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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Correlation of antioxidant and NADPH generating enzymes activities with tissues inflammation during smoke exposure of rats and smoke cessation

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Abstract

A modified smoking machine has been constructed in our lab and used for monitoring the effects of cigarette smoke on albino rats. Long-term administration of cigarette smoke lowers the activity of antioxidant enzymes and NADPH generating enzyme in liver, lung and kidney. In comparison with control animals the cigarette smoke exposure caused a significant decrease in catalase specific activity compared with control by 23, 18 and 50% in liver, kidney and lung tissues respectively. However, during the recovery period this enzyme activity retained almost normal levels by 97, 99 and 95% respectively. Similarly, smoking caused a significant reduction in glutathione peroxidase (GPx) specific activity compared to control by 16, 13 and 35% in liver, kidney and lung tissues, respectively. After the recovery period glutathione peroxidase (GPx) activities in liver, kidney and lung retained almost normal levels 97, 99 and 96% respectively. Also, smoke exposure caused a significant reduction in G6PD specific activities compared to control by 29, 18 and 38% in liver, kidney and lung tissues respectively. However, during the recovery period, the enzyme activity retained almost normal levels in liver, kidney and lung by 98, 97 and 96 % respectively. Long-term administration of cigarette smoke impairs the enzymatic antioxidant defence system in liver, lung and kidney. These alterations may be one of the responsible factors for cigarette smoke induced inflammation in these organs. Cigarette smoke causes severe histological effects especially in lung alveoli. It induces apoptosis only in lung alveoli. Smoking has more severe inhibitory and damaging effects on lung due to the differential load of metabolites of cigarette smoke in correlation with the alterations in enzymes activities. Present study demonstrated an induction of inflammatory changes in tissues due to the effect of chronic smoking exposure on rats. These changes involved an interstitial inflammation comprised of lymphocytes and plasma cells in lung, portal tract inflammation in liver and mesangial cell proliferation in kidney corpuscles. All these inflammatory changes almost diminished in tissues after the recovery period from smoking effects, indicating reversible effects of smoking on tissues and enzymes of the rat.

Keywords: Cigarette smoke; glutathione peroxidase; catalase; glucose-6-phosphate dehydrogenase (G6PD); inflammation; apoptosis

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Introduction

Smoking is the act of inhaling and exhaling the fumes from burning plant materials especially tobacco. It is consumed in the form of cigars, cigarettes, chew, water-pipe (Hoffmann and Wynder, 1986).

Cigarettes contain more than 4,000 identified chemical compounds including 60 known carcinogens (Borgerding and Klus, 2005). The gaseous components of mainstream smoke (92% of the total smoke) involve 400-500 different gases which include carbon-monoxide, nitrogen oxide, hydrogen cyanide, formaldehyde and ozone. Particulate matter (8% of mainstream smoke) contains tar product such as naphthalene, pyrene and nitrosamine (Kaiserman and Rickkert, 1992; Yarnell, 1996; Borgerding and Klus,

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2005; Ding et al., 2008) and metal such as cadmium, polonium, selenium, mercury, lead and arsenic (Borgerding and Klus, 2005; Galażyn-Sidorczuk et al., 2008). According to a report by Public Health Laboratories, Maryland, USA, 1997, the Jordanian cigarettes contain about twice the amount of nicotine and tar which is found in non-Jordanian cigarettes.

Cigarette smoke contains and generates various reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as superoxide radical, hydrogen peroxide, hydroxyl radical, and peroxynitrite (Aoshiba and Nagay, 2003). The highly reactive radicals can act as initiators of carcinogenesis, cause DNA damage, activate procarcinogens and alter the cellular antioxidant defence system. The antioxidant defence system, which includes superoxide dismutase, glutathione peroxidase and catalase, can work sequentially with glucose-6-phosphate dehydrogenase (G6PD) enzyme to dispose free radicals (Carnevali, et al, 2003; Avti et al., 2006).

