



Araştırma Makalesi /Research Article

Haemolymph Biochemistry Parameters of Scorpions: *Aegaeobuthus gibbosus*

Akreplerin Hemolemf Biyokimya Parametresi: *Aegaeobuthus gibbosus*

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Öz

Amaç: Akrepler genellikle tropikal ve tropik altı iklim kuşaklarında yayılış gösterirler. Sudan karaya geçen ilk hayvan gruplarından olan akrepler evrimsel olarak dayanıklıdır. Taşıdıkları zehir iğnesi ile avlanırlar. Akrep zehri yüzyıllardır hastalıkların tedavisinde alternatif tedavi yöntemi olarak kullanılmıştır. Buthidae familyasına ait Anadolu sarı akrebi *Aegaeobuthus gibbosus* (Brulle, 1832) geniş yayılış alanına sahiptir. Halk sağlığı açısından önemli bir türdür. Akreplerde biyolojik veya metabolik aktivite doğrudan hemolemf ile ilişkilidir. Bu çalışmanın amacı, akrep hemolemf oksidan-antioksidan durumları incelenerek bilimsel çalışmalarda kullanılabilir olduğunun gösterilmesidir.

Gereç ve Yöntem: Çankırı ilinden toplanan akrep örnekleri laboratuvar ortamına getirildikten sonra hemolemf dokuları enjektör ile alınmıştır. Hemolemf örneklerinin toplam antioksidan ve toplam oksidan durumları spektrofotometrik olarak incelenmiştir.

Bulgular: Akrep hemolemlerinde TAS değerleri TOS değerlerinden daha düşük çıkmıştır. Oksidatif stres indeks değerleri ise 12.76 olarak tespit edilmiştir.

Sonuç: Akrep zehri birçok medikal alanda kullanılmaktadır. Akrep zehrine ek olarak bu çalışmada hemolemf sıvısının da medikal alanda bir çok hastalığa ilaç geliştirmede kullanılabileceği görülmüştür.

Anahtar Kelimeler: *Aegaeobuthus gibbosus*, hemolemf, TAS, TOS, Akrep

Abstract

Objective: Scorpions are generally distributed in tropical and sub-tropical climatic zones. It is the first animal group to move from water to land, scorpions are evolutionarily hardy. They are hunted with their venom stinger. Scorpion venom has been used as an alternative treatment method for treating diseases for centuries. The Anatolian yellow scorpion *Aegaeobuthus gibbosus* (Brulle, 1832) belonging to the Buthidae family has a wide distribution area. It is an important species for public health. Biological or metabolic activity in scorpions is directly related to hemolymph. This study aims to examine scorpion hemolymph's biochemical parameters and show that it can be used in scientific studies.




Materials and Methods: After the scorpion samples collected from Çankırı province were brought to the laboratory environment, haemolymph tissues were taken with a syringe. The hemolymph samples' total antioxidant level (TAS) and total oxidant levels (TOS) parameters were examined.

Results: TAS values in scorpion haemolymphs were lower than TOS values and oxidative stress index values were determined as 12.76.

Conclusion: Scorpion venom is used in many medical fields. In addition to scorpion venom, this study showed that hemolemf fluid can also be used in the medical field to develop drugs for many diseases.

Key Words: *Aegaeobuthus gibbosus*, haemolymph, TAS, TOS, Scorpion

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INTRODUCTION

Scorpions are one of the first groups of animals to move from water to land. *Paleophonus*, known as the first scorpion genus was lived in the seas¹. The first terrestrial scorpions appeared 420 million years ago in the late Silurian period². Scorpions have not changed much since their first appearance in geological times. The secret of the evolutionary success of scorpions, which have been so durable throughout geological periods, has attracted the attention of scientists³.

Scorpions has an important role in the food chain in nature. They especially contribute to the stabilization of insect populations. Some scorpion species live in very close proximity to humans. Medically important species such as *Androctonus crassicauda* are very important for human health as they tend to enter the interior of houses⁴.

