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Effect of olanzapine antibody on body physiology and enzymatic system of *Tilapia Mozambique* fish (*Oreochromis mossambicus*)

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Abstract

Tilapia (Mozambique) fish, belonging to the family Cichlidae and order Cichliformes, are native to freshwater in Africa. Olanzapine, a second-generation antipsychotic drug (APD), is known for its improved efficacy and reduced side effects compared to first-generation APDs and is commonly used for treating psychiatric disorders and brain cancer. This study aimed to assess the effects of Olanzapine on the growth, behavior, haematology, and histopathology of *Tilapia* fish. The fishes were collected from the selected fish farm in Pattoki. The fish were acclimatized for one week and then treated with 0.06 mg/l of Olanzapine daily for 28 days. They were divided into control, treated, and untreated groups. Morphometric measurements, haematological analyses, and histological evaluations of kidney, liver, and gill tissues were conducted. The results indicated significant changes in morphometric characteristics and an increase in movement in treated fish compared to controls. MCH and MCHC levels were significantly higher in the treated group ($P < 0.005$), while other haematological parameters, including WBCs, RBCs, HGB, HCT, MPV, PDW, PCT, and MCV, showed no significant changes ($P > 0.05$). Histopathological analysis revealed notable changes in the kidneys, liver, and gills of Olanzapine-treated fish. Despite non-significant increases in total body weight, Olanzapine positively affected morphometric traits and feeding behavior while causing observable haematological and histological alterations in *Tilapia* Mozambique. To conclude, the Olanzapine is relatively safe for *Tilapia*'s liver but may harm the gills and kidneys. It had minimal effects on behavior, morphology, and hematology, with significant damage only to gill structure and mild changes in the kidneys.

Keywords: *Tilapia* fish, Growth, Behavior, Hematology, Histopathology

Introduction

Tilapia Mozambique (Oreochromis mossambicus) fish belong to the family Cichlidae and the order Cichliformes, and are native to freshwater regions of Africa. They are part of the mouthbrooding genera, such as *Sarotherodon* and *Oreochromis*, and are known for substrate spawning. *Tilapia* species have been introduced worldwide, particularly in tropical and subtropical regions, to advance the freshwater aquaculture sector. Global production of tilapia is approximately 659,000 metric tons annually, with *Oreochromis niloticus* being the most widely cultivated species (1).

In 1997, 91% of global aquaculture production, amounting to 36 million metric tons, was generated by Asian countries. However, the economic value of this production saw a decrease: China's contribution dropped from 68% to 45%, while India's share decreased from 5% to 4%. Between 1991 and 1997, aquaculture production increased by 12%, indicating rapid growth in the sector. Currently, the top 10 countries in aquaculture production are all located in Asia (2). China has long history of production in majority fish from fish pond approximately (3).

Aquaculture systems are predominantly utilized in Bangladesh, China, India, Indonesia, Malaysia, and Thailand. Fish meat is a rich protein source, containing 16-20% protein, which is higher than that in eggs (12%), meat (3.5%), milk (3.5%), and rice and wheat (6.6%).

Additionally, fish meat is highly nutritious and easily digestible, with an assimilation rate of 85-95% (4). Additionally, fish meat is rich in essential minerals, including vitamins D and A, and contains beneficial unsaturated fatty acids (5). It is estimated that fish provides a major source of animal protein for approximately one billion people worldwide. As a result, the fish industry has experienced rapid growth and is now one of the fastest-growing food sectors globally (6).

Olanzapine is a second-generation antipsychotic (SGA) that offers improved efficacy and reduced side effects compared to first-generation antipsychotics. However, it can cause significant weight gain and other adverse effects. Currently, SGAs like Olanzapine (OLZ) are commonly used to treat schizophrenia and other brain and bipolar disorders due to their reduced side effects compared to older medications. Despite its benefits, OLZ is associated with weight gain, increased adipose tissue, elevated blood leptin and insulin levels, insulin resistance (type 2 diabetes), high blood pressure, and disruptions in fat metabolism (7). Among SGAs, OLZ the most commonly cause weight gain (8). Compared to typical antipsychotics, atypical

antipsychotic drugs are more effective in addressing both negative and positive symptoms of schizophrenia (9) . In general, the side effects of Olanzapine in humans often include weight gain, which is linked to the drug's sedative effects. This weight gain is primarily due to increased appetite and enhanced food consumption. Consequently, the observed weight gain and increased adiposity may result from higher food intake combined with reduced physical activity (10). Despite the known association between Olanzapine and weight gain, the underlying mechanisms driving this effect and metabolic imbalances remain uncertain (11). The present study aims to investigate Olanzapine's impact on fish by examining its effects on growth, body physiology, histopathology, and the enzymatic system, including changes in body weight.

