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Research Article

Effect of gamma irradiation on biochemical properties of Egyptian honey bee venom *Apis mellifera lamarckii*

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Abstract. Honey bee venom is a protein-rich secretion whose biochemical properties can be altered when exposed to ionizing radiation. Controlled irradiation has been proposed as a method for reducing allergenicity and improving handling while maintaining core biological characteristics. This study examined how moderate gamma irradiation (4 and 6 kGy) affects total protein concentration and the activities of six enzymatic components phospholipase A_2 (PLA_2), hyaluronidase, superoxide dismutase (SOD), acid phosphatase (ACP), phosphodiesterase (PDE), and acetylcholinesterase (AChE) in venom from *Apis mellifera lamarckii*. Protein quantification and native PAGE demonstrated a dose–response pattern, with minimal alterations at 4 kGy and more evident reduction at 6 kGy. Enzyme assays revealed a clear biphasic response: activities increased at 4 kGy but declined at 6 kGy. These findings indicate that moderate irradiation can modulate the biochemical activity of venom enzymes in a dose-dependent manner.

Keywords: Bee venom composition, venom proteins, venom enzymes, gamma irradiation

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Introduction

Honey bee venom is a complex natural secretion with a long history of use in traditional medicine and apitherapy (Bava*et al.*, 2023). Its modern pharmacological value lies in its intricate biochemical composition, which contains a rich array of pharmacologically active compounds(Gajski*et al.*, 2024).Bee venom is an aqueous mixture of proteins, peptides, small molecules and enzymes such as phospholipase A_2 (PLA_2), hyaluronidase, Superoxide dismutase (SOD), acid phosphatase (ACP), phosphodiesterase (PDE), and acetylcholinesterase (AChE) (Wehbe*et al.*, 2019; Yaacoub*et al.*, 2023).These components provide a wide range of biological properties, including anti-inflammatory, antimicrobial, anticancer, and immunomodulatory effects, positioning bee venom as a promising candidate for novel therapeutics (Grinn-Gofroń*et al.*, 2025).PLA2 is highly significant as it disrupts cell membranes, leading to cell lysis and inflammation (Darwish *et al.*, 2021).Hyaluronidase acts as a "spreading factor" by breaking down hyaluronic acid (Abdel-Monsef *et al.*, 2020; de Graaf *et al.*, 2021). In cancer treatment, co-administering hyaluronidase with chemotherapy help in drug penetration and enhancing treatment efficacy while allowing for lower drug doses (El-Wahed*et al.*, 2021; Lee & Bae, 2023). SOD is a key antioxidant in bee venom that neutralizes harmful free radicals. This action stabilizes the venom's components and contributes to its anti-inflammatory, antimicrobial, and anticancer properties (Abdel-Monsef *et al.*, 2023).ACP in bee venom breaks down phosphoric acid compounds, beyond defense, it shows antitumor and anti-inflammatory effects, making it a promising candidate for treating chronic diseases and allergies (Abdel-Monsef *et al.*, 2025).PDE modulates inflammatory responses by regulating cyclic nucleotides, this contributing to anti-inflammatory and neuroprotective effects (Hossen*et al.*, 2016).