Scorpions are invertebrates in the class Arachnida. They feed as carnivores and neutralize their prey with their venom. Nocturnal scorpions hide in their burrows, rock crevices, or under stones during the day. Some scorpion species are also dangerous for humans. According to the World Health Organisation (WHO), around 5.000 people die annually from scorpion stings.

In the 21st century, cancer ranks high among the main health problems⁵. Classical chemotherapy drugs are used in cancer treatment. These drugs damage healthy cells while fighting cancerous cells. Therefore, the search for new and effective treatment methods to combat cancer is ongoing all over the world. Various toxins in the scorpion venom component are the poisons whose pharmacological aspects are the most investigated and have significant effects on humans^{3,6}. Although scorpion venoms cause poisoning in humans, these toxins are also seen as an important potential source for the discovery and development of new and specific effective drugs by controlled use⁷. In addition, the effects of scorpion haemolymph on some types of cancer are known⁸. Studies have shown that the peptides in the scorpion venom composition have cytolytic and cytotoxic activities, and some peptides in the venom composition also affect the body's immune system by strengthening it⁹.

Aegaeobuthus gibbosus (Brulle, 1832), which has a wide distribution in Türkiye, prefers hot and arid habitats. It is an important species for public

health. Scorpion haemolymph is bright blue. Haemolymph makes up 34% of the scorpion's body weight and does not clot when it leaves the body. Its composition includes proteins, free amino acids, carbohydrates, lipids, and ions such as sodium, potassium, calcium, magnesium, chlorine, copper, iron, manganese, and zinc⁸.

Oxidative stress is a condition that can occur as a result of reactions occurring in cells. Oxidative stress occurring in a cell suppresses oxidant elements (enzymatic or non-enzymatic) by activating antioxidant defense mechanisms. As a result of excessive oxidative stress, undesirable effects can be observed in normal cellular functioning such as cell signaling^{10,11}. This study aimed to investigate the TAS and TOS parameters in the hemolymph fluid of scorpions and to investigate the usability of scorpions as target or non-target organisms in the studies.

MATERIALS AND METHODS

Test organisms

Aegaeobuthus gibbosus (Brulle, 1832) was collected from Ballica locality of Çankırı province in August 2023. The specimens (n=10) were taken from under the stones with the help of pens. The collected scorpions were brought alive to Çankırı Karatekin University Zoology Laboratory in storage boxes. No anesthetic was used during haemolymph collection. The haemolymph of scorpions was drawn into the syringe by puncturing the heart located at the dorsal segments 7-13 (Figure 1).



Figure 1 Haemolymph from a scorpions.

Biochemical parameters of haemolymph

Within the scope of haemolymph biochemistry parameters, total antioxidant status (TAS; RL0017) and total oxidant status (TOS; RL0024) levels were measured spectrophotometrically using the Rel Assay brand commercial kit (Gaziantep, Turkey). TAS level as mmol Trolox equiv/L and TOS level was expressed as $\mu\text{mol H}_2\text{O}_2$ equiv/L. Oxidative stress index (OSI), expressed as the ratio of TOS levels to TAS levels, was also calculated.

Statistical analysis

After entering the haemolymph fluid TAS, TOS, and OSI values into the Microsoft Excel file, data were evaluated using the GraphPad Prism program.

RESULTS AND DISCUSSION

This study is the first study to determine the biochemical parameters TAS, TOS, and OSI of scorpion hemolymph fluid, as far as the current literature is concerned.

Table 1. Biochemistry analysis results of scorpion haemolymph (mean \pm SEM)

Parameter	Value
TAS (mmol Trolox equiv/L)	0.65 \pm 0.07
TOS ($\mu\text{mol H}_2\text{O}_2$ equiv/L)	83.6 \pm 21.64
OSI	12.76 \pm 1.94

Arthropods including scorpions play an important role in the food chain in soil ecosystems¹²⁻¹⁴. In addition, scorpions have been used in medical uses due to the anticancer^{15,16} and antimicrobial¹⁷⁻¹⁹ effects of their venom²⁰. In this study, TAS, TOS, and OSI parameters of haemolymph samples of scorpions collected from nature and adapted to laboratory conditions were investigated. TOS levels were higher than TAS levels in healthy scorpion haemolymph fluid.

