

**EFFECT OF ANTIBIOTICS EXPOSURE ON GILLS, LIVER, KIDNEY AND
BRAIN OF COMMON CARP (*CYPRINUS CARPIO*)**



SUPERIOR UNIVERSITY

Thesis Submitted to

The Superior University Lahore

In Partial Fulfillment of the

Requirement for the Degree of

MS Zoology

By

MUHAMMAD WAQAR

SU92-MSZGW-F22-012

Session: 2022-2024

Faculty of Sciences

2022-2024

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FOS

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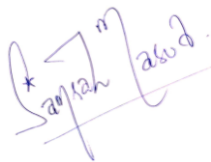
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DEDICATION

Dedicated to my Parents

My loving Father, **Altaf Hussain** whose inspiration towards knowledge served me as a beacon of light, he made me what I am today. My loving Mother Who always inspired and encouraged me, who always wish for my success and prosperity.

ACKNOWLEDGEMENT

In the name of **ALLAH**, the most Gracious, the most Merciful. I am extremely thankful to Almighty ‘ALLAH’ Who is the entire source of knowledge and wisdom endowed to mankind.

I would like to express my profound gratitude to **Holy Prophet MUHAMMAD (Peace Be Upon Him)** who has guided his "Ummah" to seek knowledge from cradle of grave...!!!

I am sincerely grateful to the Dean **Prof. Dr. Muhammad Naveed Babur** for their overall support and encouragement throughout my research journey.

I would like to express my heartfelt thanks to the Head of Department **Prof. Dr. Uqba** for their encouragement and insightful feedback.

I would like to express my profound gratitude to my Supervisor **Dr. Usman Elahi** for his wise counseling and encouraging attitude towards this study. This project would not have been possible without his support and expert guidance.

I would like to express my profound gratitude to my Co-supervisor **Dr. Samrah Masud** for her invaluable guidance and support towards this study. This project would not have reached completion without her dedicated efforts.

I would like to thank the Program Leader **Dr. Noor Ul Ain** for their continuous support and helpful coordination during my research work.

In the end, I would like to extend my deepest gratitude to my family members. Without their encouragement, I would not have been able to complete this endeavor.

MUHAMMAD WAQAR

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LIST OF ABBREVIATIONS

AChE	Acetyl cholinesterase
AMX	Amoxicillin
ANOVA	Analysis of variance
DFZ	Difenoconazole
DNFB	2,4-dinitro-1-fluorobenzene
GPx	Glutathione peroxidase
GST	Glutathione S-transferase
MAO	Monoamine oxidase
MNZ	Metronidazole
MPs	Micro plastics
NPs	Nano plastics
OTC	Oxytetracycline
ROS	Reactive oxygen species
SD	Standard deviation
SOD	Superoxide dismutase
SS	Sum of square

ABSTRACT

Common carp locally known as Gulfam fish (*Cyprinus carpio*) is a widely cultured fish species that is often exposed to antibiotics used by humans for bacterial infections e.g. Amoxicillin (AMX) and Metronidazole (MNZ). It is reported that AMX and MNZ are severely maims the fish organs. A study was designed to investigate the histopathological effects of AMX and MNZ exposure on the internal anatomy (brain, gills, liver, and kidneys) of common carp and to compare the severity of damage caused by these antibiotics. Common carp samples of similar size and weight were randomly divided into three groups: control, amoxicillin-treated, and metronidazole-treated. Fish were exposed to AMX and MNZ at a concentration of 20mg/l in their water for the specified duration. After exposure dissected organs were collected to take tissue samples. Hematoxylin and Eosin (H&E) staining was done to reveal the histopathological changes. Result indicated significant ($P \leq 0.05$) damage to gill, liver, kidney, and brain tissues of common carp, when exposed to either of antibiotics for 7 and 14 days. The exposure of MNZ for 14-days caused significant ($P \leq 0.05$) histopathological damage in all organs compared to AMX (7 and 14 days). The exposure of common carp to AMX and MNZ revealed significant effects on alteration of tissue structure, epithelial hyperplasia, and lamellar fusion vacuolization, presence of inflammatory and degenerative cells. Compare to the AMX, MNZ demonstrated greater harmful impacts across various organ systems and life forms. Our study confirms that AMX and MNZ have detrimental effects on the health of common carp which emphasizes their responsible use in aquaculture.

Keywords: *Cyprinus carpio*, gills, histopathology, amoxicillin, metronidazole

CHAPTER 1

INTRODUCTION

The term antibiotic is comes from the word "antibiosis," which means literally to "against life." Originally it was believed that Antibiotics were chemical substances made by one microbe that were harmful to other microbes [1]. This idea led to the initial broad definition of an antibiotic being a chemical produced by a single bacterium [2] or of biological origin that, at low doses, can either kill or restrict the growth of other germs [3]. In the modern era, this term has been modified to include antimicrobials that are also made entirely or in part artificially. While some medicines can kill other germs entirely, others can just stop them from growing. Although the term "antibiotic" generally refers to antibacterial, antibiotic compounds are differentiated as antibacterial, and antivirals to reflect the group of microorganisms they antagonize [4]. Bacteriostatic are those that inhibit bacterial growth, while bactericidal agents kill bacteria [5].

In aquaculture, Antibiotics are used to avoid or treat bacterial infections and can be used to improve the growth and productivity of stocks. These antibiotics can leach and skip into different water resources after being used by humans and can be found in different environmental compartments, including surface water, groundwater, and sediment. The presence of antibiotics in the environment is due to their extensive use in human and veterinary medicine, as well as in agriculture. It is mostly used in the pharmaceutical industrial facilities, hospitals and household which participate to the release of antibiotics into the marine system. A small amount of antibiotics may be released into the rivers and other water bodies. It can also be used in aquaculture to avoid and treat bacterial infections in the fish. Waste material can be discharged into the water, which affects the both aquatic and other environments. It may participate to the improvement of antibiotic-resistance bacteria. These compounds may have serious impact on the non -target organisms in aquatic environment [6].

It can be used for their antimicrobial effects by targeting specific cellular processes in bacteria. It mostly targets the pathogenic bacteria. At low concentration, it may effect on non -target organisms such as algae, invertebrates and fish in aquatic environment.

These effects include the changes in behavior, growth, reproduction and overall ecological balance. Extended exposure to low concentrations of antibiotics in aquatic environments provides selective pressure for the development and proliferation of antibiotics-resistance bacteria. Antibiotics play an important role in aquatic systems. Some antibiotics are more resistance and persistence in degradation processes, which may lead to the longer presence in the ecosystems. The effectiveness of antibiotics can be influenced by the factors such as solubility and adsorption to the sediments [7].

In human and veterinary medicine, AMX is a common β -lactam antibiotic used to treat and prevent bacterial infections, which include skin, urinary tract, and respiratory infections. Antibiotics like AMX are utilized as growth promoters in food animals by extension of their application in the veterinary field. Between 2000 and 2010, the two most often used antimicrobial drugs in 71 countries were ampicillin and amoxicillin [8]. Between 2007 and 2011, amoxicillin was the most prescribed antibiotic in Brazil and one of the two most regularly prescribed antibiotics in China, with over 6 billion units sold. Post administration, AMX is eliminated unaltered from the body and ends up in sewage. Its existence in the environment is a result of wastewater treatment's incomplete removal of this released and unaltered AMX [9]. Because of this, if this antibiotic is used improperly, it may end up in aquatic bodies and residues in animal tissues that are not needed. Due to the introduction of new strains of bacteria that are resistant to antibiotics, these residues create a risk to human health by resulting in allergic reactions in certain people and, more significantly, decreasing the effectiveness of antibiotics in treating illness [10].

The primary ingredient of nitroimidazole antibiotics, metronidazole (MTZ; 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol), is used extensively across the world to treat a variety of human and animal infections caused by protozoa or anaerobic bacteria [11]. There are more and more signs that MNZ produces a wide range of toxicity [12]. MNZ is highly genotoxic; it is activated intracellular by reduction, and the resulting intermediates bind DNA, which is crucial for strand smashing, helical structural damage, and DNA function damage [13]. It is a potential carcinogen, and rats and mice develop many tumors when high doses of byproducts of around MNZ are administered over an

extended period of time [14]. Furthermore, MNZ significantly inhibits the rise of anti-TAB (anti-Salmonella tophi, *S. paratyphoid* A, and *S. paratyphoid* B) antibody levels to TAB vaccine in rabbits and stops mice from developing ear enlargement caused by 2,4-dinitro-1-fluorobenzene (DNFB) following sensitization [15]. Additionally, MNZ disrupts male fertility and induces liver toxicity in experimental animals [16] [17].

The main family of freshwater is Common carp (*Cyprinus carpio*) which goes to the order Cypriniformes and the family Cyprinidae. In freshwater environments, particularly ponds, lakes and rivers, the common carp lives. It also infrequently occupies brackish-water environments [18]. It is extensively disseminated in nearly all countries of the world but is very common in Asia and approximately European countries [19]. Because of its high status, its spreading has been broadly lengthy by human introduction. The third utmost commonly announced species worldwide is common carp. In Asia and some European countries, common carp is measured as a latent candidate for commercial aquaculture and it has a very high adaptive aptitude to both environment and food [20]. From common carp, more than 80% of total fish creation originates, in some European countries [21]. By the fish species, the nutrient turnover of the aquatic environment is powerfully disposed; particularly planktivorous and benthivores fish inhabitants. In both organic and inorganic methods, the common of nutrients in ponds are kept. Sediment can be accumulated more than 100 times extra nutrients than the water column [22].

Amoxicillin, as an antibiotic, can disrupt the microbial balance within the aquatic environment. This disruption can affect the delicate balance of microorganisms in the gills of common carp, potentially leading to dysbiosis and increased susceptibility to secondary infections. The gills are an important site for immune defense in fish. Exposure to amoxicillin can modulate immune function in the gills, leading to altered cytokine production, impaired leukocyte recruitment, and compromised immune surveillance against pathogens. Long-time structural and functional changes can induced from amoxicillin exposure during important developmental stages, which may also intact with gills development and cellular differentiation. The fishes have ability to adjust to changes in its surroundings may be compromised, and respiratory efficiency may be affected [23]. Antibiotic, metronidazole can affect common carp (*Cyprinus*

carpio) gills through different ways particularly when given in large quantities or over long period of time. The gills are very important for breathing, excretion and osmoregulation making them highly susceptible to environmental stresses, such as antibiotics [24].

Exposure of fish to metronidazole induces histopathological alterations in the gill tissues. These changes may be lamellar hypertrophy, epithelial hyperplasia, and fusion of gill lamellae, which can reduce the fish's ability to normally perform gas exchange. This may results in respiratory disorder and lesser oxygen uptake. Antibiotics may cause an inflammatory response in the gill tissues, as a result of immune system activation. This inflammation may result in thickening of the gill epithelium and minimizing functionality. Continuous inflammation could damage the gill filaments and lamellae, which may affect the carp's respiratory performance and overall health of fish [25].

By changing metabolic pathways involved in drug metabolism and detoxification, antibiotics like amoxicillin and metronidazole may disrupts liver function. Longtime exposure to antibiotics can change hepatic enzyme activity, impairing the liver's ability to metabolize and remove toxins from the body. Exposure to antibiotics can induced histopathological changes in the liver of common carp. These changes may be hepatocyte degeneration, vacuolation, inflammation, fibrosis and necrosis. Such changes can disrupt liver function and overall health. Some antibiotics may induce oxidative stress in the liver by promoting the generation of reactive oxygen species (ROS) or impairing antioxidant defense mechanisms. Hepatocytes can sustain damage from longtime exposure to oxidative stress, which can cause to liver disease. Common carp gut microbiota can be disturbed by antibiotics, which may result in dysbiosis and altered signaling of the gut-liver axis [26]. By changing the makeup and function of the gut microbiota, many signaling pathways can affect liver function and metabolism. The fish's immunological response is significantly controlled by its liver. Exposure to antibiotics may affect hepatic immune function, resulting in a change in cytokine production, a reduced in leukocyte recruitment, and a weak immunological response to infections. Antibiotic exposure during important stages of liver development can interfere with hepatic organogenesis and cellular differentiation, resulting in the liver

structural and functional changes that last a lifetime. Growth, energy homeostasis, and nutrition metabolism in juvenile and adult fish may be impacted by antibiotics exposure. Antibiotic resistant bacteria may grow in common carp liver and gut as a result of ongoing antibiotic exposure. The liver tissue may become affected by these resistant bacteria, which could result in chronic infections that are challenging to cure with traditional antibiotics [26].

