

Fractionation and enzymes activity measurement of the scorpion *Androctonus crassicauda* (Scorpionida: Buthidae) venom

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Abstract

The scorpion of *Androctonus crassicauda* is one of the Buthida family members whose neurotoxic venom is deadly to humans. Enzymes are abundant components of animal venom that play roles in local and systemic symptoms following envenoming. The venom of this scorpions contains various pathological enzymes, among which studies have reported the toxic effects of venom's hyaluronidase and phospholipase A2 enzymes. This study aimed to measure the activity of venom enzymes of hyaluronidase and phospholipase A2 from scorpion of *A. crassicauda* and its fractions. For this purpose, the venom from scorpion of *A. crassicauda* were collected and lyophilized. The whole venom was fractionated on a gel filtration (Sephadex G-50) column. Then protein concentration, hyaluronidase and phospholipase A2 activity of the crude venom and its fractions were determined. Gel filtration on Sephadex G-50 and SDS-PAGE electrophoresis of *A. crassicauda* crude venom revealed four protein picks and 15 protein bands with the majority of molecular masses between 10.0 and 15.0 kDa, respectively. The supernatant obtained from crude venom clarification showed protein content 71.80%, high hyaluronidase (92.40%) and low PLA2 (302±2.1 mg/U) enzyme activity. Fraction 1 showed highest hyaluronidase and phospholipase A2 activities. We found that pH 5 is the optimum pH for hyaluronidase activity and phospholipase A2 activity increases with increasing incubation time. In conclusion, venom of *A. crassicauda* displayed the hyaluronidase and phospholipase A2 enzymes activity, which were separated by gel filtration chromatography in fraction I. Such information is important both for predicting the biological activity of venom components and useful for developing effective antivenoms based on these venom components.

Key words: *Androctonus crassicauda*, Hyaluronidase, Phospholipase A2, Gel filtration chromatography, SDS-PAGE chromatography

