Original Article

Toluene-induce changes in lung tissue and white blood cells

Adeli Hamid Reza[1], Zare Kobra[2]*, Naeimi Nasim [3], Alamdar Maryam[4]

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1. Dept of Developmental Biology, Faculty of Science, Branch of Sience and Research of Tehran Azad University, Tehran, Iran
2. Dept of Animal Physiology, Faculty of Science, Branch of Sience and Research of Tehran Azad University, Tehran, Iran
3. Dept of Biology, Faculty of Science, University of Sistan and Baluchestan,Zahedan,Iran
4. Dept of Biology, Pishvaofthe University ofVaramin, Varamin, Iran

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Abstract

Introduction:
Toluene (C7H8), an aromatic constituent of petroleum, is mainly used as a chemical solvent in industry. Given the harmful effects of toluene on the environment, the present study aimed to investigate its effects on lungs and blood.
Material and Methods:
Thirty mature mice were randomly divided into three groups of treatment 1, treatment 2 and sham. Treatment group 1 and 2 received 0.1 ml toluene 1700 mg/kg bw, and 1000 mg/kg bw, respectively for 25 days. After weighting, lung tissue and blood parameters were studied macroscopically and microscopically using Mutic software.
Result:
The percentage of neutrophils reduced and the number of degenerated lymphocytes and other leukocytes increased in the treatment group 1. The mean thickness of bronchial walls, the overall diameter of terminal bronchioles, respiratory bronchioles, and the thickness of the alveolar septa improved in treatment group 2. Furthermore, pneumocytes type 1increased and pneumocytes type2 decreased. The difference between treatment group 1 and treatment group 2, and the difference between sham group and the treatment groups were significant for all the above-mentioned parameters ($P<0.05$).
Conclusion:
Toluene changed the percentage of white blood cells, increases the thickness and diameter of lung parameters, caused inflammation and constriction of alveoli, and hence weakened respiratory system and reduced its performance. These effects are dose-dependent, so higher concentrations of toluene cause much more damage.

Keywords: Toluene, Hematology, Lung

Introduction

Petroleum and coal are two immense natural sources of organic matter, from which, various aromatic compounds are obtained (1). With the chemical formula C_7H_8, toluene belongs to a group of aromatic hydrocarbons, also known as methyl benzene, phenyl methane, toluene met-acid, and methyl benzoyl (2). It is one of the toxic aromatic compounds in petroleum that harm the environment (3). According to a report by Green Wire in 1995, chemical industries release profuse

* Corresponding author, Address: Dept of Animal Physiology, Faculty of Science, Branch of Sience and Research of Tehran Azad University, Tehran, Iran
Tel:+989127925603 Email: kobra.zare@yahoo.com

amounts of three toxic compounds, namely, toluene, methanol, and ammonia into the environment by (4). Alkyl toluene is a benzene that causes death or unconsciousness in copious amounts (5). It has a melting point of -95 °C and a boiling pint of 110.6 °C. Toluene is inflammable, colorless, transparent, volatile, and aromatic, and in liquid state at room temperature (2). Its solubility is low in water, but high in fats (6).

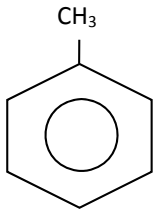


Figure 1: Chemical structure of toluene

As additives, toluene, together with benzene, and xylene make up 15.4% of petrol. It is a naturally occurring chemical in crude oil; it is also obtained from coal in the process of coke production. Toluene has applications in the production of paints, adhesives, acrylic paints, rubber, resin, aviation glue, tanning, shoe polish, hair and skin oils, perfume, antifreeze, antirust, and detergents (7). Toluene-containing compounds such as paints, adhesives, paint thinners, polishes, and petrol easily release toluene into the environment, which mixes with the air people breathe and can enter surface and underground waters, and contaminate the soil (8).

People (especially children) may be exposed to toluene and its complications in various ways, including, sniffing glues (intentionally or inadvertently), drinking contaminated water, breathing chemical vapors from industrial centers and workshops, breathing petrol vapor, and cosmetic products. Moreover, people working with petrol and other petroleum products, or with cosmetics at home such as nail varnishes, paints, thinner, stain-removers …, may be exposed to this substance (9). Of the toluene entering the body, 75% is lost in the first 12 hours; however, due to its poor solubility in water, it cannot be excreted through perspiration, urination, or defecation (10 and 11).

