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# Effect of the lethal dose of *Naja naja* venom snake on the lung tissue of male albino rats

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## Abstract

Snake venoms are known to cause different metabolic disorders, altering cellular and enzymatic activities in animals and releasing pharmacological substances. The objective of this study is to explore the effect of the lethal dose of Indian cobra (*Naja naja*) venom snake on the lung tissue of albino rats. The rats were divided into three groups, the first group served as a control group, while the second and third group were treated with the snake venom injected intraperitoneal (i.p) as LD50 (o.o5µg/g body weight) and sacrificed after 3 and 24hrs of snake venom injection respectively, tissue sections of the lab. Animal's lung were prepared and examined Microscopically. Histopathological changes was seen after 3hr from injection as thickening of alveolar septa with focal dilatation of alveoli producing emphysema. While those changes after 24hrs from envenoming were showed same changes with more prominent congestion and more abundant thickening of alveolar septa with focal dilatation of alveoli producing emphysema. This study proved of LD50 of cobra *(Naja naja*) venom had the same effect on lung tissue after 3hrs and 24hrs.

Keywords: *Naja naja*, Snake venom, Lung structure, Tissue changes.

## Introduction

The study of structure and function of snake venom toxins is carrying out with medical application purposes. Snake venom is a complex mixture of many substances, such as toxins, enzymes, growth factors, activators and inhibitors with a wide spectrum of biological activities (Rahmy and Hemmaid, 2000; Al-Sadoon *et al*., 2013; Cherifi and Laraba-Djebari, 2013). They are also known to cause different metabolic disorders by altering the cellular inclusions and enzymatic activities of different organs (Aiesenberg,1981). Cobra snakes are widely distributed in Africa and the Middle East. Envenomation causes local pain and swelling hemorrhage, coagulation disturbances, edema and myotoxicity. These enzymes are peptidases (Harris, 1991) Metallo-peptidases and non-enzymatic proteins/peptides like cardiotoxins, that caused cardiac muscles arrest (Henrikson *et al*., 1977), and small amounts of organic and inorganic molecules (Gold *et al*., 2002) Neurotoxic and systemic symptoms develop within few hours, and deaths have occurred within 6–16hrs after large snakes' bites, despite the use of antivenom and mechanical ventilation. Cobra envenoming is known to induce multiple-organ failure, leading to death in case of severe envenoming (Gold *et al*., 2005). Victims of snake bite quickly succumb to severe respiratory failure, which can be fatal if left untreated. One of the most toxic components of snake venom is phospholipase A2. There are reports showing the effects of various snake venom on liver tissues in rat that the venom causes damage of the hepatocytes (Chu *et al*., 2002; Agrawal *et al*., 2001; Morad, 20014).

## Materials and Methods

Venom: Lyophilized crude venom of snake *Naja naja* venom was obtained from India (Sigma loeate Ltd). The crude venom was dissolved in phosphate buffered saline (PBS), pH 7.2. The determination of the median lethal dose LD50 of the snake *Naja naja* venom by intraperitoneal (i.p). The injected dose was 0.05 µg/g body weight which was calculated according to the method of (Meier and Stocker, 1991; Morad, 2014).

Animal and experiment design: Healthy adult albino rats of same age group (80±5 days) and weight (190±10g) were taken from the High Institute for Infertility Diagnosis and Assisted Reproduction Technologies, Al-Nahrain University and the animals were housed in standard condition and fed with normal diet and water *Ad libitum*. Animals were divided into three groups of 3 animals each. The first group, control, animals were injected i.p. with 0.1ml

in phosphate buffered saline and sacrificed 24hrs after injection. Groups two and three were injected i.p. with LD50 (0.05µg/g body weight) of cobra venom and sacrificed at 3 and 24hrs after envenomation respectively. All animals were sacrificed, then lung was isolated and cut to small pieces from each experimental rats then transferred immediately to 10% formalin for 24hsr and dehydrated in ascending grades of ethanol (50-100%). Clearing was done in xylene and embedded in paraffin wax. Sections (4-5µm thick) were prepared and then stained with hematoxylin and eosin (H & E) to be examined with light microscope.

