ejpmr, 2020,7(6), 682-686

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATIONOFOXIDATIVESTRESSBYXANTHINEOXIDASEIN INDIAN MAJOR CARPS FROM LAKES OF AJMER

Dr. Sudha Summarwar* and Dr. Harendra Kumar (IPS)

Research Associate, UGC-PDF, School of Basic & Applied Science, Sangam University, Bhilwara, Rajasthan.

***Corresponding Author: Dr. Sudha Summarwar**

Research Associate, School of Basic & Applied Science, Sangam University, Bhilwara, Rajasthan.

Article Received on 06/04/2020 **Article Revised on 27/04/2020** **Article Accepted on 18/05/2020**

ABSTRACT

In order to assess the impact of pesticide in Indian major carps of lakes of Rajasthan, a study was conducted at the tissue level of antioxidant enzymes xanthine oxidase. Two type's fishes i.e. Labeo rohita & Catla catla were collected from Ana Sagar & Foy Sagar Lake of Ajmer, Rajasthan. Fishes were collected from different areas of lakes namely Ana Sagar site 1, Ana Sagar site 2, Ana Sagar site 3, Ana Sagar site 4, Ana Sagar site 5, and Foy Sagar. The activity of Xanthine Oxidase was observed and analyzed in both the fishes from all six sites. The study revealed that Xanthine Oxidase activity was higher in Catla catla.

KEYWORDS; Xanthine Oxidase, Oxidative stress, fish health, lakes of Rajasthan.

INTRODUCTION

Fishes serve as bio indicators of environmental pollution and therefore can be used for the assessment of the quality of aquatic environment as they are directly exposed to chemicals resulting from agricultural production via surface runoff of water or indirectly through the food chain of the ecosystem (Ateeq et al., 2002). Fishes are endowed with defensive mechanisms to counteract the impact of reactive oxygen species (ROS) resulting from the metabolism of various chemicals. These mechanisms include various antioxidant defense enzymes such as superoxide dismutase which catalyze the dismutation of superoxide radicals to hydrogen peroxide, catalase acting on hydrogen peroxide, glutathione S-transferase family possessing detoxifying activities towards lipid hydroperoxides generated by organic pollutants such as heavy metals (Tjalkens et al., 1998).

Several studies have associated the involvement of xanthine oxidase (XO) activity, asource of uric acid and reactive oxygen species (ROS), to pro-oxidative and pro-inflammatory effects during pathological conditions. Considering this, this study aimed to evaluate whether up regulation on seric XO activity may be a pathway involved in the oxidative stress in fish. Several studies have shown that xanthine oxidase activity has been associated with pro-oxidative and pro-inflammatory pathogenic conditions.

Taking cue of this fact, the present study contemplates whether xanthine oxidase activity can be attributed to oxidative stress among fishes, as there is a want of study in this regard for Indian major carps. The present study was devised to ascertain the impact and correlation of xanthine oxidase and oxidative stress in major carps of India and especially from the two lakes of Ajmer, Rajasthan.

MATERIAL AND METHOD

Sample collection

To appraise the impact of pesticides on the tissue level of the antioxidant enzyme Vitamin E in major Indian carps, two types of fishes were collected from the lakes of Ajmer, Rajasthan. The fish type included for this purpose were Labeo rohita and Catla catla. The collection sites included two lakes situated in the Ajmer city of Rajasthan. These lakes were Ana Sagar and Foy Sagar Lake. Fishes were collected from different areas of lakes namely Ana Sagar site 1, Ana Sagar site 2, Ana Sagar site 3, Ana Sagar site 4, Ana Sagar site 5, and Foy Sagar.

Xanthine oxidase

It was determined by the colorimetric method as described by Joshi (2012). The colorimetric method is based on the detection of substrate disappearance. In this method protein is precipitated from the sample and supernatant is taken for colorimetric analysis of xanthine oxidase.

