ vs PH (+) NAC group; $^{e}P<0.05$ vs sham control group; $^{f}P<0.05$ vs PH (-) control group; $^{g}P<0.05$ vs PH (-) CCl_4 group; $^{h}P<0.05$ vs PH (-) NAC+CCl_4 group; $^{i}P<0.05$ vs PH (-) NAC group.

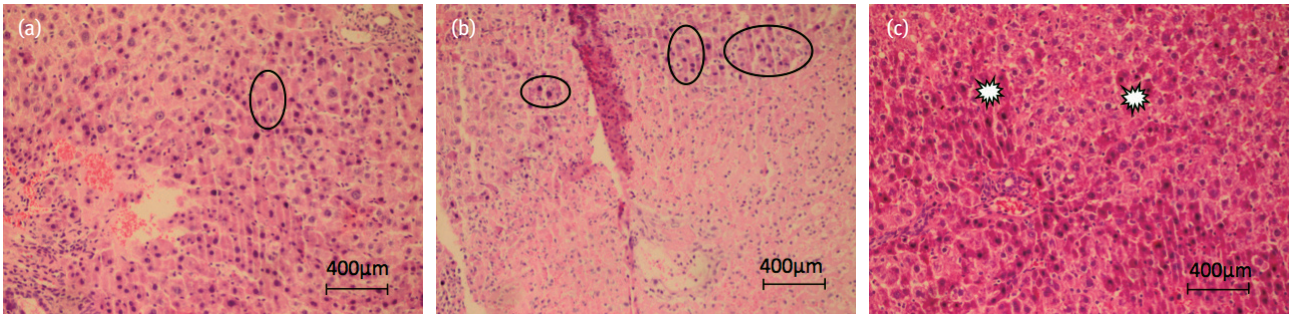

Figure 1: Histopathological examination of liver tissue of rats under various treatment conditions. Livers were sectioned and stained with hematoxylin&eosin by standard techniques (200x). (A) PH (+) control, (B) PH (+) CCl_4, (C) PH (+) NAC+CCl_4. According to microscopic examinations, severe hepatic destruction induced by CCl4 was remarkably reduced by the administration of NAC, which was in good correlation with the results of hepatic lipid peroxidation.

cantly suppressed by NAC treatment. SOD activity increased significantly in the PH (+) CCl_4+NAC (0.199±0.012, p=0.001) and PH (-) CCl_4+NAC groups (0.185±0.015, p=0.001).

CAT activity decreased significantly in the PH (+) CCl_4 (1.030±0.164, p=0.015) and PH (-) CCl_4 (1.756±0.164, p=0.034) groups when compared with their control groups. The decrease in CAT activity was significantly suppressed by NAC treatment. CAT activity increased significantly in the PH (+) CCl_4+NAC (2.516±0.276, p=0.001) and PH (-) CCl_4+NAC groups (2.377±0.210, p=0.035).

A significant statistical difference of GSH-Px level was not found when the subgroups of PH (+) group compared with the subgroups of PH (-) group (P>0.05, Table 1).

Lipid peroxidation

We measured MDA level in the liver and the results are shown in Table 2. Liver MDA level in the PH (-) CCl_4 group was significantly higher than PH (-) control group (94.531±3.752, p=0.001). The elevation of MDA induced by CCl_4 was significantly inhibited by the treatment with NAC in the PH (+) CCl_4+NAC (61.210±2.816, p=0.001) and PH (-) CCl_4+NAC groups (53.018±4.547, p=0.001).

GSH

A significant statistical difference of GSH level was not found when the subgroups of PH (+) group compared with the subgroups of PH (-) group (P>0.05, Table 2).

PC

We measured PC level in the liver and the results are shown in Table 2. Liver PC level increased in the PH (+) CCl_4 (0.836±0.088, p=0.044) and PH (-) CCl_4 (0.846±0.165,