AChE, which modulates cholinergic signaling, shows neuroprotective potential and suggests therapeutic relevance for neurodegenerative diseases such as Alzheimer's and Parkinson's (Lee *et al.*, 2014; Chen *et al.*, 2022).Gamma irradiation can modulate the biological activity of honey beevenom because ionizing radiation induces dose-dependent chemical modifications (oxidation, limited backbone cleavage and cross-linking) that can reduce allergenicity and acute toxicity while preserving structural fragments suitable for biochemical and immunological analysis (Abbasi *et al.*, 2023). Also, gamma irradiation has emerged as a promising sterilization method (Hussein *et al.*, 2014). Moderate absorbed doses (4 and 6 kGy) were selected on the basis that these doses markedly enhance venom bioactivity compared with non-irradiated venom, while higher doses (≥8 kGy) can produce inconsistent or excessive degradation of protein components (Abbasi *et al.*, 2023). In addition, protein-irradiation studies indicate that doses below ~10 kGy typically produce limited, tractable changes in protein solubility and secondary structure, whereas higher doses cause more extensive oxidation and fragmentation; therefore 4 kGy and 6 kGy provide a pragmatic attenuation window that balances safety/attenuation with retention of venom components. (Hassan *et al.*, 2018; Shawrang*et al.*, 2022; Stanca*et al.*, 2023).This study aims to examine how gamma irradiation at 4 kGy and 6 kGy influences the biochemical characteristics of *Apis mellifera lamarckii* venom. Specifically, the work evaluates changes in total protein content and the activities of key venom enzymes PLA_2, hyaluronidase, SOD, ACP, PDE, and AChE following exposure to moderate irradiation doses known to modify bioactivity without fully degrading protein structure. By comparing irradiated and non-irradiated venom, the study seeks to clarify how controlled irradiation can modulate biological properties relevant to therapeutic and bioprocessing applications.

Materials and methods

Collection of Honey bee Venom

Honey bee venom from *Apis mellifera lamarckii* was obtained from colonies maintained at the Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt. Venom was collected from multiple healthy colonies to minimize colony-specific variation and to ensure that the final sample represented the typical biochemical profile of this subspecies. Colonies selected for sampling were comparable in strength, age structure, and disease-free status, based on routine inspection.Venom extraction was carried out using a standardized electrical stimulation technique (de Graaf *et al.*, 2021). A glass plate covered with a sterile paraffin membrane was positioned at the hive entrance, and a low-voltage pulsed current was applied to induce stinging behavior without causing harm to the bees. Workers deposited venom onto the membrane surface, where it was allowed to dry under ambient conditions, the dried venom was gently scraped from the membrane and collected as a raw powderuntil used for subsequent analyses.

Irradiation Protocol

Gamma irradiation of bee venom samples was carried out using a sealed Cesium-137 gamma cell (Gammacell-40, Atomic Energy of Canada Ltd.) located at the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. The collected venom was portioned into separate aliquots and exposed to 4kGy and 6 kGy**,** delivered at a dose rate of 0.67 kGy/h. An additional aliquot remained unexposed and was used as an untreated control. Two doses, 4 kGy and 6 kGy, were selected because previous studies have shown that irradiation within this range effectively reduces toxicity and microbial load while maintaining the structural and functional integrity of venom proteins. Higher doses such as 8–10 kGy were avoided, as they are known to cause excessive oxidation, fragmentation, and loss of bioactivity in protein-based venoms (Shawrang*et al.*, 2022; Abbasi *et al.*, 2023). All treated and untreated samples were stored at −20 °C until biochemical analysis.

Chemicals

Phosphatidylcholine, phenol red, hyaluronic acid, cetyltrimethylammonium bromide, Cytochrome C, xanthine sodium salt, xanthine oxidase, p-Nitrophenyl phosphate (p-NPP), Bis(p-nitrophenyl) phosphate, Acetylthiocholine iodide (AcSChI), 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) and chemicals for electrophoresis were purchased from Sigma-Aldrich Chemical Co. The other chemicals were of analytical grade.

Protein determination

Protein concentration was determined using the Bradford method (1976), with bovine serum albumin (BSA) employed as the standard (Goldring, 2012; Nielsen, 2024).

Native polyacrylamide gel electrophoresis (PAGE)

Polyacrylamide gels consist of polymerized acrylamide chains cross-linked by a bifunctional agent, typically N,N′-methylenebisacrylamide. Native gel electrophoresis separates proteins based on both their size and charge. The pore size of the acrylamide gel acts as a molecular sieve, while proteins with higher net charge at the gel's pH exhibit greater mobility (Arndt*et al.*,2012; Li & Arakawa, 2019).

Gel Documentation

Gel images were captured using the Syngene InGenius3 Gel Documentation System (Syngene, UK), equipped with a high-resolution 3-megapixel CCD camera and a manual zoom lens (6.5–39 mm, f/1.4). White light illumination was used for imaging Coomassie-stained gels inside the system's integrated darkroom to minimize ambient light.GeneSys software was used for image acquisition, and GeneTools software was employed for band intensity analysis.