Exposure to antibiotics amoxicillin and metronidazole can induced many effects on the kidney of common carp, which play an important role in maintaining osmotic balance, regulating ion concentrations, and removal of metabolic wastes. Some antibiotics can cause direct nephrotoxic effects on the kidneys of common carp. This may be structural damage to renal tubules, glomeruli, and other renal structures, impairing kidney function and compromising overall health [27]. Common carp kidney shows histopathological alterations when it is exposed with antibiotics. Tubular degeneration, interstitial inflammation, glomerular enlargement, and fibrosis are some examples of these alterations. These changes may affect the fish capacity to maintain homeostasis and damage kidney function [28]. By changing glomerular filtration rate (GFR) and tubular secretion, antibiotics exposure may affect common carp's renal filtration and excretory processes. This can lead to kidney damage and dysfunction by causing toxins, metabolic wastes, and antibiotics themselves to direct in the bloodstream. Fishes immunological response is controlled by the kidneys. When kidney exposed to antibiotics it may alter immune function, resulting in a change in cytokine production, a reduction in leukocyte recruitment, and a weak immune response to all infections [29]. Exposure to antibiotics during critical periods of kidney development can affect nephrogenesis and cellular differentiation, leading to prolonged structural and functional changes in the kidneys. This can disrupts renal function, urine production, and osmoregulation in juvenile and adult fish [30]. Antibiotics may also disturb electrolyte balance in the kidneys by disrupting ion transport mechanisms and renal tubular function. Different changes in electrolyte concentrations, such as sodium, potassium, and calcium, can results in metabolic disturbances and osmoregulatory dysfunction in common carp. Some antibiotics can cause oxidative stress in the kidneys by promoting the generation of reactive oxygen species (ROS) or impairing antioxidant defense mechanisms. Chronic

exposure to oxidative stress can result in renal cells and contribute to kidney dysfunction [31].

In brain organ of fish, amoxicillin may alter the neural tissues, which may results in the neuronal degeneration. It can also change the neurotransmitter levels. It may also associate with the behavioral changes in fish. In liver organ of fish, amoxicillin may change the liver tissue. Like other antibiotics amoxicillin may be able to cross the blood-brain barrier which results in affecting the neuronal function in the brain of common carp. This may cause neurotoxic effects which may be neuronal damage, changed neurotransmission, and impaired synaptic plasticity [32]. An important channel of communication between the central nervous system and the gastrointestinal tract is the gut-brain axis. Exposure to amoxicillin may alter the gut microbiota of common carp, altered gut-brain communication and perhaps results in affecting behavior and neurological function. Common carp brain function and behavior may be controlled by oxidative stress, which can cause neuronal death, inflammation, and decreased neurogenesis [33]. Antibiotics can directly influence neurotransmitter systems in the brain of common carp. Changes in neurotransmitter levels or receptor sensitivity could results in various physiological processes, including cognition, motor function, and stress responses. When it is exposed to amoxicillin during critical periods of brain development in common carp could lead to long-term structural and functional changes. Later in the life this could result in behavioral abnormalities, cognitive impairments, or modifications in brain activity. In common carp, amoxicillin exposure can induce systemic effects like immune system changes, inflammation, or metabolic disturbance that may have an indirect effect on brain function [34].

Exposure metronidazole may modify the gut microbiota in common carp, leading to changes in gut-brain signaling and potentially affecting neurological function and behavior. Antibiotics like metronidazole may involve into the generation of reactive oxygen species (ROS), which results in causing oxidative stress in the brain [35]. The accumulation of ROS can affect brain cells, change in neuronal function, and impact cognitive processes. Antibiotics can also affect neurotransmitter systems. In common carp, metronidazole exposure may disrupt the balance of neurotransmitters such as

serotonin, dopamine, and acetylcholine, potentially affecting the fish's behavior and cognitive functions. Prolonged exposure to metronidazole might cause neuroinflammation. Antibiotics have been shown to induce cellular stress responses, which could trigger an inflammatory reaction in the brain tissues, possibly leading to cytotoxicity and damage to neurons and glial cells [36].

Antibiotics can enter the environment through a variety of sources, including wastewater treatment plants, manufacturing facilities, hospitals, and residential municipal effluents; the disposal of unused or expired compounds down the drain; runoff from fields where antibiotic-treated animals or their manure have been applied; the direct dosage of antibiotics in aquaculture water, and more. Antibiotics come from a wide range of sources and are spread to humans, animals, and the environment through a cycle of delayed biotransformation, bioaccumulation, and slow deposition in soil and ground water [37]. Since most chemical pollutants moved into the sea, rivers, lakes, and wetlands and aquatic pollution is the most common type of contamination. In surface and ground water, wastewater treatment plant effluent, and municipal sewage, antibiotics have been found in recent years at concentrations in the $\mu\text{g/L}$ range [38][39]. These chemicals are a possible environmental hazard, which is why their existence in freshwater systems has drawn more attention. Because antibiotics are made to have a biochemical effect on microorganisms and on the emergence and spread of antibiotic resistance genes brought on by repeated exposure to bacterial agents, they are dangerous to ecosystems even at low concentrations and persistence rates, raising the risks to human health [40]. Aquatic ecosystems are constantly exposed to antibiotics since they are not entirely eliminated following wastewater treatment. Although fish are less frequently reported to be affected than microorganisms, low dosages of antibiotics can induce non-target toxicity to aquatic creatures. As a result, antibiotics have become new environmental contaminants that need to be closely examined by the environmental science community [41].

Despite the current environmental issues, the topic of ecotoxicology and water contamination is still important and relevant. Water contamination becomes worse by a variety of harmful pollutants, including pesticides, chemical compounds, heavy metals,

and other anthropogenic residues, as anthropogenic activity on Earth rise. Fish which are sensitive indicators of the condition of aquatic environments, and aquatic ecosystems may suffer because of these pollutants. The main difficulties in this discipline include determining the effects of different water pollutants on the health of aquatic life, such as fish, in addition to recognizing them [42]. In literature the term "ecotoxicology" has been used Since 1970s to describe the scientific study how chemical and physical agents effect on living organisms, especially on populations and communities in specific ecosystems, including the pathways of these agents' transport and their interaction with the environment. Ecotoxicological research helps us to understand the mechanisms of water resource management strategies and helps us in the understanding of the mechanisms underlying the effects of contaminants on biological systems [43]. There are many definitions of ecotoxicology, but they all agree that it is a scientific field that examines how harmful substances interact with the abiotic elements of ecosystems and the people who live there. Both the individual and the biological community levels are examined in relation to these relationships. Aquaculture is greatly impacted by ecotoxicology since it influences the survival, growth, and health of aquatic organisms by exposing them to dangerous pollutants. Pollutants such as heavy metals, pesticides, and pharmaceuticals accumulate in aquatic habitats, potentially leading to bioaccumulation and biomagnification in farmed animals. These pollutants can affect fish and shellfish physiological processes, reproductive outcomes, and disease susceptibility, which can ultimately affect aquaculture systems' productivity and sustainability. Additionally, long-term exposure of aquaculture species to sub-lethal concentrations of contaminants can affect consumer safety because seafood products may contain residual toxins [44].

AIMS AND OBJECTIVE

- To assess the overall health status of common carp in exposure to AMX and MNZ
- To investigate the histopathological effects of AMX and MNZ on the organs of common carp
- To compare the impacts of AMX and MNZ on the tissue integrity and physiological health of common carp

CHAPTER 2

LITERATURE REVIEW

It was reported that Amoxicillin (AMX) is an antibiotic which is mostly used in human and veterinary medicine all over the world. These antibiotics are produced in a large number and have been used in pharmaceutical which is released in the environment. Its concentration ranges from ng L^{-1} to $\mu\text{g L}^{-1}$. In recent research, it has been shown that this antibiotic produces lethal effects, which mostly change the embryonic development of the fish. However, it is important to illustrate the danger that this antibiotic shows the economic concentration such as *Cyprinus carpio*, in a specific method. The main objective of this study was to shown if amoxicillin, at environmentally-relevant concentrations, is able to produce the genotoxic and cytotoxic changes in *C. carpio*. In order to determine genotoxicity, the comet assay and micronucleus test were carried out; in addition to elevate cytotoxic effects, TUNEL assay and caspase-3 activity were used. The results presented that in micronucleus frequency raised by 204% in C1 and by 310% in C2 when associated to control; compared to control, caspase-3 activity raised 261% in C1 and 786% in C2; for the TUNEL assay, pieces of DNA raised by 85% in C1 and 121% in C2 associated to control and in the comet assay, DNA damage occur due to effect of biomarkers at 72h, $0.038 \mu\text{g L}^{-1}$ resulted in a 28% rise as associated to control, and $1.66 \mu\text{g L}^{-1}$ produced a 42% increase. Damage of the DNA and cytotoxic effects in the blood cells were produced by the amoxicillin which is due to environmentally-relevant concentrations [45].

Most frequently used antibiotic is AMX all over the world due to its broad-spectrum action as compared to the different bacterial strains. It is mostly used in the growth promoter in animal husbandry. While in different countries, the remnant of the antibacterial agent has been present in the aquaculture, however this is not too much evidence on tis probable poisonous to water bodies. The main objective of this research is to determine amoxicillin produce oxidative stress in liver, kidney, gills and brain in the common carp. Three various concentrations of amoxicillin (9 ng/L , 11 $\mu\text{g/L}$, 10 mg/L) for 14, 26, 52, 70, and 92 h, were given to the carp. Different biomarkers were determined such as protein carbonyl content lipid, peroxidation, and hydroperoxide

content. By the action of the antioxidant enzymes such as glutathione peroxidase, superoxide dismutase and catalase were also noted. Amoxicillin and its main product amoxicilloic acid were observed by the high performance of liquid chromatography attached with electrochemical detection and UV detection. In all study organs, especially kidney, the most important alteration occurred due to the antioxidant enzyme action, which was due to the important increase in LPX, HPC, and PCC ($P < 0.05$). The highest alteration occurred in the kidney organ of the fish due to oxidative stress which is mainly induced by the Amoxicilloic acid in water. The biomarkers which used for the valuation of the factors effect of this agent on aquatic species were effective [46].

Metronidazole is most common antibiotic for its lethal effects on the liver of the common carp. Three different doses of AMX (0.2, 0.6, and 2.6 mg/L AMX) in aquatic for 30 days were given to the fish. It was observed that these doses showed the liver mutilation and oxidative stress in the organ of fish. AMX amplified liver ethoxyresorufin-O-deethylase, malondialdehyde, and glutathione levels, and determined the action of superoxide dismutase, catalase, aspartate aminotransferase, and alanine aminotransferase. These doses commonly evaluated that the main differences in the 0.6 and 2.6 mg/L AMX groups associated to the control group ($P \leq 0.05$). These results showed that AMX produce oxidative stress in the fish organ. This antibiotic causes the main alteration in the liver of common carp. This result showed that all these parameters should be taken to prevent from pollutant of surface water with AMX [47].

It was reported that the first organ of a fish to encounter environmental watery stimuli is its gills. It is therefore the first location where harmful compounds are absorbed and bio transformed, making it susceptible to a variety of traumatic, toxicological, and viral assaults. The majority of teleost have gills that are normally made up of four pairs of gill arches that are supported by a bone skeleton. From these gill arches, filaments that are supported by cartilage (primary lamellae) originate; these filaments serve as the secondary lamellae's anchoring point. As a result, their sensitive structure reacts rapidly to changes in the environment by exhibiting structural changes that are not specific to any one pollutant but rather depend on the severity and length of exposure. Any degradation in their structure and function may result in physiological and metabolic

discomfort, which could have a negative impact on the fish's survival and biology. This is why histological changes in the gills were employed in this study as crucial biomarkers to evaluate the toxicity of antibiotics since they are directly linked to the negative effects of pollutants [48].

