

Toxicological Effects of Amitriptyline on Growth, Blood Parameters, and Liver and Kidney Functions of the Freshwater Carp *Cirrhinus mrigala*

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Abstract

Background: Pharmaceuticals are meant to treat diseases and promote human health. Antibiotics, anti-inflammatory medications, lipid-lowering compounds and anticonvulsants are the most often found pharmaceuticals in aquatic environments. Amitriptyline is a tricyclic antidepressant widely used to treat depression, chronic pain, anxiety, migraines, insomnia and neuropathic pain. It can enter aquatic ecosystems through wastewater effluents, potentially affecting non-target organisms including fish. *Cirrhinus mrigala* (mrigal carp) is an essential commercial and food fish in South Asia, widely cultivated in fish farms due to its quick development rate and adaptability to a wide range of environmental conditions.

Objective: The current study was designed to investigate the toxicological impacts of amitriptyline on growth performance, hematological parameters, liver function, and renal functions of *Cirrhinus mrigala*.

Methods: The *Cirrhinus mrigala* used in the experiments were obtained from a fish hatchery in Punjab, Pakistan. Fish were acclimated to room temperature in 80-liter glass aquariums for two weeks before receiving amitriptyline treatment. Three groups of fish were created: Group A served as control, while Groups B and C received amitriptyline at 0.125 mg/L and 0.25 mg/L, respectively. Amitriptyline was administered through water, and water was changed after 24 hours. After 28 days of treatment, three fish from each group were randomly selected for sampling. Blood samples were collected via cardiac puncture using heparinized needles and placed in EDTA tubes. Growth parameters (weight and length) were measured before and after the trial. Hematological analysis included WBC, RBC, HGB, PLT, MPV, PDW, PCT, HCT, MCV, MCH, MCHC, RDW, LYM, MON, and GRA. Serum was extracted by centrifugation for biochemical analysis of SGPT, SGOT, urea, and creatinine to assess liver and kidney functions. Data were analysed using GraphPad Prism (V. 9.3) with one-way ANOVA and t-tests applied to determine statistical significance.

Results: The results indicated that Weight significantly increased from (0.91±0.31g) of control group to (3.61±1.57g) of low dose group and (4.12±0.60g) of high dose group. Length significantly increased from (0.89±0.15cm) of control group to (1.79±0.29cm) of low dose group and (2.87±0.38cm) of high dose group. Hematological analysis showed that WBC, Platelets and PCT increased with increasing the concentration of amitriptyline. While, RBCs, HGB, HCT, LYM and MCV increased in low dose of amitriptyline and decreased I high dose and MPV, PDW, MON, GRA, MCHC and RDW decreased in low dose of amitriptyline and increased I high dose.

Conclusion: Amitriptyline exposure significantly altered growth performance, hematological parameters and hepatorenal functions in *Cirrhinus mrigala*. The observed paradoxical growth enhancement alongside multi-organ toxicity indicates that amitriptyline poses significant toxicological risks to this commercially important fish species.

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1. Introduction

Pharmaceuticals have become indispensable to modern society, yet their pervasive presence in aquatic ecosystems represents a profound and growing environmental challenge. A diverse array of pharmaceutical compounds including antibiotics, anti-inflammatory drugs, lipid regulators, and neuroactive substances is continuously introduced into waterways, where they are

detected at concentrations ranging from nanograms to micrograms per liter. [1] A primary pathway for this contamination is the incomplete removal of these compounds by conventional wastewater treatment plants (WWTPs), which are not designed to eliminate complex pharmaceutical residues. Recent monitoring studies confirm that despite removal efficiencies exceeding 75% for some pharmaceutically active compounds, many compounds persist in effluent and are released into receiving rivers, giving them a pseudo-persistent character and posing risks to the entire river ecosystem. [2]

Among the most concerning classes of emerging contaminants are antidepressants, whose consumption has risen dramatically worldwide. Amitriptyline, a tricyclic antidepressant, is one of the most widely prescribed medications globally, used not only for depression but also for chronic pain, migraine prophylaxis and anxiety disorders. Its extensive use inevitably leads to environmental release, and amitriptyline has been repeatedly detected in WWTP effluents and surface waters. The maximum reported concentrations of amitriptyline in effluent and river water in the United Kingdom are 0.243 μg L^{-1} and 0.0716 μg L^{-1}, respectively, with local hotspots reaching low μg L^{-1} levels in specific watercourses. [3] More recent monitoring has identified amitriptyline as the most abundant analyte in water and sediment from cold-region rivers, reaching low μg/L concentrations downstream of WWTPs. These environmentally relevant concentrations are sufficiently high to interact with the conserved neuroendocrine systems of aquatic vertebrates, raising substantial concerns about ecological consequences. [4]

To evaluate the risks posed by such contamination, it is essential to study sentinel species that are both ecologically and economically significant. *Cirrhinus mrigala* (mrigal carp), a cyprinid native to the Indo-Gangetic riverine system, is one of the three Indian major carps and a cornerstone of freshwater aquaculture across South Asia. [5] It is an economically important fish in aquaculture, and its culture has spread due to its suitable stimulated reproduction and rapid growth, it is an important animal for polyculture systems in India and other parts of South Asia, contributing significantly to fish production in the region. As a bottom-dwelling, detritus-feeding species, *C. mrigala* occupies a distinct ecological niche that may render it particularly vulnerable to sediment-associated and waterborne contaminants. [6] Despite its commercial and ecological importance, the toxicological impact of pharmaceutical pollutants on this species remains almost entirely unexplored, creating a critical knowledge gap in the risk assessment of antidepressants in South Asian aquatic environments.

