Evaluation of the effect of gamma rays on Echis Coloratus snake venom through toxicological, immunological and biological studies.

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Abstract- The present study was performed to evaluate the effectiveness of gamma radiation in detoxification of *Echis Coloratus* snake venom without affecting their immunogenic properties. This was carried out by studying the toxicological, immunological and biological properties of the crude venom. Data revealed that the toxicity of venom after (1.5 and 3 KGy) gamma irradiation was reduced 6.4 and 7.2 times, respectively, compared to the native venom. Immunogenicity was evaluated by performing the double immunodiffusion test; same number of visible lines joined smoothly at the corner was observed with the non-irradiated, as well as, the two dose levels gamma irradiated (1.5 and 3 KGy) snake venom; this result indicating that there was no change in antigenic reactivity. The effect of gamma radiation on some venom enzymes was studied; the phospholipase A_2 specific activity of native, 1.5 and 3 KGy gamma irradiated venom was 6.25, 4.8 and 2.7 U/mg; respectively. The proteolytic specific activity of native, 1.5 and 3 KGy gamma irradiated venom was 13, 7.5 and 5.5 U/mg; respectively. This means that proteolytic and phospholipase A_2 activities were inhibited by irradiation. The study confirmed that native *Echis Coloratus* snake venom showed high edema forming activity accompanied by apparent hematoma; peak volume was achieved after 30 min and percent edema reached 41.8 % from the initial diameter of the rat paw. This criteria greatly reduced by radiation as gamma irradiated (1.5 KGy and 3 KGy) venoms induced edema with peak volume achieved after 30 min and percent edema reached 29.7 and 21 % respectively, from the initial diameter of the rat paw. *Echis Coloratus* snake venom showed a high concentration dependant coagulant activity; The clotting time of native, 1.5 and 3 KGy gamma irradiated venom at 200 µg/ml was 4.8, 16.8 and 26.6 seconds. So, gamma irradiation decreased the coagulant activity of the venom. These results indicate that 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 antivenin production and extending the lifespan of immunized animals.

Index Terms- *Echis Coloratus*, gamma radiation, LD_{50}, double immunodiffusion, edema.

I. INTRODUCTION

Snake bites are a public health problem that deserves attention from health authorities ***(De Souza et al., 2012)***. Venomous snakes are capable of inject venom, modified saliva, into another creature for the purpose of prey immobilization or self-defense ***(Meier and White, 2007).***

Echis coloratus belongs to the family *Viperidae*; it is the most familiar family in Egypt ***(Mallow et al., 2003).*** Its venom induces complex biological alterations and multisystem affection that may result in a death. Vipers' venoms typically contain an abundance of protein-degrading enzymes, called proteases, which produce symptoms such as pain, strong local swelling and necrosis. Another important enzyme in *Echis Coloratus* snake venom is phospholipase A_2. It is a small enzyme which causes havoc by interfering in the normal physiological processes of the victim and inducing a variety of pharmacological effects ***(Kini, 2003).***

Generally, the crude venom of viper *Echis Coloratus* causes swelling, pain, respiratory failure, arrhythmia, hypotension, circulatory collapse which leads to acute renal failure ***(Osman and Gumma, 1974; Boviatsis et al., 2003).*** Liver is severely affected by *Echis coloratus* envenomation; elevation of serum levels of glucose, hepatocytes glycogen depletion and elevation of AST, ALT and ALP indicates hepatocellular damage ***(Al-Jammaz, 2003)***. Antivenins are the only effective treatment for snakebites and envenomations by other venomous animals ***(Morais and Massaldi, 2009).*** In order to improve antivenin production, extend the lifespan of the immunized animals and decreasing the production cost, several trials have been used to detoxify venoms and maintaining its immunogenicity. One method that has been shown to be effective for attenuating venom toxicity and maintaining immunogenicity is gamma radiation ***(Shabaan, 2003)***.

The present study was performed to evaluate the effectiveness of gamma radiation (1.5 and 3 KGy) in detoxification of *Echis Coloratus* snake venom without affecting their immunogenic properties. This was carried out by studying the toxicological, immunological and biological properties of the crude venom before and after exposure to radiation.

II. MATERIALS AND METHODS

Animals

Animals used in the present study included Swiss albino male

mice (20-25 g) and Wistar albino male rats (150-180 g). Animals were obtained from institute of ophthalmology (Giza, Egypt). The animals were acclimatized in the animal facilities of the National Center for Radiation Research and Technology under appropriate conditions of temperature, humidity and light. They were allowed free access to food consisting of standard pellet obtained from El-Nasr chemical company (Cairo, Egypt) and water ad libitum. Study was conducted in accordance with the regulations approved by the ethics committee at Faculty of Pharmacy, Cairo University. Animals were purchased from the National Research Center (Giza, Egypt).

