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CONTENTS

Effect of metronidazole on hematological parameters in Common Carp (*Cyprinus carpio*)

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ABSTRACT: The potential hemotoxic effect of the commonly utilized antibiotic metronidazole (MTZ) was investigated in the common carp (*Cyprinus carpio*). The fish were exposed to sublethal concentrations of MTZ, 5 mg/L and 10 mg/L in water for duration of 96 hours along with one control group. No macroscopic pathology or death of fish was observed at these concentrations. Various hematological parameters were evaluated, including Hemoglobin (Hb), Packed Cell Volume (PCV), Red blood cell (RBC), White blood cell (WBC), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Volume (MCV), and Mean Corpuscular Hemoglobin Concentration (MCHC). Under the tested concentrations, MTZ- exposed fish at 5 mg/L dosage exhibited a gradual increase in Hb and RBC values, while a decrease in values of WBC, PCV, MCV, MCH and MCHC from control to 96 hours was observed. However, the fish exposed at 10mg/L dosage exhibited a gradual increase in RBC, MCHC values, while a variation in values in Hb, WBC, PCV, MCV and MCHC from control to 96 hours was seen. Moreover, hematological parameters have the potential to act as appropriate biomarkers for assessing fish health and can be utilized as bio-indicators in environmental bio-monitoring.

Key words: Metronidazole, Common Carp, Pharmaceuticals, Haematology

Antibiotics are a category of pharmaceutical compounds that are widely used and consumed on a worldwide. The worldwide usage of antibiotics is steadily on the rise, with a substantial 80% growth, mainly due to the escalating demand in low and middle-income countries. Aquatic contaminants originating from pharmaceuticals used in human health, agriculture, and aquaculture have garnered significant attention in recent years (Li *et al.,* 2011; Anoob *et al.,* 2024; Nwokediegwu *et al.,* 2024). Metronidazole (MTZ) is a widely used antibiotic as an antiparasitic and antibacterial compound in veterinary and medical use. Metronidazole (MTZ; 2-(2-methyl-5- nitro-1H-imidazol-1-yl) ethanol) classified under the 5-nitroimidazole class of drugs. It is widely utilized globally to treat various infections in humans and animals caused by protozoa or anaerobic bacteria (Rizzo *et al.,* 2010). Nevertheless, the utilization of this drug in the treatment of food fish has been prohibited in both Europe and the USA. This prohibition is primarily attributed to its potential to cause cancer, its ability to persist in the environment, and its harmful effects on aquatic organisms. Despite this, metronidazole is available for the treatment of ornamental fish through veterinary prescription, and is widely uses in some specific areas of South East Asia. MTZ is highly soluble in water (Wu and Fassihi, 2005), but it also exhibits hydrolytic and photostable properties (Sánchez-Polo *et al.,* 2012), potentially resulting in accumulation in aquatic ecosystems (Richardson and Bowron, 1985; Rediguieri *et al.,* 2011). Being soluble in water and not easily biodegradable, the discharged MTZ is assumed to be fairly persistent in water, where it may be taken up by aquatic animals (Rodriguez *et al.,* 2011). Moreover, this drug has been found to possess carcinogenicity, mutagenic and toxic properties. However, the effective removal of MTZ from waste water from sewage treatment plants still remains problematic. Hence, this drug has the potential to significantly impact non-target aquatic organisms and could lead to the creation of bacterial strains that are resistant to its effects (Johnson, 1993; Kümmerer, 2004). Jousimies-Somer *et al.* (1988) investigated and found that the metronidazole concentration in water before and after treatment at Spanish sewage plants was 164 ng/L and 167 ng/L, respectively. Through the

detection of the effluent from the sewage treatment plant, it was found that the concentration of MTZ had reached a level that could cause harmful effects towards humans and the ecological environment (Marta *et al.,*2015). MTZ metabolites are carcinogenic and mutagenic in some animals because original nitromidonic ring is retained. This may pose the possibility for long-term effects upon these animals. MTZ used at standard dose has been shown to suppress the growth of bone marrow, which is the primary hematopoietic organ and central immune organ (Fararjeh *et al.,* 2008).

The analysis of fish hematological characteristics has become a crucial tool in gaining insights into both normal and pathological processes (Singh and Srivastava, 2010; Li *et al.,* 2011). Hematological analysis is a valuable tool for monitoring the health and condition of wild and cultured fish. Hematological indices vary based on factors such as fish species, age, sexual maturity cycle, and overall health status (Blaxhall, 1972). Furthermore, haematological assessments and serum component analyses have proven to be beneficial in identifying and diagnosing metabolic disruptions and diseases in fish (Aldrin and Messager, 1982; Jamalzadeh *et al.,* 2009). It is known that blood represents 1.3–7% of fish body mass and represents one of the most active components that with hematopoietic organs contribute to constant metabolic processes ensuring gas and nutrient changes between animal body and environment. The value of standard hematological indices plays a crucial role in evaluating the overall physiological condition of fish, with a specific emphasis on their health status (Radu *et al.,* 2009). The Common carp (*Cyprinus carpio*) is categorized within the order Cypriniformes and the family Cyprinidae, which is recognized as the largest family of freshwater fish. It is commonly found in freshwater habitats like ponds, lakes, and rivers, and can also be occasionally found in brackish-water environments (Barus *et al.,* 2001). Common carp is considered to be a very important aquaculture species in many Asian and some European countries. It affects the aerobic decomposition of organic matter and nutrient availability in the water column via bioturbation of benthic sediment during feeding on benthic organisms (Rahman, 2015). According to the FAO (2013), the common carp is the third most widely farmed and commercially valuable freshwater fish species worldwide. In Asia, common carp are normally cultured in various aquaculture systems but the most common is the semi-intensive pond polyculture system (FAO 2012).