## Results and Discussion

Light microscopic observation revealed that the control lung tissue (group 1) showed normal alveoli and thickness have very thin epithelial walls and are surrounded by capillaries (Figures 1). In the second group showed some histopathological changes were recorded after 3hrs from envenoming showing thickening of alveolar septa with focal dilatation of alveoli producing emphysema, and few cases reveled interstitial pneumonia (Figures 3, and 4) histopathological changes were recorded after 24hrs from envenoming. These changes include more prominent congestion and more abundant thickening of alveolar septa with focal dilatation of alveoli producing emphysema with inflammatory infiltrate and the thickened wall has compressed the alveolar sac (Figures 5, 6 and 7).

Snake venoms are chemically complex mixtures of pharmacologically active proteins Many of which also have enzymatic properties (Meier and. Stocker, 1991). Because venoms serve not only as a source of digestive enzymes but also as a defense mechanism, these proteins can target multiple tissues, causing a simultaneous poisoning of multiple physiological systems. From these venoms, hundreds of toxins have been purified and characterized one of the components that contributes significantly to the lethality of snake venoms

Phospholipase A2 (PLA2) ( Meier and Stocker, 1991), digestive function, venom (PLA2) exhibits antiplatelet, anticoagulant, hemolytic, neurotoxic (presynaptic) myotoxic, hemorrhagic, inflammatory and cardiotoxic activities. In the present study we explored that sub lethal dose of *Naja naj* snake venom induced potent histopathological alternation in the lung of the rats these results were in accordance with (El-Fiky, 1999; Rahmy and El-Fiky, 2001; Morad, 2014). Who reported that sub lethal dose of *Naja haj*e venom induced potent histopathological, histochemical, and patho-physiological alternation in the heart, liver, kidney and brain of rats these pathological changes included severe degree of cellular damage concomitant with marked sings of both myolytic and coagulative necrosis (Rahmy and Hemmaid, 2000; *Nair et al*., 1993) reported the mains cause of death upon cobra envenomation is peripheral respiratory paralysis because of both presynaptic and postsynaptic neurotoxin present in the venom. This study showed that prolonged period of envenomation for 24hrs more sever effect on lung alveoli with thickening of alveolar septa and focal dilatation of alveolar which lead to emphysema which play important role in inducing respiratory failure, these results come in accordance with (Zamuner *et al*., 2004), who investigate pro-inflammation and edema inducing properties of PLA2. Also (Lee,1971; Costa *et al*., 2002; Loghmani *et al*., 2002) recorded the molecular determinants which involved in venom PLA2 induced inflammation and edema especially in the lung and agree with (Vishwanath,1986). Zamuner *et al*. (2004) which conclude that snake bite succumb severe respiratory failure due to phospholipase A2 which lead into alternation in pulmonary gene expression involved in cytokine and chemokines responsible for inflammation.

## Conclusion

In conclusion it could be suggested that *Naja naja* snake venom could lead to sever histopathological effect of lung architecture causing respiratory failure and death of animal.

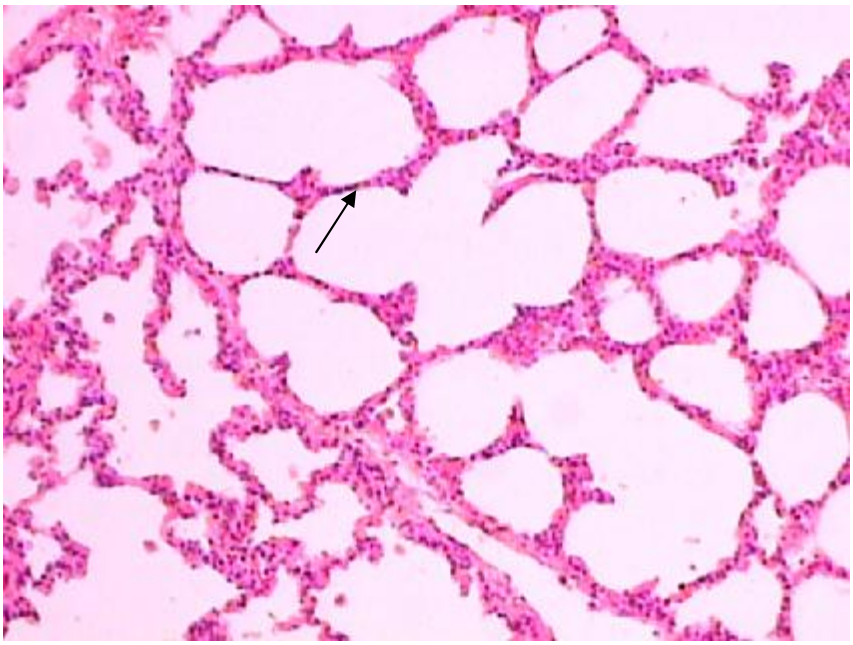

Figure (1): Section from rat lung in control group. This view shows the normal of these alveoli and thickness have very thin epithelial walls and are surrounded by capillaries (⟶) H&E stain X100