The toxicological effects of amitriptyline on fish are multifaceted, targeting behavioral, physiological, and biochemical endpoints. Recent research on model species such as zebrafish (*Danio rerio*) has demonstrated that exposure to environmentally relevant concentrations can disrupt neurotransmitter systems, leading to altered locomotor activity and anxiety-like behaviours. [7] For instance, combined exposure to amitriptyline and microplastics significantly elevated locomotor activity and altered social behaviour in zebrafish, accompanied by decreased ocular levels of superoxide dismutase, catalase, and glutathione, indicating oxidative stress. [8]

Beyond neurobehavioural toxicity, amitriptyline exposure has been shown to impair xenobiotic metabolism and reduce the activity of critical detoxification enzymes. Furthermore, emerging evidence indicates that these effects may not be confined to the exposed generation; parental exposure to amitriptyline at environmentally relevant concentrations (0.8 and 8 μg/L) significantly reduced locomotor activity in both adult zebrafish (F0) and their unexposed offspring (F1), with these behavioural alterations linked to changes in monoamine neurotransmitter levels. [9] Similarly, parental amitriptyline exposure has been shown to affect survival, development, behaviour, and gene expression in zebrafish offspring, highlighting the multigenerational risk of this contaminant. [9] However, the vast majority of this mechanistic work has been conducted on small model fish, and there is a conspicuous absence of data on how amitriptyline affects the growth, haematology, and organ function of commercially important aquaculture species like *C. mrigala*. [10] While toxicological studies on *C. mrigala* have begun to employ multiple biomarker approaches for other contaminants such as triclosan, no study has yet applied an integrated biomarker framework to assess amitriptyline toxicity in this species.

2. Rationale and Objectives

The present study was therefore designed to address this critical gap by evaluating the sub-lethal toxicity of amitriptyline in *Cirrhinus mrigala*, using an integrated suite of biomarkers.

The specific objectives were:

- To investigate the impacts of amitriptyline on growth of *Cirrhinus mrigala*
- To investigate the impacts of amitriptyline on hematology of *Cirrhinus mrigala*
- To study the impacts of amitriptyline on the functions of liver of *Cirrhinus mrigala*
- To check the impacts of amitriptyline on the function of kidney of *Cirrhinus mrigala*

3. Materials and Methods

3.1. Collection and Transportations of Experimental Fish

Fish seedlings were obtained from the aquaculture pond Manga Mandi Lahore, Punjab, Pakistan. The specimens weighed an average between 15-20g. The young plants were safely transported in plastic bags with plenty of oxygen and water. Specimens were then placed in tanks with aerators and aquarium heaters to keep the water at the appropriate temperature and humidity. The trial will begin after seven days of acclimating fish seedlings in glass tanks. The electrical conductivity meter was used to periodically check the pH and other water quality parameters.

3.2. Experimental Design

The *Cirrhinus mrigala* used in the experiments came from a fish hatchery in Punjab that had been given permission to breed the species. The fish were acclimated to their 80-liter glass aquariums at room temperature for around two weeks before receiving amitriptyline treatment. During acclimatization fish were fed two times per day. After acclimatization fish were divided into 03 groups. Group A was used as control group while group B and C were treated with 0.125 mg/l and 0.25 mg/l of amitriptyline respectively. Amitriptyline was given to fish through water and water was changed after 24 hours.

3.3. Growth

Body length of fishes was measured one by one with the help of measuring tape before and after the trial both in control and treatment groups.

Weight Gain (G) = Final Body Weight (G) – Initial Body Weight (G)

Length Gain (Cm) = Final Body Length (Cm) – Initial Body Length (Cm)

3.4. Sampling

After 28 days of therapy, three fish from each group were chosen at random for sampling. Heparinized 2-ml needles were used to collect blood samples from a heart puncture, and the blood was then placed in EDTA tubes with separator gel. The fish's organs were removed and preserved in formalin, including its liver, kidneys, heart, intestines, and gills. Fish from the control group and both treatment groups had blood and tissue samples taken on the same day for haematological and histopathological analysis.

3.5. Blood Sample Analysis

After 28 days of experimental investigation, three blood samples of *C. mrigala* were taken from each group and were examined. The blood samples were used for studies of HGB, RBCs, WBCs, MPV, PDW, PCT, HCT and no. of platelets.

3.5.1 Haemoglobin (Hgb)

Haemoglobin is a protein found in red blood cells (RBCs) that carries oxygen to tissues that need it and returns carbon dioxide to the lungs. The general fisheries-lab at the University of Okara quantitatively measured HGB. This was accomplished by combining 5ml of HGB solution with 20l of whole blood using an equipment set to a wavelength of 540nm. After letting the mixture sit for five minutes at room temperature, the HGB content was determined in g/dl.

