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Effect of Molluscicidal and Cercariacidal Permethrin Preparation on Acid Phosphatase of Molluscs *Planorbis planorbis* and *Melanopsis praemorsa*

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ABSTRACT. Acid phosphatase activity of the freshwater molluscs *Planorbis planorbis* both uninvaded and naturally invaded by cercariae of *Skrjabinoeces similis* was determined in experiments *in vitro*. It is statistically true that acid phosphatase activity of infected snails was 2.56 times less than in the uninfected ones with cercariae. Permethrin at concentrations of $10^{-3} \cdot 10^{-4}$ and 10^{-5} mg/l activated acid phosphatase in both infected and uninfected *P. planorbis* in comparison to the control without permethrin. The effect of permethrin was more expressed in infected molluscs and was valid only at concentration of permethrin of 10^{-5} mg/l. The effect of permethrin was investigated on acid phosphatase of uninfected molluscs *Melanopsis praemorsa*. High concentrations of permethrin (0.1, 10^{-2} mg/l) reduced the enzyme activity, compared to the control without permethrin. At concentration of 10^{-3} the peak of enzymatic activity was marked. With the decrease of permethrin concentration to 10^{-4} and 10^{-5} mg/l, the acid phosphatase activity of *Melanopsis praemorsa* also decreased approaching the control value. The possible mechanism of permethrin action on the acid phosphatase of gastropods infected with cercariae is discussed. *© 2019 Bull. Georg. Natl. Acad. Sci.*

Key words: molluscs, *Planorbis planorbis*, *Melanopsis praemorsa*, permethrin, acid phosphatase

Wide distribution of molluscs in nature enabled many helminths to exploit their bodies for resettlement and going through certain stages of their development. Therefore, the role of molluscs as intermediate or reservoir hosts of dangerous helminths is very significant. In this regard, the study of the impact of molluscicidal preparations on such gastropod species is of particular interest having practical importance. This is a number of plant-based preparations and synthetic molluscicides as well as pyrethroids, including permethrin [1-3]. In the fight against trematodoses, permethrin was investigated *in vivo* as cercariacidal substance on snails family Planorbidae [4, 5] and experiments *in vitro* on alkaline phosphatase [6]. However, its impact on other enzymes of molluscs, in particular, acid phosphatase is unstudied. This enzyme catalyses a number of hydrolysis reactions in a living organism and it is often investigated as targer for effect evaluation of toxicants on molluscs [3]. Acid phosphatase is present in lysosomes. Its activity is observed in the organs and hemolymph

of gastropods [7]. Acid phosphatase is histochemically identified in hepatopancreatic cells of *Lymnaea auricularia* infected with *Cercaria pigmentosa*. It is also observed in sporocysts, fully developed cercariae and rediae [8]. When *Lymnaea luteola* gets infected with larvae of *Prosthogonimus sp*., acid phosphatase activity continuously increases that, according to Jyothirmayi and Rao P. Venkateswara [9], may have diagnostic implication.

There is a dearth of studies on the effect of permethrin on molluscan enzymes. The results of testing of its effects on acid phosphatase enable us to define the role of this pyrethroid as a cercariacidal agent. For this purpose, we studied the effect of permethrin on acid phosphatase of the snails of the genera Planorbis and Melanopsis.

Materials and Methods

The collection of the freshwater gastropods was carried out in the vicinity of the towns of Tbilisi and Rustavi. The snails of the genus Melanopsis (*M. praemorsa*) were not infected, while among Planorbis (*P. planorbis*) there were both infected and uninfected specimens. Under laboratory conditions the infected ones discharged the cercaria *Skrjabinoeces similis*. After parasitological examination, we were provided the materials for further biochemical analysis. Taking of the cochlea from the body of mollusc the 1% of water homogenate centrifuged at 3000 rev/min for 5 min was prepared. The amount of protein in oversedimentary liquid was determined by Lowry [10] method, while acid phosphatase activity by the method of Bodansky [11]. β-glycerophosphate 1% solution served as substrate. The experiments were conducted at pH 4.5. For experiments with permethrin basic 0.1% solution on 96° ethanol was prepared first out of which after certain dissolving distilled water received working solutions of the necessary concentrations were obtained. Homogenates were preincubated with permethrin for 15 minutes. The effect of permethrin was examined in experiments *in vitro* at different concentrations on both infected and uninfected molluscs. The activity of the enzyme was expressed in phosphorus (mg) per/mg by protein (mgPh/mgP).

