ISSN: 0976-3376

Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol.07, Issue, 04, pp. xxxxxx, April, 2016

RESEARCH ARTICLE

STUDIES ON THE ACTIVITY OF OXIDATIVE ENZYMES IN MUSCLE TISSUE OF FRESHWA TER FISH *OREOCHROMIS MOSSAMBICUS* EXPOSED TO AZO DYE METHYL RED

***[1]Sasikala, M., [1]Savitha, R., [1]Saravanan, R., [1]Chitra, N. and [2]Jothi Narendiran, N.**

[1]Department of Zoology, Dr Ambedkar Government Arts College, Vyasarpadi, Chennai- 600039, Tamil Nadu
[2]Department of Zoology, Government Arts College for Men, Nandanam, Chennai-600035, Tamil Nadu

ARTICLE INFO

Article History:
Received xxxxxx, 2016
Received in revised form
xxxxxxxxxxxx, 2016
Accepted xxxxxxxxxxxxxx, 2016
Published online xxxxxxxxxx, 2016

Key words:

Oreochromis mossambicus,
Muscle, Methyl red,
Metabolic pathways, Hexokinase,
LDH, SDH and MDH.

ABSTRACT

The potential toxicity of dyes produces enzymatic changes in metabolic tissues due to alteration in metabolic pathways in these animals. Muscle tissue of fish was analysed for the effects of methyl red, since it is a major target tissue involved in key metabolic activity and form the edible part of the fish. The present study unravels the activity of enzymes involved in muscle oxidative metabolism in fishes exposed to methyl red dye. The fishes were maintained for a period of 40 days at one- fifth sub lethal concentration. The present study revealed no significant change in hexokinase activity, an increased activity in lactate dehydrogenase and (LDH), a decline in activity of succinate dehydrogenase (SDH) and an increase in activity of malate dehydrogenase (MDH).

INTRODUCTION

Wastewater from the textile industry is a complex mixture of many polluting substances ranging from organochlorine-based pesticides to heavy metals associated with dyes and the dyeing process (Barot and Bahadur, 2013). Inefficiencies in dyeing result in discharge of large amounts of the dyestuff and contamination of surface water with these effluents from the textile-dye and dyestuff industry represent a serious ecological problem (Abdel Moneim *et al.,* 2008). Extensive usage of commercial dyes has been observed in various rural sectors of India. Unfortunately, these commercial dyes do not have any information with respect to their chemical structure, purity or composition; continuous use of such dyes can lead to serious consequences to exposed organisms (Afaq and Rana, 2008). During textile processing, inefficiencies in dyeing result in large amounts of the dyestuff (varying from 2% loss when using basic dyes to a 50% loss when certain reactive dyes used) is being directly lost to the wastewater, which ultimately finds its way into the environment. Among all the chemical classes of dyes, azo dyes are considered to be recalcitrant, non-biodegradable and persistent (Saratale *et al.,* 2011). Moreover, azo dyes as well as their breakdown products are cytotoxic or carcinogenic (Khehra *et al.,* 2006). The azo group of dyes binds to an aromatic ring, through mineralization, this dye is suspected to be carcinogenic. Most of the azo dyes are water soluble and readily to absorb through skin contact and inhalation leading to the risk of cancer and allergic reactions, an irritant for the eyes and highly toxic, if inhaled or consumed (Oliveira *et al.,* 2007; Sudha *et al.,* 2014). Some azo dyes are linked to human cancer, splenic sarcomas, hepatocarcinomas, and nuclear anomalies in experimental animals and chromosomal aberrations in mammalian cells (Puvaneswari *et al.,* 2006). Dyes may also significantly affect photosynthetic activity in aquatic life by reducing light penetration intensity and may also be toxic to some aquatic fauna and flora due to the presence of aromatics, metals and chlorides (Dhaneshvar *et al.,* 2007). Certain acid and basic azodyes are acutely toxic to aquatic organisms particularly fish, crustaceans, algae and bacteria (Material Safety Data Sheet, Methyl Red, 2010). Methyl red is an anionic azo dye (Sahoo *et al.,* 2005). The chemical structure of methyl red (Fig A). The IUPAC name 2-[4(dimethylamino) phenyl] diazonyl benzoic acid. It is also refered to as C1 Acid red 2. It is odourless and dark red in colour. pH for various colour ranges differently. It is red in colour under pH 4.4, yellow in pH 6.2 and orange with pH 5.1. The present study was undertaken to assess the effect of methyl red dye on fingerlings of *Oreochromis mossambicus* as no report is available on juvenile or developmental toxicity. The objective of the study was to assess the activity of metabolic enzymes in muscle tissue during the stages of growth.

