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Effect of Antibiotics Exposure on Gills, Liver, Kidney and Brain of Common Carp (*Cyprinus carpio*)

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ABSTRACT

This work specifically assesses histopathological alterations in the organs of common carp (*Cyprinus carpio*) exposed to two frequently prescribed and broadly administered antibiotics, amoxicillin and metronidazole. Since these antibiotics affect human health, its effects on aquatic lives needs to be understood. Common carp samples of similar size and weight were randomly divided into three groups: the control group, another group treated with amoxicillin for 7 and 14 days, and the last group treated with metronidazole for 7 and 14 days. Usually the fish were treated with antibiotics at a concentration of 20 mg/L in their water for the duration stated. After exposure, the fish were killed and organs such as the brain, gills, liver, and kidneys were dissected out and processed for histopathological examination using the H&E staining. Diaphragmatic tissues were examined for histological alterations using light microcopy at 10x and 40x objectives and the quantitative analyses were performed with Image software. In the present study, statistical measurements were made by employing the software named as SPSS version 23.0 and analyzing nutrient through ANOVA. The investigation established marked histological alterations in the gill, hepatic, renal, and cerebrum tissues of the experimentally challenged common carp with either of the antibiotics at 7 and 14 days post-treatment. Stress response alterations several reins were observed under metronidazole treatment with significant differences ($P<0.05$) across all analyzed organs compared to amoxicillin. Thus, the particular impact of these antibiotics on common carp is exposed in regard to the main potential threat to aquatic environment and to human health due to fish consumption. Thus, the present work confirms the potential of amoxicillin and metronidazole to induce histological alterations, as well as showing that metronidazole is more toxic to common carp. Finally, these findings underscore the significance of responsible use of antibiotics that are used in fish production and beckon for constant surveillance of antibiotic remnants in water in order to completion the probable adverse effects on fish health and human beings. More studies are needed to capture the enduring impact of antibiotics on fish and the environment as well.

INTRODUCTION

The Impact of Antibiotics on Aquatic Ecosystem

Antibiotics are prescribed to cure bacterial infections in human and veterinary medicine and are used as feed supplements in intensive livestock farming, with resulting fears as to their effects on the environment, particularly on the aquatic environment [1]. Fish and other water dwelling organisms are actually now gaining exposure to these pharmaceuticals via a number of routes, including through agriculture pollution, sewage outflow, and littering. Of all, the common carp (*Cyprinus carpio*) is used due to its relevance to ecosystems and aquaculture management [2].

The term 'antibiotics' is a composite of two terms 'anti' and 'biosis', the literal rendering for antibiotics is substances that operate against microbes. In the past, they were described as substances manufactured by one microbe to neutralize others but today, they are described as both natural and synthetic [3]. These substances target a variety of microorganisms, with a primary focus on bacteria, although they also affect fungi and viruses.3 Antibiotics are classified into two main categories: antimicrobial preservative which slows microbial growth and antimicrobial agent that kills microbes. Despite using antibiotics as important tools for curing

infections and enhancing the quality of population health, negative consequences of their discharge on aquatic environment which influences the aquaculture mainly bother the scientists and health authorities [4].

Pathways of Environmental Contamination

It is well established that antibiotics regularly find their way into the environment through wastewater effluent originating from human, livestock, and fish farming. These substances are not removed effectively by most of the conventional treatment systems in water and wastewater treatment plants, hence find their way into surface waters and sediments [5]. Thus, it is stated that antibiotics easily penetrate water environment and remain there for long duration depending on their chemical stability solubility and interaction with sediments. Such a persistence of chemicals can cause lethal effects on other forms of life in a given ecosystem such as algae, invertebrates, and fish [6].

Substances like antibiotics can hinder the development and productivity of the undesirable species within a water system leading to upsetting of the aquatic life structures. For example, the increase in the numbers of certain bacteria strains is capable of depriving the balance of beneficial microbial production of space, and in the process reduce the health of the ecosystem even further [7]. Furthermore, anti-bacterial agents are potent in engendering the formation of antibiotics resistant bacterium which is considered an almighty threat to public health since it hinders the treatment of many infections in animals and man alike. Especially, the development of antibiotic resistance poses some threats such as; treatment failure and rise in healthcare costs [8].

Case Studies: Amoxicillin and Metronidazole

Lactam antibiotics such as amoxicillin and nitroimidazole antibiotics such as metronidazole are two of the most famous and most risky of the pollutants. Amoxicillin is a popular β-lactam antibiotic that has been identified often in wastewater and aquatic environments; it affects the microbial flora that maintain important fish organs – such as the bronchial arches – of common carp (*Cyprinus carpio*) [9]. This disruption impacts the gills, liver, kidneys, as well as the brain, which decrease respiratory ability, changes immunological reactions, and intensifies disease vulnerability that forms constant health challenges for fish [10].

In a similar way, metronidazole causes unfavorable histopathological effects on aquatic organisms as well. These antibiotics have negative consequences not only for people's health but also for the organism's functions of fish and destabilization of the species as well as aquatic environments. Additive effects of chronic antibiotic usage may decrease the abundance of important species in a body of water, and this disrupts the web and ecosystem of such water systems [11].