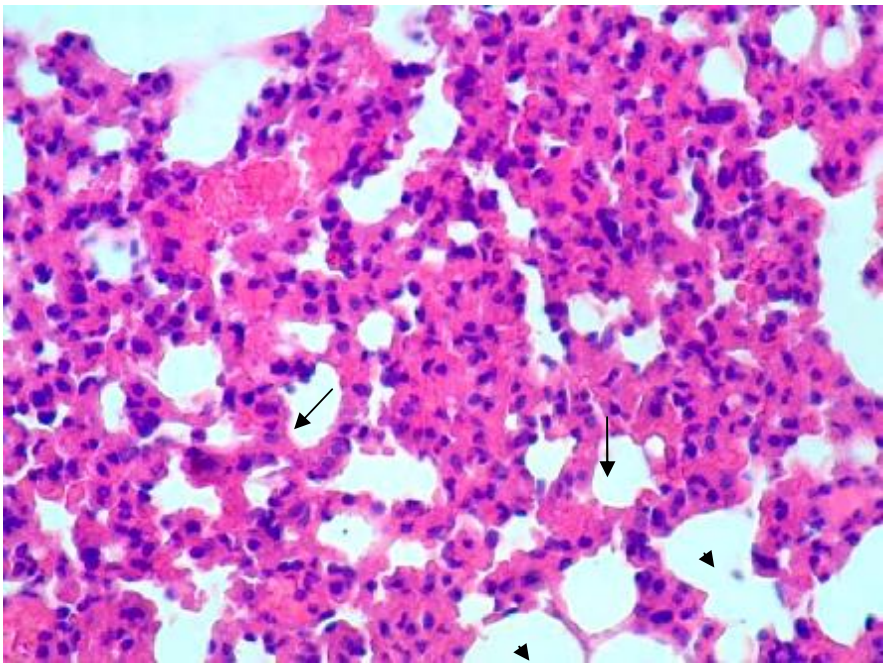

Figure (2): Section from rat lung injection with *Naja naja* snake venom after 3hrs showing thickening of alveolar (↘) with focal dilation of alveolar production emphysema(◂) H&E stain X 400

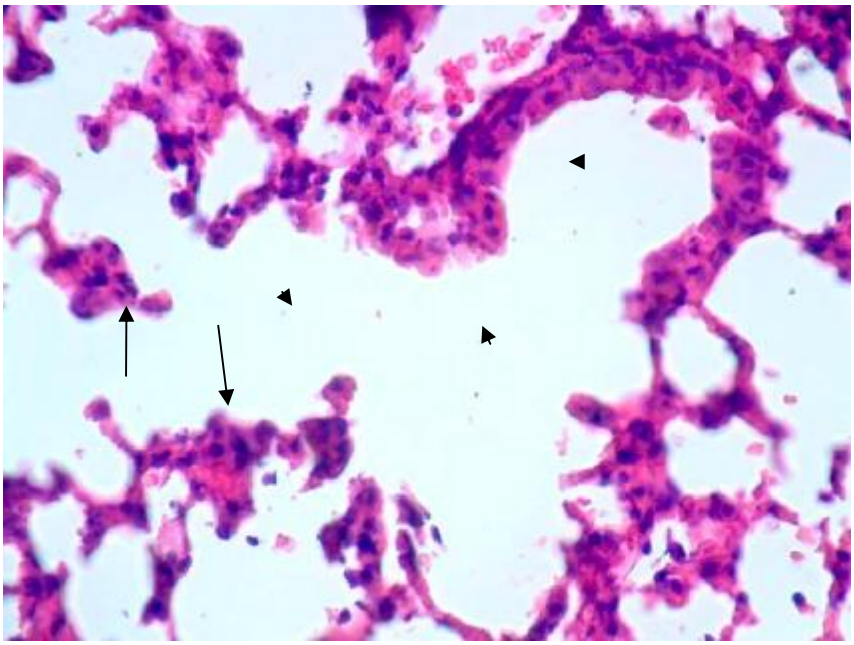

Figure (3): Section from rat lung injection with *Naja naja* snake venom after 3hrs showing thickening of alveolar wall(↘ ) and emphysema (▼) H&E (X400).