In small amounts, toluene causes signs of fatigue, confusion, and loss of memory and appetite, and its high concentration can cause brain damage in the long-term. Continuous inhalation of toluene can cause brain damage, and vision, hearing, and speech impairment, and it can also cause various syndromes in pregnant mothers by crossing from the placenta to the fetus such as microcephaly and growth retardation (12 and 13).

Toluene-induced changes are examined by explaining the physiology of blood and lung tissues as follows. Blood is a fluid medium containing white cells, red cells and platelets. About 55% of blood is composed of plasma (14 and 15). White blood cells (WBC) are divided into poly-nucleus granulocytes and mono-nucleus agranulocytes according to the shape of nucleus and type of granules in cytoplasm. Leukocytosis refers to the rise in number of peripheral WBC caused by intense physical activity, anoxia, pregnancy, childbirth and stressors (16). Lymphocytosis is an abnormal rise in the number of lymphocytes. Abnormal and large lymphocytes with abnormal nucleus morphology are observed in peripheral blood during viral infections and stressful immune reactions. Lymphocytes are swollen and their nucleus and cytoplasm are destroyed during poisoning. Neutrophils are the most important highly mobile phagocytic polymorphonuclear WBCs (17 and 18). The respiratory system is divided into conductive and respiratory

zones. Terminal bronchioles are fine ducts that lead to large collections of alveoli, and respiratory bronchioles are the starting point of respiratory exchanges. Alveolar ducts and alveoli are both covered by fine squamous alveolar cells. Alveoli are the air sacs where blood and air exchange gases (19). Generally, a septum between two alveoli called inter-alveolar septum separates alveolar space and is in contact with capillaries. Type 1 pneumocytes are thin cells that line alveolar surface, and comprise 97% of cells. Type 2, which are called large alveolar cells or septal cells, comprise the remaining 3% of alveolar surface (20).

Given all the above, and also contact with toluene in a wide range of people including painters, construction workers, and those that work with petroleum products, the effects of this chemical need to be further studied. To this end, the present study was conducted on blood and lung tissue in male Albino NMRI rats.

Materials and Methods

The present experimental study was conducted on 30 male Albino NMRI rats procured from Pasteur Institute in Iran (21). Rats weighed between 25 and 30 grams and were kept in laboratory conditions as follows:

1. Stainless steel cages of 45×30×15 cm dimensions
2. Free access to food and water for a sham and two treatment groups. Food was procured from Pars-Tehran Animal Feed Company, and water from piped municipal drinking water.
3. Cages were cleaned and disinfected once a week using washing-up liquid and ethylic alcohol 70%. Feed remains were collected daily and replaced with fresh feed.
4. Appropriate room temperature (22±2 °C) was maintained using an electric heater and an evaporative cooler. Temperature was monitored using a mercury thermometer.
5. Alternate 12 hourly light-dark periods. Sources of light included room light and a fluorescent lamp, which was switched on from 7am to 7pm.

Experiment groups:

Prior to the tests, every five rats were kept in one cage under controlled conditions in terms of temperature and light and free access to food and water in an animal room for a week. Animals were randomly divided into the following groups:

Sham:

This group received 0.1 cc of saline by gavage once every day for 25 days.

Treatment 1:

This group received 0.1 cc of toluene plus saline (35% toluene plus 65% saline) of 1700 mg/kg bw by gavage once every day for 25 days.

Treatment 2:

This group received 0.1 cc of toluene plus saline (35% toluene plus 65% saline) of 1000 mg/kg bw once every day for 25 days. These compounds were administered into rats' stomach using an insulin syringe and gauge 20 gavage needle (purchased from Iran Razi-Rad Company) at a specified time. In the course treatment, rats were weighed every day (22).