Enzymatic Activity Assays

The enzymatic activities within the honey bee venom samples were evaluated using established spectrophotometric procedures. All assays were conducted in triplicate to ensure statistical reliability, with mean values and standard errors reported. The activity of (PLA2, hyaluronidase, SOD, ACP, PDE and AChE) enzymes were determined using a spectrophotometer of JASCO, Tokyo, Japan, as follows:

1- Phospholipase A2 (PLA2) Activity

Phospholipase A2 activity was determined by monitoring the acidification resulting from the hydrolysis of phosphatidylcholine, following an adapted turbidimetric procedure (Darwish *et al.*, 2021). The reaction medium was prepared to a final volume of 2.5 mL in 7.5 µmol Tris/HCl buffer (pH 7.9), containing 15 µmol phosphatidylcholine as the substrate, 18 µmol Triton X-100, 5 µmol$CaCl_2$ as an essential cofactor, and 80 µmol phenol red as a pH indicator. Following the measurement of the initial absorbance at 558 nm, the reaction was initiated by adding the venom sample. The mixture was subsequently incubated for 60 minutes at 37°C, after which the final absorbance was recorded. The enzymatic activity, calculated from the decrease in absorbance, was defined in units (U), where one unit corresponds to the amount of enzyme that catalyzes the complete hydrolysis of 1 µmol of phosphatidylcholine per hour under the assay conditions.

2- Hyaluronidase Activity

Hyaluronidase activity was assessed using a turbidimetric method based on the degradation of hyaluronic acid (Abdel-Monsef *et al.*, 2020). The assay mixture, with a total volume of 0.5 mL, consisted of 0.2 M acetate buffer (pH 5.5) containing 0.15 M sodium chloride, 50 mg of hyaluronic acid, and the venom sample. After a 15-minute incubation at 37°C, the reaction was terminated by adding 1.0 mL of a stopping solution containing 2.5% cetyltrimethylammonium bromide in 2% sodium hydroxide. The turbidity generated by the complex between undegraded hyaluronic acid and the stopping reagent was measured at 400 nm. Enzyme activity is inversely related to the resulting turbidity. One Turbidity Reducing Unit (TRU) is defined as the amount of enzyme that hydrolyzes 50% of the hyaluronic acid substrate, leading to a 50% reduction in turbidity.

3- Superoxide Dismutase (SOD) Activity

The activity of Superoxide Dismutase was evaluated by measuring its capacity to inhibit the superoxide-mediated reduction of cytochrome C (Abdel-Monsef *et al.*, 2023). The superoxide radicals were enzymatically generated in a 1.0 mL reaction system containing 20 mmol L^{-1} potassium phosphate buffer (pH 7.8) with 0.1 mM EDTA, 0.01 mM cytochrome C, and 0.05 mM sodium xanthine. The reaction was started by adding 21 milliunits of xanthine oxidase. The rate of cytochrome C reduction was tracked by the increase in absorbance at 550 nm. One unit of SOD activity is defined as the quantity of enzyme required to achieve 50% inhibition of the cytochrome C reduction rate under the specified conditions.

4- Acid Phosphatase (ACP) Activity

Acid Phosphatase activity was quantified by measuring the hydrolysis of p-nitrophenyl phosphate (p-NPP) to p-nitrophenol (p-NP) (Abdel-Monsef *et al.*, 2025). A reaction mixture containing 0.8 mL of 20 mmol L^{-1} sodium acetate buffer (pH 5.0), 0.1 mL of 0.05 M p-NPP, and 0.1 mL of the venom sample was incubated at 37°C for 15 minutes. The reaction was stopped with 1 mL of 0.1 M sodium hydroxide, and the released p-nitrophenol was measured at 405 nm using a JASCO V-730 spectrophotometer. One unit of ACP activity is defined as the amount of enzyme that liberates 1 µmol of p-nitrophenol per minute.