Two tubes were taken and labeled as control and sample. In each tube, 1 ml serum and 0.3 ml buffer solution were added. In sample tube 0.6 ml xanthine solution and in control tube 0.6 ml distilled water was added. Both the tubes were incubated at 37oC for 40 minutes in a water bath. Then in each tube 1 ml of 40% sodium tungstate, 5 ml distilled water and 1 ml 2N H_2SO_4 were added. Each was centrifuged for 5 minutes at 2000 rpm to get

supernatant.

Then 0.5 ml of supernatant from each tube was taken into two respectively labeled clean dry test tubes. In each test tube 2.5 ml of distilled water was added, followed by 1 ml of the diluted Folin-Ciocalteu reagent. The color was developed by the addition of 5 ml of saturated sodium carbonate solution. A reagent blank was prepared by taking 1 ml of 40 percent sodium tungstate, 5 ml of distilled water, and 1 ml of 2 N H2SO4. From this mixture, 0.5 ml was taken and mixed with 2.5 ml of distilled water, 1 ml of the diluted Folin Ciocalteu reagent, and 5 ml of saturated sodium carbonate. The optical densities of sample and control were determined at 660 mμ wavelength in a spectrophotometer against the reagent blank.

Xanthine oxidase activity was determined directly in mUL^{-1} by the formula given below:

$$= \frac{\text{Test OD-Control OD X 1000 X 1 X5 X 1000}}{1.22X10^{4} \text{ X } 0.6}$$

*Here, 5 is dilution factors,1000 is correction factor, 1 is amount of serum taken, 1000 is unit convertor,$1.22X10^{4}$ is the molar absorbance and 0.6 is the ml of xanthine taken.

**Then units were calculated for mg of protein as for other enzymes

RESULTS AND DISCUSSION

Mean± SEM values of XO of heart, kidney, liver, and gills of male and female fishes i.e. Labeo rohita (Rohu, Lr) and Catla catla (Catla, Cc) collected from different areas of Ana Sagar lake (site 1, site 2, site 3, site 4 and site 5) and Foy Sagar lake is presented in table 1 and depicted in figures 1 and 2. In each gender, fishes were further grouped as low weight (LW) and high weight (HW). The data is based on 20 observations each as specified in the section of materials and methods.

