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ORIGINAL ARTICLE

Acid and Alkaline Phosphatase Activity in the Indian Apple Snail *Pila globosa* (Swainson) During Aestivation

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The Indian apple snail *Pila globosa* (Swainson) experiences an annual cycle of aestivation (summer sleep) as a survival strategy during a hot and dry period to avoid damage from high temperatures and the risk of desiccation. Alterations in the external environment reflect in their haemolymph. Changes in enzyme levels because of any type of stress are immediately reflected in the functional responses of animals. These environmental factors can be simulated in the laboratory so as to study enzymatic alterations occurring in the haemolymph of snails to overcome the adverse features during aestivation. Phosphatase enzymes are important for many biological functions. Acid and alkaline phosphatase activities have been studied in the haemolymph of three months aestivated and active snails. The activity level of enzymes ACP decreased and ALP increased significantly in the haemolymph of aestivated *P. globosa* when compared to active snails. The significance of these findings is discussed. This investigation explains the adaptability of *P. globosa* to overcome the adverse features during aestivation. This study also reveals that *Pila globosa* is a suitable model for studies on aestivation and provides an interesting case of aestivation.

Key words: *Acid Phosphatase, Aestivation, Alkaline Phosphatase, Haemolymph, Pila globosa*

Invertebrate and vertebrate animals like poriferans (Loomis, 2010), nematodes (Hu, 2007), arthropods (Tovi Lehmann *et al.*, 2010), molluscs (Krishnamoorthy, 1968), amphibians (Hudson *et al.*, 2004), fishes (Sturla, 2002), reptiles (Seidel, 1978), mammals (Vivier and van der Merwe, 2007; Warnecke, 2008) and birds (Thomas and Fritz, 2015; Lyman, *et al.*, 1982) are known to enter prolonged torpor, dormancy, quiescence or diapause during a hot and dry period to avoid damage from high temperatures and the risk of desiccation. Aestivation is a very ancient phenomenon. The fossil record gives evidence of structures related to aestivation, such as earthworm chambers in the Pleistocene epoch, lungfish burrows in the Devonian to Cretaceous period, and lysorophid amphibian burrows in the Permian period (Hembree, 2010).

The Indian freshwater apple snail *Pila globosa*, which is mainly distributed around equatorial and tropical regions of the world, aestivates when its natural habitat like ponds, pools, streams, ditches, canals, and paddy fields dry up in summer (Meenakshi, 1956, 1958, 1964; Shylajakumari, 1975). The *Pila globosa* typically experience an annual cycle of aestivation (summer sleep) as a survival strategy in hot, arid and semi-arid regions of the world where conditions include high temperature, low humidity, little rainfall, low oxygen conditions, and scanty green vegetation.

In this dormant state, the organism can survive for long periods without water, food, or oxygen. The concerns for an aestivating snail are to conserve energy and body fuel reserves, to retain water in the body, to maintain oxygen level, to regulate the nitrogenous end products, and to stabilize bodily organs, cells, and macromolecules over many weeks or months of dormancy. During this unfavourable situation, they develop a variety of morphological, biochemical, physiological, and behavioural adaptations to withstand adverse conditions (Riddle, 1983; Tal Mizrahi, 2010). During this aestivation period, snails hide in the ground, withdraw their bodies into the shell, tightly close the shell aperture with the operculum, reduces all vital functions to a necessary basic level, and remains in a state of torpor until the advent of rain. Key features of aestivation or dryness sleep are reduced metabolic activity, a decrease in body temperature below normal level (adaptive hypothermia), low oxygen consumption, and a reduced heart rate.

Snails have an open circulatory system, so alterations in the external environment reflect immediately in their haemolymph. Changes in enzyme levels because of any type of stress are immediately reflected in the functional responses of animals. These environmental factors can be simulated in the laboratory so as to study enzymatic alterations occurring in snails to overcome the adverse features during aestivation.

