Available online on 15.08.2021 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

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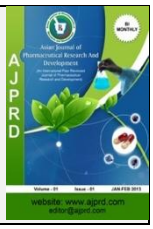

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Review Article

Oxidative Stress in Fish *Cyprinus Carpio* Caused By Oxytetracycline

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A B S T R A C T

Environment pollution is posing threat to humans and animals. Environmental pollution is caused by anthropogenic activities and is a major problem in many countries. The present study was investigated to find the oxidative stress induced by oxytetracycline in fish *Cyprinus carpio*. The acute exposure was given for 24, 48, 72 and 96 hrs of exposure. The sublethal concentration decided was 80 mg/l. Under oxidative stress, parameters such as glutathione-S-transferase (GST), catalase (CAT) and superoxide dismutase (SOD) were studied and a significant reduction in the activity of GST, CAT and SOD was observed in the treated group as compared to control. A significant decrease in SOD and CAT is at 72 and 96 hours of exposure while, a significant decline trend after 24 hours of exposure in GST activity was observed. Acute exposure of OTC enhanced reactive oxygen species formation and inhibited antioxidant capacities in the fish. Based on the results it is evident that oxytetracycline is causing oxidative stress in fish. It is also demonstrated that oxidative stress biomarker may play important in biomonitoring assessment protocol.

Keywords: Oxidative stress, pharmaceuticals, reactive fish species, oxytetracycline

A R T I C L E I N F O: Received 25 April 2021; Review Complete; 27 July 2021 Accepted; 10 August 2021 Available online 15 August 2021

Cite this article as:

Sharma M*, Daundkar P, Rani D, Oxidative Stress In Fish *Cyprinus Carpio* Caused By Oxytetracycline., Asian Journal of Pharmaceutical Research and Development. 2021; 9(4):118-121. **DOI:** http://dx.doi.org/10.22270/ajprd.v9i41006

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INTRODUCTION

The concern for antibiotics is increasing due to their increasing consumption, inappropriate dosage, high discharge, relentlessness nature, low efficiency of removal from sewage, toxic properties and promotion of antibiotic resistance in bacteria [1]. Thus, they are present in all aquatic compartments ranging from nanograms per liter to microgram per liter in effluents, rivers, coastal water, wastewater treatment plants, ground and drinking water sources [2-4].

Oxytetracycline (OTC) is the broad-spectrum antibiotics of tetracycline group and an antibacterial agent which is used as human and veterinary medicines for prophylactic and therapeutic purposes [5]. OTC inhibits the protein synthesis that interacts with 30 s ribosomal unit [6].

Oxytetracycline was first isolated from the bacteria *Streptomyces rimosus* in 1948 from soil and 2nd antibiotics to be discovered. OTC is used as animal and human medicine because it has low price and excellent antimicrobial activity with very less side effects [7].

Oxidative stress develops when there is an imbalance between production of reactive oxygen species (ROS) and reduced enzymatic and non-enzymatic antioxidants present in animal body. When generation of free radicals exceeds the body's antioxidant defence capacity after exposure to environmental pollutants, it leads to oxidative stress and subsequently injury to the cells [8]. Alterations in the antioxidant enzyme activities were reported in the fish tissues [9-13] and also in the blood [9,10] after administration of OTC through medicated diet.

Fish represent the largest group of vertebrates and they inhabit a broad range of ecosystem where they are exposed

to different aquatic contaminants. *Cyprinus carpio* is a hardy fish and consumers' preference in this region. In number of toxicity studies this fish has been used[14-16]. So the present study was planned to observe the oxidative stress in fish *Cyprinus carpio* in response to oxytetracycline.