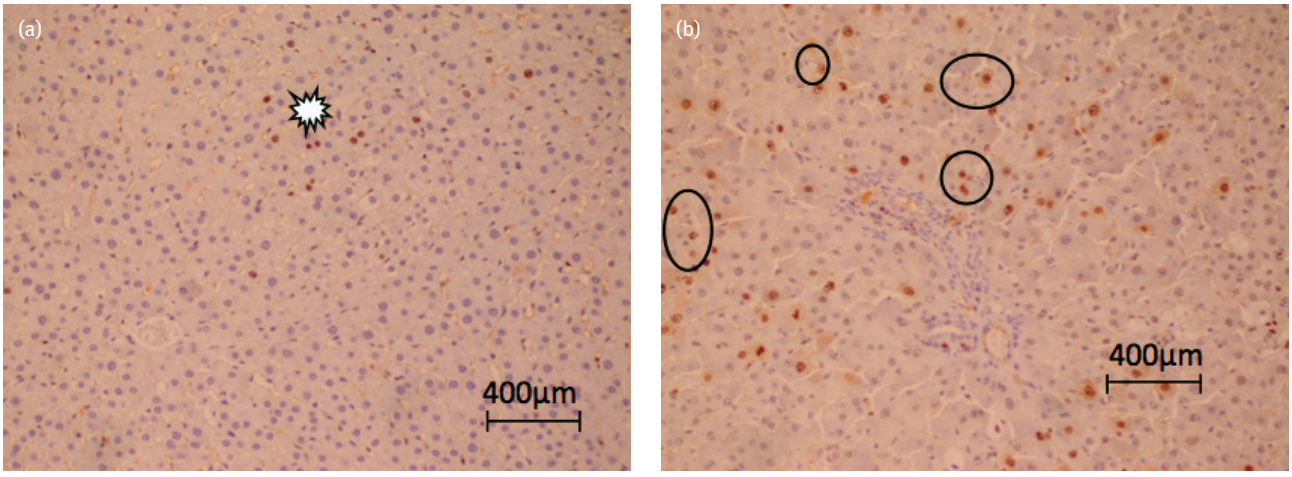

Figure 2: Immunohistochemical analysis of Ki-67 in PH rats (n=8). Original magnification x 200. (A) PH(+)NAC, (B) PH(+)NAC+CCl_4. Severe hepatic destruction induced by CCl_4 was remarkably reduced by the administration of NAC. The relative number of Ki-67 positive hepatocytes was higher in the NAC treated group.

Table 3: Ki-67 levels in liver tissue of Carbontetrachloride (CCl_4) intoxicated rats. The reduction of proliferation activity induced by CCl_4 was inhibited by the treatment with N-Acetylcysteine (NAC) and improved higher than the values of control groups.

Group		Grades
PH(+)control	(n=8)	++
PH(+)CCl4	(n=8)	+
PH(+)NAC+CCl4	(n=8)	+++
PH(+)NAC	(n=8)	++
Sham control	(n=7)	++
PH(-)control	(n=7)	++
PH(-)CCl4	(n=7)	+
PH(-)NAC+CCl4	(n=7)	++++
PH(-)NAC	(n=7)	+

Grades are follows:-= absent, + = 0.1–1.9%, ++ = 2.0–3.9%, +++ = 4.0–5.9%, ++++ = 6.0–7.9%.

p=0.001) groups when compared with their control groups. The elevation of PC induced by CCl_4 was significantly inhibited by the treatment with NAC in the PH (+) CCl_4+NAC (0.398±0.060, p=0.001) and PH (-) CCl_4+NAC groups (0.480±0.062, p=0.003).

Histopathologic examination

According to microscopic examinations, severe hepatic destruction induced by CCl_4 was remarkably reduced by the administration of NAC, which was in good correlation with the results of hepatic lipid peroxidation (Fig. 1). The relative number of Ki-67 positive hepatocytes was higher in the NAC treated groups (Fig. 2). The reduction of proliferation activity induced by CCl_4 was inhibited by the treatment with NAC and improved higher than the values of control groups (Table 3).

Discussion

The liver is an important organ which plays a central role in the metabolism, detoxification and excretion of various endogenous and exogenous substances such as xenobiotics. Liver injury can be caused by bacterial and viral infections, alcohol abuse, environmental pollutants, and several other factors [21].

Hepatic injury induced by CCl_4, a classic experimental model, has been extensively used for evaluation of hepatoprotective activity. CCl_4 has been used for years to induce acute liver damage in rats [22]. Liver injuries induced by CCl_4 are the best-characterized system of the xenobiotic-induced hepatotoxicity and is a commonly used model for the screening the anti-hepatotoxic/hepatoprotective activity of drugs [23]. The typical hepatotoxin CCl_4 is known to be rapidly transformed by cytochrome P4502E1 of the hepatocyte endoplasmic reticulum to a trichloromethyl radical (CCl_3*) which is converted into a peroxyl radical (CCl_3O_2*) in the presence of oxygen [24].