Material and methods

Collection of samples

Samples were obtained from a fish farm in Pattoki. The specimens had an average weight ranging from 20 to 30 grams and measured 12 cm in length. To prevent any fatalities during transportation, the fish fry were kept in plastic bags filled with fresh water, ensuring adequate oxygen supply.

Ethical concern

The study was conducted according to the declaration of Helsinki and the rule to work on animals was followed.

Rearing and Behaviour study

Around 40 grams of each fish were imported from Manu Fish Farm in Pattoki and housed in a separate aquarium at room temperature, each containing 80 L of water. Initial weight and length measurements were taken before dividing the fish into six groups after two weeks. The first group served as a control (no olanzapine), while the experimental group received 0.06 mg/L of olanzapine. We observe fish behaviour before and after meals and gain weight on a daily basis and weekly after olanzapine treatment in controlled and treated groups.

Study design and treatment with olanzapine

The 28-day experiment involved keeping the fish in 80-liter aquariums with dimensions of 45.74 x 60.97 x 45.74 cm. Control and experimental groups were established for observation. After 24 hours, 0.8 mg of olanzapine per liter was administered. The fish were fed commercial feed daily at 2:00 p.m. Before each treatment, 80% of the aquarium water was replaced to remove waste. The fishes were divided into six groups: control, treated, and untreated. After 28 days of

olanzapine treatment, three fish from each group were randomly selected for sampling. Blood samples were obtained via cardiac puncture using a 2-ml needle and collected in EDTA tubes. The liver and gills were isolated from the same fish and preserved in formalin. Blood and tissue samples were processed on the same day.

Determination of hematological parameters

Blood samples were analyzed for hemoglobin, red blood cells, white blood cells, mean cell hemoglobin, platelet count, and cell hemoglobin concentration. Platelet Distribution Width (PDW) was measured to evaluate morphometric changes in platelets, including activation and size, using histograms. Hemoglobin (Hb), which transports oxygen from the lungs to body tissues and returns carbon dioxide to the lungs, was quantified using a MICRO-LAB 3000 at a wavelength of 540 nm. A 5 ml Hb solution was mixed with 20 µl of whole blood, left at room temperature for five minutes, and then measured in g/dl. Additionally, Mean Corpuscular Volume (MCV), Mean Cell Hemoglobin (MCH), and Mean Cell Hemoglobin Concentration (MCHC) were assessed (12).

Study of cortisol level and antioxidant enzymes analysis

Five types of antioxidant enzymes were studied in *Tilapia Mozambique* which were catalase (CAT) (13), superoxide dismutase (SOD) (14), glutathione (GSH) (15), glutathione S-transferase (GST) (16) and MDA (17) an oxidative stress maker. At the end of the trial, three fish were randomly selected from each group (control and both fluoxetine-treated groups), then beheaded and frozen at -80°C for cortisol extraction. The frozen tissues were thawed, washed with ice-cold 1.15% KCl solution, blotted, weighed, and homogenized separately in an ice-cold buffer containing 1.15% KCl and 50 nM Tris-HCl (pH 7.4). Approximately 1 g of tissue from each sample was homogenized using a piston mortar, and the homogenate was centrifuged at 10,000 g for 20 minutes using a Sigma 2-16K centrifuge. The supernatant was then separated, decanted, and stored at -20°C (18).

Study of the Morphometric characters

To measure the morphometric parameters of fish, an inch tape was used to assess 12 features: Total Length (TL), Total Weight (TW), Body Depth (BD), Standard Length (SL), Fork Length (FL), Snout Length (SnL), Dorsal Fin Length (DFL), Pectoral Fin Length (PtFL), Pelvic Fin Length (PvFL), Caudal Fin Length (CFL), Anal Fin Length (AFL), and Head Length (HL). Total Weight included the gonads or gut. Total Length was measured from the mouth tip to the end of

the longest caudal fin. Body Depth was the maximum vertical distance. Standard Length was the distance from the snout to the base of the caudal fin, excluding the tail. Fork Length was measured from the snout to the end of the central caudal ray, while Snout Length was the distance from the snout to the foremost edge of the fleshy orbit. Dorsal Fin Length was taken from the base to the tip of the longest ray, Pectoral Fin Length from the origin to the tip of the largest ray, and Pelvic Fin Length from the origin to the tip of the largest ray. Caudal Fin Length was the maximum tail fin length, Anal Fin Length was from the origin to the tip of the largest ray, and Head Length was measured from the snout to the end of the operculum (19).