At the end of the 25th day, and after dissection, blood parameters were assessed using blood samples taken from the right atrium, and WBC count using Neubauer slide (16 and 22). Structural changes in the lung sections and lung tissues were identified according to histological studies conducted after various stages including tissue passage, fixation, dehydration, clearing, embedding in paraffin, blocking, sectioning, fixing sections on slide, staining with hematoxylin and eosin, dehydration and clearing, and labeling them for microscopic examination. Also, using a microscope equipped with a digital camera, images of tissues were prepared, and then target sections were measured in micrometers using Motic software.

Data relating to various sections of the respiratory system were analyzed using One-Way Variance Analysis for sham and

treatment groups (under toluene stress). Intra-group differences and comparison of mean values were determined using Tukey post hoc test. The results obtained were presented as Mean ± SEM. Significance level was considered P<0.05. Charts were plotted using Excel, dimensions of cells were measured using Motic software.

Results

Mean WBC count in male rats was 3800 and 5600 per ml of blood in treatment groups 1 and 2, and 4300 in the sham group, with a significant reduction in treatment group 1 of 11.6%, and a significant increase in treatment group 2 of 30% compared to the sham. Variance analysis revealed a significant increase in treatment group 2 compared to treatment group 1 (P<0.05) (Chart 1).

Small lymphocytes comprised 20% of WBC in the sham group, and reached 25% and 32% in treatment groups 1 and 2, respectively. Medium-sized lymphocytes were 30% in sham, reaching 17% and 23% in treatment groups 1 and 2, respectively. Large lymphocytes increased from 20% to 25% in group 1, and 22% in group 2. Mean degenerated lymphocytes were 7% in the sham group and 20% and 12% in treatment groups 1 and 2, respectively. Mean neutrophil was 23% in the sham and 8% and 12% in treatment groups 1 and 2, respectively. Treatment group 1 had a significant relationship with group 2 and sham. There was a significant relationship between two treatment groups (P<0.0) (Chart 2).

Changes in different lymphocytes and neutrophils and their degeneration in treatment groups 1 and 2 compared to the sham group can be observed in Figures 2.

Microscopic examination showed that bronchial wall thickness increased 7.3% in group 1 and 2.7% in group 2. The internal diameter of terminal bronchiole increased by 30.7% in group 1 and by 14.1% in group 2. The diameter of respiratory bronchial also increased to 35.4% in group 1 and 11.5% in group 2. Thickness of the septa significantly increased in groups 1 and 2 compared to the sham, but showed a significant reduction in group 2 compared to group 1. Treatment groups 1 and 2 had a significant increase in the four lung tissue parameters, compared to the sham, but treatment group 2 showed a significant decrease compared to treatment group 1 (P<0.05) (Chart 3).

Mean reduction in the number of pneumocyte type 1 was 50% in group 1 and 16.62% in group 2. Examination of lung tissue sections showed a significant decrease in mean number of pneumocyte type 1 in alveoli in groups 1 and 2 compared to the sham, and a significant increase in group 2 compared to group 1 (P<0.05).

Unlike type 1, mean type 2 cells increased by 1200% in group 1 and by 275% in group 2. Mean number of pneumocyte type 2 in one alveolus significantly increased in groups 1 and 2 compared to the sham, but significantly decreased in group 2 compared to group 1 (P<0.05) (Chart 4).

