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ORIGINAL ARTICLE

Effect of Tannery Effluents on the Antioxidant Enzymes of a Fresh Water Fish *Channa striatus*

Sivachandran Ramachandran [1], Navaneetha Lakshmi Krishnan[2], Priya Ponmudi [*3]

[1]*Department of Advanced Zoology and Biotechnology, Ramakrishna Krishna Mission Vivekananda College, Mylapore, Chennai -600004, India*

[2]*PG and Research Department of Zoology, Ethiraj College for Women, Chennai-600 008, India*

[3]*Corresponding Author, PG and Research Department of Zoology, Pachaiyappa's College, Chennai-600 030, India*

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KEYWORDS

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ABSTRACT: Tannery effluents add pollutants to the aquatic environment. The characterization of responses to toxic exposure at the molecular level of biological systems is a major challenge in ecotoxicology because it enables the unraveling of mechanisms of toxicity, the discovery of novel biochemical markers, and early diagnoses of exposure and effects. The three major classes of antioxidant enzymes viz. superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (Gpx) were analyzed in the liver, testes, and ovary of *Channa striatus* to delineate the impact of tannery effluents on these enzymes. The oxidative stress biomarkers showed a significant reduction ($p<0.05$) in their activities among the control and experimental groups exposed to both 10 and 1 % concentrations of the tannery effluents. The SOD activity was reduced to 96.13 % in the ovary compared to the liver (60.73 %) and testes (47.89 %) after 30 days of exposure to a 10 % concentration of the tannery effluents. However, the activities of catalase and glutathione peroxidase decreased to 58.59 % and 43.64 % in the testes when compared to the liver and ovary.

INTRODUCTION

Environmental degradation is referred to as pollution. An undesirable change in the physical, chemical or biological properties of the land, air, and water will have an adverse effect on living organisms [1]. Understanding the mechanisms of toxicity and discovering novel biochemical biomarkers may aid in the early diagnosis of toxic exposure and effects at the molecular level of biological systems. The unbiased and non-hypothesis-driven approaches of functional genomics techniques, like proteomics, allow simultaneous analysis of large groups of biomolecules. This makes them powerful tools for comprehensive molecular effect screening. But, they have only recently been introduced in the field of ecotoxicology. The extension of these techniques towards well-known ecotoxicological model organisms like zebrafish embryos could offer a high potential for a more thorough understanding of toxicity. With the chance to find novel biological markers, they may also promote rapid and sensitive toxicity screening. Industrial effluents from a variety of industrial and commercial entities pose major environmental risks, especially in urban and semi-urban areas [2]. The untreated effluents by industrial facilities are primarily responsible for the water pollution problems. The Indian sector is ranked as the tenth biggest in the world in gross industrial output [3]. Biological or physiological variations in fish caused due to exposure to pollutants are used as biochemical markers of environmental pollution and are frequently used to assess the health of aquatic ecosystems [4-5]. One benefit of using these biological markers in

*Corresponding author: priyaponmudi79@gmail.com (P. Priya)
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environmental assessment is that they allow for the examination of particular target organs, such as the brain, liver, kidney, and gills which carry out vital functions like nervous coordination, detoxification process, excretion, and respiration. Also, the biological accumulation and transformation of xenobiotics in fish can be determined [6]. Besides any change or alteration found in the organs would help to determine their functional capability and serve as early warning indicators of damage to an animal's health [7-9]. Many studies have been made on various biochemical parameters of the fishes [10]. However, the deleterious impact of various concentrations of distillery effluent on biological markers of reproductive tissues has not been worked out. Therefore, the current investigation was carried out to evaluate the toxic effects of tannery effluents on the important antioxidant enzymes from liver, testes, and ovary of freshwater fish *C. striatus.*

MATERIALS AND METHODS

Collection and maintenance of experimental fishes

The experimental model used to assess the toxicity consisted of healthy fish that weighed 200–250 g and measured 30–35 cm in length. The fish were procured from Double Lake Chennai and were accustomed to laboratory settings for three weeks prior to the experiment. Commercial fish feed was fed to them twice daily. The fish were kept in a rectangular plastic container measuring 64 x 44 x 29.5 cm and was filled with 20 litres of dechlorinated tap water. Before introducing the fish, the containers were thoroughly cleaned and disinfected with potassium permanganate solution to avoid fungus growth.