MATERIALS AND METHODS

Fish (*C. carpio)* taken from the Fish farm of Department of Fisheries, DGCN COVAS, CSKHPKV, Palampur, Himachal Pradesh, India were used for the experiment. Fish of average length of 19±0.5 cm and weight 103.9±6.5 grams were given a prophylactic treatment by bathing the fish specimen twice in 0.05% potassium permangnate ($KMnO_4$) for two minutes to avoid any dermal infection. Fish were acclimatized under laboratory conditions for two weeks and were fed with pelleted feed during this period. The antibiotic oxytetracyclin immersion ZYDUS AH manufacturer (Steclin injection 100%) was taken from the local medical store. Exposure was semi-static, and every 24 hours and the drug was replenished to complete the initial concentration. The experimental glass aquaria used were of 100 liters capacity each. Control animals were submitted to the same water change schedule without the addition of OTC. Exposure was given for 96 hours. Sub-lethal concentration of OTC was decided as 80 mg/l of water for the exposure according to the survival to the mortality ratio value given by Ambili et al[17]. Blood sampling was done from the caudal vein with a heparinized syringe after 24, 48, 72 and 96 hours of exposure from both treated as well as control groups. Approximate 1ml of blood was collected from each fish and from each group blood was collected from five fish. All the samples were collected from 5 fish in each replicate. The experiment was conducted in triplicate.

After each duration of exposure five fish were used for blood sample collection. Blood was collected from caudal vein in anticoagulant free centrifuge tubes. Serum was obtained by centrifugation of blood at 3000 rpm for 10 min. Serum samples were stored at -80 ^{o} C until the analysis. After centrifugation the erythrocytic pellets left were washed twice with normal saline solution after removing the buffy layer to prepare10% erythrocyte lysate in distilled water. Then haemolysate was used for the estimation of superoxide dismutase (SOD) (Marklund and Marklund [18], catalase (CAT) (Aebi 1983[19]) and glutathione S-transferase (GST) (Habig et al [20]).

STATISTICAL ANALYSIS

The results are expressed as mean ± S.E. and to study the significance of the difference in the values of different parameters between treated and control groups a one-way analysis of variance (ANOVA) and Tukey-HSD test using the statistical software Graph pad was conducted.

RESULTS

There were alterations in the activities of antioxidant enzymes after exposure to oxytetracyclines (OTC) in *C. carpio* as evident by significant reduction in the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase.

There was non-significant reduction in the superoxide dismutase activities in treatment groups from 24 hours onward, that became significant at 72 hours of exposure (Table-1). The maximum reduction (37.02%) was noted at 96 hrs of exposure. The levels of catalase decreased significantly from 24 hrs until 96 hrs of OTC treatment in common carps as compared to control. Maximum reduction (78.99 %) was observed at 72 hrs of exposure. The activities of GST also showed a similar trend as that of CAT with the greatest decrease (89.82 %) at 24 hrs of OTC exposure (Table-1).

Table 1: Effect of acute exposure of Oxytetracycline (80mg/l) on Superoxide dismutase (SOD), Catalase (CAT) and Glutathione-S-transeferase activities in the blood of *C. carpio*.

Parameter	Control	24 hrs	48 hrs	72 hrs	96 hrs
SOD	22.42 ± 1.11	19.41 ± 0.86	16.78 ± 1.12	14.21 ± 1.93**	14.12 ± 1.71 **
CAT	76.92 ± 2.26	21.12 ± 1.53	18.43 ± 1.34	16.16 ± 1.39 ***	22.42 ± 0.65 ***
GST	11104.5 ± 744.6	1130.4 ± 241.0 **	2313.1 ± 326.1**	5594.7 ± 511.3***	3011.2 ± 509.2***

Values are expressed as the amount of enzyme causing 50% inhibition of auto-oxidation of pyragallol (SOD), µmole H_2O_2 decomposed/min/mg Hb (Catalase) and µmole of conjugate of GSH and CDNB formed/min/g Hb (GST). Values given are mean ± SE, n = 3

** & *** means values differ significantly with P< 0.01 & P<0.001 respectively.

DISCUSSION

The oxidative stress produced in the organism depends on various factors *viz.* type of oxidant, its composition, site and degree of exposure and interaction with antioxidants and their capability to repair systems [8]. Oxytetracycline form complex with divalent cations (iron & copper) which in turn generate reactive oxygen species (ROS) through the Fenton reaction [11]. These free radicals are responsible for irreversible conformational changes in the protein structure making it more susceptible to the action of proteases resulting in a significant decrease in antioxidant enzyme activities in common carps [13].