The histological alterations seen in the gills can be divided into two primary categories: those linked to adaptive changes (such as mucous cell proliferation, lamellar lifting, epithelial hyperplasia, and lamellar fusion) and those reflecting the direct effects of the toxicant (such as aneurysms, oedema, and filament epithelium thinning). The surface area of the gill is decreased, the diffusion distance is increased, and the interlamellar distance is decreased by this latter group. However, some morphological changes—like necrosis and epithelial degradation, which can cause inflammatory reactions—are severe and irreversible. The presence of leucocytes, or inflammatory cells, however, may indicate a mild to moderate inflammatory response brought on by necrotic regions [49].

It was reported that the liver of fish has a role in xenobiotic metabolism, enzyme synthesis, and nutrition processing and storage. Because of its extensive blood supply, which results in obvious exposure to toxins, the liver is also a target organ. Additionally, oxygen shortage linked to gill degeneration may put additional strain on the induction of alterations in the fish liver. As a result, the liver is typically the organ most damaged, and a variety of environmental stresses, including medications, can cause histological changes. As seen in several salmonids, *S. aurata* exhibits a hepatopancreas, in which the exocrine portion of the pancreas encircles the liver's portal canal. We saw hepatocyte cytoplasmic vacuolization in histologic sections, which may indicate the storage of lipids and glycogen. Tetracycline exposure has already been shown to cause vacuolization in fish. Lipid typically appears as one or more clear circular vacuoles with crisp edges, while glycogen is thought to be associated with vacuoles that have loose borders and include slightly flocculent material. Increased lipid-like vacuolization accompanied by considerable nuclear displacement in gilthead seabream livers was the most regressive modification in this study across all assays, and it was much higher in those who had been exposed to OTC acutely [50].

A stress trial was carried out with common carp, which is the most common species in aquatic environments all over the world. This trial was used to detect the gene regulation pathways in various part of the brain. The most dangerous stress is due to contact with the action of expression of the direct gene *c-fos* in the telencephalon. In the previous study, the bonding between regulations of the two corticotropin-releasing factor genes with their binding protein. Due to direct contact with the air were determined by the main observation, which allows the estimate of a single value. The stress trial observed the main unknown impact of stress in various areas in carp. All these impacts were completely described by this stress trial. However, all these stress trial were done to identify the possible regulation gene in the various parts of the fish brain [51].

Investigation of neurotoxic effects of polyethylene (PE) plastics on the brain using a Common carp as a organism. After exposing carp brain to 100 mg/L of either microplastics (MaP), microplastics (MPs), or nano plastics (NPs) for 15 days, we assessed the activity of acetylcholinesterase (AChE), monoamine oxidase (MAO), and the amount of nitric oxide (NO) in comparison to an unexposed group. Each biochemical indicator saw a 30–40% decrease after exposure; the larger the drop, the smaller the particle size (NPs > MPs > MaPs). Plastic particles exposed the carp tectum for 15 days, causing necrosis in big cerebellar neurons and ganglion cells, fibrosis, alterations in blood capillaries, tissue separation, edema, and deteriorated connective tissues in variable degrees. Necrosis, degeneration, vacuolation, and curvature in the inner layer were seen in the carp retina. Here, we present evidence linking neurotoxicity in common carp to exposure to plastic particles [52].

One of the most frequently given antibiotics in the whole world, amoxicillin (AMX), has been identified in an assortment of environmental water bodies, which has caused concerns about potentially toxicological effects on aquatic nontarget species. The comet test was used in this investigation to determine any possible genotoxic effects of AMX in the peripheral blood of common carp (*Cyprinus carpio*). Different concentrations (10 ng/L, 10 µg/L, and 10 mg/L) were introduced to teleost fish for varying exposure durations (12, 24, 48, 72, and 96 hours). A UV and an electrochemical detector were used in conjunction with high-performance liquid chromatography (HPLC-EC) techniques to determine the AMX and its primary breakdown product, amoxicilloic acid

(AMA). At 24 and 48 hours, the results demonstrate a highly significant time concentration-dependent increase in DNA damage. Amoxicilloic acid has been found and even evaluated in water and blood from the experiment's start, but no amoxicillin was identified in any system analyzed at any point in time or concentration. During hypersensitivity actions, amoxicilloic acid can increase the quantity of reactive oxygen species, which is responsible for DNA damage in *Cyprinus carpio* blood cells [53].

Antibiotics are frequently prescribed to treat veterinary and human illnesses, and they are also utilized extensively in agriculture for promoting the growth of cattle. Aquatic organisms, however, are poisonous to many antibiotics that survive in aquatic habitats. Because wastewater treatment plants can not eliminate antibiotics completely, they end up releasing them into aquatic ecosystems. Because of their non-target effects, this raises concerns about the potential catastrophe of the ecosystem. Since antibiotics are intended to remain in the body and function gradually, research has been done on the long-term toxicity of these drugs in aquatic bacteria. Nonetheless, there is lack of information regarding the toxicity of antibiotics to fish at the top of the aquatic food chain. This document provides an overview of what is known at present about the reported toxicity studies. It has been reported that four antibiotic classes—quinolones, sulfonamides, tetracyclines, and macrolides—which are regarded to be genetically hazardous to fish, bioaccumulation in fish tissues and in aquatic settings like rivers and surface water. It is widely recognized that the negative effects of these antibiotics harm fish's immune system, antioxidant defenses, and developmental, cardiovascular, and metabolic systems. As a result, there are valid worries regarding the toxicity of antibiotics to fish, and more studies and prevention measures are required in various parts of the globe [54].

It was reported that now a days Antibiotics are used in aquaculture to treat and prevent from diseases. Currently, there is clear evidence in the literature regarding the advantages and disadvantages of different antibiotics in cultured fish as well as the possible health risks to humans from eating the fish. On the other hand, by eliminating or preventing the growth of harmful bacteria, antibiotics is given in aquaculture contribute to the improvement of fish health. However, they have a number of negative

effects. According to the review, 44 distinct antibiotics—of which oxytetracycline dominated—were investigated in fish species across the globe. Fish growth and survival rates are either decreased or increased by the use of antibiotics in aquaculture worldwide. They generate oxidative stress, which alters detoxification and antioxidant responses and leads to dysbiosis of the host microbiota. Fish immunity is weakened by these alterations, which eventually cause cellular harm. By starting gluconeogenesis and anaerobic glycolysis, the toxins enhance energy demand while suppressing lipogenesis, fatty acid β -oxidation, and fish aerobic glycolysis. Antibiotic-treated fish poses a risk to human health when consumed. Future study is needed to determine what agents can be used to repair the dysfunctions caused by antibiotics in cultured fish, and further research and prevention efforts are needed in many parts of the world to minimize the use of antibiotics in aquaculture production [55].

Antibiotics are substances, either synthetic or natural, that have the power to either eradicate or stop the growth of microorganisms. Chemotherapeutic agents are antibiotics that are sufficiently non-toxic to the host to treat infectious diseases in people, animals, and plants. They have been a part of the ecosystem for a very long time and have been very important in the conflict between microbes and humans. Since many bacterial species proliferate so quickly—they can double in number every 20 to 30 minutes—their capacity to adapt to environmental changes and endure unfavorable circumstances frequently leads to the emergence of mutations that allow the species to endure shifting external environments. The fact that individual cells don't rely only on themselves is another aspect of their flexibility. A vast array of mobile genetic components, such as plasmids and transposable elements, allow bacteria to travel from one cell to another and proliferate throughout bacterial populations. Many, if not all, of these elements are available to them. Resistance is the ability of bacteria to adapt to changes in their environment and therefore survive. Due to the evolution of drug resistance in bacteria and fungi, there are fewer, more expensive, and often non-existent pharmacological options for treating common infectious diseases. This resistance poses a threat to undo a lot of the past 50 years' worth of medical progress. It is possible for resistant microorganisms to spread throughout communities and hospitals. It is acknowledged that a primary means of transmission for small organism. Antibiotics have mostly been

employed in aquaculture as preventative and therapeutic measures. Using a risk analysis methodology, the impact of antibiotics used in aquaculture to antimicrobial resistance is reviewed here. To lessen the risk of antibiotic resistance building up, some guidelines for appropriate behavior in this situation are put forward [56].

It was reported that the harmful effects of the commonly used antibiotic metronidazole (MTZ) on the liver of common carp (*Cyprinus carpio* L.) were studied. After the fish were submerged in water containing 0.1, 0.5, and 2.5 mg/L MTZ for 30 days, indicators of oxidative stress and liver damage were examined. MTZ boosted the activities of superoxide dismutase, catalase, aspartate aminotransferase, and alanine aminotransferase as well as liver ethoxyresorufin-O-deethylase, glutathione, and malondialdehyde. When compared to the control group, these metrics often revealed significant differences ($p < 0.05$) between the 0.5 and 2.5 mg/L MTZ groups. These results suggested that action should be done to prevent MTZ contamination of surface waters since it produced oxidative stress and liver damage in common carp [57].

One triazole fungicide that is frequently used to combat fungi that cause water pollution in crops is called difenoconazole (DFZ). The effect of silybin (SYB), a flavonolignan obtained from the seeds of the silymarin plant and used to treat liver diseases on DFZ-induced kidney damage in carp was examined using the H&E assay and ROS levels were measured. In these results it showed that SYB decreased the elevation of kidney ROS levels in carp as well as the DFZ-induced degradation of kidney tissue structure in carp, and that SYB+DFZ co-treatment increased mRNA levels of interleukin. (L) SYB+DFZ co-treatment increased mRNA levels of interleukin-1 β (il-1 β) and decreased mRNA levels of inflammation-related markers such as interleukin-6 (il-6), interleukin-10 (il-10), and tumour necrosis factor α (tnf- α). It also reduced nuclear factor- κ B (NF- κ B) protein expression and inducible nitric oxide synthase (iNOS) levels. The nuclear factors E2-related factor 2 (Nrf2) and NAD(P)H:quinone oxidoreductase 1 (NQO1) protein expression levels were also decreased by the SYB+DFZ group. Additionally, the oxidative stress-related mRNA markers catalase (cat) and superoxide dismutase (sod) were increased by DFZ. SYB+DFZ counteracted the effects of DFZ on the elevation of B cell lymphoma-2 (bcl-2) mRNA and protein expression levels and Bcl-2-associated X protein (bax) mRNA and protein expression levels. In conclusion, SYB may lessen the

oxidative stress, inflammatory response, and apoptotic kidney damage that DFZ pollution causes in carp aquatic habitats. It may also provide a theoretical basis for the foundation for the therapeutic development of freshwater carp diets [58].

It was stated that common carp (*Cyprinus carpio*) were utilized to see quercetin's protective effects on growth performance, immunity, and biochemical markers. Fish exposed with quercetin + malathion for 21 days, fish provided with 200 mg/kg diet, fish exposed to doses of 1.04 and 2.08 mg/l malathion, and fish treated with the control group were the six experimental groups chosen for the experiment. Following the feeding phase, the hepatocyte's activities of glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), and glutathione S-transferase (GST) were dramatically reduced, whereas malondialdehyde (MDA) concentration rose in response to malathion. Upon exposure to malathion, there was a considerable increase in the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as in the levels of glucose, cortisol, and urea. Other characteristics changed in different ways depending on the malathion concentration. When fish were given quercetin supplements, the malathion-induced alterations in mucosal lysozyme and protease activity did not improve. However, quercetin reduced the suppressive effects of malathion on biochemical and immunological markers. Growth performance and hematological parameter variations indicated malathion's negative effects. Lastly, quercetin may successfully mitigate the detrimental effects of malathion on the immunological, hematological, and biochemical traits of common carp [59].

It was observed in pervious study that Metronidazole's acute toxicity was examined in both freshwater and marine creatures. The experiments revealed effects on *Selenastrum capricornutum* and *Chlorella* sp. Among the results were 72-hour EC₅₀ values of 12.5 mg/l and 40.4 mg/l, and 72-hour EC₁₀ values of 2.03 mg/l and 19.9 mg/l, respectively. Neither *Brachydanio rerio* nor *Acartia tonsa* showed any signs of acute mortality. The study indicates that metronidazole may have an ecotoxic effect and calls for more research on the effects of pharmaceuticals on the environment [60].