Phosphatases have the physiological role of dephosphorylating compounds. Phosphatase enzymes are important to many biological functions because phosphorylation and dephosphorylation serve diverse roles in cellular regulation and signaling. Various functions of phosphatases include the synthesis of fibrous protein (Johnson and McMinn, 1958), mucopolysaccharides (Kroon, 1952) and nucleic acid (Cox *et al.*, 1967), regulation of intracellular phosphate concentrations (Gutman, 1959), hydrolysis of dead cells, and permeability processes. Phosphatases are of two types, based on their optimal pH for catalysis activity: acid and alkaline. Acid phosphatases show maximum activity at an acidic pH around 6, whereas alkaline phosphatases show maximum activity at an alkaline pH around 11 (Chin -Yin and Hiroyuki, 1987).

Detailed research work on the activity pattern of acid phosphatase and alkaline phosphatase enzymes in molluscan haemolymph is reported by some investigators like Eble (1966), Cheng and Rifkin (1970), Feng *et al.*, (1971) and Frankboner (1971). *Pila globosa* is a suitable model for studies on aestivation and provides an interesting case of aestivation. However, no published information is available regarding acid and alkaline phosphatase enzymatic activities in haemolymph of the snail, *Pila globosa,* during aestivation. Therefore, an investigation was conducted to determine acid and alkaline phosphatase activity in the Indian apple snail, *Pila globosa* (Swainson), during aestivation.

MATERIALS AND METHODS

Collection, holding, and acclimation of test

animals

Live specimens of freshwater Indian apple snails (*Pila globosa*) were hand-picked from the River Godavari in Nashik, Maharashtra state and immediately transferred from the collection site to the laboratory for acclimation. Organisms were handled gently, carefully, and quickly as possible to minimize stress. Organisms that were dropped or injured during handling were discarded. They were cleaned and kept in glass aquaria containing dechlorinated tap water. During the acclimation period of 2 weeks, snails were fed daily with their natural foods like *Hydrilla*, *Valisneria*, *Nymphaea*, and green algae (Haniffa, 1980), and water was changed every 24 hours. Before the daily renewal of water, uneaten algae and other debris were removed from the acclimation tank. No disease treatments were administered during acclimation period (APHA, 2017; OECD, 2019).

Simulation of conditions to induce aestivation

Healthy, active, acclimatized snails of approximately the same weight and size were kept on filter paper for around four hours to remove water from the mantle cavity. After drying, snails were aestivated experimentally for a duration of three months (Srinivasa Reddy *et al.*, 1977). The test animals were exposed to simulated laboratory conditions, viz. a wooden box of size (10 cm x 10 cm x 10 cm) with a bed of dry sand. A heat source was provided by keeping a 10-watts bulb covered with silver foil to ensure only heat and not light is come out. The boxes were covered with lid & fixed with a thermometer and maintained at a temperature of 23 - 27°C. The wooden containers were placed in an undisturbed area of the laboratory where there was plenty of ventilation. After regular intervals (30 days, 60 days, and 90 days), the aestivating snails were taken out of wooden boxes to collect haemolymph for the estimation of acid and alkaline phosphatases. At each time interval, 10 snails were used as an experimental for study of activity levels of haemolymph enzyme. Freshly collected animals (acclimated to laboratory conditions for one week) were used as control.

Collection of Haemolymph

For sampling purposes, snails were quickly sacrificed by breaking their shells. An opening was made on the body whorl of the shell at the left-hand top side of the operculum. Haemolymph was obtained by puncturing the heart with a needle and collected in test tubes packed with ice.

Quantitative Estimation of Acid and Alkaline Phosphatases

Acid phosphatase and alkaline phosphatase activities were assayed by the colorimetric end-point pNPP method described in sigma technical bulletin 104 with slight modifications and Tenniswood *et al.*, (1976).

Statistical Analysis

The statistical analysis of collected data was carried out by using student's 't' test (Croxton *et al.*, 1975 and data analysis package, Microsoft Excel, windows 10, 2020). Differences between the control and experimental animal group were considered significant at $p<0.05$, $p<0.01$ and $p<0.001$. Data was presented as the mean ± standard deviation.

