

# Ability of Gamma Radiation to Detoxify Black-necked Spitting Cobra (*Naja Nigricollis*) Snake Venom

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**Abstract-** In the current study, the lethality as well as the immunological, biochemical and histological effects of *Naja nigricollis* (Black-necked spitting cobra) venom at a sublethal dose has been investigated before and after exposure to gamma radiation (1.5KGy and 3KGy). Data revealed that the toxicity of irradiated venom (1.5KGy & 3KGy) decreased as compared to that of the native one. The LD<sub>50</sub> for native and gamma-irradiated (1.5KGy & 3KGy) *Naja nigricollis* venom was 0.440 mg/kg, 4.79 mg/kg and 5.38 mg/kg, respectively. Irradiation of the whole venom with (1.5kGy & 3kGy) reduced its lethality 10.8 and 12.22 times as compared to its native venom, respectively. The immunodiffusion technique showed same number of precipitin bands against polyvalent antivenin with the native and the two dose levels gamma-irradiated venoms. There was no change in the antigenic reactivity between both native and irradiated *Naja nigricollis* snake venom. As for the biochemical responses of *Naja nigricollis* venom, the effect of half LD<sub>50</sub> of native or irradiated (1.5KGy) was studied on the activities of heart enzymes: CPK, CK-MB, LDH and AST after (1, 2, 4, 24 hours) of envenomation. The present study showed that snake venom envenomation caused significant ( $p \leq 0.05$ ) elevation in serum CPK, CK-MB, LDH and AST levels. In contrast, the 1.5KGy gamma-irradiated *Naja nigricollis* venom recorded no significant changes compared to that of the non envenomated normal rats. These results are in accordance with the histological findings of the heart that showed severe degeneration of the cardiac muscle with loss of striations and extensive haemorrhage inbetween the myocardial bundles. These results indicate that 1.5KGy gamma irradiation of venoms offer an effective method for reducing the chronic toxic effect of venom in immunized animals for preparing the best toxoids and vaccines, facilitating antisera production and extending the useful life of immunized animals.

**Index Terms-** double immunodiffusion, gamma radiation, heart enzymes, *Naja nigricollis*, snake venom.

## I. INTRODUCTION

Snakebite is a serious medical problem worldwide, especially in the tropics. The incidence of snakebite mortality is particularly high in Africa, Asia and Latin America (*Gutierrez et al., 2006*). In Africa, snakebites cause more than one thousand deaths each year and thousands of cases of permanent physical disability (*Theakston et al., 2003*). In Egypt, the Black-necked Spitting Cobra; *Naja nigricollis* (*N. nigricollis*) is one of the most venomous snakes distributed in the south part of Egypt (*Saleh, 1997*). 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 (*Mady, 2002; Badr et al., 2012*). They are also known to cause different metabolic disorders by altering the cellular inclusions and enzymatic activities of different organs. Snake bite is an important cause of mortality and morbidity. Elapidae family such as *Naja nigricollis* is characteristic of the African spitting cobras. Its' venom retains the typical elapid neurotoxic properties while combining these with cytotoxins. The lethal effect of cobra bites is mainly neurotoxic. This is explained by the presence of highly potent  $\alpha$ -neurotoxin in their venoms (*Meenatchisundarama & Michael, 2010*). The myocardial effect of snake venoms is considered as one of the most common pathogenesis in many cases of snake envenomation (*Saadeh, 2001; Maheshwari & Mittal, 2004; Gaballa et al., 2005*). To improve antisera production and extend the useful life of immunized horse much effort has been devoted to decrease chronic venom toxicity. Towards more effective and safer antivenins, one method that has been shown to be venom toxicity effective for attenuating venom toxicity while maintaining its' immunogenicity is gamma irradiation (*Shaaban, 1990; Shaaban et al., 1996; Clissa et al., 1999; Souza et al., 2002; Oussedik-Oumehdi & Laraba-Djebari, 2008*). The aim of the present study was to evaluate the effectiveness of gamma irradiation using 1.5KGy in the detoxification of *Naja nigricollis* snake venom without affecting their immunogenic properties. This was carried out by studying the toxicological, immunological, biochemical and histological parameters induced by envenomation.

## II. MATERIALS AND METHODS

### Animals

White male Swiss albino mice, weighing 20-25 g were used for LD<sub>50</sub> study. Also adult male Wister albino rats, weighing 120-150 g, were used. Mice & rats were obtained from the Institute of Ophthalmology (Giza, Egypt). The animals were kept under suitable laboratory conditions of temperature, humidity and light throughout the period of investigation. They were allowed free access to food consisting of standard pellets obtained from El-Nasr Chemical Company (Cairo, Egypt) and water ad libitum. The study was carried out according to the approval of Ethics Committee for Animal Experimentation at Faculty of Pharmacy, Cairo University and in accordance with the guidelines set by the EEC regulations (revised directive 86/609/EEC) at the National Center for Radiation Research and Technology.

## Venom

*Naja nigricollis* venom was obtained from laboratory unit of Medical Research Center, Faculty of Medicine, Ain Shams University. The venom was obtained by milking healthy snake, dried and kept in desiccators at 4°C till used.

## Antivenin

Egyptian polyvalent antivenin prepared against *Cerastes cerastes*, *Echis pyramidum*, *Naja haje*, *Naja nigricollis*, *Cerastes vipera* and *Echis carinatus* obtained from the Egyptian Organization of Biological Products and Vaccines, Agouza, Cairo, Egypt, (VACSERA) was used. The polyvalent antivenin produced in horses was kept at 4°C till used.

