Biospectra : ISSN: 0973-7057, Vol. 18(2), September, 2023, pp. 27-34

An International Biannual Refereed Journal of Life Sciences

Animal Science

ISSN : 0973-7057

Biochemical responses of the fresh water Snail *Pila globosa* expose to urea

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Received : 05th May, 2023 ; Revised : 06th June, 2023

DOI:-https://doi.org/10.5281/zenodo.10718877

Abstract- The apple snail *P. globosa* is commonly known as Indian apple snail which is ectothermic invertebrates. Recently, it has received a lot of attention in the search for physiological responses measured by altered biochemical or enzyme activities. The present study was undertaken to study its various life cycle-associated issues in relation to metabolism, physiology, adaptation, etc. under various environmental factors including pollutants. *Pila globosa* were exposed to three sub lethal concentration urea - 0.8, 0.9, and 1.0 gm/L for a period of 15 days. The enzyme assay for the enzymes LDH, GOT, GPT, and AcP are done by different methods by calculating OD values by LABTRONICS model LT - 2900 spectrophotometer. The study of activities of these four enzymes appears that the exposure of *Pila globosa* to sub lethal concentrations (0.8, 0.9. and 1.0 g/L) of urea for 15 days caused significant changes in the activities of these enzymes altering many metabolic processes.

Key words: *P. globosa*, organophosphorus, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, acid phosphatase

INTRODUCTION

Ectothermic invertebrates have recently received a lot of attention in the search for physiological responses measured by altered biochemical or enzyme activities.[1-4] Environmental contaminants, such as metals, pesticides, and herbicides, have negative effects on a variety of organisms.[5-8] The apple snail *P. globosa* is commonly known as Indian apple snail. It is an ectothermic mollusc that can survive both in Water and on land and prefers location with a lot of aquatic flora.[9] In winter and summer seasons, this snail can even stay inactive for months. At such point, the organism may be extremely susceptible to environmental extremes and even a small amount of exposure to low-dose contaminants. As a result, apple snails can be a good model since they are important to both freshwater and grassland ecosystems, and because it is simple to measure and detect metabolic changes in this organism under controlled laboratory conditions.[10] The freshwater snail *P. globosa* is extremely susceptible to organophosphates like phorate (250%), formothion (130%) and trichlorfon (64%) and carbamates like aldicarb (198%), herbicides like butachlor (54.79%) and metals like mercury (44.3%) and nickel (101.55%).[11-13] The genus *Pila* was believed to have originated in Africa before spreading into Asia, perhaps as a result of continental drift.[14] The *Pila* genus has about 30 species of snail, and *P. globosa* is one of the most significant ones due to its value as a food, as a fish diet in aquaculture, and because of its semi-sessile, ectothermic nature, which makes it an ideal model for studying environmental physiology. The widespread

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species in India is *P. globosa*. The shell of *P. globosa* measures 46.5 mm in length and is univalve like those of other gastropods, and has an apex at its top. The growth of the shell is initiated from the apex, which is formed initially. The apple snail's delicate body is shielded by a thick, globular, univalvate shell that is brown or black in hue.[10] Three separate layers can be seen in the anatomy of the shell. The periostracum is the thin outer layer. It is made of homogenous, uncalciûed organic material containing glycoproteins and macromolecules that act as a resin to prevent shell breakage and help nucleate mineral component.[15] The gastropod *P. globosa* experiences periods of dormancy similar to aestivation and hibernation, just as other lower animals. In order to prevent hyper- or hypothermia and desiccation during these times, nearly all physiological activities are stopped. To avoid the water deprivation situation during that time, *Pila* withdraws its soft parts within the shell and is covered by the operculum by the secretion of dry mucus known as the epiphragm. The protein content of epiphragm ranges from 17 to 23 dry weight % while the carbohydrate content is only 0.4 to 2 dry weight %.[16] Under the hard winter or summer conditions, the freshwater snail *P. globosa* spends about 80 days either hibernating or aestivating.[10] And therefore, this snail's physiological reactions change as well.[10] Most physiological functions cease and many others are controlled by variations in enzyme activitiy during the dormant phase. *P. globosa* can be used as a food source since it is less expensive than other animal protein sources, has a high amount of protein, and is particularly beneficial for patients and individuals who are malnourished.[17] Due to its economic significance, it is necessary to study how the organism reacts to various abiotic and biotic insults at the biochemical and molecular levels in order to better understand potential for using this snail as bio- indicator species. The present study was undertaken to review its various life cycle-associated issues in relation to metabolism, physiology, adaptation, etc under various environmental factors including pollutants.

