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Histopathology of Inhalation of Industrial Bleach and Detergent Mixture on Epithelial Layer of Trachea in Mice

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Abstract: This study is the effects of inhalation toxic mixture of bleach and detergent was examined on the epithelial layer of trachea in the mice. In this study, 42 adult male mice NMRI race weighing 35-40 gr and from age 8 to 10 weeks were divided into 6 experimental groups and one control group. Experimental groups 1-2-3 with the using of chamber, as inhalation 20 minutes were exposure to spray the amount 1 cc of mixture of bleach and detergent by nebulizer. Experimental groups 4-5-6 were for 35 minutes to inhale the same amount of material. Mice killed at 24-48-72 hours after inhalation and the trachea was studied pathology. In microscopic sections of tissue taken from the trachea the experimental group compared with the control group was changed to include: stimulation and activation of the respiratory epithelium hungarian (Mocusa layer), reducing the length of ciliated columnar cells, reducing the number of goblet cells ,loss of cilia, chaos and clutter on the order of tissue. In addition, statistically, the changes in length of ciliated columnar epithelium cells in experimental groups 2 and 6 seen significantly decreased than control group and the number of experimental groups 2 and 4 goblet cells significantly increased compared to control group, experimental group 6 was significantly decreased than the control group. As a result discussion, increasing the inhalation time of mixing bleach and detergent, also as time passed, cause to increase the tissue damage and changes.

Keywords: bleach, detergents, chlorine, trachea, goblet cell, inhalation, mice

INTRODUCTION

Today, products of industrial bleach and detergents being used and sometimes people for more cleaning use of bleach and detergents mixture that for the reason of chemical interaction release chlorine gas and thereby Increase the severity of the symptoms of poisoning. Detergents can clean the fat and dirt particles in various forms (Grime & Class, 1990). There are different types of bleach: chlorinated or household bleach containing sodium hypochlorite (NaClO) which is also called Javel water or vaytex. The bleach solution in water to forming 5 or 10 percent will be offered. Vaytex is an oxidizer. Other types of non-chlorine bleach contain hydrogen peroxide or sodium perborate or sodium is percarborate and finally the calcium hypochlorite is a bleaching powder (Dence & Douglas, 1996). People for more cleaning use of mixed bleach and detergents, it lead to more release of chlorine gas and thereby increase the severity of the symptoms of poisoning (Trotman, 1968). Most cases of poisoning were caused by

inhaling these compounds in the space causing chlorine gas that could irritate the airways and can cause symptoms of poisoning individuals (Das & Blanc, 1993).

Chlorine was discovered by Scheele in 1774, at atmospheric pressure and room temperature. Elemental chlorine is a gas. It is yellowish-green and the characteristic smell of bleach. The combined chlorine bleach with detergent could cause more and more harmful effects(Hedges & Morrissey, 1979).

The most important effects of chlorine gas in the body caused by free radicals of oxygen. Oxygen is an essential element of life can be severely harmful effects on the body, under certain conditions. Many of the harmful effects of oxygen due to the formation and activity of chemical compounds is known as reactive oxygen species (ROS) (Bagchi & Puri, 1998).

Free radicals are the agent of many diseases and fatal cancers (Harman, 1992). Oxygen free radicals, are molecules that in the reduction of molecular oxygen are generated. These compounds are produced in cells from several

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directions. But most of the activities are performed in mitochondria, because mitochondria are the main places in which oxygen are reduced (Grime & Class, 1990). And cause damage in 4 points of to the body, including lipid compounds, proteins, DNA and is the lysosome and thus can lead to cell damage and eventually to cell death (Han *et al*, 2001).

The trachea extends from the larynx to about the middle of the thorax where it divides into two bronchi, the primary bronchi. Wall of the trachea has three layers of mucosa, submucosa and adventitia (Rojhan, 1992). The aim of this study is examined the effects of inhalation toxic mixture of bleach and detergent on the epithelial layer of trachea in the mice.

MATERIALS AND METHODES

In this study of 42 adult male mice, NMRI race was used with weighing 35-40 gr, and age range from 8 to10 weeks, its were purchased from Karaj Anistito Pastor. Animals at 23- 25 °C temperature and light conditions under the standard 12 hours light and 12 hours of darkness and a relative humidity of 40 to 60 percent water and food were kept as same. Food of Plates prepared from mice that had been prepared from Pastor Institute of kraj. The animals randomly divided into 6 experimentalthe group 1-2-3-4-5-6 and one control group. Test group 1-2-3 included 18 mice in the chamber that dimensions were 30 \times 30 cm³ for 20 minutes. Value 0.5 cc of bleach and detergent material (Bleach and detergent were obtained from kondor (ABC) Company) was poured into the nebulizer to spray and mice were exposed to it. Mice in groups of six teeth

were returned to cages and food and water were available to them. Experimental groups 1-2-3 of mice were killed 24, 48 and 72 hours after inhalation and the trachea was pathologically studied. Experimental groups 4-5-6 included 18 mice in the chamber inhale for 35 minutes to the same amount of material. Mice in groups of six teeth were returned to cages and food and water were available to them. Experimental groups 4, 5 and 6 of mice were killed 24, 48 and 72 hours after inhalation and the trachea was pathologically studied.

