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Effects of gamma irradiation on allergen compounds and lethality of bee venom in a hamster animal model

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Introduction

Honey bee venom has long been accepted for use in the treatment of many diseases. In vitro and in vivo studies have provided substantial insights into the biological activities of the distinct components of honey bee venom and highlighted their potential for treating inflammatory diseases. Moreover, abundant pieces of evidence have indicated their positive effects on a variety of human diseases such as viral and microbial infections, neurodegenerative diseases, diabetes mellitus, and cancer, in addition to inflammatory disorders. Unprocessed honey bee venom has allergenic compounds and people's sensitivity to bee venom is different, so it is difficult to determine the optimal dose for treatment. New knowledge of honey bee venom safety is of great importance for clinical practitioners to avoid the negative aspects and hazardous consequences of this fundamental bee product. Accordingly, different procedures have been proposed to detoxify venoms. Among these techniques, gamma irradiation is considered one of the most effective methods for attenuating venom toxicity besides retaining its immunological properties intact. Hence, this study, for the first time, focuses on evaluating the effects of gamma irradiation on the reduction in the allergen compounds of honey bee venom. Gamma irradiation can be used to chang the structure of bee venom allergens and changing their function. This study was done to investigate the effects of gamma irradiation on lethal dose 50% (LD50) and allergen compounds of honey bee venom.

Methods

Venom samples were irradiated at doses of 0,2,4,6 and 8 kGy by the Gammacell 220 Cobalt 60 irradiation facility. Malondialdehyde level and true protein concentration were determined pre- and post-irradiation by Thiobarbituric acid assay and Bradford assay Respectively. Protein subunits of venom were detected by polyacrylamide gel electrophoresis In the Lumley method. Allergen compounds were measured using HPLC technique. The chromatogram was recorded at a wavelength of 214 nm with a flow intensity of 1 mL/min. A lethal dose of 50% (LD50) was determined using in vivo trial. Eighty hamsters were allocated to 5 treatments and 4 replicates in a CRD design. Venom solution at a dose of 0.5, 0.75, 1, and 2 mg/Kg BW was injected intra peritoneal and mortality recorded then LD50 was computed by Spearman–Karber method. In the final of study, hamsters liver samples were collected and fixed in 10% formalin. Liver samples were fixed in formalin 10%, blocked with paraffin wax, cut into 5-µm sections using a senior rotary microtome (Leica Rm 2255, Germany), and stained with hematoxylin and eosin. The sections were observed under a high-power light microscope (Nikon E100) equipped with a digital camera and Dino capture version 2 software. Data were statistically analyzed by GLM procedure of SAS (SAS Institute, 2008) software in a completely randomized design.

Result & Discussion*:* The results showed that true protein content and malondialdehyde level in irradiated samples had no difference from the control group ($P>0.05$). Electrophoresis patterns and HPLC results showed that irradiation at doses of 4 and 6 kGy decreased phospholipase amount and increase the low subunits of protein ($P<0.05$). Irradiation at doses of 6 and 8 kGy increased LD50 by 34%. This increase in the LD50 after gamma irradiation of the honey bee venom at 4, 6, and 8 kGy indicates that irradiation by reducing the amount of allergen compounds including PLA2 decreases venom toxicity. These results were in accordance with other studies who reported a dose-dependent rise in the LD50 after gamma irradiation and a reduction in toxicity of venom. Based on the histology results, irradiation of honey bee venom at doses of 4 and 6 kGy could decrease the inflammation of hepatocytes and vein hyperemia in the liver ($P<0.05$). Ionizing rays lead to changes in the function and integrity of biomolecules, including proteins. Exposure to low doses of ionizing radiation leads to changes in the first, second and third structures of proteins. Irradiation at a dose of 6 kGy can be applied to reduce the toxicity of bee venom by removing allergen factors.

Conclusion: The use of gamma irradiation with doses of 6 and 8 kGy increased the lethal dose of bee venom by 50%, but considering that the dose of 8 kGy caused the breakdown of heavy subunits and increased the light subunits of bee venom and had negative effects on the liver tissue, it can be concluded that dose of 6 kGy is suitable for reducing the toxicity of bee venom. In the continuation of this research, it is necessary to study other anticancer and antiviral properties of irradiated honey bee venom.

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