Catalase is a common enzyme present in the peroxisomes of nearly all aerobic cells, which functions in catalyzing the decomposition of hydrogen peroxide to water and oxygen without the production of free radicals (Chelikani et al., 2004). Glucose-6-phosphate dehydrogenase, found in animal tissues, plant tissues and microorganisms (Igoillo-Esteve and Cazzulo, 2006; Scharte et al., 2009; Abboud and Al-Awaida, 2010). Glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: NADP+1-oxidoreductase, E.C.1.1.1.49) catalyzes the conversion of glucose-6-phosphate to 6-phosphogluconate in the pentose phosphate cycle. This house keeping enzyme provides reductive potential in the form of NADPH to maintain several cellular biosynthetic pathways (Luzzatto and Battistuzzi, 1985), and monitoring the cellular redox regulation (Salvemini et al., 1999). The isolated clones of defective G6PD gene were very sensitive to oxidizing agents like H_2O_2, indicating the importance of this enzyme in protecting cells against oxidative stress (Pandolfi et al., 1995). Glutathione peroxidase (GPx), which can metabolize a wide range of organic hydroperoxides as well as hydrogen peroxide. This enzyme contains four selenium ions and found in different cell fractions and tissues of the body (Arthur, 2000).

Cigarette smoking is associated with 400,000 deaths annually from cardiovascular diseases in the United State alone (U.S. Public Health Service, 2005). There is a clear relationship between the degree and duration of exposure to cigarette smoking and incidence of cardiovascular events (Fitzgerald, 1997; Leone, 1993; 1994; Ambrose and Barua, 2004).

There are two types of cell death; apoptosis and necrosis (Zimmermann et al., 2001). Necrotic cell death is considered an accidental type of death caused by gross cell injury and results in the death of groups of cells within a tissue. In contrast, apoptotic cell death may be induced or is preprogrammed into death of individual cells (Zimmermann et al., 2001). The oxidative stress was the initial event in the lung of guinea pig exposed to cigarette smoke, which was followed by inflammation, apoptosis and lung injury (Banerjee et al., 2007).

Aims of the work

1- To study the effect of cigarette smoke on specific activity of antioxidant enzymes including catalase, Glucose-6-phosphate dehydrogenase (G6PD) and glutathione peroxidase enzymes in the lung, liver and kidney.
2- To study the recovery of antioxidant enzymes activity three months after stopping the rat exposure to mainstream cigarette smoke.
3- To examine the histopathological changes that may occur after chronic exposure of rats to mainstream cigarette smoke in lung, liver and kidney
4- To study the recovery of histopathological changes in lung, liver and kidney tissues three months after stopping the rat exposure to mainstream cigarette smoke
5- To detect the presence of apoptotic nuclei in lung, liver and kidney tissues

The understanding of mechanisms for cigarette smoke induced cellular damage may offer therapeutic opportunities and ways to modify the adverse effects of cigarette smoke

Materials and methods

Chemicals

Bovine serum albumin (Sigma-Aldrich, USA), Hematoxylin (Sigma-Aldrich, USA), Eosin Y (Sigma-Aldrich, USA), Folin - Ciocalteau phenol reagent (Sigma-Aldrich, USA), Glutaraldehyde (BDH Chemicals, England), Glutathione reductase (Sigma-Aldrich, USA), Hydrogen peroxide 37% (Gainland chemical company, UK), L-Glutathione (Sigma-Aldrich, Japan), LM brand (Jordan cigarettes company), NADPH (Sigma-Aldrich, USA), Paraformadehyde 4% (Fluka AG, Buchs SG, Switzer land), Sodium azide (Research organic, USA), Sodium potassium tartarate (Scharlau chemie, European union), ß-NADP (Sigma-Aldrich, USA).

Experimntal design

Sixty male Albino rats (*Rattus norvegicus*), obtained from the animals room, Department of Biological Science, University of Jordan, with an average weight of 100-150g were divided into two groups. The first group (test) was exposed to cigarette smoke while the second group (control) was left

untreated. The exposure to smoking was carried out as one daily dose for a period of 90 days, followed by a period of three months of non-exposure to smoking as a recovery stage from the effects of cigarette smoking. Following each period, a histological and biochemical studies were performed. Control animals were placed in the chamber and were exposed to fresh air instead of cigarette smoke.

The digital Smoking Machine

Exposure of animals to cigarette smoke has been done using a digital smoking machine ((Shraideh et al., 2011).