It was reported that the effects of diesel oil on fish defensive mechanisms are the main topic of this essay. In biological systems, reactions that produce free radicals—

particularly oxygen free radicals—are intricate and frequently entail chain reactions that produce a variety of radical types. Aerobic organisms have several defense mechanisms in place to prevent the unintentional generation of oxygen radicals, which can occur from autooxidation processes or uncoupling at different electron transfer sites. The antioxidant defense system is vital to the maintenance of cell homeostasis since it is the major system in the body of aerobic organisms that removes reactive oxygen [61].

It was observed that the effects of the antiepileptic medication carbamazepine (CBZ) on lactate dehydrogenase (LDH) activities in the liver, muscle, and gills of the freshwater fish *Cyprinus carpio*, as well as its toxicity on transaminases such as glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) were studied. The median lethal dose (LC50) of CBZ to *C. carpio* was determined to be 59.70 mg l⁻¹ over a 24-hour period. A concentration of 5.97 mg/l, or one tenth of the LC50 value, was deemed sublethal. Fish were exposed to acute and sublethal amounts of CBZ at weekly intervals of 24 hours and 35 days, respectively. GOT activity was reduced in the gill, liver, and muscle; GPT and LDH activity were elevated in the liver and muscle, and GOT activity was reduced in the gill as a result of acute therapy. While GPT activity rose in the liver and muscle with sublethal therapy, GOT activity reduced in these two organs. GOT and GPT activity in the gills as well as LDH activity in the gills, liver, and muscle showed a biphasic tendency. The current study shows that CBZ changed the activities of GOT, GPT, and LDH in different fish organs; these enzymes could be a reasonable way to track how hazardous drugs are in aquatic environments [62].

CHAPTER 3

METHODOLOGY

Effect of antibiotics exposure on brain, gill, liver, and kidney of common carp (*Cyprinus Carpio*) was the present study goals. The investigation was carried out at the Institute of Zoology, Bahaudin Zakariya University, Multan.

3.1 Fish Samples

A total number of 30 common carp (*Cyprinus carpio*) individuals of with an average body weight 20g to 50g were employed in the present study. Fish samples were obtained from urban ponds of KotAddu, located in Southern Punjab, during the summer season of July 2024, wisely transported in oxygenated plastic bags to laboratory at Bahaudin Zakariya University for the experimental procedure. Upon arrival, the fish were acclimatized for 15 days in the laboratory setting to ensure stable conditions and adaptation before the initiation of the experimental trials. The fish were housed in 3 separate aquaria, with ten individuals per aquarium provided with aerator. Fish were fed once daily with commercial pellet feed, during the accommodation. The tank water was renewed every day in order to maintain optimal water quality conditions along with antibiotics concentration determined. Physicochemical parameters of the water were estimated regularly. The water temperature was maintained at 26 °C under whole day and night cycle. Dissolved oxygen and pH were maintained 7-8 milligram per liter and 7-7.5 respectively.

3.2 Antibiotics Exposure

The study included 3 experimental groups. The control group (C1) was kept in untreated water under the same environmental conditions, while the treatment groups (T1 and T2) were exposed to antibiotics. T1 was treated with amoxicillin at a concentration of 20 mg/L, and T2 was treated with metronidazole at the same concentration of 20 mg/L. Both antibiotics were obtained from a local pharmacy. The exposure duration ranged from 7 to 14 days to assess the effects of these antibiotics on the fish. These exposure levels were selected based on the concentrations comparable to its natural habitat as well as previous research studies on antibiotic toxicity in fish. Each tank waste and 35 % of water will be drain off on daily basis.

Table 3.1: Properties and Administration of Amoxicillin and Metronidazole

Parameters	Amoxicillin	Metronidazole
Proprietary name	Amoxll, trimox	Acea, Anabact, Flagyl, Metrogel
Molecular formula	C ₁₆ H ₁₉ N ₃ O ₅ S	C ₆ H ₉ N ₃ O ₃
Molecular weight	419.45g/mol	171.156g/mol
Manufacturer Company	GlaxoSmithKline	Abbott
Route of administration	Exposure	Exposure

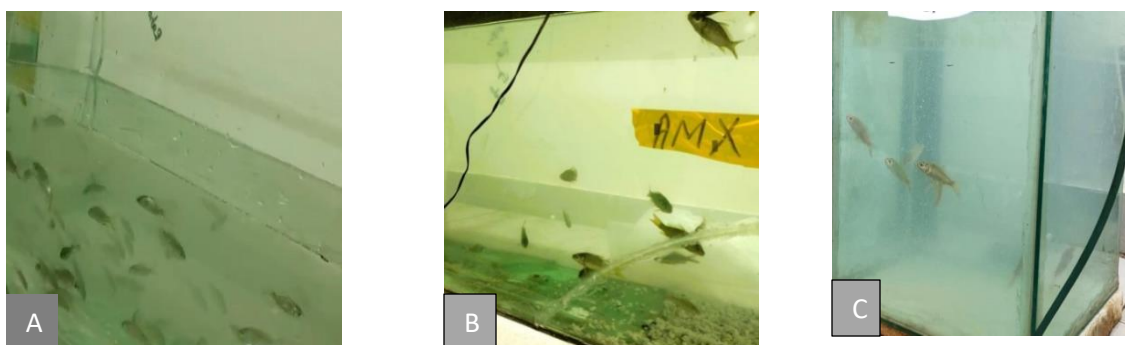


Figure 3.1: Fish Groups Fig3.1.1A Control Group (T0), Fig3.1.2B Amoxicillin treatment (T1)and Fig 3.1.3C Metronidazole (T2) treatment

Table 3. 2: Overview of experimental groups, species, weight, antibiotics and exposure time

Groups	Species	N	Weight	Antibiotics	Dosage	Route of administration	Exposure time	Exposure time
GROUP A Control (C1)	Common Carp	10	30g-50g	No antibiotics	-	-	-	
Group B (T1)	Common carp	10	30g-50g	Amoxicillin	20 mg/L	Exposure	7 days	14 days
Group C(T2)	Common carp	10	30g-50g	Metronidazole	20 mg/L	Exposure	7 days	14 days

The samples were randomly allocated to three groups and exposed with the antibiotics for the period of 7-14 days.

3.3 Water Quality Parameters

3.3.1 Temperature Measurement

The water temperature was consistently maintained at 26°C. A digital thermometer (FT-710 Lutron Electronics, Taiwan) with a precision of $\pm 0.1^{\circ}\text{C}$ was used for continuous monitoring. The thermometer was placed within the experimental tanks to ensure real-time, accurate temperature readings.

3.3.2 Dissolved Oxygen and pH observance

A calibrated pH meter (AZ8685 Instrument Corp, Taiwan) was used to maintain pH of water between 7.0 and 7.5 which was checked regularly to ensure stability. Oxygen meter (AR8210 Smart Sensor, China) used to check dissolved oxygen (DO) levels, a dissolved) ensuring that DO concentrations ranged between 7 to 8 mg/L. Both pH and DO measurements were recorded daily to ensure stable water conditions throughout the experimental period.

Table 3. 3: Water quality, Parameters of Experimental groups

Parameters	Group A (C1)	Group B(T1)	Group C (T2)	Standard values
Water temperature WT (whole day & night cycles)	26°C	26°C	26°C	22-32°C
PH	7-7.5	7-7.5	7-7.5	6.5 -8.5
Dissolved oxygen DO mg/L	7-8	7-8	7-8	>6.0

3.4 Fish Dissection

The dissection procedure of common carp (*Cyprinus carpio*) involved several steps to ensure accurate organs collection and preservation. First of all, the fish were anesthetized by using a solution of Chloroform to minimize the stress and movement. All fishes were checked with no obvious abnormalities or diseases were found on fish sample. After that, the fish were set up ventral side up on a dissection board, and the internal organs were accessible by means of a longitudinal incision performed along the midline, from the anal fin to the pectoral fin. To prevent harm, the cranium was carefully lifted and the brain was carefully removed using forceps. The liver was extracted without breaking apart after the gills were gently cut free from the gill arches. With the use of scissors, the kidneys were cut away from the backbone. To maintain tissue integrity, each organ was stored in 10% formalin solution as soon as it was removed. Every sample has the proper labeling and storage as described by Sweeney and Wong (2012) [63].

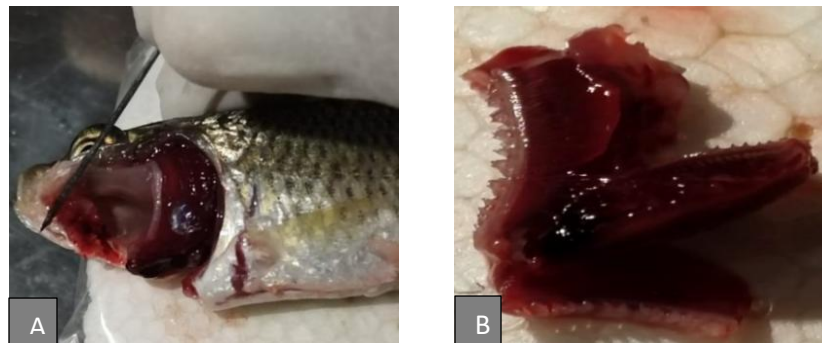


Figure3. 1: Different Organs of Fish after dissection: **Figure 3.3A, Figure 3.4B:** Gills of Fish

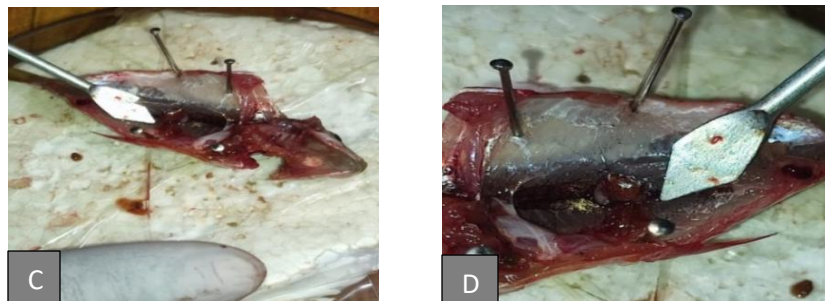


Figure 3.5 C, D: kidney of Fish



Figure 3.7E: Liver of Fish & **Figure 3.8F: Brain of Fish**

3.5 Histological procedure

Three major steps were taken for histological procedure of fish.

3.5.1 Fixation:

First procedure of histological analysis is fixation in which fish organs were preserved, hardened and avoid from changes in the tissues of the fish. The chemical which was used for the fixation of fish is 10% formalin. Water was adjusted to that chemical.

3.5.1.1 Procedure

After removal from the body of fish, these organs were placed in fixative. In a short time, the organ blocks were cut to thickness so that the fixative urgently penetrate in tissues.

3.5.2 Tissue processing:

This was the second step in the histological procedure of fish. It includes the cleaning, dehydration and infiltration of the fish organ with paraffin. 50-70% alcohol was used for the dehydration of fish organ which prevent from distortion. Clearing helps in bringing about miscibility between alcohol and paraffin. Organs of fish were impregnated and embedded with paraffin and alcohol.

3.5.3 Procedure

Organs of fish were washed over night in running water. Alcohol con was taken for the aqueous medium. After that, organ of fish was preserved 70% alcohol. Then, organ of fish was dehydrated by transferring them alcohol. After that, this organ of fish was transferred to the paraffin and xylene for 30 minutes. These organs were infiltrated in 2-

3 changes. Care was taken for this procedure. Excess air from getting trapped in the blocks was carefully avoided. The wax was permitted to solidify on a cold plate.

3.5.4 Section cutting:

Tissues were cut and floated in a water bath. Then moved to the glass slides with egg albumin. Then, dehydrate on a hot plate. Then transferred to the staining.

3.6 Microtome Protocol

A rotary microtome (Manual Microtome YR420 – Kalstein) was used for the fish. All histologic samples were organized for light microscopy investigation. For each fish, the liver, kidney and gill were sampled. All these samples washed in tap water, using a semi auto-mated microtome and placed on sterilized glass slides. Histological changes observed and photographed by using a microscope.

3.7 Image analysis

By the use of image J, image analysis was obtained. In the image analysis, slides of the organs of fish which were obtained from microtome are analyzed under Olympus Compound microscope different magnification 4X, 10X and 40X. Photoshop is also used for image crops and editing for text.