Toxicity tests

The location site and collection of tannery effluents were from Chromepet (12°58'22.6"N, 80°07'00.6"E), Chennai, Tamil Nadu, India. The mean lethal tolerance limit of experimental fishes exposed to tannery effluents was done for 96 days by preliminary toxicity tests [11]. Based on this, two sub-lethal concentrations of 10 % and 1 % were selected for further investigation. In order to study the effect of tannery effluents on the antioxidant enzymes the fish were divided into three groups and the course of the experiment was for 30 days. Group, I served as the control (kept in tap water alone), while groups II and III were exposed to 10 % and 1 % sub-lethal concentrations of tannery effluent respectively. Each group comprised ten fish. For enzymatic studies, the liver, ovary, and testis were carefully dissected and kept in an ice box until taken for homogenization. The tissue samples after being homogenized in 0.62 % NaCl (1:5w/v) were subjected to centrifugation at 5000 rpm for 5 min. The supernatant was collected and used as the enzyme source.

Assay of Antioxidant enzymes

Superoxide Dismutase (SOD)

The standard protocol was followed for measuring SOD [12]. 0.25 mL of ethanol and 0.15 mL of chloroform were added to 0.5 mL of enzyme preparation (tissue homogenate supernatant), centrifuged, and the supernatant was used for the assay. The supernatant was added to tubes containing 1.5 mL of carbonate buffer and 0.5 mL of EDTA solution, and the final volume was made to 2.5 mL by adding distilled water. A Shimadzu-UV-visible spectrophotometer was used to measure the increase in absorbance at 480 nm after the addition of 0.4 mL of epinephrine, which started the reaction. In a control tube lacking the enzyme, 50% auto-oxidation of epinephrine to adrenochrome was accomplished. The specific activity of SOD was measured in units/min/mg protein. Protein concentration in the supernatant was also estimated following the standard method [13].

Catalase (CAT)

The standard procedure was followed for assessing the CAT activity [14]. The reaction mixture was made up of 0.2 mL of enzyme source (tissue homogenate supernatant) and 1.2 mL of phosphate buffer. The enzymatic reaction was initiated by the addition of 1.0 mL of diluted hydrogen peroxide (H_2O_2) and a decrease in absorbance was measured at 240 nm over the course of three minutes at intervals of 30 seconds in a Shimadzu-UV-Spectrophotometer. The specific activity of CAT was measured in micromoles of H_2O_2 decomposed per minute per mg of protein. Protein

concentration in the supernatant was also estimated following the standard method [13].

Glutathione Peroxidase (Gpx)

Gpx was analyzed following the standard technique [15]. Reduced glutathione (0.2ml), an enzyme source (0.1 mL), and H_2O_2 (0.1 mL) were added to 0.4 mL of 0.3 molar sodium phosphate buffer (0.3 molar, pH 7.4) and made up to the final incubation volume of 2.0 mL with distilled water. The tubes were incubated for 10 minutes at 37 ºC. The reaction was stopped after 2 minutes intervals by the addition of 0.5 mL of 5 % trichloroacetic acid (TCA). To determine the residual glutathione content, the contents were centrifuged and 2.0 mL of supernatant was added to 8.0 mL of disodium hydrogen phosphate solution and 1 mL of 5, 5' Dithio-bis-2-nitrobenzoic acid (DTNB) reagent. The color was read at 412 nm in a Shimadzu-UV-Spectrophotometer. The specific activity of Gpx was expressed as micrograms of GSH oxidized/min/mg protein. Protein concentration in the supernatant was also estimated following the standard method [13].

Estimation of Protein

Lowry's reagent was prepared by adding 50ml of reagent A to 0.5 ml of reagent B and 1.0 ml of reagent C, just before use. Reagent A is prepared by dissolving 2 g of sodium carbonate in 100mL of 0.1N Sodium hydroxide solutions. Reagent B contains 2.5g Copper sulfate dissolved in 100 mL of distilled water and Reagent C contains 4g Sodium potassium tartrate dissolved in 100mL of distilled water [13]. To measure the total protein 0.5mL of the supernatant was taken and to this 4.5 mL of alkaline copper reagent (Lowry's reagent) was added. The contents were mixed and left to stand for 10 minutes at room temperature. To this solution, 0.5 mL of diluted Folin's-Phenol reagent was added and mixed rapidly and allowed to stand for 20 minutes. Blank and standard solutions were also prepared simultaneously. The blue color developed was read at 640 nm using Shimadzu-UV-visible spectrophotometer. The protein content was expressed as mg/g of wet tissue.

Statistical analysis

All the data obtained in the present study were statistically analyzed using Students't-test between the control and the experimental groups.