Cell Extraction

Rat tissues of liver, lung and kidneys were excised and perfused with ice-cold perfusion solution (0.15 M KCl, 2 mM EDTA, pH-7.4). Tissues were homogenized in Tris-HCl buffer (50 mM, pH 7.4), and the homogenates were centrifuged at 10,000xg and 4°C for 30 min to obtain the supernatant. The latter was used for measurement of enzyme activities and estimation of protein concentration (Avti et al*.,* 2006).

Assay of Catalase (CAT) Activity

Catalase activity was measured in the tissue supernatant by the method of Luck (1963). The reaction mixture contained 0.05 M Tris-buffer, 5 mM EDTA (pH 7.0), and 10 mM H_2O_2 (in 0.1 M potassium phosphate buffer, pH 7.0). The supernatant was added to the above mixture in a final reaction mixture of 3 ml. The rate of change in absorbance per minute was recorded at 240 nm. Catalase activity was calculated by using the molar extinction coefficient of 43.6 M^{-1} cm^{-1} for H_2O_2. The level of CAT was expressed in terms of µmoles H_2O_2 consumed/min /mg of protein**.**

Assay of Glucose-6-phosphate Dehydrogenase (G6PD) Activity

The G6PD activity was measured by the method of Tian et al*.,* (1994). The supernatant was added to a mixture of 1 mM $MgCl_2$, 1 mM sodium azide, 50 mM Tris-HCl buffer (pH 7.6), and 0.25 mM NADP in a final volume of 3 ml. The reaction was started in cuvette at 37°C by adding glucose 6-phosphate (0.6 mM). The increase in absorbance per min at 340 nm due to reduction of $NADP^+$ to NADPH was measured in a spectrophotometer. Enzyme units were expressed as number of µmoles NADPH formed using the extinction coefficient 6.22 x 10^3 M in 1cm path at 340 nm. The levels of G6PD activity were expressed in terms of µmoles NADPH produced/min /mg of protein in crude extract.

Assay of Glutathione Peroxidase (GPx) Activity

The GPx activity was measured by the method of Paglia and Valentine (1967). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM sodium azide, 0.2 mM NADPH, 1 U glutathione reductase, and 1 mM reduced glutathione. The mixture (2.9 ml) was allowed to equilibrate for 5 min at 25°C before initiating the reaction with 0.1 ml of 2.5 mM H_2O_2. The linear activity was recorded as absorbance at 340 nm. Units of enzyme activity were expressed as µmoles of NADPH oxidized to NADP by using the extinction coefficient of 6.22 x 10^3 M^{-1} cm^{-1} at 340 nm. The levels of GPx were expressed in terms of µmoles NADPH consumed/min/ mg of protein in crude extract.

Histopathological Examination

Following an overnight recovery from the last smoke exposure, rats were sacrificed by ether anesthesia and tissues of interest (trachea, alveoli of the lung, aorta, and ventricles of the heart) were gently dissected out, washed well with normal saline (0.9% NaCl), and fixed in 10% saline buffered formalin for at least 24 hrs. To ensure adequate fixation, the formalin fixative was used at volume 10-20 times more than the volume of tissue pieces. Dehydration was achieved by passing tissues through a graded series of alcohol followed by two changes of xylene. After infiltration in paraffin wax, tissues were embedded in pure paraffin wax (Avti et al., 2006).

Sections (5µm thick) were obtained by a microtome (Spencer 50). Finally sections were mounted on glass slides and stained with hematoxylin and eosin. Sections were examined and photographed using Zeiss photomicroscope1, equipped with Moticam 2300 digital camera (3.0 Mega pixels).

Detection of Apoptosis in Tissue Sections

The presence of apoptotic cell death in tissue sections of trachea, alveoli of the lung, aorta, and ventricles of the heart, was examined using Dead End Colorimetric TUNEL (TdT-mediated dUTP Nick-End Labeling) kit (Promega, USA).

