SPECIES-WISE AND ORGAN-WISE CHANGES IN SUPEROXIDE DISMUTASE ACTIVITY IN TILAPIA, GREY MULLET AND SPOTTED SCAT FROM COCHIN BACKWATERS

Reynold Pranoy et al.,

KEYWORDS
Superoxide dismutase
Organs
Species
ABSTRACT

An attempt has been done to investigate the Superoxide dismutase activity in four organs viz., gills, liver, kidney, muscle in three species of fish viz., Tialpia (Oreochromis mossambicus), Grey mullet (Mugil cephalus) and Spotted scat (Scatophagus argus) collected from a traditional brackish water farm in Kochi to understand the species-wise and organ-wise alterations in Superoxide dismutase activity in fish. The results showed a decreasing trend in enzyme activity as liver > gills > kidney > muscle in all the species of fish selected. In Tilapia (Oreochromis mossambicus) the organ-wise trend was 0.1 ± 0.006 in liver, 0.06 ± 0.004 in gills, 0.04 ± 0.002 in kidney and 0.01 ± 0.004 in muscle U/mg protein. In Grey mullet (Mugil cephalus) Superoxide dismutase activity in liver, gills, kidney and muscles was 0.26 ± 0.007 U/mg protein, 0.23 ± 0.01, 0.13 ± 0.002 and 0.07 ± 0.004 respectively. In Spotted scat (Scatophagus argus) the organ-wise variation was 0.27 ± 0.01 U/mg protein in liver, 0.16 ± 0.009 in gills, 0.15 ± 0.009 in kidney and 0.12 ± 0.006 in muscle. Hepatic, Renal and Muscular Superoxide dismutase showed a highest activity in S. argus, least in O. mossambicus and the activity in M. cephalus lies in between. On the other hand Branchial Superoxide dismutase showed a different trend i.e., highest in M. cephalus lowest in O. mossambicus and S. argus lies in between.

INTRODUCTION

Oxygen is absolutely necessary for the life processes, in particular cell respiration. However, the metabolism of oxygen may generate reactive elements called free radicals, in particular the superoxide ion (O₂⁻) and the hydroxyl ion (OH⁻) (Joanny and Menvielle-Bourg, 2005). These short-lived and highly reactive oxygen species (ROS) such as O₂⁻ (superoxide); OH (hydroxyl radical) and H₂O₂ (hydrogen peroxide) are continuously generated in vivo. These chemically unstable compounds carry free electrons that react with other molecules, in turn destabilizing them and thereby inducing a chain reaction. In particular, free radicals damage DNA, essential cellular proteins and react with the unsaturated fatty acid of cellular or subcellular membranes. Therefore, they lead to peroxidation of membrane lipids (Łukaszewicz-Hussain and Moniuszko-Jakoniuk, 2004), which may lead to cell death (Joanny and Menvielle-Bourg, 2005).

In the resting state, the balance between antioxidants and oxidants is sufficient to prevent the disruption of normal physiologic functions (Liocher and Fridovich, 2007; Imlay, 2008). These antioxidant mechanisms mainly involve specific enzymes (superoxide dismutase or SOD, catalase, glutation peroxidase or Gpx) as well as radical scavengers that trap free radicals ((antioxidant vitamins A, C, E), thiols and ß-carotene) (Vouldoukis et al., 2004). Either increases in oxidants or decreases in antioxidants can disrupt this balance giving rise to elevated levels of ROS (Liocher and Fridovich, 2007; Imlay, 2008), condition termed as Oxidative stress. Oxidative stress affects cellular integrity only when antioxidants are no longer capable of coping with ROS (Łukaszewicz-Hussain and Moniuszko-Jakoniuk, 2004).

The detrimental role of superoxide dismutase (SOD) in the antioxidant defense systems has been known since 1968. It is well known that superoxide ion (O₂⁻) is the starting point in the chain production of free radicals. At this early stage, superoxide dismutase inactivates the superoxide ion by transforming it into hydrogen peroxide (H₂O₂). The later is then quickly metabolised by catalase and peroxidases into dioxygen (O₂) and water (H₂O) (Joanny Menvielle-Bourg, 2005). SOD is reported to be an indicator of oxidative stress and is an important enzyme of the antioxidant system of the cell against free radicals (Hayes et al., 1997).

Gregory et al. (1974) indicates it to be present in all oxygen-metabolizing cells, the present work is designed to analyse the organ wise and species wise changes in superoxide dismutase activity in a group of fish since fishes are often at the top of the aquatic chain and is one of the most appropriate organisms to study the physiological influence of changes in aquatic system because they can serve as bioindicators of environmental pollution (Dautremepuit et al., 2004).

