Seasonal Alterations in Oxidative Stress Biomarkers of Freshwater Snails: *Bellamya bengalensis* and *Lymnaea acuminata* from Malangaon Reservoir of Dhule District, Maharashtra, India

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**Abstract**

**Objectives:** Two freshwater snails *Bellamya bengalensis* and *Lymnaea acuminata* from Malangaon reservoir of Dhule district (Maharashtra, India) were examined to study biomarkers of oxidative stress with the investigation of antioxidant enzyme activities. **Methods:** The experimental snail species were collected seasonally and acclimatized in the laboratory. Their digestive glands were used to study the activities of oxidative biomarker enzymes like Lipid Peroxidation (LPO), Glutathione-S-Transferase (GST), reduced glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx). All investigations were carried out on double beam spectrophotometer (Elico BL 200). **Findings:** It was observed that, LPO level in *B. bengalensis* was 0.63, 0.55 and 0.60 (nmol of MDA formed/mg protein) while in *L. acuminata* 0.74, 0.67 and 0.70 respectively in summer, monsoon and winter seasons. GST activity was 23.41, 15.73 and 19.61 in *B. bengalensis* while 26.29, 18.34 and 22.29 in *L. acuminata*. Level of GSH was 0.083, 0.117 and 0.094 (µM/g wet tissue) in *B. bengalensis* while in *L. acuminata* it was 0.018, 0.056 and 0.026 (µM/g wet tissue) in three seasons. The level of SOD was 20.87, 24.83 and 23.33 (U/mg of protein) in *B. bengalensis* while in *L. acuminata* it was 18.32, 22.35 and 20.73 in three seasons. Level of CAT was 9.23, 13.20, and 11.56 (U/mg of protein) in *B. bengalensis* while in *L. acuminata* it was 7.02, 10.53 and 8.86 (U/mg of protein) respectively during three seasons. GPx movement was 6.57, 8.50 and 7.48 (mg of GSH utilized/min/mg protein) in *B. bengalensis*. It was 5.05, 8.07 and 7.04 (mg of GSH utilized/min/mg protein) in *L. acuminata* respectively in three seasons. The obtained data also showed the lowest levels of LPO and activities of GST and uppermost doings of SOD, CAT, and GPx and uppermost heights of GSH in two snail types in rainy period than summer and winter period. This designates that in summer, the snails were under more conservation stress than winter and rainy periods. **Application:** It was decided that changes in antioxidant enzymes and LPO and GST activity can be used as tool in ecological nursing packages.

**Keywords:** *Bellamya bengalensis*, Biomarkers, *Lymnaea acuminata*, Malangaon Reservoir, Oxidative Stress, Seasonal Alterations

1. Introduction

In water faunae, numerous ordinary and anthropogenic issues encourage unevenness among the ROS and their elimination. As a consequence of this oxidative stress occurs. Educations of the oxidative pressure response in water organisms have been projected as a basis of significant material that could be secondhand as gears to control...
the excellence of the environment\textsuperscript{3}. Cellular antioxidant enzymes respond to augmented ROS stages with unalike planes of magnitude\textsuperscript{4} and their goings-on alteration below the inspiration of periodic rhythmicity\textsuperscript{5}, revision to low ecological temperatures\textsuperscript{6} and site-specific conservation impact\textsuperscript{7}. Modifications in antioxidant enzyme activities can afford valuable statistics for characteristic sandwiched between different groups of plants conferring to the indigenous fundamental conservational scene and biological factors\textsuperscript{8}. The oxidative stress may give a good indication of the atmosphere's indigenous effluence position.

The antioxidants structure comprises in the sanitization of xenobiotics\textsuperscript{9}. Antioxidant enzymes arrangements are regularly determined by the limitations like SuperOxide Dismutase (SOD), CATalase (CAT), concentrated glutathione (GSH) Glutathione Peroxidase (Gpx), and Glutathione S-Transferase (GST)\textsuperscript{10,11,12}. These limitations deliberate quantifiable of well-designed complaint of a bacterium are regularly previously owned in biomonitoring studies as biomarkers. They were described in a certain number of aquatic creatures such as snails\textsuperscript{13}, annelida\textsuperscript{14}, mussels\textsuperscript{15}, and fish\textsuperscript{16}. These antioxidant enzymes have been proposed to use as biomarkers for exposure to ROS-mediating contaminants, such as weighty metals\textsuperscript{17}.