TUNEL Assay Procedure (TUNEL kit, Promega)

The DeadEnd™ Colorimetric TUNEL System is a modified TUNEL Assay designed to provide accurate detection of apoptotic cells at the single-cell level. The DeadEnd™ Colorimetric TUNEL System measures nuclear DNA fragmentation, an important biochemical indicator of apoptosis. The system can be used to assay apoptotic cell death in tissue sections. The DeadEnd™ Colorimetric TUNEL System end-labels the fragmented DNA of apoptotic cells using a modified TUNEL (TdT-mediated dUTP Nick-End Labeling) assay. Biotinylated nucleotide is incorporated at the 3´-OH DNA ends using the enzyme Terminal Deoxynucleotidyl Transferase (TdT). Horseradish-peroxidase-labeled streptavidin (Streptavidin HRP) is then bound to these

biotinylated nucleotides, which are detected using the hydrogen peroxide, and the stable chromogen, diaminobenzidine (DAB). Using this procedure, apoptotic nuclei are stained dark brown.

Protein Content Determination

The protein concentration in the tissue homogenates was determined using Lowry method (Lowry et al., 1951).

Statistical analysis

Values for specific enzyme activity are expressed as mean ± SEM. Statistical analysis was performed by the unpaired Student's t-test. P value less than 0.05 was considered significant.

Results

Catalase Specific Activity of Cigarette Smoke-exposed Rats and Recovery Rats

The cigarette smoke exposure caused a significant decrease in catalase specific activity compared to control by 23, 18 and 50% in liver, kidney and lung tissues respectively. However, during the recovery period this enzyme activity retained almost normal levels by 97, 99 and 95% respectively. Smoking has more severe inhibition effects on catalase specific activity in the lung (Table 1).

Glutathione Peroxidase Specific Activity of Cigarette Smoke-Exposed Rats and Recovery Rats

Smoke exposure caused a significant reduction in glutathione peroxidase (GPx) specific activity compared to control by 16, 13 and 35% in liver, kidney and lung tissues respectively. After the recovery period GPx activities in liver, kidney and lung retained almost normal levels 97, 99 and 96% respectively. Smoking showed severe inhibition effects on GPx specific activity in lung (Table 2).

G6PD Specific Activity of Cigarette Smoke-Exposed Rats and Recovery Rats

Smoke exposure caused a significant reduction in G6PD specific activities compared to control by 29, 18 and 38% in liver, kidney and lung tissues respectively. However, during the recovery period, the enzyme activity retained almost normal levels in liver, kidney and lung by 98, 97 and 96% respectively. Smoking showed severe inhibition effects on G6PD specific activity in the lung (Table 3).

Tissues Inflammation during Smoke Exposure and Recovery in Liver, Lung, and Kidney

Long period smoking exposure of rats, produced inflammatory changes in liver, lung, and kidney tissues. These changes involved interstitial inflammation involving lymphocytes and plasma cells in lung (Figure 2), portal area inflammation in liver (Figure 5) and mesangial cell proliferation in corpuscles (Figure 8). After the recovery period, all these inflammatory changes from smoking effects almost diminished in tissues (Figure 3, 6 and 9).

Table 1: Catalase specific activity compared to control in the cigarette smoke-exposed rats and after recovery period in liver, kidney and lung tissues of rats

Tissues	Control rats[b]	Smoke exposed rats[b]	Smoke recovered rats[b]
Liver	31.67±1.6	24.33±1.08*	30.87±1.33
Kidney	8.95±0.53	7.3±0.28*	8.84±0.48
Lung	1.95±0.19	0.96±0.08*	1.82±0.21

Values are expressed as means ± SEM of 6 experimental animals. *Significant value (P<0.05); [b]The level of CAT activity was expressed in terms of µmoles H_2O_2 consumed/min /mg of protein in crude extract.

Table 2: Glutathione peroxidase specific activity compared to control in the cigarette smoke-exposed rats and after recovery period in liver, kidney and lung tissues of rats

Tissues	Control rats[b]	Smoke exposed rats[b]	Smoke recovered rats[b]
Liver	86±2.67	72.67±4*	83.33±3.33
Kidney	53±2.3	45.3±2.1*	52±1.9
Lung	35±2.5	22±1.6*	33±2.1

Values are expressed as means ± SEM of 6 experimental animals. *Significant value (P<0.05); [b]The levels of GPx activity were expressed in terms of µmoles NADPH consumed/min/mg of protein in crude extract

