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Original Article

Temporal Dynamics of Ovotesticular Biochemical Depletion in Semperula maculata under Acute Boric Acid Stress

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Abstract

The present study investigates the temporal effects of acute boric acid exposure on the biochemical composition of the ovotestis in the terrestrial slug Semperula maculata. The experiment revealed significant depletion in glycogen, lipid, and protein contents under lethal concentration exposure over varying time durations. These findings provide insight into the toxicological impact of boric acid and highlight the ovotestis as a sensitive biomarker organ under chemical stress. The results contribute to understanding the reproductive toxicity mechanisms in pulmonate gastropods.

This study evaluates the impact of acute boric acid exposure on the ovotestis of the terrestrial slug Semperula maculata, with emphasis on temporal alterations in key biochemical constituents. Experimental slugs exposed to a lethal concentration (LC_{50} = 5459.45 ppm) of boric acid for 24–96 hours showed a progressive decline in protein, glycogen, and lipid levels compared to controls. Protein concentration decreased sharply from 6.53 mg/100 mg at 24 hours to 1.14 mg/100 mg at 96 hours, while glycogen and lipid contents followed a similar depletion trend. These results indicate that boric acid stress induces enhanced metabolic mobilization of organic reserves, likely triggered by oxidative stress and cytotoxic damage. The findings highlight the ovotestis as a highly sensitive biomarker organ for reproductive toxicity assessment in pulmonate gastropods. Overall, this work contributes to understanding the biochemical mechanisms underlying molluscan responses to xenobiotic stress and supports the use of S. maculata as a potential bioindicator species for ecotoxicological evaluations.

Keywords: *Semperula maculata, ovotestis, boric acid, reproductive toxicity.*

Introduction

The survival and physiological integrity of animals rely heavily on key metabolic substrates such as proteins, carbohydrates, and lipids, which are obtained through dietary intake and mobilized under environmental stress (Awati and Nanaware, 2004). Monitoring changes in biochemical profiles provides critical insights into the sublethal effects of toxicants, as such alterations often indicate cellular damage and adaptive metabolic responses (Sousa, 2003). The accumulation of environmental pollutants can induce metabolic reprogramming aimed at detoxification, as demonstrated in aquatic organisms exposed to heavy metals, where measurable shifts in biochemical constituents serve as reliable biomarkers of toxicity (Zhang et al., 2010; Rajkumar and Milton, 2011).

Waykar and Shinde, (2019) highlighted that these responses can manifest at the molecular, biochemical, physiological, or histological levels. Biochemical biomarkers, defined as measurable changes in metabolic or cellular components triggered by environmental stressors, are widely recognized as sensitive indicators of pollutant exposure (Benford et al., 2000). Experimental studies have shown progressive depletion of internal biochemical reserves—including lipids, proteins, carbohydrates, and nucleic acids—following toxicant exposure, reflecting their mobilization to meet energy demands during prolonged stress (Jagtap et al., 2011). The generation of reactive oxygen species (ROS) by pesticides, particularly insecticides, contributes to lipid peroxidation and oxidative stress, resulting in structural and functional cellular damage (Yang et al., 2018; Geter et al., 2008; El-Gendy et al., 2010). Phospholipids, critical for membrane integrity, are particularly susceptible to oxidative degradation (Martinez-Pita et al., 2012).

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Manduzio et al., (2005) emphasized that all major classes of biomolecules such as polysaccharides, lipids, proteins, and nucleic acids are vulnerable to ROS-mediated oxidative modification. The present study aims to evaluate contaminant-induced biochemical alterations in the gonadal tissues of three molluscan species—Semperula maculata (terrestrial slug), Macrochlamys indica (terrestrial snail), and Bellamya bengalensis (freshwater snail)—as indicators of sublethal toxic stress.

Objectives

1. To evaluate the temporal effects of acute boric acid exposure on the biochemical composition (glycogen, lipid, and protein) of the ovotestis in Semperula maculata.
2. To determine the extent of biochemical depletion in the ovotestis under varying durations of lethal boric acid stress.
3. To assess the ovotestis as a sensitive biomarker organ for detecting reproductive toxicity in terrestrial pulmonate gastropods.
4. To contribute to understanding the mechanisms of boric acid-induced reproductive toxicity by analyzing changes in key biochemical constituents.

