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Histopathologic Effects of Sulfur Dioxide in Mouse Liver Following the Chronic and Acute Exposure

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Abstract: The present study examined the histopathologic effects of this gas on the mouse liver in acute and chronic exposure. Twenty eight male mice were divided into 4 groups. Group 1 (G1) as control (not exposed to gas), group 2 (G2) were exposed to high dose of (100 ppm) and group 3 (G3) and group 4 (G4) were exposed to low dose of sulfur dioxide for 5 min for 1 and 3 weeks (20 ppm). Finally the mice were sacrificed and liver were assessed macroscopically and then the biopsy of the liver were assessed microscopically. The data were analyzed statistically. The results showed that the mean number of kupffer cells in G2, G3 and G4 significantly increased (9 ± 1.0 , 10 ± 1.0 , 11 ± 1.2 vs 7 ± 1.4 and $p<0.0001$). Qualitative observations also showed significantly increase in the rate of liver cell necrosis and deformation of hepatic cords and sinusoids with an increase of inflammatory reactions in experimental mice. Present study was indicated that the liver can be damaged by the chronic and acute exposure of sulfur dioxide.

Key words: Sulfur dioxide, mouse, liver

INTRODUCTION

Rapid growth and development of cities created various bio-environmental problems for inhabitants. One of the most important problems of living in big cities in present and future for small cities is pollution.

Air pollution is a permanent and serious threat for cities and leads to dangerous effects on health and society health and cause considerable economic suffers (Zhang *et al.*, 2007).

Given the statics and data use of fossil fuel had an ascending growth in transport and to the same extant is the growth of the various pollutant in the air.

In 2000 transport section played important great role in pollution and had spread to 60.2% Azot oxides, 22% Sulfur dioxide, 98.3% Carbon mono oxide, 90% Hydrocarbons and 75% dust.

Sulfur dioxide (SO₂) is one of pollutant of the city air that in low density in city air and in high density in industrial environments was found (Qin and Meng, 2006; Bai and Meng, 2005; Meng and Liu, 2007).

In 1992 international cancer research agency classified SO₂ to be one of carcinogen materials (Pesatori *et al.*, 2006).

Pollution produced by fossil fuel rich with sulfur increase the death rate especially death of inspiratory and cardiovascular disease (Hedley *et al.*, 2002).

Exposure to high amount of SO₂ is dangerous. Exposure to gas with the density of 100 ppm threat the health. Exposure to air pollutant may cause irreversible changes in activity of inspiratory system and dose effected with asthma are more in danger (Islam and Oberbarnsheidt, 1994).

Apart from cases above, effect of other synthetics of SO₂ like sulfite and sulfate on hypo cay neurons has been studied and results revealed that SO₂ synthetics cause increase of irritability of theses neurons (Du and Meng, 2004).

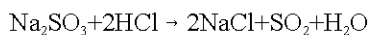
Exposure to SO₂ cause chronic disorder and change of activity of lungs. Although air pollutant enter the body by inspiratory system and the major effect of them is on lungs but other organs harm, too. Disease of cardiovascular (Pope *et al.*, 2003), lung cancer (Zhang *et al.*, 2007; Matsumoto *et al.*, 2007) especially in women (Hwang *et al.*, 2007; Soll-Johanning *et al.*, 1998), kidney and bladder pharynx and larynx (Soll-Johanning *et al.*, 1998), gaster and prostate (Berndt, 1977), skin and rectum even in some cases liver (Soll-Johanning *et al.*, 1998), decrease of body abilities,

decrease of IQ in children mostly for precipitation of Pb and decrease of life span and miscarry (Hafez *et al.*, 2001) in pregnant women are among the result of the air pollution on health of human. The aim of present study was to investigate the histopathological effect of sulfur dioxide (SO₂) on the mouse liver in acute and chronic exposure.

MATERIALS AND METHODS

First 28 suri mice with the average weight of 30 g were randomly selected and divided into four groups. One as control group that mice were not exposed to gas. Second groups were exposed to gas with a high dose (100 ppm) just once. Third and fourth groups selectively for one and 3 weeks were exposed once in a day and for 5 min with the low dose of gas (20 ppm).

Mice of each group in a special cage with the sizes of 10×20×40 cm. Gas was produced outside of the cage in special system and by an elastic pipe was led inside the cage (Sheal method). SO₂ gas was produced by this chemical reactions:



Weight of the salt and the volume of the acid given the specialities and condition of the materials like concentration degree, acid percentage was determined and condition like temperature and pressure, were considered.

In all groups mice were unconscious by the dislocation of the cervical vertebral and their liver was removed. First macroscopic observation and then microscopic evaluation was carried out. For microscopic evaluation samples of liver was prepared and kept in formalin 10%. After fixation and embedding, serial sectioning was done and from each sample 5 section were selected (section number 5, 8, 11, 14 and 17). After staining with H and E, microscopic slide were prepared.

Statistical tests: Among prepared sections in each group 10 slide and from each slide randomly 5 microscopic field were selected. Macrophages in each microscopic fields were counted; then information of the acute and chronic one and three week by t-test with the control group were compared.