3.5.2 White Blood Cells (WBC)

WBCs are involved in protecting body against foreign microorganisms and infectious diseases. Following formula was used for the measurement of WBCs:

WBC/µL = (Average of cells × Correction for dilution) / (No. of squares × Volume of one square)

3.5.3 Total Red Blood Cells Count (TRBC)

Red blood cells (RBCs) are the most common form of blood cell and are responsible for transporting oxygen throughout the body via blood flow to the various tissues. TRBCs were measured by following:

$$\text{RBC (\%)} = \frac{50\ (\text{volume factor}) \times 200\ (\text{Dilution Factor}) \times \text{Number of cell counted}}{100}$$

3.5.4 Platelets (PLT)

Platelets are in involved in blood clotting and their total count was measured by following formula:

$$\text{Total Platelets Count (per Liter)} = \frac{\text{Cells count (N)} \times \text{Dilution Factor (20)} \times 10^6}{Area\ (0.2\ mm^2)} \times Volume\ coated\ (0.1\ mm)$$

3.5.5 Mean Platelet Volume (MPV)

Platelets are in involved in blood clotting and their total count was measured by following formula:

$$\text{Total Platelets Count (per Liter)} = \frac{\text{Cells count (N)} \times \text{Dilution Factor (20)} \times 10^6}{Area\ (0.2\ mm^2)} \times Volume\ coated\ (0.1\ mm)$$

3.5.6 Platelets (PCT)

It is average volume of platelets in blood and is expressed in femtoliters. The formula which was used to calculate Mean Platelet Volume (MCV) is:

$$\text{MPV}\ (fL) = \text{Plateletcrit (\%)}\text{Platelets count}\ (\times 10^9/L) \times 10^5$$

Here, platelets is the ratio between total platelet volume and complete blood volume.

3.5.7 Platelet Distribution Width (PDW)

It is actually involved in the measurement of different morphometric changes occurred in platelets, regarding activation and size. A histogram for each blood sample was used to measure the platelet distribution width.

3.5.8 Haematocrit (HCT)

It is the part of blood that is composed of packed RBCs and is expressed in percentage. Following formula was used to count it:

$$\text{HCT (\%)} = \text{RBCs count}\ (\times 10^{12}/L) \times MCV(fL)/10^6$$

3.9. Enzyme Analysis

As an indicator, nitrotetraazolium blue chloride (NBT) was used in this procedure. There was 10 mM of NBT and 10 mM of hypoxanthine in the reaction mixture. The buffer comprised 100 mM phosphate and phosphate at a pH of 7.0. Liver and kidney enzyme extracts were combined with 0.023 U/mol of xanthine oxidase at 20 degrees Celsius to kick off the reaction. Inhibition of NBT served as a proxy for the enzyme's activity, which was then represented as units of activity per milligramme of protein.

The reaction mixture for GST activity was 2 ml of 100 mM chloro-dinitro benzene (CDNB) and 100 mM potassium phosphate buffer (pH 7.2). Before adding the sample, 100 mM GSH was mixed with both reagents at 25° C. The protein concentration was calculated from the 340 nm absorbance reading. After adding 500 ml of 4% sulphosalicylic acid to 100 ml of hemolysate, incubating the mixture at 4 °C for about an hour, and then centrifuging it at 12,000 RPM while maintaining a temperature of 4 °C for 15 minutes, we used this standard DTNB reagent. A mixture of 0.4 ml of the collected supernatant and 0.1 M of potassium phosphate (pH 7.4) was created in a test tube. The mixture was then supplemented with 0.4 cc DTNB. The spectrophotometer reading for the after-reaction of DTNB with GSH was a

brilliant yellow at 412 nm. The results were expressed as moles of protein per milligramme of sample.

Catalase enzyme activity was measured by following the process. The substrate for the H_2O_2 solution was prepared by mixing 50 mM of potassium phosphate buffer with 50 mM of peroxide. Spectrophotometric analysis of H_2O_2 breakdown produced by catalase was performed at a wavelength of 240 nm. Throughout, we maintained a 7.0 pH in the CAT.

Measuring MDA (Malondialdehyde content) is essential for studying lipid peroxidation in membranes. The method was used to calculate the MDA values. The reaction mixture consisted of.01 ml of tissue extracts and 2 ml of TBA-TCA-HCl. After 15 minutes in a boiling water bath, the mixture was allowed to cool to room temperature before being centrifuged at 4000 rpm for 10 minutes. The absorbance at 535 nm was analysed against a blank solution that contained all the reagents besides the sample. Micromoles per milligramme of protein were reported as the MDA concentrations.

3.10. Statistical Analysis

Data was analysed by using GraphPad (V. 9.3). Descriptive statistics was applied. To find P value and F value one-way ANOVA was applied. Coefficient R was measured in order to find the relationship between haematology and morphometry.

4. Results

This study was conducted to study the impact of amitriptyline on Growth, Blood Parameters, Liver Function and Renal Functions of *Cirrhinus mrigala.*

4.1. Growth Performance

4.1.1 Weight Gain

Weight significantly increased (0.0036**) from (0.91±0.31g) of control group to (3.61±1.57g) of low dose group and (4.12±0.60g) of high dose group.