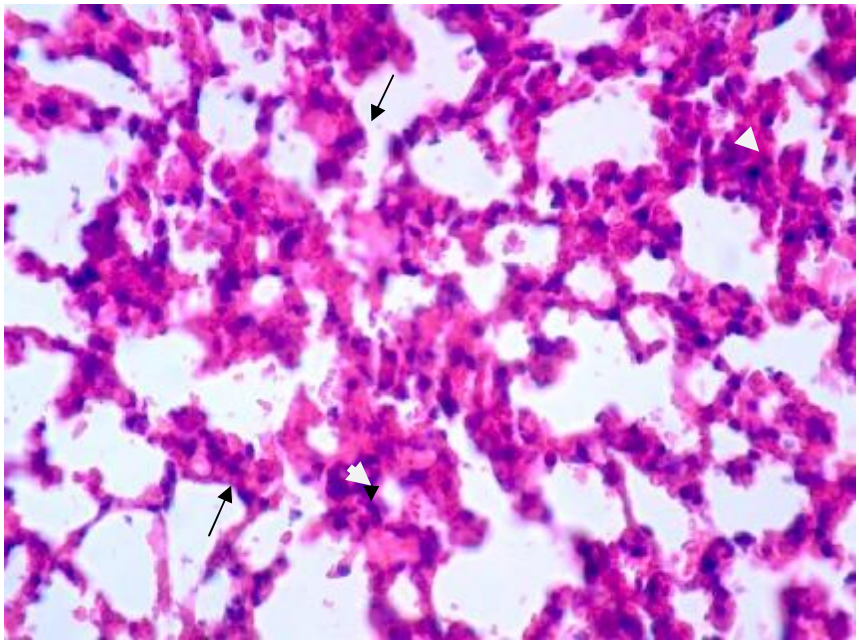

Figure (4): Section from rat lung injection with *Naja naja* snake venom after 3hrs showing sever thickening of alveolar walls(↖) ,and interstitial pneumonia ( ▼) The thickening walls have compressed the alveolar .H&E stain. X250

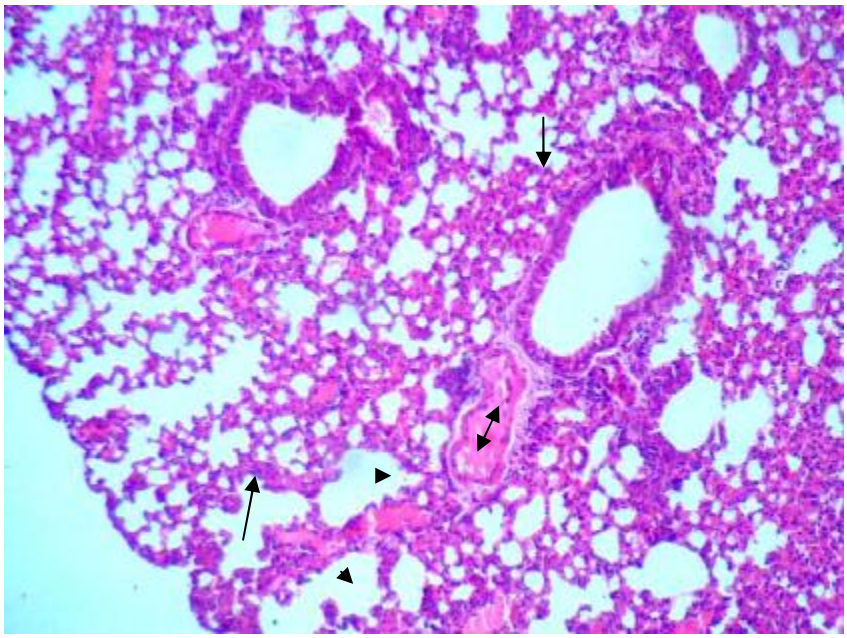

Figure (5) : Section from rat lung injection with *Naja naja* snake venom after 24hrs showing thickening of the alveolar walls ( ↘ ) emphysematous changes ( ▲ ) and also seen congestion( ↕ ) H&H stain (X100).