## Irradiation of the venom

The venoms were irradiated with 1.5K Gy and 3K Gy gamma rays in the National Center for Research and Radiation Technology, Cairo, Egypt, using cobalt-60 Indian gamma cell (GE 4000A). The radiation dose rate was 1.26 Gy per second. In this study, a saline solution of *Naja nigricollis* snake venoms were subjected to integral radiation dose levels (1.5 and 3K Gy).

## Lethality

Toxicity of *Naja nigricollis* snake venom was studied before and after exposure to 1.5K Gy or 3K Gy gamma radiation. The LD<sub>50</sub> was determined according to the method of Spearman and Karber (*WHO, 1981*) by intraperitoneal (i.p) injection of the venom in different doses into swiss albino mice. Six mice were used for each dosage. The LD<sub>50</sub> was determined from the formula:

$$M = X_k + \frac{1}{2} d - \frac{dr}{N}$$

M=log LD<sub>50</sub>

X<sub>k</sub>=log dose causing 100% mortality (log LD<sub>100</sub>).

d=logarithmic interval of doses.

r=Sum of the number of animals dead at each of the individual doses.

N=Number of animal in each group.

## Immunodiffusion technique

Double immunodiffusion technique was carried out as described by *Ouchterlony (1948)*. They were carried out using 1.7 Nobel Agar (Difeco. Lab. Detroit. Mich.) in 0.9% NaCl solution, sodium azide was added in a concentration of 0.05%, to retard bacterial growth. The wells were filled with 20 µl volumes. The venom samples in concentration of (20 mg/ml) were added in the peripheral wells, while the antivenin was added in the central well. After developing of the precipitation bands (48 h), the plates were washed for 24 h in saline, dried and stained using Amidoschwartz stain 10B (0.5 % in 5 % acetic acid) for 7 min, washed with methanol acetic acid (9:1) dried in air and photographed.

## Experimental Design

Mice were used for determination of median lethal dose (LD<sub>50</sub>) of native and gamma-irradiated (1.5K Gy and 3K Gy) *Naja nigricollis* snake venoms.

Mice were divided into three sets as follows:

**Set 1:** was used to determine the LD<sub>50</sub> of native *Naja nigricollis* venom.

**Set 2:** was used to determine the LD<sub>50</sub> of 1.5K Gy gamma-irradiated *Naja nigricollis* snake venom.

**Set 3:** was used to determine the LD<sub>50</sub> of 3K Gy gamma-irradiated *Naja nigricollis* snake venom.

Each set was subdivided into several groups and each group included six animals, according to the method of Spearman & Karber for LD<sub>50</sub> determination (*Finney, 1964*). The 1.5K Gy γ-radiation is the selected dose to carry out the biochemical experiments of the present study, as it gets rid of venom toxicity while maintains immunogenicity.

ii. Rats were used for measuring the biochemical parameters; the equivalent rat dose was calculated according to *Paget & Barnes (1964)* to be used in the following biochemical experiments. Half value of LD<sub>50</sub> of native and 1.5K Gy irradiated *Naja nigricollis* venom were the selected doses to carry out the biochemical experiments of this study in rats. The animals were classified into three groups each group included eight rats and were treated as follows:

**Group I:** received 0.3 ml saline i.p injected and served as normal non-venomated.

**Group II:** received native *Naja nigricollis* snake venom (0.154 mg/kg) i.p injected.

**Group III:** received gamma-irradiated (1.5K Gy) *Naja nigricollis* (1.67 mg/kg) i.p injected.

Rats in groups (I, II and III) were sacrificed by decapitation (1, 2, 4, 24 hours) respectively after venom injection.

## Determination of cardiotoxicity of native & irradiated (1.5K Gy) *N. nigricollis* venom

Determination of serum creatine kinase (CK) according to (*Szasz et al., 1976*), serum isoenzyme creatine kinase-MB (CK-MB) was determined according to the method of the **IFCC, (1989)** while lactate dehydrogenase was determined according to the method of (*Wacker et al., 1956*) and aspartate aminotransferase (AST) activity. AST was determined in serum colorimetrically according to the method of (*Reitman & Frankel, 1957*). Enzymatic activity was expressed as units per liter.

## Histopathological Study

Following the animals being sacrificed the abdomen was opened, the specimens from heart of experimental rats were collected and immediately fixed in 10% formalin solution, kept until become hard enough to be sectioned. Samples were embedded in paraffin blockers. Sections of 5 µm were obtained and stained with Hematoxylin and Eosin (H&E) for standard histological examination using light microscope according to the method of (*Bancroft & Stevens, 1996*).

### Statistical Analysis

Values were calculated as mean  $\pm$  standard error (S.E) of the mean. Comparisons between different groups were carried out by one way analysis of variance = (ANOVA) followed by Tukey-Kramer multiple comparison test, using Instant software, version 3 (Graph pad software, Inc., San Diego, USA). The P value was set at  $\leq 0.05$ .

The figures were drawn using instant software program (Microsoft Office Excel 2010).

## III. RESULTS AND DISCUSSION

### 1- Lethality

The LD<sub>50</sub> for native and gamma-irradiated (1.5KGy & 3KGy) *Naja nigricollis* venom was 0.440 mg/kg, 4.79 mg/kg and 5.38 mg/kg, respectively. Toxicity of gamma-irradiated venom was reduced 10.8 and 12.22 times as compared to the native venom, respectively. Table (1) showed the LD<sub>50</sub> of *Naja nigricollis* venom before and after irradiation by 1.5KGy and 3KGy at 95% confidence limits. There was a dose dependent increase in the LD<sub>50</sub> after gamma irradiation and decrease in the toxicity of *Naja nigricollis* venom.