Considering the importance of fish as a source of food for humans, as well as their value in reflecting the quality of the aquatic environment (Kollner *et al.,* 2002), it is useful to further assess the effects of MTZ on fish health. The aim of this study was to evaluate the effects of MTZ on the hematological parameters in Common Carp.

MATERIALS AND METHODS

Experimental organisms

The present study was conducted on Common carp (*Cyprinus carpio*) obtained from the Department of Fisheries farm at CSK Himachal Pradesh Agricultural University, Palampur (India). The removal of dermal infection was done with 0.2% $KMnO_4$ for 2 minutes in a stock tank. The fish were transferred to the laboratory and kept in three different aquaria of 200 liters capacity half filled with dechlorinated water for acclimatization. The fish were fed on the farm-made pelleted feed once in a day. The trial was conducted for duration of 96 hrs. Fresh dechlorinated water was regularly introduced on a daily basis to replace the existing water throughout the duration of the trial. The total number of 24 fish were divided into three different groups, *i.e.,* Treatment groups (Group I & II) and control group with 8 fish in each treatment group and in control. The treatment groups were subjected to addition of MTZ at the rate 5mg/L and 10mg/L concentrations in respective treatment group. The average length and weight of the fish was 19±0.5 cm and 103.9±6.5 g respectively.

Drug and dosage

The drug MTZ was purchased from a local pharmacy manufactured by Denis Chem Lab Ltd. The liquid formulation had a concentration of 500 mg/100ml. The sublethal concentrations of MTZ were decided as 5mg/L and 10mg/L of water for the exposure

according to the value given by Cavas and Ergene (2005).

Collection of samples

The blood samples were collected from the caudal vein with heparinized syringe storage vials at 24, 48, 72 and 96 hours intervals form treatment as well as the control groups. The samples were transported in an ice box to the laboratory and were analyzed immediately.

Hematological analysis

The blood samples were collected and analyzed for Haemoglobin, Packed Cell Volume, Red Blood Cell (RBC) White Blood Cell (WBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC). The haemoglobin (Hb) concentration was determined using the Cyan Methemoglobin method (Sahli's Hemoglobinometer method) (Jain, 1986). The red blood cells (RBC) and white blood cells (WBC) counts were determined by the Haemocytometer (Neubauer Chamber) method. The packed cell volume (PCV) was determined by the Micro-haematocrit method. MCV, MCH and MCHC were computed according to formulas'

$$\text{MCV (fl)} = \text{PCV} \times 10/\text{RBC}$$
$$\text{MCH(pg)} = \text{Hb} \times 10/\text{RBC}$$
$$\text{MCHC (g/dL)} = \text{Hb} \times 100/\text{PCV}$$

Statistical Analysis

The results obtained in the study are expressed as mean ± S.E and to study the significance of difference in values of different haematological parameters between treated and control groups a one-way analysis of variance (ANOVA) followed by Post Hoc Tukey's Test using statistical software 'Graph Pad' was applied.

RESULTS AND DISCUSSION

The values of all the hematological parameters are given in Table 1. Significant variations ($p<0.05$) were observed between various blood parameters with different concentrations of MTZ. The data shows that change in hematological indices of fish may be due to MTZ and are determined both by concentration of MTZ and time of exposure. The hemoglobin (g %) of fish treated with MTZ show an increase at both the concentrations however a greater increase in values was seen at 10mg/L concentration.

In comparison to the control group, a decline in values of PCV (%) was observed at 5 mg/L concentration while an increase was observed at 10mg/L concentration. In comparison to the control group, an increase was seen in the values of RBC count at both concentrations. However, a greater increase was observed at 10 mg/L concentration. The WBC count (10^3cells/μL) of fish treated with MTZ results in decline in values at both the concentrations. In comparison to the control group, a major decline in values of MCV was observed at both concentrations. The MCH values of fish treated with MTZ showed a decrease in the value at both the concentration. The MCHC values were observed to increase at both concentrations as compared to the control group. However, the increase was more significant at 10 mg /L.

MTZ is an antibiotic used for parasitic infections in a number of species. Its interaction with fish of enormous value makes this drug particularly important. In the present study, the influences of MTZ on some humoral parameters of common carp were investigated. MTZ is used as an antibacterial and antiparasitic antibiotic, there is little information about its potential influence on the immune system cellularity and function.