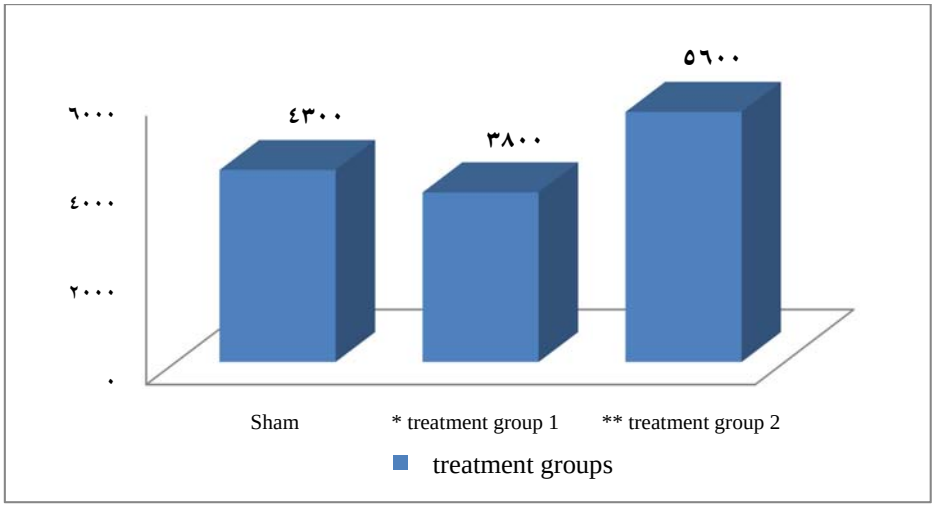


Chart 1: Mean WBC count in sham and treatment groups 1 and 2 (P<0.05)

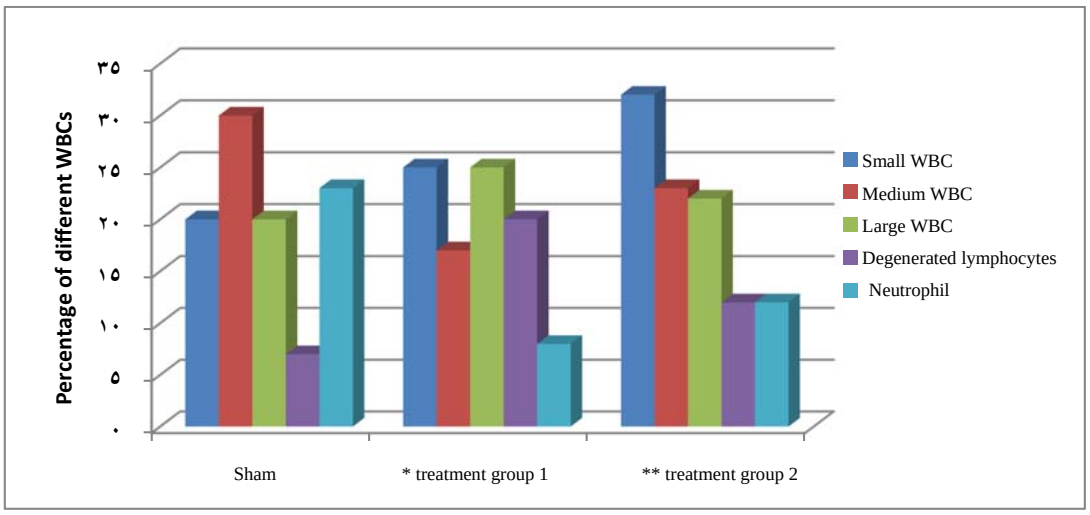


Chart 2: Percentage of different WBCs in the sham and treatment groups 1 and 2
*Indicates the significant relationship of group 1 with sham and group 2 (P<0.05)
** Indicates a significant relationship between treatment groups 1 and 2 (P<0.05)

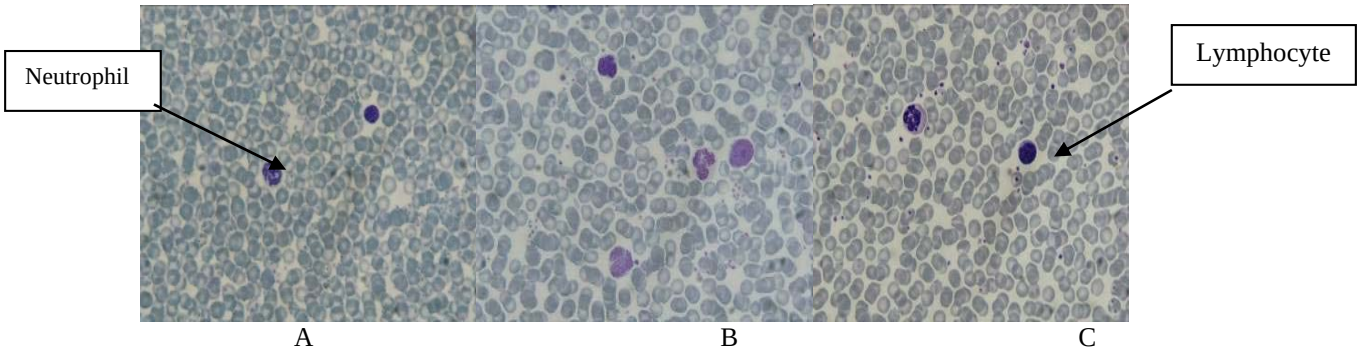


Figure 2: Lymphocytes and neutrophils in A) sham group; B) treatment group 1; C) treatment group 2 (×1000)

