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Trend of organ-wise change in selected antioxidant enzyme activity in spotted scat from Cochin backwaters

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ABSTRACT

Reactive oxygen species generation and enzymatic and non-enzymatic antioxidant profiling have been emerged as an important area of research due to its correlation with environmental stress where the organism inhabit and as a way to recover from ROS induced damages. Here an attempt was done to investigate the antioxidant enzymes like Catalase, Superoxide dismutase, Glutathione peroxidase activity in four organs viz., gills, liver, kidney, muscle in a species of fish, Spotted scat (Scatophagus argus) collected from a traditional brackish water farm in Kochi to understand the organ-wise alterations in these enzyme activity in fish. The results showed a similar trend of organ-wise variation in all the antioxidant enzyme activity like liver > gills > Kidney > muscle in the fish species selected.

Keywords: Spotted scat, Catalase, Superoxide dismutase, Glutathione peroxidase.

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 (O_2^-) and the hydroxyl ion (OH^-) (Joanny and Menvielle-Bourg, 2005). These short-lived and highly reactive oxygen species (ROS) such as $O2\cdot^-$ (superoxide), $\cdot OH$ (hydroxyl radical), and H_2O_2 (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 (Lukaszewicz-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, gluthation peroxidase or Gpx) as well 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 (Lukaszewicz-Hussain and Moniuszko-Jakoniuk, 2004).

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 (H_2O_2). The latter is then quickly metabolised by catalase and peroxidases into dioxygen (O_2) and water (H_2O) (Joanny

Menvielle-Bourg, 2005). Mainly Catalase (CAT) and Glutathione Peroxidase play a significant role in the elimination of hydrogen peroxide. Catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less-reactive gaseous oxygen and water molecules (Gaetani *et. al*., 1996; Yoshpe-purer and Henis, 1976, Chelikani, *et. al.* 2004). Glutathione Peroxidase (GSHPx), a selenoenzyme that catalyses the reduction of hydrogen peroxide to water, with the simultaneous conversion of reduced glutathione to oxidised glutathione (Michiels *et. al*., 1994).

The present work is designed to analyse the organ wise changes in Catalase, Superoxide dismutase and Glutathione peroxidase activity in a species 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. Salinity, season, as well as the feeding habitat, difference in fish species induce modifications in the peroxisomal enzymatic activity (Fahimi and Cajaraville, 1995; Rocha *et. al*.., 2003).

A lot of field studies based on the influence of various chemical substances on the catalase, superoxide dismutase and Glutathione peroxidase activity in sanguine, hepatic, renal and branchial (Bainy *et. al*.., 1996; Otto and Moon, 1996; Spolarics and Wu. 1997; McFarland *et. al*.., 1999; Varanka *et. al*.., 1999; Sóle *et. al*.., 2000; Livingstone *et. al*., 2000; Bindu and Philip, 2001; Filho,*et. al*.,2001; Ikic *et. al*.., 2001; Pandey *et. al*.., 2001; Jena *et. al*.., 2002; Achuba and Osakwe, 2003; Buet *et. al*.., 2005; Gulcin *et. al*.., 2005; Ramazan,*et. al*.,2006; Lima *et. al*.., 2006; Sun *et. al*.., 2006; Farombi.,*et. al.* 2007; Rajamanickam and Muthuswamy., 2009; 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 Jastrzębska, 2010; Kandemir *et. al*.,2010; Nogueira *et. al*.., 2010; Modesto and Martinez 2010; Radovanovic *et. al*.., 2010; Kandemir *et al*., 2010; Nogueira *et. al*.., 2010; Neeraj Kumar *et. a.*, 2011; Rekha and Joseph, J., 2011; Anushia *et. al*., 2012; Saliu and Bawa-Allah 2012; Obaiah 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 organ- wise changes in catalase, superoxide dismutase and Glutathione peroxidase enzyme by investigating its activity in liver, gills, kidney and muscles of *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 homogenizer. The prepared homogenate were centrifuged at 3500 rpm for 10 minutes in a cooling centrifuge kept at 4^{o} C. The supernatant was collected after centrifugation and were kept in ice until the enzyme assay.