Snake bite envenomation is an important neglected disease in many tropical and subtropical developing countries (Warrell, 1993; Chippaux, 1998; Kasturiratne et al., 2008), most severe cases of snake bite envenomation are inflicted by species of the family Elapidea and the family Viperidea (Gutierrez et al., 2006). In the present study, the LD<sub>50</sub> of the *Naja nigricollis* venom was found to be 0.440 mg/kg, whereas toxicity of gamma irradiated (1.5KGy & 3KGy) *Naja nigricollis* venom was reduced 10.8 and 12.22 times as compared to its native venom, respectively. Thus, progressive dose dependent increase in the LD<sub>50</sub> after gamma irradiation indicates decrease in toxicity of *Naja nigricollis* venom. The present result agrees with Murata et al., (1990) who showed that irradiation with 2000Gy crude venom of *Crotalus durissus terrificus* was the ideal irradiation dose, promoting venom detoxification with maintenance of its immunogenicity. Souza et al., (2002) investigated the ability of gamma radiation from <sup>60</sup>Cobalt (2 KGy) to attenuate the toxic effects of *Bothrops jararacussu* venom. It was concluded that <sup>60</sup>Co gamma radiation is able to abolish toxic effects of *Bothrops jararacussu* venom. Also, Shaaban, (2003) showed that irradiated *Naja haje* and *Cerastes cerastes* venoms were 28.1 % and 30.8 % less toxic respectively than the native ones. In addition, these results were in accordance with results of Abib & Laraba-Djebari (2003) who reported that the toxicity of irradiated *Cerastes cerastes* venoms (1 and 2KGy) decreased as compared with that of native venom. Similar researches on influence of identification of gamma radiation on venom of snakes (*Cerastes cerastes*, *Bothrops jararacussu* and others) are given in researches of some authors. After irradiation of poison of snakes to gamma radiation <sup>60</sup>Co to doses 1 and 2kGy, decrease in toxicity of venom was noted. However decrease in immunogenic properties of toxins of snake poison was not noted. Authors revealed changes of spectral characteristics of the irradiated samples of toxins (Boni-Mitake et al., 2001; Oussedik-Oumehdi & Laraba-Djebari, 2008; Jessica et al., 2009). Moreover, Bennacef-Heffar & Laraba-Djebari (2003) showed that the effect of gamma irradiation on the venom of *Vipera lebetina* (one of the two widespread snakes in Algeria). *Vipera lebetina* venom

was irradiated with two doses of gamma rays (1 and 2 KGy) from a <sup>60</sup>Co source, and the venom's toxic, enzymatic, and structural properties were analyzed. Intraperitoneal injection of the irradiated venoms (100–500  $\mu$ g/20 g mouse body mass) revealed a significant decrease in its toxicity. Irradiated venoms with 1 and 2KGy doses were four and nine times less toxic, respectively, than the native venom. These results indicate that irradiation of *V. lebetina* venom with a dose of 2KGy can promote a significant detoxification, keeping the immunological properties intact. Both chromatographic and electrophoretic profiles of the irradiated venom were drastically changed as compared with that of the native venom (Shaaban et al., 2010). Loss of function of protein by irradiation is not usually due to breaking peptide bonds, or otherwise, disrupting the primary skeletal structure of peptide chain. It may result from a break in the hydrogen or disulfide bonds which in turn can result in a disorganization of the internal relationships of side chain groups, or an exposure of amino-acid groups, resulting in change in biological activity (Hayes & Francis, 2001).

It was well known that the doses of the order of kilograys used in detoxification of the venom influenced both the physiochemical properties and the biological activity of macromolecules, when irradiated in aqueous media or even in the solid state (Antoni, 1973; Hayes & Francis, 2001), since there is a close interrelationship between the structure and the biological activity of macromolecules, some alteration appeared to be the most possible explanation for the radiation effects.

**Table (1): LD<sub>50</sub> for Native, 1.5kGy and 3kGy Gamma-Irradiated *Naja nigricollis* Snake Venom:**

Venom	LD <sub>50</sub> (mg/kg)	95% Confidence limits
Native <i>Naja nigricollis</i>	0.44	0.42 $\pm$ 0.46
Irradiated (1.5KGy) <i>Naja nigricollis</i>	4.79	4.04 $\pm$ 5.69
Irradiated (3KGy) <i>Naja nigricollis</i>	5.38	4.91 $\pm$ 5.92

### 2-Immunodiffusion technique

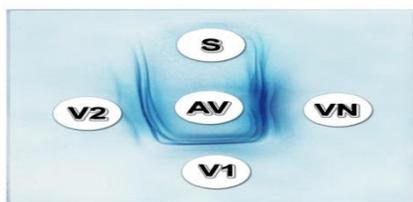
The result of double immunodiffusion test of native, 1.5KGy and 3KGy gamma irradiated venoms against the commercial polyvalent Egyptian antivenin, showed similar precipitin patterns using venom concentration of 20 mg/ml. Only faint bands were seen with a concentration of 10 mg/ml of *Naja nigricollis* snake venom. Exposure to 1.5KGy or 3KGy gamma radiation did not affect the immunological properties of the used snake venom.

Visible lines were observed with the non- irradiated, as well as, the two dose levels gamma-irradiated (1.5KGy and 3KGy) *Naja nigricollis* snake venom (fig. 1). These visible lines were identical, continuously joined smoothly at the corners, indicating that there was no change in antigenic determinants.

These results demonstrate that the ability of the venom antigens to react with its corresponding antibodies was maintained in spite of being exposed to radiation doses of 1.5KGy and 3KGy.

