Future Biochemistry and Bioscience
Yenilikçi Biyokimya ve Biyobilim

FBB 2024;6(1):15-19
http://doi.org/10.48086/ASD0061

Araştırma Makalesi / Research Article

Benzalkonyum Klorürün Tatlı Su Istakozları Üzerindeki İmmünolojik ve Biyokimyasal Etkileri

The Immunological and Biochemical Effects of Benzalkonium Chloride on The Narrow-Clawed Freshwater Crayfish

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Öz

Amaç: Benzalkonyum klorürler (BAC'ler), evsel, klinik ve çevresel ortamlarda mikrobiyal enfeksiyonları önlemek amacıyla kullanılan bir maddedir. Suda çözünebilme yeteneği nedeniyle sucul ekosistemlere karışması durumunda sucul canlılar üzerinde istenmeyen etkilere neden olabilirler. Bu çalışmanın amacı BAC'ın sucul omurgasız bir tür olan tatlı su ıstakozları üzerindeki etkisini araştırmaktır.

Gereç ve Yöntem: Bu çalışmada kullanılan dar pençeli tatlı su ıstakozları Eğirdir Gölü (Isparta, Türkiye)'den temin edilerek laboratuvara getirilmiş ve laboratuvar koşullara iki hafta süre ile aklime edilmiştir. Aklimasyon sonunda ıstakozlar, 7 gün boyunca 10, 25 ve 50 mg/L BAC50'ye maruz bırakılmışlardır. Maruziyet süresinin sonunda ıstakozlardan toplam hemosit sayısı için hemolemf sıvısı ile biyokimyasal parametreler için solungaç ve hepatopankreas dokusu alınmıştır.

Bulgular: Solungaç ve hepatopankreas dokuları MDA ve AOPP değerleri arasında önemli bir fark vardır ($p<0.05$). Hepatopankreas değerleri her iki parametre için de yüksek elde edilmiştir. Glutatyon değerlerinde ise herhangi önemli bir değişim gözlenmemiştir ($p>0.05$). Toplam hemosit sayısında ise konsantrasyon arttıkça hemosit sayısında bir artış olduğu gözlenmiştir ($p<0.05$).

Sonuç: Bu çalışmanın sonuçlarına göre, dezenfektan amaçlı kullanılan BAC'ın sucul omurgasızlar üzerinde toksik etkileri vardır.

Anahtar Kelimeler: *Astacus leptodactylus*, Benzalkonyum klorür, Biyokimya, Hemosit

Abstract

Aim: Benzalkonium chlorides (BACs) are substances used to prevent microbial infections in domestic, clinical, and environmental settings. Due to its ability to dissolve in water, it may cause undesirable effects on aquatic organisms if mixed into aquatic ecosystems. This study aimed to investigate the effect of BAC on the freshwater crayfish, an aquatic invertebrate species.

Material and Method: The narrow-clawed freshwater crayfish used in this study were obtained from Lake Eğirdir (Isparta, Türkiye), brought to the laboratory, and acclimated to laboratory conditions for two weeks. At the end of acclimation, crayfish were exposed to 10, 25, and 50 mg/L BAC50 for seven days. At the end of the exposure period, the hemolymph fluid was taken from the crayfish for the total number of hemocytes, and the gill and hepatopancreas tissues were taken for biochemical parameters.

Results: There was a significant difference between MDA and AOPP values of gill and hepatopancreas tissues ($p<0.05$). Hepatopancreas values were obtained high for both parameters. No significant change was observed in glutathione values ($p>0.05$). As for the total number of hemocytes, it was observed that there was an increase in the number of hemocytes as the concentration increased ($p<0.05$).

Conclusion: According to the results of this study, BAC used for disinfectant purposes has toxic effects on aquatic invertebrates.

Keywords: *Astacus leptodactylus*, benzalkonium chloride, biochemistry, hemocyte

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Geliş Tarihi/Received:02 Nisan 2024. Kabul Tarihi/Accepted:15 Nisan 2024. Çevrimiçi Yayın: Published Online: 30 Nisan 2024.

INTRODUCTION

Quaternary ammonium compounds easily bind to negatively charged surfaces such as mud, sediment, and soil, thanks to the positive charges they contain. When they come into contact with cells, they can destroy cell membranes by binding to the fatty acids in the cell membranes[1,2]. Benzalkonium chloride (BAC, also known as Actinicide), a quaternary ammonium compound, is used in human and veterinary health as a biocidal agent against organisms such as viruses, fungi, and bacteria[3,4]. It is also used as an algicide in areas such as swimming pools. Therefore, it can potentially impact other living organisms in the aquatic environment due to mixing with aquatic ecosystems[5,6].