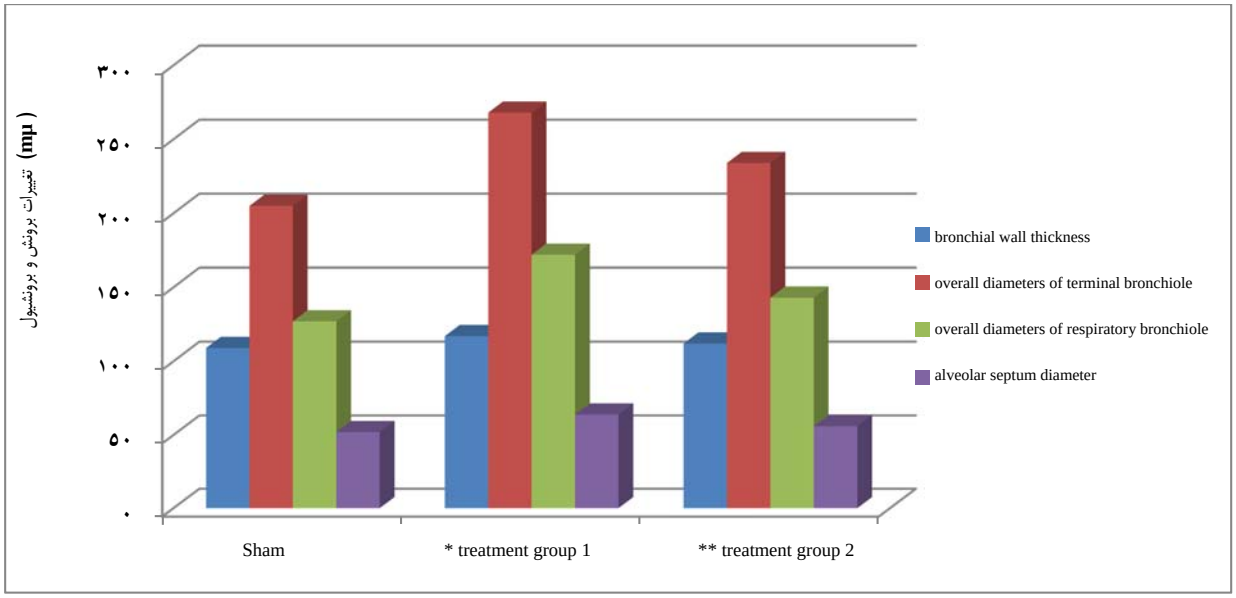


Chart 3: Mean bronchial wall thickness, overall diameters of terminal bronchiole and respiratory bronchiole, and alveolar septum diameter in the sham and treatment groups 1 and 2
*Indicates a significant difference between groups 1 and 2 compared to sham
** Indicates a significant reduction (P<0.05) in group 2 compared to group 1