4.1.2 Length Gain

Length significantly increased (0.010*) from (0.89±0.15cm) of control group to (1.79±0.29cm) of low dose group and (2.87±0.38cm) of high dose group.

Table 1: Comparison of weight gain and length gain of control group and treated groups

Group	Weight Gain	Length Gain
Control	0.91±0.31	0.89±0.15
Low Dose (5mg/L)	3.61±1.57	1.79±0.29
High Dose (10 mg/L)	4.12±0.60	2.87±0.38
F value	9.342	5.625
P value	0.0036**	0.019*

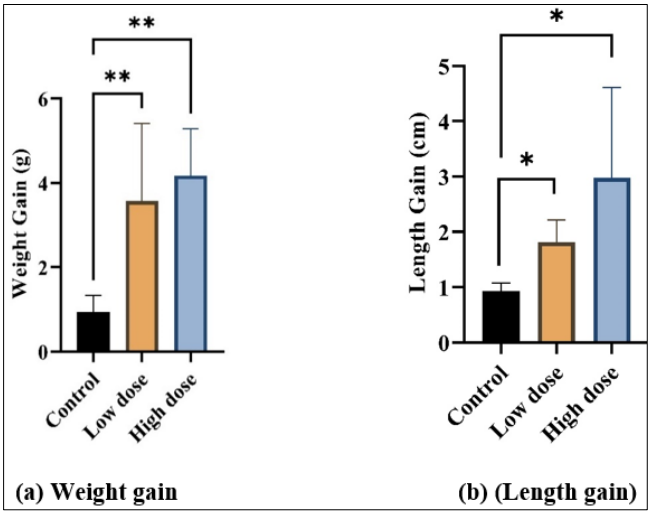

Fig 1: Comparison of growth performance of Cirrhinus mrigala exposed to amitriptyline: (a) weight gain and (b) length gain.

4.2. Haematological Analysis

Three experiments were performed with an increasing concentration of amitriptyline preservative in each experiment for 30 days trial. Those exposed to a low dose of 5 mg/L amitriptyline were labelled as T1, and those exposed to a high dose of 10 mg/L amitriptyline were tagged as T2. The blood samples were taken after the completion of the exposure periods to compute the increase and decrease in the various blood parameters.

4.2.1 Haematological Changes in Treatment 1

Comparison of various hematological parameters between *Cirrhinus mrigala* exposed to 5 mg/L (Treatment-1), with the untreated control group after 30-Days of the trial is shown in (Table 1). The Data is expressed as Mean ± Standard Deviation of Mean. P-value indicates the result of the 2-sample t-test calculated for each comparison in Table 1.

The white blood cell count (WBC) of fish given 5 mg/L amitriptyline was significantly higher than that of the control group (2.48 1.58). The RBC value of fish given 5 mg/L amitriptyline was found to be higher than that of the control group (0.81 0.32), at 2.21 1. 03.. Platelets increased in treated fish, in contrast to control group from 60.67 ± 12.91 to 77 ± 1.5. An increase in Haemoglobin concentration was recorded in fish exposed to 5 mg/L amitriptyline as mean of Haemoglobin was found to be 7.9 ± 0.7 in the treated group while the mean concentration of Hemoglobin in untreated control group fish was 5.85 ± 3.18.

MPV and PDW concentration increased to 7.3 ± 1.6, 8.2 ± 1 in treated fish as compared to untreated control group fish in which MPV and PDW concentration was 7.10 ± 0.42, 8.76 ± 0.28 respectively. PCT percentage increased from 0.03 ± 0.01 in control group to 0.55 ± 0.1 in the treatment group. In treatment group, increase in HCT percentage was recorded.

The values of HCT % in control group were 11.00 ± 4.514, and in treatment group was 33.9 ± 1.3. There was increase recorded in Lymphocytes percentage (99 ± 1) of fish exposed to 5 mg/L amitriptyline as compared to the control group (86.57 ± 8.534).

MON percentage decreased in treated fish, in contrast, to control group from 3.200 ± 2.156 to 0.7 ± 0.1. Granulocytes percentage also decreased in treated fish as compared to control group fish from 5.833 ± 1.750 to 0.3 ± 0.05. MCH concentration in untreated control group was 81.18 ± 12.32 and decreased to 35.3 ± 2.60 in treatment group. MCHC showed a decrease in treatment group as compared to the control group. In the control group, MCHC was recorded at 55.52 8.021, while in the treatment group, it was recorded at 23 1. Compared to the untreated control group, the MCV concentration was observed to be higher in the treatment group. The mean CV was 151.31.794 in the control group and 153.41.2 in the treatment group. In contrast to the treatment group's RDW% of 8.2 0.7, the control group's RDW% of 28.83 2.858 was quite high. The mean RDWa values in the treatment group dropped from 158.0 29.45 in the control group to 68.2 2.

4.2.2 Haematological Changes in Treatment 2

Comparison of various haematological parameters between *Cirrhinus mrigala* exposed to 10 mg/L (Treatment-2) amitriptyline with the untreated control group after 30-Days of the trial is shown in (Table 2). The data is expressed as Mean ± Standard Deviation of Mean. P-value indicates the result of 2-sample t-test calculated for each comparison in Table 2.