5- Phosphodiesterase (PDE) Activity

Phosphodiesterase activity was assayed using bis(p-nitrophenyl) phosphate as the substrate (Balestrieri*et al.*, 2020). The 1 mL reaction system contained 50 mM Tris-HCl buffer (pH 8.5), 1 mM substrate, and 5 mM $MgCl_2$. Following a 30-minute incubation at 37°C, the reaction was terminated with 1 mL of 0.5 N sodium hydroxide. The amount of p-nitrophenol released was determined by measuring the absorbance at 410 nm. Enzyme activity was calculated and expressed as µmol of p-nitrophenol generated per minute per milliliter. Blank reactions without the enzyme were used to correct for non-enzymatic hydrolysis.

6- Acetylcholinesterase (AChE) Activity

Acetylcholinesterase activity was determined spectrophotometrically according to the method of Ellman*et al.* (1961). The 1.0 mL assay system contained 60 mM Tris-HCl buffer (pH 8.0), 1 mM 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), 1 mM acetylthiocholine iodide, and the enzyme solution. After a 30-minute incubation at 37°C, the increase in absorbance at 412 nm, resulting from the reaction of DTNB with thiocholine, was recorded. One unit of AChE activity is defined as the amount of enzyme that hydrolyzes 1 µmol of acetylthiocholine iodide per minute, using a molar extinction coefficient of 13.6 $mM^{-1}{\cdot}cm^{-1}$ for the 5-thio-2-nitrobenzoate anion (Sawires*et al.* 2025).

Statistical analyses

The data underwent analysis using one-way analysis of variance (ANOVA). If variances were detected among treatments, means were compared using Tukey's test with the assistance of Minitab statistical software (Minitab, Coventry, UK). Significance was established at a level of $P< 0.05$.

Results

Protein determination in Honey bee venom

Analysis of total protein content revealed a measurable reduction in protein levels following gamma irradiation (Table. 1). The non-irradiated control venom exhibited the highest protein concentration (12.98 ± 0.235 mg/mL). Exposure to 4 kGy produced a moderate decrease (12.23 ± 0.241 mg/mL), which was not statistically distinct from the control. In contrast, the 6 kGy treatment resulted in a lower protein concentration (11.14 ± 0.220 mg/mL) compared with the control ($P< 0.05$). These findings indicate a dose-dependent reduction in total protein, with the higher irradiation dose.

Electrophoretic analysis of honey bee venom protein on PAGE

The native polyacrylamide gel electrophoresis (PAGE) profile of honey bee venom proteins provided a visual representation of the changes induced by gamma irradiation, corroborating the measured protein data (Fig. 1). In the venom sample irradiated at 4 kGy (Lane 2), the banding pattern remained largely similar to the control; however, a slight decrease in the intensity of the bands was observed, consistent with the measured reduction in total protein content. A more pronounced effect was evident in the 6 kGy sample (Lane 3). This lane showed a reduction in the intensity of bands. The protein profiles of honey bee venom before and after irradiation were analyzed using native polyacrylamide gel electrophoresis (PAGE), and the band intensities were assessed with the Syngene InGenius3 Gel Documentation System software. The results are summarized in (Fig. 2). By comparing intensity of lanes, control without irradiation (a) and 4kGy (b) intensity almost the same but 6kGy (c) shows lower intensity.

Table (1): Effect of Gamma irradiation on the total protein concentration of the honey bee venom

Bee venom	Protein mg/ mL ± SE
Venom, Non-Irradiated (Control)	12.98 ± 0.235 [a]
Venom, Irradiation dose 4 kGy	12.23 ± 0.241 [ab]
Venom, Irradiation dose 6 kGy	11.14 ± 0.220 [b]

* Each value represents the mean of a triplicate test ± SE
* Different letters indicate statistically significant differences at $P< 0.05$

Fig. 1: Electrophoretic analysis of honey bee venom protein before and after radiation exposure on 7% native polyacrylamide gel; protein pattern, (lan1) control without irradiation, (lan2) 4kGy and (lan3) 6kGy.