The present results showed that the antigenic response was not changed as judged by the capacity of irradiated venom to react with the antivenin. The immunodiffusion technique showed identity between native and irradiated samples. These results are in agreement with the data of other studies that reported that ionizing radiation is able to detoxify *Androctonus amoreuxi* without affecting the immunogenic properties (Shaaban, 1990). Also, the recorded results are in accordance with those concluded by Shaaban et al., (1996). This indicated that, there was no change in the antigenic reactivity of native, 1.5KGy and 3KGy for *Naja nigricollis* snake venom.

Furthermore, Nascimento et al., (1998) reported that ionizing radiation is able to detoxify several venoms, including snake venoms, without affecting their immunogenic properties significantly. It has been shown that gamma irradiation is an effective technique for attenuating venom toxicity and maintaining venom immunogenicity (Shaaban, 2003; Abib & Laraba-Djebari, 2003). Irradiation of *Vipera lebetina* venom with a dose of 2KGy can promote a significant detoxification while keeping the immunological properties intact (Shaaban et al., 2010).



S = Saline.

AV= polyvalent antivenin.

VN=Native *Naja nigricollis* venom.

V1 = 1.5KGy irradiated *Naja nigricollis* venom.

V2 = 3KGy irradiated *Naja nigricollis* venom.

**Figure (1): Immunodiffusion Reaction of Horse Serum Polyvalent Antivenin with Native, 1.5KGy and 3KGy Gamma-Irradiated *Naja nigricollis* Venoms.**

### 3-Effect of ½ LD<sub>50</sub> of Native & Gamma-Irradiated (1.5KGy) *N. nigricollis* Snake Venoms (0.154 mg/kg i.p) and (1.67 mg/kg i.p) respectively on Heart Enzymes (CK, CK-MB, LDH & AST) Activities in Male Albino Rats

In the present study, a dose of 1.5KGy gamma rays was selected, showing significant increase in LD<sub>50</sub> for *Naja nigricollis* snake venom. The results are shown in Table (2) and illustrated in Figures (2), (3), (4) and (5):

CPK levels of normal (non-envenomed) rats were 444.10±25.43, 371.60±18.86, 478.10±42.14 and 833±54.96U/L after (1, 2, 4, and 24) h respectively. *Naja nigricollis* native venom in a dose equal to ½ LD<sub>50</sub> (0.154 mg/kg) showed highly significant elevation in CPK levels by 63%, 240.2 %, 132.3%

and 71.30 % compared to the normal group after (1, 2, 4, 24) h respectively. Gamma-irradiated (1.5KGy) *Naja nigricollis* venom groups did not cause any significant change in CPK levels after 1 & 4 h, but significant increase was observed in CPK levels by 46.20 % & 28 % after 2 & 24 h respectively compared to non-envenomed control.

As regard to CK-MB levels of non-envenomed rats were 576±47.76, 451.30±33.18, 398±15.30 and 673.2±45.92 U/L after (1, 2, 4, and 24) h respectively. *Naja nigricollis* native venom in a dose equal to ½ LD<sub>50</sub> (0.154 mg/kg) showed highly significant elevation in CK-MB levels by 67.46 %, 82 %, 81 % and 78.7% compared to the normal group. Gamma-irradiated (1.5KGy) venom groups did not cause any significant change in CK-MB after 1 & 4 h, but significant increase was observed in CK-MB levels by 37.3 % & 32 % after 2 & 24 h respectively compared to non-envenomed control.

However, LDH levels of normal rats were 893.5± 61.01, 902.7±41.66, 962.2 ±28.76 and 898.1±4.60 U/L respectively. The group injected i.p. with native *Naja nigricollis* venom in a dose equal to ½ LD<sub>50</sub> did not cause any significant change in LDH after 1, 2 & 4 h, but showed significant increase in LDH level by 46.2 % after 24 h compared to the normal group. Also, gamma-irradiated (1.5KGy) *Naja nigricollis* venom groups did not cause any significant change in LDH levels.

AST levels of normal rats were 103.4±2.21, 99.11±2.35, 101.3±0.87 and 102±0.69 U/L respectively. *Naja nigricollis* native venom did not cause any significant change in AST levels compared to control after 1 & 2 h, while showed highly significant elevation in AST level by 32.4 % & 27 % after 4 & 24 h respectively compared to the normal group. Also, gamma-irradiated (1.5KGy) venom groups did not cause any significant change in AST levels compared to non-envenomed control.

The impact of  $\gamma$ -irradiation on *Naja nigricollis* snake venom induced changes on some biochemical markers. As for the effect on heart enzymes, the present study indicated that injection of native *Naja nigricollis* snake venom in a dose equal ½ LD<sub>50</sub> (0.154 mg/kg i.p) caused a highly significant increase of Aspartate aminotransferase (AST), Lactate Dehydrogenase (LDH), Creatine Phosphokinase (CPK) and Creatine Phosphokinase isoenzyme (CK-MB) compared to the normal control. The obtained results are in agreement with those previously reported by other investigators. Tressler (1988) mentioned that elevation in serum AST results from conditions causing injury to cardiac muscle. Also, Fernando et al., (1989) reported that *B. asper* venom caused serum AST, LDH and CPK to increase significantly. Moreover, Aguiyi et al., (2001) reported an elevation in the levels of Lactate Dehydrogenase (LDH) and Creatine Phosphokinase (CPK) following administration of *Echis carinatus* venom.