****Corresponding author: Sasikala, M.,***
Department of Zoology, Dr Ambedkar Government Arts College, Vyasarpadi, Chennai- 600039, Tamil Nadu

MATERIALS AND METHODS

Maintenance of experimental animals

Freshwater fish *Oreochromis mossambicus* was used as the experimental model to evaluate the toxicity of methyl red. The fish used in this experiment were transferred from natural ponds around Tiruvallur district and brought to the laboratory and acclimatized for 15 days to laboratory conditions in tub aquaria each measuring (60 cm ×30 cm × 30 cm) filled with 25 litres of dechlorinated tap water with aerator fitted to the aquaria for continuous oxygen supply. The aquaria were disinfected with potassium permanganate solution and washed thoroughly prior to introduction to prevent any fungal infection. Feeding was stopped 24 hours before the commencement of the toxicity test to keep the animals more or less in the same metabolic state. Initial mean weight and length of the fish were 20-28 gm and 8-12 cm respectively. The fishes were maintained in normal light dark period and optimal temperature. The fishes were fed twice (5% of body weight) a day with artificially prepared imported fish feed available in the market. The protein content of the feed was around 40%.

Methyl red dye toxicity and Determination of lethal concentration (LC_{50})

Acute toxicity experiments were conducted for 96 hours using a static bioassay technique. Five groups of 10 fishes each were set for the LC_{50} bioassay method. The fishes were exposed to a range of six different concentrations (1.10, 0.9, 0.7, 0.5, 0.3, 0.1, 0.0 mg/L) of methyl red. The fishes were maintained in a narrow range concentration. The 96 hour LC_{50} was determined by Probit analysis method (Finney, 1971). The Safe Application Factor Equation (SAFE) was calculated from the LC_{50} values. The concentration at which 50% mortality occurred after 96 hours was taken as the median lethal concentration. The dead fishes were removed immediately from the aquaria to avoid oxygen depletion. Mortality, behavioral and morphological changes were recorded during the 96 hr LC_{50} observation. LC_{50} was found to be 0.5 mg/L. The chosen mean concentration for methyl red was one fifth (0.10 mg/L) sub lethal concentration to study the effect of the dye. Experiments were conducted with sub-lethal and toxicologically safe concentrations of methyl red for a period of 40 days.

Experimental Design: Group I served as the control while Group II was exposed to methyl red at sub-lethal concentration.

Group I: Control fishes maintained in toxicant free water.
Group II: Fishes maintained at 0.10 mg/L of methyl red for a period of 40 days

The control and the experimental animals were fed with normal fish feed. Commercial food pellets with ingredients consisting of fish meal, wheat flour, soybean meal, yeast, vitamins and minerals were fed. Water was changed daily at 8.00 hours which facilitated the removal of unconsumed food. After renewal of water the required quantity of methyl red was added to maintain the concentration of the toxicant in water. At the end of 40^{th} day five fishes were sacrificed by cervical dislocation. Muscle tissues were dissected out and washed thoroughly with 0.9N saline solution. Tissues were weighed and homogenized in Tris 0.1M HCL buffer using a homogenizer. The homogenate of the tissue were centrifuged at 2500 rpm for 15 minutes in a high speed centrifuged and clear supernatant was used for biochemical analysis.

Enzyme analysis: Activity of oxidative enzymes were analysed by using standard procedures. Lactate dehydrogenase (King 1965), Hexokinase (Brandstrup *et al* 1957), MDH (Mehler *et al*., 1948) and SDH (Slater and Bonn, 1952).

Statistical Analysis: The data collected on the different parameters of the control and experimental study were subjected to statistical analysis by using statistical software SPSS version 6.0. The statistical significance was tested at 1% and 5% levels using Paired Sample 't' test.