Preparation of tissue samples

Samples after separation were placed in 10% formalin fixative solution for one week. After preparing the tissue by processing system and molding samples using microtom system, 5-micron thick sections were cut. For histology studies, sections were stained with hematoxylin and eosin staining method.

Statistical analysis

The results were evaluated in the studies between experimental and control groups considering mean and standard deviation (Mean \pm SD). Statistical calculations were performed to evaluate significant differences between experimental and control groups using the test (ANOVA) followed by Tukey complementary test (P \leq 0.05).

RESULTS

The ciliated columnar epithelium cells of the trachea in a dose of 1 cc, during inhalation of 20 and 35 minutes showed in fig 1 and 3.



Figure 1: Comparison of mean \pm SD Length of ciliated columnar epithelium cells of the tracheal, 24-48-72 hours after inhalation of a mixture of bleach and detergent. The amount of 1 cc, 20 and 35 minutes in duration Between experimental and control groups(*P \leq 0.05 and ** P \leq 0.01 compared with control group) (n=6) (B+D \rightarrow Bleach + Detergent)



Comparing the results of the mucous layer of the trachea epithelium goblet cells in doses of 1 cc

,during inhalation of 20 and 35 minutes, showed in Fig 2 and 4.

Figure 2: Comparison of mean \pm SD Number of goblet cells in the trachea 24-48-72 hours after inhalation of a mixture of bleach and detergent, The amount of 1 cc, 20 and 35 minutes in duration Between experimental and control groups(*P ≤ 0.05 and ** P ≤ 0.01 \jmath *** P ≤ 0.001 compared with control group) (n=6)(B+D \rightarrow Bleach + Detergent)



Figure 3: The photomyograph of tracheal epithelial layer in group 2 (coloring: H & E microscopic magnification: 100 ×) Increase in the number of goblet cells can be seen in this picture. CCC: ciliated cylindrical cells, GC: Goblet cells, Epi: layer of mucous Epithelium.

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Figure 4: The photomyograph of tracheal epithelial layer in group 5 (coloring: H & E microscopic magnification: $100 \times$) what can be seen in this picture: Reduction in the length cylindrical ciliated cells, and the number of goblet cells, from loss of cilia and the disorder and clutter on the order of tissue.

CCC: ciliated cylindrical cells, GC: Goblet cells, Epi: layer of mucous Epithelium.

DISCUSSION

According to studies, inhalation of chlorine gas and its reaction with existed water in body tissues to releasing of free oxygen cause radicals(Florian, 2003; Han et al, 2001). This molecule is highly unstable and the tendency to take electrons from other molecule, the molecules self-sacrifice this process causes the flow to become a free radical chain reactions can be production of free radicals (Han et al, 2001). External stimuli can have effect on mitochondrial electron transport chain of cell it's original position and reduce oxygen free radical production and increase free radical production (Florian, 2003). The creation of free radicals,4 point of the body that are damage to lipid compounds, proteins, DNA and have harmful effects are Lysosome (Han et al, 2001). Since the cell membranes of unsaturated fat are made. these molecules are susceptible to damage by the free radicals are these lipid molecules so that cause hardening of cell membranes, which blocks the ability of cells to receive nutrients and cellular exchanges will lose this is accompanied by cell death (Han et al, 2001). DNA structure in an open rupture in the sequence of purine and the DNA structure are destroyed. In protein building caused degradation amino acids of pre-protein and thus the degradation structure has to follow

and free radical damage to membrane Lysosomes, and the enzyme produced in these been released into the cell cytoplasm cellular components and thus can digest (Florian, 2003). The study of microscopic sections of mouse trachea taken many histopathological changes seen in experimental animals compared with controls was significant. Changes include the stimulation and activation of the respiratory epithelium hungarian (Mucosa layer), reducing the length of ciliated columnar cells, reducing the number of goblet cells, loss of cilia, chaos and clutter on the order of tissue. In addition, statistically, the changes in length of ciliated columnar epithelium cells in experimental groups 3 and 6 seen significantly decreased than control group and the number of experimental groups 2 and 4 goblet cells significantly increased compared to control group, experimental group 6 was significantly decreased than the control group.

This can be explained by the to the effects of free radicals generated during this study extended According to findings by the come the highly reactive free radicals, these molecules are can reaction side cellular companies also have devastating effects on their own. As a result, increasing the inhalation time of mixing bleach and detergent, as time passed, increasing in the mean show that the sections. Thus number of goblet cells due to increased secretion of mucus to enter a foreign agent in to airway the increased (Rojhan, 1992) but over time the number of the damaging effects of free radicals decreased in goblet cells.

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