Materials and Methods:

Animal Collection and Maintenance -

Adult specimens of *Semperula maculata* were maintained under controlled laboratory conditions in the Department of Zoology, Shivaji University, and Kolhapur. The slugs were acclimated in ventilated plastic troughs and provided with a continuous supply of tomatoes, cauliflower, cabbage, mulberry leaves and water (Figure 1).

Tissue Sampling -

Biochemical analysis was conducted on the ovotestis of *S. maculata*. Both control and experimental groups were exposed to boric acid to assess tissue-level toxicity.

Biochemical Analyses -

Protein Estimation (Lowry *et al.*, 1951):

Protein content was estimated using the Lowry method, a colorimetric assay based on the reaction of proteins with Folin–Ciocalteu reagent. Tissue homogenates (0.1 mL) were mixed with 1.4 mL distilled water, followed by 3.0 mL of Lowry's reagent C and 0.5 mL Folin-Ciocalteu reagent. Absorbance at 660 nm was measured following a 15-minute reaction period. The calibration curve was generated using bovine serum albumin (BSA) as the protein standard.

Quantification of Glycogen Using the Anthrone Method (Modified from Hassid and Abraham, 1957):

Known-weight tissues were digested in 30% KOH, followed by ethanol precipitation, centrifugation, and addition of Anthrone reagent. After heat treatment and cooling, absorbance was measured at 720 nm. Glucose served as the standard for glycogen conversion.

Colorimetric Estimation of Lipids Following the Vanillin–Phosphoric Acid Protocol (Barnes and Stock, 1973):

A 100 mg tissue sample was extracted with a 2:1 chloroform–methanol mixture; 1 mL of the filtrate was evaporated, treated with concentrated H_2SO_4, and reacted with vanillin reagent. The pink chromogen was detected at 540 nm, and cholesterol served as the standard for calibration.

Statistical Analysis -

All data were analyzed using SPSS software. LC_{50} values were determined through probit analysis. Reductions in protein, glycogen, and lipid levels in response to toxicant exposure were assessed using one-way ANOVA and Student's *t*-test ($p < 0.05$).

Results:

The terrestrial slug Semperula maculata, an experimental molluscan species, exhibited notable alterations in its biochemical composition including protein, glycogen, and lipid content following exposure to boric acid. The extent of biochemical alterations depended on both the exposure duration and concentration of boric acid.

Effect of Boric acid exposure on protein, glycogen, and lipid content from ovotestis of *Semperula maculata*:

Exposure to a lethal concentration of boric acid (LC_{50} = 5459.45 ppm) induced significant alterations in these biochemical contents in the ovotestis of S. maculata.

Protein content in the ovotestis of *S. maculata* showed marked alterations following boric acid treatment:

As shown in Table 1, the protein content in the ovotestis of control S. maculata remained relatively stable across time points: 8.3156 ± 0.040 mg (24 hrs.), 8.3033 ± 0.035 (48–72 hrs.), 8.2913 ± 0.020 mg/100 mg (96 hrs.). The experimental group of slugs exhibited a decrease in protein content as the boric acid exposure period increased from 24 to 96 hours. At 24 hours of boric acid exposure, the protein level declined to 6.5333 ± 0.035 mg/100 mg, significantly lower than in the control group. The protein concentration decreased even further to 5.2333 ± 0.023 mg/100 mg when the exposure period was extended to 48 and 72 hours. After 96 hours of exposure, the protein content in ovotestis tissue markedly decreased to 1.1456 ± 0.020 mg/100 mg. As demonstrated in Graph 1, the protein concentration of the ovotestis of Semperula maculata was significantly reduced at various time intervals between 24 and 96 hours due to boric acid.