Antibiotic Use in Aquaculture

Intake of antibiotics in aquaculture has been found to pollute water systems in a very big way. With the increasing trend of fish and seafood consumption, aquaculture practices have developed greatly, and diseases which occur frequently due to high density in fish farms are treated with antibiotics [12]. However, the removal of such compounds is never total when the wastewater undergoes treatment which opens room for such contaminants to flow into surrounding water bodies and alter the biochemical balances and exert selective pressure for antibiotic-resistant organisms. Consuming fish that is polluted with antibiotic resistant bacteria means that people are transferring antibiotic resistance through food chain hence posing great threats to their lives [13].

Ecotoxicology and the Need for Comprehensive Studies

Thus, antibiotic and other pollutants influence the inhabiting water-acoustic organisms, especially important in ecotoxicology as the study of the effects of pollutants on living organisms and ecosystems. These contaminants can have negative impacts on the health and yield of farmed species, potential dangers to human health through bioaccumulation of pathogenic elements in commercial species [14].

Further studies should focus on the sustained impact of antibiotics in the aquatic environments so as to establish legal interventions, measures, which will prevent harm, but not keep antibiotic usage in healthcare and aquaculture industries from benefits. [15]. Consequently, whenever determining the effectiveness of effective antimicrobial compounds in handling diseases in human and veterinary practices, environmental consequences such as the release of the antibiotics through aquaculture practices should be taken into consideration. Interactions between antibiotic application, environmental pollution, and antibiotic-resistant bacteria challenging the sustainability of aquaculture and aquatic ecosystem balance are vital to future generations.

MATERIAL AND METHODS

The biological aims of the present study were to determine the effect of antibiotics exposure on the brain, gill, liver, and kidney of common carp (*Cyprinus Carpio*). This work was conducted at the Institute of Zoology, Bahaudin Zakariya University, Multan.

Fish Samples

In this study, thirty common carp (*Cyprinus carpio*) fish measuring between 20g and 50g for each fish were used. Fish collections were made from urban ponds in Kot Addu, Southern Punjab, during July 2024 and transported in oxygenated plastic bags to the laboratory of Bahauddin Zakariya University. On arrival, fish were

subjected to acclimatization for 15 days to balance out the environment before experimentation. They were placed in three aquaria, ten to each, with water aerators placed in the water tanks. It also anchored the fish with commercial pellet presented to the fish daily and also changed the water in the tanks daily. Physical and chemical characteristics were documented frequently, water temperature was kept at 26°C. DO ranges were maintained at between 7 and 8 mg/L while the pH was maintained at between the values of 7 and 7.5.

Antibiotics Exposure

The study included three experimental groups: an unexposed control group (C1) in tap water without antibiotics and an exposed control group (T1 and T2) in the same tap water but containing antibiotics. Treatment for T1, which was initiated at 20 mg/L of amoxicillin purchased at a local pharmacy, T2 received metronidazole at the same concentration. The exposure time required was 7 to 14 days in order to observe the responses of the fish to the antibiotics. These concentrations were chosen based on the natural habitat level of the fish species and from prior scientific investigations into the toxicity of antibiotics to fish. In a day, they took out 35% of the water from the tank and filled the tank with fresh water again.

Antibiotic Characteristics and Administration: Amoxicillin and Metronidazole

Amoxicillin which is promoted with the brand names of Amoxll and Trimox has the chemical formula of C16H19N3O5S and molecular weight of 419.45 g/mol. It is produced by GlaxoSmithKline and is offered through exposure as medicated feed. Also, metronidazole sold under the trade names Acea, Anabact, Flagyl and Metrogel with the Chemical formula; $C_6H_9N_3O_3$ and molecular Weight; 171.156 g/mol manufactured and distributed through medicated feed and exposure by Abbotts

Fish Groups

Figure 2.1

A Control Group

Figure 2.1 B

Amoxicillin Treatment

Figure 2.1 C

Metronidazole Treatment

Table 2.1

Description of groups

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

They randomly divided the samples into three groups and placed them under exposure with the antibiotics for 7-14 days.

Water Quality Parameters

Experimental Conditions and Measurements

The water temperature was stable at 26°C with the help of a digital thermometer (FT-710, accuracy± 0.1 °C) which was immersed in the experimental tanks. A calibrated pH meter AZ8685 was employed in establishing the water pH of between 7.0 and 7.5 and checked frequently, for stability. DO levels were measured using an oxygen meter (AR8210) ensuring they ranged between 7 and 8 mg/L. The water pH and dissolved oxygen levels were monitored daily in order to

stabilize the experimental conditions during the course of the study.

Table 2. 2
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	22-32C
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

Fish Dissection
The anatomical procedure followed in order ensure that there was correct organ pickup and preservation include the following on common carp (*Cyprinus carpio*). Firstly, the fish was anaesthetized using MS-222 just to reduce as much stress as possible during the experiment. Once no pathologies had been identified, the fish were placed with their ventral surface in contact with the dissection board and its pectoral fin, and an IOP from the anal fin was made to expose internal organs. They used forceps to loosen the cranium before lifting it to remove the brain; with gill branches free, the liver was also taken out. With scissors, the kidneys were disconnected from the backbone. Each organ was immediately placed in 10% formalin solution to ensure that the tissues are preserved for each sample, appropriate labels were on them.