Invertebrates are one of the model organism groups used in ecotoxicological studies. Since they show high sensitivity to cationic surfactants, aquatic invertebrates are preferred in investigating the effects of these substances on aquatic ecosystem health[2,4].

The narrow-clawed freshwater crayfish (*Astacus leptodactylus* Eschscholtz, 1823) is an invertebrate found in the natural environment in many countries, including Turkey, and has been introduced to many other countries[7]. These organisms have important value for the aquatic ecosystem and are used in nutrition by people in many countries. Therefore, these organisms are good model organisms used in ecotoxicological studies[7-9]. This study aimed to examine the effects of BAC, which can cause toxic effects on aquatic crustaceans, on the crayfish from a biochemical and immunological perspective.

MATERIAL AND METHODS

Freshwater crayfish (average weight 53.37 ± 1.5 g and average length 9.05 ± 0.5 cm) were obtained from local fishermen in Lake Eğirdir (Isparta, Türkiye) during the intermolt and hunting period. At the end of the two-week adaptation and acclimatization period to laboratory conditions, the crayfish were taken to the experimental aquariums (10 organisms/aquarium). There was a control group in the experiment. The concentrations of BAC50 (Thor, Tellerini, Italy) were applied as 10, 25 and 50 mg/L. Exposure experiments were carried out for 7 days. After the exposure period, the hemolymph fluid was taken to determine the total hemocyte counts[10]; the gill and hepatopancreas tissues were taken to determine the lipid peroxidation (MDA)[11], glutathione[12] and advanced oxidative protein products (AOPP)[13] parameters.

All data were distributed normally, and therefore, statistical significance was determined using a one-way ANOVA comparison test using the GraphPad Prism program. Statistical significance was defined as p values <0.05.

RESULTS AND DISCUSSION

This study examined the effect of benzalkonium chloride, which enters aquatic ecosystems after its use in domestic, hospital, and animal husbandry[14], on the narrow-clawed freshwater crayfish, one of the aquatic model organisms.

The narrow-clawed freshwater crayfish used in aquatic toxicology studies resist environmental changes. They give physiological responses to the pressures of different species[15]. When exposed to various xenobiotics, their antioxidant mechanisms come into play against the reactive oxygen species produced, and they try to protect macromolecules such as lipids, proteins, and DNA from damage[7,16].

The MDA values of the hepatopancreas and gill tissue of the crayfish are given in Figure 1. According to the results, MDA values of hepatopancreas tissue were approximately 10 times higher than MDA values of gill tissue ($p<0.05$). On the other hand, hepatopancreatic MDA values increased slightly compared to the control groups; only a significant difference was observed between BAC 25 mg/L-exposed and control groups ($p<0.05$). Besides, there was a significant difference between the BAC 50 mg/L-exposed and the control groups of gill tissues of crayfish ($p<0.05$).

MDA is one of the indicators of cellular damage as the end product of lipid peroxidation occurring in the cell[16]. In studies conducted with crayfish, results such as increases or decreases in tissue MDA values were observed. Shehata et al.[17] reported that hepatopancreatic MDA values increase in crayfish with different feeding activities. Ou-Yang et al.[18] showed that an increase in MDA values was observed in crayfish exposed to cyhalofopbutyle. Like these studies, the current study's observation that MDA values increased in hepatopancreas tissues was compatible with the literature.

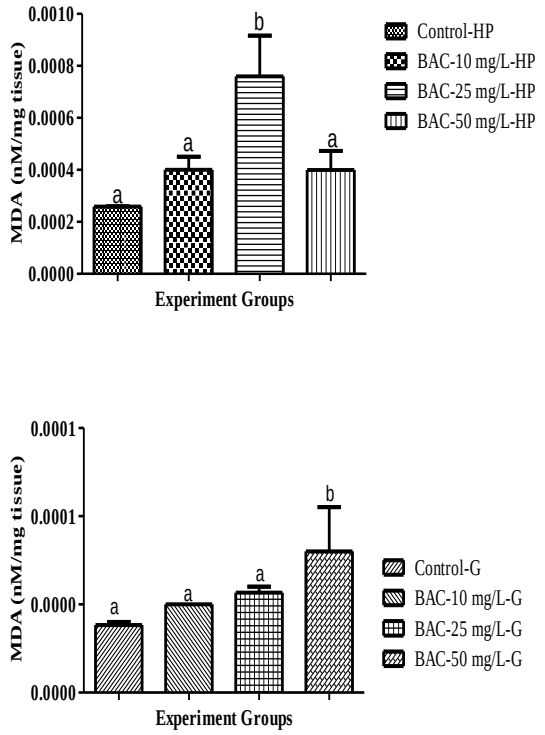

Figure 1. The MDA values (mean ± SEM) of hepatopancreas (HP) and gill (G) of control and BAC50-exposed groups (different letters showed significance; p<0.05)