WBC increased from 2.48 ± 1.58 in untreated control group to 93 ± 1 in treatment-2. RBC concentration in untreated control group was 0.81 ± 0.32 and decreased to 0.04 ± 0.01 in treatment group. Platelets increased in treated fish, in contrast to control group from 60.67 ± 12.91 to 174 ± 0.4. An increase in Haemoglobin concentration was recorded in fish exposed to 10mg/L amitriptyline as mean of Haemoglobin was found to be 5.9 ± 0.1 in the treated group while the mean concentration of Heamoglobin in untreated control group fish was 5.85 ± 3.18.

MPV and PDW concentration increased to 7.7 ± 1.2, 9.7 ± 1.01 in treated fish as compared to untreated control group fish in which MPV and PDW concentration was 7.10 ± 0.42, 8.76 ± 0.28 respectively. PCT percentage increased non-significantly (P>0.05) from 0.03± 0.01 in control group to 0.134 ± 0.01 in the treatment group. In treatment group, increase in HCT percentage was recorded. The values of HCT % in control group were 11.00±4.514, and in treatment group was 23.9 ± 0.6. There was increase recorded in Lymphocytes percentage (95 ± 0.5) of fish exposed to 10mg/L amitriptyline as compared to the control group (86.57 ± 8.534). MON percentage decreased in treated fish, in contrast, to control group from 3.200 ± 2.156 to 2.1 ± 0.6. Granulocytes percentage also decreased in treated fish as compared to control group fish from 5.833 ± 1.750 to 1.8 ± 0.4. MCH concentration in untreated control group was 81.18 ± 12.32 and decreased to 28.9 ± 1.3 in treatment group.

MCHC showed a decrease in treatment group as compared to the control group. In the control group, MCHC values were recorded at 55.52 8.021, whereas those in the treatment group were reported at 24.7 1.2. The mean concentration of MCV was lower in the treatment group than in the control group. MCV was 151.3 1.794 in the control group, but only 117.2 0.9 in the treatment group, and the RDW% was 28.83 2.858 in the control group but only 18.4 1.16 in the treatment group (P 0.001).

4.2.3 Comparison of Haematological Profile Between Control and All Treatments

After 30 days of the trial, the table below compares the haematological parameters of *Cirrhinus mrigala* subjected to 5 mg/L (T1) and 10 mg/L (T2) of bacterriocin with those of the untreated control group. Mean Standard Error of the Mean is the form in which the data is presented. The p-value represents the overall comparison result from a "One way ANOVA" analysis.

One-way Analysis of Haematological results of untreated control group fish and those exposed to 5 mg/L (T1), and 10 mg/L bacteriocin revealed that means of platelets, PDWa, PCT percentage, MCHC concentration varied significantly (**P<0.05**) when compared between all treatments of amitriptyline and untreated control group. Similarly, MCV percentage, and RDW percentage and P-LCR% also varied high-significantly between treated groups and untreated control group as shown in Table 4.

Table 2: Comparison of Hematological profile between control and all treatments

Parameters	Control	T1 (Low Dose)	T2 (High Dose)	F-Value	P-Value
	Mean ± SD	Mean ± SD	Mean ± SD		
WBC (x 10^9L^{-1})	2.48 ± 1.58	53.6 ± 0.7	93 ± 1	4651	<0.0001
RBC (x$10^{12}L^{-1}$)	0.81 ± 0.32	2.21 ± 1.03	0.04 ± 0.01	9.363	0.0143
Platelets (x10^9L^{-1})	60.67 ± 12.91	77 ± 1.5	174 ± 0.4	199.8	<0.0001
HGB (gdl^{-1})	5.85 ± 3.18	7.9 ± 0.7	5.9 ± 0.1	1.16	0.3751
MPV [11]	7.10 ± 0.42	7.3 ± 1.6	7.7 ± 1.2	0.2011	0.8231
PDW (%)	8.76 ± 0.28	8.2 ± 1	9.7 ± 1.01	2.464	0.1655
PCT (%)	0.03± 0.01	0.55 ± 0.1	0.134 ± 0.01	66.8	<0.0001
HCT (%)	11.00 ± 4.514	33.9 ± 1.3	23.9 ± 0.6	52.89	0.0002
LYM (%)	86.57 ± 8.534	99 ± 1	95 ± 0.5	4.891	0.0549
MON (%)	3.200 ± 2.156	0.7 ± 0.1	2.1 ± 0.6	2.816	0.1373
GRA (%)	5.833 ± 1.750	0.3 ± 0.05	1.8 ± 0.4	22.85	0.0016
MCH (pg)	81.18 ± 12.32	35.3 ± 2.6	28.9 ± 1.3	45.68	0.0002
MCHC (gdl^{-1})	55.52 ± 8.021	23 ± 1	24.7 ± 1.2	45.16	0.0002
MCV	151.3 ± 1.794	153.4 ± 1.2	117.2 ± 0.9	679.6	<0.0001
RDW (%)	28.83 ± 2.858	8.2 ± 0.7	18.4 ± 1.16	95.73	<0.0001

P > 0.05 = Non-significant; P < 0.05* = Least Significant; P < 0.01** = Significant; P < 0.001*** = highly significant

4.3. Graphical Comparison of Haematological Profile

Graphical Comparison of MCV, HCT percentage, platelets, PDWa, PCT percentage and PLCR percentage between different concentrations of treatments and control group of *Cirrhinus mrigala* is plotted. Parameters are at X-Axis and Treatments with concentration are Y-Axis. Bars length shows mean of WBC in control and treated while error bars show standard deviation. The significance level is denoted by least significant with *, significant with ** and highly significant with ***.