A lot of field studies based on the influence of various chemical substances on the superoxide dismutase activity in sanguine, hepatic, renal, branchial, neural and cardiac (Spolarics and Wu, 1997; Livingstone et al., 2000; Bindu and Philip, 2001; Filho, et al., 2001; Ramazan et al., 2006; Farombi et al., 2007; Matos et al., 2007; Metwally and Fouad, 2008; Al-Kahtani and Fathi 2008; Padmini et al., 2008; Soundararajan et al., 2009; Rajamanickam and Muthuswamy., 2009; Kavitha and...
Venkateswara Rao 2009; Radovanovic et al., 2010; Brucka and Jastrzébska, 2010; Kandemir et al., 2010; Nogueira et al., 2010; Modesto and Martinez 2010; Neeraj Kumar et al., 2011; Rekha and Joseph, 2011; Anushia et al., 2012; Saliu and Bawa-Allah 2012; Obiaah and Usha 2012; Peixoto et al., 2013) reported a wide spectrum of inter-site differences (higher, equal or lower activities of various antioxidant enzymes with tissue peculiarities and disbalance) in polluted compared to clean areas.

The present study is an attempt to analyse the results of species-wise and organ-wise changes in superoxide dismutase enzyme by investigating its activity in liver, gills, kidney and muscles of Oreochromis mossambicus, Mugil cephalus and Scatophagus argus.

MATERIALS AND METHODS

The fish were collected from a traditional aquaculture farm at Chellanam, Kochi, Kerala, India using traditional cast net. Ten fish samples coming under similar size group were selected from the catch. The collected fishes were transported to the laboratory in living condition by keeping in polyethylene bags. On reaching the laboratory the fishes were immediately dissected and the organs viz., kidney, liver, gills and muscle were taken, washed in ice-cold Alsevers ringer solution, kept in plastic containers with screw cap lid and refrigerated in freezing condition. The refrigerated tissues were taken out, dried using blotting paper and the organs were weighed for the preparation of 5% of the tissue homogenate in ice-cold Tris-Hcl buffer pH 7.5 in a glass homogeniser. The prepared homogenate were centrifuged at 3500 rpm for 10 minutes in a cooling centrifuge kept at 4ºC. The supernatant was collected after centrifugation and were kept in ice until the enzyme assay.

Estimation of SOD activity was carried out according to the procedure suggested by Das et al. (2000). The reaction mixture consist of 50mM Phosphate buffer pH 7.4, 20 mM Methionine, 1 % (v/v) Triton X-100, 10 mM Hydroxylamine hydrochloride and 50mM EDTA was incubated for 5 minutes at 30°C and the homogenates was added to this and a control was prepared in a similar manner but instead of homogenate phosphate buffer was added. After that 50 mM riboflavin was added and the reaction mixture was kept under fluorescent light of 40 W CFL for 15 minutes. After incubation Greiss reagent was added and the absorbance of the colour developed was measured at 543 nm against phosphate buffer as blank in a UV-VIS spectrophotometer (Systronics, 118). Total protein of the homogenate was also measured using the Kit provided by Randox based on the Biuret method. At last the results were statistically interpreted by the ANOVA test, the unifactorial pattern using SPSS version 20.

RESULTS AND DISCUSSION

Oxidative stress, the natural consequence of the oxygen metabolism, is normally controlled by antioxidant endogenous defense systems. When these prove to be insufficient, cellular lesions develop that result in ageing but also in some pathological processes. The powerful natural antioxidant enzyme superoxide dismutase (SOD) acts at the very source of the chain reaction resulting in reactive types of oxygen and therefore constitutes the first and one of the main links of the defense process against free radicals. (Joanny Menivielle-Bourg, 2005).

The specific activity of Superoxide dismutase in different organs like liver, gills, muscle and kidney of Tilapia (Oreochromis mossambicus), Grey mullet (Mugil cephalus) and Spotted scat (Scatophagus argus) takes the form of graph (Fig. 1, 2, 3 and 4).

In Tilapia (Oreochromis mossambicus) Superoxide dismutase activity in liver, gills, kidney and muscles are 0.1 ± 0.006, 0.06 ± 0.004, 0.04 ± 0.002 and 0.01 ± 0.004 U/mg protein respectively. Branchial Superoxide dismutase activity is 60 % of hepatic; renal is 40 % of hepatic and 66.67 % of branchial; muscular is 10 % of hepatic, 16.67 % of branchial and 25 % of renal Superoxide dismutase activity.

A one-way within subjects (or repeated measures) ANOVA was conducted (using SPSS version 20) to compare the organ wise variation in Superoxide dismutase activity in Oreochromis mossambicus.