Shaaban & Hafez (2003) reported that the intraperitoneal injection of a sublethal dose of *Naja haje* venom (0.2mg/kg) in rats induced a significant elevation in the activities of LDH and CK-MB as compared to normal control. Furthermore, Lucas de Oliveira et al., (2007) evaluated the muscular enzymes creatine kinase (CK) and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) following envenomation with natural and <sup>60</sup>Co-irradiated *bothropic* venom (*Bothrops jararaca*). The authors stated that animals injected with natural venom exhibited serum levels of these enzymes higher than those

observed in animals injected with irradiated venom. In other side, the irradiated venom caused increasing in the serum levels of muscular enzymes CK, AST, and ALT, although animals inoculated with natural venom exhibited higher serum levels of such enzymes.

The obtained results are in accordance with those previously reported by other investigators (*Bhagwat & Amar, 2013*) who studied that serum activities of enzymes (AST), (ALT), (LDH) and (CK) was found to be specifically elevated in patients of snake bite. The increase in serum activities of above enzymes is due to secretion of the enzyme in the serum as a result of muscular damage, (rhabdomyolysis), produced by toxins from venom. AST and CK found elevated due to cardiac as well as skeletal muscle damage. ALT found elevated due to skeletal muscle damage. While LDH increases due to hemolysis and cardiac as well as skeletal muscle damage.

Moreover, *Al-Sadoon et al., (2013)* showed that, mice envenomation with *Cerastes cerastes gasperetti* crude venom at LD<sub>50</sub> dose for the 1st, 3rd and 6th hr. caused different changes of the selected biochemical parameters. *Cerastes cerastes gasperetti* crude venom induced a highly significant elevation in serum AST & LDH activities.

Interestingly, gamma-irradiated (1.5KGy) *Naja nigricollis* venom in a dose equal ½ LD<sub>50</sub> (1.67 mg/kg i.p.) did not cause any observed significant change in CPK, CK-MB, LDH and AST serum levels compared to non-envenomed control. This was in contrast to the native *Naja nigricollis* venom that induced a highly significant increase of CPK, CK-MB, LDH and AST serum levels compared to the normal control or the 1.5KGy  $\gamma$ -irradiated *Naja nigricollis* venom.

Also, *Shaaban et al., (1996)* recorded that there was non-significant increase following 2KGy  $\gamma$ -irradiated *Cerastes cerastes* and *Echis pyramidum* snake venom. This is attributed to the disorganization of the molecular structure of venom after exposure to gamma radiation resulting in a change in its biological activity (*Hayes, 2001*). Thus, radiation is able to detoxify snake venoms and decrease its harmful effects. In addition, *Abib & Laraba-Djebari (2003)* reported that the creatine phosphokinase (CPK) activity was significantly increased in the serum and decreased in the myocardium after envenomation with native *Cerastes cerastes* venom, but no significant enzymatic changes were observed in mice envenomated with irradiated venom. These results indicate that irradiation of venom with a 2KGy dose may offer an effective method for reducing the chronic toxic effects of venom in immunized animals.

These results were in accordance with results of *Shaaban et al., (2010)* who showed that 1.5KGy  $\gamma$ - irradiated *Echis pyramidum* venom caused a non- significant change (when used at a dose equal to that used for the native venom; 27.69  $\mu$ g/mouse) of ALT, AST, LDH, CPK and CK-MB compared to the normal control, while the native *Echis pyramidum* venom (27.69  $\mu$ g /mouse) that induced a highly significant increase of ALT, AST, LDH, CPK and CK-MB compared to the normal control or the 1.5 KGy  $\gamma$ - irradiated *Echis pyramidum* venom.

**Table (2): Effect of Native & Gamma-Irradiated (1.5KGy) *N. nigricollis* Snake Venoms on Heart Enzymes (CK, CK-MB, LDH & AST) Activities in Male Albino Rats at Different Times:**

Parameters	Normal (non-envenomed)	Native <i>N. nigricollis</i> (non-irradiated) (0.154 mg /kg i.p)	Gamma-irradiated (1.5KGy) <i>N. nigricollis</i> (1.67 mg/kg i.p)
<b>CPK (U/L)</b>			
1 h	444.10±25.43	724.10±44.96*	409.50±27.20 #
2 h	371.60±18.86	1264±19.20*	543.30±41.48
4 h	478.10±42.14	1111±41.57*	*#
24h	833±54.96	1427±65.86*	490.40±40.86 # 1064±79.16 *#
<b>CK-MB(U/L)</b>			
1 h	576±47.76	964.6 ±56.58*	469.3±39.55 #
2 h	451.30±33.18	820.9 ±38.26*	619.5±22.23 *#
4 h	398±15.30	719.1±22.61*	517.7±21.53 #
24h	673.2±45.92	1203 ±14.65*	887.1±41.18 *#
<b>LDH(U/L)</b>			
1 h	893.5±61.01	1096±63.39	961.7±49.23
2 h	902.7±41.66	1061±83.37	969.5±45.34
4 h	962.2±28.76	1052±55.25	1056±40.60
24h	898.1±4.60	1313±46.69 *	901.7±23.17 #
<b>AST(U/L)</b>			
1 h	103.4±2.21	103.7±2.79	103.7±4.87
2 h	99.11±2.35	114.1±2.82	108.4±2.31
4 h	101.3±0.87	134.2±4.61 *	108.9±0.19 #
24h	102±0.69	130±1.27 *	107±2.95 #

Three groups of animals each consisting of 8 rats received saline (0.3 ml i.p) served as normal group, native *Naja nigricollis* venom (0.154 mg/kg i.p) and gamma-irradiated (1.5KGy) venom (1.67 mg/kg i.p), blood was collected (1, 2 4, 24) h after envenomation, for estimation of heart enzymes activities.