3.8 Statistical analysis

All the data was analyzed using SPSS developed by IBM version 23.0. One way ANOVA was used to compare quantitative data / mean difference while the exact Fischer test was used to compare qualitative data between the groups. A ($P \leq 0.05$) was considered statistical significant. Quantitative data was presented as mean $\pm$ standard deviation (SD). While the categorical / qualitative data was presented in the form of frequency/percentage or graphs.

CHAPTER 4

RESULTS

4.1 Histopathological Effect of Antibiotics on Gills of Common Carp

Antibiotics have diverse effects on the gills of common Carp. The gill tissues of the control group had well developed secondary lamellae and intact epithelial layers without any sign of hyperplasia or lamellar fusion.

4.1.1 Effect of Amoxicillin 7 days Exposure:

In the group treated with amoxicillin (AMX) for 7 days, a very slight epithelial hyperplasia and minimal lamellar fusion with normal gill structure were noted, which suggest only a mild stress response at this time point (Fig 4.1- B).



Figure 4.1: The photomicrograph of gills of *C. Carpio* 7 days exposure: Fig 4.1-A: AMX Control 7 day's Normal tissue with intact epithelial cells EC, Mucus cell MC and secondary gills lamellae GL Fig 4.1-B: AMX treatment 7 days post treatment showing slight epithelial hyperplasia EH and disorganization of lamellar fusion DOL.

4.1.2 Effect of Metronidazole 7 days Exposure:

There were moderate epithelial hyperplasia, partial lamellar fusion and initial epithelial lifting in gills exposed to metronidazole (MNZ) for 7 days which indicated that the fish had a more severe response to the drug than AMX at the same exposure time (fig 4.1-C).



Figure 4.1: The photomicrograph of gills of *C. Carpio* 7 days exposure: Fig 4.1-C: Normal tissue with intact epithelial Cells EC, Mucus cell MC and secondary gills lamellae GL. Fig 4.1-D: 7 days post treatment showing Mild epithelial hyperplasia EH and lamellar fusion is seen.

4.1.3 Effect of Amoxicillin 14 days Exposure:

The gill tissue of the fish exposed to AMX for 14 days showed a marked epithelial hyperplasia characterized by the thickening of the epithelial layer and increased extent of lamellar fusion. Further, there was an increase in the number of mucous cells signifying a stronger tissue reaction on account of chronic exposure (Fig 4.1-D).



Figure 4.1: The photomicrograph of gills of *C. Carpio* 14 days exposure 4.1-E: AMX 14dy control showing intact epithelial cells EC and mucus cells MC Fig 4.1-F AMX 14 days post treatment shows gills with increased epithelial hyperplasia and lamellar fusion.

4.1.4 Effect of Metronidazole 14 Exposure days:

At 14 days, the MNZ-exposed gills showed a more severe epithelial hyperplasia, total lamellar coalescence and mucous cell proliferation, which suggests that the tissue has been severely affected (Fig 4.1-G). The findings of the present study indicated that MNZ is more toxic to gill tissue than AMX at all exposure periods with a further increase in toxicity with increase in exposure time (Fig 4.1-H)



Figure 4.1: Fig: 4.1G 14 dy MNZ control showing intact structures. Fig 4.1-H MNZ 14 days treated section indicate severe epithelial hyperplasia SEH, mucous cell proliferation MCP, total lamellar coalescence TLC and Advanced tissue damage ATC

4.2 Comparison of AMX and MNZ Exposure on Gill Tissues

Differences in histopathological responses are observed when comparing the effects of AMX and MNZ on gill tissues at both 7-day and 14-day exposure periods. The Image J software was used for comparison of the histopathological section images and t test was used to compare the statistical significant difference between the two groups (7 day and 14 day) of each antibiotic.

The gills of fish treated with 7 day AMX showed only a slight epithelial hyperplasia and a slight lamellar fusion (Figure 4.1-B). The tissue damage at this stage was small. However, gills of the MNZ-treated fish exhibited moderate epithelial hyperplasia, higher degrees of lamellar fusion and epithelial lifting (Figure 4.1-D) indicating higher pathological response to MNZ at a preliminary level. While comparing effects of two antibiotics, the disparity was even more pronounced after 14 days in both cases. AMX exposure resulted in a marked epithelial hyperplasia, epithelial layer became thicker and lamellar fusion was more prominent. There was also increased mucous cell proliferation as shown (Figure 4.1-C) which shows moderate tissue reaction to sustained exposure. However, significantly higher degree of damage was observed in the gills of fish treated with MNZ as the p value was < 0.05 . The total lamellar coalescence, the advanced epithelial hyperplasia, and the extensive mucous cell proliferation were observed (Figure 4.1-E). MNZ exerts the more severe tissue degradation and damage than the AMX at least over the same period. The antibiotics affected gill tissues histopathological but MNZ was more damaging to the tissues than AMX and this was

most apparent at 14 days where coalescent and extensive tissue damage were observed.

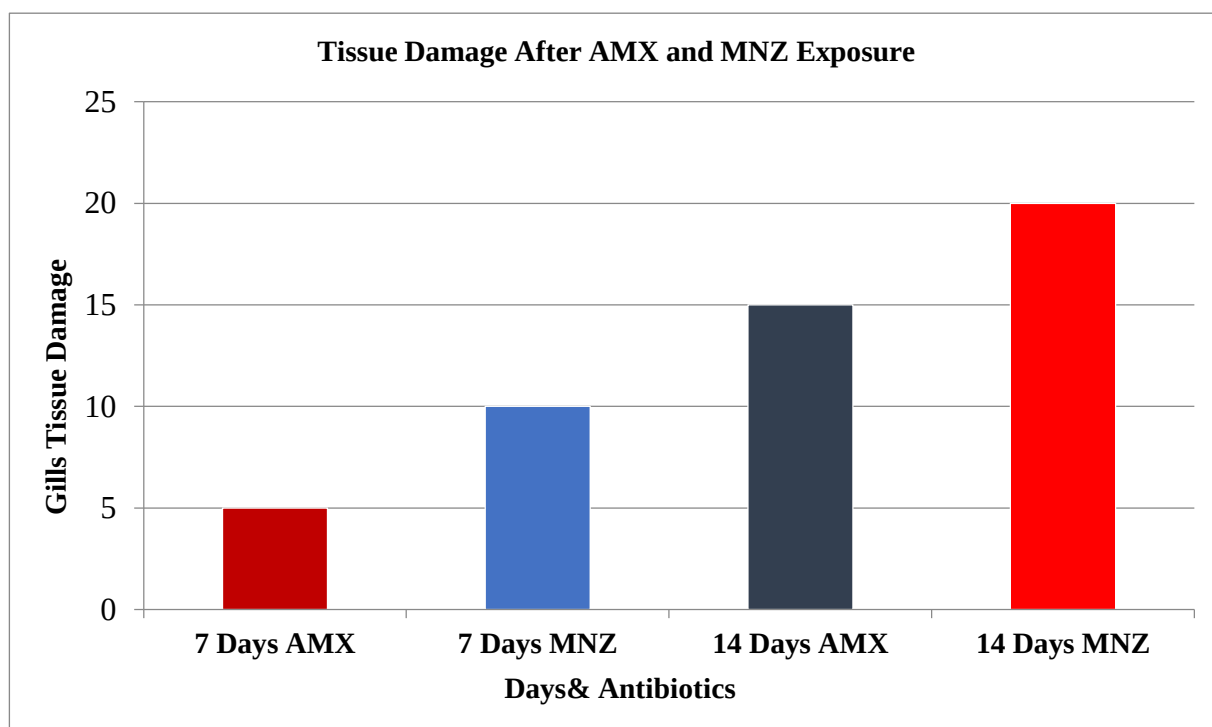


Figure 4.2: Comparative Analysis showed MNZ had more toxic effects as compared to AMX in context of tissue damage

Control was compared with the antibiotic groups and Anova was applied to check the statistical difference. Tissue damage parameters in context of histopathological changes were examined under microscope. Metronidazole 14 day caused the most tissue damage with significant difference from the control group, while the AMX for 14 days also caused significant tissue damage compared to the control group. Metronidazole (MNZ) for 7 days and Amoxicillin (AMX) for 7 days both caused significant tissue damage compared to the control group, but to a lesser extent than the 14-day exposures. Thus, appears to be more potent, especially at longer exposure times.

4.3 Histopathological Effect of Antibiotics on Liver of Common Carp

The liver tissues from the control group included intact hepatic cells without any degenerative or pathological changes in their structure.

4.3.1 Effect Amoxicillin 7 days Exposure:

In the group treated with amoxicillin (AMX) for 7 days, slight hepatocellular alterations were noticed in the form of minor vacuolation in the liver parenchyma. Nevertheless, the level of cellular stress was not very high and there was no sign of tissue damage at this point (Figure 4.3-B).



Figure 4.3: Figure 4.3 A: AMX Control 7 Days intact hepatic cells HC, without any degenerative Hepatic pathology HP. Figure 4.3-B: AMX 7 days post treatment liver showing Mild vacuolization in liver parenchyma MVLP.

4.3.2 Effect Metronidazole 7 days Exposure:

Livers treated with metronidazole (MNZ) for 7 days were characterized by moderate grade of vacuolization, bile stagnation and cytoplasmic degeneration comparing with AMX at the same time (Figure 4.3-C).



Figure 4.3: Figure 4.3C MNZ 7 days control showing normal hepatic cells. Figure 4.3-D: MNZ treatment 7 days tissue reveal moderate bile stagnation MBS and Moderate vocalization MV.

4.3.3 Effect Amoxicillin 14 days Exposure:

The liver tissues showed significant changes of vacuolization and hepatocellular degeneration after the fish was exposed to AMX for 14 days. This indicates that there is enhanced stress response after exposure to AMX for some time (Figure 4.3-D).



Figure 4.3: Photomicrograph of liver of *C. carpio* 14 days exposure. Figure 4.3 E: Control tissue indicate normal liver structure with hepatic cells H, Blood vessel BV Figure 4.3-D: AMX 14 days post treatment showing increased vacuolization IV and hepatocellular degeneration.

4.3.4 Effect Metronidazole 14 days Exposure:

After 14 days of exposure to MNZ, severe hepatocellular degeneration was noted characterized by extensive vacuolization and bile stasis. There were also changes in the liver tissues such as cytoplasmic vacuolization which were more pronounced in MNZ than AMX over time (Figure 4.3-E).



Figure 4.3: Photomicrograph of liver of *C. carpio* 14 days exposure. Figure 4.3 G: Control with intact liver structure comprising Blood vessel BV and hepatic Cells HC. Figure 4.3-H. MNZ 14 days treated tissue showing severe vacuolization or cytoplasmic vacuolization and severe hepatocellular degeneration SHD.

4.4 Comparison of AMX and MNZ Exposure on Liver Tissues

The histopathological changes of the livers of Fish exposed to AMX and MNZ are different at both 7 days and 14 days of treatment. After 7 days, liver tissues from AMX-treated groups showed only slight vacuolization and little evidence of cellular stress; there were no severe hepatocellular degeneration; thus, AMX induced only mild liver changes at this time point (Figure 4.3-B). However, liver tissues of the group treated with MNZ showed moderate vacuolization, early bile stasis and cytoplasmic degeneration; this indicates that MNZ had a more severe toxic impact on the liver than

AMX at the same time period (Figure 4.3-C). This was so at the 14th day where the differences were more evident. Hepatocytes in livers of AMX treated groups displayed increased vacuolization and initial signs of hepatocellular degeneration suggesting moderate but evident effect of AMX on liver health after prolonged administration (Figure 4.3-D). Nevertheless, the liver tissues treated with MNZ showed extensive hepatocellular degeneration along with marked vacuolar changes and bile stasis. The extent of cytoplasmic degeneration in livers of MNZ treated groups was significantly higher than in AMX group suggesting that MNZ is more toxic to the liver tissues over the prolonged time of exposure as compared to AMX (Figure 4.3-E).