In recent decades, the pharmaceutical application potential of NAC supplement has attracted much interest from researchers in treating liver damage. NAC exerts its antioxidant action by facilitating glutathione biosynthesis and scavenging the reactive oxygen species (ROS) formed during oxidative stress [25]. The NAC, the *N*-ace-

tyl derivative of the amino acid L-cysteine, is a sulfhydryl group donor that crosses the cell membrane and restores glutathione levels by providing cysteine for its synthesis [26]. NAC has antioxidant, anticytotoxic and antiapoptotic properties and may therefore be useful in counteracting liver injuries induced by CCl_4 and treatment of liver. For comparison, we used NAC, which has been known for its therapeutic effects linked to the antioxidant and free radical scavenging action, and is commonly used as an antidote against drug-induced hepatopathies [27].

Liver regeneration is a physiological response to the loss of hepatic tissue as in the case of surgical resection of the liver [28]. This regeneration after injury with CCl_4 followed by PH is a complex model involving toxicological, inflammatory, and necrotic processes [29]. PH is generally regarded as the strongest reproducible stimulus to hepatocyte proliferation. It is well known that the remnant liver after 70% PH can regenerate and restore its original mass within 7–10 days in rats [30].

The enzymatic antioxidant defense system is the nature protector against excessive free radicals. Several studies have demonstrated that antioxidant enzymes such as SOD, and CAT represent one protection against oxidative tissue-damage and contribute to oxygen radicals scavenging activity [31]. Previous studies have indicated that CCl_4 decreased activities of antioxidant enzymes [32,33]. In our study, NAC markedly elevated the levels of SOD and CAT, and protected the liver from CCl_4, indicating that inhibition of the oxidative cascading stress was one of the main mechanisms in CCl_4-induced liver injury in the rats. Our results show that NAC treatment may contribute to the antioxidant system in the liver following CCl_4 administration. Therefore, the regeneration may be assumed to be related with increasing CAT and SOD enzymatic activities.

TBARS are indicators of the oxidative stress, since they are generated from the breakdown of lipid peroxyl radicals [34]. Earlier it was shown that NAC treatment during CCl_4 administration was able to prevent liver necrosis, to decrease partially the covalent binding of CCl_4 reactive metabolites and to diminish an increase in lipid peroxidation [35]. Smilarly, in an experimental model of 10-week long-term rat CCl_4 intoxication, NAC (150 mg/kg, starting 7 days before CCl_4 administration) inhibited liver malondialdehyde formation [36]. Elevated levels of MDA reflect an enhanced lipid peroxidation leading to liver tissue damage and failure of antioxidant defense mechanisms. In our study, the elevation of MDA induced by CCl_4 was significantly inhibited by the treatment with NAC. Therefore, NAC treatment may decrease the MDA content, may diminish an increase in lipid peroxidation and may contribute to the antioxidant system in the liver following CCl_4 administration.

Another indicator of oxidative stress is the PC content which is the oxidation products of specific amino acid residues [37]. Previous studies have indicated that NAC treatment in rats with CCl_4-induced liver injury decreased the PC accumulation and partially attenuated mixed protein-glutathione disulphide levels due to thiol-disulphide exchange between the incoming thiol and oxidized glutathione [38]. Our findings indicated that the increase of PC formation might be a result of oxidative stress and decrease of detoxification process. Therefore, NAC treatment may increase the generation of sulfhydryl groups and may contribute to the antioxidant system in the liver. Thus, the regeneration may be assumed to be related with decreasing PC level by NAC.

Ki-67 immunostaining, a marker for hepatic cell proliferation, was performed to evaluate the proliferation of hepatocytes. Previous studies indicated that hepatocyte proliferation inhibited by CCl_4 [39]. In our study, treatment with NAC increased the amount of Ki-67 positive hepatocytes. Accordingly the reduction of proliferation activity induced by CCl_4 was inhibited by the treatment with NAC. This positive effect of NAC may contribute to the liver regeneration. Thus, the regeneration may be assumed to be related with increasing Ki-67 level.