RESULTS AND DISCUSSION

The current study determines how tannery effluents affect important antioxidant enzymes such as SOD, CAT, and GpX in *C. striatus*. The study of these biochemical targets provides a deeper understanding of various underlying toxicity mechanisms in living organisms. Further, it also assesses the exposure and effects of chemicals, or the susceptibility of individuals to respond to these toxic chemicals. The resulting information can be used for monitoring, clinical diagnosis, or even risk assessment for health.

Table 1 shows the mortality percentage of the *C. striatus* exposed to tannery effluent concentrations ranging from 100-10 % and 1 % respectively for 96 days. Nearly 50% and 30% mortality were observed in 10 % and 1 % concentrations. Hence, these two sub-lethal concentrations viz. 10% (chronic) and 1% (acute) were selected to analyze the effect of tannery effluents on the important antioxidant enzymes viz. SOD, CAT and Gpx of *C. striatus*.

Table 1. Manifestation and survival time of *Channa striatus* exposed to an acute and chronic concentration of tannery effluent

Effluent concentration (%)	No. of Fish used	No. of Fish Dead	No. of Fish survived	Mortality (%)
100	10	10	0	100
90	10	10	0	100
80	10	10	0	100
70	10	9	1	90
60	10	8	2	80
50	10	8	2	80
40	10	8	2	80
30	10	7	3	70
20	10	7	3	70
10	10	5	5	50
1	10	3	7	30

*Values are means of 5 individual observations

The SOD activity of the control and experimental groups exposed to 10% and 1% concentrations of tannery effluents is shown in Table 2. The catalytic activity of SOD was significantly ($p<0.05$) reduced in the liver, testis, and ovary exposed to both chronic and acute concentrations of tannery effluent. However, the reduction percentage of SOD was high in the ovary (96.13%), when compared to the liver (60.73%) and testes (47.89). In acute concentration, the SOD activity decreased to 10.82%, 20%, and 27.52% in the liver, testes, and ovary respectively.

Table 2. Effect of acute and chronic concentrations of tannery effluent on the Superoxide Dismutase of *Channa striatus*

SOD (mg l^{-1})		Tissues					
Tissue		**Liver**		**Testes**		**Ovary**	
Concentration	**Days**	**Control**	**Experiment**	**Control**	**Experiment**	**Control**	**Experiment**
Chronic (10%)	0 day	6.23 ± 0.135	5.32 ± 0.015	0.19 ± 0.132	0.20 ± 0.011	1.49 ± 0.180	1.47 ± 0.352
	30^{th} day	6.0 1± 0.012	2.36 ± 0.003	0.18 ± 0.094	0.099 ± 0.057	1.42 ± 0.063	0.055 ± 0.052
Percentage change			**60.73%**		**47.89**%		**96.13%**
Acute (1%)	0 day	6.23 ± 0.135	6.23 ± 0.135	0.19 ± 0.132	0.20 ± 0.011	1.49 ± 0.180	1.47 ± 0.352
	30^{th} day	6.01 ± 0.135	5.36 ± 0.103	0.18 ± 0.094	0.152 ± 0.046	1.42 ± 0.063	1.08 ± 0.214
Percentage change			**10.82%**		**20%**		**27.52%**

(Values are shown as Mean ± SD values, n=3, and percentage change values shown in bold) (* $P<0.05$)

Tables 3 and 4 shows the chronic (10%) and acute (1%) effect of tannery effluents on the catalase and glutathione peroxidase activity of *C. striatus* after 30 days of exposure. It is clear from the results that both the CAT and Gpx activity in the experimental groups decreased significantly ($P<0.05$) at 10 and 1% concentrations. The percentage reduction in the catalase (58.59%) and Gpx activity (43.64%) was higher in the testes at 10% chronic concentration of tannery effluent when compared to the liver and ovary.

Table 3. Effect of acute and chronic concentrations of tannery effluent on the Catalase activity of *Channa striatus*

Catalase (mg l^{-1})		Tissues					
Tissue		**Liver**		**Testes**		**Ovary**	
Concentration	**Days**	**Control**	**Experiment**	**Control**	**Experiment**	**Control**	**Experiment**
Chronic (10%)	0 day	52.4 8 ± 0.452	50.31 ± 0.361	7.99 ± 0.537	7.38 ± 0.205	6.20 ± 0.006	6.12 ± 0.016
	30^{th} day	50.12 ± 1.029	35.69 ± 0.352	7.80 ± 0.041	3.23 ± 0.046	6.11 ± 0.013	3.0 2 ±1.024
Percentage change			**28.79%**		**58.59%**		**50.57%**
Acute (1%)	0 day	52.48 ± 1.423	50.31 ± 0.361	7.99 ± 0.537	7.38 ± 0.205	6.20 ± 0.011	6.12 ± 0.016
	30^{th} day	52.48 ± 1.423	45.69 ± 1.022	7.80 ± 0.041	6.63 ± 0.206	6.11 ± 0.013	5.02 ± 0.045
Percentage change			**12.94%**		**15%**		**17.84%**