Superoxide dismutase (SOD) a metallo-enzyme, convert enzyme dismutase's superoxide radicals ($O_2^{\cdot}$) to hydrogen peroxide which is then used as a substrate by CAT and GPx. Thus, it plays an important role as a primary defense system against the toxic effects of reactive oxygen species (ROS) generated by environmental pollutants in aerobic organisms [9-11]. H_2O_2 produced by SOD is then either disintegrated by catalase or reduced by GSH dependent mechanism [8]. Significant reduction in

SOD activities observed in present study after OTC exposure in the fish blood are in agreement with observations of previous authors after oral administration of this antibiotic [9,10]. The similar alterations in the activities of this enzymes were also noted in various tissues such as liver, kidney, spleen, heart, gut, muscles etc. following oral OTC treatment [9-13] The decreased activities of SOD in OTC exposed common carps can be due to excessive accumulation of reactive oxygen (O_2^- and H_2O_2) and nitrogen species (peroxynitrite and hydroxyl radicals) in the blood and tissues resulting in structural alterations in the protein structure[9-13].

Catalase is an important antioxidant enzyme which metabolizes hydrogen peroxide (H_2O_2) produced in the body to non toxic molecular oxygen and water [9-10] The enzyme, thus plays a key role in the acquisition of tolerance to oxidative stress [8]. Significant decrease in catalase activities found in the blood as well as different tissues of OTC treated fish were similar to the findings of the present investigation [10]. These findings are also in tune with declined SOD activities after OTC exposure. The reduced CAT in the present study indicates excess production of H_2O_2 in the blood and tissue through dismuatation of O_2^- resulting depletion antioxidant enzyme during the metabolic process [9-13]. Another mechanism behind this decline may be an alteration of the secondary structure of this enzyme after covalent bond formation with OTC as observed in the *in vitro* trials [21].

Some authors, however, reported a significant elevation in the catalase activities in various tissues as a part defense mechanism to trigger detoxification of OTC when administered by the oral route[11-12]. The contradictory findings in the present investigation might be due to the inability of the body's antioxidant defense system to counteract peroxidative damage produced in response to excessive production of free radicals after OTC exposure in the environment. Another reason may be due to different species used during these experimental trials.

Glutathione-S-transferase (GST) is a dimeric enzyme that plays an important role in the detoxification of endogenous (intracellular metabolites) as well as exogenous substances (drugs, pesticides and other pollutants) thus protecting tissue from xenobiotic induced oxidative stress. The antioxidant, helps in conjugation of the reactive lipophilic molecules to reduced glutathione (GSH) which in turn converts them into a water-soluble harmless conjugate that can be easily excreted from the body [8,10,2]. Significant decrease in the activities of GST observed during the present study after OTC exposure is in agreement with previous findings in the muscles of silver catfish[13]. This decline may be due to utilization of this enzyme to neutralize excessive blood levels of OTC and inability of the body's antioxidant defense system to counter the excessive production of free radicals in the blood [9,10,13]. In some previous studies significant increments in GST activities were found in different tissues and blood after oral OTC treatment [10-13]. The induction of this enzyme in these trials indicates the adaptive response against OTC induced stress in the body of various fish species [8-11].

Furthermore, tetracycline has antioxidant activity which is demonstrated by a number of previous studies [11]. OTC act as chelating agents and can bind to divalent cation such as copper and iron salt. Free radicals are formed after binding of tetracycline Cu II to DNA that lead to oxidative process. Certain biochemical parameters and oxidative stress parameters follow a non-linear pattern which may be due to gene activation p450 which activate the metabolizing enzymes that provide a defensive mechanism against toxicants [23].

Acknowledgments: Authors are thankful to the Dean COVAS for providing laboratory facilities for performing the experiment.

Conflict of interest: Authors don't have any conflict of interest.

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