Table 3: G6PD specific activity compared to control in the cigarette smoke-exposed rats and after recovery period in liver, kidney and lung tissues of rats

Tissues	Control rats[b]	Smoke exposed rats[b]	Smoke recovered rats[b]
Liver	3.4±0.2	2.4±0.13*	3.33±0.16
Kidney	3.7±0.17	3.5±0.19*	3.62±0.2
Lung	2.27±0.16	1.35±0.06*	2.09±0.11

Values are expressed as means ± SEM of 6 experimental animals. *Significant value (P<0.05); The levels of G6PD activity were expressed in terms of µmoles NADPH produced/min /mg of protein

The Ability of Cigarette Smoke to Induce Apoptosis in Lung Sections

Cigarette smoke induced apoptosis only in lung section. There were characteristic dark-brown apoptotic nuclei in the examined sections (Figures 11 and 12). Cigarette smoke didn't induce apoptosis in liver and kidny (Data not shown).

Discussion

In comparison with normal tissues, present data showed that the smoke exposure caused a significant

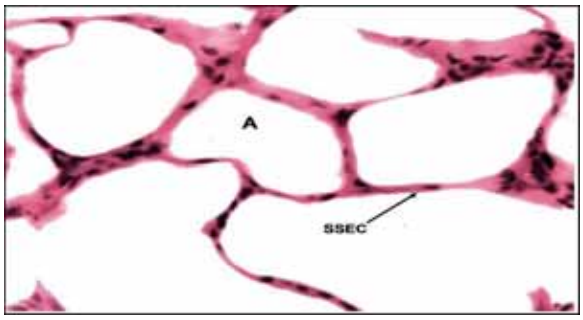

Fig. 1: Normal morphology of lung alveoli in a control rat. A: alveolus, SSEC: simple squamous epithelial cell. Magnification: 815x. H&E stain.

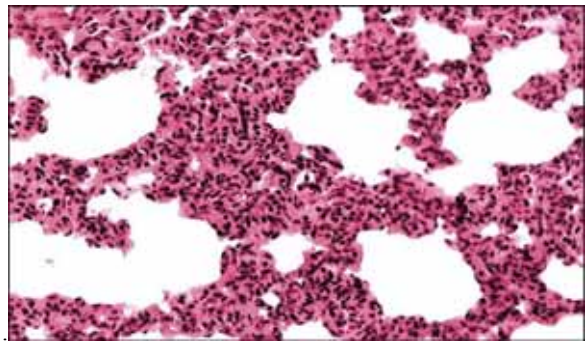

Fig. 2: Lung alveoli of cigarette smoke-exposed rat, showing some degree of collapsed alveoli and inflammatory cel.l infiltration. Magnification: 435x. H&E stain

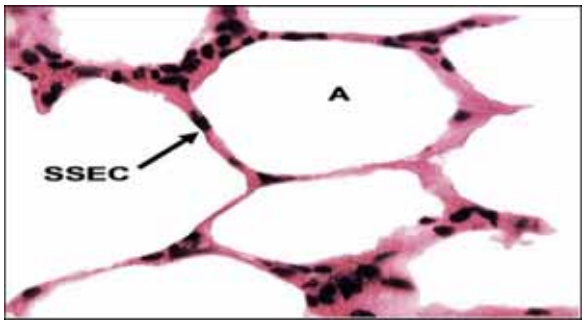

Fig. 3: Lung alveoli of cigarette smoke-exposed rat after the recovery period. Much less thickening in the alveolar wall and no inflammatory cell infiltration was observed. A: alveolus, SSEC: simple squamous epithelial cell. Magnification: 1380x. H&E stain

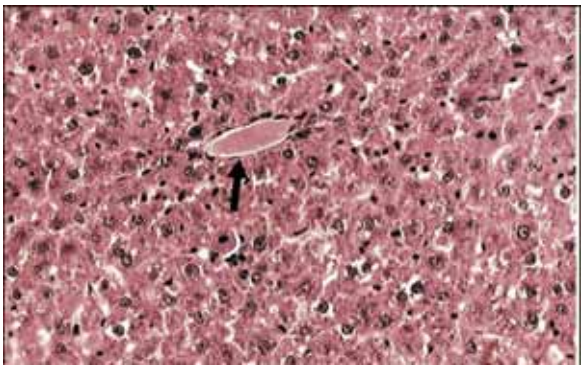

Fig. 4: Normal morphology of liver in a control rat. Arrow: Portal area. Magnification: 375x. H&E stain.

