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Original Research

Sublethal Dose of Diazinon Induces Pulmonary Toxicity in Rat: Histopathological Evidence

Gholamreza Najafi[a], Ali Asghar Tehrani[b], Ali Shalizar Jalali[a], Mohammad Babaei[a], Ali Najafi[a]

Departments of Basic Sciences[a] and Pathobiology[b], Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

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Corresponding Author:
Ali Shalizar Jalali
Department of Basic Sciences,
Faculty of Veterinary Medicine,
Urmia University, Urmia, Iran
a.shalizar@urmia.ac.ir,
ali_shalizar@yahoo.com

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Abstract

Objective: Diazinon (DZ) is a widely used contact organophosphorous pesticide with broad spectrum insecticide activity. The extensive use of DZ has caused great concern due to the hazardous side effects on human beings as well as wild and domestic animals. The aim of this study was to investigate the effect of sublethal dose of DZ on rat lung.

Material and methods: Seven groups of male Wistar rats were used comprising control and test groups. The control group received corn oil (0.3 ml/day) for 60 days by oral gavages. The test groups received DZ at a dose of 60 mg/kg body weight orally for 2, 10, 24, 30, 54 and 60 days, respectively.

Results: The histopathological analysis of the lungs in DZ-treated groups revealed congestion on day 2, pulmonary edema and emphysema on day 10, congestion and atelectasia on day 24, infiltration of mononuclear cells on day 30 and pulmonary hemorrhage along with bronchial glands hyperplasia on days 54 and 60. DZ administration also caused a significant decrease in serum cholinesterase activity in a time-dependent manner.

Conclusion: These findings indicate that sublethal dose of DZ can induce severe lesions in the lung of rat.

INTRODUCTION

Diazinon (DZ) is a contact organophosphorus pesticide with broad spectrum insecticide activity [1]. It has been widely used throughout the world to control flies, lice and other insect pests of ornamental plants and food crops [2] as well as a veterinary ectoparasiticide [3]. The extensive use of this compound has caused great concern due to the hazardous side effects on human beings as well as wild and domestic animals [4]. Toxic effects of DZ are associated with the inhibition of acetylcholinesterase (AChE) activity, an enzyme which hydrolyzes the neurotransmitter acetylcholine (ACh) in cholinergic synapses and neuromuscular junctions [5]. The mechanism of DZ-induced toxicity in non-target organisms is similar to the mode of action for target organisms [6]. Non-target organisms can be exposed to DZ by inhalation, ingestion and/or dermal exposure [7].

It has been reported that DZ causes hematological [8], pancreatic [9] and hepatic damages [10] as well as reproductive toxicity in humans and experimental animals [11, 12]. Also, DZ has been found to induce DNA strand break leading to a type of genetic damage called micronuclei [13]. Based on this concept, the present study was undertaken to evaluate the histopathological effects of exposure to sublethal dose of DZ on rat lung tissue.

MATERIALS AND METHODS

Animal Model

49 adult sexually mature male (4 months of age weighing 200.75 ±14.68 g) albino rats of Wistar strain were obtained from the animal house of faculty of Veterinary Medicine, Urmia University. The animals were housed in a specific pathogen-free environment, given water *ad libitum* and fed standard pellet diet. Rats were exposed to a 12h light: 12h dark cycle at a room temperature of 25 ± 2°C. Animal work was conducted in compliance with guidelines for the humane care and use of laboratory animals using protocols approved by the university.

Experimental Protocol

After 7 days of acclimation to the environment, the rats were randomly segregated into seven groups, each group having seven animals. Group I served as control receiving corn oil (0.3 ml/day) for 60 days by oral gavages. Group II-VII (experimental groups) received DZ at a dose of 60 mg/kg body weight orally for 2, 10, 24, 30, 54 and 60 days, respectively.

Determination of LD50

DZ was applied as a commercial emulsifiable concentrate formulation containing 60% active ingredient. It was diluted in deionized water for the final concentration. 25 male albino rats of Wistar strain (divided into five groups) were used to determine the acute oral LD50 of DZ. The animals were observed for five days and the number of dead animals was recorded. LD50 was later calculated according to the equation of Behren and Karber [14]. It was found oral LD50 in rats ranges from 300 to 850 milligrams per kilogram (mg/kg).

Sampling

At the appropriate times, animals were euthanized by CO_2 exposure in a special device following anesthesia with ketamine (5%, 40 mg/kg, intraperitoneally) and xylazine (2%, 5 mg/kg, intraperitoneally). The chest was rapidly opened and the two lungs of each animal, in the experimental and control groups were carefully dissected out.