- The mean values of XO from each site revealed a significant difference ($p \leq 0.05$) among themselves. Fishes collected from Ana Sagar Lake Site 4 revealed significantly ($p \leq 0.05$) the highest values of XO in all the tissues respectively, as compared to the rest of the other sites. This revealed that maximum oxidative stress was developed in fishes collected from Ana Sagar Lake Site 4. Extremely high or low pH impinged on the antioxidant status of fishes of both genders. This also existed in the value of XO of the tissues of fishes collected from different areas.
- At each collection site, Catla catla fish revealed higher XO activities. This exhibited that Cc fish built up a greater degree of oxidative stress than Lr fish. In both the category of fishes, females revealed higher activities of XO than males. This pointed up that females of both the type of fishes had developed a higher scale of oxidative stress. Furthermore, it was found that low-weight male and female fish had a larger amount of oxidative stress than high-weight male and female fish. SOD was significantly ($p \leq 0.05$) greater in low-weight fish than the high-weight fish.
- Xanthine oxidase activity was reported to be linked with the pH of water samples from where the fishes were collected for the study. Alterations in the pH level of water samples indicated pollution in the water. It divulged that pollution of the water altered the antioxidant status of fishes of both the types i.e. Lr and Cc.
- It appeared that the pH level of water, presence of pollutants in water, XO activity of tissues, and antioxidant status of fish were having husky relation. Oxidative stress is considered to play a major role in affecting the rejoinders of fish to variations in the environment (Gauvin et al., 2017).
- The mean values of XO in various tissues obtained in the present investigation showed more or less similar precedent of distribution in body tissues and values obtained from Ana Sagar site 1 were taken as control values. Based on available control values, it was concluded that the mean values of XO in all the tissues of both the types of fish divulged oxidative stress. Alterations in antioxidant status are connected with the development of oxidative stress (Kataria et al., 2016).
- A higher concentration of XO in fish indicated the presence of oxidative stress. In animals also, higher XO activity is a reflection of the development of oxidative stress (Kataria and Kataria, 2013). In each area, the XO activity significantly differed among all the tissues collected i.e. heart, kidney, liver, and gills. In each area, the activity of XO was highest in gills for both the fishes i.e. Lr and Cc. The activity was reported lowest in the heart of both the types of fishes i.e. Lr and Cc, collected from all the six areas.
- In each area, the XO activity in every tissue was significantly higher in Catla catla than in Labeo rohita. In mammals the increased activity of XO proposed the aptitude of the animals to offer defense against free radicals. It is to fight against oxidative stress (Kataria et al., 2010a)
- Observations of the present endeavor divulged that the stressful conditions in the water medium could be the motive for higher XO activity leading to the vast production of free radicals. This most likely resulted in oxidative stress and an inequity between oxidant and antioxidant systems. Oxidative stress can be incited by any sort of stress (Kataria et al., 2010b). The consequence of variations in the environment can lead to the development of oxidative stress in animals (Kataria et al., 2010b). The increased activity of XO was also correlated with oxidative stress in infected cases (Kataria et al. 2010a).
- It can be conjectured that higher XO activities exhibited activation of the defense system of fish due to oxidative stress. Therefore, Cc developed greater oxidative stress compared to Lr. Females of

each species developed greater oxidative stress than males. Low-weight fish developed greater oxidative stress than high-weight fish in each gender and species. A higher concentration of XO in fish marked the presence of oxidative stress.

- Xanthine oxidase acts as a biomarker of oxidative stress in animals (Kataria et al., 2010a). A higher concentration of XO in fish indicated the presence of oxidative stress. Pandey et al. (2003) reported higher activity of XO in the tissues of fish i.e. gills, kidney, and liver collected from the polluted site. Xanthine oxidase is a form of xanthine oxidoreductase that generates reactive oxygen species (Ardan et al., 2004). In stressed animals higher xanthine oxidase may indicate oxidative stress; therefore, it can be used as a marker of oxidative stress (Joshi, 2012).

Table 1: Mean values of Xanthine Oxidase in the tissues of fishes collected from different areas of Ana Sagar and Foy Sagar lakes.