Venom

The crude venom was obtained from *Echis Coloratus* snakes kept in laboratory unit of Medical Research Centre, Faculty of Medicine, Ain Shams University. The venom was pooled by milking healthy snakes collected from Sinai, Egypt and it was dried and kept in the refrigerator at 4°C until used.

Antivenin

Egyptian polyvalent antivenin, produced in horses, was obtained from the Holding Company for Biological Products and Vaccines (VACSERA), Agouza, Giza, Egypt. The polyvalent antivenin was kept at 4°C till used.

Irradiated Facilities

The venom was irradiated with gamma rays in the National Center of Radiation and Research Technology, Cairo, Egypt, using **(cobalt-60 gamma cell 220, Atomic Energy of Canady Limited (AECL), Canada).** The radiation dose rate was 1.2 Gy per second. In this study, a saline solution of *Echis Coloratus* snake venom was subjected to radiation dose level of 1.5 KGy or 3 KGy.

Determination of lethal dose fifty (LD_{50}) of native and (1.5 KGy and 3 KGy) gamma irradiated venom

Toxicity of *Echis Coloratus* snake venom was studied before and after exposure to 1.5 KGy or 3 KGy gamma radiation. The LD_{50} was determined according to the method of Spearman and Karber ***(Finney, 1964)*** by intraperitonial (i.p) injection of the venom in different doses into swiss albino mice. Six mice were used for each dosage. The LD_{50} was determined from the formula:

$$M = X_k + {}^1/_2 d - dr_1/N$$

M = log LD_{50}
X_k= log dose causing 100% mortality.
d = logarithmic interval of doses.
r = Number of dead animal in each group.
r_1 = Sum the number of dead animals at each of the individual dose levels.
N = Number of animals in each group.

Immunodiffusion technique for native and (1.5 KGy and 3 KGy) gamma irradiated venom

Double immunodiffusion technique of native and (1.5 KGy and 3 KGy) gamma irradiated *Echis Coloratus* snake venom 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 0.5 % Amidoschwartz stain (0.5 % in 5 % acetic acid) for 7 min, washed with methanol acetic acid (9:1) dried in air and photographed.

Determination of phospholipase A_2 (PLA_2) activity of native and (1.5 KGy and 3 KGy) gamma irradiated venom

Venom phospholipase A_2 activity of native and (1.5 KGy and 3 KGy) gamma irradiated *Echis Coloratus* snake venom was determined according to the method of ***Marinetti (1965).*** A stock of egg yolk suspension was prepared by shaking one egg yolk in 0.9% NaCl to give a final volume of 100 ml suspension, stable for at least 2 weeks when stored at 0 °C. The working suspension of egg yolk was prepared by making 5-fold dilution of this stock suspension in 0.9% NaCl. To 1 ml of the working egg yolk suspension, 4.9 ml of isotonic saline was added. This suspension was mixed, and equilibrated to a temperature of 41°C for 5 minutes. 0.1 ml of the sample in saline was added to this suspension. Then, the contents were rapidly mixed and the absorbance at 925 nm was recorded each 5 min for 10 min. The control was prepared by adding 1 ml of working solution to 5 ml 0.9% NaCl and was adjusted to give an absorbance of 0.7 (at 925 nm). One unit of enzyme is defined as the amount of venom in µg that will cause 50% turbidity reduction per 10 min.

Determination of proteolytic activity of native and (1.5 KGy and 3 KGy) gamma irradiated venom

Proteolytic activity of native and (1.5 KGy and 3 KGy) gamma irradiated *Echis Coloratus* snake venom was measured using azocasein as substrate according to the method of ***Lemos et al. (1990).*** In a test tube, 2.5 % azocasein substrate solution dissolved in distilled water (w/v) was added to reaction mixture containing 0.05M tris Hcl Bufer (PH=8.5) and different concentrations of native venom and (1.5 and 3 KGy irradiated) venom, The mixtures were incubated at 37 °C for 60 minutes. Trichloroacetic acid (15%, 0.5ml) was added to the mixtures. The reaction mixtures were cooled in an ice bath for 10 minutes; the mixtures were clarified by centrifugation. The absorbance of supernatants was determined at 366 nm against blank, using quartz cuvette. One unit of enzyme is defined as the amount of venom in µg which cause a reduction in absorbance by 50% at 366 nm.