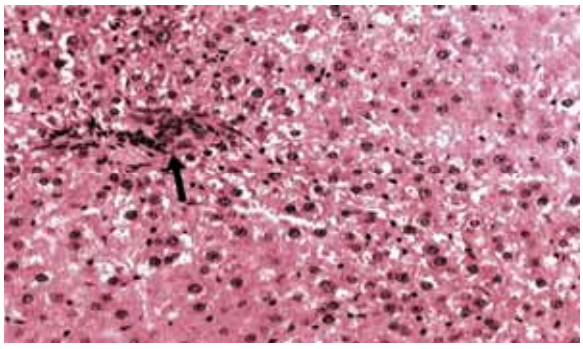

Fig. 5: Liver tissue of cigarette smoke-exposed rat. Showing portal area inflammation in liver. Arrow: phagocytic cells. Magnification: 400x H&E stain.

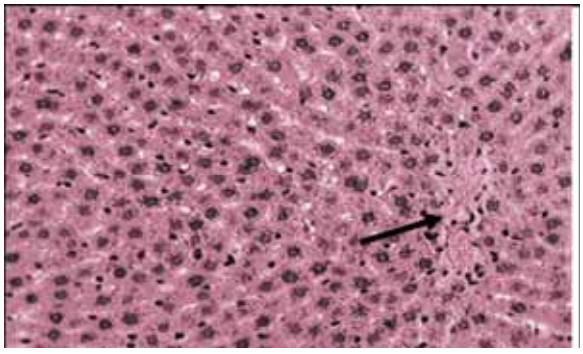

Fig. 6: liver tissue of cigarette smoke-exposed rat after the recovery period showing the absence of portal area inflammation. Arrow: Portal area. Magnification: 440x H&E stain

decrease in catalase specific activity by 23, 18 and 50% in liver, kidney and lung tissues respectively. However, during the recovery period, th**e** enzyme activities retained almost normal levels (97%, 99% and 95% respectively). Similarly, smoking caused a significant reduction of G6PD specific activities by 29, 18 and 38% in liver, kidney and lung tissues, respectively. However, during the recovery period, the enzyme activities retained almost normal levels 98, 97 and 96% respectively. Also, smoke exposure caused a decrease in GPx by 16, 13 and 35% in liver, kidney and lung tissues respectively. In the recovery period, GPx the enzyme activities retained almost normal levels (97, 99 and 96% respectively). These data suggest different effects of cigarette smoke on different organs which

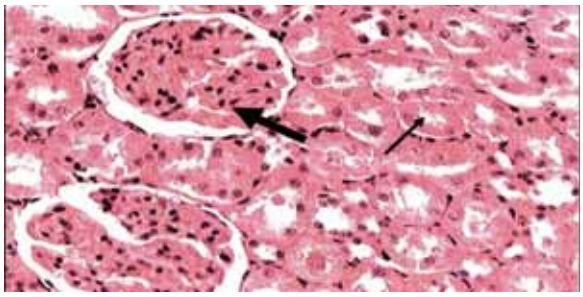

Fig. 7: Normal morphology of kidney in a control rat. Thick arrow: Renal corpuscle .Thin arrow: Proximal tubule. Magnification: 440x. H&E stain

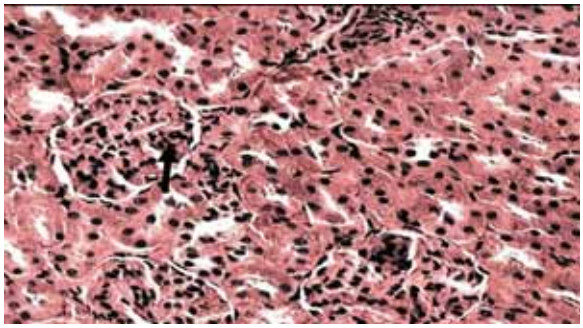

Fig. 8: Kidney tissue of cigarette smoke-exposed rat. Showing mesangial cell proliferation. Arrow: mesangial cell.Magnification: 440x. H&E stain