Name of Area	Type of fish			Mean ± SEM (mUmg-1 protein)			
				Heart	Kidney	Liver	Gills
Ana Sagar Site 1	Lr Overall value (80)			1.59b±0.004	1.74b±0.003	2.06b±0.004	2.84b±0.005
	Lr (80)	M (40)	LW(20)	1.58c±0.001	1.73c±0.001	2.05c±0.001	2.83c±0.001
			HW(20)	1.55d±0.001	1.70d±0.001	2.02d±0.001	2.80d±0.001
		F (40)	LW(20)	1.62c±0.001	1.78c±0.001	2.10 c±0.001	2.88c±0.001
			HW(20)	1.60d±0.001	1.75 d±0.001	2.07 d±0.001	2.85 d±0.001
	Cc Overall value80)			1.64b±0.004	1.79b±0.004	2.11 b±0.004	2.89b±0.005
	Cc (80)	M(40)	LW(20)	1.62c±0.001	1.78c±0.001	2.10 c±0.001	2.88c±0.001
			HW(20)	1.60d±0.001	1.75d±0.001	2.07d±0.001	2.85d±0.001
		F (40)	LW(20)	1.68c±0.001	1.82c±0.001	2.15 c±0.001	2.93c±0.001
			HW(20)	1.65d±0.001	1.820d±0.001	2.12 d±0.002	2.90 d±0.001
Ana Sagar Site 2	Lr Overall value (80)			2.89b±0.004	3.04b±0.001	3.16 b±0.001	3.44b±0.001
	Lr (80)	M (40)	LW(20)	2.88c±0.001	3.03c±0.001	3.15 c±0.001	3.43c±0.001
			HW(20)	2.85d±0.001	3.00d±0.001	3.12d±0.001	3.40d±0.001
		F (40)	LW(20)	2.93c±0.001	3.08c±0.001	3.20 c±0.001	3.48c±0.002
			HW(20)	2.90d±0.001	3.05d±0.0014	3.17 d±0.001	3.45 d±0.002
	Cc Overall value (80)			2.94b±0.001	3.09b±0.005	3.21 b±0.007	3.49b±0.004
	Cc (80)	M (40)	LW(20)	2.93c±0.001	3.08c±0.001	3.20 c±0.002	3.48c±0.001
			HW(20)	2.90d±0.001	3.05d±0.001	3.17d±0.001	3.45d±0.001
		F(40)	LW(20)	2.08c±0.001	3.13c±0.002	3.25 c±0.001	3.53c±0.001
			HW(20)	2.95d±0.001	3.10 d±0.001	3.22 d±0.001	3.50 d±0.001
Ana Sagar Site 3	Lr Overall value (80)			4.21b±0.003	4.36b±0.005	4.68 b±0.006	5.46b±0.006
	Lr (80)	M (40)	LW(20)	4.20c±0.001	4.35c±0.001	4.67.00c±0.001	5.45c±0.002
			HW(20)	4.17d±0.001	4.32d±0.001	4.64d±0.001	5.42d±0.001
		F (40)	LW(20)	4.25c±0.001	4.40c±0.002	4.72 c±0.001	5.50c±0.001
			HW(20)	4.22d±0.001	4.37 d±0.001	4.69 d±0.001	5.47 d±0.001
	Cc Overall value (80)			4.26b±0.004	4.41b±0.005	4.73 b±0.004	5.51b±0.005
	Cc (80)	M (40)	LW(20)	4.25c±0.001	4.40c±0.001	4.72 c±0.001	5.50c±0.001
			HW(20)	4.22d±0.001	4.37d±0.002	4.69d±0.001	5.47d±0.001
		F(40)	LW(20)	4.20c±0.001	4.35c±0.002	4.67 c±0.001	5.45c±0.001
			HW(20)	4.27d±0.001	4.42 d±0.001	4.74 d±0.001	5.52 d±0.001
Ana Sagar Site 4	Lr Overall value(80)			5.42b±0.004	5.57b±0.004	5.89 b±0.004	6.17b±0.004
	Lr(80)	M (40)	LW(20)	5.41c±0.001	5.56c±0.001	5.88 c±0.002	6.16c±0.001
			HW(20)	5.38d±0.001	5.53d±0.001	5.85d±0.001	6.13d±0.001
		F (40)	LW(20)	5.46c±0.001	5.61c±0.001	5.93 c±0.001	6.21c±0.001
			HW(20)	5.43d±0.001	5.58d±0.002	5.90 d±0.001	6.18 d±0.001
	Cc Overall value(80)			5.47b±0.007	5.62b±0.008	5.94 b±0.008	6.22b±0.007
	Cc(80)	M (40)	LW(20)	5.46c±0.001	5.61c±0.001	5.93 c±0.001	6.21c±0.001
			HW(20)	5.43d±0.001	5.58d±0.001	5.90d±0.001	6.18d±0.001
		F (40)	LW (20)	5.51c±0.001	5.66c±0.002	5.98 c±0.001	6.26c±0.002
			HW(20)	5.48d±0.001	5.63 d±0.002	5.95 d±0.002	6.23 d±0.001
Ana Sagar Site 5	Lr Overall value (80)			1.82b±0.005	1.97b±0.004	2.29 b±0.005	3.07b±0.005
	Lr (80)	M (40)	LW(20)	1.81c±0.001	1.96c±0.002	2.28 c±0.001	3.06c±0.001
			HW(20)	1.78d±0.001	1.93d±0.001	2.25d±0.001	3.03d±0.001