Histological Procedure
The histological procedure for fish involves three main steps: A global fixation involves the use of 10% formalin for the purpose of not allowing tissue changes to occur; tissue processing entails washing of the tissues, dehydration through gradual steps of alcohol dilution, infiltration of the tissues with paraffin; and section cutting where the tissues are cut into sections floated in water bath, placed on glass slide after dehydration using hot plate.

Microtome Protocol
Paraffin embedded tissues of the liver, kidney and gill were sectioned using a rotary microtome for light microscopy. Samples were washed in tap water and arranged on sterilized glass slides for histological study under microscope for histological changes.

Image Analysis
Image analysis was carried out from the electron microscopic examination of the slides having been averted through Olympus compound microscope with a 4x, 10x and 40x zoom lens and ImageJ from National Institute of Health. Adobe Photoshop was used for cropping and fixing the images as well.

Statistical Analysis
Data were analysed by one-way analysis of variance (ANOVA) and data presented as mean ± standard deviation (SD).

DATA ANALYSIS & RESULTS
Effect of Antibiotics on Various Organs of *Cyprinus carpio*
Histopathological Effect of Antibiotics on Gills of Common Carp
The investigation on the effects of metronidazole (MNZ) and amoxicillin (AMX) on common carp (*Cyprinus carpio*) reveals notable histological alterations, especially in the gills, a vital respiratory organ. The untreated fish's control gills showed normal, healthy structures with intact epithelial layers and secondary lamellae that were well organized shown in (Figure 3.1-A). Nonetheless, exposure to the two medications showed unique damage patterns that differed in duration and severity.

Following a 7-day exposure to AMX, the gills showed only minor changes. A small lamellar fusion and epithelial hyperplasia (an increase in the number of epithelial cells) were noted, indicating mild stress but no significant tissue injury. These alterations were minor and most likely reflected the fish's adaptive reaction to the medication rather than widespread damage shown in (Figure 3.1-B).

On the other hand, MNZ exposure for the same amount of time resulted in more serious harm. A noticeable marker of tissue damage and an early warning sign of impaired gill function was epithelial hyperplasia, which was accompanied by lamellar fusion and epithelial lifting shown in (Figure 3.1-D).

Comparison of AMX and MNZ Exposure on Gills Tissues
The distinctions between the two treatments become even more noticeable when the exposure period increased to 14 days. While the effects of MNZ grew considerably more severe, fish treated with AMX did not exhibit a substantial worsening of the histological damage, with epithelial hyperplasia and lamellar fusion being controlled.

Extensive epithelial hyperplasia in the gills of fish exposed to MNZ for 14 days resulted in the complete coalescence of lamellae and the proliferation of mucous cells, which is frequently a defensive reaction to irritation or harm. These results suggest that MNZ caused substantial, long-lasting damage to gill tissue, resulting in notable structural and functional abnormalities, especially over extended exposure times (Figure 3.1-H).

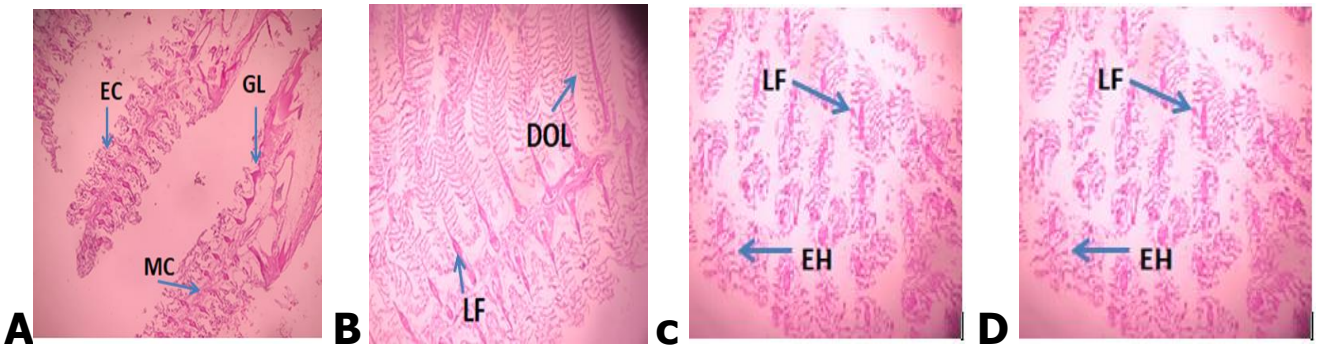
The gills of fish treated with 7 day AMX showed only a slight epithelial hyperplasia and a slight lamellar fusion (Figure 3.1-B). The tissue damage at this stage was small. However, gills of the MNZ-treated fish exhibited moderate epithelial hyperplasia, higher

degrees of lamellar fusion and epithelial lifting (Figure 3.1-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 3.1-E) 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 shown in (Figure3.1-H).