As compared to untreated control, values of Platelets decreased least significantly in 5mg/L amitriptyline treatments as shown in Figures.

4.4. Serology

After complete blood count, blood serum was extracted by centrifugation to perform biochemical analysis to check the concentration of SGPT and SGOT to check liver functioning. Urea and creatinine concentration was measured to check kidney functioning.

4.4.1 Comparison of Serology Profile Between Control and All Treatments

Comparison of various serological parameters between *Cirrhinus mrigala* exposed to 5 mg/L (T1), and 10 mg/L (T2) amitriptyline with control group after 30-Days of the trial is shown in table. The Data is expressed as Mean ± Standard deviation of Mean. P-value indicates the result of "One-way ANOVA" calculated for overall comparison. One-way Analysis of serological results of untreated control group fish and those which exposed to 5 mg/L (T1), and 10 mg/L (T2) amitriptyline revealed that means of creatinine, and Sugar varied highly significantly ($P<0.001$) while means of AST and sugar varied least significantly ($P<0.05$) when compared between all treatments of amitriptyline and untreated control group. While urea varied non-significantly ($P>0.05$) between treated groups and untreated control group.as shown in Table 2.

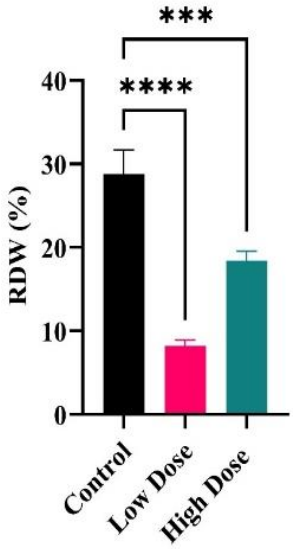

Fig 2: Comparison of RDW between control group and amytrityline tread groups

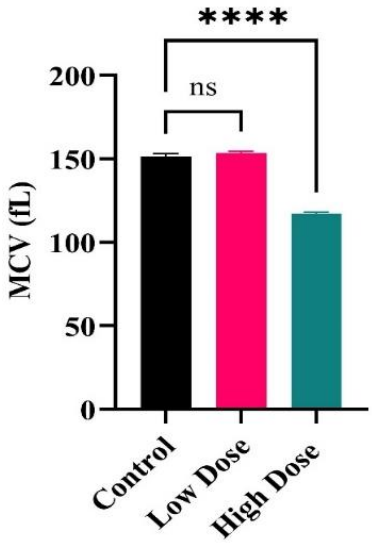

Fig 3: Comparison of MCV between control group and amytrityline tread groups

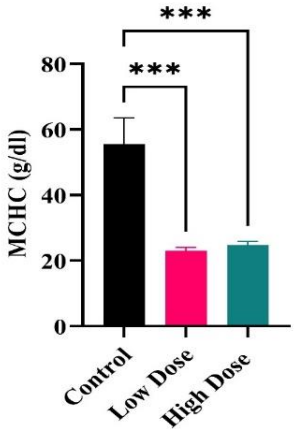

Fig 4: Comparison of MCHC between control group and amytrityline tread groups

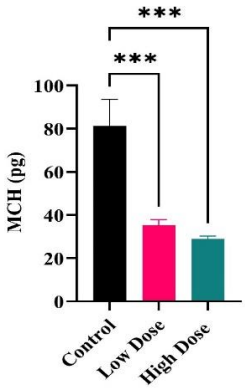

Fig 5: Comparison of MCH between control group and amytrityline tread groups

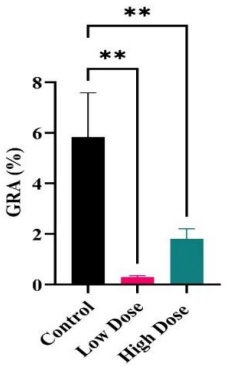

Fig 6: Comparison of GRA between control group and amytrityline tread groups

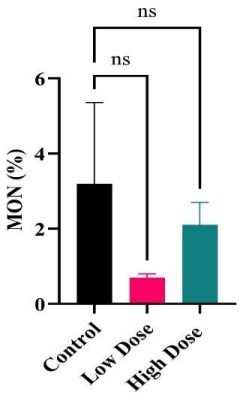

Fig 7: Comparison of MON between control group and amytrityline tread groups