There was a significant variation in hepatic, branchial, renal and muscular Superoxide dismutase activity in O. mossambicus (variation in Superoxide dismutase activity with organ type), Wilks’ Lambda = 0.001, F (1, 5) = 8400.000, p < .001

Multivariate Tests

<table>
<thead>
<tr>
<th>Effect</th>
<th>Value</th>
<th>F</th>
<th>Hypothesis df</th>
<th>Error df</th>
<th>Sig. df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilks’ Lambda</td>
<td>.001</td>
<td>8400.</td>
<td>1.000</td>
<td>5.000</td>
<td>.000</td>
</tr>
</tbody>
</table>

a. Design: Intercept Within Subjects Design: tilapia; b. Exact statistic:

In Grey mullet (Mugil cephalus) the organ-wise trend is similar as mentioned in Tilapia, 0.26 ± 0.007, 0.23 ± 0.01, 0.13 ± 0.002, 0.07 ± 0.004 U/mg protein respectively. Branchial Superoxide dismutase is 88.46 % of hepatic, renal is 50 % of hepatic and 56.52 % of branchial, muscular is 26.92 % of hepatic, 30.43 % of branchial and 53.85% of renal Superoxide dismutase activity.

A one-way within subjects (or repeated measures) ANOVA was conducted (using SPSS version 20) to compare the organ wise variation in Superoxide dismutase activity in Mugil cephalus.

There was a significant variation in hepatic, branchial, renal and muscular Superoxide dismutase in M. cephalus (variation in Superoxide dismutase activity with organ type), Wilks’ Lambda = 0.022, F (2, 4) = 2809.974, p < .001

Multivariate Tests

<table>
<thead>
<tr>
<th>Effect</th>
<th>Value</th>
<th>F</th>
<th>Hypothesis df</th>
<th>Error df</th>
<th>Sig. df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilks’ Lambda</td>
<td>.001</td>
<td>2809.</td>
<td>2.000</td>
<td>4.000</td>
<td>.000</td>
</tr>
</tbody>
</table>

a. Design: Intercept Within Subjects Design: Grey mullet; b. Exact statistic:

In Spotted scat (Scatophagus argus) also the organ-wise trend is similar to Tilapia and Grey mullet, 0.27 ± 0.01 U/mg protein in liver, 0.16 ± 0.009 in gills, 0.15 ± 0.009 in kidney and
There was a significant variation in hepatic Superoxide dismutase activity in three species of fish selected. A one-way within subjects (repeated measures) ANOVA was conducted (using SPSS version 20) to compare the specieswise variation in hepatic Superoxide dismutase activity with type of species, Wilks’ Lambda = 0.000, F (2, 4) = 7058.057, p < .001

Multivariate Tests

<table>
<thead>
<tr>
<th>Effect</th>
<th>Value F</th>
<th>Hypothesis df</th>
<th>Error df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spotted scat</td>
<td>.004</td>
<td>1249. 1.000</td>
<td>5.000</td>
<td>.000</td>
</tr>
</tbody>
</table>

a. Design: Intercept Within Subjects Design: spotted scat; b. Exact statistic

A comparison of hepatic Superoxide dismutase activity in Oreochromis mossambicus, Mugil cephalus, Scatophagus argus shows a similar trend to that of hepatic Superoxide dismutase, i.e., highest activity in S. argus (0.15 ± 0.009), and lesser activity in M. cephalus (0.13 ± 0.002), which is 86.67% of S. argus and the least in O. mossambicus (0.04 ± 0.002) which is 30.77% of M. cephalus and 26.67% of S. argus renal Superoxide dismutase activity.

A one-way within subjects (repeated measures) ANOVA was conducted (using SPSS version 20) to compare the specieswise variation in renal Superoxide dismutase activity in three species of fish selected. There was a significant variation in renal Superoxide dismutase activity in O. mossambicus, M. cephalus, S. argus (variation in renal Superoxide dismutase activity with type of species), Wilks’ Lambda = 0.004, F (1,5) = 1249.714, p < .001

Multivariate Tests

<table>
<thead>
<tr>
<th>Effect</th>
<th>Value F</th>
<th>Hypothesis df</th>
<th>Error df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal</td>
<td>.004</td>
<td>1249. 1.000</td>
<td>5.000</td>
<td>.000</td>
</tr>
</tbody>
</table>

a. Design: Intercept Within Subjects Design: renal SOD; b. Exact statistic

A comparison of muscular Superoxide dismutase activity in Oreochromis mossambicus, Mugil cephalus, Scatophagus argus shows a similar trend to that of hepatic and renal Superoxide dismutase activity i.e., highest activity in S. argus (0.12 ± 0.006), and lesser activity in M. cephalus (0.07 ± 0.004), which is 58.33% of M. cephalus and the least in O. mossambicus (0.01 ± 0.004) which is 14.29% of M. cephalus and 8.33% of S. argus renal Superoxide dismutase activity.