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References

- Amozegari, Z., Abyaz, S., Nour Behbahani, M., & Mohammadi, A. (2016). Measurement of Hyaluronidase Enzyme Activity in Venom of Iranian *Vipera Lebetina*. *Jundishapur Scientific Medical Journal*, 15(3), 363-370.
- Batista, C. V. F., Román-González, S. A., Salas-Castillo, S. P., Zamudio, F. Z., Gómez-Lagunas, F., & Possani, L. D. (2007). Proteomic analysis of the venom from the scorpion *Tityus stigmurus*: biochemical and physiological comparison with other *Tityus* species. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 146(1-2), 147-157.
- Bradford MM. (1997). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal Biochem*. 72(1-2):248-54.
- Cala-Riquelme, F., & Colombo, M. (2011). Ecology of the scorpion, *Microtityus jaumei* in Sierra de Canasta, Cuba. *Journal of Insect Science*, 11(1), 86.
- Dehghani, R., Vazirianzadeh, B., Nasrabadi, M. R., & Moravvej, S. A. (2010). Study of scorpionism in Kashan in central of Iran. *Pak J Med Sci*, 26(4), 955-8.
- Díaz-García, A., Ruiz-Fuentes, J. L., Yglesias-Rivera, A., Rodríguez-Sánchez, H., Garlobo, Y. R., Martínez, O. F., & Castro, J. A. F. (2015). Enzymatic analysis of venom from Cuban scorpion *Rhopalurus junceus*. *Journal of venom research*, 6, 11.
- Dorce, V. A. C., da Rocha, M. M. T., Candido, D. M., Nencioni, A. L. A., Auada, A. V. V., Barbaro, K. C., & Lebrun, I. (2018). Influence of different processing techniques on the toxicity and biochemical characteristics of *Tityus serrulatus* scorpion venom. *Toxicon*, 156, 41-47.
- Firoozfar, F., Saghaipour, A., & Jesri, N. (2019). Scorpions and their human mortality report in Iran: a review article. *Iranian journal of public health*, 48(12), 2140.
- Firoozziyan, S., Sadaghianifar, A., Rafinejad, J., Vatandoost, H., & Bavani, M. M. (2020). Epidemiological Characteristics of Scorpionism in West Azerbaijan Province, Northwest of Iran. *Journal of Arthropod-Borne Diseases*, 14(2), 193.
- Gorham, S. D., Olavesen, A. H., & Dodgson, K. S. (1975). Effect of ionic strength and pH on the properties of purified bovine testicular hyaluronidase. *Connective tissue research*, 3(1), 17-25.
- Jafari, H., Salabi, F., Navidpour Sh & Forouzan, A. R. (2020). Phylogenetic and morphological analyses of *Androctonus crassicauda* from Khuzestan Province, Iran (Scorpiones: Buthidae). *Archives of Razi institute*, 75(3), 405.
- Ketelhut, D. F. J., De Mello, M. H., Veronese, E. L. G., Esmeraldino, L. E., Murakami, M. T., Arni, R. K., & Sampaio, S. V. (2003). Isolation, characterization and biological activity of acidic phospholipase A2 isoforms from *Bothrops jararacussu* snake venom. *Biochimie*, 85(10), 983-991.
- Khataminia, A., Jalali, M. R., Jalali, S. M., & Jafari, H. (2020). The Effect of Various Fractions of *Hemiscorpius lepturus* Scorpion (Scorpionida: Hemiscorpiidae) Venom on Hemostatic System in Peripheral Blood of Rats in Comparison to Whole Venom. *Jundishapur Journal of Health Sciences*, 12(3).
- Kodkhodaei Eliadrani, M., Amouzgari, Z., & Hanifi, H. (2006). The activity of phospholipase A2 and hyaluronidase in the venom of the scorpion *Mesobutus opius opius*. *J Kashan Univ Med Sci-Feyz*. 10(4):24-30.
- Morey, S. S., Kiran, K. M., & Gadag, J. R. (2006). Purification and properties of hyaluronidase from *Palamneus gravimanus* (Indian black scorpion) venom. *Toxicon*, 47(2), 188-195.
- Nazari, M., Rooshanfekr, H. A., & Salabi, F. (2023). Isolation, Characterization, and Biological Activity of Phospholipase A2 (PLA2) and Hyaluronidase from Iranian Honey Bee Venom (*Apis Mellifera meda*). *Agricultural Biotechnology Journal*, 15(2), 141-156.
- Nevalainen, T. J., Peuravuori, H. J., Quinn, R. J., Llewellyn, L. E., Benzie, J. A., Fenner, P. J., & Winkel, K. D. (2004). Phospholipase A2 in cnidaria. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 139(4), 731-735.
- Pessini, A. C., Takao, T. T., Cavalheiro, E. C., Vichnewski, W., Sampaio, S. V., Giglio, J. R., & Arantes, E. C. (2001). A hyaluronidase from *Tityus serrulatus* scorpion venom: isolation, characterization and inhibition by flavonoids. *Toxicon*, 39(10), 1495-1504.