The glutathione activities of the gill and hepatopancreas tissue of crayfish are given in Figure 2. Glutathione, one of the cell antioxidant defense mechanisms, is a non-enzymatic biomarker. In this study, no significant decrease or increase in GSH activity occurred. Lin et al.[19] showed that GSH activity in crayfish had a decrease in ammonia exposure. Changes in GSH levels indicate ROS production associated with increased or decreased detoxification capacity[16].

The AOPP values of the gill and hepatopancreas tissue of crayfish are given in Figure 3. AOPP results showed a significant increase in the BAC-exposed groups compared to the control groups (p <0.05). In addition, the hepatopancreas AOPP values were 10-16.7 times higher than gill AOPP values (p<0.05). The increase or decrease in AOPP values obtained as a result of the study was compatible with the change in AOPP values of freshwater mussels as a result. of exposure to xenobiotics[20].

The THCs of the crayfish are given in Figure 4. As the BAC50 concentration increased, it was

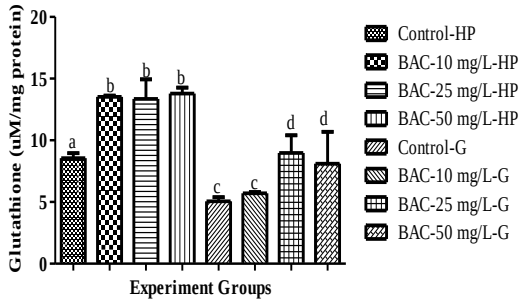

Figure 2. The GSH values (mean ± SEM) of hepatopancreas (HP) and gill (G) of control and BAC50- exposed groups (different letters showed significant; p<0.05)

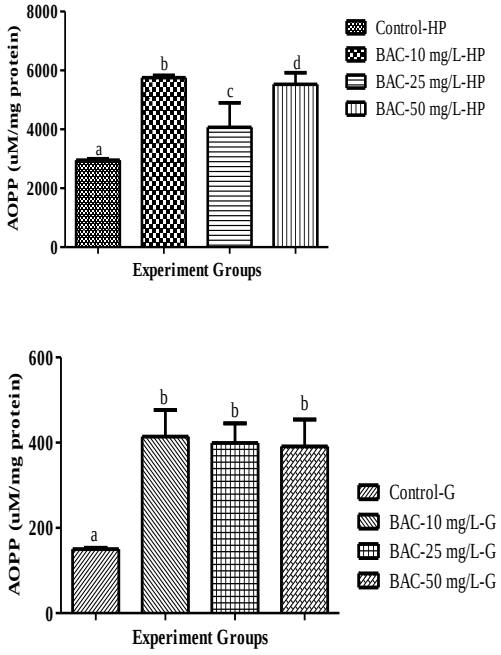

Figure 3. The AOPP values (mean ± SEM) of hepatopancreas (HP) and gill (G) of control and BAC50-exposed groups (different letters showed significance; p<0.05).

observed that there was an increase in THC values compared to the control groups. The THC values of crayfish exposed to the highest concentration increased significantly compared to the control groups (p<0.05). It is observed that the substance applied in this study stimulates the immune system of the organism by causing an increase in the number of hemocytes.

Hemolymph tissue is the body fluid of invertebrates and is similar to the blood tissue of mammals. When there is a change in the

environment in which invertebrates live or when there is a change in the organism's health, an increase or decrease occurs in the hemocyte cells in the hemolymph tissue, creating an immunological defense mechanism[7,10,21]. The study results are similar to studies that observed changes in the number of hemocytes due to exposure to xenobiotics in freshwater mussels[21], freshwater crayfish[7], oysters[22], crabs[22], and clamb[23].