Figure 3.1

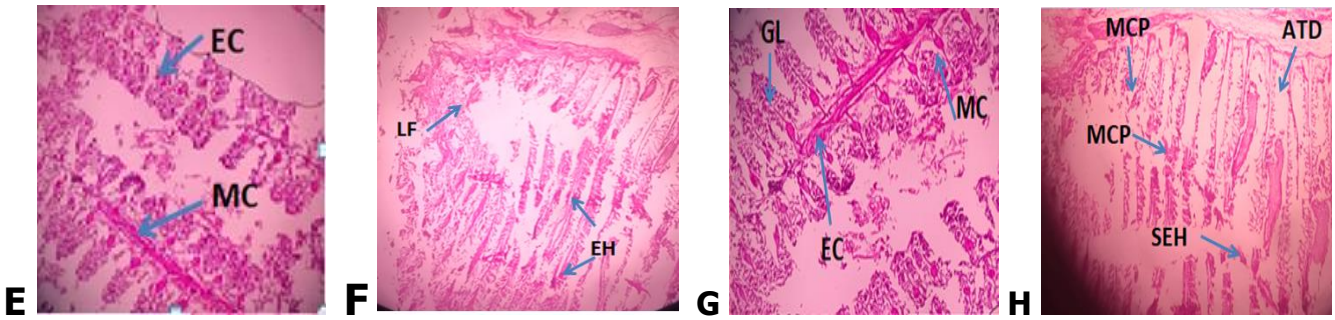
Representative H&E 10 Images (control and post treatment Gills sections)

A: AMX Control 7 days	B: AMX treatment 7 days post treatment	C: MNZ Control 7 days	D: MNZ treatment 7 days post treatment



The photomicrograph of gills of *C.Carpio* 7 days exposure: **(A)** Normal tissue with intact epithelial cells EC, Mucus cell MC and secondary gills lamellae GL. **(B)** slight epithelial hyperplasia EH and disorganization of lamellar fusion DOL. **(C)** normal epithelial cells EC and gill lamellae GL AMX 7 dy control. **(D)** Mild epithelial hyperplasia EH and lamellar fusion is seen

E: AMX Control 14 days	F: AMX treatment 14 days post treatment	G: MNZ Control 14 days	H: MNZ treatment 14 days post treatment



The photomicrograph of gills of *C.Carpio* 14 days exposure **(E)** AMX 14dy control showing intact epithelial cells EC and mucus cells MC.(**F)** gills with increased epithelial hyperplasia and lamellar fusion**.(G)** 14 dy MNZ control showing intact structures**.(H**) MNZ 14 dys treated section indicate severe epithelial hyperplasia SEH, mucous cell proliferation MCP ,total lamellar coalescence TLC and Advanced tissue damage ATC.

Histopathological Effect of Antibiotics On Liver of Common Carp

The normal liver tissues comparisons were represented by intact hepatic cells without signs of degenerative or pathological transformations in their structure (Figure 3.2-A).Mild changes in the hepatic cells were observed in the group receiving amoxicillin at a dose of 20 mg/l for 7 days; the groups showed moderate fatty changes in the liver without much damage to the tissue and also no high cellular stress as seen in (Figure 3.2-B). However, livers from animals treated with metronidazole (MNZ) for the same duration showed moderate degree of vacuolation, bile duct congestion and cytoplasmic vacuolation, which suggest a rather adverse effect than that observed in AMX (Fig 3.2-D). After the AMX exposure for 14 days, the hepatocytes exhibited a centrilobular distributed degeneration and vacuolization in the liver tissue sections indicating both adaptation

(Figure 3.2-F). After 14 days of treatment with MNZ hepatocytes revealed severe pathological changes: extensive bacillar vacuolation and bile stasis with higher grade of cytoplasmic vacuolization compared to AMX (Figure 3.2-H).

Comparison of AMX and MNZ Exposure on liver Tissues

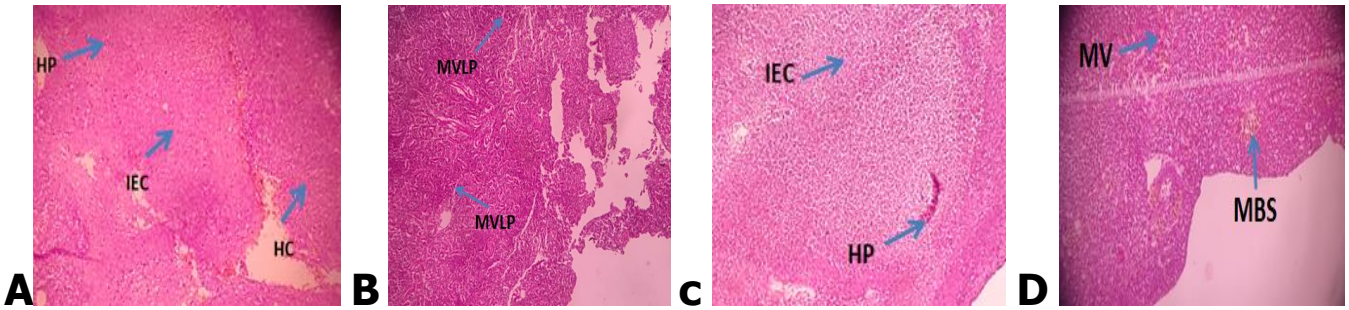
The histopathological changes in the liver of fish when exposed to AMX and MNZ are different at both 7 days and 14 days of treatment. After 7 days, liver tissues of AMX-exposed groups showed only slight vacuolization and little evidence of cellular stress; there were no severe hepatocellular degeneration; so AMX induced only mild liver changes at this time point (Figure 3.2-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 3.2-D).