The action of antioxidant defense enzymes other biomarkers variations significantly with admiration to the accessibility of nutrients, generative station, season-related evolution rate and other clothes, all over the year. Seasonal alterations have also been observed in the levels of contaminant and in natural exposure to oxidative stress\textsuperscript{18}. Particular features of cyclical vicissitudes in antioxidant protection have been observed in the tissues of many aquatic animals, such as thin-lip gray mullet, Liza ramada\textsuperscript{19}, mussel, Mytilus galapog ii\textsuperscript{20} and blue mussel, Mytilus edulis\textsuperscript{21}.

Ponder of cancer prevention agent safeguard in the view of biological danger is not totally caught on. Freshwater snails are found in streams, waterways, tidal ponds, and lakes, and are enter interfaces in the natural way of life chief from supplements in the water and buildup to trawl and ducks that are used by individuals. They are moderately simple to gather and identify\textsuperscript{21,22}. Also, snails fulfill every one of the states of a decent organic indicator\textsuperscript{23}. In this paper, we describe changes in antioxidant defense enzyme levels in whole soft body tissues of the freshwater snail, Bellamya bengalensis and Lymnaea acuminata summer, monsoon and winter dated from Malangaon reservoir.

2. Materials and Methods

2.1 Morphometry of Study Area
Malangaon artificial lake is positioned on Kan River. It is one of the tributary of Panzara river of North Maharashtra area of spagetti western India. Geographically it is located at 21°05’16.72” N and 74°04’52.98” E. The storage volume of the tank is 11.32 million sq. meter. The Basin is stone type and its span is 1091 patterns. The decided height is 23 meter. The run of the mill territory of the tank is 1587 hectares. It has left trench of 25 kms in length which covers around 9 towns. The water of this store is held for drinking and water system purposes.

2.2 Experimental Setup
The freshwater snails Bellamya bengalensis and Lymnaea acuminata\textsuperscript{24} were collected seasonally from Malangaon reservoir of Dhule district. They were dissected discretely and the intestinal glands were uninvolved systematically and eroded by phosphate buffer (50 mM; pH 7.3). 1 gm of gastric gland was standardized with 50 mM phosphate buffer (pH 7.4) and centrifuged at 10000 rpm for 20 min at 4°C. The supernatant was recycled for enzyme activities. Numerous oxidative biomarker enzymes like Lipid peroxidation (LPO)\textsuperscript{25}, Glutathione-S-transferase (GST)\textsuperscript{26}, Reduced glutathione (GSH)\textsuperscript{27}, Superoxide dismutase (SOD)\textsuperscript{28}, Catalase (CAT)\textsuperscript{29} and Glutathione peroxidase (Gpx)\textsuperscript{30} were estimated. All the enzyme events were unhurried by overwhelming second beam spectrophotometer (Elico BL 200).

3. Results

3.1 Water Quality
Infection and softened oxygen of water were projected seasonally and exposed in Table 1 and High temperature diverse from maximum 22.48°C in straw-hat and the lowest as 17.55°C in season. It was reasonable (19.51°C) in rainy season. The melted oxygen attentions were highest (12.24 mg/l) throughout taciturn weather of wintertime and lowest (7.36mg/l) during summertime. It was judicious (10.521 mg/l) in monsoon.

3.2 Biomarkers
The oxidative pressure pointer limitations like doings of antioxidant protection enzymes, the levels of antioxidant
scavenger molecules and Lipid Peroxidation (LPO) were projected seasonally from peptic glands of pond snails, *B. bengalensis* and *L. acuminata* collected from Malangaon reservoir. The obtained results are summarized in the Table 2.

### 3.2.1 Lipid Peroxidation (LPO) (MDA Formation) Level

The LPO levels in *B. bengalensis* were found 0.63, 0.55 and 0.60 (nmol of MDA shaped/mg protein) while in *L. acuminata* 0.74, 0.67 and 0.70 (nmol of MDA formed/mg protein) correspondingly in summer, monsoon as well as winter seasons.