Statistical Analysis

Data was being analyzed using Minitab Version 18. (Mean, standard error of mean and range) we used one-way ANNOVA (20).

Results

This study investigated the hematological profile, morphometric characteristics, and the effects of olanzapine on the histopathological profile of *Oreochromis mossambicus* fish.

Effect of olanzapine on haematological profile Tilapia (*O. mossambicus*)

After treating the fishes with olanzapine the complete blood count of fishes were analyzed. The study compared hematology profiles between control and experimental groups, focusing on WBCs, RBCs, platelets, and related factors. The control group had higher WBC, RBC, HCT, MPV, PDW, and MCV values, while the experimental group showed higher MCH, MCHC, RDW-CV, and PCT values. Significant differences were observed in platelet counts (higher in control, $P=0.024$), MCH (higher in experimental, $P=0.004$), and MCHC (borderline higher in experimental, $P=0.053$). Overall, the control group had generally higher blood cell counts, while the experimental group exhibited elevated MCH and MCHC, suggesting treatment effects on blood composition. These results are shown in Table 1.

Table 1: Hematological profile of the tilapia affected by the olanzapine

Parameters	Groups	Mean	SD	SE	F value	P-value
WBCs	Control	12.7	0.08	0.05	3.00	0.159
	Experimental	6.37	5.175	2.99		
RBCs	Control	0.45	0.03	0.02	3.27	0.145

	Experimental	0.25	0.153	0.09		
Platelets	Control	19.13	0.12	0.07	12.51	0.024*
	Experimental	78.33	23.669	13.67		
HGB	Control	2.13	0.12	0.07	0.28	0.625
	Experimental	1.6	0.726	0.42		
HCT	Control	5.33	0.12	0.07	2.7	0.176
	Experimental	2.87	2.12	1.22		
MPV	Control	8.23	0.54	0.31	1.03	0.368
	Experimental	7.83	0.125	0.07		
PDW	Control	17.27	0.12	0.07	1.89	0.241
	Experimental	16.9	0.356	0.21		
PCT	Control	0.02	0	0	0.72	0.443
	Experimental	0.06	0.019	0.01		
MCHC	Control	38.37	0.21	0.12	7.42	0.053*
	Experimental	48.9	5.463	3.15		
MCH	Control	48.93	0.12	0.07	37.47	0.004**
	Experimental	58.75	2.265	1.31		
MCV	Control	128.53	0.29	0.17	2.26	0.207
	Experimental	121.63	6.483	3.74		

NS= non-significant ($p > 0.05$); *= significant ($p < 0.05$); **=highly significant ($p < 0.01$)SD= Standard deviation; SE= Standard error

Effect of olanzapine on Tilapia behaviour (*Oreochromis mossambicus*)

Tilapia fish movement on the left side was 23.94 ± 7.92 in the control group and 26.27 ± 9.01 in the experimental group ($P=0.473$). On the right side, movement was 25.01 ± 5.27 in the control group and 37.00 ± 9.35 in the experimental group, with a highly significant P value of 0.000. Surface and center movements were 8.73 ± 2.98 and 6.67 ± 2.39 in the control group, and 25.36 ± 8.20 and 30.33 ± 11.89 in the experimental group. Column-side and feeding movements were 28.87 ± 8.04 and 27.80 ± 10.13 in the control group, and 5.01 ± 1.62 and 6.33 ± 2.09 in the experimental group. Base movement was 5.87 ± 1.45 in both groups. The right-side movement was the only statistically significant difference ($P=0.000$). These findings are detailed in Table 2.