RESULTS

Variable changes were observed in the enzymes associated with glycolytic and Kreb's cycle. No significant changes was seen in the activity of hexokinase, while an increase was noticed in lactate dehydrogenase activity of muscle tissue which was significant ($P<0.001$) during 40 days exposure of the fishes to the methyl red when compared to control fishes (Table 1). Activity of succinate dehydrogenase declined while activity of malate dehydrogenase of muscle tissue was significantly increased ($P < 0.001$) during 40 days of exposure of fishes to methyl red dye (Table 2).

Table 1. **Effect of methyl red dye on muscle hexokinase and lactate dehydrogenase activity in freshwater fish *Oreochromis mossambicus***

Glycolytic Enzyme activity	Control	Experimental group (0.10mg/l)	t -value	p -value
Hexokinase	0.44 ± 0.01	0.49 ± 0.04	1.952	0.123^{NS}
Lactate dehydrogenase	9.42 ± 0.61	16.47 ± 0.94	489.069	< 0.001**

Values are expressed - Hexokinase - µ moles of glucose-6-phosphate formed /min/mg protein
Lactate dehyrogenase - µ moles of pyruvate produced /min/mg protein
Values are Mean ± SD (n=5) observations ;** denotes significance at 1% level ;NS-Non-Significant

Table 2. **Effect of methyl red dye on muscle succinate and malate dehydrogenases in freshwater fish *Oreochromis mossambicus***

Kreb's cycle Enzyme activity	Control	Experimental group (0.10mg/l)	t -value	p -value
Succinate dehydrogenase	0.28 ± 0.01	0.12 ± 0.01	50.596	< 0.001**
Malate dehydrogenase	1.76 ± 0.34	4.79 ± 0.12	22.953	< 0.001**

Values are expressed- Succinate dehydrogenase - µ moles of succinate formed /min/mg protein
Malate dehydrogenase - µ moles of NADH oxidised /min/mg protein
Values are Mean ± SD (n=5) observations : ** denotes significance at 1% level

DISCUSSION

In the present study *Oreochromis mossambicus* was exposed to azo dye methyl red used basically in textile industries for dying purpose. The study will help in determining the muscle oxidative enzyme activity in fingerlings of *Oreochromis mossambicus.* Glycolytic enzyme hexokinase showed slight increase in activity but there was no significant change observed in the experimental groups. Increase in hexokinase was reported in muscle of *Channa punctatus* exposed to sevin for 96 hr and 120 days (Sastry *et al*., 1988). Increased activity of LDH may specify a shift towards anaerobiosis resulting in boosted production of lactic acid. Our studies from this laboratory show that there was an decrease in pyruvate and an increase in lactate contents in *Oreochromis mossambicus* exposed to methyl red (Chitra, 2015). Following methyl red exposure elevated activity of LDH in the present study indicates increased anaerobic respiration to meet the energy demands where aerobic oxidation is lowered. An increase in LDH in muscle tissue of fishes was reported after exposure to pesticidal stress (Neelima *et al.,* 2015). LDH was found to be elevated in muscle tissue after 24 and 96 hr of exposure and after 8 days of exposure in *Channa punctatus* exposed to methyl parathion (50% EC) (Padmavathi *et al*., 2015).

The present study shows reverse trends in two of the key enzymes studied in Krebs cycle pathway. Inhibition of SDH activity could be due to the depression of cellular oxidation and derailment in metabolic cycle. A decline in muscle SDH was reported in muscle tissue of *Labeo rohita* exposed to sodium cyanide (Dube *et al*., 2013). Similar decline in SDH activity was reported in *Cyprinus carpio* after 60 days of exposure to fenthion (Leena Muralidharan, 2014). Antagonistic activity of LDH and SDH activities indicate the blockage of anerobic and aerobic metabolism to meet the energy demand due to toxic stress. The increase in the activity of malate dehydrogense in the present study during the end of the exposure period could be due to the shuttling of amino acids through gluconeogenesis via Kreb's cycle (Chitra, 2015).

REFERENCES

Abdel Moneim, M., Abou, Sabana, N. M., Khadre, S.E.M. and Abdul Khader, H.H. 2008.Physiological nad histological effects in catfish exposed to dyestuff and chemical wastewaters. *Int J Zoo Res*, 4: 189-202.

Afaq, S. and Rana, K.S. 2008. Effect of leather dyes on packed cell volume of fresh water teleost *Cirrhinus mrigala* (Ham.). *Asian J.Exp. Sci.,* 22: 347-350.