Concisely, AMX and MNZ elicited histopathological alterations in the liver tissues with MNZ exhibiting more severe effects compared to AMX especially at 14 days exposure period where sever vacuolation, bile stasis and hepatocellular degeneration were observed (Figure 4.4)

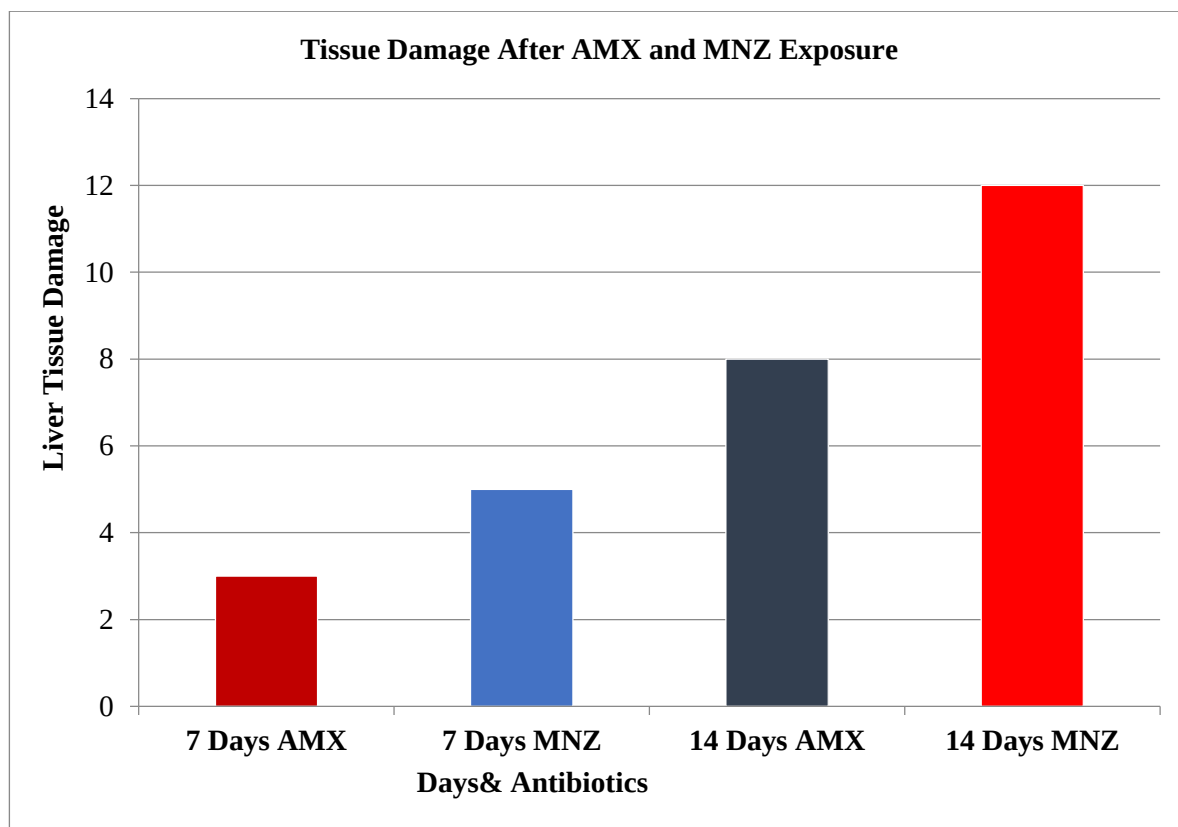


Figure 4.4: Comparative Analysis showed MNZ had more toxic effects as compared to AMX in context of tissue damage

Control was compared with the antibiotic groups and ANOVA was applied to check the statistical difference among groups of liver sections in context of histopathological changes Metronidazole 14 day caused liver damage with significant difference from the control group ($p < 0.0001$).

4.5 Histopathological Effect of Antibiotics on kidney of Common Carp

The control group kidney tissue had intact renal structure without vacuolization or other pathological changes in the kidney tissue sections (Figure 4.5-A).

4.5.1 Effect Amoxicillin 7 days Exposure:

In the group treated with amoxicillin (AMX) for 7 days, there was mild renal tubule vacuolization which could be considered as initial sign of tissue injury. Nonetheless, the general disposition of the renal structure was preserved after 7 days of exposure with only slight evidence of kidney injury (Figure 4.5-B).



Figure 4.5: Figure 4.5-A: AMX 7 days Control Normal renal structure (control) showing renal tubules RT and proximal Tubules PT. Figure 4.5-B AMX 7 days post treatment of kidney showing mild vacuolization showing the mild renal tubule vacuolization MRTV

4.5.2 Effect of Metronidazole 7 days Exposure:

The kidneys of fish treated with metronidazole (MNZ) for 7 days revealed moderate degree of tubular vacuolization and early signs of cytoplasmic degeneration of the renal tubules which suggest that the tissue had a more severe response to MNZ than to AMX at this stage (Figure 4.5-D).



Figure 4.5: Figure 4.5C: MZN 7 days control showing intact renal corpuscle RC, Renal tubule RT and proximal tubule PT. Figure 4.5-D: MNZ 7 days post-treatment showing moderate proximal tubule degeneration MPTD seen.

4.5.3 Effect Amoxicillin 14 days Exposure:

Prolonged exposure to AMX for 14 days led to increased vacuolization and apparent signs of renal tubule degeneration indicating moderate increase in tissue stress (Figure 4.5- F).



Figure 4.5: Photomicrograph of kidney of *C. Carpio* 14 days exposure. Figure 4.5- E: AMX Control showing normal renal cells RC, Renal tubule RT and proximal tubule PT Figure 4.5- F: AMX 14 days kidney post-treatment section showing pronounced vacuolization PV and cytoplasmic degeneration of renal tubules CD indicate irregular arrangement of tubular cells

4.5.4 Effect of Metronidazole 14 days Exposure:

In the kidneys, there was vacuolar degeneration of the tissues after 14 days of exposure to MNZ and renal structures were almost completely destroyed, with wide-spread cytoplasmic damage indicating that MNZ has a more potent pathological impact (Figure 4.5-H).



Figure 4.5: Photomicrograph of kidney of *C. Carpio* 14 days exposure. Figure 4.5G: MNZ 14 day control showing normal renal structure with renal tubules RT and renal corpuscles RC. Figure 4.5-H: MNZ 14 day's post-treatment section showing pronounced vacuolization PV and cytoplasmic degeneration of renal tubules CD indicate irregular arrangement of tubular cells.

4.6 Comparison of AMX and MNZ Exposure on Kidney Tissues

Comparing the effects of AMX and MNZ on the kidney tissues, it is found that MNZ has a more detrimental effect on the renal tissues than AMX especially when exposed for a long time. In the AMX-treated kidney tissues, after 7 days of exposure, only a mild vacuolization was observed, and other signs of stress as well as significant structural changes were not detected (Figure 4.5-B). On the other hand, kidneys treated with MNZ showed moderate vacuolation and initial evidence of cytoplasmic pathology suggesting that MNZ was more toxic to renal tissues than AMX at this time point (Figure 4.5-D).

After 14 days the difference in the efficacy of both the antibiotics was more prominent. Hematoxylin and eosin staining of the kidney sections also revealed that the AMX-treated kidney tissues showed greater vacuolization and initial signs of renal tubule damage indicating that though the tissue stress had increased due to AMX treatment the kidney tissues were not severely damaged (Figure 4.5-C). Conversely, kidneys treated with MNZ displayed extensive vacuolar changes and signs of renal tissue degeneration with prominent cytoplasmic damage indicating that MNZ has a higher pathological effect than AMX in the same duration (Figure 4.5-F). Thus, although both AMX and MNZ induced vacuolization in the kidney tissues, MNZ was found to exert a more potent toxic effect on the renal tissues within the shortest time of 14 days as compared to AMX.

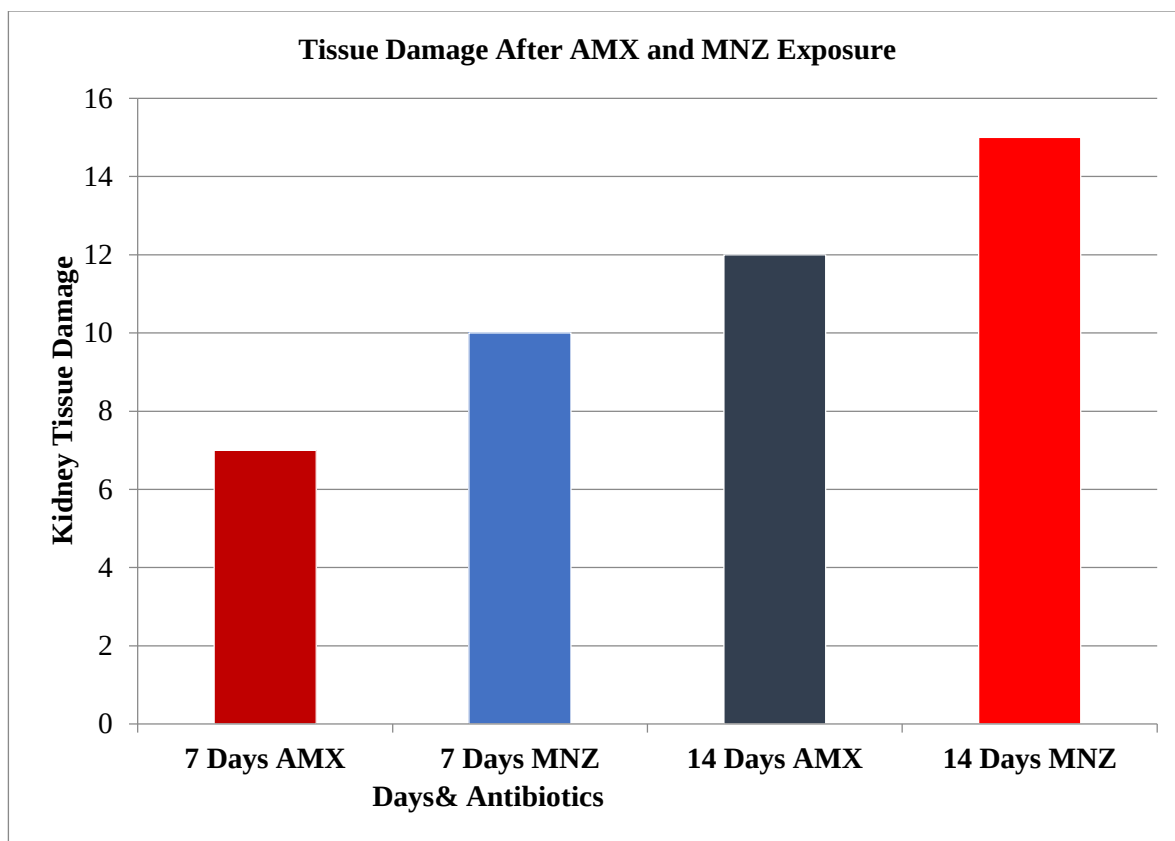


Figure 4.6: Comparative Analysis showed MNZ had more toxic effects as compared to AMX in context of tissue damage

Control was compared with the antibiotic groups and ANOVA was applied to check the statistical difference. Renal parameters in context of histopathological changes were examined. Metronidazole 14 days caused the renal damage with significant difference from the control group ($p < 0.0001$), while the AMX for 14 days compared to the control group ($p < 0.001$). MNZ had more potent, especially at longer exposure times.

4.7 Histopathological Effect of Antibiotics on brain of Common Carp

The H&E staining of the brain tissues from the control group showed that there was no necrosis, vacuolization or pathological changes and the neurons were intact.

4.7.1 Effect of Amoxicillin 7 days Exposure:

In the group treated with amoxicillin (AMX) for 7 days, there was a moderate neuronal damage with little vacuolation. The brain tissue appeared to be relatively preserved,

which suggested that AMX exposure elicited only a slight early-stage stress response (Figure 4.7-B).



Figure 4.7: Photomicrograph of brain of *c. carpio* 7 days exposure. Figure 4.7A: Control showing the normal histological structure of brain of fish with cerebellum CL, olfactory lobe FL connected with cerebrum CR . Figure 4.7-B: AMX 7 days exposed tissue section showing mild neuronal degeneration MND

4.7.2 Effect of Metronidazole 7 days Exposure:

On the other hand, the brains treated with metronidazole (MNZ) for 7 days showed mild neuronal damage, increased vacuolization and early signs of necrosis. The pathological response to MNZ was higher than that of AMX at the same exposure level or phase (Figure 4.7-C).