In general, organophosphorus molecule known as organophosphate is utilized as an insecticide. The hydrolytic activity of acetylcholinesteraseis inhibited by organophosphate substance phorate by 175%–325% by forming phosphorylated enzyme complexes, and this effect was generally shown to be time-dependent but not dose-dependent. Similarly, phosphorothioates, another organophosphate, also blocks acetylcholinesterase function by forming an oxygen analog.[18] Similar to phorate, the effects of pesticides such as formothion and trichlorfon on the acetylcholine function of *P. globosa* are shown to be time-dependent. For instance, the maximum effectiveness of formothion is reached 55 min longer than that of trichlorfon. As a result, different organophosphates effect differently in a time or dose- dependent manner on *P. globosa,* which demonstrate the mechanism of the effects of organophosphates. Under some circumstances, some organophosphates selectively cause toxicity. For instance, a high concentration of two pesticides formothion and trichlorfon inhibit the activity of acetylcholinesterase on excitatory receptors only by 130% and 64%, respectively. It indicates that organophosphates act on two different types of acetylcholine receptors in a selective manner.[19] Similarly, organophosphates such as methyl parathion and fenitrothion have an effect on carboxy esterase in the hepatopancreas of *P. globosa*. It is suggested that *P. globosa* be used as potential biomarker of organophosphate toxicity in light of the facts presented above, which points to the selective active action of organophosphates

Another class of insecticides called carbamate has a structure and function very similar to those of organophosphates. Metcalf (1971) proposed that carbamate compounds having N-methyl and N, N - dimethyl groups have anti-acetylcholinesterase activitiy. Aldicarb 107%–289%, however, despite not having either of the above chemical groups, inhibits acetylcholinesterase activity.[19]

METHODOLOGY

Pila globosa were exposed to three sub lethal concentration urea – 0.8, 0.9, and 1.0 gm/L for a period of 15 days. The snails were maintain in a fresh water at room temperature 28±2°C. control group was also maintain simultaneously for 15 days. After 15 days muscles, digestive gland, and gills were isolated from the control and urea treated groups. The tissue were homogenized in buffer solution and centrifuged at 8000 rpm for 30 minutes. The supernatant were isolated for enzyme assay appropriate dilution were done for supernatants protein concentration was also obtain by LORY METHOD. Enzyme assay were done according to given methods –

1. **Lactate dehydrogenase (LDH)**
   The method recommended by Bergmeyer *et al.* (1965)[20] was used for assay of LDH in different tissues of control and treated groups of snails. The OD were measured by LABTRONICS model LT – 2900 spectrophotometer at 340 nm.
2. **Aspartate aminotransferase (AAI or GOT)**
   The method recommended by Bermeyer and Bernt (1965)[21] was used for assay of GOT in different tissues of control and treated groups of snails. The OD were measured by LABTRONICS model LT – 2900 spectrophotometer at 540 nm.
3. **Alanine aminotransferase (AIAT or GPT)**
   The method recommended by Bermeyer and Bernt (1965)[21] was used for assay of GPT in different tissues of control and treated groups of snails. The OD were measured by LABTRONICS model LT – 2900 spectrophotometer at 540 nm.
4. **Acid phosphatase (AcP)**
   Acid phosphate activity was determined essentially according to the procedure of Champbell *et al.* (1978)[22]. By measuring the amount of p-nitro phenol (p-NP) released from the substrate p-nitrophenyl phosphate (p-NPP) disodium salt (Champbell *et. al.*1978)[22].