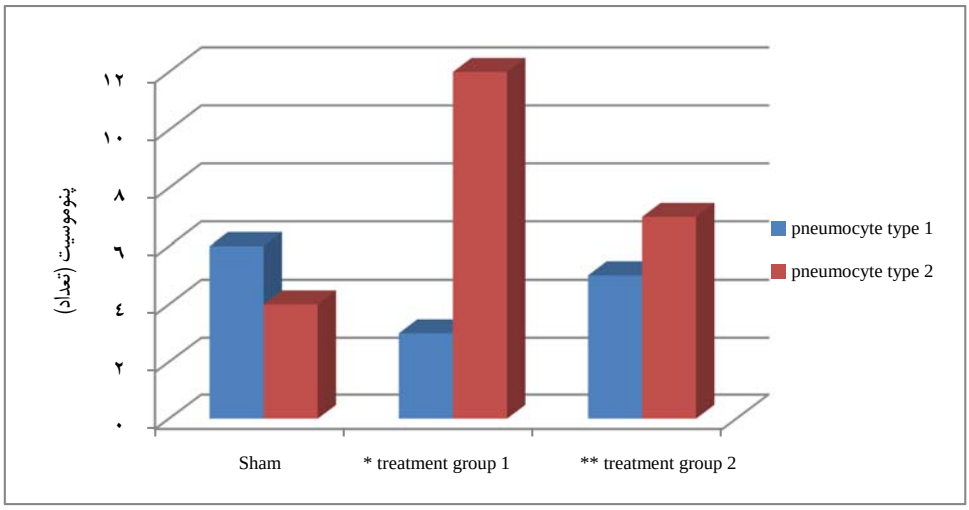
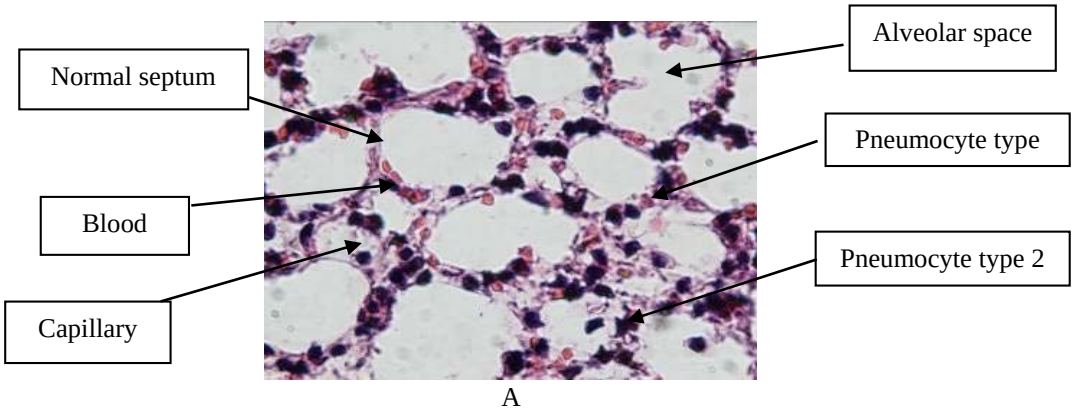


Chart 4: Mean number of pneumocyte type 1 and 2 in one alveolus in the sham and treatment groups 1 and 2
*Indicates a significant difference between sham group and treatment groups 1 and 2 (P<0.05)
** Indicates a significant difference between treatment groups 1 and 2 (P<0.05)
NB: Changes in pneumocyte type 1 and 2 were opposite



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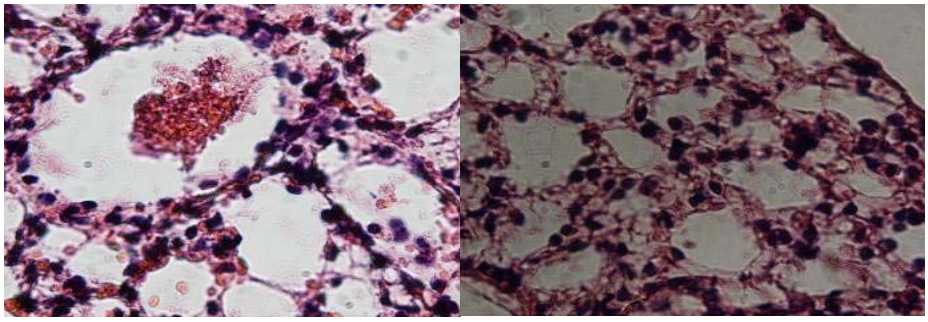

C B

Figure 2: Changes in the structure of alveoli in rats' lung
A) Sham group B) Treatment group 1 C) Treatment group 2 (×1000)

Table 1: Mean changes in lung tissue parameters (Mean ± SEM) in the sham and treatment groups 1 and 2