This was so at the 14th day where the differences were more evident. Hepatocytes in livers of AMX treated groups displayed increased vacuolization and initi.al signs of hepatocellular degeneration suggesting moderate but evident effect of AMX on liver health after prolonged administration (Figure 3.2-F). 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 3.2-H).

Figure 3.2

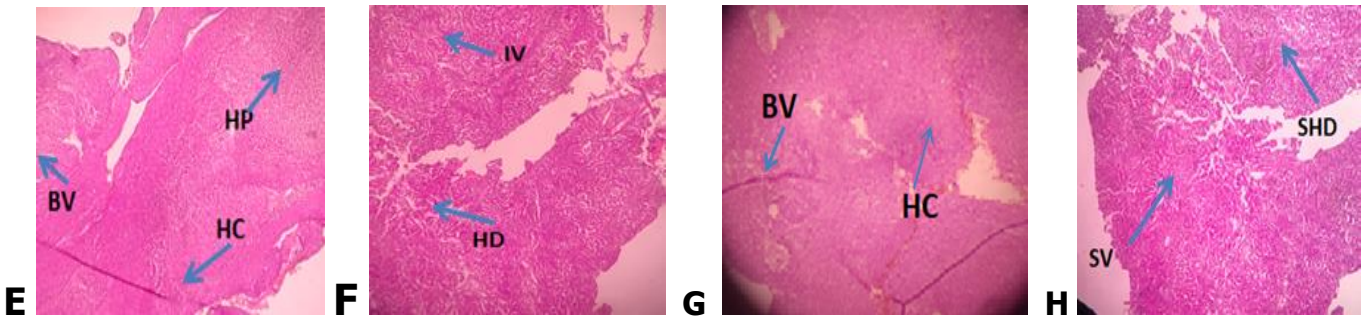
Representative H&E 10X images (control and post treatment Liver sections)

A: AMX Control 7 days	B: AMX treatment 7 days post treatment	C: MNZ Control 7 days	D: MNZ treatment 7 days post treatment



Photomicrograph sections of liver of *C.carpio* 7 days exposure **(A)** intact hepatic cells HC, without any degenerative Hepatic pathology HP. **(B**) Mild vacuolization in liver parenchyma MVLP**.(C)** MZN 7 days control showing normal hepatic cells.(**D**) tissue reveal moderate bile stagnation MBS and Moderate vocalization MV.

E: AMX Control 14 days	F: AMX treatment 14 days post treatment	G: MNZ Control 14 days	H: MNZ treatment 14 days post treatment



Photomicrograph o sections of liver of *C.carpio* 14 days exposure **(E)** control tissue indicate normal liver structure with hepatic cells HC, Blood vessel BV**.** (**F**)AMX 14 days treated tissue showing increased vacuolization IV and hepatocellular degeneration HD.(**G**) control with intact liver structure comprising Blood vessel BV and hepatic Cells HC**. .(H)** MNZ 14 days treated tissue showing Severe vacuolization or cytoplasmic vacuolization and severe hepatocellular degeneration SHD

Histopathological Effect of Antibiotics on kidney of Common Carp

Kidney tissue of the control group was normal with no vacuolization or witness of any pathological changes on renal structure shown in (Figure 3.3-A). At day 7 of AMX treatment, there was slight renal tubule

vacuolization in the tissue indicating some degree of tissue damage but the architectures remain relatively preserved shown in (Figure 3.3-B). On the other hand, histopathological changes in MNZ exposed fish observed showed moderately severe response with features such as tubular vacuolation and early cytoplasmic degeneration in kidneys shown in (Figure 3.3-D). At the end of 14 days exposure of AMX the nuclei in the renal tubules were larger and more vacuolated and therefore their tissue stress score was moderate shown in (Figure 3.3-F).However, in the comparison with AMX exposure to MNZ for 14 days there was severe vacuolar degeneration, and virtually disintegration of the renal structures, pointing to higher pathogenicity of MNZ degeneration shown in (Figure-3.3-H).

Comparison between groups of AMX and MNZ

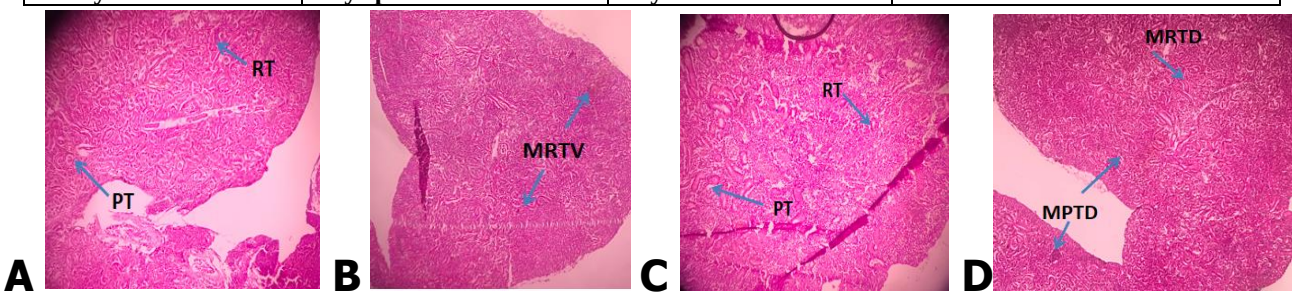
Comparing the effects of AMX and MNZ on the kidney tissues, it is shown that MNZ has a more pronounced effect on the renal tissues than AMX specially when its exposed for a long time. In the AMX-treated tissues,7 days of exposure, only a mild vacuolization was observed, and other signs of stress as well as significant structural changes were not detected (Figure 3.3-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 3.3-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 3.3-F). 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 3.3-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 3.3-H).