Each value represents Mean± S.E

Statistical analysis was carried out by one way ANOVA followed by Tukey- Kramer Multiple Comparison Test:

\*Significant difference from Normal group ( $P \leq 0.05$ ).

# Significant difference from Native group ( $P \leq 0.05$ ).

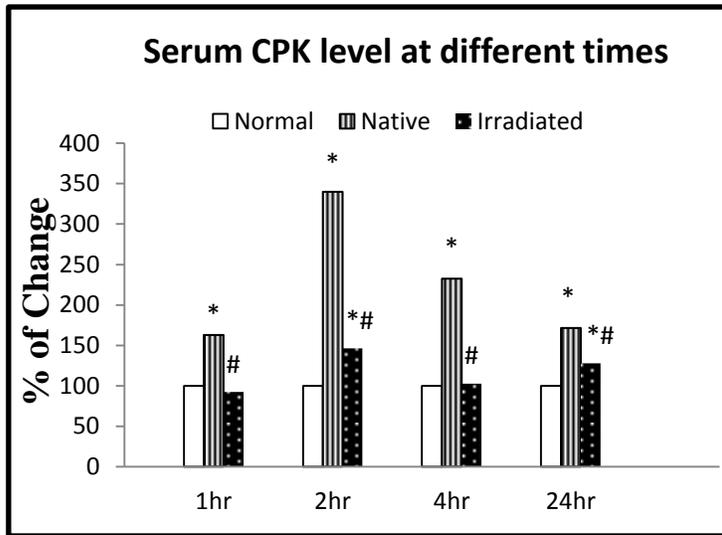


Figure (2): Effect of Native (0.154 mg /kg i.p) & Gamma-Irradiated (1.5KGy) (1.67 mg/kg i.p) *N. nigricolis* Snake Venoms on Creatine Kinase (CPK) in Male Albino Rats:

\*Significant difference from Normal group ( $P \leq 0.05$ ).  
# Significant difference from Native group ( $P \leq 0.05$ ).  
% of change: The percentage change of control.

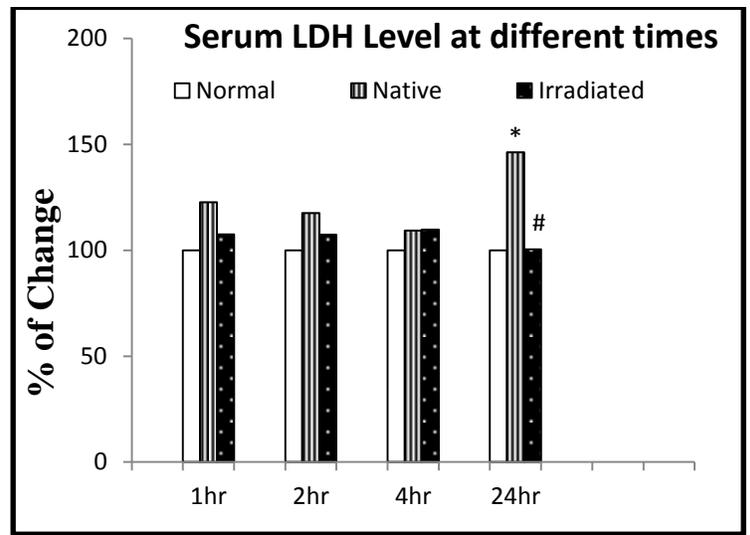


Figure (4): Effect of Native (0.154 mg /kg i.p) & Gamma-Irradiated (1.5KGy) (1.67 mg/kg i.p) *N. nigricolis* Snake Venoms on Lactate Dehydrogenase (LDH) in Male Albino Rats:

\*Significant difference from Normal group ( $P \leq 0.05$ ).  
# Significant difference from Native group ( $P \leq 0.05$ ).  
% of change: The percentage change of control.

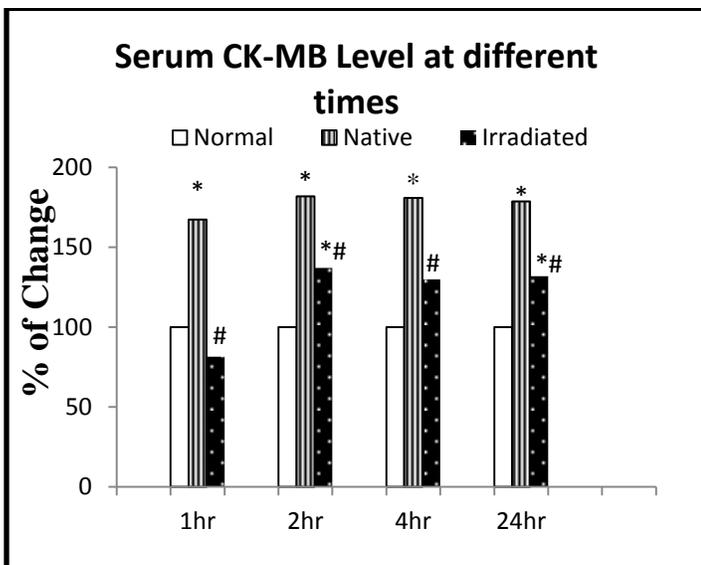


Figure (3): Effect of Native (0.154 mg /kg i.p) & Gamma-Irradiated (1.5KGy) (1.67 mg/kg i.p) *N. nigricolis* Snake Venoms on Creatine Kinase-MB isoenzyme (CK-MB) in Male Albino Rats:

\*Significant difference from Normal group ( $P \leq 0.05$ ).  
# Significant difference from Native group ( $P \leq 0.05$ ).  
% of change: The percentage change of control.