RESULT & DISCUSSION

The level of LDH, GOT, AcP and GPT enzyme were studied in gills, digestive glands and muscles of control and urea exposed snails which are present in the table 1, 2, 3, and 4. The changes in enzyme levels percentage of urea exposed snails in comparison to that of control are indicated in the same tables. From the data presented in the table's it is clear that sub lethal concentration of urea caused changes to different values in LDH activities in these three tissues of *Pila globosa*. However the effect of urea appears to be different on LDH and AcP levels. AcP was inhibited and LDH was activated in all three tissues of the *Pila globosa*. At all three exposure concentration – 0.80, 0.90, and 1.0 g/m. maximum inhibition was of -52.64% of AcP while maximum activation of LDH activity was of 95.65 was found in muscle and gills respectively. The minimum activation of LDH (12.56%) was found in digestive glands. (Table 1 & 2) the inhibitory effect of urea on SDH activity in *Pila globosa* tissue appears to be concentration dependent. The AcP activity was decreased in all the three tissue of all urea treated snails with maximum inhibition of enzyme in the 1.0 g/L exposed group. Reduction in the activity of the two transaminases AAI, GOI, and AIAT is cleared from table 3 and 4. GOT levels reduced significantly in all the selected tissues in snails to 0.8, 0.9, and 1.0 g/L urea exposed in the gills snail except to 0.80 g/L. The AIAT activity also showed significantly reduced (- 16 % to – 34%) in the gills of snails at all expose concentration while the decrease (2.2 % to 25 %) in the muscle. It was significant at these exposure concentrations in the digestive glands.

Table-1- Lactic dehydrogenase (LDH) activity (unit/0.1mg Protein) in different tissues of *P. globosa* exposed to urea for 15 days

Exposed to urea concentration gm/L	LDH activity (unit/ 0.1mg. Protein) in different tissues of *Pila globosa*		
	Gills	Digestive gland	Muscle
Control (0.00)	6.21 ± 0.53	7.66 ± 0.63	7.01 ± 0.56
0.80	8.63 ± 0.74	8.76 ± 1.36	6.68 ± 1.16
0.90	10.76 ± 1.36	12.53 ± 1.06	7.28 ± 0.76
1.00	12.15 ± 0.83	12.89 ± 1.56	8.85 ± 1.36

Table 2- Aspartate Aminotransferase (AAT or GOT) activity (unit/ 0.1mg. Protein) in different tissues of *P. globosa* exposed to urea for 15 days

Exposed to urea concentration gm/L	AAT activity (unit/ 0.1mg. Protein) in different tissues of *Pila globosa*		
	Gills	Digestive gland	Muscle
Control (0.00)	4.53 ± 0.34	5.16 ± 0.16	4.76 ± 0.28
0.80	4.43 ± 0.46	4.73 ± 0.22	4.26 ± 0.26
0.90	3.54 ± 0.63	3.26 ± 0.18	4.68 ± 0.66
1.00	3.38 ± 0.24	3.84 ± 0.56	3.82 ± 0.26

Table 3- Alanine Aminotransferase (AIAT or GPT) activity (unit / 0.1mg. Protein) in different tissues of *P. globosa* exposed to urea for 15 days