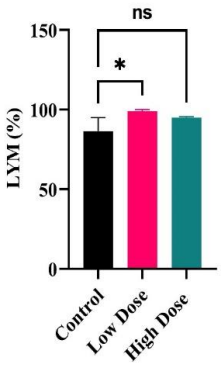

Fig 8: Comparison of LYM between control group and amytrityline tread groups

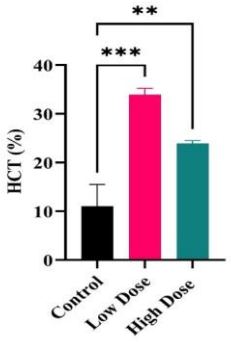

Fig 9: Comparison of HCT between control group and amytrityline tread groups

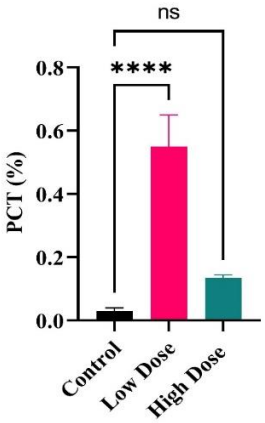

Fig 10: Comparison of PCT between control group and amytrityline tread groups

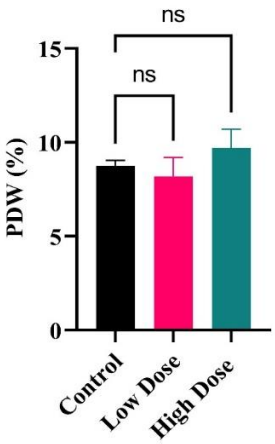

Fig 11: Comparison of PDW between control group and amytrityline tread groups

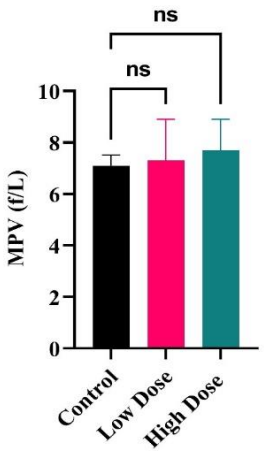

Fig 12: Comparison of MPV between control group and amytrityline tread groups

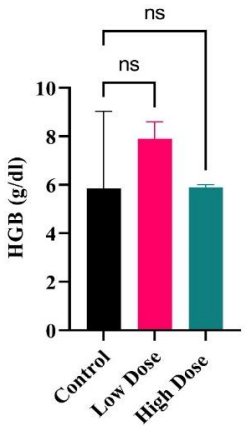

Fig 13: Comparison of HGB between control group and amytrityline tread groups

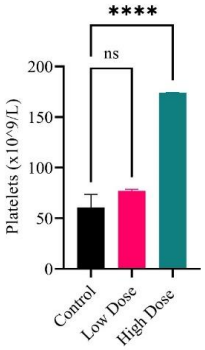

Fig 14: Comparison of Platelest between control group and amytrityline tread groups

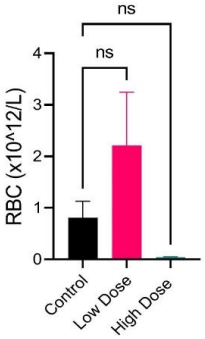

Fig 15: Comparison of RBCs between control group and amytrityline tread groups

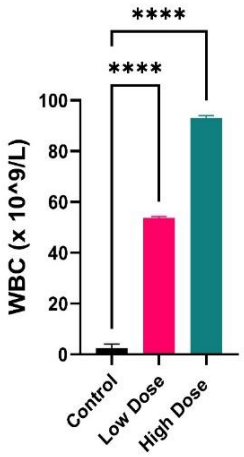

Fig 16: Comparison of WBCs between control group and amytrityline tread groups

Table 3: Statistical analysis of serology

Parameters	Control	Low Dose (T1)	High Dose (T2)	F value	*p* value
	Mean± S. D	Mean± S. D	Mean± S. D		
SGPT	320.0 ± 299.9	4 ± 1.6	7 ± 1.4	2.79	0.09
SGOT	1723 ± 1415	5 ± 0.25	6 ± 1	4.70	0.026*
Urea (mg/dl)	17.67 ± 1.506	26 ± 1.8	48 ± 1.1	0.81	0.46
Creatinine (mg/dl)	0.45 ± 0.09	117 ± 1	288 ± 1.9	8.65	0.0032**

P > 0.05 = Non-significant; P < 0.05* = Least Significant; P < 0.01** = Significant; P < 0.001*** = highly significant

4.6.2 Graphical Comparison of Serology Profile

Graphical grouped Comparison of SGPT, SGOT, Urea and Creatinine between different concentrations of treatments with control group is plotted for each parameter. Parameters are at X-Axis and Treatments with concentration are Y-Axis. Bars length shows mean of parameter in control and treated while error bars show standard deviation. The significance level is denoted by least significant with *, significant with ** and highly significant with ***.