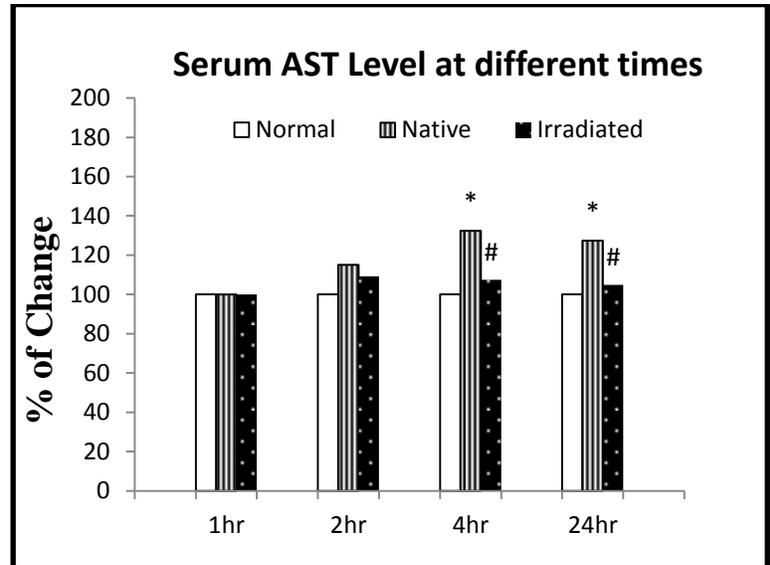


Figure (5): Effect of Native (0.154 mg /kg i.p) & Gamma-Irradiated (1.5KGy) (1.67 mg/kg i.p) *N. nigricolis* Snake Venoms on Aspartate Aminotransferase (AST) in Male Albino Rats:

\*Significant difference from Normal group ( $P \leq 0.05$ ).  
# Significant difference from Native group ( $P \leq 0.05$ ).  
% of change: The percentage change of control.

### Histopathological Study:

Histopathological examination of heart was carried out to adjoin the biochemical changes of heart enzymes. Certain histopathological changes were observed after injection with native and gamma irradiated (1.5KGy) *Naja nigricollis* venom. The effect of native *Naja nigricollis* venom in a dose equal to  $\frac{1}{2}$  LD<sub>50</sub> (0.154 mg/kg i.p) and gamma-irradiated (1.5KGy) *Naja nigricollis* venom  $\frac{1}{2}$  LD<sub>50</sub> (1.67 mg/kg i.p) on heart muscle after 24 h envenomation was illustrated as following:

#### a)- Control group:

Heart of control, untreated rats showing normal appearance of cardiac tissues could be observed in Fig. (I&II) , in these figures, longitudinal section of cardiac muscle illustrating the variable diameter of the fibers and the central position of their nuclei. The ends of the fibers are split longitudinally into a small number of branches the end of which abuts onto similar branches of adjacent cells giving the impression of a continuous three-dimensional cytoplasmic network.

#### b)- Native venom-injected group:

Heart of rats injected with native venom at a dose of (0.154 mg/kg i.p) exhibited highly degenerated muscle fibers with loss of striations. Also massive extended haemorrhagic areas. Intramuscular oedema and haemorrhage, vacuolation of sacroplasm of cardiac myocytes and inflammatory cells infiltration were apparently seen Figs. (III & IV & V).

#### c) - Gamma -irradiated (1.5 KGy) venom injected group:

Heart of rats injected with  $\gamma$ - irradiated (1.5KGy) venom at a dose of (1.67 mg/kg i.p) showed restoration of the characteristic myocardium appearance, also showing no histopathological changes, but in some areas, few inflammatory cells between cardiac myocytes were apparently seen Figs. (VI&VII&VIII).

Histological examination demonstrated highly degenerated muscle fibers with loss of striations of cardiac muscles following injection with native *Naja nigricollis* venom in a dose equal  $\frac{1}{2}$  LD<sub>50</sub> (0.154 mg/kg i.p) after 24 h. Also massive extended haemorrhagic areas, intramuscular oedema, haemorrhage, vacuolation of sacroplasm of cardiac myocytes and inflammatory cells infiltration were observed.

This might be due to a large number of toxins have been isolated, purified and characterized from such venoms including cardiotoxins, cytotoxins (*Jeyaseelan et al., 1998*), neurotoxin (*Afifiyan et al., 1999*) and phospholipases (*Jeyaseelan et al., 2000*) that responsible for inducing these changes (*Abdel Ghani et al., 2010*).

On the other hand, the present findings show that the restoration of the characteristic myocardium appearance, also showing no histopathological changes, but in some areas, few inflammatory cells between cardiac myocytes were apparently seen in case of injection with  $\gamma$ - irradiated (1.5KGy) venom in a dose equal  $\frac{1}{2}$  LD<sub>50</sub> (1.67 mg/kg i.p) after 24 hours. Treatment of the venom by gamma radiation in a dose (1.5KGy) markedly reduced the degree of damage induced by the venom in the heart

which appeared more or less normal except for minor abnormalities that were still present.

Similar results were recorded by *Tu & Homma (1970)*; *Rahmy et al., (1995) & Hanafy et al., (1999)*. The haemorrhage observed inbetween the cardiac muscles of animals receiving the venom may be due to increased intravascular tension or venous congestion (*Willoughly, 1960*). In addition, *Rahmy et al., (1991)* reported that the crude venom of *Cerastes cerastes* induced severe haemorrhage as well as extensive myofilament damage at the electron microscopic level.