Figure 3.3

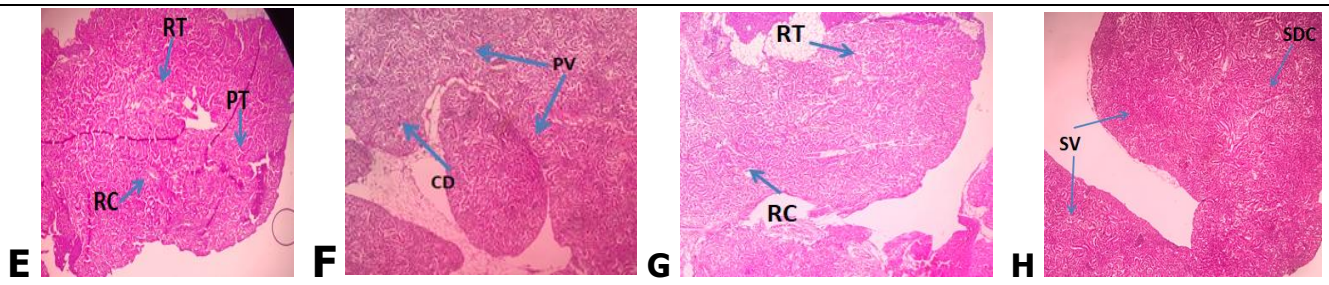
Representative H&E 4& 10X images (control and post treatment Kidney sections)

A: AMX Control 7 days	B: AMX treatment 7 days post treatment	C: MNZ Control 7 days	D: MNZ treatment 7 days post treatment



Photomicrograph of kidney of *C.Carpio* 7 days exposure **(A)** Normal renal structure (control) showing renal tubules RT and proximal Tubules PT**.(B**) showing the mild renal tubule vacuolization MRTV**.(C)**MZN7 dy control showing intact renal corpuscle RC ,Renal tubule RT and proximal tubule PT**.(D**) moderate proximal tubule degeneration MPTD seen

E: AMX Control 14 days	F: AMX treatment 14 days post treatment	G: MNZ Control 14 days	H: MNZ treatment 14 days post treatment



Photomicrograph of kidney of *C.Carpio* 14 days exposure **(E)** AMX Control showing normal renal cells RC , Renal tubule RT and proximal tubule PT.**(F)** section showing pronounced vacuolization PV and cytoplasmic degeneration of renal tubules CD indicate irregular arrangement of tubular cells**.(G)** MNZ 14 day control showing normal renal structure with renal tubules RT and renal corpuscles RC (H) MZN 14 day section showing the degenerative kidney with severe vacuolization SV and severe degenerative renal cells SDC.

Histopathological Effect of Antibiotics on brain of Common Carp

In addition, from the H&E staining, the brain tissues of the control group displayed no necrosis, vacuolization as well as no pathological alterations in neuronal cells (Figure 3.4-A).

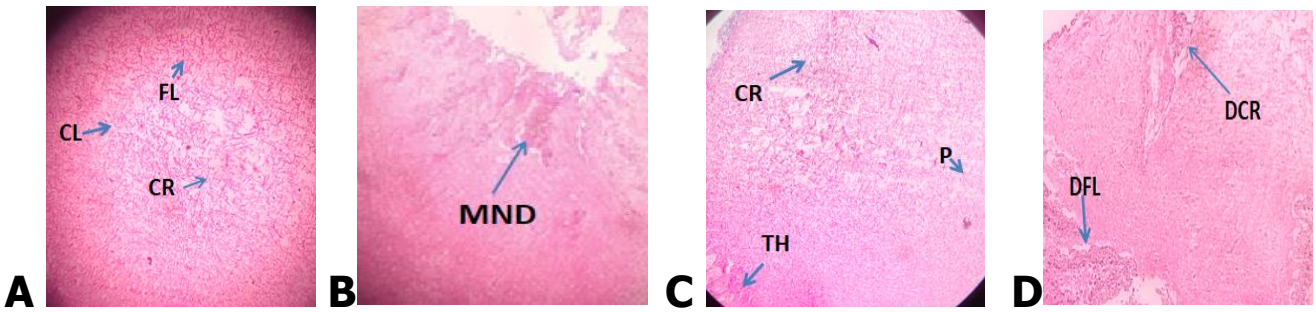
In the group which received amoxicillin (AMX) for 7 days, moderate neuronal damage along with slight vacuolation showed that the animals had a mild early stage of stress (Figure 3.4-B). In contrast, brains from rats treated with metronidazole (MNZ) for 7 days showed a low grade of neuronal damage, greater vacuolation and signs of the beginnings of necrosis; there were phenomena of a higher pathological reaction than from AMX (Figure 3.4-D).