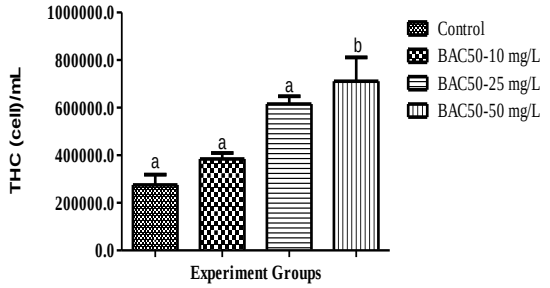

Figure 4. The THCs values (mean ± SEM) of control and BAC50-exposed groups (different letters showed significant; $p<0.05$)

CONCLUSION

A higher rate of lipid peroxidation occurred in the hepatopancreas tissues of freshwater lobsters exposed to different BAC concentrations than the gill tissues. Glutathione activities were increased in the hepatopancreas tissues of freshwater lobsters and in AOPP values in the hepatopancreas and gill tissues. Higher AOPP values were detected in the hepatopancreas tissues of freshwater lobsters than in the gill tissues. As the concentration of the BAC50 increased, the number of hemocytes increased. Thus, it has been revealed that BAC may have adverse effects on the health of aquatic organisms.

Etik Onay:
Çıkar Çatışması: Bu çalışma ile ilgili olarak yazarların çıkar çatışması bulunmamaktadır.
Finansal Destek: Bu çalışma için herhangi bir kurum ya da firmadan maddi ve/veya manevi herhangi bir destek alınmamıştır.
Ethical Approval:
Conflict of Interest: The authors have no conflict of interest regarding this study.
Financial Support: No financial and/or moral support was received from any institution or company for this study.

Acknowledgments: Gülsüm Batmaz was supported by TÜBİTAK 2211-A Domestic Doctoral Scholarship Program 2022/1.

REFERENCES

1. Ferk, F., Misík, M., Hoelzl, C., Uhl, M., Fuerhacker, M., Grillitsch, B., et al. Benzalkonium chloride (BAC) and dimethyldioctadecyl-ammonium bromide (DDAB), two common quaternary ammonium compounds, cause genotoxic effects in mammalian and plant cells at environmentally relevant concentrations. Mutagenesis, 2007;22(6):363-70. https://doi.org/10.1093/mutage/gem027.
2. Chen, Y., Geurts, M., Sjollema, S.B., Kramer, N.I., Hermens, J.L., Droge, S.T. Acute toxicity of the cationic surfactant C12-benzalkonium in different bioassays: how test design affects bioavailability and effect concentrations. Environmental Toxicology Chemistry. 2014;33(3):606-15. https://doi.org/10.1002/etc.2465.
3. Intorre, L., Meucci, V., Di Bello, D., Monni, G., Soldani, G., Pretti, C. Tolerance of benzalkonium chloride, formalin, malachite green, and potassium permanganate in goldfish and zebrafish. Journal of the American Veterinary Medical Association. 207;15;231(4):590-5. https://doi.org/10.2460/javma.231.4.590.
4. Lavorgna, M., Russo, C., D'Abrosca, B., Parrella, A., Isidori, M. Toxicity and genotoxicity of the quaternary ammonium compound benzalkonium chloride (BAC) using *Daphnia magna* and *Ceriodaphnia dubia* as model systems. Environmental Pollution. 2016;210:34-9. https://doi.org/10.1016/j.envpol.2015.11.042.
5. Pérez, P., Fernández, E., Beiras, R. 2009. Toxicity of Benzalkonium Chloride on Monoalgal Cultures and Natural Assemblages of Marine Phytoplankton. Water Air Soil Pollut. 2009;201:319–330. https://doi.org/10.1007/s11270-008-9947-x.
6. Oh, S., Kurt, Z., Tsementzi, D., Weigand, M. R., Kim, M., Hatt, J. K., Tandukar, M., Pavlostathis, S. G., Spain, J. C., Konstantinidis, K. T. Microbial Community Degradation of Widely Used Quaternary Ammonium Disinfectants. Applied and Environmental Microbiology, 2014;80(19):5892-5900. https://doi.org/10.1128/aem.01255-14.
7. Günal, A.Ç., Tunca, S.K., Arslan, P. *et al.* How does sublethal permethrin effect non-target aquatic organisms?. Environmental Science and Pollution Research 2021; 28:52405–52417. https://doi.org/10.1007/s11356-021-14475-4
8. Gholamhosseini, A., Banaee, M., Sureda, A., Timar, N., Zeidi, A., & Faggio, C. Physiological response of freshwater crayfish, *Astacus*

leptodactylus exposed to polyethylene microplastics at different temperature. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 2023;267, 109581. https://doi.org/10.1016/j.cbpc.2023.109581.

