Iranian Journal of Military Medicine Winter 2011, Volume 12, Issue 4; 203-209

Effect of sulfur mustard on the epithelial cell necrosis of urinary duct of kidney in rat

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Abstract

Aim: Kidney is a high vascularized organ which can be affected by sulfur mustard. Sulfur mustard causes tubular necrosis and urinary epithelial cell sloughing. The aim of this study was to evaluate the cellular and tissue damages in rats exposed to sulfur mustard.
Methods: This experimental study was performed on 100 rats. 10mg/kg was assigned as the desirable dose which rats could live at least 10 weeks while receiving it. 2.5mg/kg and 5mg/kg were also assigned as doses in which tissue changes were studied. Tissue changes were evaluated after 2, 14, 28 and 56 days in 12 experimental groups (3 groups for each time period each one receiving one of the 3 mentioned doses). 4 natural groups (one for each time period) received no injection and 4 control groups only received Tyrode's solution. Prepared slides of all groups were compared and histological changes were evaluated. Data was analyzed using one-way ANOVA and Tukey's Post-hoc test by SPSS 13 software.
Results: Data of this study revealed no remarkable difference between natural and control groups. Tubular necrosis and urinary epithelial cell sloughing were observed in all experimental groups. The rate was statistically significant only in group which received 10mg/kg sulfur mustard for 56 days compared to the control group.
Conclusion: Exposure to sulfur mustard cause tubular necrosis and urinary epithelial cell sloughing in rats and its increase in statistically significant in rats receiving 10mg/kg for 8 weeks.

Keywords: Sulfur Mustard, Tubular Necrosis, Epithelial Cell Sloughing

Introduction

Sulfur mustard, is an alkylation agent that creates a spectrum of toxic responses in individuals exposed to it. This material causes cytotoxicity [1]. Sulfur mustard causes destruction of normal control processes of cells by creating a series of reactions due to alkylation of DNA [2]. This material creates sulfonium ion in the body that causes alkylation of DNA and consequently, the break of the DNA strings and creation of transverse joints in two DNA strings and interference in DNA synthesis and cellular division or death [1, 2, 3].
A considerable amount of blood flows in kidneys. Regarding the existence of mustard toxic element in the blood flow and due to filtration of blood tissue while passing different stages of secretion, resorption and excretion of this material can have destructive effect on epithelial cells of nephron urinary tube. According to available research works and evidences on the effect of mustard gas on human and animals, kidney and liver are affected by mustard gas [4, 5].
It has been observed that sulfur mustard causes creation of apoptosis and necrosis [6] and the rate of apoptosis depends on time. When cells are in contact with high dosages of sulfur mustard in a short while, the rate of apoptosis is high. Direct contact with sulfur mustard causes necrosis in long time. This material causes induction of apoptosis at average rate and necrosis at extreme level [7].
In drug toxicity, there are different stages of cellular degeneration in urinary tubes. Epithelial cells loss and urinary dilation diameter and duct wall tear have been observed in most cases. Degeneration changes of epithelial cell in urinary tube and coronary hyperemia have been reported as the complications of diclofenac drug in parts of interstitial space in cortex and central district [8]. The aim of this study was to analyze the tissue and cellular destructive effects and complications including cellular death and epithelial cell loss in urinary tubes, in order to understand the role of protective materials and pre-treatment of mustard gas effects through recognizing the rate and type of tissue destruction.

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Received 2010/06/16; Accepted 2010/09/29

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Methods

This study was performed on 225 Albino-Wistar male rats with an average weight of 200±40 grams. They were selected randomly and food and water distribution were regularly controlled. In all stages of working with chemical agent, immunity issues, and personal protection were tightly controlled. Therefore, in all operational procedures, using protective gloves, dress gown, mask, cover for shoes, and applying compensating materials of chemical agent were necessary.

In order to determine the considerable dosage of mustard agent at which, the animal can continue his life and not to die because of fatal complications of the agent, the low dosage was determined. In order to do this, 125 rats were used. The animals did not tolerate the duration of time at the dosages of 20, 40, and 60mg/Kg and died. Therefore, 10mg/kg was selected as the desirable dosage at which the animal could survive for more than 10 weeks. Two lower dosages of 2.5 and 5mg/Kg were also determined for analyzing the tissue changes and comparing the agent intensity.