Determinations of the rat's hind paw edema induced by native and (1.5 KGy and 3 KGy) gamma irradiated venom

Inflammatory response caused by *Echis Coloratus* snake venom was investigated by measuring the rat hind paw edema. Rat hind paw edema induced by native and (1.5 KGy and 3 KGy) gamma irradiated *Echis Coloratus* snake venom was determined by the method of ***Faria et al. (2001).*** 60 male albino rats weighing (180-200 gm) were selected for the study and divided into 10 groups, 6 rats each. Each sample was injected into sub-plantar region of the right hind paw. The paw diameter was measured using vernier caliper initially and at ½ h. after sample

administration. The percent of induction of edema in the rat hind paw was calculated from the formula: **$(D_f - D_i/D_i) \times 100$**
D_f is the final diameter in each time interval
D_i is the initial diameter at 0 h

Screening of coagulant properties of native and (1.5 KGy and 3 KGy) gamma irradiated venom (Plasma recalcification time)

Coagulant activity of *Echis Coloratus* snake venom before and after 1.5 & 3 KGy gamma irradiations was determined according to the method of ***Biggs and Macfarlene (1962).*** Rabbit blood (4.5 ml) was withdrawn by a sterile plastic syringe from the marginal vein of the ear and immediately transferred to a clean test tube containing 0.5 ml sodium citrate solution (3.8%); the citrated blood was centrifuged for 20 minute at 2000 rpm. The supernatant plasma was pipetted into a clean tube; 0.l ml aliquots of the normal plasma were pipette into a number of clotting glass tubes incubated at 37°C. Different sample concentration was added to the tubes. Then 0.1 ml of 25mM calcium chloride was added to each tube and the clotting time was immediately recorded with stop watch. The tubes were kept in the water bath and tilted every 10 seconds until a clot was formed.

Statistical Analysis:

Values was expressed as mean ± standard error (S.E), Statistical analysis was performed using instant software, version 2 (Graph pad software, Inc., San Diego, USA), One way analysis of variance (ANOVA) followed by Tukey- Kramer multiple comparison test were used for Comparisons between different groups, For all the carried statistical tests, the level of significance was set at $P \leq 0.05$.
Graphical representation and regression analysis were conducted using Prism computer program.

III. RESULTS AND DISCUSSION

1- LD_{50} of native and (1.5 KGy and 3 KGy) gamma irradiated snake venom

LD_{50} for native and gamma irradiated (1.5 and 3 KGy) *Echis Coloratus* venom is 2.88 mg/Kg, 18.47 mg/Kg and 22.42 mg/Kg respectively**.** There was a dose dependant increase in the LD_{50} after gamma irradiation and decrease in toxicity for *Echis Coloratus* venom. The present LD_{50} and 95% confidence limits for *Echis Coloratus* venom before and after irradiation by 1.5 and 3KGy are shown in (**Table 1 and figure 1).**

Envenoming by *Viperidae* family leads to a complex combination of systemic and local symptoms and up to 20% mortality rates without antivenin treatment ***(Casewell et al., 2009).***

In the present study, The LD_{50} of *Echis Coloratus* venom was 2.88 mg/Kg, whereas the toxicity of *Echis Coloratus* venom after (1.5 and 3 KGy) gamma irradiation was reduced 6.4 and 7.2 times, respectively, compared to the native venom. This dose dependant increase in the LD_{50} after gamma irradiation indicates decrease in toxicity of *Echis Coloratus* venom.

Table (1): LD_{50} for Native, 1.5 KGy and 3 KGy Gamma Irradiated *Echis Coloratus* Snake Venom:

Venom	LD_{50} (mg/Kg)	95% Confidence Limits
Native *Echis Coloratus*	2.88	2.35 – 3.52
Irradiated (1.5 KGy) *Echis Coloratus*	18.47	16.95 – 20.60
Irradiated (3 KGy) *Echis Coloratus*	20.77	18.63 – 23.61

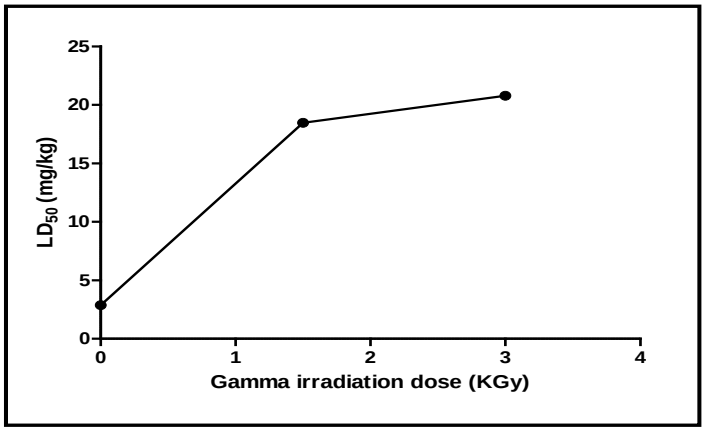


Figure (1): Effect of Gamma Irradiation on Median Lethal Dose (LD_{50}) for *Echis Coloratus* Snake Venom