9. Mercugliano, E., Messina, V., Bonelli, M., Manenti, R., Scaccini, D., Ferrante, L. et al. Human Dimensions and Visitors' Perspective in Freshwater Crayfish Conservation: The Case of a Protected Area in Italy. Diversity, 2023; 15(9), 999. https://doi.org/10.3390/d15090999.
10. Yavuzcan H. Y., & Benli, A. C. K. Nitrite toxicity to crayfish, *Astacus leptodactylus*, the effects of sublethal nitrite exposure on hemolymph nitrite, total hemocyte counts, and hemolymph glucose. Ecotoxicology and Environmental Safety, 2004;59(3), 370-375. https://doi.org/10.1016/J.ECOENV.2003.07.007.
11. Mihara, M. and Uchiyama, M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Analytical Biochemistry, 1978;86(1): 271-278. https://doi.org/10.1016/0003-2697(78)90342-1
12. Ellman, G. L. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics, 1959;82, 70–77.
13. Witko-Sarsat, et al. Kidney International, 1996;49(5), 1304-1313
14. Pereira B, M, P, Tagkopoulos I. Benzalkonium Chlorides: Uses, Regulatory Status, and Microbial Resistance. Applied Environmental Microbiology. 2019;17;85(13):e00377-19. https://doi.org/10.1128/AEM.00377-19.
15. Arslan, P., Gül, G. & Günal, A.Ç. The effects of invasive epibiont *Dreissena polymorpha* on the narrow-clawed crayfish (*Astacus leptodactylus*, Astacidae) in Eğirdir Lake, Turkey: a case study. Environmental Monitoring and Assessment, 2022;194:590. https://doi.org/10.1007/s10661-022-10302-6
16. Li, S., Lin, Y., He, N., Fang, L., Wang, Q., & Ruan, G. Antioxidation, immunity and hepatopancreatic histology of red swamp crayfish (*Procambarus clarkii*) subjected to low-temperature aerial exposure stress and re-immersion. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 2023;282, 111441. https://doi.org/10.1016/j.cbpa.2023.111441.
17. Shehata, A. I., Alhoshy, M., Wang, T., Mohsin, M., Wang, J., Wang, X. et al. Dietary supplementations modulate the physiological parameters, fatty acids profile and the growth of red claw crayfish (*Cherax quadricarinatus*). Journal of Animal Physiology and Animal Nutrition, 2023;107(1):308-328. https://doi.org/10.1111/jpn.13704.
18. Ou-Yang, K., Feng, T., Han, Y., Li, J., & Ma, H.Cyhalofop-butyl and pyribenzoxim-induced oxidative stress and transcriptome changes in the muscle of crayfish (*Procambarus clarkii*). Science of The Total Environment, 2023;864:161170. https://doi.org/10.1016/j.scitotenv.2022.161170
19. Lin, W., Wu, J., Luo, H., Liu, X., Cao, B., Hu, F., et al. Sub-chronic ammonia exposure induces hepatopancreatic damage, oxidative stress, and immune dysfunction in red swamp crayfish (*Procambarus clarkii*). Ecotoxicology and environmental safety, 2023;254:114724. https://doi.org/10.1016/j.ecoenv.2023.114724.
20. Arslan, P.How does cyphenothrin affect the freshwater mussel as in vitro and in vivo models?. Water, Air, & Soil Pollution, 2022;233(9), 386. https://doi.org/10.1007/s11270-022-05860-x.
21. Arslan P, Günal AÇ. Does Fipronil Affect on Aquatic Organisms? Physiological, Biochemical, and Histopathological Alterations of Non-Target Freshwater Mussel Species. *Water*. 2023; 15(2):334. https://doi.org/10.3390/w15020334
22. Hurtado, M. Á., da Silva, P. M., Le Goïc, N., Palacios, E., & Soudant, P. Effect of acclimatization on hemocyte functional characteristics of the Pacific oyster (*Crassostrea gigas*) and carpet shell clam (*Ruditapes decussatus*). Fish & shellfish immunology,2011; 31(6), 978-984.
23. Matozzo, V., Ballarin, L., Pampanin, D. M., & Marin, M. G. Effects of copper and cadmium exposure on functional responses of hemocytes in the clam, *Tapes philippinarum*. Archives of environmental contamination and toxicology, 2001;41:163-170. https://doi.org/10.1007/s002440010234.