Statistical Analysis

The results are presented as means ± S.D. The Student t-test was used to determine the statistical significance.

Results and Discussion

The results of the effect of different concentrations of permethrin on the acid phosphatase of snails of

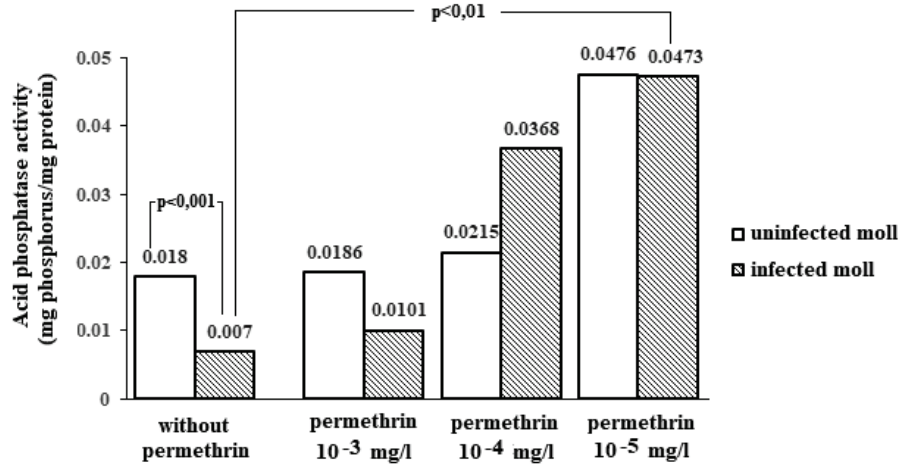

Fig. The effect of permetrin on the activity of acid phosphatase of infected and uninfected mollucks *P.planorbis* (n = 3-10).

the *P. planorbis* uninfected and infected with cercaria of *S. similis* are presented in Fig.

As shown in Fig., the acid phosphatase activity of uninfected *P. planorbis* was 2.56 times higher than that of the infected ones and amounted to 0.018±0.003 and 0.007±0.0016 mgPh/mgP, respectively. The difference between them was statistically true ($p<0.001$). The data obtained served as control when studying the effect of permethrin on those two groups of molluscs.

Experiments show that at concentration of permethrin (10^{-3}mg/l) the infected *P. planorbis* acid phosphatase activity increased almost by 1.44% in comparison to control and amounted to 0.0101±0.0016 mgPh/mgP. The activity of uninfected ones was equal to 0.0186±0.0038 mgPh/mgP. At concentration of permethrin of 10^{-4}mg/l the activity of infected ones increased by 5.25 times and was equal to 0.0368±0.013 mgPh/mgP, while that of uninfected ones – only by 1.19 times and made 0.0215±0.00045 mgPh/mgP. At concentration permethrin by 10^{-5}mg/l the growth of acid phosphatase activity in the uninfected prevailed the activity in control by 2.5 (2.64) and was equal to 0.0476±0.024 mgPh/mgP and in uninfected *P. planorbis* in the same conditions the enzyme activity grew by 6.74 times and was equal 0.0473±0.008 mgPh/mgP (Fig.). As can be seen, at concentrations 10^{-5} mg/l the average values of the activities of molluscs are identical, but the difference in activity of infected ones with the respective control turned out to be statistically true ($P<0.01$). All concentrations of permethrin had activating effect on the acid phosphatase of both infected and uninfected snails. It was especially significant in homogenates of the infected *P. planorbis.*

The effect of permethrin was studied also on the acid phosphatase of the uninfected molluscs genus Melanopsis and the results are presented in Table. It should be noted that in the experiments with *M. praemorsa* the notable variation of the specific activity of enzymes was observed (0.01 up to 0.119 mgPh/mgP) that was reflected on the average values of acid phosphatase.

Table. Acid phosphatase activity of uninfected mollusks *M. praemorsa* under the influence of permethrin

Permethrin concentration (mg/l)	Acid phosphatase activity (mg Ph/mg P)	number of experiments (n)	Influence %
0.1	0/0339 ± 0.0097	4	- 17.79
10^{-2}	0.0378 ± 0.0056	5	- 8.34
10^{-3}	0.0578 ± 0.0019	6	+ 40.3
10^{-4}	0,0519 ± 0,01	5	+ 25.8
10^{-5}	* 0.0415 ± 0.0077	5	+ 0.63
Control without permethrin	0.0412 ± 0.0064	5	

* statistical confidence indicator P< 0,02.