- Pukrittayakamee, S., Warrell, D. A., Desakorn, V., McMichael, A. J., White, N. J., & Bunnag, D. (1988). The hyaluronidase activities of some Southeast Asian snake venoms. *Toxicon*, 26(7), 629-637.
- Radmanesh M. (1990). *Androctonus crassicauda* sting and its clinical study in Iran. *J Trop Med Hyg*. 93(5):323-26.
- Ramanaiah, M., Parthasarathy, P. R., & Venkaiah, B. (1990). Purification and properties of phospholipase A2 from the venom of scorpion, (*Heterometrus fulvipes*). *Biochemistry international*, 20(5), 931-940.
- Rodríguez-Ravelo, R., Coronas, F. I., Zamudio, F. Z., González-Morales, L., López, G. E., Urquiola, A. R., & Possani, L. D. (2013). The Cuban scorpion *Rhopalurus junceus* (Scorpiones, Buthidae): component variations in venom samples collected in different geographical areas. *Journal of venomous animals and toxins including tropical diseases*, 19, 1-10.
- Ruppert, EE., Fox, R., & Barnes, RD. (2004). *Invertebrate zoology. 7th ed, Belmont, CA: Thomson-Brooks.*
- Salabi, F., Baghal, M.L., Kordzangene, A.R., & Mohamadian, A. (2023). Production of monovalent antivenom effective against *Androctonus crassicauda* scorpion venom. *Journal of Arak University of Medical Sciences*, 0-0.
- Salabi, F., & Jafari, H. (2023). New insights about scorpion venom hyaluronidase; isoforms, expression and phylogeny. *Toxin reviews*, 42(1), 69-84.
- Salabi, F., & Jafari, H. (2022). Differential venom gland gene expression analysis of juvenile and adult scorpions *Androctonus crassicauda*. *BMC genomics*, 23(1), 636.
- Salabi, F., Jafari, H., Navidpour, H., & Sadr, A.S. (2021). Systematic and computational identification of *Androctonus crassicauda* long non-coding RNAs. *Scientific reports*, 11(1), 4720.
- Salabi, F., & Jafari, H. (2024). Whole transcriptome sequencing reveals the activity of the PLA2 family members in *Androctonus crassicauda* (Scorpionida: Buthidae) venom gland. *The FASEB Journal*, 38(10), e23658.
- Senthilkumaran, S., Meenakshisundaram, R., & Thirumalaikolundusubramanian, P. (2015). Problems and Paradoxes of Animal Toxins and the Heart. In *Heart and Toxins* (pp. 133-149). Academic Press.
- Shahi, M., Habibi-Masour, R., Salehi, M., Ghasemi-Nang, M., Rafizad, E., Abbasi, M., & Hanafi-Bojd, A. A. (2019). Scorpions and scorpionism in Roudan County, southern Iran. *Journal of Arthropod-Borne Diseases*, 13(4), 353.
- Shanaki BavArsad, M., Amoozgari, Z., & Noor Behbahani, M. (2009). Measurement of phospholipase A2 enzyme activity in crude venom and isolated fractions from the venom of *Leptina viper* in Iran. *Jundishapur scientific medical (JSMJ) journal*. 8(3):355-60.
- Sharifinia, N., Gowhari, I., Hoseiny-Rad, M., & Aivazi, A. A. (2017). Fauna and geographical distribution of scorpions in Ilam Province, South Western Iran. *Journal of Arthropod-Borne Diseases*, 11(2), 242.
- Shirmardi, S. P., Gandomkar, M., Shamsaei, M., ZARE, M. A., GHANADI, M. M., Shafiei, M., & Vahidfar, N. (2010). Preparation and biodistribution study of a ^{99m}Tc-labeled toxic fraction of Iranian mesobuthus eupeus scorpion venom. *Iran J Nucl Med*. 18(1):37-44.
- Sutti, R., Tamascia, M. L., Hyslop, S., & Rocha-e-Silva, T. A. A. (2014). Purification and characterization of a hyaluronidase from venom of the spider *Vitalius dubius* (Araneae, Theraphosidae). *Journal of Venomous Animals and Toxins including Tropical Diseases*, 20, 1-7.
- Tibballs, J., A Yanagihara, A., C Turner, H., & Winkel, K. (2011). Immunological and toxinological responses to jellyfish stings. *Inflammation & Allergy-Drug Targets (Formerly Current Drug Targets-Inflammation & Allergy) (Discontinued)*, 10(5), 438-446.
- Topchiyeva, T., & Mammadova, F. Z. (2016). The seasonal activity of hyaluronidase in venom of a honey bee (*Apis mellifera* L. caucasica) in various regions of Azerbaijan. *J. Entomol. Zool. Stud*, 4, 1388-1391.
- Valdez-Cruz, N. A., Batista, C. V., & Possani, L. D. (2004). Phaiodactylipin, a glycosylated heterodimeric phospholipase A2 from the venom of the scorpion *Anuroctonus phaiodactylus*. *European journal of biochemistry*, 271(8), 1453-1464.