CONCLUSION

In scientific studies, especially in ecotoxicological studies, the search for organisms that can be alternatives to model

organisms is being carried out rapidly. The use of invertebrates, which are lower than vertebrates within the framework of the 3R rules, is a popular study model. This study shows that haemolymph tissues of scorpions collected from nature can be used in other studies in addition to their venoms used in medical studies.

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REFERENCES

- Thorell, T., Lindström, G. Sur un scorpion du terrain Silurien de Suede. CR Acad. Sci. Paris, 1884;99, 984.
- Legros, C., Martin-Eauclaire, M.F., Cattaert, D. 1998, The myth of scorpion suicide: are scorpions insensitive to their venom?, J. Exp. Biol. 1998;201 (Pt 18): 2625-36. <https://doi.org/10.1242/jeb.201.18.2625>
- Kayhan, N. Y., Öcal, İ. Ç., Büyükkartal, O. Mesobuthus gibbosus (Scorpiones: Buthidae) Akrep Ham Zehrinin Kanser Hücre Hatları Üzerine Sitotoksik ve Antiproliferatif Etkilerinin Araştırılması. Abant Medical Journal. 2023;12(1), 19-31. <https://doi.org/10.47493/abantmedj.1094466>
- Crucitti, P., Vignoli, V. Gli scorpioni (Scorpiones) dell'Anatolia sudorientale (Turchia), Bollettino del Museo Regionale di Scienze naturali. Torino. 2002; 19 (2), 433-480. <http://dx.doi.org/10.1016/j.toxicon.2015.06.029>.
- Sağlık Bakanlığı Tedavi Hizmetleri Genel Müdürlüğü. Türkiye Onkoloji Hizmetleri Yeniden Yapılanma Programı 2010-2023. Ankara: T.C. Sağlık Bakanlığı. 2010.
- Santibáñez-López CE., Possani L. D. Overview of the Knottin scorpion toxin-like peptides in scorpion venoms: Insights on their classification and evolution. Toxicon. 2015;107: 317-326. <https://doi.org/10.1016/j.toxicon.2015.06.029>
- Kayhan, N. Y., Ocal, I. C., Akdeniz, K. Investigation Of Cytotoxic And Anti-Proliferative Effects Of Crude Spider Venom Of Agelena Orientalis On The Breast Cancer Cell Line. Fresenius Environmental Bulletin. 2022; 31(8), 7751-7759.,
- İşcan, A., Çalışkan, F., Kutlu, H. M., Sezer, C. V., Çalışkan, H. Akrep Hemolenfinin Kanser Hücrelerinde Antiproliferatif ve Morfolojik Etkilerinin Araştırılması. Bilecik Şeyh Edebali Üniversitesi Fen Bilimleri Dergisi. 2020;7(100. Yıl Özel Sayı), 363-372. <https://doi.org/10.35193/bseufbd.682149>.