### 3.2.2 Glutathione-S-Transferase (GST)

During seasonal study period summer, monsoon and winter seasons GST activity was 23.41, 15.73 and 19.61 (nmol CDNB conjugate formed/min/mg of protein) in *B. bengalensis* while 26.29, 18.34 and 22.29 (nmol CDNB conjugate formed/min/mg of protein) in *L. acuminata*.

### 3.2.3 Reduced Glutathione (GSH)

The level of GSH was 0.082, 0.117 and 0.094 (µM/g wet tissue) in *B. bengalensis* while in *L. acuminata* it was 0.018, 0.056 and 0.026 (µM/g wet tissue) respectively throughout summertime, rainy season and wintertime.

### 3.2.4 Superoxide Dismutase (SOD)

The level of SOD was 20.87, 24.82 and 23.33 (U/mg of protein) in *B. bengalensis* while in *L. acuminata* it was 18.32, 22.35 and 20.73 (U/mg of protein) correspondingly throughout summertime, rainy season and wintertime.

### 3.2.5 CATalase (CAT)

The level of CAT was 9.23, 13.20 and 11.56 (U/mg of protein) in *B. bengalensis* while in *L. acuminata* it was 7.02, 10.53 and 8.86 (U/mg of protein) respectively.

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Table 1. Seasonal alterations in physico-chemical parameters of water from Malangaon reservoir of Dhule district.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Parameters</th>
<th>Seasons</th>
<th>Monsoon</th>
<th>Summer</th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dissolved oxygen (mg/l)</td>
<td></td>
<td>10.51 ± 0.49</td>
<td>7.35 ± 0.44</td>
<td>12.24 ± 0.41</td>
</tr>
<tr>
<td>2</td>
<td>Temperature (°C)</td>
<td></td>
<td>19.50 ± 0.75</td>
<td>22.48 ± 0.64</td>
<td>17.55 ± 0.70</td>
</tr>
</tbody>
</table>

(±) indicates standard deviations.

Table 2. Biomarker enzyme activities in freshwater snails from Malangaon reservoir.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Snail species</th>
<th>Season</th>
<th>GST nmol/min/mg protein</th>
<th>SOD U/mg protein</th>
<th>GPx Mg/min/mg protein</th>
<th>LPO nmol/mg protein</th>
<th>GSH µM/g wet tissue</th>
<th>CAT U/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Bellamya bengalensis</em></td>
<td>Summer</td>
<td>23.41 ± 0.76</td>
<td>20.87 ± 0.56</td>
<td>6.57 ± 0.20</td>
<td>0.63 ± 0.20</td>
<td>0.083 ± 0.026</td>
<td>9.23 ± 0.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Monsoon</td>
<td>15.73 ± 0.53</td>
<td>24.83 ± 0.60</td>
<td>8.50 ± 0.32</td>
<td>0.55 ± 0.021</td>
<td>0.117 ± 0.020</td>
<td>13.20 ± 0.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Winter</td>
<td>19.61 ± 0.56</td>
<td>23.33 ± 0.47</td>
<td>7.48 ± 0.30</td>
<td>0.60 ± 0.018</td>
<td>0.094 ± 0.005</td>
<td>11.56 ± 0.46</td>
</tr>
<tr>
<td>2</td>
<td><em>Lymnaea acuminata</em></td>
<td>Summer</td>
<td>26.29 ± 0.74</td>
<td>18.32 ± 0.33</td>
<td>5.05 ± 0.13</td>
<td>0.74* ± 0.020</td>
<td>0.018 ± 0.002</td>
<td>7.02* ± 0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Monsoon</td>
<td>18.34 ± 0.42</td>
<td>22.35 ± 0.41</td>
<td>8.07 NS ± 0.15</td>
<td>0.67** ± 0.021</td>
<td>0.056 ± 0.004</td>
<td>10.53** ± 0.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Winter</td>
<td>22.29 ± 0.68</td>
<td>20.73 ± 0.55</td>
<td>7.04 NS ± 0.10</td>
<td>0.70** ± 0.037</td>
<td>0.026 ± 0.003</td>
<td>8.86 NS ± 0.44</td>
</tr>
</tbody>
</table>

(±) values indicates standard deviations.