Figure 4.7: Figure 4.7-D: Control of MNZ 7 days indicate the normal brain cells and thalamus TH, cerebrum CR and pituitary gland P. Figure 4.7-D: MNZ 7-day post-treatment MNZ treated showing moderate degenerated cerebrum DCR and degenerated olfactory lobe DFL

4.7.3 Effect of Amoxicillin 14 days Exposure:

At the end of the 14 days of exposure, neuronal degeneration was further observed characterized by increased vacuolization and mild necrosis in some areas of the brain. This indicates that chronic toxic effects of AMX cause mild damage to the brain tissue, but to a moderate extent only (Figure 4.7-D).



Figure 4.7: Photomicrograph of brain of *C. carpio* 14 days exposure. Figure 4.7E AMX 14 dy control shows normal hypothalamus HT & olfactory lobe FL connected with cerebrum CR Figure 4.7-F: AMX 14 days Showing increased degeneration in olfactory DFL, degenerative cerebrum DCR and degenerative hypothalamus DTH

4.7.4 Effect of Metronidazole 14 days Exposure:

After 14 days, extensive neuronal degeneration, vacuolization and necrosis were observed in the brain tissues of MNZ-treated group. This was even worse than in the AMX-treated group, indicating that MNZ had a worse impact on the brain tissue over the same period (Figure 4.7-H).



Figure 4.7: Figure 4.7-G. MNZ 14 days control showing the normal olfactory lobe FL and hypothalamus HY. Figure 4.7-H: MNZ 14 days control showing the normal olfactory lobe FL and hypothalamus HY showing severe neuronal degeneration SND and necrosis N

4.8 Comparison of AMX and MNZ Exposure on Brain Tissues

Thus, the comparative analysis of the impact of AMX and MNZ on the brain tissues indicates that MNZ has greater neurotoxic effect than AMX. AMX-treated brain tissues exposed for 7 days also demonstrated only mild neuronal degeneration and minimal vacuolization, which suggests that AMX induces only a slight stress response with no significant morphological changes in the brain (Figure 4.7-B). On the other hand, there was moderate neuronal damage and vacuolation, early signs of necrosis in the brain sections treated with MNZ as compared to AMX (Figure 4.7-D).

It is quite evident that after 14 days the differences were more profound. After AMX treatment, there was an increase in vacuolation and mild necrosis in the brain sections; however, there was more profound neuronal degeneration, which showed a moderate neurotoxic effect of AMX when exposed for an extended period (Figure 4.7-C). However, the brain tissues treated with MNZ showed a much higher degree of damage as evidenced by neuronal degeneration, extensive vacuolation and necrosis. The comparison shows that MNZ has a greater and more toxic impact on the neurons than AMX after 14 days of exposure (Figure 4.7-E).

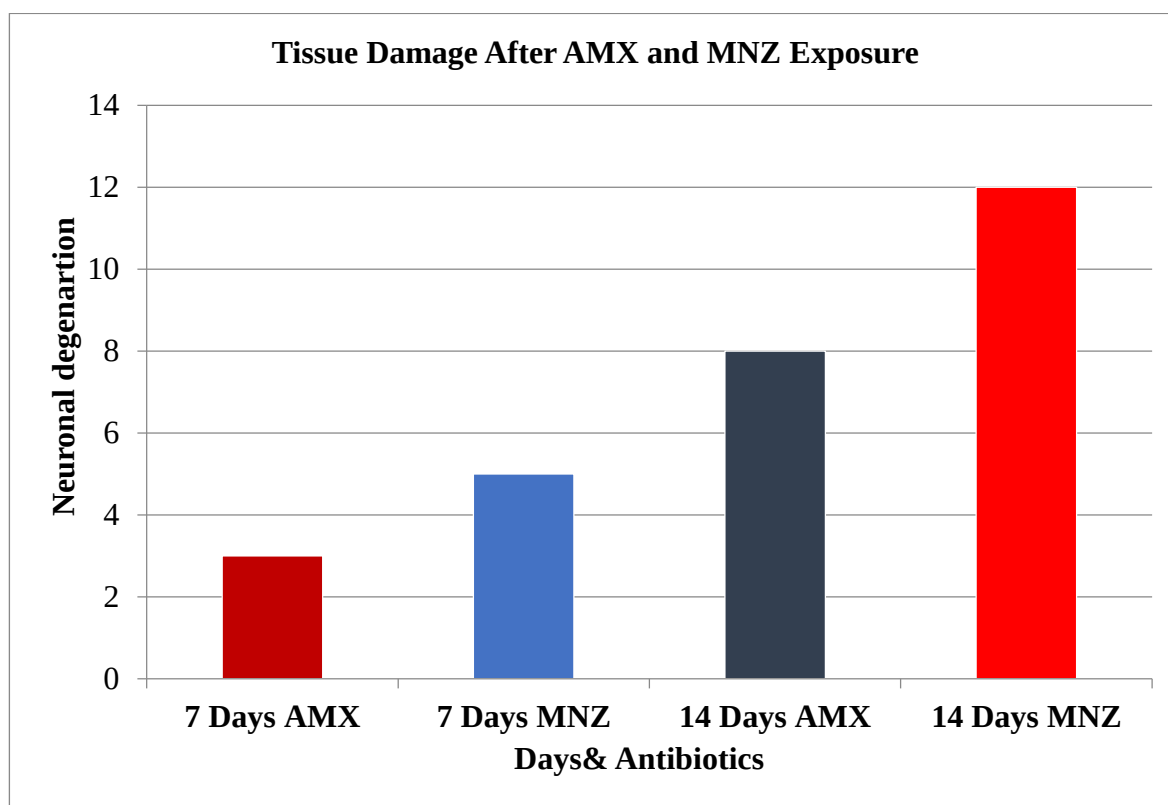


Figure 4.8: Comparative Analysis showed MNZ had more toxic effects as compared to AMX in context of tissue damage

Control was compared with the antibiotic groups and Anova was applied to check the statistical difference. Neurological parameters in context of histopathological changes were examined under microscope. Metronidazole 14 day caused the most tissue neuronal degeneration with significant difference from the control group ($p < 0.0001$).

MNZ for 7 days and Amoxicillin (AMX) for 7 days also caused significant tissue damage compared to the control group, but to a lesser extent.

Table 4.1: Description of the Histopathological alteration in different tissues of Common carp

Histopathologic al parameters	Gills	Liver	Kidney	Brain
Respiratory effect	Lamellar Fusion	-	-	-
Circulatory effect	dilated vessels and lamellar congestion	-	-	-
toxic effect	-	Cell death in liver cells, indicating toxic damage	-	-
Vacuolization	-	Formation of vacuoles within hepatocytes	-	-
Necrosis	-	-	Necrosis and degeneration of kidney tubule cells.	-
Glomerular changes	-	-	Alterations in the structure of the glomeruli	-
Inflammation	-	Cell infiltration lymphocytes and macrophages	Presence of inflammatory cells in kidney tissues	-
Neuronal damage	-	-	-	Signs of degeneration.
Glial Cell Proliferation	-	-	-	Increased presence of glial cells

4.9 Comparison of the AMX & MNZ group

We want to analyze the effect of two antibiotics (Amoxicillin and Metronidazole) administered for 7 days and 14 days on different tissues (brain, liver, kidney) of fish. The dependent variable is tissue damage score, which is continuous, while the independent variable is antibiotic type and duration.

The tissue damage scores for each groups were calculated on H/E stains while microscopy and scored according to mild, moderate or severe damage. Mean scores were calculated.

Calculated group means

Table 4.2: Comparison of the AMX & MNZ group

Group	GILLS	Brain	Liver	kidney
Amoxicillin (7 days)	13.0	11.0	9.0	8.0
Amoxicillin (14 days)	17.0	19.0	15.0	16.0
Metronidazole (7 days)	19.0	23.0	21.0	20.0
Metronidazole (14 days)	30.0	33.0	29.0	27.0

Sum of Squares Between Groups (SSB)= Measures variability due to differences between group means

Sum of Squares Within Groups (SSW)=Measures variability within each group.

Degrees of Freedom= df between groups=3

df within groups = 8

Critical F-value (dfB=3, dfW=8, α =0.05 = 5 .08 (from F-distribution table).

Standard Error Mean =SEM (per group) = $SD/\sqrt{n}$

Table 4.3: ONEWAY ANOVA

Source	SEM	SS (Sum of Squares)	DF (Degrees of Freedom)	MS (Mean Square)	F-value	P-value
BETWEEN GROUPS	1.9	510	3	169	170	0.0001
WITHIN GROUPS	0.47	8	8	1	-	
Total		518	11			

$F=171 > 4.07 \rightarrow$ Reject the null hypothesis.

Accept the alternative that there is statistically significant difference between the groups in term of antibiotic effects on tissues.

There is a **statistically significant difference** in tissue damage scores between the antibiotic groups ($F(3,8) = 170$, $p \leq 0.05$).

Table 4.4: Difference between the AMX and MNZ groups

Variation	Df	MS	F value	P value
Between groups	2	59.5	8.3	0.002

There was a statistical significant difference among the three groups in context of histological alterations due to antibiotic exposure as the p value calculated was less than 0.05 .Both antibiotics had lethal or toxic effects on the tissues of fish . Increased intake of the antibiotics or exposure of 14 days was related to more severe pathological changes in tissues as compared to the AMX 7 days treatment.

Table 4.5: Comparative analysis of Histopathological alteration in treatment groups exposed to Antibiotics

Histopathological parameters	Respiratory effect	Circulatory effect	Toxic effect	P value
AMX 7 day	No	No	No	0.37
MNZ 7 day	Yes	Yes	No	0.021
AMX 14 Day	yes	Yes	No	0.01
MNZ 14 day	Yes	Yes	Yes	0.001

Table 4.6: Fischer exact test for qualitative data

Antibiotic type	Vacuolization	P value	Inflammation	P	Degeneration	P	Necrosis	P value
AMX	4	0.45	3	0.04	0	0.01	0	0.001
MNZ	2		5		2		2	

A Fisher's exact test showed no significant association between antibiotic type (AMX vs. MNZ) and vacuolation presence ($p = 0.45$). More frequent inflammation in one group over another resulted in $p \leq 0.05$ suggesting a statistically significant difference depending on antibiotic type used.

CHAPTER 5

DISCUSSION

The exposure of common carp (*Cyprinus carpio*) to antibiotics has induced significant histopathological changes in various organs. The histopathological effects of metronidazole (MNZ) and amoxicillin (AMX) on the common carp reveal critical insights into the toxicity and physiological responses of fish to these antibiotics over varying exposure durations. The current study indicated that MNZ generally had severe toxic effect on the tissues of common carp compared to AMX. MNZ exposure has been associated with moderate to extensive vacuolation, necrosis, and cellular degeneration in various organs. In contrast, AMX induced milder changes, such as slight vacuolization and minimal cellular stress, particularly during 7 day exposure periods. This differential impact suggests that while both antibiotics can affect fish health while MNZ poses a greater risk of acute toxicity. The research findings are compatible with the previous international studies that revealed antibiotics had various effects on the fish organs that were being investigated in diverse species of the aquatic organisms particularly targeting the fish species.

Amoxicillin (AMX) and metronidazole (MNZ) induced significant histopathological changes across various organ systems, including the gills, liver, kidneys, and brain.

Effect on the gills of Common carp

The findings on the gills, especially 7 days after amoxicillin exposure, provide insight into the subtle but far-reaching effects of antibiotics on aquatic life. Gills from the control group showed normal histology, characterized by well-developed secondary lamellae and an intact epithelial layer.. The presence of mild epithelial hyperplasia indicates a response of the gill tissue to antibiotic exposure. Minimal lamellar fusion results in less efficient breathing because it may reduce the surface area available for gas exchange. Although the observed convergence was not extensive, it raises concerns that long-term exposure or higher concentrations of amoxicillin may lead to more severe pathological changes. The effect of MNZ on gill tissue is more significant. The moderate epithelial proliferation, partial lamellar fusion, and initial epithelial elevation

observed in gills exposed to MNZ for 7 days indicate a significant stress response. This contrasts with the relatively mild changes observed with AMX exposure during the same period, where only mild hyperplasia and minimal lamellar fusion were noted. More severe reactions to MNZ indicate greater toxicity, possibly due to its mechanism of action, which involves disrupting cellular processes and inducing oxidative stress in tissues.