This study has revealed that MTZ caused significant increase in the RBC, PCV, Hb values. The probable reason for this can be that MTZ has the potential to stimulate erythropoietin release from the kidneys with a resultant increase in fish RBC production (erythropoiesis) which could ultimately induce polycythemia. Increased oxygen demand at tissue due to stress may lead to increase in RBC and Hb as they are directly involved in oxygen transportation. WBCs play a pivotal role in fish immunology they constitute the cellular component of innate

Table 1: Value of different hematological Parameters in fish after treatment with 5mg/l and 10mg/l concentration of MTZ and control group (Mean±S.E.)

Hematological Parameters	MTZ	Control	24 hours	48 hours	72 hours	96 hours
Hb	5mg/l	6.55±0.03	8.05 ± 0.38*	8.15±0.14*	9.35±0.70***	9.7±0.05***
	10 mg/l	6.35±0.03	9.5± 0.05***	8.4±0.11***	12.75±0.40***	10.50±0.30***
PCV	5mg/l	33.5±0.3	29 ±0.5	25±2.8*	31.5±3.7	28±1.10
	10 mg/l	28.5±0.3	39 ±0.44***	31.5±1.10	40.60±0.40***	30.6±0.30
RBCs Count	5mg/l	1.8±0.03	2.2 ±0.10	4.1±0.20***	4.15±0.03***	6.8±0.10***
	10 mg/l	2.2±0.03	2.45 ±0.03	3.70±0.11***	4.60±0.40***	7.25±0.03***
WBCs count	5mg/l	23.7±0.3	21.5 ±0.30	22.6±1.7	19.60±0.30	17.40±0.50**
	10 mg/l	21.8±0.3	23.80± 0.50	23.2±1.20	18.50±0.30**	17.1±0.20***
MCV	5mg/l	187.5±0.2	133.30 ±9.60	62.70±10.50***	74.70±8.80***	41.10±1.00***
	10 mg/l	126.5±0.3	156.00 ±2.30	85.70±6.5***	89±7.80***	41.3±0.20***
MCH	5mg/l	35.6±0.24	36.60 ±0.50	19.95±0.80***	22.20±1.60***	14.2±0.2***
	10 mg/l	28.1±0.3	38.00 ±0.20*	23.40±0.03***	28.10±1.60	13.70±0.03***
MCHC	5mg/l	18.6±0.4	27.80 ±1.60	34±4.50*	37.40±2.60**	38.70±1.20*
	10 mg/l	22±0.3	24.30 ±0.20	27.00±1.70**	31.90±1.10***	33.80±0.30***

*-(pd≤0.05); **-(pd≤0.01); ***-(pd≤0.001)

immunity. MTZ has been proven to elicit suppression in the primary lymphoid organ bone marrow. The hemotoxic effects obtained in the current study can be explained on the basis that, MTZ when used in the higher dose may lead to the suppression of the bone marrow (El-Nahas and El Ashmawy, 2004). Further Tomaszewski (1997) have observed that change in hematopoietic organ hinder granulopoisis and lymphopoisis. In a study, the lymphocytes proliferation assay revealed that MTZ has been reported to impart inhibitory effects on blood lymphocytes proliferation. (Fararjeh *et al.,* 2008). WBC count is a cellular innate immunity parameter of common carp and is an indicator of cellular defense. The MTZ acts as antibacterial and antiparasitic agent, thus leads to lesser infection load which is probably in agreement with declined WBC count in this study. The decrease in white blood cell (WBC) count observed in the treatment groups could potentially be attributed to the secretion of epinephrine in response to stress. Epinephrine has the ability to induce spleen contraction and subsequently lead to a reduction in the number of leukocytes. This decline in leukocyte count may ultimately compromise the effectiveness of the immune system (Svoboda, 2001; Witesta, 2003). This supports the finding of our study w.r.t WBC count, which showed a decline trend towards 96 hours of drug administration. Similar decrease is also observed by Han *et al.* (2014) in MTZ-treated fish in a concentration-dependent manner compared to the control. Decrease in the value of MCV has been observed which may be due to defective hemoglobin synthesis which results in micro cystic anemia. Increased value of MCHC may be due membrane loss and consequent spherical shape attained by the cell.

These consequences indicate immunosuppressive effects subsequently increase fish susceptibility to bacterial, viral and fungal diseases. Finally, the current investigation demonstrates that MTZ inhibits both humoral and cell-mediated immune responses. The current study's findings suggest that MTZ modifies immune function since it caused alterations in these parameters. Additionally, the study's findings reveal the hemotoxicity of MTZ in fish *C. carpio* when exposed to concentrations of 5mg/L and 10mg/L. Significant dose dependent changes in the values of the assessed haematological parameters are observed. However, the changes are more severe at 10 mg /L concentration.

CONCLUSION

The current investigation results show that MTZ exhibits inhibitory properties on both humoral and cell-mediated immune responses. Findings of the present study would be useful to demonstrate the adverse effects of MTZ use, emphasizing the

importance of the effect on fish which could be very important concerning public health.

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