Table 2: Comparison in hematological parameters between experiment and control group due to

Olanzapine

Observations	Control		Experimental			
	Mean± S. D	Range Minimum- Maximum	Mean± S. D	Range Minimum- Maximum	F value	P value
Left side	23.94±7.92	15-45	26.27±9.01	13-39	0.53	0.473 ^{NS}
Right side	25.01±5.27	19-35	37.00±9.35	22-45	17.48	0.000**
Surface	8.73±2.98	03-13	6.67±2.39	03-11	4.11	0.05*
Centre	25.36±8.20	14-45	30.33±11.89	11-45	1.66	0.208
Column	28.87±8.04	17-39	27.80±10.13	10-44	0.1	0.758
Feeding	5.01±1.62	03-08	6.33±2.09	03-09	3.52	0.071
Base	5.87±1.45	04-09	6.60±2.03	04-10	1.21	0.281

NS= non-significant ($p > 0.05$); *= significant ($p < 0.05$); **=highly significant ($p < 0.01$)SD= Standard deviation; SE= Standard error

Comparison of morphometric Characters *Tilapia mozambique* (*Oreochromis mossambicus*) due to Olanzapine

We observed 10 morphometric characteristics in the experiment. The body weight in the control group was 24.82 ± 4.08 , slightly higher than the 23.59 ± 6.80 observed after treatment and 21.94 ± 6.73 before treatment ($P=0.863$). Body length was similar across groups, with 10.82 ± 0.72 in the control group, 10.98 ± 1.08 after treatment, and 10.91 ± 0.97 before treatment ($P=0.996$). Pelvic fin length increased significantly in the treated group, measuring 5.67 ± 4.40 , compared to 2.14 ± 0.21 in the control group and 2.60 ± 0.37 before treatment ($P=0.121$). Pectoral fin length was 3.22 ± 0.19 in the control group, decreasing to 2.71 ± 0.28 before treatment and increasing to 3.04 ± 0.37 after treatment ($P=0.356$).

Caudal fin length was 2.14 ± 0.16 in the control group, increasing to 2.50 ± 0.41 before treatment ($P=0.24$). Anal fin length increased from 2.50 ± 0.06 in the control group to 3.29 ± 0.99 after treatment ($P=0.126$). Dorsal fin length decreased from 6.38 ± 0.49 in the control group to 5.98 ± 1.35 after treatment ($P=0.121$). Body circumference was greater in the treated group (7.52 ± 1.21) compared to the control group (6.21 ± 1.49) ($P=0.192$). Head circumference was similar between groups, with 3.32 ± 1.96 in the treated group and 3.21 ± 2.23 in the control group ($P=0.916$). Head size was larger in the treated group (6.69 ± 0.71) compared to the control group (5.54 ± 1.71) ($P=0.145$). These results are detailed in Table 4.

Table 4: Effect of olanzapine on morphometric parameters

Measurements	Control		Experimental					
			Before treatment		After treatment			
	Mean $\pm$ SD	SEM	Mean $\pm$ SD	SEM	Mean $\pm$ SD	SEM	F Value	P value
Body weight	24.82 $\pm$ 4.08	1.58	21.94 $\pm$ 6.73	2.74	19.59 $\pm$ 6.80	2.77	0.15	0.86
Body length	10.82 $\pm$ 0.72	0.25	10.91 $\pm$ 0.97	0.39	10.98 $\pm$ 1.08	0.44	0	0.996
Pelvic fin	2.14 $\pm$ 0.21	0.074	2.60 $\pm$ 0.37	0.15	5.67 $\pm$ 4.40	1.795	2.38	0.121
Pectoral fin	3.22 $\pm$ 0.19	0.2	2.71 $\pm$ 0.28	0.11	3.04 $\pm$ 0.37	0.149	1.1	0.356
Caudal fin	2.14 $\pm$ 0.16	0.057	2.50 $\pm$ 0.41	0.16	2.46 $\pm$ 0.46	0.18	1.55	0.24
Anal fin	2.50 $\pm$ 0.06	0.069	2.49 $\pm$ 0.42	0.17	3.29 $\pm$ 0.99	0.404	2.33	0.126
Dorsal fin	6.38 $\pm$ 0.49	0.204	5.42 $\pm$ 0.45	0.18	5.98 $\pm$ 1.35	0.55	2.38	0.121
Body circumference	6.21 $\pm$ 1.49	0.606	6.06 $\pm$ 1.04	0.42	7.52 $\pm$ 1.21	0.493	1.81	0.192
Head circumference	3.21 $\pm$ 2.23	0.803	3.33 $\pm$ 1.92	0.78	3.32 $\pm$ 1.96	0.801	0.09	0.916
Head size	5.54 $\pm$ 1.71	0.59	5.36 $\pm$ 1.05	0.42	6.69 $\pm$ 0.71	0.289	2.15	0.145

NS= non-significant ($p > 0.05$); *= significant ($p < 0.05$); **=highly significant ($p < 0.01$)SD=