		F (40)	LW(20)	1.86c±0.001	2.01c±0.001	2.33 c±0.002	3.11c±0.001
			HW(20)	1.83d±0.001	1.98 d±0.001	2.30 d±0.001	3.08 d±0.001
	Cc Overall value(80)			1.87b±0.004	2.02b±0.005	2.44 b±0.006	3.12b±0.004
	Cc (80)	M (40)	LW(20)	1.86c±0.001	2.01c±0.002	2.44 c±0.001	3.11c±0.001
			HW(20)	1.83d±0.001	1.98d±0.001	2.39d±0.001	3.08d±0.001
		F (40)	LW(20)	1.91c±0.001	2.06c±0.001	2.48 c±0.001	3.16c±0.001
			HW(20)	1.88d±0.001	2.03 d±0.001	2.45 d±0.002	3.13 d±0.001
Foy Sagar	Lr Overall value(80)			2.10b±0.005	2.25b±0.004	3.07 b±0.005	3.65b±0.005
	Lr (80)	M (40)	LW(20)	2.09c±0.001	2.24c±0.001	3.06 c±0.001	3.64c±0.001
			HW (20)	2.06d±0.001	2.21d±0.002	3.03d±0.001	3.61d±0.001
		F (40)	LW(20)	2.14c±0.001	2.29c±0.001	3.11 c±0.002	3.69c±0.001
			HW(20)	2.11d±0.001	2.26 d±0.001	3.08 d±0.001	3.66 d±0.001
	Cc Overall value (80)			2.15b±0.004	2.30b±0.005	3.12 b±0.004	3.70b±0.004
	Cc (80)	M (40)	LW(20)	2.14c±0.001	2.29c±0.001	3.12 c±0.001	3.69c±0.001
			HW(20)	2.11d±0.001	2.36d±0.001	3.08d±0.002	3.66d±0.001
		F (40)	LW(20)	2.39c±0.001	2.34c±0.001	3.16 c±0.001	3.74c±0.001
			HW(20)	2.16d±0.002	2.31 d±0.001	3.13 d±0.001	3.71 d±0.001

Figures in the parentheses indicate number of observations in each case Lr= Labeo rohita fish
Cc= Catla catla fish
M = Male
F = Female
b = Significant (p≤0.05) difference in overall mean values of Lr andCc
c = Significant (p≤0.05) difference for LW in a fish type
d = Significant (p≤0.05) difference for HW in a fish type

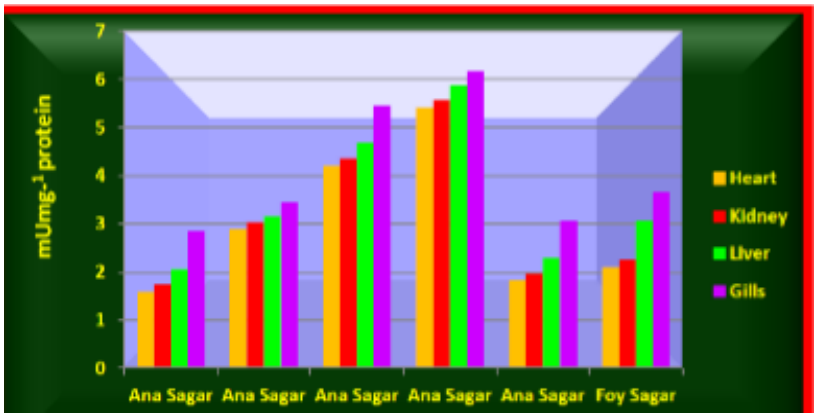

Fig.1: Illustration of mean changes in values of xanthine oxidase in the tissues of Labeo rohita fish from different areas of Ana Sagar and Foy Sagar lakes.