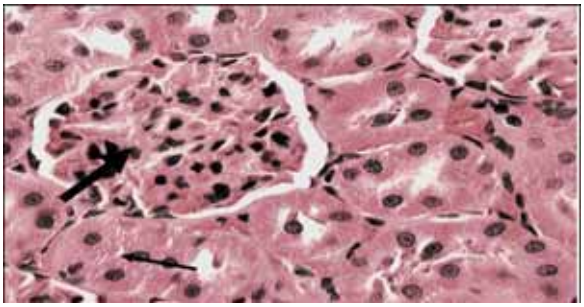

Fig. 9: Kidney tissue of cigarette smoke-exposed rat after the recovery period, showing absence of mesangial cell proliferation in the renal corpuscle. Thick arrow: Renal corpuscle. Thin arrow: Proximal tubule. Magnification: 900x. H&E stain

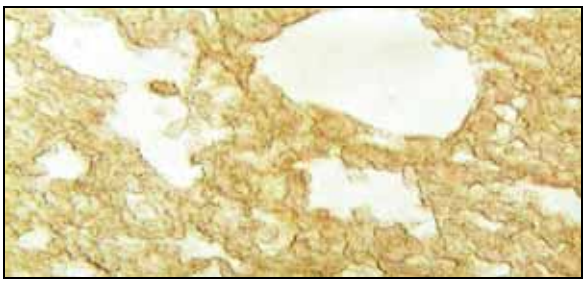

Fig. 10: Normal lung alveoli (TUNEL staining, original magnification: 160x)

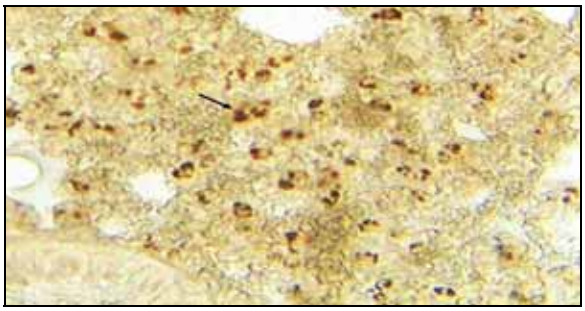

Fig. 11: Lung alveoli from cigarette smoke-exposed rat, with the presence of many dark-brown apoptotic nuclei. The arrow indicates one apoptotic nucleus (TUNEL staining, original magnification: 160x)

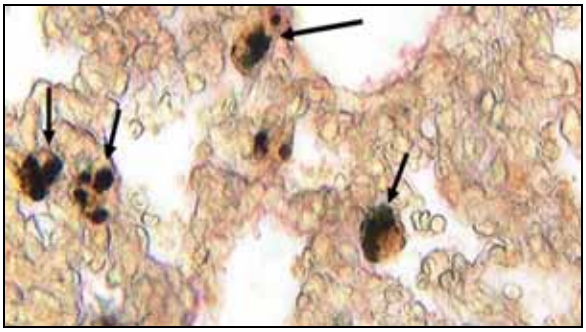

Fig. 12: Magnified picture of several typical apoptotic cells, as observed in tissue sections of lung alveoli from cigarette smoke-exposed rat. The arrows indicate typical apoptotic nucleus (TUNEL staining, original magnification: 400x).

could be due to the differential load of metabolites of cigarette smoke in these organs (Hecht, 2002; Ebbert et al., 2004; Stepanov and Hecht, 2005). That can change the antioxidant defence system leading to an oxidative stress with a variable effect on these organs.

An imbalance between cellular pro-oxidant and antioxidant levels leads to the oxidative stress resulting in tissue damage. The antioxidant enzyme interacts directly with reactive oxygen species (ROS) to convert them to non-radical products. Overproduction of these radicals has an inhibitory effect on the enzymes responsible for removal of ROS such as CAT, G6PD and GPx. It has been reported that superoxide radicals suppress CAT activity and that H_2O_2 inhibits SOD activity (Hassan and Fridovich, 1978), and the toxicity of aqueous extract of smokeless tobacco (AEST) in various organs like liver, lung, and kidney due to the formation of the radical species (Avti et al., 2006), which might explain the inhibition of these enzymes after the long-term administration of cigarette smoke in the present study.

