EFFECTS OF GAMMA RADIATION ON BEE VENOM: PRELIMINARY STUDIES

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ABSTRACT

Africanized honeybees are very common insects in Brazil and frequently cause accidents followed by important immunological reactions and even deaths. Their venoms are composed of a complex mixture of substances of general biological actions. Several works utilizing ionizing radiation showed that it is able to modify protein structures, and successfully detoxify snake venoms toxins, although maintaining its immunological properties. The main objective of this paper was to study the effects of gamma radiation on bee venom, regarding some biochemical and toxicological aspects. Africanized *Apis mellifera* whole venom (2 mg/ml) in 0.15M NaCl solution was irradiated with 2 kGy in a ^{60}Co source. Preliminary studies has been carried out in order to identify some biochemical changes after irradiation. Concerning this, irradiated and native venom were submitted to a molecular exclusion chromatography (Sephadex G-100), UV absorption spectrum and protein concentration analysis. It could be seen that irradiated bee venom spectrum presented differences when compared to native bee venom, suggesting that some structural alterations has occurred. Protein concentration and chromatography profiles were not changed after irradiation. In order to evaluate the toxicity a lethality assay (LD_{50}) has been performed with both venoms, and irradiated venom showed to be less toxic than native one.

Key words: bee venom, gamma radiation, toxicity.

I. INTRODUCTION

Africanized honeybees (*Apis mellifera*) have a high colony defensive behavior and now they are prevalent in South and Central America and Mexico being very common insects in Brazil. Their venoms are a complex mixture of substances of general biological actions, presenting a wide range of high and low molecular weight toxins and biologically active amines. They can present a threat to individuals regardless of allergy status [1]. It has been estimated that toxic reactions from bee stings may occur with as few as 50 stings [2,3]. Toxic reactions include vomiting, diarrhea, shock, hemoglobinuria, and renal failure [4,5]. Many works have been done, with snake venom toxins, trying to obtain a less toxic product that preserves its immunological properties, improving horse antisera production, extending immunized animals useful life. Regarding to this several chemical and physical methods have been used [6,7,8,]. Among these techniques, ionizing radiation showed to be a very promising tool in detoxifying snake venoms although maintaining their immunological properties. Previous works carried out in our laboratory showed that 2 kGy dose was the most effective one regarding these aspects [9,10,11]. Most of the damages caused by radiation on proteins in water solution are indirectly, consisting of the formation of free radicals such as OH°, H , e^{-}_{aq}, H_2O_2 and other species from the water radyolisis. These free radicals can react with polypeptide chains inducing alteration in protein primary, secondary and tertiary structure [12,11]. This structural changes are related with the modifications of toxic properties of venoms. The main objective of this paper was to study the effects of gamma radiation on bee venom, regarding some biochemical and toxicological aspects.

II. MATERIAL AND METHODS

Bee venom was obtained by eletrostimulation, and all venom was obtained in a single day. It was supplied by CEIS/CEVAP-UNESP-Rio Claro/SP. The venom was solubilized in the appropriate buffer, centrifuged at 10,000 rpm, for 15 minutes at 4°C and the supernatant used as crude venom.

Swiss mice used in the experiments were obtained from the colony housed in Department of Bioengineering at IPEN/CNEN-USP-SP.

Protein Determination

Protein concentration was estimated by the Lowry Method, modified by MILLER [13], using bovine serum albumin to build a standard curve.

Bee Venom Irradiation

Crude venom was irradiated in saline solution (0.15M NaCl) at a concentration of 2mg/ml, with a 2 kGy dose. Gamma rays were derived from a ^{60}Co source Gammacell (Atomic Energy Agency of Canada Ltd), in presence of O_2, at room temperature, with dose rate of 800 Gy/h. After irradiation samples were immediately submitted to biological and biochemical assays.

UV-Absorption Spectrum

Crude native or irradiated bee venom in a concentration of 1mg/ml in saline solution (0.15M NaCl) were recorded on a Ultrospec III spectrophotometer, using a range of UV wavelengths from 230 to 350nm. Absorption spectra were automatically registered and absorbance were obtained using saline solution (0.15M NaCl) as blank.

Size Exclusion Chromatography

Native or irradiated bee venom, in a concentration of 2mg/ml, in a total volume of 10 ml was applied to a 2.6 x 70 cm column of Sephadex G-100 (Pharmacia), previously equilibrated in 5 mM ammonium acetate buffer, pH 6.8. Proteins were eluted in the same buffer at a flow rate of 12 ml/h. Fractions of 3 ml were collected, at 4 °C and protein elution profile was monitored by absorbance at 280 nm.

Tricine-SDS- PAGE

Slab electrophoresis was performed following the method described by SCHÄGGER & JAGON [14], using a discontinuous system. Low molecular weight markers were obtained by Sigma Chemical Company USA. After running, proteins were stained with Coomassie Brilliant Blue G-250 (Sigma).

Lethality assays

Toxic activities of native and irradiated venoms were evaluated by the determination of LD_{50} in mice, calculated by the Spearman-Karber Method [15].

Male Swiss mice (18-22g), were divided in groups of 5 animals and were injected intraperitoneally (i.p)., with 200 µl of native or irradiated venom in 7 different doses, using a dilution factor of 1.5. Doses were calculated from protein concentrations of both venoms. Control group were injected i.p., with 200 µl of saline solution (0.15M NaCl). Survival was registered 48 hours after injection.