According to *Shaaban & Hafez (2003)*, it was shown that the injection of *Naja haje* venom in a sublethal dose (0.2 mg/kg) produced severe degeneration of muscle fibers and loss of striations. Also, haemorrhage and extravasated red blood cells were seen inbetween the myocardial bundles.

In addition, *Abib & Laraba-Djebari (2003)* indicated that histopathologic evaluation of heart showed that native *Cerastes cerastes* venom caused severe degenerative changes in the myocardium. In the case of 2KGy irradiated venom, no tissue alterations were observed.

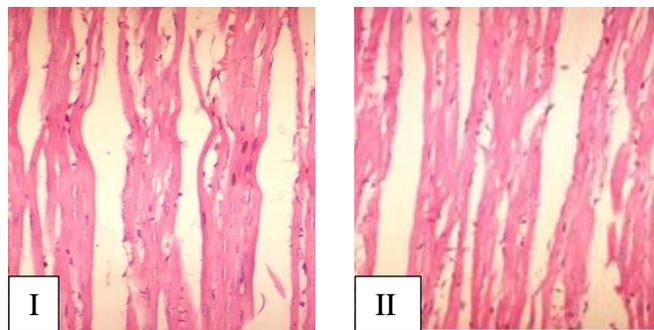
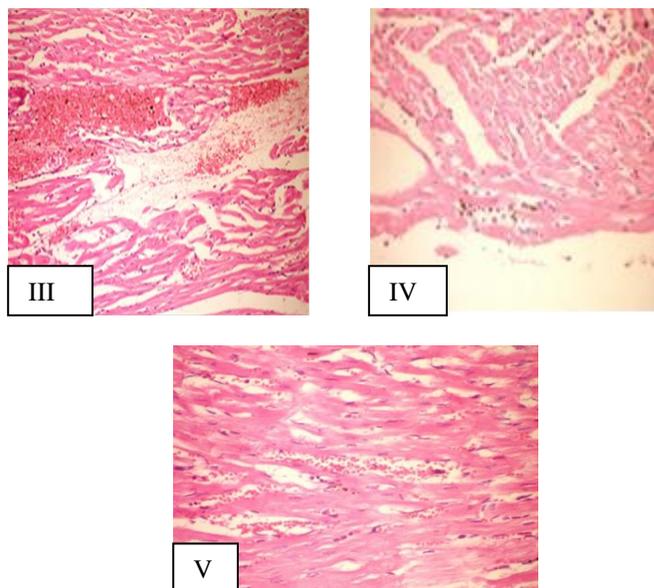


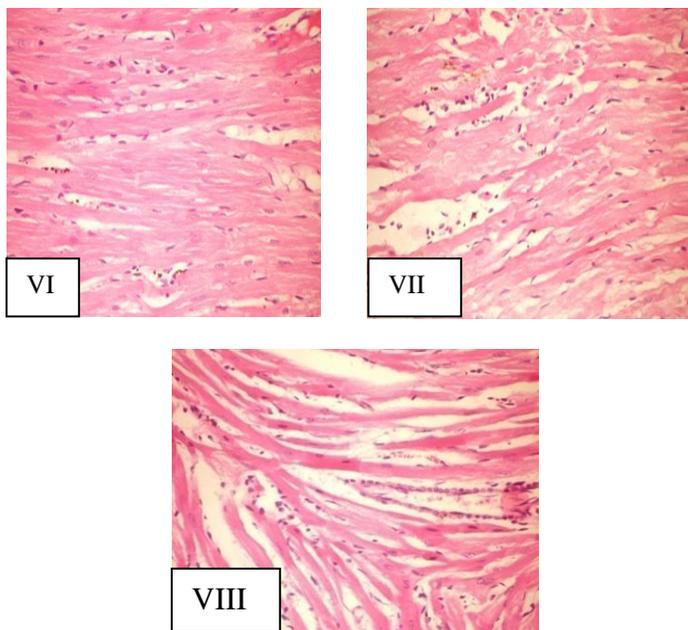
Fig. I &II: Longitudinal section of the myocardium of untreated control rat (H & E; X400).



**Fig.III:** Section in the heart of a rat injected with native venom (0.154 mg /kg i.p.) showing intramuscular oedema and haemorrhage (H&E; X400).

**Fig.IV:** Section in the heart of a rat injected with native venom (0.154 mg /kg i.p.) showing degenerated muscle fibers with loss of striations (H&E ; X400).

**Fig.V:** Another section of the myocardium of a rat injected with native venom (0.154 mg /kg i.p.) showing extensive congestion of myocardial blood vessels (H & E; X 400).



**Fig.VI:** Heart section of a rat injected with  $\gamma$ -irradiated (1.5KGy) (1.67 mg/kg i.p.) venom showing obvious return to normal myocardial appearance (H & E; X 400).

**Fig. VII:** Section in the heart of a rat injected with  $\gamma$ -irradiated (1.5KGy) (1.67 mg/kg i.p.) venom showing restoration of the characteristic appearance of cardiac muscle with few inflammatory cells inbetween the myocardial bundles (H & E ; X 400).

**Fig. VIII:** Another heart section of a rat injected with  $\gamma$ -irradiated (1.5KGy) (1.67 mg/kg i.p.) venom showing restoration of the characteristic appearance of cardiac muscle with minimal extravasation of blood cells inbetween the myocardial bundles (H & E; X 400).

#### IV. CONCLUSION

Collectively, the present data support the conclusion that gamma radiation is an effective venom detoxification method. Thus, the present data support that, gamma irradiation of *Naja nigricollis* snake venom with 1.5KGy dose offer an effective method for reducing the chronic toxic effect of venom in immunized animals for preparing the best toxoids and vaccines.

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