Tissue changes were studied for all three dosages under study for two days as acute, 2 weeks (14 days), four weeks (28 days), and also 8 weeks (56 days) as chronic. Five rats were considered for each time group at determined dosages. 5 rats were considered as control (to which, tirodos was injected) and 5 other rats as the natural group (to which nothing was injected) beside each groups. Each group was placed in 5-piece shelves. The atmosphere, temperature, lighting type, and animals' nutrition in all groups were the same. The profile of each group indicating the amount of agent dosage and maintenance time after the injection was written on each cage. Moreover, every animal was marked in order to be recognized at different stages. The injection and departure date of animal were regarded as another criterion. For the animal to get used to laboratory conditions, it was kept for one week after being delivered from the University animal house situated at the complex and then, it went under injection and was studied.

The used sulfur mustard was an oily liquid at 99% purity and specific weight of 1.270g/µl. Therefore, in order to produce the dosages of 2.5, 5, and 10µl/Kg, the following measurements were taken:

Based on the specific weight of liquid sulfur mustard, the required volume for dosage of 2.5µl/Kg was determined to be 1.96µl (0.00196µl), 3.9µl (0.0039µl) for dosage of 5µl/Kg and 7.8µl (0.0078µl) for dosage of 10µl/Kg. A solvent first dissolves the chemical mustard agent to be transferred to the body. Among the different solvents available for this agent, buffer tirodos was selected. The reason for choosing this buffer was non toxicity and lack of immunological stimulation and frequent application of this solvent in the available studies. 2.5µl/Kg of sulfur mustard equals to 0.4µl for each 200-gram animal, 5µl/Kg equal to 0.8µl, and 10µl/Kg equal to 1.6µl was used. 0.4µl sulfur mustard was dissolved in 99.6µl and 0.8µl of sulfur mustard was dissolved in 99.2µl, and 1.6µl of sulfur mustard was dissolved in 98.4µl of tirodos.

The groups of each period included:

1. Control group: in this group, tirodos was injected intraperitoneally by insulin syringe in the left iliac based on the animal's weight according to the formula at the determined rate.
2. Natural group: no drug was injected to the animal.
3. Sulfur mustard 1 (HD1): that sulfur mustard 0.4% was injected intraperitoneally in left iliac.
4. Sulfur mustard 2 (HD2): that sulfur mustard 0.8% was injected intraperitoneally in left iliac.
5. Sulfur mustard 3 (HD3): that sulfur mustard 1.6% was injected intraperitoneally in left iliac.

For each time group, after being weighted, the animal was transmitted to a large glass container containing some cotton stained to chloroform.

The animal was immediately transmitted to anatomy table after being killed. Through a length cutting the middle line of abdomen from xiphoid to the pelvic area, the animal's abdomen opened, and after pushing into the abdominal viscera, animals' kidneys were completely removed. Then, the kidneys were transmitted to the appropriate containers, and kept for 24 hours in formalin 10%. After 24 hours, the stabilizing fluid was changed and a longitudinal cut was made in the tissue to let the fluid permeate in whole part of kidney tissue. To prevent the stiffness of kidney tissue, after 48 hours the type of stabilizing fluid was changed and alcohol 70% was used, and the sample got ready for the next stage. After tissue fixation, the next stage was the tissue turnover. In this stage, samples were placed in the basket based on their special texture number.

The samples were placed as kidney tissues cut in two pieces through a longitudinal cut. Tissue passage operation was done by machine. In this stage, the sample tissues were molded by liquid paraffin in proper aluminum molds. The mold was placed on a clean smooth glass and a thin layer of liquid paraffin was poured in the mold by paraffin dispenser machine. Then the sample tissue was placed into the mold by forceps and the mold was filled by liquid paraffin.

After the molds got cold at the room temperature and liquid paraffin solidified, the blocks were separated

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from mold and kept in refrigerator. Using microtome, 5-microni cuttings were prepared from molded tissues. Then these cuttings were overspread by warm bath on prepared lams. After that, and when lams got dry, they got prepared for staining. Hematoxylin- Eosin painting was used for histological studies. For this purpose, hematoxylin Harris Alum and saved Eosin solution 1% were used. Then the prepared lams from kidney tissue were studied histologically. The tissue slide samples of experimental groups and available morphologically and histology changes in these lams were compared with witness and control groups. The type and rate of these tissue changes were analyzed in the experimental group for different dosages and the days in which the tissue has faced chemical agents.