Estimation of CAT activity was carried out according to the procedure suggested by Sinha A.K. (1972). To the reaction mixture consist 0.2M hydrogen peroxide, 0.01M Phosphate buffer pH 7.0, distilled water, homogenates was added to initiate the reaction of H_2O_2 decomposition and the activity of catalase was stopped at 0 seconds, 30 seconds, 60 seconds and 90 seconds interval with 2 mL dichromate acetic acid solution. A control was also prepared in a similar manner but instead of homogenate phosphate buffer was added. Tubes heated for 10 minutes in boiling water bath and the absorbance of the colour developed was measured at 610 nm against phosphate buffer as blank in a UV-VIS spectrophotometer (Systronics 118).

Estimation of SOD activity was carried out according to the procedure suggested by Das *et. al*.(2000). The reaction mixture consist of 50 mM Phosphate buffer pH 7.4, 20 mM Methionine , 1 % (v/v) Triton X-100 , 10 mM Hydroxylamine hydrochloride and 50 mM EDTA was incubated for 5 minutes at 30^{o}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 againt phosphate buffer as blank in a UV-VIS spectrophotometer (Systronics 118).

Estimation of GPx activity was carried out according to the procedure suggested by Rotruck (1973). To a reacting mixture of 0.4 M phosphate buffer pH 7.0, 10mM Sodium azide, 4mM reduced glutathione, 2.5 mM hydrogen peroxide added 200 µL of 5% homogenate solution of the sample tissue to initiate H_2O_2 utilization . Then the reaction of enzyme is arrested by the addition of 10% TCA at various time intervals (0 seconds, 30 seconds, 60 seconds, and 90 seconds). The test tubes were centrifuged at 3500 rpm for 10 minutes and to the supernatant 0.3 M phosphate solution and 0.04% DTNB in 1% sodium citrate were added. Optical density (OD) of colour developed was measured using a UV-Visible spectrum of spectrophotometer at 412 nm.

Total protein of the homogenate was also measured using the Kit provided by Randox based on the Biuret method.

RESULTS AND DISCUSSION

The specific activity of catalase, superoxide dismutase and Glutathione peroxidase in different organs like Liver, gills, muscle and kidney of Spotted scat (*Scatophagus argus*) takes the form of graph (figure 1- 3)

Figure 1: Trend of organ wise variation of Catalase activity in liver, gills, kidney and muscle of *Scatophagus argus*

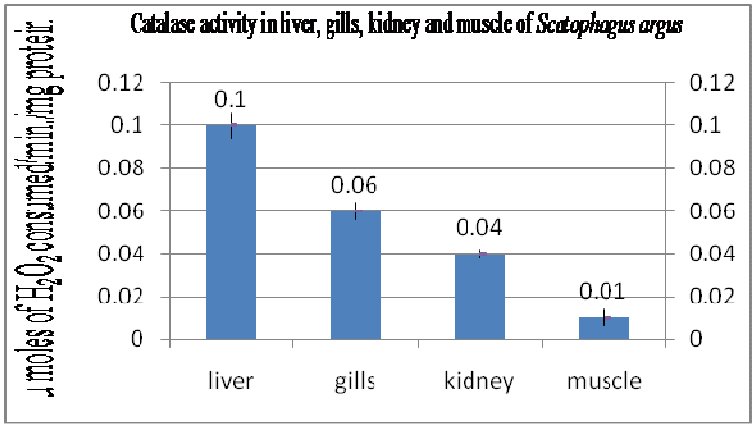


Figure 2: Trend of organ wise variation of Superoxide dismutase activity in liver, gills, kidney and muscle of *Scatophagus argus*