9. Nabi, G., Ahmad, N., Ullah, S., Khan, S. Therapeutic Applications of Scorpion Venom in Cancer: Mini Review. *J Biol Life Sci.* 2015; 6(1): 57. <http://dx.doi.org/10.5296/jbls.v6i1.641>
10. Hanan, Taha, S. E. D., Eman., Radwan, M. M. Insect growth regulator insecticides toxicity evaluation in relation to catalase, pyrooxidase, lactate-dehydrogenase and total antioxidant activities in field population of *Pectinophora gossypiella* (Lepidoptera: Gelechiidae). *Egypt. J. Plant Prot. Res. Inst.* 2023; 6 (1): 84 – 95.
11. Kaya, S. T. Age- and Sex- Dependent Changes in Serum Levels of TAS, TOS, TLR2, TLR4, HSP60, HSP90, and HMGB1. *Konuralp Medical Journal.* 2023;15(1), 105-115. <https://doi.org/10.18521/ktd.1214575>
12. Brito-Almeida, T. R., Salomão, R. P., Teles-Pontes, W. J., Lira, A. F. Comparison of desiccation resistance in the litter-dwelling scorpion *Tityus pusillus* Pocock, 1893 (Scorpiones: Buthidae) from dry and wet tropical forests. *The Journal of Arachnology.* 2023; 51(2), 118-122. <https://doi.org/10.1636/JoA-S-22-018>.
13. Feitosa, M. L. B., Barbosa-da-Silva, H. R., Salomão, R. P., Desouza, A. M., de Moura, G. J. B., Lira, A. F. D. A. Effects of landscape metrics on scorpion (Arachnida: Scorpiones) assemblage in a tropical urban ecosystem. *Ecology and Evolution.* 2024;14(2). <https://doi.org/10.1002/ece3.11026>
14. Naumova, M. Survey of the Moroccan arachnids (Araneae, Scorpiones & Solifugae) in the collections of the Institute of Biodiversity and Ecosystem Research, Bulgaria. *Arachnologische Mitteilungen: Arachnology Letters.* 2023;66(1), 72-78. <https://doi.org/10.30963/aramit6608>.
15. Hassan, H., Mirza, M. R., Jabeen, A., Alam, M., Kori, J. A., Sultan, R., ... & Choudhary, M. I. Yellow scorpion (*Buthus sindicus*) venom peptides induce mitochondrial-mediated apoptosis in cervical, prostate and brain tumor cell lines. *Plos one.* 2024;19(2). <https://doi.org/10.1371/journal.pone.0296636>.
16. Setayesh-Mehr, Z., Asoodeh, A., Ghasemi, L. V. The Anti-cancer Effect of Two Extract Fractions from the *Hemiscorpius lepturus* Scorpion Venom. *Applied Biochemistry and Microbiology.* 2023;59(6), 850-857. <https://doi.org/10.1134/S0003683823060145>
17. Jahangirian, E., & Zargan, J. Investigating the Antibacterial Effects of 3 Novel Peptides Isolated from the Venom of Iranian *Odontobuthus doriae* and *Buthotus saulcyi* Scorpions on *Escherichia coli* (UTI89) and *Enterococcus faecalis* Causing Urinary Tract Infection. *International Journal of Peptide Research and Therapeutics,* 2023;29(4), 55. <https://doi.org/10.1007/s10989-023-10529-y>.
18. Mechkarska, M., Cunning, T. S., Taggart, M. G., Ternan, N. G., Leprince, J., Coquet, L., ... Conlon, J. M. Identification of an antimicrobial peptide from the venom of the Trinidad thick-tailed scorpion *Tityus trinitatis* with potent activity against ESKAPE pathogens and *Clostridioides difficile*. *Antibiotics.* 2023;12(9), 1404. <https://doi.org/10.3390/antibiotics12091404>.
19. Xia, Z., Wang, H., Chen, W., Wang, A., Cao, Z. Scorpion Venom Antimicrobial Peptide Derivative BmKn2-T5 Inhibits Enterovirus 71 in the Early Stages of the Viral Life Cycle In Vitro. *Biomolecules.* 2024; 14(5), 545. <https://doi.org/10.3390/biom14050545>.
20. Anandhan Sujatha, V., Gopalakrishnan, C., Anbarasu, A., Ponnusamy, C. S., Choudhary, R., Saravanan Geetha, S. A., Ramalingam, R. Beyond the venom: Exploring the antimicrobial peptides from *Androctonus* species of scorpion. *Journal of Peptide Science.* 2024;e3613. <https://doi.org/10.1002/psc.3613>