The conducted studies show that tissue complications in kidney toxins include cellular death, cell loss in urinary tube walls, vascular hemorrhage, cellular infiltration in interstitial space, dissolution (integration) of walls urinary tubes and losing epithelial cell order in ordering urethras, maintaining renal skeleton and upsetting glomerular hank and availability of multi and single-cells in interstitial space [8]. To study and compare each of the characteristics, pathologic changes were studied in separation of calibration. To analyze the rate of destructive changes (++++), (+++), (++), and (+) signs were used to show severe, high, average, and slight respectively, also, 0 meant natural. Optical microscope by zoom 400x square micrometer was used to calibrate the cellular order. Desired level for study was 141000 square micrometers. Cortical area and renal medullary changes were observed and registered in each tissue sample in three points (high, average, and low). Vision area was lens space. In each vision area, the desired element including bowman capsule, initial curved tube, secondary curved tube, Henley arch, urine duct colleting, and finally interstitial space.

In analyzing the data, one-way ANOVA statistical test was used through the method of Tukey's Post-hoc using software SPSS 13. The results are shown in Figures based on average±standard deviation.

Results

Cellular death was observable in experimental samples. Rate of cellular necrosis in experimental cases were more than witness samples, the rate of cellular death increases as mustard agent concentration increases. In samples that were polluted by 2.5mg/Kg dosage, the cellular necrosis was not observed in 2-day and 2-week periods. Cellular necrosis was observed in samples with a concentration of 2.5mg/Kg in 4-week, and 8-week periods as + (slight). The increase of cellular death rate was observed in 5 and 10mg/Kg dosages, yet the death cellular density with increasing the concentration of mustard agent was not observable. The density of cellular death increased with increasing the concentration of mustard agent in 4, and 8-week periods. Severe cellular death (++++) was not seen in any sample. High cellular death (+++) was 10mg/Kg concentration in experimental samples and in an 8-week period. According to the statistical studies, the rate of cellular death increased with the increasing concentration of mustard agent. Increasing necrosis cells increased with increasing the conflict period. Comparing the experimental results with witness and natural groups' results showed that cellular death has happened in experimental samples. Dead cells did not have any significant difference in experimental samples among all groups. The significant difference remained between 2.5 and 5mg/Kg groups with 10mg/Kg group for 56 days, and between 2.5mg/Kg group for 2 days, and for 2.5mg/Kg for 56 days with 5mg/Kg groups for 2 days and 5mg/Kg for 56 days (Diagram 1). Situations in which the intensity concentration of mustard agent was more or in which the duration of conflict period increased, this increase was statistically significant.

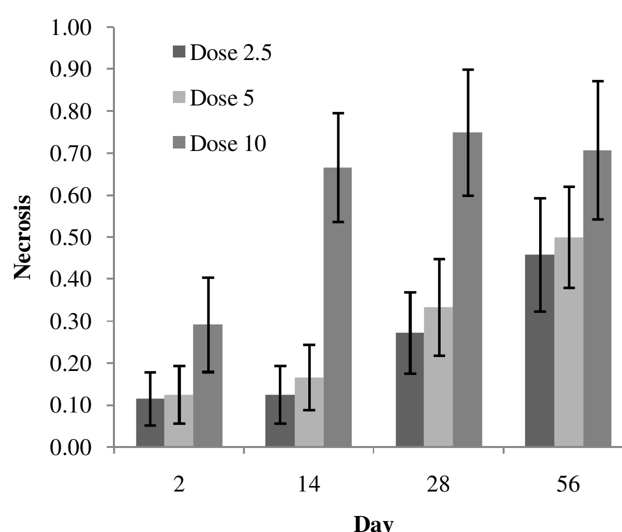

Diagram 1- Analysis of created necrosis in studying samples in accompany with increasing the intensity of mustard agent.