Glycogen levels within the ovotestis of *S. maculata* were significantly affected by boric acid-induced stress:

Observable changes in the ovotestis glycogen content of the control and experimental S. maculata are presented Table 2. The glycogen content in the ovotestis of the control group of slugs was 3.346 ± 0.017 mg/100 mg at 24 hours and remained constant at 3.336 ± 0.017 mg/100 mg at 48, 72 and 96 hours. Exposure of the experimental group to a lethal concentration of boric acid resulted in a significant, time-dependent depletion of glycogen content from 24 to 96 hours. The experimental group showed substantially lower ovotestis glycogen levels compared to the control group during the 24 to 96 hour exposure period to the lethal boric acid dosage. The quantity of glycogen decreased to 2.698 ± 0.013 mg/100 mg and 2.193 ± 0.013 mg/100 mg after exposure to boric acid for 24 and 48 hours, respectively. Comparative analysis of the ovotestis glycogen

content in Semperula maculata revealed a marked reduction at 72 hours (1.557 ± 0.0068 mg/100 mg) and 96 hours (0.551 ± 0.0063 mg/100 mg) following boric acid exposure. As shown in Table 2 and Graph 2, exposure to boric acid significantly reduced glycogen content in the ovotestis of S. maculata.

Table 1: Time-Dependent Effects of Boric acid on Protein content in Ovotestis Of Semperula maculata.

Exposure Duration (hours)	Control Group (Mean ± SEM)	Treated Group (Mean ± SEM)
24	8.3156 ± 0.040	6.5333 ± 0.035 ***
48	8.3033 ± 0.035	5.2333 ± 0.023 ***
72	8.3033 ± 0.035	5.2333 ± 0.023 ***
96	8.2913 ± 0.020	1.1456 ± 0.020 ***

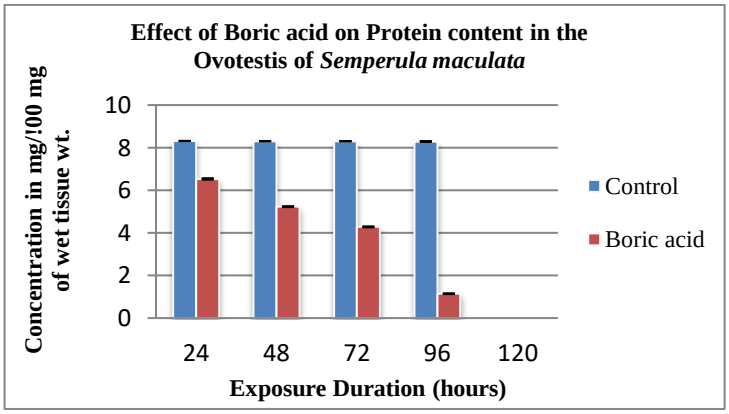
Table 2: Time-Dependent Effects of Boric acid on Glycogen content in Ovotestis Of Semperula maculata.

Exposure Duration (hours)	Control Group (Mean ± SEM)	Treated Group (Mean ± SEM)
24	3.346 ± 0.017	2.698 ± 0.013 ***
48	3.336 ± 0.017	2.193 ± 0.013 ***
72	3.336 ± 0.017	1.557 ± 0.0068 ***
96	3.336 ± 0.017	0.551 ± 0.0063 ***

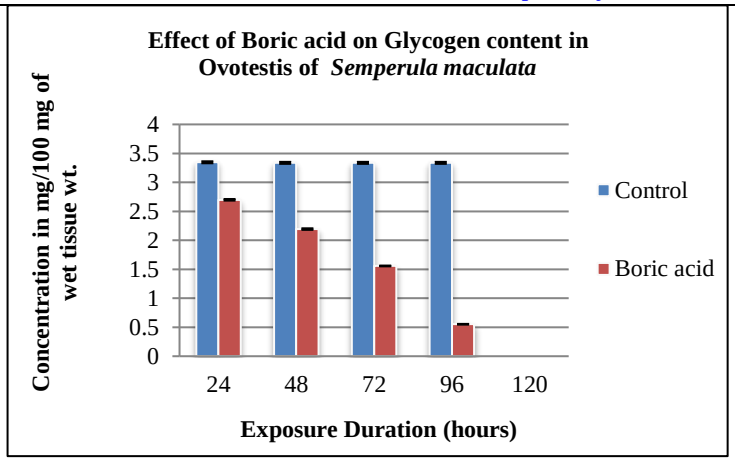
Table 3: Time-Dependent Effects of Boric acid on Lipid content in Ovotestis Of Semperula maculata.

