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EVALUATION OF TOXIC ACTIVITY LOSS AND MAINTENANCE OF IMMUNOLOGICAL PROPERTIES OF CROTOXIN AFTER GAMMA IRRADIATION

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ABSTRACT

Snake venoms are complex mixture consisting mainly of proteins, peptides, carbohydrate, lipids, nucleotides, amino acids e inorganic compounds. The main toxic components are proteins and enzymes.
Crotoxin is a β-neurotoxin that can act pre or post synoptically. It is composed for two sub units: The crotapotin a 9 kDa (acidic sub unit) and a 13 kDa phospholipase A_2 (basic sub unit).
The protein irradiation, in aqueous solution, has showing studied to cause chemical changes, physics chemistry alterations and disorder of secondary and tertiary structure of proteins. The main products generated from water radiolysis are hydroxyl radicals ($OH^{\cdot}$) and hydrated electron ($e^{-}_{aq.}$). In the present report, crotoxin from used to has been investigate the structure alterations caused by ionizing radiation, as well as, the loose of toxicity and immunological properties. In this case, the crotoxin was irradiated with 400, 2000 and 10000 Gy in the presence or absence of "scavengers". Our results in uv spectra indicate that irradiation leads to progressive changes in the structure of the toxin. The LD 50 presents a decreasing of toxicity when the toxin was submitted to gamma rays effects ionizing radiation. This results suggest that hydrated electrons are related with lost of toxicity. On the other hand, the immunological properties are preserved either 2000 Gy doses and when, just, hydroxyl radicals are presented in solution, showing the importance of participation this free radical. Maintenance crotoxin immunological properties.

1. INTRODUCTION

Crotoxin is a β-neurotoxin that can act pre or post synoptically. It is composed for two sub units: The crotapotin (acidic sub unit) with 9 kDa and phospholipase A_2 (basic sub unit) with 13 kDa. This toxin present highly specific toxicity toward neuromuscular junction. At this level, the crotoxin acts primarily by causing a triphasic change (depression, facilitation and final blockage) of acetylcholine the motor nerve terminal. Secondarily, crotoxin induces a postsynaptic blockage of neuromuscular by stabilizing a desensitized state of nicotinic receptor to acetylcholine [(1)].
The irradiation of proteins in aqueous solution generally results in a loss or decrease of biological activity. This fact may be ascribed to the interaction of free radicals generated during water radiolysis with the protein, as well as to direct action of the ionizing radiation upon the protein. The direct action the ionizing radiation interacts directly on target of

biomolecules and the indirect effects occur when the products generated during water radiolysis interact with molecules target. In addition, the main species formed, with income respectively, for 100 eV of absorbed energy (G value), are: [2]

$$H_2O \rightarrow 2{,}7\ e^-_{aq.} + 0{,}45\ H_3O^+ + 3{,}2\ OH^- + 0{,}6\ H^+ + 0{,}45\ H_2 + 0{,}7\ H_2O_2 \qquad (1)$$

Thus, the two main products are: hydrated electron and hydroxyl radical. Both of them have similar action, leading hydrogen abstraction of alpha carbon in relationship with carboxylic[3]. Some substances can be used with "scavenger", these substances react with radiolysis products, protecting the molecule[4].
The species more frequently employed, to hydrated electron, are nitrate ions and to hydroxyl radicals, are the alcohols, more specifically the t-butanol alcohol[5].
In present report, crotoxin was irradiated in the presence or absence "scavenger" substances in order to investigate the effects of gamma radiation on the toxin structure of toxin, toxicity and immunological properties.

2. MATERIALS AND METHODS

2.1. Crotoxin irradiation

Crotoxin was dissolved in a 0,15M NaCl solution to a 2mg/mL final concentration, with or without "scavengers" and irradiated with 400, 2000 and 10000 Gy doses with 5,17 kGy/h dose rate using gamma rays emitted by a ^{60} Co source (Gamma Cell 220 Canada). The "scavengers" employed were t-butanol and sodium nitrate.

2.2. Ultra violet spectra of crotoxin

The native and irradiated crotoxin, with or without "scavengers", were submitted to UV scanning from 200 to 360 nm. The blank employed for baseline subtraction consisted of 0,15M NaCl.