In this set of experiments the effects of five different concentrations of permethrin (0.1; 10^{-2}; 10^{-3}; 10^{-4} and 10^{-5} mg/l) were examined. According to the results, high concentrations of permethrin (0.1 and 10^{-2} mg/l) reduced acid phosphatase activity in comparison with control by 17.79 and 8.34% (percent effect) respectively. Together with decreasing permethrin concentration by 10^{-3} mg/l, enzyme activities increased amounting to 40.3%. This concentration was accounted as the peak of activity (0.0578±0.019 mgPh/mgP). The subsequent low concentrations of permethrin (10^{-4}mg/l) also activated enzymes this time by 25.8 percent. All above the values obtained of enzyme activity were invalid. Only at concentrations 10^{-5} mg/l the result was statistically true ($P<0.02$). The effect of permethrin amounted only to 0.63% and acid phosphatase activity in experiment was equal to that of the control.

When discussing the obtained results, it may be noted that *M. praemorsa* snails were markedly different from *P. planorbis* by external signs and acid phosphatase activity value as well. The existing material made it possible to compare the

enzyme activity of uninfected specimens only with each other. As it appeared, the acid phosphatase activity of *M. praemorsa* prevailed more than twice that of *P. planorbis* under the same conditions. However, the effects of low concentrations of permethrin were identical in both species.

The presence of parasites disturbs all metabolism processes in the body of molluscs and effects the activity of a number of enzymes. Thus, acid phosphatase activity increased in the hemolymth *Lymnaea luteola*, infected with *Prosthogonimus sp.* [12], in hemolymph and hepatopancreas of the molluscs *Mytilus edulis,* infected with partenites *Proctoeces maculatus* [13], in hemolymph of *Lymnaea stagnalis*, *Planorbis corneus* and *Viviparus viviparus*, infected with partenites and the larvae of trematodes [7], in Lymnaea acuminata infected with the redias and echinostome cercarias [14]. However, it should be noted that Kuzmovich and Kostinik [15] histochemically detected pronounced acid and alkaline phosphatase activities in hepatic cells of uninfected *Helicopsis instabilis*, while during the invasion of molluscs by the larvae of *Dicrocoelium lanceatum* low activities of the unspecific phosphatases took place. As noted above (Fig.), low acid phosphatase activity in cercaria-infected *P. planorbis* was also detected. In our opinion, lower acid phosphatase activity in our material is related to such an important factor as the intensity of invasion. The infection of planorbis by cercariae *S. similis* equalled only 1.8%. It is possible that in that case the destructive processes occurring in hepatopancreas and other organs have not yet resulted in significant pathological changes that could be reflected in metabolic activity, particularly in the acid phosphatase activity of the host. Yadav B. B. and Karyakarte P. P. [8] detected through the histochemical study of the hepatopancreas of infected and uninfected *Lymnaea auricularia* that when molluscs became infected by *Cercaria pigmentosa* Porter, 1938, the rate of the increase in phosphatase activity was correlated with the intensity of the infection of molluscs, trematode developmental stage and alkaline phosphatase activity rose far more markedly than that of the acid phosphatase. As can be seen, the activity of acid phosphatases of infected molluscs can manifest itself to both increase and decrease, depending on different factors. The intensity of invasion is one of the major factors noted above. Different localizations of parasites within the body of the host, developmental stages as well as the invasion into the host's body by various species of trematodes also play significant part in the manifestation of pathological processes [7].

Permethrin as molluscicidal preparation was investigated by Lomidze, Nikolaishvili [6] in experiments *in vitro* on the alkaline phosphatase of snails of the genus Planorbis. The authors show that all the tested concentrations of permethrin, especially low ones, some decrease the initial activity in the infected molluscs, while in the controll snails alkaline phosphatase was more labile. Both enzyme activation and inhibition were observed here. Vegetable and synthetic molluscicides, according to El-Emam and Ebeid [16], increased acid phosphatase activity and common protein content in *Biomphalaria alexandrina* snails infected by *Schistosoma mansoni*. Comparing these data with the general activation of acid phosphatase of infected and uninfected *P. planorbis* and uninfected *M. praemorsa* discovered by us, with low concentrations of permethrin, it can be concluded that acid and alkaline phosphatises of the aforementioned gastropods are very sensitive to that preparation, especially if they are infected by cercariae. The absence of credible differences in a number of cases does not exclude participation of the lysosomal apparatus in molluscan cells. In view of the fact that acid phosphatise is one of the most important hydrolysing lysosomal agents, it is possible that tissue phosphatases could be subjected to the activation by permethrin. Studying *in vivo* effects of permethrin on the cercariae