In conclusion, these results indicate that CCl_4 increased oxidation products, reduced liver enzymatic activity and reduced proliferation activity in both hepatectomised and non-hepatectomised liver. In addition, these findings indicate that the liver injury of CCl_4 and the protective effect of NAC were similarly in above both groups. Consequently, making PH not created a negative effect and additional health problems in the liver donors. Thus, these results can positively affect the decision of the donors. On the other hand, no difference was observed in the sensitivity of regenerated liver tissue to CCl_4 toxic and in response to the protective effect of NAC. The absence of an increase of hepatic sensitivity in the regenerated liver against hepatotoxic agents can be positively affect volunteers decisions for donation.

In addition, the present study can contribute to the formation of data about the sensitivity of the donors with regenerated liver against hepatotoxic agents. Thus, both health professionals and community can raise awareness of such an issue, but knowledge about this is not sufficient. Nonetheless, more studies are needed to fully comprehend the issue.

Conflict of Interest: The authors have no conflict of interest.

References

[1] Uemoto S, Inomata Y, Sakurai T, Egawa H, Fujita S, Kiuchi T, et al. Living donor liver transplantation for fulminant hepatic failure. Transplantation 2000; 70(1):152–7.

[2] Higgins GM, Anderson RM. Experimental pathology of the liver. I:Restoration of the liver of the white rat following partial surgical removal. Arch Pathol 1931; 12:186–202.

[3] Szymonik-Lesiuk S, Czechowska G, Stryjecka-Zimmer M, Słomka M, Madro A, Celiński K, et al. Catalase, superoxide dismutase, and glutathione peroxidase activities in various rat tissues after carbon tetrachloride intoxication. J Hepatobiliary Pancreat Surg 2003; 10(4):309–15.

[4] Castillo T, Koop DR, Kamimura S, Triadafilopoulos G, Tsukamoto H. Role of cytochrome P-450 2E1 in ethanol-, carbon tetrachloride- and iron-dependent microsomal lipid peroxidation. Hepatology 1992; 16(4):992–6.

[5] Kovalovich K, DeAngelis RA, Li W, Furth EE, Ciliberto G, Taub R. Increased toxin-induced liver injury and fibrosis in interleukin-6-deficient mice. Hepatology 2000; 31(1):149–59.

[6] Kelly GS. Clinical applications of N-acetylcysteine. Altern Med Rev 1998; 3(2):114–27.

[7] Aruoma OI, Halliwell B, Hoey BM, Butler J. The antioxidant action of N-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. Free Radic Biol Med 1989;6(6):593–7.

[8] Office of Drinking Water. Final draft criteria document for carbon tetrachloride. Washington, DC, US. Environmental Protection Agency 1985 (TR-540-131A).

[9] SAX Dangerous Properties of Industrial Material, 8th ed. 1993 update and vol III (1992). Lewis RJ Sr, ed. New York: Van Nostrand Reinhold 1993;52:1149.

[10] Gopal N, Sengottuvelu S. Hepatoprotective activity of Clerodendrum inerme against CCl_4 induced hepatic injury in rats. Fitoterapia 2008; 79(1):24–6.

[11] Richard J. Lewis, Sr. Sax's Dangerous Properties of Industrial Materials, Wiley-Interscience, John Wiley & Sons, Hoboken, NJ, USA, 11th edition, 2004. J. Am. Chem. Soc 2005; 127(8):2794.

[12] Taniguchi E, Sakisaka S, Matsuo K, Tanikawa K, Sata M. Expression and role of vascular endothelial growth factor in liver regeneration after partial hepatectomy in rats. J Histochem Cytochem 2001; 49(1):121–30.

[13] Aebi H. Catalase: In Bergmeyer HU ed. Methods of Enzymatic Analysis. New York: Academic Press 1974. p. 673–7.

[14] Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988; 34(3):497–500.

[15] Durak I, Yurtarslanl Z, Canbolat O, Akyol O. A methodological approach to superoxide dismutase (SOD) activity assay based on inhibition of nitroblue tetrazolium (NBT) reduction. Clin Chim Acta 1993; 214(1):103–4.

[16] Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70(1):158–69.

[17] Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990;186:421–31.

[18] Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82(1):70–7.

[19] Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, et al. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 1990; 186:464–78.

[20] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193(1):265–75.

[21] Rutherford A, Chung RT. Acute liver failure: mechanisms of hepatocyte injury and regeneration. Semin Liver Dis 2008; 28(2):167–74.