2.3. Toxicity analyses LD 50% of crotoxin

To verify the toxic activity from native and irradiated crotoxin, with or without "scavengers", it was carried out a lethal doses 50% test, which analyzes the capacity of toxin killed 50% of animals. The lethal dose used was 0,06 μg/g of animal as described by AIRD (1985)[6]. And to evaluate the lower of toxicity it was used 1, 5 and 10 amounts of LD 50 value. The animals' mortality was observed for 48hs after injection.
The animals employed were Swiss male mice from IPEN with body mass 20 g.

2.4. Antibodies production

Specific antibodies anti native and anti irradiated crotoxin, in presence or absence of "scavenger", were produced by immunizing swiss mice with native or irradiated crotoxin in the presence or absence of "scavenger" following the immunization classical protocol [7]. Blood samples were collected and after centrifugation, the plasma was separated and frozen.

2.5. Enzyme linked immunosorbent assay

Plasma collected from immunized animals, with native or irradiated, crotoxin in the presence or absence of "scavenger" were analyzed by Elisa [8]. The dilution of plasmas was 1/1000 in phosphate saline buffer. For negative control, plasma was collected before immunization. The microplates were analysed at 450 nm.

3. RESULTS

In order to evaluate the conformational alterations the crotoxin in the presence or absence of "scavenger", before and after irradiation were performed analyze of u.v. spectra. Analyzes was carried out the lost of toxicity was detected by LD 50 assay, and antibodies production, evaluated by using enzyme linked immunosorbent assay (ELISA).
Ultraviolet spectra of native and irradiated crotoxin, with or without "scavengers", are showed in the figures 1, 2, 3 and 4. Results that present exposition of chromophores at irradiated toxins.

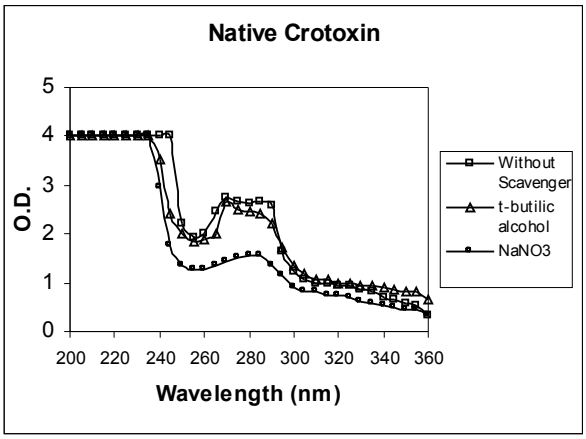

Figure 1. Ultraviolet spectra of native crotoxin with or without "scavenger".

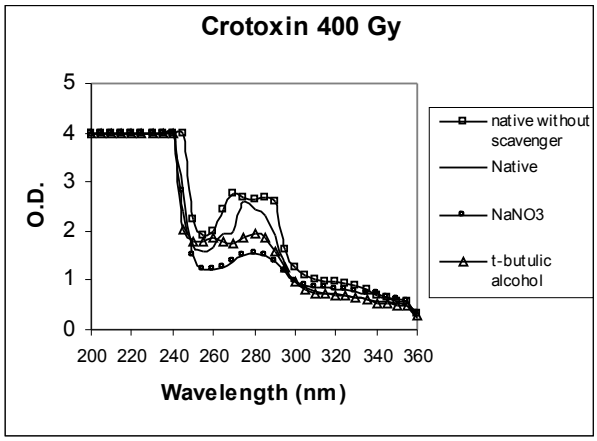

Figure 2. Ultraviolet spectra of native and 400 Gy irradiated crotoxin with or without "scavenger".

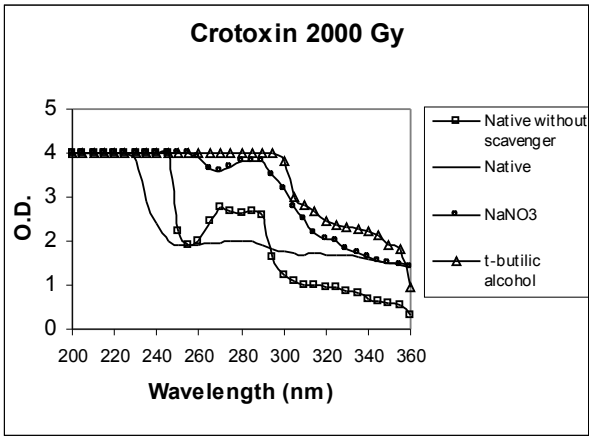

Figure 3. Ultraviolet spectra of native and 2000 Gy irradiated crotoxin with or without "scavenger".