Souza et al., (2002) showed that *Bothrops jararacussu* venom irradiated with a dose of gamma rays (2000 Gy) from a source of Cobalt-60 (^{60}Co) reduces the acute toxicity of the venom, preserving the antigenic and immunogenic properties. Also, ***Netto et al. (2002)*** examined the toxicity of native and 2000 Gy gamma irradiated *Crotalus durissus terrificus* venom from a Cobalt 60 source; the LD_{50} values were 0.09 (0.05-0.14) µg/g and 0.40µg/g mouse for native venom and irradiated venom, respectively. So, the LD_{50} for irradiated venom was 4.4 times less toxic than native venom. Moreover, ***Shaaban, (2003)*** showed that the effect of gamma irradiation (15 KGy) on *Naja haje* and *Cerastes cerastes* venoms were 28.1 % and 30.8 % less toxic respectively than the native ones.

Moreover, according to ***Bennacef-Heffar and Laraba-Djebari, (2003)*** *Vipera lebetina* venom was irradiated with two doses of gamma rays (1 and 2 KGy) from a ^{60}Co source and Intraperitoneal injection of the irradiated venoms (100-500 µg/ 20 g mouse body mass) were analyzed for the venom's toxic, enzymatic, and structural properties, this revealed that the

toxicity of irradiated venoms with 1 and 2 KGy doses were four and nine times less toxic, respectively, than the native venom. Similarly, ***Oussedik-Oumehdi and Laraba-Djebari, (2008)*** describes gamma irradiation effects on Cerastes cerastes venom after treating with Doses of 1 KGy and 2 KGy gamma radiations. These treated venoms did not have any residual lethal effects until 10 LD_{50}.

Loss of function of protein by irradiation is not usually due to breaking peptide bonds, or otherwise, disrupting the primary skeletal structure of the peptide chain. It may result from a change in a critical side chain or 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 a change in biological activity ***(Hayes, 2001).***

2-Immunodiffusion technique of native and (1.5 KGy and 3 KGy) gamma irradiated venom

The result of double immunodiffusion test of native, 1.5 KGy, 3 KGy gamma irradiated venoms against the commercial polyvalent Egyptian antivenin, showed similar precipitin patterns using venom concentration of 20 mg/ml.

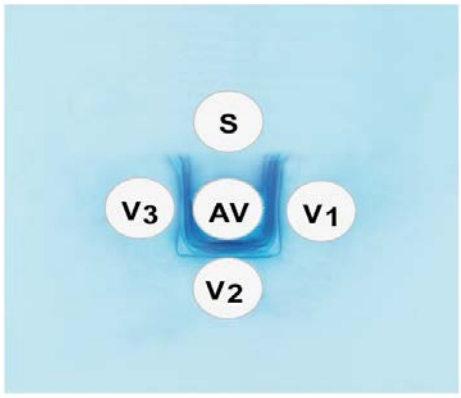
Exposure to 1.5 KGy or 3 KGy gamma radiation did not affect the immunological properties of the crude venom, same number of visible lines joined smoothly at the corner was observed with the non-irradiated, as well as, the two dose levels gamma irradiated (1.5 and 3 KGy) *Echis Coloratus* snake venom **(Figure 1).** These visible lines were identical, continuous and joined smoothly at the corners, indicating that there was no change in antigenic determinants.

The most efficient treatment known until the present moment for accidents caused by venomous snakes is the antivenin. Venoms employed for immunizations are fairly toxic and some venoms present low immunogenicity. Thus, having modified antigens with lower toxicity and preserved or improved immunogenicity would be useful.

The result of double immunodiffusion test of native, 1.5 KGy and 3 KGy gamma irradiated *Echis Coloratus* venoms against the commercial polyvalent Egyptian antivenin, showed similar reactivity. The visible lines obtained in the immunodiffusion reaction were identical, continuous and joined smoothly at the corners, indicating that there was no change in antigenic determinants, i.e. the antivenin cannot distinguish between the native, 1.5 KGy and 3 KGy gamma irradiated venoms, as they are immunologically identical. So, ionizing radiation has proven to be a powerful tool to attenuate snake venoms toxicity without affecting their immunogenic properties.

Shaaban (2003) performed the double immunodiffusion test for non-irradiated and 15 KGy gamma irradiated Naja haje and Cerastes cerastes venoms against a commercial polyvalent Egyptian antivenin , the tested venoms showed similar patterns; the visible lines obtained in the immunodiffusion reactions were identical and join smoothly at the corners, indicating that there was no change in antigenic determinents. Moreover, ***Ferreira Junior et al. (2005)*** observed that the optical density values in the ELISA test for the several dilutions of native and 2000 Gy irradiated venoms of *Bothrops jararaca, Bothrops jararacussu and Bothrops moojeni* did not show statistical difference between the groups studied. Serum titers produced with native venom were similar to those produced with irradiated venom. This means that these venoms before and after irradiation immunogenically identical.