Previous studies have shown hyperplasia is often a compensatory mechanism in response to an irritant or stressor, suggesting that although the gills are still functioning, they are under some form of stress. This may be attributed to the pharmacological effects of antibiotics or their effects on the fish microbiome, which plays a role in maintaining gill health [64]. Previous studies have shown metronidazole causes more pronounced immunosuppressive effects and tissue damage in fish than amoxicillin. For example, metronidazole has been shown to adversely affect innate immune parameters in carp, leading to a decrease in important immune functions such as complement activity. Further investigation into the long-term effects and potential impacts on fish health and aquatic ecosystems is needed. Continuous monitoring is essential to mitigate adverse effects on non-target organisms in the aquatic environment. These findings are consistent with previous studies conducted [65].

Regarding the effects of chronic exposure, fish treated with AMX for 14 days showed significant epithelial hyperplasia, which was characterized by thickening of the epithelial layer and increased lamellar fusion, suggesting that long-term exposure to AMX can lead to more significant changes in gill structure, possibly Impairs respiratory efficiency. An increase in the number of mucus cells further indicates a chronic response aimed at protecting the gill surface from irritants or pathogens, but it may also imply a maladaptive response to ongoing stress. In contrast, gills exposed to MNZ for 14 days exhibited severe epithelial hyperplasia, complete lamellar coalescence, and extensive mucous cell proliferation. These findings suggest that MNZ not only exacerbates tissue damage over time but also causes complete changes in gill morphology. Complete lamellar coalescence may severely impair gas exchange capabilities, posing risks to fish survival in environments where oxygen supply is critical.

Previous studies have shown many changes have also been seen after an long exposure, they were considerably more pronounced effect on gills and more frequent after a chronic test. These alterations were the main contributors to the overall histopathological changes that were noted [66]. Progressive alterations such gill cell hyperplasia and enlargement might be viewed as typical defense mechanisms against many contaminants. The possible explanation for the observed epithelial thickening (increase of basal epithelial thickness, or BET) is the emergence of certain defense cells, such as leucocytes and macrophages, as a compensatory method of tissue repair. The mucus, a coating of glycolipids and glycoproteins which may function as a protective barrier over the gill epithelium, the proliferation and hypertrophy of mucous cells—observed is another protective mechanism. Several general theories have been proposed to explain causes of histological damages on fish resulting from pollutants, including cumulative effects of oxidative stress. One consequence is organelle/cellular damage, which can directly affect key cellular responses [67].

Effects on liver of common carp

Histopathological examination of liver tissue exposed to amoxicillin (AMX) and metronidazole (MNZ) revealed significant differences in the severity of liver injury during 7- and 14-day exposure. The findings highlight the different toxicological effects of these antibiotics on liver health. The liver tissue of AMX-treated fish showed only mild vacuolation and minimal cellular stress, indicating that AMX induced minor liver changes at this early stage. The lack of severe hepatocellular degeneration suggests that AMX may be relatively safe for short-term use in aquatic environments. In contrast, the liver tissue of MNZ-treated fish showed moderate vacuolation, early cholestasis, and cytoplasmic degeneration. These changes indicate that MNZ has a more pronounced toxic effect, possibly due to its mechanism of action, which can lead to oxidative stress and cell damage in liver tissue. This suggests that, although the initial risk of AMX may be low, long-term exposure may result in significant hepatic changes.

In contrast, MNZ treatment resulted in extensive hepatocellular degeneration, marked vacuolar changes, and marked bile stasis, suggesting that MNZ is more toxic to liver tissue over time.

Numerous studies have shown hepatic damage, including nuclear anomalies linked to exposure to various chemical substances, and have connected cytochrome activity in fish liver to tissue degeneration and necrosis [68]. One possible explanation for this could be that the liver is a target organ of metabolism that receives a lot of blood supply [69]. The liver's cellular deterioration and necrosis may also be brought on by vascular lesions in the blood vessels that cause blood to stasis. This is consistent with previous studies showing that MNZ can induce oxidative stress and impair cellular integrity in various fish species, resulting in severe histopathological changes [70]. It was observed that the most common antibiotic used was metronidazole due to its lethal effect on the liver of carp. Severe damage caused by MNZ may impair liver function, affecting metabolic processes and overall health. Given that the liver plays a crucial role in detoxification and metabolism, severe liver damage may increase the susceptibility of diseased fish and reduce the growth rate of affected fish [71].

Effects on kidneys of common carp

Cyprinus carpio showed significant differences in the severity of renal injury during 7-day and 14-day exposure. The findings suggest that MNZ has more harmful effects on kidney tissue than AMX, especially with long-term exposure. Minimal effects on kidney health were observed with short-term exposure.

MNZ exhibit moderate vacuolation and initial signs of cytoplasmic pathology, Disruption of cellular integrity and function may lead to further complications and, if exposure continues, greater toxic effects. This moderate effect may reflect the body's ability to cope with lower levels of toxicity over time, but the increased changes suggest that MNZ has a significantly higher pathological impact on kidney tissue compared with AMX. The severe degeneration observed may lead to impaired renal function, as the kidneys play a crucial role in filtering waste and maintaining homeostasis. These results are similar to previous studies that reported the highest changes in the renal organ of fish due to oxidative stress caused primarily by amoxicillin acid in the water .

Previous studies have shown alterations in enzyme activity and biomolecule damage, the kidney was the organ most susceptible to the exposure of AMX. This reaction could be linked to oxidative stress and immunological response. The thymus, kidney, and

spleen are the primary lymphoid organs found in teleost fish. The primary hematopoietic organ the primary location for erythrocyte, granulocyte, B lymphocyte, and monocyte development and the primary source of antibodies is the kidney. The kidney's displayed the second-highest significant alterations in enzyme activity and biomolecule destruction after gills. The latter are recognized as an area where increased enzyme activity favors the formation of ROS and oxidative metabolism [72].

Effects on brain of common carp

Brain damage caused by amoxicillin was mild, especially after seven days of exposure. Although there were some signs of neuronal degeneration and vacuolation, the overall effects were less severe. However, long-term exposure to amoxicillin and metronidazole for 14 days resulted in a modest increase in brain tissue damage. AMX induced a mild stress response without causing significant morphological changes in the brain, but brain slices treated with MNZ showed signs of moderate neuronal damage, vacuolation, and early necrosis.

The presence of necrotic changes suggests that MNZ may induce cellular mechanisms leading to oxidative stress and inflammation, known factors responsible for neurotoxicity in different species. Metronidazole consistently has more harmful effects on the brain than amoxicillin. Even within the shorter exposure period of 7 days, metronidazole-treated fish showed higher neuronal damage. The widespread damage observed may impair critical functions such as neurotransmission and overall brain health, potentially leading to behavioral deficits and reduced survival. These findings are consistent with previous studies using the carp model to study the effects of antiepileptic drugs on neuronal tissue.

Previous studies have shown direct exposure to AMA and direct exposure to AMX Brain displayed alterations in enzyme activity, biomolecule destruction, and Histopathological changes. Even when there is no meningeal inflammation, the latter substance can pass through the blood-brain barrier [73]. The nervous system needs a lot of oxygen and neuronal cell membranes contain a lot of polyunsaturated fatty acids, as well as because the brain contains a lot of iron, which causes free radical reactions, the

nervous system is especially susceptible to ROS. Common carp after 15 days of exposure to 100 mg/L of either macro-plastics (MaP) microplastics (MPs), or nanoplastics (NPs) in contrast to an control group. From this exposure, there was a 30–40% drop in each biochemical indicator, the greater the drop, the smaller the particle size (NPs > MPs > MaPs). For 15 days, the carp was exposed to plastic particles, which resulted in fibrosis, changes to blood capillaries, tissue separation, edema, necrosis in large cerebellar neurons and ganglion cells, and varying degrees of degraded connective tissues. The carp showed signs of necrosis, degeneration, vacuolation, and curvature in the inner layer. Here, we provide evidence that exposure to plastic particles causes neurotoxicity in common carp [74].

Comparative analysis of the tissue effects of amoxicillin (AMX) and metronidazole (MNZ) revealed significant differences, particularly after long-term exposure. Statistical analysis data showed that antibiotic exposure time had an impact on tissue damage, and the difference between the two groups was significant, $p < 0.05$. The results showed that MNZ had a more obvious toxic effect than AMX, and cell morphology changed significantly over time.

The differences between the two antibiotics became more pronounced after 14 days of exposure. The extensive damage observed could impair critical functions such as neurotransmission and overall brain health, potentially leading to behavioral deficits and reduced survival rates.

The data derived from the fischer and t test indicate that antibiotic type and exposure duration have an impact on the tissue damage and the interaction between these two factors is also statistically significant. This also compatible with the literature data that revealed significant results .The exposure of common carp (*Cyprinus carpio*) to antibiotics such as amoxicillin (AMX) and metronidazole (MNZ) has been shown to induce significant histopathological changes across various organ systems, including the gills, liver, kidneys, and brain. The comparative analysis of these effects reveals critical insights into the safety and ecological implications of antibiotic use in aquaculture and raises the concerns about its use in aquaculture settings. The findings underscore the need for careful monitoring of antibiotic usage in aquatic environments to mitigate

adverse effects on fish populations. Additionally, the differences observed between AMX and MNZ highlight the importance of selecting appropriate therapeutic agents based on their toxicity profiles. While AMX may present a lower risk at shorter exposure times, its chronic effects cannot be overlooked, especially in aquaculture practices where long-term antibiotic administration is common. The accumulation of antibiotics like MNZ in aquatic ecosystems poses significant ecological risks. As these substances can alter not only the target species but also non-target organisms and microbial communities, there is an urgent need for regulations governing their use in aquaculture and environmental management practices.

Comparative analysis of these effects reveals important insights into the safety and ecological impacts of antibiotic use in aquaculture and raises concerns about their use in aquaculture settings. The findings highlight the need to use optimal concentration in aquatic environments to mitigate adverse effects on fish populations. Furthermore, the differences observed between AMX and MNZ highlight the importance of selecting appropriate therapeutic agents based on toxicity profiles. Although AMX may pose a lower risk during shorter exposure times, its chronic effects cannot be ignored, especially in aquaculture practices where antibiotics are administered over long periods of time. The accumulation of antibiotics such as MNZ in aquatic ecosystems poses serious ecological risks. Because these substances can alter not only target species but also non-target organisms and microbial communities, regulations are urgently needed to govern their use in aquaculture and environmental management practices.

This is consistent with previous findings showing that MNZ can induce oxidative stress and impair cellular integrity in a variety of fish species, leading to severe histopathological changes. Severe damage caused by MNZ may impair gills, liver, kidney and brain function, affecting metabolic processes and overall health. Given that the liver plays a crucial role in detoxification and metabolism, severe liver damage may increase the susceptibility of diseased fish and reduce the growth rate of affected fish.

The findings highlight the importance of monitoring antibiotic use in aquaculture and natural waters. Research also shows that antibiotics can damage the immune function of carp. Studies have shown that MNZ can suppress innate immune parameters, including

complement activity and leukocyte count. This immunosuppression may make fish more susceptible to infection and disease, exacerbating the negative effects of antibiotic exposure.

CHAPTER 6

CONCLUSION

The exposure of common carp to amoxicillin and metronidazole reveals significant differences in toxicity and histopathological effects. MNZ showing harmful impacts across various organs. The histopathological effects observed in common carp gills following exposure to metronidazole and amoxicillin underscore the varying degrees of toxicity associated with these antibiotics. Metronidazole demonstrates a consistently higher level of toxicity across both short- and long-term exposures compared to amoxicillin, emphasizing the need for judicious use of antibiotics in aquaculture settings to preserve fish health and ecological balance. Future studies should focus on understanding the underlying mechanisms of these effects and exploring alternatives to antibiotic treatments in fish farming practices. Future research should focus on understanding the mechanisms underlying these toxic effects and exploring alternative treatments that minimize harm to aquatic organisms while effectively managing disease outbreaks.

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