By day 14, AMX exposure elicited additional effects on neuronal damage through increased vacuolation and mild signs of necrosis, which points to chronic toxicity which imparts moderate toxicity (Figure 3.4-F). In contrast, brains from MNZ-treated group exhibited complicated lesions: widespread neuronal loss, vacuolar change and necrosis suggesting that MNZ has a greater toxic effect on the brain tissue (Figure 3.4-H).

Comparison of AMX and MNZ Exposure on brain Tissues

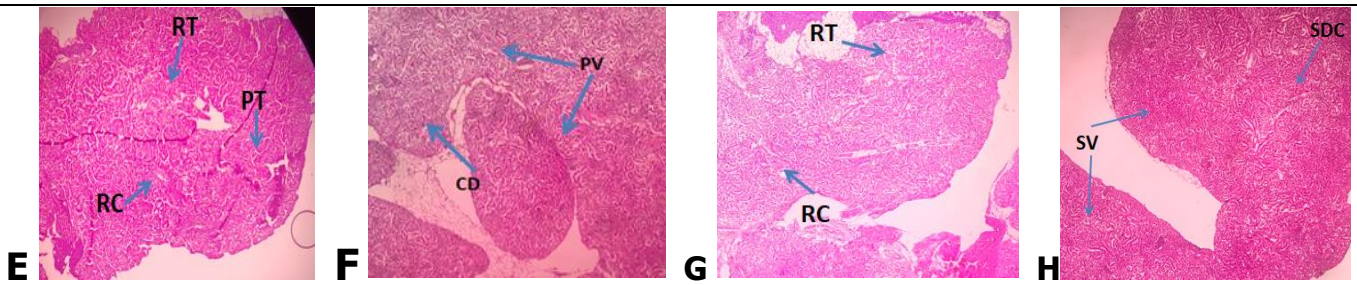
The comparative analysis of the impact of AMX and MNZ on the brain tissues indicates that MNZ has greater neurotoxic effect than AMX. AMX-treated brain tissues exposed for 7 days also demonstrated only mild neuronal degeneration and minimal vacuolization, which suggests that AMX induces only a slight stress response with no significant morphological changes in the brain (Figure 3.4-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 3.4-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 3.4-F). 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 3.4-H).

A: AMX Control 7 days	B: AMX treatment 7 days post treatment	C: MNZ Control 7 days	D: MNZ treatment 7 days post treatment



Photomicrograph of brain of *c.carpio* 7 days exposure **(A)** Control showing the normal histological structure of brain of fish with cerebellum CL ,olfactory lobe FL connected with cerebrum CR.**(B**) AMX 7 days exposed tissue section showing mild neuronal degeneration MND.**(C**) control of MNZ 7 days indicate the normal brain cells and thalamus TH , cerebrum CR,and pituitary gland P.**(D**)MNZ treated showing moderate degenerated cerebrum DCR and degenerated olfactory lobe DFL.

E: AMX Control 14 days	F: AMX treatment 14 days post treatment	G: MNZ Control 14 days	H: MNZ treatment 14 days post treatment



Photomicrograph of brain of *c.carpio* 14 days exposure **(E)** AMX 14 dy control show normal hypothalamus HT &olfactory lobe FL connected with cerebrum CR.**(F)** Showing increased degeneration in olfactory DFL , degenerative cerebrum DCR and degenerative hypothalamus DTH.**(G**) MNZ 14 day control showing the normal olfactory lobe FL and hypothalamus HY**(H)** showing severe neuronal degeneration SND and necrosis N.

Figure 3.4 *Representative H&E 10& images (control and post treatment Brain sections)*

Analysis of The Groups (ANOVA) Both AMX and MNZ induced neuronal lesion in both the cerebral cortex and hippocampus but MNZ was more toxic causing more necrosis and vacuolization than AMX with chronic exposure.

Table 3
ANOVA

Source	SS (Sum of Squares)	DF (Degrees of Freedom)	MS (Mean Square)	F-value	P-value
Antibiotic Type	85.6	1	85.6	23.64	**0.0001
Exposure Duration	120.4	1	120.4	33.25	***0.00001
Antibiotic Type × Exposure Duration	32.5	1	32.5	8.97	*0.003
Error	420.0	116	3.62		
Total	658.5	119			

Significance level =p <0.05 , ***p= 0.00001 , highly significant P = *0.003 > **0.0001 > ***0.00001
ANOVA results indicate that both antibiotic type and exposure duration significantly impact tissue damage, with a significant interaction between these factors. The main effect of antibiotic type (AMX vs. MNZ) showed an F-value of 23.64 and a p-value of 0, highlighting that MNZ likely causes greater tissue damage than AMX.