RESULTS

In normal condition liver has hyaline tissue that is rather soft. In microscopic observation and slide of control group, hepatocytes in a radial style they are placed from centra lobular to the periphery and most of them were mononucleous and sometimes dinucleous forms were seen among them (Fig. 1).

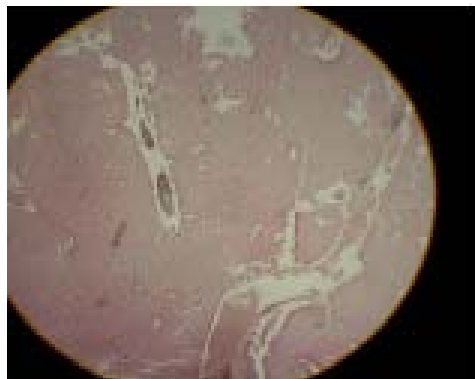


Fig. 1: Microscopic section of chronic group radicalic hepatocyte (x4)



Fig. 2: Microscopic section of acute liver group the resells were hyperaemic (x4)

In macroscopic studies of acute group, hemorrhagic spots on the surface of liver was seen. Lobules were morphologically changed in microscopic evaluation.

In each control and acute groups 50 samples were studied and compared in number of kupffer cells.

Average number of cells in control group was 7 ± 1.4 and in acute group was 9 ± 1 and statistical test revealed significant difference between them ($p < 0.0001$), hyperemia of vessels was seen noticing the photomicrographs (Fig. 2). Results in chronic stages 1 and 3 weeks were almost similar. LM studies showed darkening of the liver tissue and increase of the stiffness was observed.

Hemorrhagic spots on the liver surface were seen. In this group the lobules were morphologically changed, number and diameter of the blood vessels were increased, hyperemia and angiogenesis that are symptoms of important of the harms caused by gas, were seen clearly in the photomicrographs. Diameters of the blood vessels in both chronic groups were considerably increased (Fig. 3).

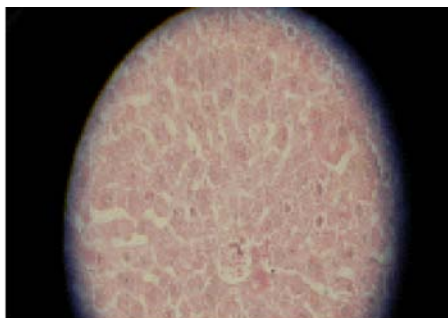


Fig. 3: Microscopic section of chronic 1 week liver group comparative diameter (x100)

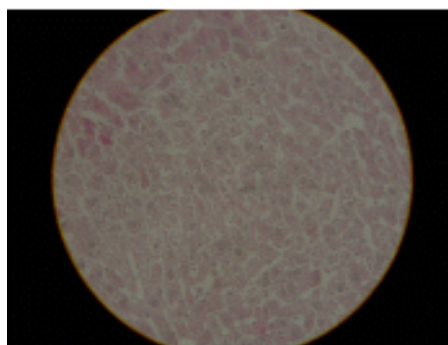


Fig. 4: Microscopic section of chronic 3 weeks liver group hepatocyte with big light nuclei indicator of malignancy (x100)

Morphologic change of hepatocytes and increase in becoming eosinophil that was the sign of cellular necrosis was observed. Hepatocytes with large and clear and extant euchromatin region was observed. It showed beginning of the malignant and precancer stages in that tissue (Fig. 4).

Evaluation of the number of kupffer cells between two control and chronic 1 week group, in each group 50 microscopic field with the magnification of the 100 were observed. Average of cell in control group were 7 ± 1.4 and in chronic group of 1 week were 10 ± 1 . The statistical test showed difference between average cells in this two groups ($p < 0.0001$).

Average number of the kupffer cells in control group 7 ± 1.4 and in chronic group of 3 weeks were 11 ± 1.2 . Statistical tests showed significant difference between average cells in these two groups ($p < 0.0001$).

DISCUSSION

Here, acute group lobules were morphologically changed and lost their normal structure. Hyperemia of vessels were observed.

This study corresponded of the results of histopathologic effects of the sulfur mostart on large mouse of laboratory.

The number of kupffer cells increased considerably in acute group. It may be due to increase of phagocytic activity caused by letting in the gas.

Macroscopic observation of chronic groups showed darkened liver tissue and increased stiffness. Hemorrhagic spot on the liver surface seen.

In studies of chronic groups showed that structure of the lobules were changed. Number and diameter of the blood vessels were increased, hyperemia of vessels were observed in photomicrographs.

These results also proved by other investigators. They reported hyperemia and dilation of the sinusoids, destroy of the lobules, limitation and disformation of liver cord.

This study, demonstrated angiogenesis in chronic groups that is a sign of damage of vessels under the gas inspiration.

Cellular changes similar increase of the number of macrophages and hepatocyte showed too.