Fig. 2: Illustration of mean changes in values of xanthine oxidase in the tissues of Catla catla fish from different areas of Ana Sagar and Foy Sagar lakes.

CONCLUSION

The present study concludes that the mean value of xanthine oxidase activity was recorded highest at Ana Sagar site 4. It suggests that due to the higher xanthine oxidase value at Ana Sagar site 4 the fishes were subjected to a higher level of stress owing to pollution and other factors. While analyzing the groups of fishes, it was observed that Catla was having more xanthine oxidase value in comparison to Labeo rohita suggesting, that the Catla was more symptomatic to stress. Gender analysis suggested that females exhibited a higher degree of oxidative stress compared to males. About concerning weight analysis, the male having less weight were showing the more oxidative stress along with females of low weight in comparison to high weighted male and female. The oxidative stress was seen in all the tissues of the heart, liver, kidney, and gills. PH of site also played a role in xanthine oxidase value. Variation in PH is indicative of pollution.

REFERENCE

1. Ardan T, Kovaceva J, Cejková J (2004). Comparative histochemical and immunohistochemical study on xanthine oxidoreductase/xanthine oxidase in mammalian corneal epithelium. Acta. Histochem, 106(1): 69-75
2. Ateeq, B.; Abul-Farah, M.; Niamat-Ali, M. and Ahmad, W. (2002). Induction of micronuclei and erythrocyte alterations in the catfish Clarias batrachus by 2, 4 - dichlorophenoxyacetic acid and butachlor. Mutat. Res, 518: 135-144.
3. Gauvin, K.B.; Costantini, D.; Cooke, S.J. and Willmore, W.G. (2017). A comparative and evolutionary approach to oxidative stress in fish: A review. Fish and Fisheries, 18(5): 928- 942.
4. Joshi, A.; Kataria, N.; Kataria, A.K.; Pandey, N.; Sankhala, L.N.; Asopa, S.; Pachaury, R. and Khan, S. (2012). Influence of ambient temperatures on metabolic responses of Murrahbuffaloes of varying physiological states from arid tracts in India. ELBA Bioflux, 4(2): 34-39.
5. Kataria, A.K.; Kataria, N. and Maan, R. (2010b). Correlation of serum IgE with stress in Indian dromedaries affected with skin wounds. J. Stress Physiol. Biochem, 6(3): 17-24.
6. Kataria, N.; Joshi, A.; Singhal, S.S.; Asopa, S. and Kataria, A.K. (2016). Evaluation of oxidative stress during hot dry and hot humid environmental periods in indigenous pigs from arid tracts in India. Porcine Research, 6(1): 16-23.
7. Kataria, N. and Kataria, A.K (2013). Ambiance associated variations in serum biomarkers of oxidative stress in donkey of arid tracts in India. Egyptian J. Bio, 15: 44-47.
8. Kataria, N.; Kataria, A.K.; Pandey, N. and Gupta, P. (2010a). Serum biomarkers of physiological defense against reactive oxygen species during environmental stress in Indian dromedaries. HVM Bioflux, 2(2): 55-60.
9. Pandey, S.; Parvez, S.; Sayeed, I.; Haque, R.; Bin-Hafeez, B. and Raisuddin, S. (2003). Biomarkers of oxidative stress: a comparative study of river Yamuna fish Wallago attu (Bl. & Schn.). Sci. Total Environ, 309(1-3): 105-115.
10. Tjalkens, R.B.; Valerio, L.G J.; Awasthi, Y.C. and Petersen, D.R. (1998). Association of glutathione Transferase isozyme-specific induction and lipid peroxidation in two inbred strains of mice subjected to chronic dietary iron overload. Toxicol. Appl. Pharmacol, 151: 174-81.