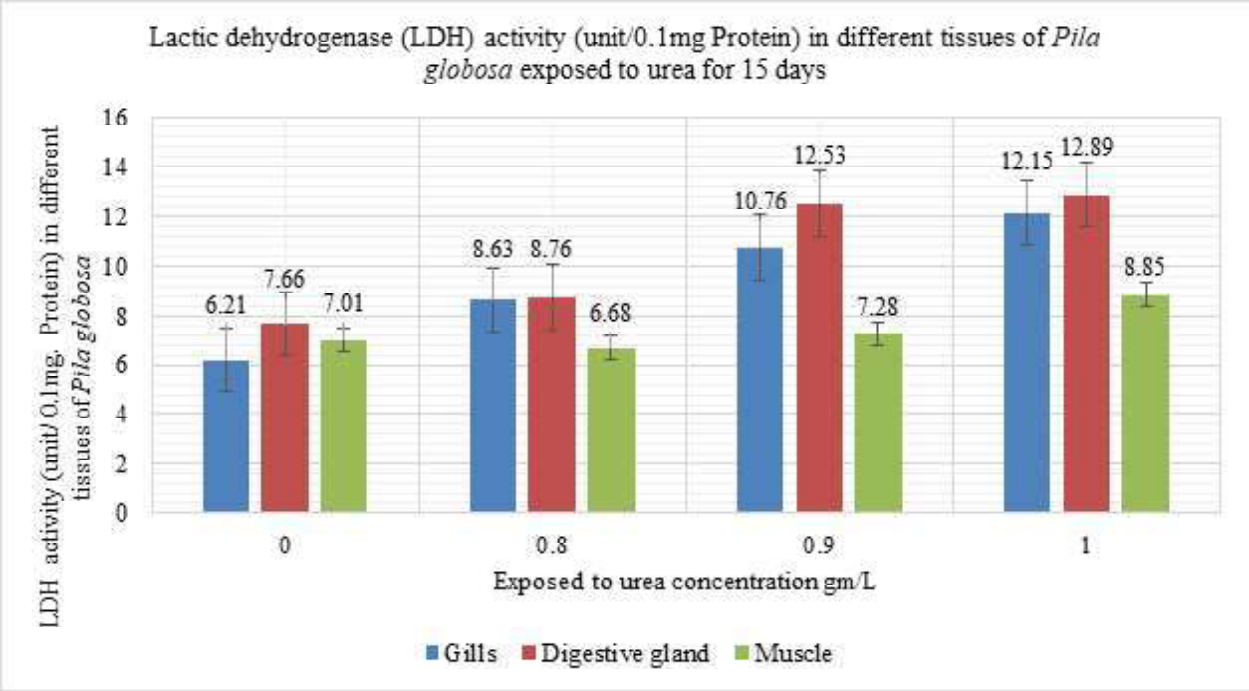
Exposed to urea concentration gm/L	AIAT activity (unit/ 0.1mg. Protein) in different tissues of *Pila globosa*		
	Gills	Digestive gland	Muscle
Control (0.00)	4.56 ± 0.24	4.86 ± 0.84	5.84 ± 0.35
0.80	3.88 ± 0.88	3.80 ± 0.46	5.42 ± 0.14
0.90	3.06 ± 0.12	2.46 ± 0.24	4.84 ± 0.54
1.00	1.38 ± 0.36	3.86 ± 0.14	4.76 ± 0.22

Values are mean ± S.D. of 5 determinations
Values in parenthesis indicate % variation from control = P< 0.05

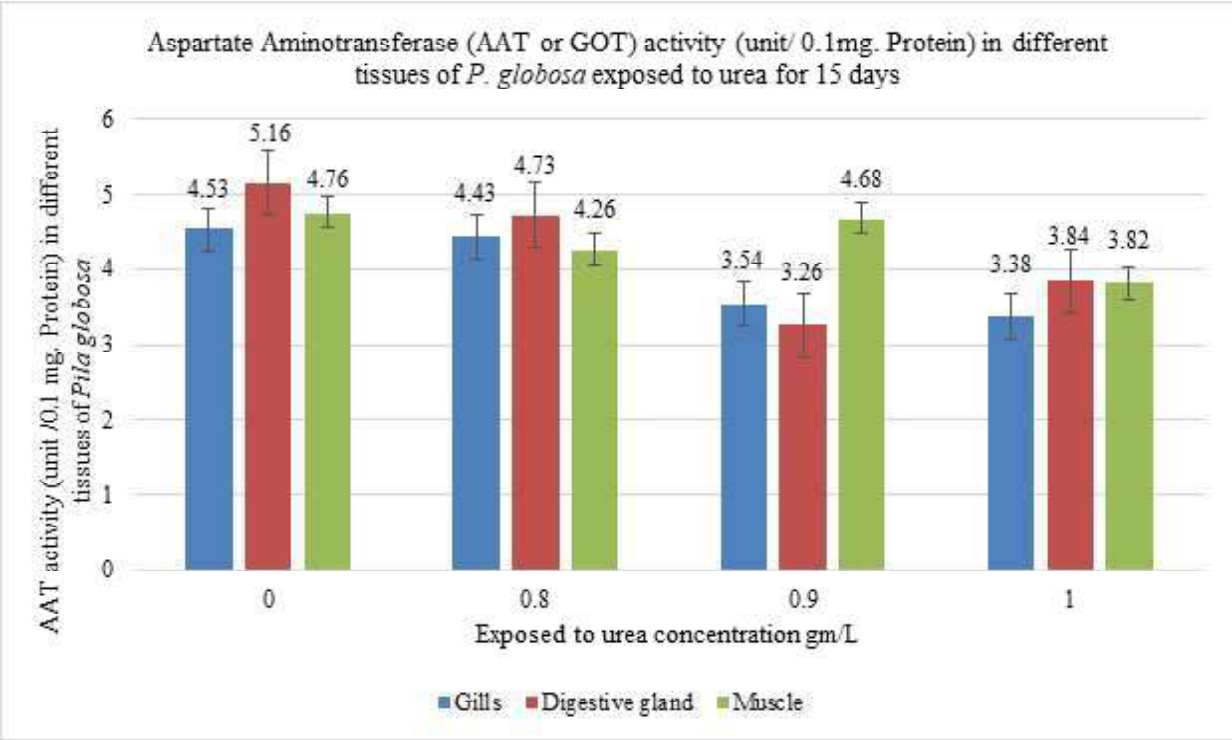
Table 4- Acid Phosphatase (AcP) activity (unit /0.1 mg Protein) activity in different tissues of *P. globosa* exposed to urea for 15 days