Tissue Preparation

For light microscopy, lungs were instilled with 8 ml 10% neutral buffered formalin to achieve full expansion of the different lobes and were fixed in formalin for at least 24 hours. Slices from all lung lobes were then dehydrated, cleared in xylene and embedded in paraffin wax. Thin sections (6 μm in thickness) were cut and stained with hematoxyline and eosin for histopathological analysis.

Measurement of Serum Cholinesterase (ChE) Activity

Blood samples were collected from the vena cava inferior of each incised animal were put immediately into ice-chilled siliconized disposable glass tubes. Serum samples were prepared by the centrifugation of the blood samples at 3000 rpm for 15 min, at 4 $^{\circ}$C. ChE activities in the serum samples were measured according to the method of Ellman and co-workers [15].

Data analysis

The results are expressed as the mean ± standard deviation (S.D.). Statistical analyses were based on comparing the data between DZ-treated groups with control group. Differences between groups were assessed by the analysis of variance (ANOVA) using the SPSS software package for Windows. Statistical significance between groups was determined by Tukey multiple comparison post hoc test and the p-values less than 0.05 were considered to be statistically significant.

RESULTS

Gross pathology

The first evidence of DZ toxicity during the exposure became evident on day 2. The normal pink coloration of the lung was replaced by a diffuse mottling with dark beefy red and purple patches due to the intense congestion. Gradually small white patches of peripheral emphysema were found (day 10). Eventually the lungs were greatly distended with edema and occupied the entire thoracic cavity (days 54 and 60).

Microscopic findings

Light microscopic examination of haematoxyline and eosin-stained lung sections from control animals revealed spongy structure of the lung with thin alveolar walls and clear alveolar cavities, normal bronchi containing cartilagenous plates in their walls, lined with respiratory epithelium and bronchioles lined with simple columnar epithelium (Figure 1A). The capillaries of alveolar walls were engorged with red blood cells on day 2 (Figure 1B). Considerable emphysema found in the alveolar ducts and marked in rats showing the greatest amount of pulmonary edema on day 10 (Figure 1C). Lung of rats on day 24 showed congested blood vessels and atelectasia (Figure 1D). The bronchioles were surrounded with clear zone of cellular infiltration on day 30. These cells composed of lymphocytes and occasionally polymorphoneuclear cells (Figure 1E). There was infiltration of mononuclear cells in the lung of rats on days 54 and 60 (Figure 1F). Pulmonary hemorrhages (Figure 1G) along with bronchial glands hyperplasia were also noted in the lungs of the rats after 54 and 60 days (Figure 1H).

Serum ChE Activity

As seen in Figure 2, treatment of rats with DZ resulted in time-dependent significant inhibition of serum ChE activity. Data exist in Figure 2 revealed that mean serum ChE activity of DZ-treated animals was significantly lower than that control.