Exposure Duration (hours)	Control Group (Mean ± SEM)	Treated Group (Mean ± SEM)
24	1.870 ± 0.051	1.536 ± 0.011 ***
48	1.883 ± 0.028	0.866 ± 0.028 ***
72	1.883 ± 0.028	0.616 ± 0.028 ***
96	1.883 ± 0.028	0.223 ± 0.015 ***

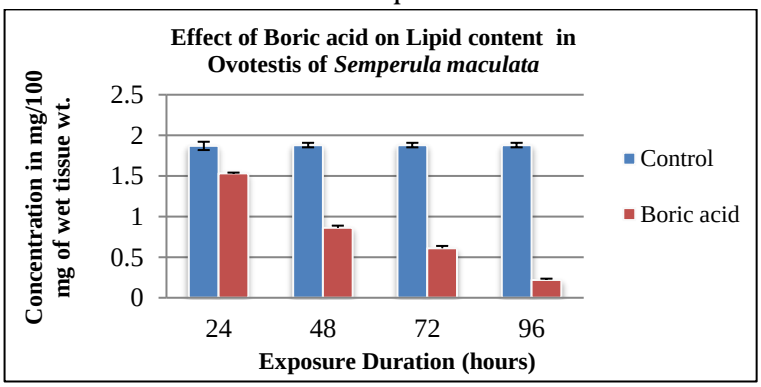
Note: Mean ± SD (n = 10). Values with ± indicates mean with standard deviation, *, **, *** and NS indicates the significance level P < 0.05 (statistically significant), P < 0.01 (statistically significant), P < 0.001 (highly significant), P > 0.05 (statistically non-significant) respective.



Graph 1



Graph 2



Graph 3

Boric acid exposure resulted in notable changes in the lipid composition of the ovotestis in *S. maculata*:

In control specimens, the ovotestis lipid content remained consistent, ranging from 1.870 ± 0.051 mg/100 mg (24 hours) to 1.883 ± 0.028 mg/100 mg (48 to 96 hours), as shown in Table 3 and Graph 3. However, as the boric acid exposure duration increased from 24 to 96 hours, the lipid concentration gradually decreased. Following 24 and 48 hours of boric acid exposure, lipid levels significantly declined to 1.536 ± 0.011 mg/100 mg and 0.866 ± 0.028 mg/100 mg, respectively, compared to controls. A further reduction in lipid concentration was observed with prolonged exposure to 72 and 96 hours, with levels markedly decreasing to 0.616 ± 0.028 mg/100 mg and 0.223 ± 0.015 mg/100 mg respectively.

Discussion:

In the present study, it was observed that exposure to boric acid resulted in a progressive and substantial depletion of organic constituents in the gonads, particularly as the exposure duration increased from 24 to 96 hours. According to Awati and Nanaware, (2004), pesticide exposure in molluscs resulted in the depletion of key biochemical constituents such as proteins, carbohydrates, and lipids, thereby reducing energy reserves, particularly in the gills and gonadal tissues. Tayade and Kulkarni, (2007) noted that total protein levels in molluscan species were lowest during the periods of reproductive activity. Kamble and Nanaware, (2007) reported that heavy metals create a toxic environment in the organs of the aquatic snail *Bellamya bengalensis* including the gills, hepatopancreas, gonads, foot, and mantle which ultimately disrupting the concentrations of proteins, carbohydrates, and lipids and causing a gradual decline to extremely low levels with prolonged exposure. This observation is consistent with the present study, wherein cells utilized their organic constituents to withstand the cytotoxic stress induced by boric acids. The depletion of these components intensifies with increasing exposure duration from 24 to 96 hours. According to Londhe and Kamble, (2014) to counteract the toxic effects of heavy metals such as mercuric chloride and zinc chloride, the body cells of the freshwater snail Bellamya bengalensis rapidly consumed these biochemical constituents for energy, resulting in a significant depletion of these nutrients. This aligns with the findings of the present study, which demonstrated that the gonadal cells of the molluscan species S. maculata utilized lipids, glycogen, and proteins for the survival, resulting in a marked reduction in these constituents upon exposure to boric acid.