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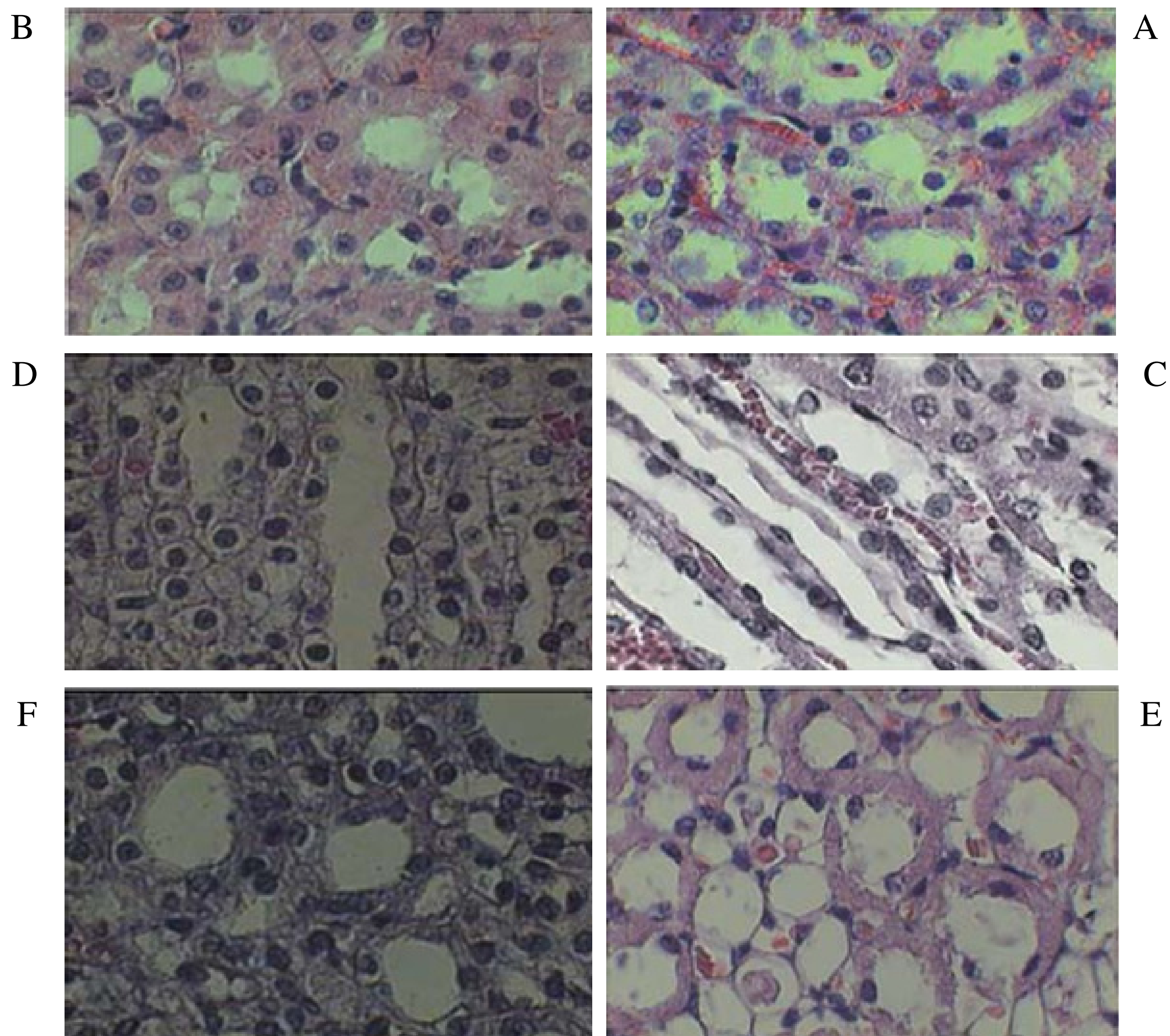

Figure 1- The images of renal tissue in different groups and cellular death in experimental groups in comparison with control and natural groups are observed (zoom 1000, painting hematoxylin-eosin). A: the experimental sample with concentration of 10mg/Kg during 8 weeks, B: the control sample with triodos injection, C: the experimental sample with a concentration of 10mg/Kg during 4 weeks, D: the natural sample after 8 weeks, E: the experimental sample with a concentration of 10mg/Kg during 8 weeks, F: the natural sample after 8 weeks.