In correlation with the alterations in enzymes activities, present data demonstrated an induction of inflammatory changes in tissues due to the effect of chronic smoking exposure on rats. These changes involved an interstitial inflammation comprised of lymphocytes and plasma cells in lung, portal tract inflammation in liver and mesangial cell proliferation in kidney corpuscles. All these inflammatory changes almost diminished in tissues after the recovery period from smoking effects, indicating reversible effects of smoking on tissues and enzymes of the rat. It has been reported that prolonged smoking causes impairments in antioxidant enzyme activities of different tissues in rat (Avti et al., 2006). This impairment was due to the inhibitory effects of ROS present in tobacco constituents on these enzymes. Also, tobacco smoke containing many toxic low molecular weight compounds that are able to activate bronchoalveolar dendritic cells (DCs) directly. Activated DCs can secrete a whole range of inflammatory chemokines, inducing the recruitment and activation of more DCs and other inflammatory cells such as neutrophils (Kantengwa, 2003).

Tobacco smoking also induces dose-dependent increases in goblet cell formation and mucin secretory capacity of rat airways (Stevenson 2004). Activation of neutrophils, in turn, releases a number of mediators and proteases that spread the inflammatory response and contribute to the destruction of the lung airways and other tissues. Therefore, it is believed that the long-term administration of tobacco smoking can impair the enzymatic antioxidant defence system of the rat liver, lung, and kidney. These alterations may be one of the responsible factors for smoking-induced inflammation in these organs (Avti et al., 2006).

The findings in the present on reversible effects of smoking support result of Pekmez et al. (2010), who found that the histopathological changes of rat kidney tissue exposed to smoking were partially disappeared after treatment with the antioxidant compound caffeic acid phenethyl ester. Many studies have reported an association between chronic cigarette smoking and reduced myeloperoxidase (MAO) activity in the brain and peripheral organs, but the inhibition of MAO-B enzyme activity in the human brain returns to normal levels after smoking cessation (Fowler et al., 2003; Lewis et al., 2007). A recent study of platelet MAO in human smokers showing no recovery of MAO during the first week of abstinence but full recovery by 4 weeks (Rose et al., 2001). *In vivo* studies on the interaction between cigarette smoke and oral peroxidase in smokers and nonsmokers showed that cigarette smoke-induced inactivation of peroxidase activity was in a reversible manner (Reznick et al., 2003). Cardellach et al. (2003) found that chronic smoking is associated with a decrease in enzyme activities of complex IV and III of mitochondrial respiratory chain, which return to normal values after cessation of tobacco smoking.

In our study, only lung tissues of smoke-exposed rats showed apoptotic cells. It is important to indicate the study by Das et al. (2009) who tried to investigate the effect of the aqueous extract of cigarette smoke on microtubules in a tissue culture. Vital cellular processes mediated by microtubules such as cell proliferation and maintenance of the cellular morphology, have been adversely affected in a dose and time-dependent manner in the extract-treated human lung epithelial cells (A549), and noncarcinoma human lung alveolar epithelial cells (L132). There was an observed disruption in the microtubule network. The damage of microtubules by the smoke extract may be correlated with the pathogenesis of cigarette smoke induced disorders, which result in cellular apoptosis and tissue damage. In another study, Kuo et al. (2005) demonstrated that the effect of CS-induced lung injury including apoptosis may be via reactive oxygen species and nitrogen oxides generation. The formation of these oxidizing agents leads either to the phosphorylation of p38/JNK MAPK pathway and then activation of Fas cascades, or to stimulate the stabilization of p53 and increase in the ratio of Bax/Bcl-2.

Long-term administration of cigarette smoke induces both apoptosis in lung tissue and impairs the enzymatic antioxidant defence system in liver, lung, and kidney. These alterations may be one of the responsible factors for cigarette smoke induced inflammation in these organs. The understanding of mechanisms for cigarette smoke induced cellular damage may offer therapeutic opportunities and ways to modify the adverse effects of cigarette smoke.

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