Exposed to urea concentration gm/L	AcP activity (unit/ 0.1mg. Protein) in different tissues of *Pila globosa*		
	Gills	Digestive gland	Muscle
Control (0.00)	12.84 ± 0.63	22.07 ± 0.86	17.76 ± 0.86
0.80	8.43 ± 0.24	26.52 ± 0.24	10.32 ± 0.34
0.90	5.86 ± 1.24	11.38 ± 0.36	6.84 ± 0.82
1.00	4.34 ± 0.34	10.86 ± 0.46	4.22 ± 1.22

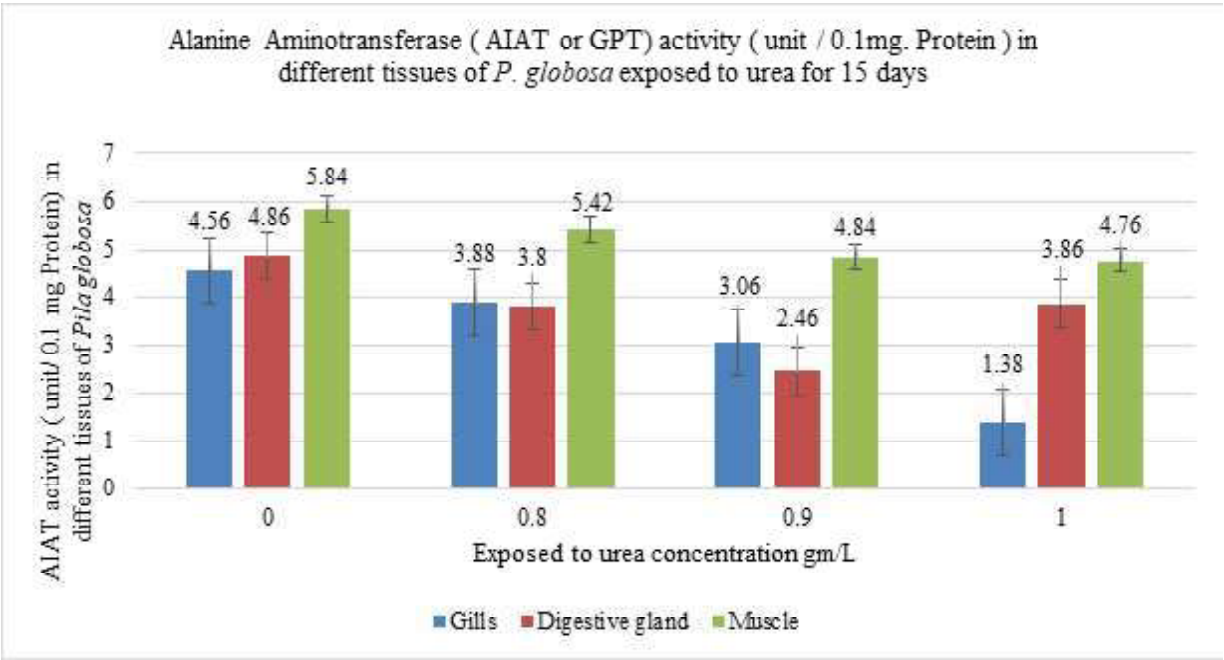
Values are mean ± S.D. of 5 determinations
Values in parenthesis indicate % variation from control = P< 0.05
Temperature 28°C ± 2