Increase of the number of macrophages may be due to the increase of phagocytotic activities caused by the gas inspiration. Macrophages producing cytokines are pre-inflammation factors. kupffer cells that is the member of the mononuclear phagocytes demonstrated more and may be due to inflammation by the gas.

Another scientist reported that polluted air increased pulmonary macrophage (Van Eeden *et al.*, 2001). This study reveled the increase of eosinophilic characteristic of hepatocytes. Probably is the due to necrosis of mentioned cells.

Hepatocytes observed with large and clear nuclei (high concentration of euchromatins). It showed the beginning of the malignant and pre-cancer mood of the tissue, probably.

These results were proved other researches, who studied the effect of SO_2 on different tissues (like: brain, lung, heart, liver, spleen, thymus, bone marrow and kidney).

They exposed the experimental group of rats during 7 days to SO_2 with density of 125, 250 and 500 $mg\ kg^{-1}$ of weight. These results of this experiment showed that SO_2 caused destroy of the DNA of these cells.

This results offered that exposure to this gas cause cancer and destroy of DNA (Meng *et al.*, 2004).

They revealed that SO_2 with the density of 28 and 56 $mg\ m^{-3}$ cause considerable increase in mRNA rate of P53 genes and bax in liver of suri mice; where as mRNA gene *bcl-2* had considerably decreased.

This results showed that exposure to SO_2 gas cause changes in gene express rate that are responsible for apoptosis and suggest this gas can induce apoptosis in liver (Bai and Meng, 2005).

Researchers in 2007 showed that, inhaling of SO₂ gas with the density of 56 mg m⁻³ caused pathologic changes of liver cells of the suri mice.

These changes were inflammation of nucleus, fat droplets, degeneration of mitochondria and dilation of rough endoplasmic reticulum rER (Meng and Liu, 2007).

Another study revealed that reduced glutathione rate to oxidate glutathione was considerably decreased in liver and other mice organs.

Decrease of the reduced glutathione rate and the ratio of the reduced glutathione to oxidate glutathione suggested that cytotoxic effects with decreased the antioxidant and weakened of the immunosystem and antioxidant in biological systems.

Evaluated the Meng (2003) effect of SO₂ on enzyme rate like catalase, glutathione peroxidase, superoxide dismutase and glutathione that has important role in decrease of free radicals.

Results showed that inspiration of SO₂ cause considerable decrease in glutathione peroxidase and superoxide dismutase activity rate in 9 organs of mice (brain, lung, heart, liver, stomach, spleen, kidney, small intestine, testis) (Meng, 2003).

Qin and Meng (2006b) studied inhalation effects of the SO₂ gas with the density of the 14.11, 28.36 and 96.25 mg m⁻³ during 7 days and daily for 6 h on activity rate of de alkinase pentoxirezophorine and nitrophenol hydroxylase of lung and liver of male rats was considerably decreased, but nitrophenol hydroxylase rate just in lung of these rats decreased (Qin *et al.*, 2006).

Gumuslu *et al.* (1998) evaluated the inhalation effects of the SO₂ with the density of the 100 ppm during 10 days and daily to 1 h on the activity rate of the erythrocyte antioxidant showed that SO₂ gas causes lipid peroxidation and also cause increase of the activity rate of erythrocyte antioxidant enzymes (Gumuslu *et al.*, 1998). Gumuslu *et al.* (2000) studied the inhalation effects of SO₂ on plasma amount of vitamin C and rat Seroluplasmin, results showed that plasma vitamin C and rat Seroluplasmin in young rats, adult and old that were exposed to the SO₂ was increased (Geng and Meng, 2003).

Based on the enzyme study and results this study concluded that cytotoxic effect of SO₂ can cause malignancy and cancer by decrease of the antioxidants and suppression of the immunosystem (Geng and Meng, 2003).

Clinic studies and laboratory evaluation showed relationship between the amount inhalation of particles and floating of polluted air and waterfall process of coagulation reactions, pellets activity and development of atherosclerosis and thrombose, these negative effects, by making free neurotransmitters of solution by lungs that

affect the coagulation parameters or by direct transfer of the particles to the circulatory and or direct effect on the heart autonome control system (Nemmar *et al.*, 2006).

Recent results of the scientists, showed considerable increase of the endothelin vasoactive peptide in circulatory in rats exposed to the polluted air (Vincet *et al.*, 2001).

Another study revealed that exposure to the polluted air with increasing the surface of the active C-protein (Peters *et al.*, 2001a), systemic inflammation that was an important sign for the cardiovascular disease (Ridker *et al.*, 2002), inflammation damages of the lungs (Ghio and Devlin, 2001; Souza *et al.*, 1998), responses of the bone marrow and blood cell (Tan *et al.*, 2000), increase of the lung macrophages cells that produce pre-inflammation cytotoxin. Increase of the blood plasma viscosity (Peters *et al.*, 1997), bad effect of the vessels endothelium activity (Brook *et al.*, 2002) and infarcts (Peters *et al.*, 2001b).

Inflammation reaction caused by floating dust of polluted air in study of the laboratory animals was seen (Mukae *et al.*, 2001).

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