(Values are shown as Mean ± SD values, n=3, and percentage change values shown in bold) (* P<0.05)

Table 4. Effect of acute and chronic concentrations of tannery effluent on the Glutathione Peroxidase activity of *Channa striatus.*

GPx (mg l^{-1})		Tissues					
Tissue		**Liver**		**Testes**		**Ovary**	
Concentration	**Days**	**Control**	**Experiment**	**Control**	**Experiment**	**Control**	**Experiment**
Chronic (10%)	0 day	34.62 ± 0.014	34.61 ± 0.024	89.34 ± 0.001	89.03 ± 0.014	22.88 ± 0.015	22.88 ± 0.015
	30^{th} day	32.58 ± 0.012	21.34 ± 0.008	88.13 ± 0.011	49.67 ± 0.016	21.13 ± 0.152	14.03 ± 0.106
Percentage change			**34.50%**		**43.64%**		**33.60%**
Acute (1%)	0 day	34.62 ± 0.014	34.61 ± 0.024	89.34 ± 0.001	81.34 ± 0.021	22.88 ± 0.015	22.78 ± 0.108
	30^{th} day	32.58 ± 0.012	30.26 ± 0.053	88.13 ± 0.011	79.67 ± 0.053	21.13 ± 0.152	20.13 ± 0.004
Percentage change			**7.12%**		**9.59%**		**4.73%**

(Values are shown as Mean ± SD values, n=3, and percentage change values shown in bold) (* P<0.05)

The findings of the current study distinctively demonstrated that tannery effluents caused oxidative stress in *C. striatus* and that this stress was accompanied by the decreased activities of SOD, CAT, and GpX in the liver, testes, and ovary of effluents-treated groups. The SOD, CAT, and GPx are potent enzymes with antioxidative properties found in all eukaryotic organisms [16]. These antioxidant enzymes either stop the uncontrollable formation of reactive oxygen species (ROS) or slow down their interactions with biological structures. Decreased activity of these enzymes in turn raises the concentration of ROS within the cell due to an imbalance between the production of ROS and its destruction by antioxidant enzymes[17]. In addition, numerous specialized antioxidant enzymes react with and detoxify oxidant compounds. The superoxide radical is dismutated by SOD to hydrogen peroxide (H_2O_2) and molecular oxygen, which is then converted to H_2O by CAT and GPx [18].

The impact of heavy metals on the antioxidant system of fish has been reported [19, 20]. Thus, the response of the fish's antioxidant system can be used as a biochemical marker to predict metal toxicity in aquatic environments. The effect of tannery effluents on the histopathologic variations in the liver, kidney, stomach, and intestine of *Channa punctatus* is on record [21].

The adverse effects of tannery effluent on the enzymes such as acid and alkaline phosphatase, SOD, and CAT in the liver, gills, and muscle of guppy fish (*Poecilia reticulata*) suggests that these toxicants interfered with fish metabolism by altering the activities of stress enzymes in fish, resulting in cellular injury [22]. The results of the present study were, however, in contrast with these findings that fish's stress enzyme activity increased when they were exposed to environmental toxins.

CONCLUSIONS

The current study showed that tannery effluents caused *C. striatus* to experience oxidative stress, which was accompanied by a decrease in the activities of SOD, CAT, and GpX. The normal functional activities of *C. striatus* are altered by the acute and chronic concentrations of tannery effluents. The variation in the

antioxidant profile that causes oxidative stress in *C. striatus* suggests that exposure to certain concentrations of tannery effluents has a significant impact on the fish's defense system. However, more study is required to standardize the measurements and methods so that these assays can be used as a biomarker of aquatic environments.

CONFLICT OF INTERESTS

The authors declare no conflict of interest regarding this manuscript

Authors contribution

RS collected the samples and carried out the experiments. KN helped with the statistical analysis of the data. PP contributed in the conceptualization and preparation of the manuscript. The final version of the manuscript is confirmed by all the authors of this manuscript.

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