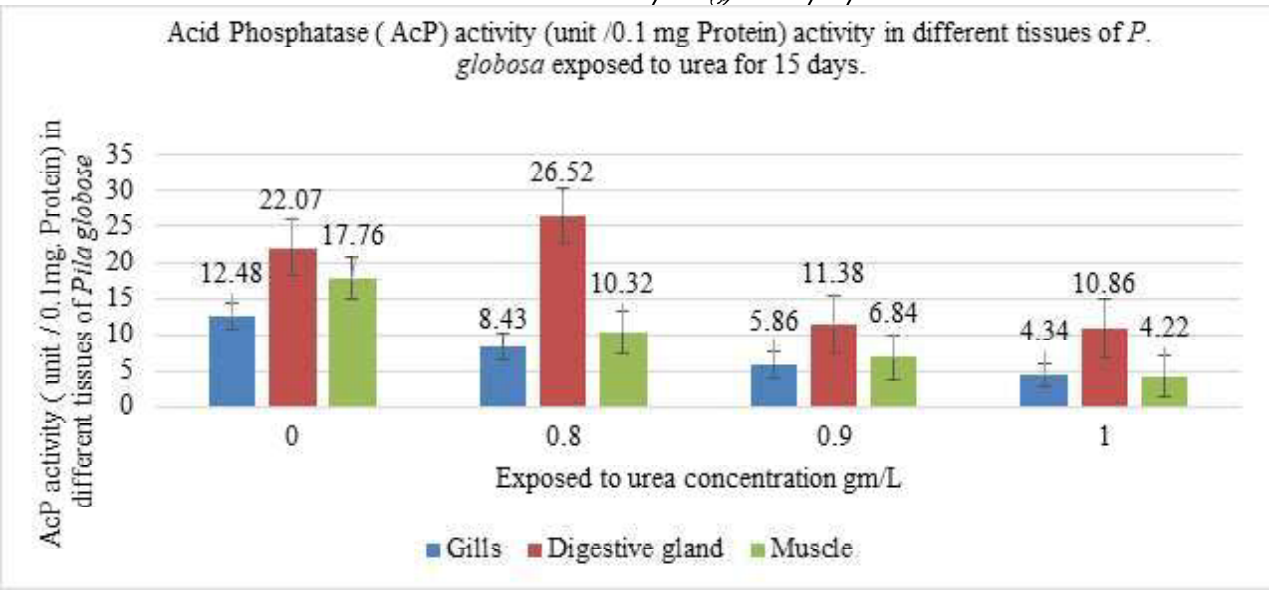
Graph 1- Lactic dehydrogenase (LDH) activity (unit/0.1mg Protein) in different tissues of *P. globosa* exposed to urea for 15 days



Graph 2- Aspartate Aminotransferase (AAT or GOT) activity (unit/ 0.1mg. Protein) in different tissues of *P. globosa* exposed to urea for 15 days



Graph 3- Alanine Aminotransferase (AIAT or GPT) activity (unit / 0.1mg. Protein) in different tissues of *P. globosa* exposed to urea for 15 days



Graph 4- Acid Phosphatase (AcP) activity (unit /0.1 mg Protein) activity in different tissues of *P. globosa* exposed to urea for 15 days.

CONCLUSION

The study of activities of these four enzymes appears that the exposure of *Pila globosa* to sub lethal concentrations (0.8, 0.9. and 1.0 g/L) of urea for 15 days caused significant changes in the activities of these enzymes altering many metabolic processes.

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