Also, ***Shaaban et al., (2010)*** tested using the double immunodiffusion the immunogenic reactivity of the native, 1, 1.5 and 3 kGy γ irradiated Echis pyramidum venoms to commercial horse polyvalent Egyptian antivenin and all tested venoms showed similar reactivity



S = Saline
AV = polyvalent antivenin
V1 = Native *Echis Coloratus* venom
V2 = 1.5KGy *Echis Coloratus* venom
V3 = 3KGy *Echis Coloratus* venom

Figure (2): Immunodiffusion reaction of horse serum polyvalent Antivenin with Native, 1.5 KGy and 3 KGy Gamma Irradiated *Echis Coloratus* Venoms.

3-Phospholipase A_2 (PLA_2) activity of native and (1.5 KGy and 3 KGy) gamma irradiated *Echis Coloratus* snake venom

Phospholipase activity of *Echis Coloratus* snake venom decreased after irradiation. The specific phospholipase A_2 activity of native, 1.5 and 3 KGy gamma irradiated venom was 6.25, 4.8 and 2.7 U/mg; respectively **(Table 2 and Figure 3).**

Venom phospholipases A_2 catalyze the hydrolysis of the sn-2-acyl bond of glycerophospholipids in a calcium-dependent fashion to release free fatty acids and lysophospholipids. These reaction products may display direct biological activities or may be transformed into other active compounds with hemostatic, cardiotoxic, convulsant, hemolytic, hypotensive, hepatotoxic, myotoxic and neurotoxic activities ***(Dessen, 2000; Kini, 2003; Fuly et al., 2004; França et al., 2009).***

The phospholipase A_2 specific activities of native, 1.5 KGy and 3 Kgy gamma irradiated venoms was 13, 7.5 and 5.5 U/mg respectively.

These results are in agreement with results of ***Shaaban (2003)*** who showed that gamma irradiation of *Naja haje* and *Cerastes cerastes* venoms leasds to decrease PLA_2 activity in both venoms. Also, ***Bennacef-Heffar and Laraba-Djebari, (2003)*** tested the effect of gamma irradiation on Vipera lebetina snake venom and they found that the phospholipase A_2 activity was abolished in the irradiated venom with a dose of 2 KGy.

Table (2): In Vitro Phospholipase specific activity (U/mg) of native, gamma irradiated (1.5 and 3 KGy) venom:

Group	Specific activity (U/mg)
	Phospholipase A_2 activity
Native venom	6.25
(1.5 KGy) ɣ Irradiated venom	4.8
(3 KGy) ɣ Irradiated venom	2.7

Figure (3): Effect of native, (1.5 and 3 KGy) gamma irradiated venom on phospholipase A_2 enzyme specific activity of *Echis Coloratus* snake venom.

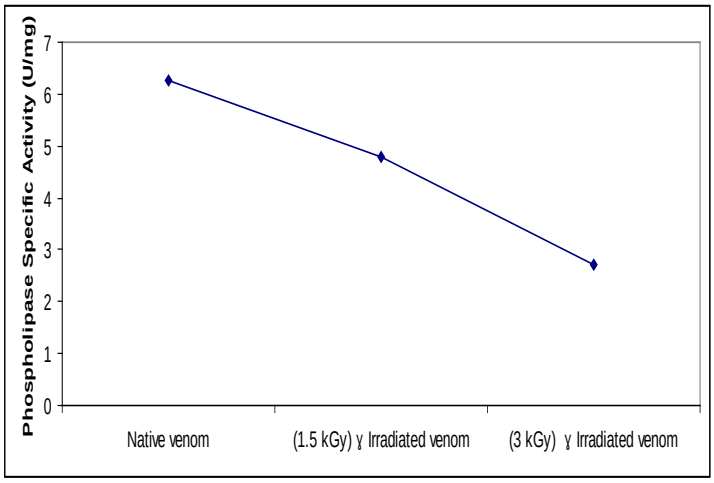
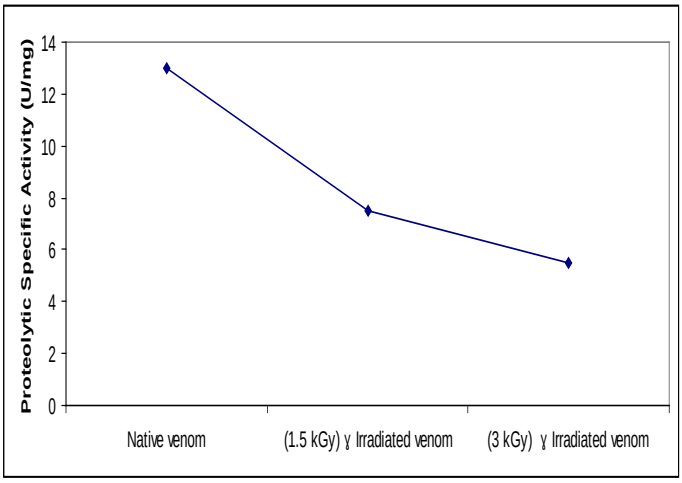