Sample/Parameter	Sham group Mean ± SEM	Treatment group 1 (1700mg/kg of Toluene) Mean ± SEM	Treatment group 2 (1000mg/kg of Toluene) Mean ± SEM	Count
Bronchial wall thickness (µm)	109.7±2.21	117.9±2.60*	112±2.60**	45
Overall terminal bronchiole diameter (µm)	205.5±3.027	268.5±3.027*	234.5±3.027**	50
Overall respiratory bronchiole diameter (µm)	127.5±3.027	172.4±2.31*	143.1±2.51**	40
Alveolar septum thickness (µm)	52.43±0.17	64.62±0.28*	56.57±0.27**	45
Pneumocyte 1 cell count	6.6±0.23	3.6±0.25*	5.4±0.18**	35
Pneumocyte 2 cell count	4.6±0.054	12.4±0.18*	7.6±0.16**	40

*Indicates a significant difference of sham and treatment 1 groups with treatment 2 group (P<0.05)
** Indicates a significant difference of treatment groups 1 and 2 with the sham group

Discussion

Toluene is a substance that is easily absorbed through the digestive and respiratory systems, and to a lesser extent through skin, and after metabolism in the liver; it enters adipose tissue, nervous system, cerebrospinal fluid, and blood. Absorbed toluene in human is excreted through urine in different forms such as hippuric acid and glucuronide. Symptoms of exposure to toluene include eye irritation, rhinorrhea, and poor neuromuscular reflexes. Olfactory deterioration has also been observed (23). Previous studies have shown that 100 ppm of toluene is harmless, 150 ppm is acceptable for 8 hours, and 200 ppm is harmful to health and life. Small amounts of toluene cause symptoms such as fatigue, confusion, lethargy, thirst, and loss of memory and appetite. However, these symptoms are temporary and diminish with distancing from the source of toluene (24). Since toluene easily passes through the placenta, exposure of pregnant women to toluene causes syndromes such as microcephaly, preterm birth, growth retardation, and head and face malformations in the fetus. Inhalation of toluene causes fetal toxicity and delayed bone development (25 and 26). Toluene intake leads to the highest concentration of toluene in the liver, followed by the brain, heart, blood, fat, and to a lesser extent, cerebrospinal fluid. Exposure of rats to 1500 ppm of toluene induces impairment in the kidney and liver functions. The relative size of the liver in male rats significantly increases by the intake of 625 mg/kg of toluene. A significant increase in the weight of the liver in female rats can also be observed by consumption of 1250 mg/kg of toluene. Swallowing even a small amount of toluene (as much as 60 mg) will cause death after 30 minutes due to liver inflammation, hyperemia, hemorrhage, and tubular necrosis (27 and 28). Contact with toluene in animals causes symptoms such

as irritation of the eye, nervous system and skin, imbalance, tremor etc. Short-term contact with 1250 mg/kg of toluene in rats reduces coordination of the nervous system. Exposure of cats to 7800 mg/kg of toluene for 80 minutes causes tremor and eventually lethargy, fatigue and sleepiness (29). In a study, rats were fed 312, 625, 1250, 2500, and 5000 mg/kg of toluene plus corn oil five days a week for 13 weeks. All rats that received 5000 mg/kg died in the beginning, and 40% of those that received 2500 mg/kg died later, and the rest suffered serious harms such as body tumors and uncoordinated movements (30). In another study, exposure of rats to 1200 ppm of toluene for 12 hours per week for 5 weeks led to irreversible hearing impairment. These impairments were deeper in older rats compared to younger ones. In a similar study, daily consumption of 620 mg/kg of toluene by rats led to hearing impairment in them (31).

In humans, contact with toluene causes depression of the central nervous system, irritation of the mucosal membranes, euphoria, and hallucination, and toluene contact with the eyes causes unstable corneal damage. Relatively chronic toluene contact with the skin causes skin inflammation (12). Different amounts of toluene have different effects on humans. For instance, exposure to 200 mg/kg of toluene causes irritation of the eyes, tears and euphoria, and exposure to 600 mg/kg of toluene causes nausea, fatigue, lethargy, sleepiness, and loss of coordination (32).