Enzymes measurements (PLA2, hyaluronidase, SOD, ACP, PDE and AChE) in honey bee venom

The specific activities of six key enzymes in honey bee venom Phospholipase A2 (PLA2), Hyaluronidase, Superoxide dismutase (SOD), Acid Phosphatase (ACP), Phosphodiesterase (PDE), and Acetylcholinesterase (AChE) following gamma irradiation are detailed in (Table 2). Gamma irradiation produced distinct, enzyme-specific alterations in venom bioactivity. Phospholipase A_2 (PLA_2) activity increased markedly at 4 kGy, showing an elevation compared with both the control and 6 kGy groups ($P< 0.05$). Hyaluronidase activity followed a similar pattern, reaching its highest level at 4 kGy, while the 6 kGy dose caused a decline but remained higher than the control. Superoxide dismutase (SOD) activity increased with irradiation, with the 6 kGy showing the greatest level relative to the other groups. Acid phosphatase (ACP) activity was maximal at 4 kGy and decreased at 6 kGy, falling below the control level. For phosphodiesterase (PDE), only the 4 kGy dose produced an increase, whereas the 6 kGy treatment did not differ from the control. Acetylcholinesterase (AChE) activity also peaked at 4 kGy, while the 6 kGy dose caused a reduction comparable to the control. Collectively, these results demonstrate a biphasic response in which moderate irradiation (4 kGy) increases several enzymatic activities, whereas higher exposure (6 kGy) tends to decrease the activity (Fig. 3).

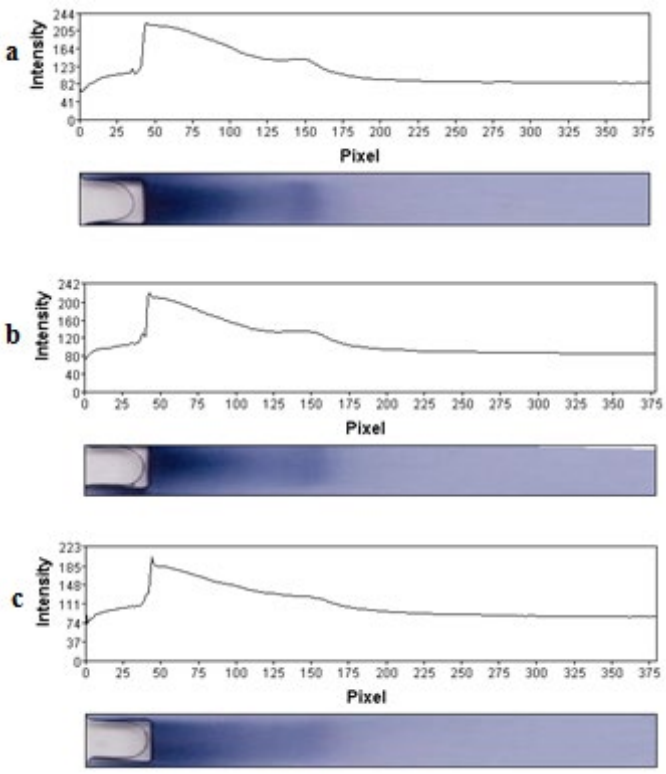

Fig. 2: Analysis of native PAGE protein gel electrophoresis of honey bee venom protein before and after irradiation exposure by Syngene Ingenius3 Gel Documentation System software, (a) control without irradiation, (b) 4kGy and (c) 6kGy.

Discussion

The present study demonstrates that gamma irradiation at moderate absorbed doses produces measurable, dose-dependent alterations in the biochemical profile of *Apis mellifera lamarckii* venom, with changes that are broadly consistent with patterns reported for irradiated proteinaceous toxins and enzyme-rich biological extracts. The modest reduction in total protein content observed at 4 kGy, followed by a more pronounced decrease at 6 kGy (Table 1), aligns with other studies showing that doses below approximately 10 kGy generally induce limited yet detectable oxidative modifications and partial fragmentation of venom proteins (Hassan *et al.*, 2018; Shawrang *et al.*, 2022). The statistically decline at 6 kGy is therefore expected, as several studies demonstrate similar reductions in protein stability and solubility when enzyme-rich materials are subjected to escalating irradiation doses (Stanca *et al.*, 2023). The PAGE analysis further corroborates these trends; the largely preserved pattern at 4 kGy, compared with the decreased intensity at 6 kGy (Fig. 1 and 2), corresponds well with observations from irradiated snake venom (Nascimento *et al.*, 1998; Bennacef-Heffar & Laraba-Djebari, 2003; El-Missiry *et al.*, 2010; Mohamed *et al.*, 2023) and scorpionvenom (Abib & Laraba-Djebari, 2003; Mohamed *et al.*, 2011).