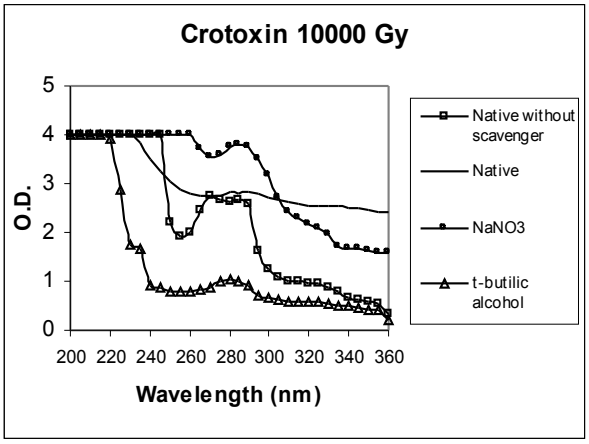

Figure 4. Ultraviolet spectra of native and 10000 Gy irradiated crotoxin with or without "scavenger".

Activity of native and irradiated crotoxin, with or without "scavenger", are presented at figures 5, 6, 7 and 8. These data showe a decreasing of mortality, suggesting the effectivity of radiation to detoxity.

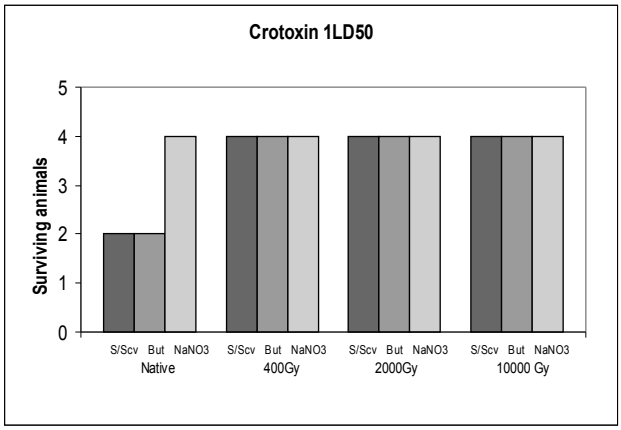

Figure 5. Toxic activities of native and irradiated crotoxin, with or without "scavenger", 1 LD50.

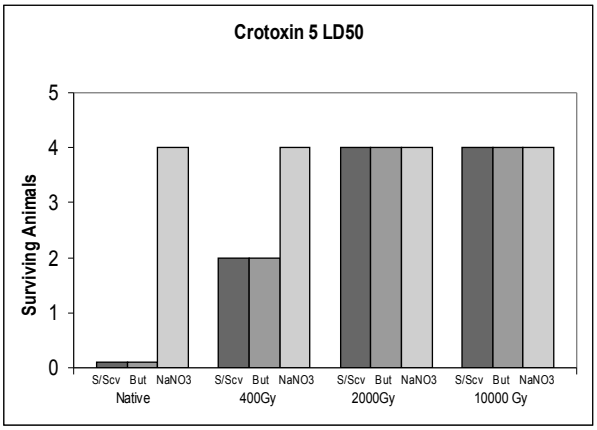

Figure 6. Toxic activities of native and irradiated crotoxin, with or without "scavenger", 5 LD50.