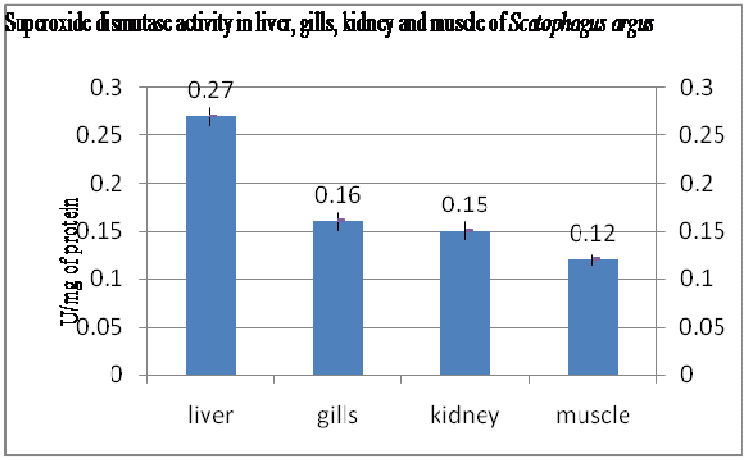
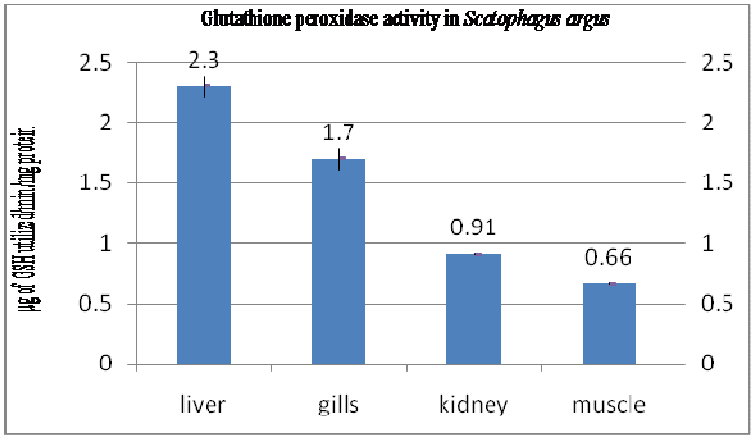


Figure 3: Trend of organ wise variation of Glutathione peroxidase activity in *Scatophagus argus*



A one-way within subjects (or repeated measures) ANOVA was conducted separately (using SPSS version 20) to compare the organ wise variation (hepatic, branchial, renal and muscular) in Catalase, Superoxide dismutase and Glutathione peroxidase activity in *Scatophagus argus.*

There was a significant variation in hepatic, branchial, renal and muscular catalase activity in *S. argus* (variation in catalase activity with organ type), Wilks' Lambda = 0.001, F (2,4) = 1342.661[b], p < .001

Multivariate Tests[a]

Effect	Value	F	Hypothesis df	Error df	Sig.
Wilks' Lambda	.001	1342.661[b]	2.000	4.000	.000

a. Design: Intercept, Within Subjects Design: hepatic, branchial, renal and muscular Catalase in S.argus , b. Exact statistic

There was a significant variation in hepatic, branchial, renal and muscular Superoxide dismutase activity in *S.argus* (variation in Superoxide dismutase activity with organ type), Wilks' Lambda = 0.001, F (2,4) = 2132.286[b], p < .001

Multivariate Tests[a]

Effect	Value	F	Hypothesis df	Error df	Sig.
Wilks' Lambda	.001	2132.286[b]	2.000	4.000	.000

a. Design: Intercept, Within Subjects Design: hepatic, branchial, renal and muscular superoxide dismutase in S.argus , b. Exact statistic

There was a significant variation in hepatic, branchial, renal and muscular Glutathione peroxidase activity in *S.argus* (variation in Glutathione peroxidase activity with organ type), Wilks' Lambda = 0 .003, F (1,5) = 1584.556[b], p < .001

Multivariate Tests[a]