RESULTS

Some of the morphological and behavioural changes observed in *Pila globosa* during aestivation were closure of the operculum, lack of movement, mucus sections, and a change in the texture of shell. The snail also becomes less responsive to external stimulation. The same changes were noted in *P. globosa* during aestivation by Basavaraju and Krupanidhi (2013).

In snails aestivated for one and two months, no significant difference in ACP enzyme activity level in haemolymph was observed when compared with the ACP enzyme level in control snails. But in snails that were aestivated for three months, a very significant decrease ($p<0.01$) in enzyme activity was observed with respect to active ones. The decrease in haemolymph acid phosphatase enzyme activity observed in three-month aestivated snails was found to be significantly lower ($p<0.05$) than that in one-month aestivated snails. Statistically significant variation was absent when ACP enzyme activity levels in snails aestivated for one & two months and two & three months were compared (Table 1).

A statistically significant increase in the alkaline phosphatase activity level was noticed in two months aestivated snails ($p<0.001$) when compared to the ALP level in the control active snail. In snails aestivated for one and three months, significant variation ($p<0.05$) in enzyme activity was noticed in comparison with that in active snails. When the alkaline phosphatase enzyme activity of three different groups of aestivated snails was compared to one another, no statistically significant difference was discovered (Table 1).

Table 1: Haemolymph acid phosphatase (ACP) and alkaline phosphatase (ALP) activity (IU/L) of *Pila globosa* aestivated for 1, 2, and 3 months

Enzyme analyzed	Control Snails	Aestivated Snails		
		1 month	2 months	3 months
ACP activity level	10.37±1.88	9.32±2.40^{NS} (↓10.13)	8.55±2.66 ^{NS} (↓17.55)	7.11±2.35** (↓31.44)
ALP activity level	3.98±2.51	9.06±4.73** (↑127.64)	10.87±5.03*** (↑173.12)	10.02±4.80** (↑151.76)

± indicates the standard deviation

significance level: * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$; NS = non significant

values in parentheses indicate percent variation over the control

DISCUSSION

The present study aims to find out the effects of aestivation on the activity pattern of acid and alkaline phosphatases in the haemolymph of *Pila globosa.* Aestivation was shown to have an impact on the level of acid and alkaline phosphatase activity in the haemolymph of *Pila* globosa.

In previous, studies mixed results have been reported. Few studies have reported similar results as the present investigation. Reju (1990), recorded a decrease in acid phosphatase level and an increase in alkaline phosphatase enzyme level in the haemolymph of aestivated snails *Pila virens*. Aruna *et al.* (1979) documented that the activity of acid phosphatase decreased in both tissues (foot & hepatopancreas) of *Pila globosa* during aestivation. During the period of dormancy, digestive, reproductive, and locomotion functions cease to operate, and the energy budget is known to change, resulting in the decreased production of orthophosphoric monoesters, which are the substrates for acid phosphatase. The present study indicates that energy demands decreased during aestivation. The possible reason for the high level of ALP in aestivating snails could be the active involvement of alkaline phosphatase enzyme in various synthetic activities meant to prepare an animal for a longer period of dormancy.

In contrast to the results of the current study, Bhunia *et al.*, recorded higher ACP activity and lower ALP activity in the haemolymph of the aestivated group when compared to the active specimens of *P. globosa*. Shylaja and Alexander (1974), reported that the activity pattern of the alkaline phosphatase enzyme in *Pila virens* during aestivating conditions was lower in all tissues examined when compared to control snails. The results of the current study disagree with the reports of Swami and Reddy (1978), who studied alkaline phosphatase activity in the tissues of active and aestivated *Pila globosa*.

CONCLUSION

In the present study, the activity level of enzymes ACP decreased and ALP increased significantly in the haemolymph of aestivating *P. globosa* when compared to active snails. This investigation explains the adaptability of *P. globosa* during aestivation. This study also reveals that *Pila globosa* is a suitable model for studies on aestivation and provides an interesting case of aestivation.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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