Values are significant at *p<0.05, **P<0.01, ***P<0.001, NS- Non significant
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10.53 and 8.86 (U/mg of protein) respectively during summer, monsoon and winter seasons.

### 3.2.6 Glutathione Peroxidase (GPx)

The GPx movement was 6.57, 8.50 and 7.48 (mg of GSH utilized/min/mg protein) in *B. bengalensis*. It was 5.05, 8.07 and 7.04 (mg of GSH utilized/min/mg protein) in *L.acuminata* respectively in summer, monsoon and winter seasons.

### 4. Discussion

Antioxidant shield classification is greatly unspoiled biochemical instrument which preserves bacteria from unhelpful possessions of touchy oxygen classes. At low meditation, ROS standardizes abundant biological methods, while at elevated consciousness they are fatal to microorganisms by way of damaging cellular utilities by oxidizing biomolecules. Seasonal variations in antioxidant fortifications variety class talent to endure their suitable ROS titer to gross changed physical professions. Oversensitive oxygen sort like superoxide radicals, hydroxyl diehards, sideways through hydrogen peroxide are created because of unfinished diminishing of atomic oxygen in electron transport chain of mitochondria and as by-products of additional biological rejoinders of metabolism. There are a few insights in writing which have unmistakably shown changes in centers of a few contaminants in air and water with profound respect to seasons. Usually, such go-betweens enhance oxidative stress. Consequently, a change in season may affect ROS status and antioxidant defense organizations in faunae and, thus, their physiology.

In the current education consequences presented that, the uppermost heights of LPO and doings of GST and deepest doings of SOD, GPx, CAT, and GSH in gastric glands of two snail’s classes were originate in straw-hat than wet season and winter seasons. The gained figures too conveyed spring variation of biotransformation enzymes counting GST in *Perna perna*. In testified vagaries in numerous biomarkers in the mangrove oyster, *Crassostrea rhizophome* collected from polluted and non-polluted sites in relation to seasonality and opportunity. Recurrent discrepancy may be cherished since of interfaces sandwiched between exogenous explanations like temperature, pH, salinity and food supply and endogenous influences such as procreative cycle. Hence it is dense to enticement precise decision as to which limitation is furthermore nominal for deviations in the antioxidant enzymes. The above results indisputably point out the status of spring changes on biomarkers in *B. bengalensis* and *L.acuminata* are helpful for elucidating...
tion of recyclable tending numbers. It is branded that the activity of antioxidative enzymes and the satisfied of low molecular weightiness antioxidants in snails expression regular fluctuations. Gradual rises in the happenings of CAT, GSH, and GST in monsoon in snail *Viviparous acerous* from Velika Morava River Serbia.

In observed GST activity in land snail, *Helix aspersa* and found higher GST activity in the summer compared to winter.

The conclusions of the existing study point out an imperative stimulus of recurrent reasons on both enzymatic and non-enzymatic antioxidants. Nevertheless it is clear from the data the amendments of antioxidant limitations and triglyceride peroxidation in *B. bengalensis* and *L. acuminata* appears to be closely related with the seasonal changes.

The general fallouts exposed the utmost heights of LPO and happenings of GST and nethermost happenings of SOD, CAT, GPx, and GSH in intestinal glands of two snail kind in travelling than cloudburst and midwinter flavor. The gained data also signposted the nethermost ranks of LPO and accomplishments of GST and determined accomplishments of SOD, CAT, GPx and GSH in digestive glands of two snail types in monsoon than seasonal and winter season. It was also experimental that the difficult level of LPO and commotion of GST and lower activity of SOD, CAT, GPx, and GSH were experimental in digestive glands of *L. acuminata* than digestive glands of *B. bengalensis* in all the three seasons.

5. Conclusion

It was strong-minded that, in travelling, snails continued further under environmental stress than winter and monsoon season. It was also concluded that, *Limaena acuninata* was under more ecological trauma than *Bellamya bengalensis*. In the existing study, we confirmed with the target of snails endures unalike equal of oxidative tension as a result of periodic dissimilarities, which proposes the stimulation of bodily apparatuses to rummage fashioned ROS.

6. References

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