Effect	Value	F	Hypothesis df	Error df	Sig.
Wilks' Lambda	.003	1584.556[b]	1.000	5.000	.000

a. Design: Intercept, Within Subjects Design: hepatic, branchial, renal and muscular glutathione peroxidase in S.argus, b. Exact statistic

Organ wise trend of all the three enzymes (catalase, superoxide dismutase and glutathione peroxidase) follow the same pattern of variation i.e., Liver>Gills>Kidney>Muscle in the species of fish selected *(Scatophagus argus).*

From the result it was clear that these selected antioxidant enzyme showed decreasing trend in the enzyme activity from Liver to muscle (Liver > Gills > Kidney > muscle). Different authors like Bindu and Philip (2001), Farombi *et al* (2008), Rajamanickam and Muthuswamy (2009), Ciornea *et al* (2009), Radovanovic *et al* (2010), Kandemir *et al* (2010), Doherty *et al* (2010), Aysel *et al* (2010), Nogueira *et al* (2010), Obaiah and Usha (2012) variously supported the present result. Bindu and Philip (2001) investigated Surfactant-induced lipid peroxidation in a tropical euryhaline teleost *Oreochromis niloticus* (Tilapia) adapted to fresh water and reported that CAT and SOD activity was found to be high in liver than in kidney even though the difference is not much significant in the case of

hepatic and renal SOD levels. An investigation done by Farombi *et al* (2008) on African cat fish (*Clarias gariepinus*) from Nigeria Ogun River, was the analysis of enzyme activity in four organs (kidney, liver, gills and heart) and was found that the CAT activity was highest in liver than kidney and gills (both showed almost similar range of activity) but the case of SOD is somewhat varied i.e., gills showed highest, liver and kidney next and least activity respectively. Rajamanickam and Muthuswamy (2009) in common carp published the result that the activity of CAT, SOD and GPx was higher in liver than in kidney. Ciornea *et al* (2009) performed a comparative determination of the hepatic and muscular catalase activity in three summer-old Cyprinids species, namely Common carp (*Cyprinus carpio*), Crucian (*Carassius auratus gibelio*) and Bighead carp (*Aristichthys nobilis*), all coming from an intensive growth system and found high hepatic CAT activity than muscular. Radovanovic *et al* (2010) carried out a study of superoxide dismutase and catalase activities in the liver and muscle of barbel (*barbus barbus*) and its intestinal parasite (*pomphoryinchus laevis*) from the Danube river, Serbia and published that the liver showed higher activity than muscle with respect to both the enzymes. In the study by Kandemir (2010) published the paper with CAT , SOD and Gpx activity showed a trend as Liver > gills > muscle of *C.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. 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 CAT and SOD was highest in liver than in gills. Nogueira *et al* (2010) reported CAT , SOD and GPx activity was found to be higher in liver than in gills of armored catfish (*Pterygoplichthys anisitsi*) and Nile tilapia (*Oreochromis niloticus*) (but in the case of Nile tilapia GPx showed slightly increased activity in Gills than in liver). Obaiah and Usha (2012) also reported a similar trend in liver and kidney SOD and CAT activity in *Oreochromis mossambicus*.

Brucka and Jastrzębska (2010) reported somewhat different observation while working with SOD activity in liver, kidney and muscle in three fish species *Cyprinus carpio* L., *Oncorhynchus mykiss* Walbaum, and A*cipenser baeri* Brandt. that the trend of variation in activity of superoxide dismutase in *Cyprinus carpio* L., was liver > kidney > muscle, kidney > liver > muscle, kidney = liver > muscle respectively. Jiang (2013) in a paper "Changes of superoxide dismutase and catalase activities in crucian carp (*Carassius auratus*) exposed to copper and recovery response" published both the CAT and SOD activity was higher in kidney than in gills, another publication found to be contradictory to the present result.

CONCLUSION

Present findings reached at a conclusion that the catalase, superoxide dismutase and glutathione peroxidase enzymes showed a decreasing trend in activity in the order of liver, gills, kidney and muscle**.**

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