[22] Chagoya de Sánchez V, Hernández-Muñoz R, Yáñez L, Vidrio S, Díaz-Muñoz M. Possible mechanism of adenosine protection in carbon tetrachloride acute hepatotoxicity. Role of adenosine by-products and glutathione peroxidase. J Biochem Toxicol 1995; 10(1):41–50.

[23] Brattin WJ, Glende EA Jr, Recknagel RO. Pathological mechanisms in carbon tetrachloride hepatotoxicity. J Free Radic Biol Med 1985; 1(1):27–38.

[24] Poyer JL, McCay PB, Lai EK, Janzen EG, Davis ER. Confirmation of assignment of the trichloromethyl radical spin adduct detected by spin trapping during 13C-carbon tetrachloride metabolism *in vitro* and *in vivo*. Biochem Biophys Res Commun 1980; 94(4):1154–60.

[25] Ocal K, Avlan D, Cinel I, Unlu A, Ozturk C, Yaylak F, et al. The effect of N-acetylcysteine on oxidative stress in intestine and bacterial translocation after thermal injury. Burns 2004; 30(8):778–84.

[26] Cotgreave IA. N-acetylcysteine: pharmacological considerations and experimental and clinical applications. Adv Pharmacol 1997; 38:205–27.

[27] Smilkstein MJ, Knapp GL, Kulig KW, Rumack BH. Efficacy of oral N-acetylcysteine in the treatment of acetaminophen overdose. Analysis of the national multicenter study (1976 to 1985) N Engl J Med 1988; 319(24):1557–62.

[28] Bolitho G, Engelbrecht G, Lotz Z, Tyler M, McLeod H, Jaskiewicz K, et al. Liver regeneration after hepatic ischemia and reduced liver autotransplantation in the rat. Hepatology 1993; 17(2):273–9.

[29] Liakos AA, Mykoniatis MG, Kokala ME, Papadimitriou DG, Liatsos GD. Levels of hepatic stimulator substance in liver regenerating process of partially hepatectomized rats pretreated with a single dose of carbon tetrachloride. Dig Dis Sci 1999; 44(5):1046–53.

[30] Malik R, Mellor N, Selden C, Hodgson H. Triiodothyronine enhances the regenerative capacity of the liver following partial hepatectomy. Hepatology 2003; 37(1):79–86.

[31] Wang BJ, Liu CT, Tseng CY, Wu CP, Yu ZR. Hepatoprotective and antioxidant effects of Bupleurum kaoi Liu (Chao et Chuang) extract and its fractions fractionated using supercritical CO2 on CCl_4-induced liver damage. Food Chem Toxicol 2004; 42(4):609–17.

[32] Ye JF, Zhu H, Zhou ZF, Xiong RB, Wang XW, Su LX, et al. Protective mechanism of andrographolide against carbon tetrachloride-induced acute liver injury in mice. Biol Pharm Bull 2011; 34(11):1666–70.

[33] Jiang W, Gao M, Sun S, Bi A, Xin Y, Han X, et al. Protective effect of L-theanine on carbon tetrachloride-induced acute liver injury in mice. Biochem Biophys Res Commun 2012; 422(2):344–50.

[34] Halliwell B. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. Am J Med 1991; 91(3C):14–22.

[35] Valles EG, de Castro CR, Castro JA. N-acetyl cysteine is an early

but also a late preventive agent against carbon tetrachloride-induced liver necrosis. Toxicol Lett 1994; 71(1):87–95.
[36] Ulicná O, Greksák M, Vancová O, Zlatos L, Galbavý S, Bozek P, et al. Hepatoprotective effect of rooibos tea (Aspalathus linearis) on CCl_4-induced liver damage in rats. Physiol Res 2003; 52(4):461–6.
[37] Di Simplicio P, Cheeseman KH, Slater TF. The reactivity of the SH group of bovine serum albumin with free radicals. Free Radic Res Commun 1991; 14(4):253–62.
[38] Grinberg L, Fibach E, Amer J, Atlas D. N-acetylcysteine amide, a novel cell-permeating thiol, restores cellular glutathione and protects human red blood cells from oxidative stress. Free Radic Biol Med 2005; 38(1):136–45.
[39] Zhou XF, Wang Q, Chu JX, Liu AL. Effects of retrorsine on mouse hepatocyte proliferation after liver injury. World J Gastroenterol 2006; 12(9):1439–42.