III. RESULTS AND DISCUSSION

The content of venom protein was estimated before and after irradiation, in order to verify if there was protein lost and it could be seen that no difference was detected, since native protein content was 2 mg/ml and irradiated one was 1.97 mg/ml

Irradiated venom had a higher absorption in all wavelengths when compared to native one (Figure 1). This suggests that irradiation may has caused changes in protein secondary and tertiary structures due to a higher exposition of peptides chromophore groups [16].

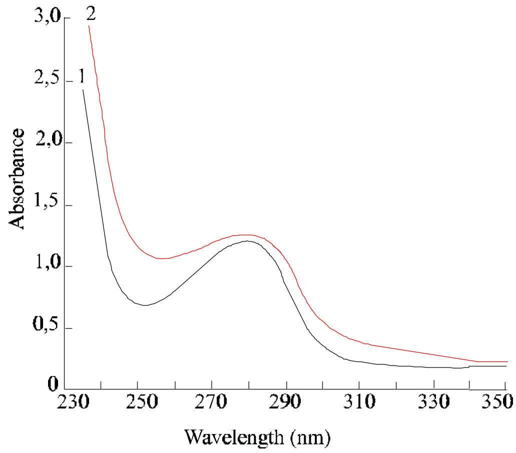

Figure 1. Absorption spectra of crude bee venom native (1) and irradiated (2) in saline solution (0.15M NaCl).

There are many peaks in chromatographic profiles from native and irradiated venoms (Figure 2), and one that presents the highest absorbance, which correspond to a molecular weight of approximately 4 kDa, probably, containing melittin and other peptides, that comprises more than 60% of the total protein in africanized bee venom. When irradiated venom profile was compared to native one it could not be observed any remarkable change. In order to verify if there is changes, both venoms will be submitted to a high performance liquid chromatography, under reverse phase (HPLC-RP).

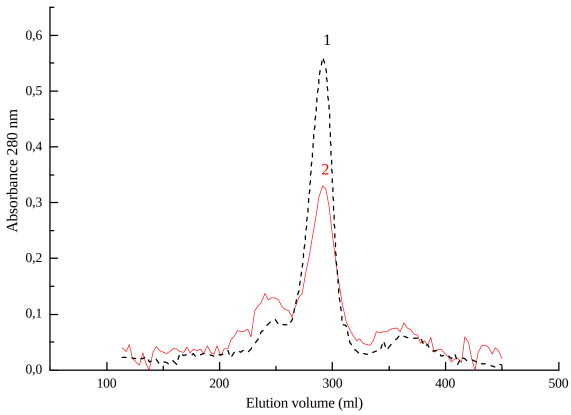

Figure 2. Chromatography profile of 2mg/ml native (1) and irradiated (2) bee venom using Sephadex G-100, eluted in 5mM ammonium acetate buffer pH 6.8. Proteins were monitored by absorbance 280 nm.

The tricine-SDS-PAGE, using a discontinuous system has been adopted because it provides a good resolution for low molecular weight proteins, that are present in a great amount in *Apis mellifera* venom. Electrophoretic profiles of native and irradiated bee venom are shown in Figure 3. No clear change could be observed in both profiles.

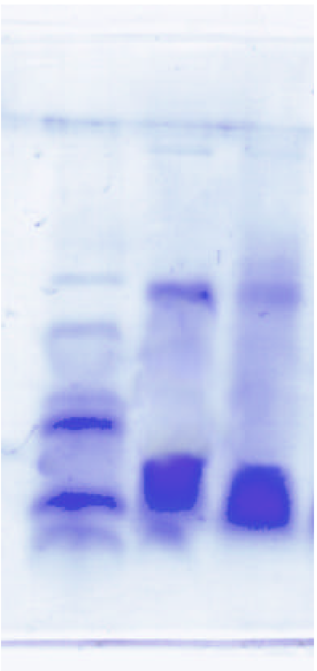

Figure 3. Native and irradiated bee venom electrophoresis profile. A-Molecular markers: myoglobin: 17 kDa, myoglobin: 14.4 kDa, myoglobin: 10.6 kDa myoglobin: 8.2 kDa, myoglobin: 6.2 kDa, glucagon: 3.5 kDa; B- native venom and C- irradiated venom.

The lethality of honeybee venom was addressed by several investigators and the results varied dramatically in early studies. Thus, lethality values varied from 3,5 to 6mg/kg depending on venom batch [17]. SCHMIDT [17] and SCHUMACHER **et al.** [18] when injected africanized *Apis mellifera* venom in mice using i.p. or intravenous (i.v.) route obtained LD_{50} value of 2.0mg/kg,. Nevertheless all this values were calculated, considering crude dry weight venom in mg per kg of animal. For this assay native venom LD_{50} in mice, after i.p. injection was 5.97 mg/kg (Table 1) based on protein concentration. The results obtained here show that irradiated venom is 2.2-fold less toxic than native. This confirms that gamma radiation can cause changes in the components of bee venom, providing its detoxification. These data are in agreement with those obtained previously [10,11,19,20,21,22,23] where snake venoms and fractions presented a reduced toxicity when submitted to gamma irradiation.

TABLE- 1 Native and Irradiated Honeybee Venom Lethality Obtained in Mice by I.p.Injection

	Native		Irradiated	
Dose (mg/kg)	Nº of deaths	LD_{50}([a]CI 95%) mg/kg	Nº of deaths	LD_{50}([a]CI 95%) mg/kg
1.84	0/5		0/5	
2.76	0/5		0/5	
4.14	1/5		0/5	
6.2	4/5	5.9(4.2 - 8.4)	0/5	13.4 (10.1-17.9)
9.3	4/5		1/5	
14	4/5		2/5	
21	5/5		5/5	

[a] Confidence intervals.

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