Table (2): Enzymes activity measurements (PLA2, Hyaluronidase, SOD, ACP, PDE and AChE) in honeybee venom.

Bee venom	PLA2	Hyaluronidase	SOD	ACP	PDE	AChE
	Specific activity is (U / mg protein ± SE)					
Venom, Non-Irradiated (Control)	237 ± 3.15 ^{c}	47.1 ± 0.96 ^{c}	221 ± 2.26 ^{c}	176 ± 1.91 ^{b}	8.12 ± 0.21 ^{b}	129 ± 2.22 ^{b}
Venom, Irradiation dose 4 kGy	328 ± 2.96 ^{a}	55.2 ± 0.84 ^{a}	234 ± 2.19 ^{b}	188 ± 1.77 ^{a}	8.67 ± 0.19 ^{a}	136 ± 2.38 ^{a}
Venom, Irradiation dose 6 kGy	279 ± 3.22 ^{b}	53.6 ± 0.94 ^{b}	257 ± 2.47 ^{a}	159 ± 1.93 ^{c}	7.93 ± 0.28 ^{b}	124 ± 2.43 ^{b}

*The specific activity is expressed as units / mg protein.
*Each value represents the mean of a triplicate test ± SE
*Different letters within each enzyme column denote statistically significant differences at $P< 0.05$.

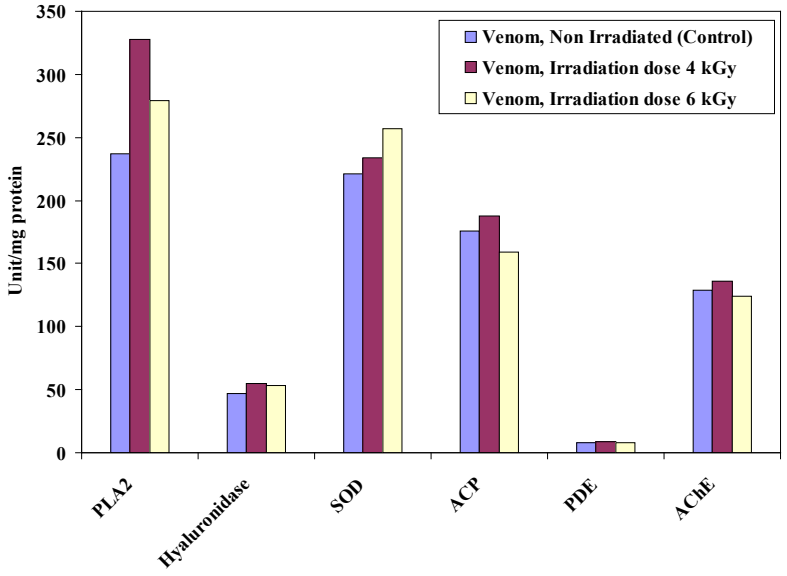

Fig. 3: Enzymes activity measurements (PLA2, Hyaluronidase, SOD, ACP, PDE and AChE) in honey bee venom.

The changes observed in protein pattern were confirmed by Gel Documentation System software analysis (Fig. 2). The dose-dependent modulation of enzymatic activities also reflects patterns reported for irradiated venom enzymes (Samy *et al.*, 2018). Enzymes activity elevation at 4 kGy (Table 2) is consistent with the notion that moderate irradiation doses preferentially induce side-chain oxidation rather than backbone cleavage. In contrast, the larger reductions at 6 kGy (Fig. 3) mirror findings in phospholipases, hyaluronidases, and esterases subjected to comparable irradiation levels (Moussa, 2008; Aly *et al.*, 2016; Rodacka *et al.*, 2016). Our findings contribute to the evidence that controlled gamma irradiation can modify the total protein content and enzymatic activity of honey bee venom in a predictable and dose-dependent manner. By integrating protein measurements, PAGE analysis, and enzymes activity assays, this work provides an experimentally supported foundation for investigations on the potential bioprocessing uses of *Apis mellifera lamarckii* venom. A task for the future, comprehensive structural, immunological, and microbiological assessment will be required for irradiation to be fully incorporated into standardized venom-processing protocols.