In the present study, significant increases or decreases in the lung tissue parameters in treatment groups 1 and 2 compared to the sham group indicate harmful effects of toluene. Ott et al. studied toluene-induced occupational asthma, and found that at concentrations in excess of 20 ppm, toluene causes pulmonary function decrement of 1% to 5% and increases thickness of different bronchial and alveolar parts (33). In the present study, bronchial wall thickened due to the increase in available air in bronchial ducts. This increase can be indicative of reduced septum elasticity and stiffness, which results in problematic changes in the diameter of airways in the lung during inhalation and exhalation. The end of bronchi reaches terminal bronchiole, pulmonary, and alveolar ducts, where negligible gas exchange occurs, and thus, regulation of air inhalation and exhalation in alveoli is important in these bronchioles. In the present study, the diameter of pulmonary bronchioles in treatment groups significantly increased due to narrowing of alveoli. Considering that air is trapped in these ducts, negligible gas exchange can be expected due to wall thickness and narrowing of alveoli, which results in little air exchange between pulmonary bronchioles and alveoli.

Increased inflammation and hyperemia of capillaries surrounding alveoli thicken the septum and fill inner alveolar space with discharge, so that thickness of the septa irregularly increases in certain alveoli, causing reduced inner alveolar space. Studies conducted in Tulane university on toluene diisocyanate production workers showed poor pulmonary function, inflammatory and irreversible effects, and severe allergic responses in these workers (34 and 35).

In the present study, congestion of the alveolar parenchyma appears to have weakened natural consistency of pulmonary parenchyma surrounding alveoli. Respiratory depression and reduced alveolar activity are signs of alveolar stenosis and increased surface tension, making it difficult for them take in air from respiratory bronchioles. Reduced internal area of alveoli, increased alveolar thickness and cell count also reduce internal alveolar space. In treatment groups 1 and 2, pneumocyte type 1 dramatically reduced, which may be due to possible reduction in gas exchange between septa, internal alveolar space and surrounding capillaries. An increase in these cells in alveolar wall causes stenosis and restriction of gas exchange from their cell wall.

Charts 1 and 2 show increased lymphocyte and degenerated cell count in WBCs, which agrees with the results obtained in other studies. Assessment of mean percentage of WBC in blood including small, medium, and large lymphocytes, degenerated neutrophils and various other blood cells can show the effect of toluene stress on blood parameters. Toluene appears to inhibit hematopoietic pathway in hematopoiesis centers for production of WBC, and reduction in medium lymphocytes and neutrophils represent cellular and phagocytic weakness in the defense system, which can be seen in most poisoning cases (36). Unusual cells were observed in the peripheral blood smears in treated rats. These cells are progenitor or precursor type cells that did not have enough time for maturity; they are immature and part of lymphoblasts, prolymphocytes, monoblasts, and promonocytes. Some immature neutrophils are seen as band cells or non-segmented, which appear as a variety of cells in relevant histograms (37 and 38).

Lung epithelial cells respond to a specific concentration of toluene (25% ppmv). Primary toxic effects are associated with glutathione concentration, so that cell DNA is damaged after one-hour exposure of lung tissue at this concentration, which is accompanied by reduction in glutathione concentration (39).

Previous studies have mentioned that inhaled toluene is easily absorbed through the lung, and 40% to 80% of pulmonary alveoli are preserved. This reduces to 10% in presence of alcohol, since solubility of toluene is highly exacerbated with alcohol (40). Moreover, absorption rate increases with increased physical activity, and thus there is a relationship between serum toluene level and physical activity (41). After absorption in lungs, toluene metabolizes into hippuric acid and cresol (only a small percentage of toluene), which is not usually found in urine. Therefore, cresol presence in urine confirms exposure to toluene (42).

Conclusion

Given damaging and stressful effects of toluene, and absence of studies on its effects on parameters of various tissues such as lung and blood, further studies in this area appear necessary.

The increasing and decreasing effects of toluene on the above parameters found in male Albino NMRI rats in the present study can be extended to humans through further experiments. Considering harmful effects of toluene compounds and its industrial applications, prolonged contact with this substance leaves irreversible damage to human health. The following measures are therefore recommended:

Use of toluene in conjunction with other compounds to reduce its harmful effects.

Development of further toxicology studies.

Identifying occupational or inhalation disorders, and the effect on various tissues and organs

Observing safety when working with this substance

Raising public awareness about harmful effects of this substance and providing strategies to reduce its complications

Conducting further studies on other organs and their changes

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The authors declare no conflict of interest in conducting the present study.

Conflict of interest

The Authors declare that there is no conflict of interest in this paper.

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