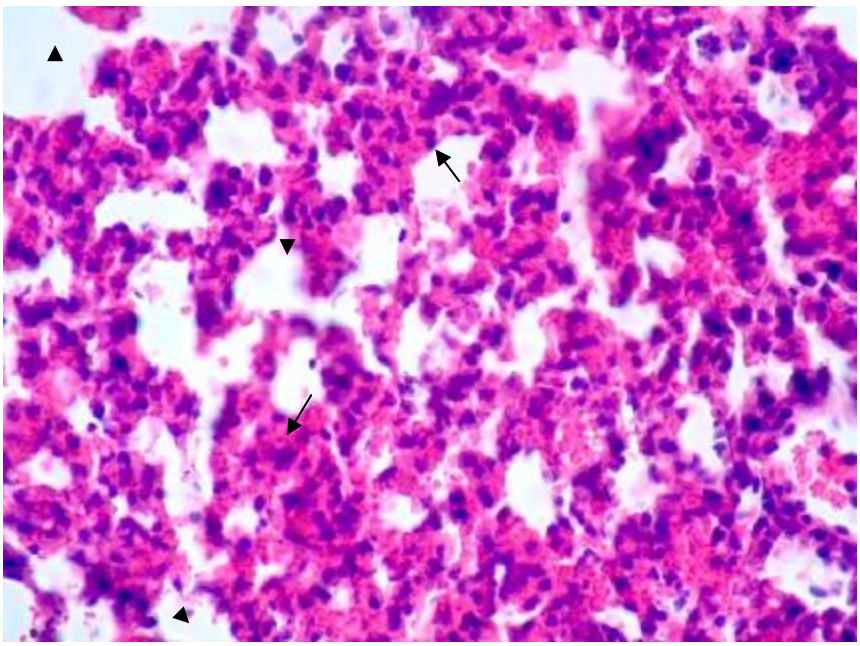

Figure (6): Section from rat lung injection with *Naja naja* snake venom after 24hsr showing thickened alveolar septa compressing on alveolar space( → ) Compensatory dilation of alveoli( ▲ ) and interstitial pneumonia H&E stain (X400).

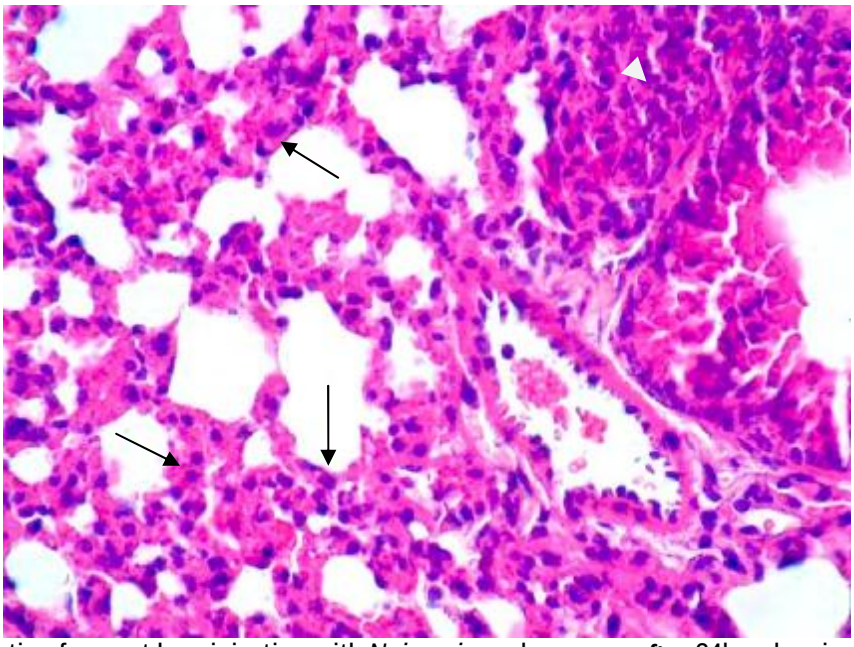

Figure (7) : section from rat lung injection with *Naja naja* snake venom after 24hsr showing thickening of alveolar septa compressing on alveolar sacs (⟶) Interstitial pneumonia is also seen ( ➤ ) H&E stain,(X400)

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