Table (3): In Vitro Proteolytic specific activity (U/mg) of native, gamma irradiated (1.5 and 3 KGy) venom:

Group	Specific activity (U/mg)
	Protease activity
Native venom	13
(1.5 KGy) ɣ Irradiated venom	7.5
(3 KGy) ɣ Irradiated venom	5.5

Figure (4): Effect of native, (1.5 and 3 KGy) gamma irradiated venom on proteolytic specific activity of *Echis Coloratus* snake venom.



4-Proteolytic activity of native and gamma irradiated (1.5 KGy and 3 KGy) *Echis Coloratus* snake venom

Proteolytic activity of *Echis Coloratus* snake venom showed a significance decrease after irradiation. The proteolytic specific activity of native, 1.5 and 3KGy gamma irradiated venom was 13, 7.5 and 5.5 U/mg; respectively **(Table 3 and Figure 3).**

Proteases are abundant in snake venoms, particularly of the *Viperidae* family, and may constitute up to 20% of total venom proteins. Proteolytic enzymes can be divided into two main groups: metalloproteases and serine proteases, which affect the hemostatic system and different steps of the coagulation cascade through different mechanisms ***(White, 2005).***

The proteolytic specific activity of native, 1.5 and 3 KGy gamma irradiated venom was 13, 7.5 and 5.5 U/mg; respectively.

This was in agreement with ***Guarnieri (1992)*** used 2,000 Gy gamma rays to detoxify *Bothrops jararaca* venom; it was the necessary dose to get the detoxification through attenuation of proteolytic activity with maintenance of immunological properties. Also, ***Shaaban (2003)*** reported the inhibition in the proteolytic activity of *Cerastus cerastes* and *Naja haje* venoms by 15 KGy gamma irradiation.

5- Rat's hind paw edema induced by native and (1.5 KGy and 3 KGy) gamma irradiated snake venom

The sub-plantar injection of 0.1 ml of native (1 mg/ml), 1.5 KGy (1 mg/ml) and 3 KGy (1 mg/ml) gamma irradiated snake venom into the right hind paw resulted in marked edema led to an increase in the paw diameters by 41.8, 29.7 and 21 % as compared to the initial paw diameter, after 30 min. The edema formed accompanied by apparent hematoma in case of native and 1.5 KGy gamma irradiated venom **(Table 4 and figures 6, 7 and 8)**.

The sub-plantar injection of 0.1 ml of native (1 mg/ml), 1.5 KGy (1 mg/ml) and 3 KGy (1 mg/ml) gamma irradiated snake venom into the right hind paw resulted in marked edema led to an increase in the paw diameters by 29.1, 23.5 and 9.5 % as compared to the initial paw diameter, after 2 h. **(Table 4).**

The sub-plantar injection of 0.1 ml of native (1 mg/ml) and 1.5 KGy (1 mg/ml) gamma irradiated snake venom into the right hind paw resulted in marked edema led to an increase in the paw diameters by 4.8 and 2.2 % as compared to the initial paw diameter, after 4 h. But, 3 KGy (1 mg/ml) gamma irradiated snake venom failed to produce any marked edema, after 4 h. **(Table 4).**

Table (4): Effect of native, gamma irradiated (1.5 KGy and 3 KGy) *Echis Coloratus* snake venom on the rat hind paw edema after ½, 2 and 4 h

Parameter / Group	% of edema after ½ an hour	% of edema after 2 hour	% of edema after 4 hour
Normal(non-envenomed)	–	–	–
Native venom	41.8±0.21 [a]	29.1±0.45 [a]	4.8±0.08 [a,b]
(1.5KGy) ɣ Irradiated venom	29.7±0.1 [a,b]	23.5±1.83 [a]	3.2±0.16 [a,b]
(3KGy) ɣ Irradiated venom	21±1.23 [a,b]	9.5±0.2 [b]	0.4±0.04 [b]

60 male albino rats weighing (180-200 gm) were selected for the study and divided into 10 groups, 6 rats each. Each sample was injected into sub-plantar region of the right hind paw. The paw diameter was measured using vernier caliper initially and at ½, 2 and 4 h. after sample administration. The percent of induction of edema in the rat hind paw was calculated from the formula: $(D_f - D_i/D_i) \times 100$

Each value represents Mean ± S.E.