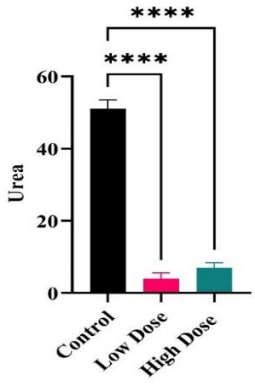

Fig 17: Comparison of urea between control group and amytrityline tread groups

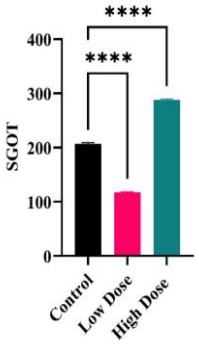

Fig 18: Comparison of SGOT between control group and amytrityline tread groups

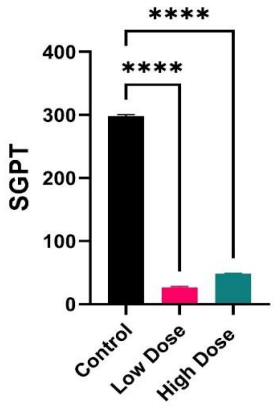

Fig 19: Comparison of SGPT between control group and amytrityline tread groups

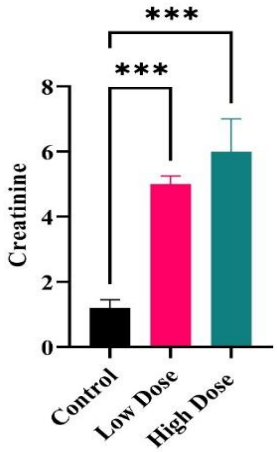

Fig 20: Comparison of creatinine between control group and amytrityline tread groups

5. Discussion

Assessment of ecotoxicity is being conducted in every region of the world in order to establish how medications impact creatures other than those that are supposed to be affected by them [12]. The presence of pharmaceutical medications in aquatic settings may result in an unexpected mechanism of interactions and effects with the molecules, cells, and organs of organisms [13]. According to Hoeger, Dietrich [14], Despite being present in relatively small amounts and having a reputation for being relatively non-toxic chemicals, medicines can still have harmful effects on species that aren't the intended beneficiaries of these therapies. This is because drugs are selective in the sorts of cells they affect.

Information on the mechanism of action and toxicity of medications can be gleaned from their acute toxicity. Pharmaceuticals have been the subject of a great deal of research on the topic of acute toxicity in aquatic creatures including algae and fishes in recent years [15, 16]. Nevertheless, a study that was conducted by Martín-Díaz, Gagné [17] stated that chronic studies that were based on particular endpoints should be utilised in risk assessment. Amytriptyline is the chemical that poses the greater risk because it has been detected in all sewage treatment plants with the greatest concentrations. Therefore, the current study was designed to evaluate the growth performance, blood parameters, liver function and renal functions of *Cirrhinus mrigala* exposed to amitriptyline.

Results of current study revealed that amitriptyline accelerated the fish growth as we found that increased weight gain in drug exposed fish groups of low dose group and of high dose group as compared to the control group. Similarly, the length gain was also observed as increased. But da Silva Santos, Oliveira [18] and Madureira, Rocha [19] reported that after 63 days acute exposure of amitriptyline did not alter the fish growth in terms of body weight gain and body length gain.

Hematology plays an important role in our research. It was found that Amitriptyline has much effect on hematology of *Cirrhinus mrigala.* White blood cells have a significant impact on the immune system. Changes in the number of white blood cells caused by toxicant exposure or stressful conditions [20]. Non-significant elevation in WBCs was observed. The number of WBCs was measured as 2.48± 1.58 $x10^3$µL in control group while in Low dose it was measured as 2.21 ± 1.03 $x10^3$µL and in high dose it was measured as 0.04 ± 0.01 $x10^3$µL. The reason behind the reduction of WBCs in amitriptyline exposed groups was the destruction of haematological centres after prolonged exposure these results are very similar to the findings of da Silva Santos, Oliveira [18] but these results are different from the findings of Saravanan, Karthika [21].

Blood platelets play a role in both general defence systems and blood coagulation. The formation of thrombocytes is typically inhibited when fish are exposed to various types of

water contaminants [22]. The number of blood platelets control group 60.67 ± 12.91 x$10^{9}L^{-1}$ was less than treated groups. In low dose group T2 77 ± 1.5 x$10^{9}L^{-1}$ the number of blood platelets was higher than T1 group 174 ± 0.4 x$10^{9}L^{-1}$ that is high dose group.

Further, it has been proposed that future ecotoxicology studies look into how pharmaceuticals at low quantities affect delicate aquatic organisms [23]. According to the results of the study, the exposure to amitriptyline changed the concentrations of liver enzymes. The levels of Alanine Aminotransferase (ALT) and Aspartate Transaminase greatly rose, but the levels of Alkaline Phosphatase (ALP) dramatically reduced. The level of bilirubin rose, although not by a substantial amount. There is no prior study in fish that is relevant and has been published.

Conclusion

In the present study effect of amitriptyline was investigated on growth and body physiology of *Cirrhinus mrigala*. Amitriptyline has dangerous effect on growth of *Cirrhinus mrigala*. On significant increase in the body weight and length was found. The haematological reports showed that number of blood platelets, PDWa, PCT percentage, MCHC concentration, MCV percentage, and RDW percentage and P-LCR% were significant ($P<0.05$) while WBC, RBC, MPV, PCT, MON, lymphocytes, GRA, and MCH. Further study should be extended on the effects of amitriptyline on body weight and haematology by long term exposure of amitriptyline on *Cirrhinus mrigala*.

Conflict of Interest

The authors declare no conflict of interest.

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