A one-way within subjects (repeated measures) ANOVA was conducted (using SPSS version 20) to compare the specieswise variation in muscular Superoxide dismutase activity in three species of fish selected. There was a significant variation in muscular Superoxide dismutase activity in O. mossambicus, M. cephalus, S. argus (variation in muscular Superoxide dismutase activity with type of species), Wilks’ Lambda = 0.0002, F (1,5) = 22083.857, p < .001

Multivariate Tests

<table>
<thead>
<tr>
<th>Effect</th>
<th>Value F</th>
<th>Hypothesis df</th>
<th>Error df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscular</td>
<td>.000</td>
<td>22083. 1.000</td>
<td>5.000</td>
<td>.000</td>
</tr>
</tbody>
</table>

a. Design: Intercept Within Subjects Design: muscular SOD; b. Exact statistic

From the result it became clear that the selected antioxidant enzyme showed decreasing trend in the enzyme activity from Liver to muscle (Liver > Gill > Kidney > Muscle). The present findings of highest hepatic Superoxide dismutase activity agree with the observations of Radovanovic et al. (2010) where the Superoxide dismutase activity in liver was recorded to be higher than in muscle of Barbeis (Barbus barbus). Rajamanickam and Muthuswamy (2009) in Common carp also supported the
Figure 1: Superoxide dismutase activity in liver, gills, kidney and muscle of Oreochromis mossambicus

Figure 2: Superoxide dismutase activity in liver, gills, kidney and muscle of Mugil cephalus

Figure 3: Superoxide dismutase activity in liver, gills, kidney and muscle of Scatophagus argus

Figure 4: Hepatic Superoxide dismutase activity in Oreochromis mossambicus, Mugil cephalus, Scatophagus argus

Figure 5: Branchial Superoxide dismutase activity in Oreochromis mossambicus, Mugil cephalus, Scatophagus argus

Figure 6: Renal Superoxide dismutase activity in Oreochromis mossambicus, Mugil cephalus, Scatophagus argus

Figure 7: Muscular Superoxide dismutase activity in Oreochromis mossambicus, Mugil cephalus, Scatophagus argus

Figure 8: Comparison of Superoxide dismutase activity in liver, gills, kidney and muscle of Oreochromis mossambicus, Mugil cephalus, Scatophagus argus
increased activity of Superoxide dismutase in liver even if he compared the hepatic Superoxide dismutase activity with renal Superoxide dismutase. Kandemir (2010) noted a decreasing trend in Superoxide dismutase activity like liver > gills > muscle in of Cyprinus carpio L. In cat fish Clarias gariepinus Doherty et al. (2010) reported increased SOD activity in gills than in liver but the reverse is in the case of tilapia (Oreochromis niloticus) collected from reference site without pollution. Nogueira et al. (2010) reported higher hepatic superoxide dismutase activity than branchial in armored cat fish (Pterygoplichthys anisitsi) and Nile tilapia (Oreochromis niloticus). Obaiah and Usha (2012) also reported a similar trend in liver and kidney Superoxide dismutase activity in Oreochromis mossambicus. Bindu and Philip (2001) investigated Surfactant-induced lipid peroxidation in a tropical euryhaline teleost Oreochromis niloticus (Tilapia) adapted to fresh water and reported that superoxide dismutase activity was found to be high in liver than in kidney even if the difference is not much significant. Aysel et al. (2010) as a part of determination of biochemical indicators in common carp (Cyprinus carpio) to the physico-chemical parameters of Ceyhan River (Adana- Turkey) reported the activity of Superoxide dismutase was highest in liver than in gills.

Jiang (2013) reported somewhat different observation while working with Superoxide dismutase activity in kidney and gill of Crucian carp (Carassius auratus) that the Superoxide dismutase activity was higher in kidney than in gills. An investigation done by Farombi (2007) on African catfish (Clarias gariepinus) from Nigeria Ogun River, on enzyme activity in four organs viz., kidney, liver, gills and heart, revealed that the Superoxide dismutase activity was highest in gills , in liver and kidney next and least activity respectively, another observation that found to be contradictory to the present result.

Literature search haven’t came across with similar type of study in these selected fishes especially Scatophagus argus for defending the present result of species-wise changes.

The present analysis reached at a conclusion that the Superoxide dismutase activity show a species-wise and organ-wise variation with a decreasing trend like liver > gills > kidney > muscle and the species-wise variation in hepatic, renal and muscular Superoxide dismutase activity showed similar trend S. argus > M. cephalus > O. mossambicus, branchial Superoxide dismutase showed a trend like M. cephalus > S. argus > O. mossambicus.

**REFERENCES**


and physiology. 89: 73-80.