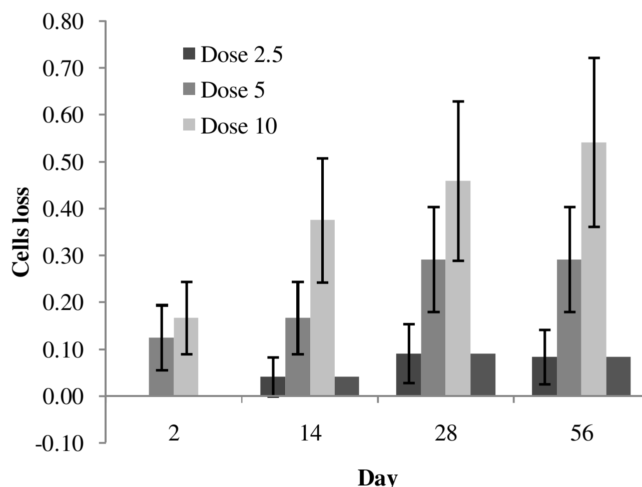

Diagram 2- The analysis of created cellular loss in studying samples with increasing the intensity of mustard agent.

Cells loss increased in studied samples accompanied by the increasing mustard agent intensity (Figure 2). The most types of cellular loss were slight (+). The increase of cellular loss rate had a direct relationship with increasing the confliction period of animal with mustard agent. High cellular loss (+++) existed in samples that were polluted by mustard agent of 10mg/Kg concentration. The cellular loss grade was not observed in samples that were polluted with 2.5 and 5mg/Kg concentrations of mustard agent. The intensity of cellular loss was observed of average degree (++) in pollution by 2.5, 5, and 10mg/Kg concentration. This rate was the most in 8-week samples and after that in 4-week experimental samples.

According to the statistical study, the cellular loss rate was increased with increasing mustard agent

concentration. The changes of epithelial cell loss increased with increasing the conflicting period in samples. Statistical difference among 2.5mg/Kg experimental groups for 14 days, 10mg/Kg group for 14 days, 2.5mg/Kg group for 56 days and 10mg/Kg group for 56 days were significant (Diagram 2).