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

[a] Significant difference from normal group ($P \leq 0.05$)

[b] Significant difference from native group ($P \leq 0.05$)

Edema is a normal response of the body to inflammation or injury ***(Noma et al., 2014)***. *Echis Coloratus* as a viper induces edema and alters vascular permeability resulting in local hemorrhage and hematoma. Native *Echis Coloratus* snake venom induced edema after venom injection; peak volume was achieved after 30 min and percent edema reached 41.8 % from the initial diameter of the rat paw, and decreased gradually within 4 h. Gamma irradiated (1.5 KGy and 3 KGy) *Echis Coloratus* snake venom induced edema but less in severity than of the native one; peak volume was achieved after 30 min and percent edema reached 29.7 and 21 % respectively, from the initial diameter of the rat paw, and decreased gradually within 4 h.

Al Asmari (2003) reported that sub- planter injection of *Echis coloratus* snake venom caused intense hemorrhage and marked paw edema compared to saline- injected paws. ***Abib and Laraba-Djebari (2003)*** studied the effects of ^{60}Co gamma radiation on edema-forming activities of *Cerastes cerastes* venom and found that the edematic and hemorrhagic activities were reduced in the detoxified irradiated samples, particularly with the 2 KGy radiation dose.

There are many inflammatory mediators which participate in the production of edema in a variety of inflammatory conditions ***(Posadas et al., 2000).*** The expected principal mediators of this inflammatory response produced by *Echis Coloratus* snake were serotonin, histamine, cyclo-oxygenase and prostaglandins (PGs) and cytokines. This was verified by ***Al-Asmari (2003)*** who tested the effect of various drugs on edema induced by the *Echis Coloratus* snake. The results showed that (H_1 and H_3) receptor antagonist (cyproheptadine) and histamine (H_1) receptor antagonist (chlorpheniramine) produced significant inhibition of edema formation, indicating the role of mast cell and histamine in this inflammatory process. PGs inhibitors (dexamethasone), Cyclo-oxygenase inhibitor (indomethacin) produced significant inhibition of edema formation.

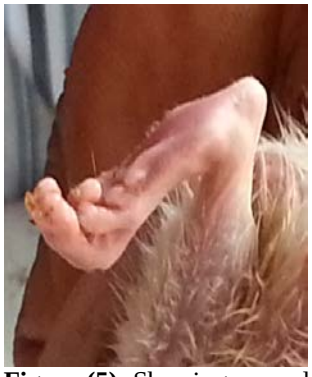


Figure (5): Showing normal non-envenomated rat hind paw.

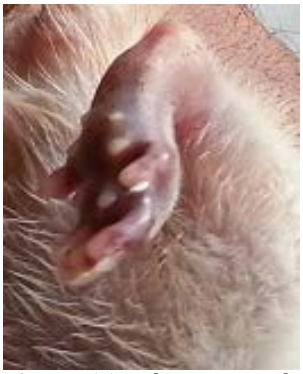


Figure (6): Showing rat hind paw edema after 30 min with hematoma after sub-planter injection of native *Echis Coloratus* venom.

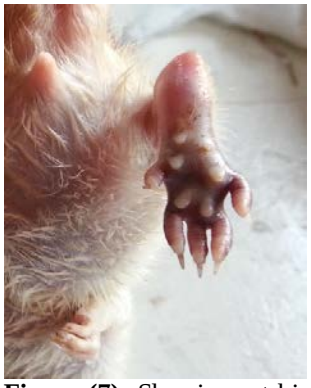


Figure (7): Showing rat hind paw edema after 30 min with hematoma after sub-planter injection of (1.5KGy) ɣ Irradiated *Echis Coloratus* venom**.**

Figure (8): Showing rat hind paw edema after 30 min after sub-planter injection of (3KGy) ɣ Irradiated *Echis Coloratus* venom.

6- Coagulant properties for native and (1.5 and 3KGy) gamma irradiated venom (Plasma recalcification time)

The clotting time of native, 1.5 and 3 KGy gamma irradiated venom at 200 µg/ml was 4.8, 16.8 and 26.6 seconds. The clotting time of native, 1.5 and 3 KGy gamma irradiated venom at 1 µg/ml was 49.4, 101.6 and 127.8 seconds **(Table 5, Figure 9).** The coagulant properties of *Echis Coloratus* snake venom was concentration dependant. As the concentration of the snake venom was increased, the clotting time was reduced indicating that the crude venom has a coagulant effect. The two doses of gamma irradiation (1.5 KGy and 3 KGy) decreased the coagulant activity of the venom.

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Moav et al. (1963) reported that *Echis Coloratus* venom contains both coagulant and anti-coagulant components. Also, ***Kornlik and Blomback (1975)*** reported that *Echis Coloratus* venom is a potent coagulant. ***Rogero and Nascimento (1995)*** used gamma rays to detoxify *Bothrops jararaca* venom and noticed attenuation of coagulant activities.