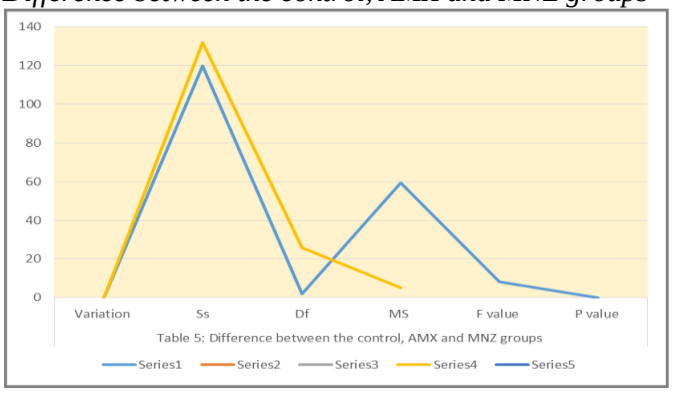
The exposure duration also had a significant effect, with an F-value of 33.25 and a p-value of 0.00001, indicating that longer exposure (14 days vs. 7 days) leads to more tissue damage. Additionally, the interaction effect had an F-value of 8.97 and a p-value of 0.003, suggesting a dose-dependent relationship, where MNZ is particularly destructive with extended exposure.

Overall, the findings confirm that both the type of antibiotic and the duration of exposure are critical determinants of tissue damage, especially for MNZ over prolonged period

Table 4
Difference between the control, AMX and MNZ groups

Variation	Ss	Df	MS	F value	P value
Between groups	119.8	2	59.5	8.3	0.002
Within groups	132	26	4.95		

Figure7
Difference between the control, AMX and MNZ groups



There was statistically significant difference among the three groups as evidenced by the p-value < 0.05 on histological changes caused by antibiotic. Both antibiotics were shown to have lethal or toxic effects to fish tissues. More treatment-related pathological changes were observed in rats with increased antibiotic intake or 14 days of AMX exposure than those of the 7-day AMX treatment.

DISCUSSION

Histological alterations in the common carp upon exposure to amoxicillin (AMX) and metronidazole (MNZ) include vacuolation and necrosis in several tissues which were more severe in the latter treated with MNZ.

Some effects of exposure to AMX were observed: after 7 days gills demonstrated mild epithelial hyperplasia and minimal lamellar fusion that may reduce respiratory efficiency. However, MNZ induced m4oderate epithelial proliferation and partial only lamellar fusion, thus it has more toxic effect. Thus, AMX led to epithelial hyperplasia and increased numbers of mucus cells by day 14 and severe epithelial hyperplasia and complete coalescence of lamellae following MNZ, which considerably impairs gas exchange.

The tissues of AMX-treated fish also showed only a slightly vacuolated kidney with no clinical presentation of severe cellular stress, leading to the conclusion that it is A.R for short term use. On the other hand, MNZ led to moderate vacuolation, early cholestasis, cytoplasmic degeneration, while after 14 days hepatocellular necrosis, total cholestasis and bile stasis indicating high toxicity.

The results showed that MNZ had more toxic effects on the renal tissue as compared to other groups, and time-dependent effects where moderate vacuolar changes as well as severe tubular degenerative changes were observed that may compromise renal function. AMX on the other hand led to very few toxic effects during the short term exposure.

Long term exposure of AMX caused slight brain damage after day seven but extreme damage after

continuous exposure. MNZ invariably led to moderate neuronal toxicity and early necrosis characterized by oxidation and inflammation that may interfere with neurotransmission.

A comparative analysis has established the differences in tissue impact and comparing the results with the statistical data, it is revealed that MNZ had shown more toxic outcomes than both the standards, especially after 14 days. Furthermore, the findings of the present study supported by ANOVA show that antibiotic type exposure time has a significant effect on tissue damage and MNZ is relatively more toxic to the aquatic environment. These studies underscore the importance of properly managed feed the fish with antibiotics in order to reduce on the effects on growth and health of fish as well as the environment. In addition, exposure to MNZ seems to have immunosuppressive effects which may lead to increased risk of infection with prolonged use.

CONCLUSION

This study aimed at assessing the toxicity and histopathological effects of amoxicillin and metronidazole in common carp; the results show that metronidazole has more effects in organ systems. These histopathological alterations in the gills of common carp show that in both acute and chronic exposure, metronidazole has significantly higher toxicity than amoxicillin. This differential toxicity is especially worrisome because gills play a crucial role in respiration and health of fish population. The moderate epithelial hyperplasia and a great deal of mucus cell hyperplasia which were caused by metronidazole not only affect the gas exchange but also indicate the dysfunction of immune system. Such effects can result in enhanced susceptibility to pathogens hence posing a threat to the fish species and the aquatic environment. Taken together, these results lay down a foundation for furthering responsible our understanding of antibiotic usage in aquaculture and promoting manage effective strategies aimed at strengthening fish health devoid of negative consequences for the aquatic environment.

Future studies hence should concentrate on identifying the causes of the toxic effect in common carp and other water animals by analyzing metronidazole and amoxicillin exposure. The comprehension of the biochemical ways that contribute to antibiotic toxicity can allow medical personnel to devise superior strategies of controlling the disease outbreaks without jeopardizing the young fishermen. Moreover, further research into searching for the effects of introducing certain probiotics, herbs or other factors implying non-antibiotic approach for the fish health would seem equally helpful in keeping fish healthy and safe for consuming while being environmentally friendly. Such research when applied could help enhance aquaculture in ways that conserve fish stock, and or ensure sustainable farming techniques that are diofriendly and safe for human consumption.

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