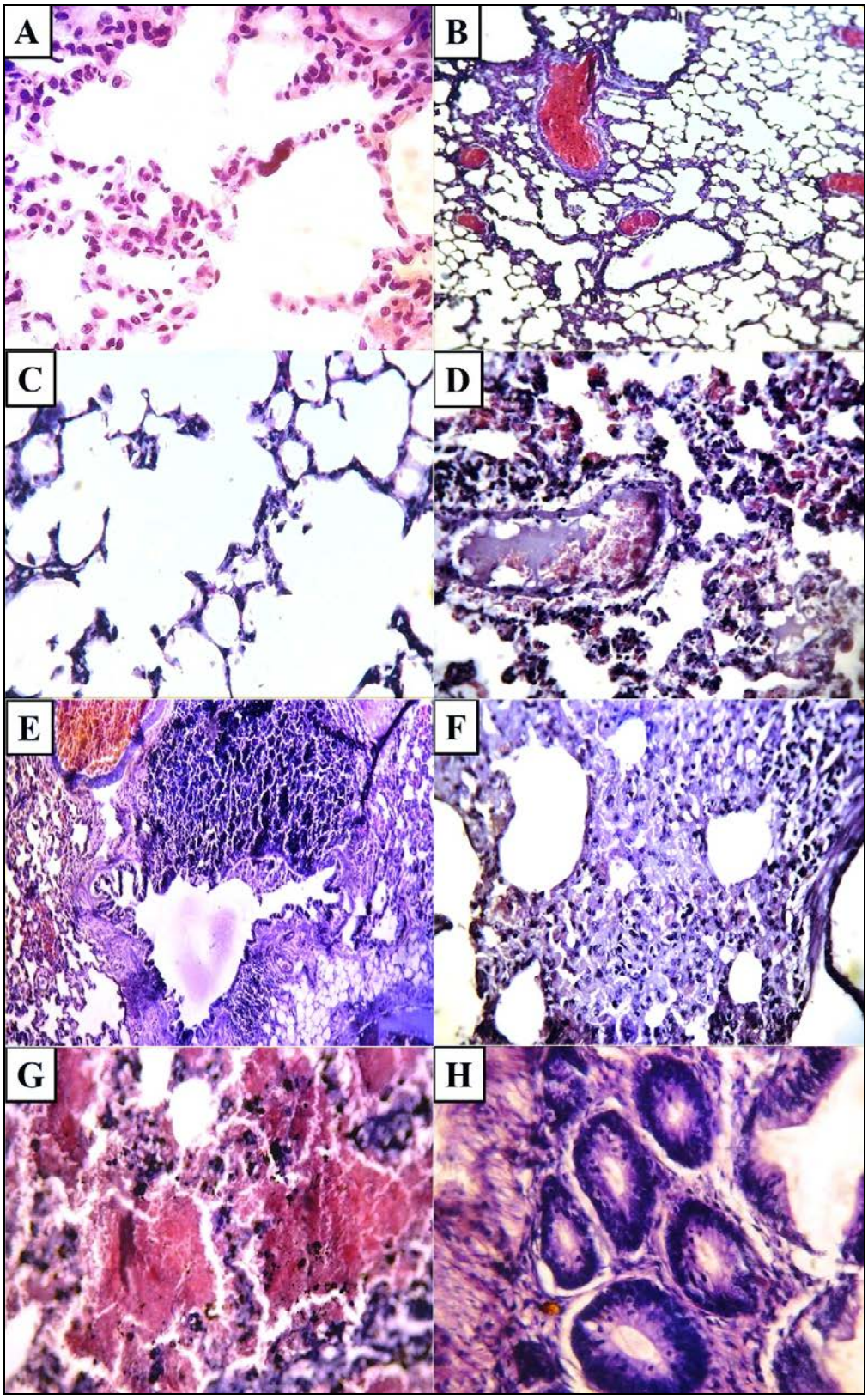

Figure 1

A: Photomicrograph of a lung section from a rat in control group showing normal alveoli.

B: Photomicrograph of a lung section from a rat treated with diazinon for 2 days showing congested blood vessels and edema.

C: Photomicrograph of a lung section from a rat treated with diazinon for 10 days showing emphysema.

D: Photomicrograph of a lung section from a rat treated with diazinon for 24 days showing congested blood vessel and atelectasia.

E: Photomicrograph of a lung section from a rat treated with diazinon for 30 days showing congested blood vessel and infiltration of lymphocytes and neutrophil.

F: Photomicrograph of a lung section from a rat treated with diazinon for 54 days showing infiltration of lymphocytes.

G: Photomicrograph of a lung section from a rat treated with diazinon for 54 days showing hemorrhages and edema in the alveoli.

H: Photomicrograph of a lung section from a rat treated with diazinon for 60 days showing bronchial glands hyperplasia

(H&E, x100 for panel B, and x400 for other all panels).