This results was in agreement with the results reported with ***Al-Saleh et al. (2002)*** who investigated the effect of *Echis Coloratus* crude venom on blood coagulation and it showed a concentration dependant strong coagulant effect, as the concentration of the snake venom was increased, the clotting time was reduced. Moreover, ***Al-Sadoon and Fahim (2012)*** tested the effect of LD_{50} of *Echis Coloratus* venom in male albino rats on blood parameters; platelet count recorded a significant rise compared to the control within one hour.

Also, ***Lucas de Oliveira et al. (2007)*** examined the anti-coagulant activity of *Bothrops jararaca* venom in vivo, goats were divided into two groups; one group inoculated with 0.5mg/Kg of crude venom and another group inoculated with 0.5mg/Kg of irradiated venom. The native venom was anticoagulant and recorded a clot formation after 55 min after one day of inoculation, while the clotting time was reduced to only 10 min after one day of inoculation by the irradiated venom.

Most vipers have been shown to have strong coagulant activity, this is may be due to indirect effects on blood components through stimulation of epinephrine release and/or direct effects on blood components especially those acting at earlier steps may yield normal thrombin and thus activate platelets ***(Bertke and Atkins, 1961).*** Since platelets can facilitate coagulation, effects of venoms on platelets may explain in part the ability of the venoms to induce thrombosis and coagulopathies such as disseminated intravascular coagulation ***(Mukherjee et al., 2000)***. According to ***(Tans and Rosing, 2001)*** the coagulopathy effect seen by *Echis Coloratus* venom could act through pro-coagulant activation of prothrombin and blood factor X.

Table (5): Effect of different concentration of native, (1.5 and 3 KGy) gamma irradiated venom on the clotting time of the plasma:

Venom (µg/ml) / Group	Clotting time in seconds						
	200	**100**	**50**	**25**	**10**	**2.5**	**1**
Normal(non-envenomed)	167.8±0.58	167.8±0.58	167.8±0.58	167.8±0.58	167.8±0.58	167.8±0.58	167.8±0.58
Native venom	4.8±0.21*	11.4±0.35*	20.2±0.56*	28.4±0.45*	35.8±0.32*	42.2±0.44*	49.4±0.68*
(1.5KGy) ɣ Irradiated venom	16.8±0.46*	28.2±0.79*	33.4±067*	41.6±0.81*	90.6±0.37*	94.8±0.65*	101.6±0.56*
(3KGy) ɣ Irradiated venom	26.6±0.33*	35.4±0.46*	44.6±0.46*	52±0.39*	100.4±0.28*	123.2±0.92*	127.8±0.83*

Each value represents Mean ± S.E

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

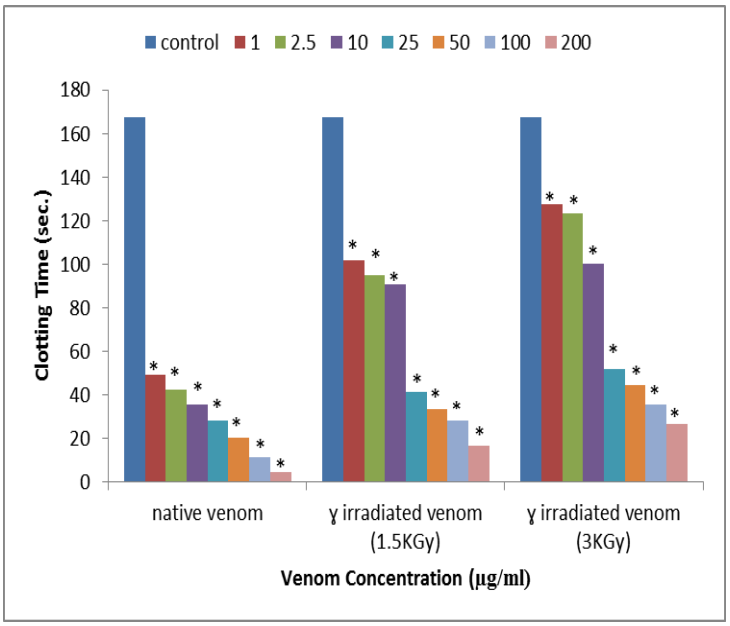

Figure (9): Effect of different concentration of native, 1.5 KGy and 3 KGy gamma irradiated *Echis Coloratus* snake venom on the clotting time of the plasma in seconds.

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

IV. CONCLUSION

This study shows that gamma radiation is a venom detoxification method that keeps the immunological aspects and this help to solve the toxic problems of using native venom in immunizing animals and have the advantage of extend the lifespan of the animals. So, this study confirms that gamma radiation is a powerful tool for having effective, safe and cheaper antivenin using the crude venom.

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