Barot, J. and Bahadur, A. 2013. Behavioural and histopathological effects of azodye on kidney and gills of *Labeo rohita* fingerlings. *J Environ Biol*, 34: 147-152.

Brandstrup, N., Krik, J. E. and Bruni, C. 1957. Determination of hexokinase in tissues. *J Gerentol*, 12: 166-171.

Chitra, N. 2015. Effect of an azo dye methyl red on biochemical constituents in muscle tissue of freshwater fish *Oreochromis mossambicus*. M.Phil Dissertation, University of Madras.

Dhaneshvar, N., Ayazloo, M., khatae, A. R. and Pourhassan, M. 2007. Biological decolourization of dye solution containing malachite green by *Microalgae cosmarium sp. Bioresource Technol.,* 29: 1-7.

Dube, N. P. Shwetha Alavandi and Dasaling B Hosetti, 2013. Effect of exposure to sublethal concentration of sodium cyanide on the carbohydrate metabolism of the Indian Major carp *Labeo rohita*. *Pesq. Vet. Bras.,* 33(7) : 914-919.

Finney, D. J. 1971. Probit analysis-A statistical treatment of sigmoid curve -3rd edn. *Cambridge University Press*, London. pp 568.

Khehra, M. S., Saini, H. S., Sharma, D. K., Chadha, B. S. and Chimni, S. S. 2006. Biodegradation of azo dye C.I. Acid Red 88 by an anoxic-aerobic sequential bioreactor. *Dyes and Pigment*. 70: 1-7.

King, J. 1965. Lactate dehydrogenase. In: Practical Enzymology. Von Nostrund Company, London.

Leena Muralidharan , 2014. Chronic impact of fenthion on the profiles of enzymes in the freshwater fish *Cyprinus carpio* (Linn). *Int J Fish Aquat Studies,* 1(4): 51-56.

Material Safety Data Sheet, Methyl Red, 2010.

Mehler, A.H., Kornberg, A., Criscolin, S. and Ochoa, S., 1948. Assay of activity of malate dehydrogenase. *J. Biol. Chem*., 174:961

Neelima, P., Govinda Rao, K., Gopala Rao, N. and Chandra Sekhara Rao Jammu, 2015. Enzymatic alterations as biomarkers of cypermethrin (25% EC) toxicity in a freshwater fish *Cyprinus carpio*(Linn.). *Int J Fish Aquat Studies,* 3(1):149-158.

Oliveira, D. P., Carneiro, P. A., Sakagami , M. K., Zanoni, M.V.B. and Umbuzeiro, G. A., 2007. Chemical characterization of a dye processing plant effluent-Identification of the mutagenic components. *Mutation Res.,* 626(1-2): 135-142.

Padmavathi, P., Veeraiah, K.,Tata Rao, S. and Vivek Ch, 2015. Methylparathion 50% EC induced changes in enzymatic activities of the fish *Channa punctatus* (Bloch). *Ind J Appl Res* 5(1): 594-599.

Puvaneswari, N., Muthukrishnan, J. and Gunasekaran, P., 2006. Toxicity assessment and microbial degradation of azo dyes. *Ind J.Exp. Biol*., 44: 618-626.

Sahoo, C, Gupta, A. K. and Anjali, P., 2005. Photocatalytic degradation of methyl red dye in aqueous solution under UV irradiation using Ag^{+} doped TiO_2. *Desalination,* 181: 91-100.

Saratale , R. G., Saratale, G. D., Chang, J. S. and Govindwar S. P. 2011. Bacterial decolorization and degradation of azo dyes: A review. *J Taiwan Inst Chemical Engineers.*, 42(1): 138-157.

Sastry, K. V., Siddique, A. and Samuel, M. 1988.Acute and chronic toxic effects of carbamate pesticide sevin on some haematological , biochemical and enzymatic parameters in freshwater teleost fish *Channa punctatus. Acta Hydrochem Hydobiol,* 16: 625-631.

Slater, E.C. and Boon, W.D., 1952. Assay of activity of succinate dehydrogenase. *Biochem. J.*, 52:185.

Sudha, M., Saranya, A., Selvakumar, G. and Sivakumar, N. 2014. Microbial degradation of azo dyes. A review Int. *J. Curr. Microbiol. Appl.Sci*., 3(2): 670-690.

****