Standard deviation; SE= Standard error

Histopathology of gills, kidney and liver treated with olanzapine

The gills of control group fish displayed normal structure (Figure 1), while those exposed to olanzapine showed fusion and disruption of primary and secondary lamellae. Olanzapine induced mild to moderate changes in the kidneys, with slight alterations in cellular proliferation and glomerular cellularity after 28 days of treatment at 0.06 mg/l. No significant adverse effects were observed in the kidneys, indicating olanzapine is relatively safe for fish kidneys. The liver, responsible for detoxifying environmental toxins, showed minimal structural changes upon olanzapine exposure, with no significant deviations from the normal liver architecture. The central lobules featured a thin-walled vein with a portal triad containing a portal vein, an artery, and a bile duct, while hepatocytes were arranged in an interconnecting network of single-cell-thick trabeculae.

Histopathological analysis of the gills in the control group showed no abnormalities. In contrast, the gills of olanzapine-treated *Tilapia* exhibited inflammation, fragmentation, hyperplasia, and hypertrophy, with the central vena cava of the gills expanding and the gill tissue thinning compared to the control group (Figure 1a). Kidney tissues of *Tilapia* treated with 0.6 mg/l olanzapine showed hyperemia, but no distinct changes were observed compared to the control group (Figure 1b). Liver variations were assessed using the method by (21), revealing hepatic cord disarrangement, hepatocyte vacuolation, and rupturing of blood vessels. Figure 1c illustrates these histopathological changes in the liver, showing normal tissue (A) and 0.6 mg/l olanzapine-treated tissue with hypertrophy and hepatocyte vacuolation (B).

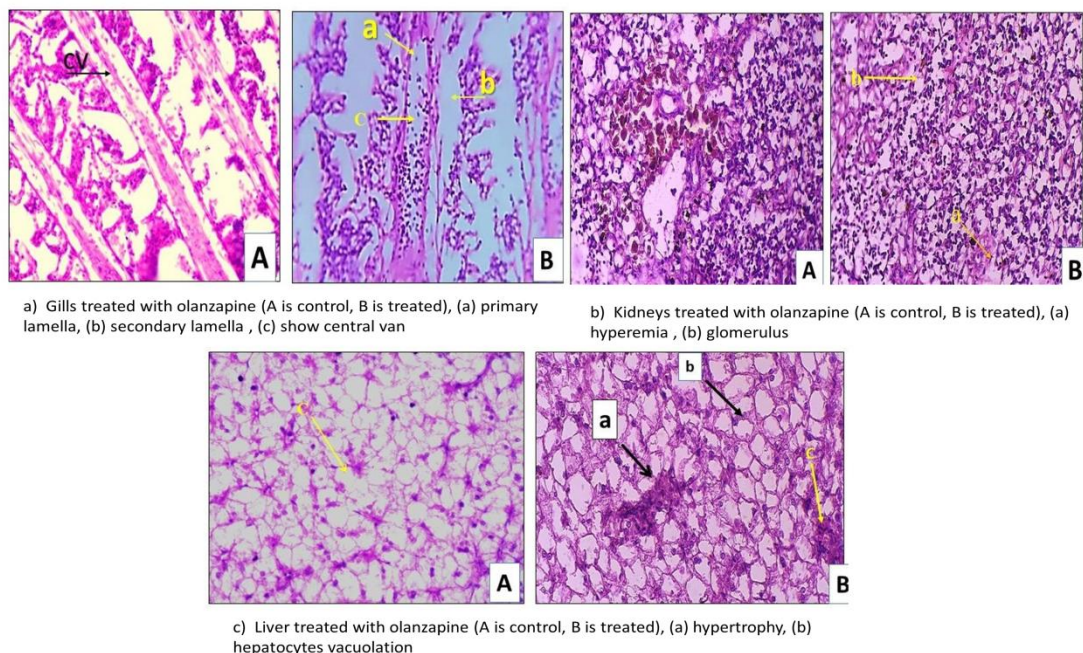


Figure 1: The histopathological analysis in different organs of fish

Effects of olanzapine on Tilapia's Cortisol Level

It was observed that, the standard error of the mean and standard deviation of cortisol levels, a key stress hormone, in the control group were 6.54 ± 1.10 μm $1.106.54 \pm 1.10$. In the group administered 0.6 mg/L, the values were 4.11 ± 0.15 μm $0.154.11 \pm 0.15$, with a P-value of 0.016 and an F-value of 8.44. These results indicate that the cortisol levels in the experimental group were significantly lower compared to the control group.