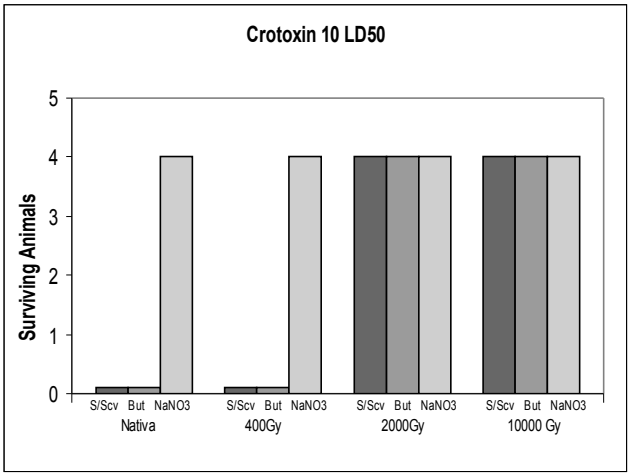

Figure 7. Toxic activities of native and irradiated crotoxin, with or without "scavenger", 10 LD50.

In the figure 8 are presented the results of analyze of antibodies producted by native and 2000 Gy irradiated crotoxin in presence or absence of "scavengers". These results suggest a improve of immunological response with irradiated form of crotoxin.

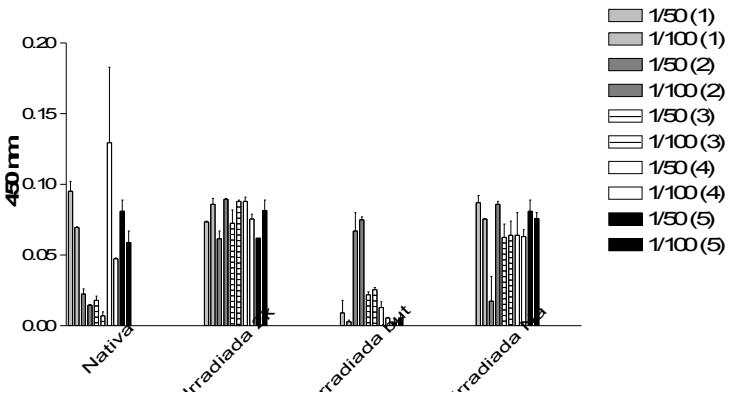

Figure 8. Antibodies production analyzes of native and irradiated crotoxin (2000 Gy) with or without "scavengers".

4. DISCUSSION

The uv spectra of native crotoxin in the presence of sodium nitrate "scavengers" did not presented significant alterations. However in t-butanol presence, it has been diminution of absorbance. Irradiation low doses (400Gy) in the presence of "scavengers" showed a decreasing of absorbance in all spectra. Two thousand Gray irradiated toxin and irradiated one with sodium nitrate presented an important increase of absorbance. And with 10000 Gy doses radiation showed increase of absorbance just without "scavengers". These data to suggest that high radiation doses can promote unfolding of protein.

Toxic activity with 1LD 50% (0,06 μg crotoxin/ animal g) demonstrated mortality of two animals only with a native form of toxin and 400 Gy radiation doses without "scavenger"and t-butanol. Other radiation doses did not showed mortality. With 5 LD 50% (0,30 μg crotoxin/ animal g), all the animals died with native form of crotoxin and two animals with 400 Gy radiation doses without "scavengers" and t-butanol presence. With 2000 and 10000 Gy doses radiation did not have mortality.

At last, with 10 LD 50% (0,60 μg crotoxin/ animal g) all animals died with native form and 400 Gy radiation doses, without "scavengers" and t-butanol presence. Newly did not mortality occurred with 2000 and 10000 Gy radiation doses. These data confirming the loss of the toxic activity of toxin in agreement with results of Baride. (1980)[9].

The antibodies production presented important results for 2000 Gy doses (Figure 8). When the toxin is in the native form, the data demonstrated a great heterogeneity. In presence of "scavenger" the results presented a bigger homogeneity. Can be verifying a decreasing of antibodies production, in the presence of t- butilic alcohol. These data allows suggest a direct participation of hydroxyl radical in antibodies production and consequently in immunological properties.

5. CONCLUSION

In the present report was verified that gamma radiation caused crotoxin structure alteration. The major effects observed were unfolding of polypeptides chain. The "scavengers" demonstrated to be effectiveness in the molecule protection, mainly the t-butanol. No mortality was observed when the animals received 2000 and 10000 Gy irradiated crotoxin. The produced antibodies products demonstrated a great improve with toxin irradiated with 2000 Gy. In conclusion, the gamma radiation has demonstrated effectiveness in abolishing toxic activity and could be useful to produce toxoids and vaccines.

ACKNOWLEDGMENTS

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