Paramphistomum skrjabini and *Xiphidiocercaria sp*., enabled Kurashvili et al. [5] at recommended concentrations, to use it as the most effective cercariacide harmless to molluscs and aquatic microorganisms. The mechanism of the effect of permethrin on molluscs remains unclear, but Sparks et al. [17] conducted the investigations on the imago of resistant flies and showed that permethrin increases the permeability of tissues and metabolism of both oxidative and hydrolytic. In our opinion, permethrin can similarly act on permeability of the tissues of the infected molluscs contributing to the activation of acid phosphatase and at the same time, indirectly, it can increase the activation of acid phosphatase granulocites. The latters lyse the membrane of sporocytes, penetrate inside and have fatal action on embryonal stages of parasites. This supposition can be proved by Cheng and Doughetty [18], who ultrastructurally showed distructive link of sporocyst *Schistosoma mansoni* with high rate of lysosomal acid phosphatase, aminopeptidase and lysozyme in molluscs *Biomphalaria glabrata.*

Conclusion

It can be said that *in vitro* experiments permethrin as molluscicido and cercariacidal preparation has general activating effect on the acid phosphatase of infected and uninfected *P. planorbis* and uninfected *M. praemorsa*. Comparing the results of a number of authors regarding acid phosphatase activity changing in infected snails and natural conditions, it can be concluded that acid phosphatase reaction is one of the responses of the mollusc organism on invasion on molecular level and may present defensive compensatory mechanism taking place in the parasite-host system.

პარაზიტოლოგია და ჰელმინთოლოგია

მოლუსკოციდურ-ცერკარიაციდური პრეპარატ პერმეტრინის გავლენა *Planorbis planorbis*-ის და *Melanopsis praemorsa*-ს სახეობების მოლუსკების მჟავე ფოსფატაზებზე

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არაინვაზირებულ და *Skrjabinoeces similis*-ის ცერკარიებით ინვაზირებულ მტკნარი წყლის *Planorbis planorbis*-ის მოლუსკებში, *in vitro* განსაზღვრულ იქნა მჟავე ფოსფატაზების აქტივობა. ცერკარიებით ინვაზირებულ მოლუსკებში მჟავე ფოსფატაზების აქტივობა, არაინვაზირებულთან შედარებით (სტატისტიკურად სარწმუნოდ) 2,56-ჯერ ნაკლებია. პერმეტრინი 10^{-3}, 10^{-4} და 10^{-5} მგ/ლ კონცენტრაციით იწვევს მჟავე ფოსფატაზას გააქტიურებას, როგორც ინვაზირებულ ასევე არაინვაზირებულ მოლუსკებში. პერმეტრინის მოქმედება უფრო მეტად გამოვლინდება *P. planorbis*-ის ინვაზირებულ მოლუსკებში. ამასთან, აქ სტატისტიკურად სარწმუნო შედეგები აღინიშნებოდა მხოლოდ პერმეტრინის 10^{-5} მგ/ლ კონცენტრაციით მოქმედებისას. მჟავე ფოსფატაზებზე პერმეტრინის მოქმედება შესწავლილ იქნა *Melanopsis praemorsa*-ს არაინვაზირებულ მოლუსკებში. აღმოჩნდა, რომ პერმეტრინი მაღალი კონცენტრაციით მოქმედებისას (0,1, 10^{-2} მგ/ლ) იწვევს ფერმენტის აქტივობის კლებას. 10^{-3} მგ/ლ-ზე აღინიშნებოდა აქტივობის „პიკი“. პერმეტრინის კონცენტაციის შემდგომი კლებისას 10^{-4} და 10^{-5} მგ/ლ-მდე ფერმენტის აქტივობა კვლავ ეცემოდა და ბოლოს მისი სიდიდე უტოლდებოდა საკონტროლო მაჩვენებელს. განხილულია ცერკარიებით ინვაზირებულ გასტროპოდებზე პერმეტრინის მოქმედების მექანიზმი.