Conclusion

The present work provides experimentally supported evidence that gamma irradiation induces measurable, dose-dependent biochemical changes in *Apis mellifera lamarckii* venom. Total protein concentration, PAGE band intensity, and the activities of PLA_2, hyaluronidase, SOD, ACP, PDE, and AChE all showed varying degrees of sensitivity to irradiation. Enzymes activity shows a biphasic response where a moderate irradiation (4 kGy) increases enzymatic activities, and higher exposure (6 kGy) tends to decreases these activities.

Author's Contributions

Mohamed M. Abdel-Monsef: Conceptualization, Supervision, Project administration, Methodology, Investigation, Visualization, Formal analysis, Draft preparation, Final review and edit. **Doaa A. Darwish**: Conceptualization, Methodology, Visualization, Investigation, Draft preparation, Final review and edit, Supervision and Project administration. **Sameh G. Sawires**: Conceptualization, Methodology, Visualization, Investigation, Final review and edit. **Hind A. Zidan**: Conceptualization, Identification, Methodology, Visualization, Investigation, Final review and edit.

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Data Availability Statement

All data generated or analyzed during this study are included in this published article.

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Ethics Approval and Consent to Participate

Insects were used in this study. All applicable international, national, and institutional guidelines for the care and use ofanimals were followed. This article does not contain any studies with human participants performed by the authors.

Conflict of Interest

The authors have no conflict of interest or competing interests, personal or financial to declare.

Generative AI statement

The authors declare that no Gen AI was used in the creation of this manuscript.

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Research Article

اثراشعه گاما برخواص بیوشیمیایی زهر زنبور عسل مصری *Apis mellifera* lamarckii

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چکیده: زهر زنبور عسل یک ترشح غنی از پروتئین است که خواص بیوشیمیایی آن می‌تواند هنگام قرار گرفتن در معرض تابش یونیزان تغییر کند. تابش کنترل‌شده به عنوان روشی برای کاهش آلرژی‌زایی و بهبود نحوه‌ی استفاده از آن در عین حفظ ویژگی‌های بیولوژیکی اصلی پیشنهاد شده است. این مطالعه بررسی کرد که چگونه تابش گاما با دوز متوسط (۴ و ۶ کیلوگری) بر غلظت کل پروتئین و فعالیت شش جزء آنزیمی فسفولیپاز A_2 (PLA_2)، هیالورونیداز، سوپراکسید دیسموتاز (SOD)، اسید فسفاتاز (ACP)، فسفودی‌استراز (PDE) و استیل کولین‌استراز (AChE) در زهر زنبور عسل *Apis mellifera* lamarckii تأثیر می‌گذارد. تعیین مقدار پروتئین و الکتروفورز بومی، الگوی دوز–پاسخ را نشان دادند، به‌طوری‌که در دوز ۴ کیلوگری (kGy) تغییرات حداقلی مشاهده شد، اما در ۶ کیلوگری کاهش آشکارتری دیده شد. آزمون‌های آنزیمی یک پاسخ دو مرحله‌ای واضح را آشکار کردند؛ به این صورت که فعالیت آنزیم‌ها در ۴ کیلوگری افزایش یافت، اما در ۶ کیلوگری کاهش پیدا کرد. این یافته‌ها نشان می‌دهد که تابش دوز متوسط می‌تواند فعالیت بیوشیمیایی آنزیم‌های زهر را به صورت وابسته به دوز تعدیل کند.

کلمات کلیدی: ترکیب زهر زنبور، پروتئین‌های زهر، آنزیم‌های زهر، تابش گاما

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