Rao and Singh, (2000) reported that the application of oils derived from Azadirachta indica, Cedrus deodara, and Allium sativum to Achatinal fulica altered the normal levels of cellular proteins, amino acids, phospholipids, and DNA content.
Satyaparameshwar, (2006) reported that the freshwater mussel Lamellidens marginalis experienced an energy deficit due to the toxic effects of copper sulphate, resulting in significant depletion of its glycogen reserves. Patil and Mane, (2004) observed biochemical alterations in various tissues of the freshwater bivalve Lamellidens marginalis exposed to mercury during the monsoon season. The freshwater prawn, Kistnensis macrobrachiuim, experiences gradual protein loss in its gills, muscles, hepatopancreas, and ovaries due to exposure to varying doses of tributyltin chloride (Kharat et al., 2009), thereby significantly disrupting normal protein metabolism (Sole et al., 2000). Similarly, Jagtap et al, (2011) found that the freshwater bivalve Lamellidens marginalis showed a significant reduction in protein concentration in the digestive glands, gills, and reproductive organ under acute tributyltin chloride exposure spanning 24 to 96 hours. Tripathi and Verma, (2004) also investigated protein

metabolic changes in the freshwater fish Clarius batrachus, where sublethal doses of endosulfan insecticides affected enzymatic metabolic processes in the brain, liver, and skeletal muscle cells, led to an approximately 30–37% reduction in protein content.

The study by Kalaimani and Kandeepan, (2017) revealed that administration of sublethal concentrations of organophosphate pesticides in Labeo rohita fish resulted in increased free radical production and oxidative damage to cellular biomolecules, including proteins, albumins, lipids, and DNA, with a marked reduction in total protein levels after thirty days. Freshwater bivalves such as Parreysia favidens, Parreysia cylindrica, and Lamellidens marginalis exhibited significant reductions in protein concentrations after exposure to distillery effluent (Shandilya et al., 2010). Additionally, the mitigating effects of ascorbic acid on profenofos insecticide toxicity (Waykar and Pulate, 2012), as well as exposure to other insecticides such as Indoxacarb and acaricide like monocrotophos (Patil, 2011), were also reported. Following exposure to arsenic, the Asian green mussel Perna viridis exhibited significant alterations in its biochemical composition, including elevated lipid peroxidation (LPO) levels up to128 ppb, increased antioxidant enzyme activity, and a noteworthy depletion in lipid content (Rajkumar, 2013**;** Bhavani and Dawood, 2003). According to Potdar and Kamble, (2014), heavy metals induced oxidative stress, promoting cells to generate reactive oxygen species (ROS) such as hydrogen peroxide, superoxide radicals, and hydroxyl radicals, which damaged proteins, carbohydrates, lipids, and nucleic acids (Monferran et al., 2008 and Vieira et al., 2009). This is further corroborated by a study by Simmons et al., (2011), which found that reactive oxygen species disrupted cellular processes, leading to necrosis and apoptosis, along with a significant reduction in nucleic acid, lipid, protein, and carbohydrate contents.

The study indicates that with increasing exposure time, there is a consistent decline in the levels of key biomolecules such as lipids, carbohydrates, nucleic acids and proteins. The study also revealed that Semperula maculata showed an increase in acidic mucosubstance levels in vital organs, while protein, glycogen, and lipid levels were reduced as a physiological response to toxic stress induced by boric acid.

Conclusion

The findings indicate that short-term exposure to boric acid at lethal concentration (LC_{50} = 5459.45 ppm) causes substantial and progressive depletion of fat, carbohydrate and protein levels in the ovotestis of the terrestrial slug Semperula maculata. Gradual declines in these biomolecules over 24 to 96 hours indicate enhanced metabolic demand and mobilization of energy reserves, likely driven by oxidative stress-induced cellular damage. The observed biochemical alterations reflect disrupted homeostasis and impaired gonadal function under toxic stress. These findings underscore the utility of protein, glycogen, and lipid contents as sensitive biochemical biomarkers for evaluating reproductive toxicity and environmental contaminant exposure in terrestrial molluscs. The study further highlights *S. maculata* as a viable model organism for ecotoxicological assessments of soil and freshwater pollutants.

Conflicts of Interest:

No potential conflicts of interest were disclosed by the authors in relation to this study.

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