Discussion

The current study examined the hematological, histopathological, and physiological effects of olanzapine exposure in *Oreochromis mossambicus*. Hematological analysis is crucial for assessing the physiological status of animals affected by various diseases and environmental toxins. In aquatic organisms like fish, hematological profiles are extensively utilized to gauge environmental pollution and assess stress effects within aquatic ecosystems (22).

Hematological values are essential for indicating the health status of both animals and mammals. They are used for diagnosing and monitoring diseases, evaluating therapy effectiveness, and assessing disease prognosis. Additionally, these values serve as physiological reference points for various species. Different physiological factors can influence the hematology of healthy animals (23).

Hematological analysis reveals an animal's health status, metabolic profile, and potential

disorders. Changes in hematological values are often associated with diseases and abnormalities. This analysis includes white blood cell (WBC) counts, which indicate infection; hemoglobin and hematocrit levels, which assess anemia; and the heterophil-to-lymphocyte ratio, which signals stress. Additionally, biological and physiological parameters such as weight and morphometry serve as indicators of health (24, 25).

A significant decrease ($P < 0.05$) in platelet counts was observed in experimental fish treated with olanzapine compared to the control group. This finding contrasts with Owoade et al. (2019), who reported increased platelet numbers in fish treated with tramadol. The observed decrease in platelet count in the current study may be attributed to secondary thrombocytosis resulting from olanzapine exposure.

Red cell indices, such as MCV, MCHC, and MCH, are crucial for assessing anemia in fish. Variations in these parameters can indicate different types of anemia, including microcytic and macrocytic anemia. In this study, a significant increase ($P < 0.005$) in MCH and MCHC was noted in fish exposed to olanzapine compared to the control group. Similar increases in MCH and MCHC values have been reported by (26) when *Clarias gariepinus* was exposed to sub-lethal concentrations of diazepam, no significant changes ($P > 0.05$) were observed in most hematological parameters, including white blood cells (WBC), red blood cells (RBCs), hemoglobin (HGB), hematocrit (HCT), mean platelet volume (MPV), platelet distribution width (PDW), platelet count (PCT), and mean corpuscular volume (MCV), compared to the control group. Olanzapine has no show significant effect on morphometric characters. It has effect on weight if we give the extra feed. It shows weight loss in condition of restrict food circumference.

Histopathological investigation is a valuable approach for detecting the effects of anthropogenic contaminants on organisms. By examining histopathological biomarkers, researchers can assess the general health of a population in an environment (27). Anthropogenic wastes dumped into water bodies negatively impact aquatic life, particularly fish. Changes observed in multiple tissues are crucial for understanding the biological consequences of a toxicant and diagnosing the resultant damage (28). The severity of the damage often inversely correlates with the toxic potential of the contaminant.

The liver plays a crucial role in detoxifying medications and harmful substances in fish.

Consequently, alterations in the liver of aquatic species, such as fish, due to environmental pollution, serve as indicators of habitat contamination (29). Liver histology provides a highly sensitive and precise method for assessing the impact of pollutants or harmful substances on fish. In the current study, *Oreochromis mossambicus* exposed to olanzapine exhibited changes in various organs, highlighting the effects of the drug on their health.

Gills are vital for respiration and ionic regulation in fish, and their high permeability and extensive surface area make them an effective tool for monitoring the impacts of contaminants on aquatic life (30). In this study, the gills of the control group fish exhibited normal structure, while the gills of fish exposed to olanzapine showed fusion and disruption of the primary and secondary lamellae. This observation is consistent with findings by other researchers, such as those in Indian major carp *Catla catla* exposed to Bisphenol-A (31). The kidneys are crucial for haematological and osmoregulatory functions in fish, and their histological condition can indicate environmental contamination because they process most of the post-branchial blood (32). In this study, no structural changes were observed in the kidneys of *Oreochromis mossambicus* treated with olanzapine, indicating that the drug did not negatively affect the kidneys of the fish.

Conclusion

The study concludes that olanzapine is relatively safe for the liver but may be toxic to the gills and kidneys of Tilapia. While the drug did not significantly impact the fish's behavior, morphometric characteristics, or hematological profile, it caused notable gill damage and mild to moderate changes in kidney structure. Most hematological parameters showed no significant differences, except for a notable change in MCH. Movement analysis revealed significant differences in right-side movement, but other behavioral and morphometric metrics remained unaffected.

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Author's contribution

All authors contributed equally in the manuscript.

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Conflict of interest

None

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