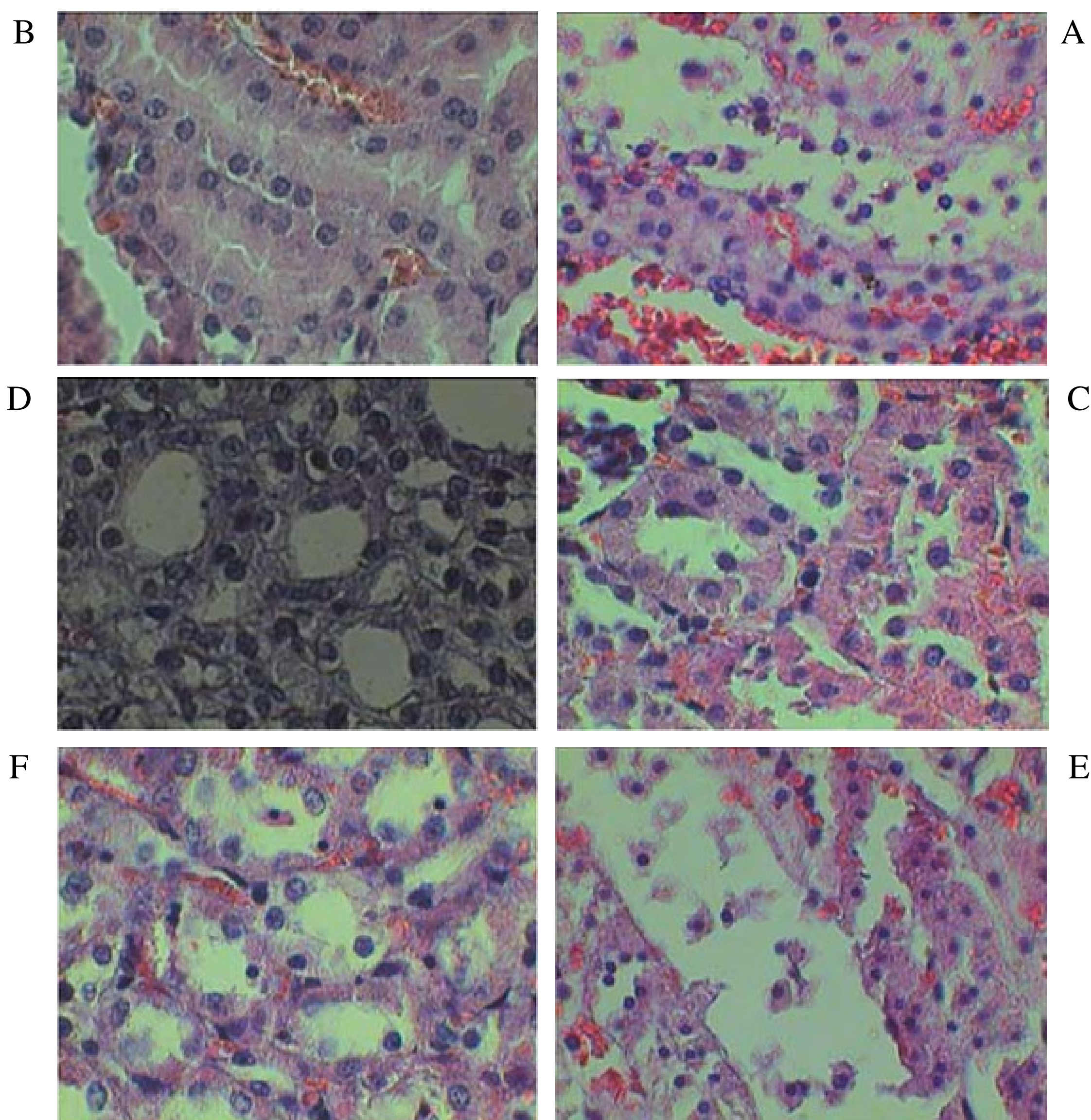

Figure 2- The images of renal images in different groups and cellular loss in the experimental groups in comparison with control and natural groups are observed (zoom 1000, painting hematoxylin-eosin). A: the control sample during 8 weeks of triodos injection, B: the experimental sample with a concentration of 10mg/Kg during 4 weeks, C: the experimental sample with a concentration of mg/Kg during 2 weeks. D: the natural sample after 8 weeks, E: the experimental sample with a concentration of 10mg/Kg during 8 weeks, F: the experimental sample with a concentration of 10mg/Kg during 4 weeks.

Discussion

Cellular necrosis is one of the destructive effects of mustard agent that can be observed in epithelial cells of urinary tubes. Diclofenac is one the most toxic drugs striking out in the process of cellular metabolism. In high dosages of this drug, degeneration changes and cellular death were observed as points. In the present study, cellular death was observed and increased in experimental group by increasing mustard agent concentration. The result of the present study is consistent with the study of Gulsen et al. [9]. The performed analysis in this study showed that the cellular death process is affected by conflicting period and this agrees with the results of studies of Ostad et al. [10]. Sulfur mustard reacts with available

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glutathione in the cell and decreases it in tissue. Therefore, those enzymes that control thiol proteins are inhibited and the level of intracellular calcium increases. The high levels of calcium in cell causes membrane failure and cell skeleton and cytokine combinations, and DNA by increasing the activity of proteases, phospholipids, and endonucleases. This operation results in inflammation process of the cell and thereupon cell death [6, 7]. Tissue changes and the cellular death in experimental samples of the present study, approves Mehrani's opinion about the research process [7].
In studying the cellular loss, separation of epithelial cells from the walls of urinary tubes and releasing in tube entry is considered. Epithelial cells loss from urinary tubes walls was among the effects of mustard agent in experimental samples and the same observations were reported in conducted studies of other researchers such as Gulson et al. [9]. In inhaling high dosages of sulfur mustard, the epithelial cells of upper respiratory tract losses, that is accompanied with edema around the bronchi, hyperemia of blood vessels, cell infiltration in submucosa layer, being vacuole, cytoplasm disorganization and core structure causes pulmonary hemorrhage, pulmonary edema, respiratory failure, and such symptoms as respiratory distress syndrome. The availability of epithelial cells in urinary tubes is an evidence for cellular loss that has been observed in the available study. This issue is consistent with the study of Ghanei et al. [11].

Conclusion

In all experimental groups, necrosis is observed in epithelial cells and epithelial cells losses in urinary tubes ducts. Therefore, this rate has a significant difference only in 10mg/Kg of mustard for 56 days in comparison to the control group.

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