REFERENCES

1. Najia A., AL- Zanbagi (2013) Review of using plants as molluscicidal, larvicidal and schistosomicidal in Saudi Arabia. *Australian J. of Basic and Applied. Sci.,* **7** (7): 110-120.
2. Singh V., Tiwari JN. (2012) Effects of synthetic and exstracts of plant on reproduction of snail *Lymnaea acuminata*. RJPBCS, **3**, 4: 705-710.
3. Ping He, Weisi Wang, Benjamin Sonogo, Xin Zeng, Xi Sun , Zhiyue Lv., Donjuan Yuan, Liping Duan, Zhongdao Wu. (2017) Molluscicidal activity and mechanism of toxicity of a novel salicylanilide ester derivative against *Biomphalaria species. Journal List Parasit Vectors,* **10**: 383. Published online PMC 5550999.
4. Kurashvili B., Matsaberidze G., Fidler Zh., Karachentseva Y., Tkachenko L. (1989) The molluscicidal property of permethrin and its effect on the fractional composition of the lipids of molluscs. Materials Scientific Conf. Parasitologists of Georgia, p. 26-28, Tbilisi (in Georgian).
5. Kurashvili B.E., Matsaberidze G. V., Fidler Zh. N. ,Tkachenko L.M. (1990) O vozmozhnosti primeneniia permetrina v kachestve cerkariocidnogo preparata pri borbe s trematodoznymi zabolevaniiami. Soobshcheniia AN GSSR, **137**, 3: 621-624 (in Russian).
6. Lomidze Ts., Nikolaishvili K. (2016) Influence of the molluscicide permethrin on the activity of alkaline phosphatases of the mollusk genus Planorbis. *Proceedings of the Institute of Zoology*, **XXV**: 139-144 (in Georgian).
7. Stadnichenko A.P. (1978) Izmenenie nekotoryx pokazatelei uglevodnogo obmena v gemolimfe presnovodnykh molluskov pri invazii ikh partenitami i lichinkami trematod. Parazitologia, **XII,** 6: 472-478 (in Russian).
8. Yadav B.B., Karyakarte P.P. (1980) Histochemical observations on phosphatase activity in the hepatopancreatic cells of *Lymnaea auricularia* infected with Gymnocephalus cercaria *Cercaria pigmentosa*, Porter, 1938. *Riv. Parasitol.*, **41**, 2.
9. Jyothirmayi G. N., Venkateswara R. P. (1987) Diagnostic value of haemolymph enzyme activity profile in infected snail *Lymnaea luteola* (Lamarck). *Indian J. Exp. Biol.,* **25**, 2: 132-134.
10. Lowry O.H., Rosenbrough N.J., Farr A.L., Randall R.J. (1951) Protein measurement with Folin – phenol reagent. *J. Biol. Chem.,* 193: 265-275.
11. Bodansky A.J. (1933) Phosphatase studies 11. Determination of serum phosphatase, factors influencing the accuracy at the determination. *J. Biol. Chem.,* 101: 93-104.
12. Jyothirmayi G.N.,Venkateswara R. P. (1988) Possible origin of hemolymph phosphatases in trematode-infected and in noninfected snails. *J. Invertebr. Pathol.* **52**, 3: 273-379.
13. Dennis E.A., Sharp M., Douglass R. (1974) Carbohydrate reserves and phosphatase activity in the mollusk-trematode relationship of *Mytilus edulis* L. and *Proctoeces maculatus* (Looss, 1901) Odhner, 1911. *J.Helminthol.,* **48**, (1): 1-14.
14. Nigan S.C., Pandey K.C., Agrawal N. (1979) Phosphatase activity in the digestive gland of *Limnaea acuminata* (L.) infected with echinostome redie and cercarie. *J.Zool.Soc. India,* **31**, 1-2: 85-88.
15. Kuzmovich L.G., Kostinik I.M. (1975) O porazhonnosti molluska *Helicopsis instabilis* lichinkami *Dicrocoelium lanceatum* Stiles at Hassall, 1896. Problemy parazitologii, 1. Mater. VIII nauchn. Conf. parazitologov Ukr. SSR. Naukova dumka, Kiev. 289-291 (in Russian).
16. EL-Emam M.A., Ebeid F.A. (1989) Effect of *Schistosoma mansoni* infection, starvation and mollucicides on acid phosphatase, aminotransferases and total protein in tissues and hemolymph of the snail *Biomphalaria alexandrina. Helminthologia,* **26,** 2: 155-161.
17. Sparks T.S., Byford R.L., Craig M.E., Crosby B.F., McKenzie C. (1990) Permethrin metabolism in pyrethroid-resistant adults of the horn fly (Muscida: Diptera.) *J. Econ. Entomol.,* **83**, 3: 662-665.
18. Cheng T.S., Dougherty W.J. (1989) Ultrastructural evidence for the destruction of *Schistosoma mansoni* sporocysts associated with elevated lysosomal enzyme levels in *Biomphalaria glabrata. J. Parasitol.,* **75**, 6: 928-941.

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