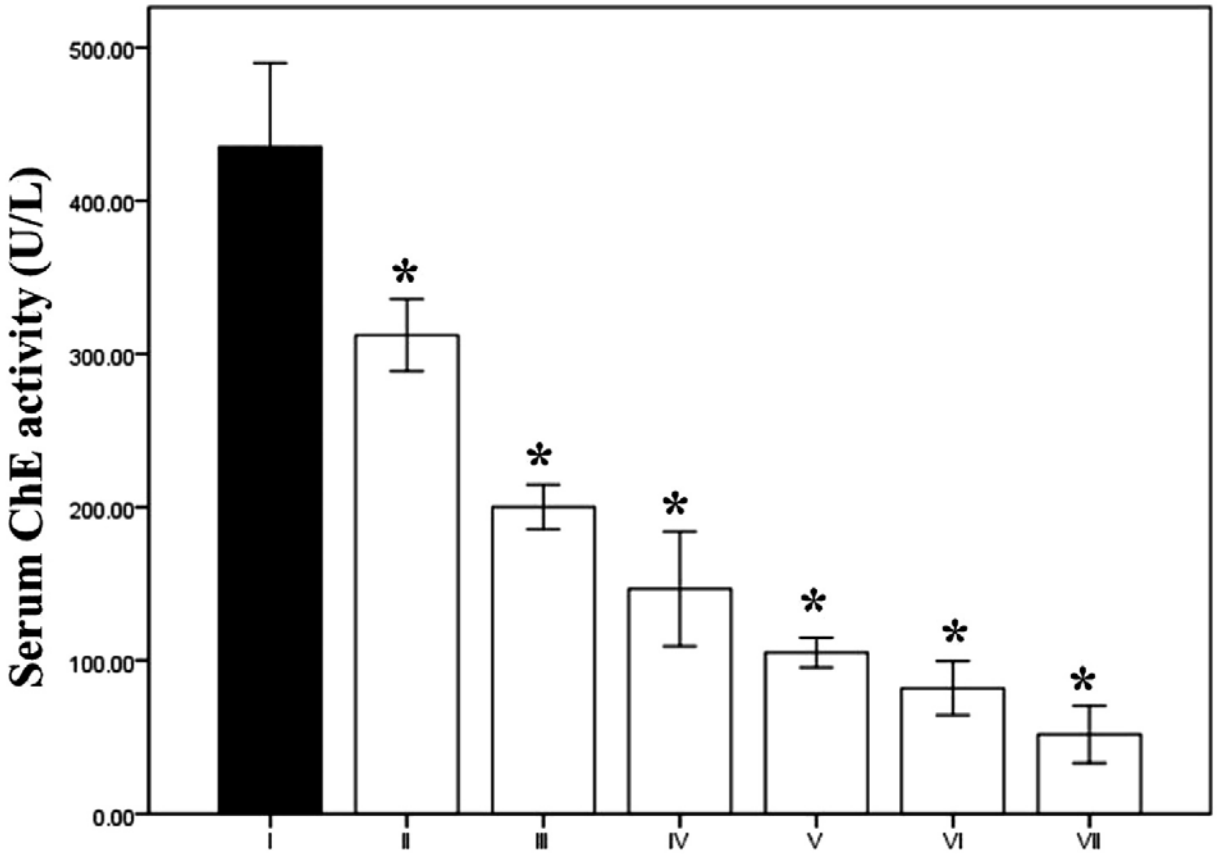

Figure 2: Serum cholinesterase activity in all experimental groups. The values are expressed as mean ± S.D. (n = 7). *Significant differences as compared with the control group at P <0.05.

DISCUSSION

Broadly indiscriminate application of pesticides is resulted in environmental pollution and therefore, is a cause of concern. Organophosphorus (OP) compounds, major component of several widely used pesticides, are efficiently absorbed by inhalation, ingestion and skin penetration and this exposure can lead to serious toxicities [16]. Several factors including dose, route of exposure and percent of absorption, physicochemical property and rate of detoxification play essential roles in severity of toxicity. Mild toxicities are seen after accidental exposure by the dermal and pulmonary routes. DZ-induced toxicities are also occurred by the multiple routes such as oral, dermal and respiratory ways. DZ is readily absorbed from the gastrointestinal tract and is rapidly metabolized within a few hours [17].

It has been reported that the acute oral LD_{50} values of DZ for rats and mice are 1160-1340 mg/kg and 80–135 mg/kg, respectively [18, 19]. Present study showed that acute oral LD_{50} value of DZ in rats ranges from 300 to 850 mg/kg body weight. Various grades of the material purity and different vehicles of administration may be involved in formation of this wide range [20].

It has been revealed that chronic DZ exposure (300 mg/kg for 45 days) causes organ pathologies, including necrotic degeneration of the spleen and thymus trabeculae, hyperplasia of thymus and lymph nodes cortex and medulla and hyperplasia of spleen white and red pulp in mice [21]. The present results showed that oral sublethal dose of DZ induces time-dependent histopathological lesions in the lung of rats. In our study, the observed histopathological alterations in the lung tissue included, edema, congestion, hemorrhage and infiltration of mononuclear cells showed similarity with those recorded in the several previous investigations [22-24]. It has been shown that these histopathological changes could be associated with the chemical-induced decreases in the antioxidant status of the animal body [25]. The congestion of the lung vasculature might impose excessive pressure on the neighboring structures leading to malnutrition, deficient oxygenation and the accumulation of the excretory products [24]. Moreover, it has been indicated that OP

compounds could induce airway hyperactivity via reduction of neuronal receptors function [26].

In conclusion, the findings of our study have shown that oral sublethal dose of DZ causes time-dependent histopathological lesions in the lung of rats.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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