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Ciprofloxacin-Induced Dysregulation of Morphogenesis, Locomotion, and Lipid Metabolism in *Caenorhabditis elegans*

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Abstract

Ciprofloxacin, a widely used fluoroquinolone, is frequently detected in water and soil due to its persistence, poor biodegradability, and continuous release from medical and agricultural sources, making it an emerging environmental contaminant. This study investigates the toxicological effects of ciprofloxacin on *Caenorhabditis elegans*. The organisms were exposed from the embryonic stage to concentrations of 0.09, 0.0009, and 0.00009 mg/mL for a total duration of 48 h. The concentrations in this study were based on the findings obtained from monitoring work carried out in the Sabarmati River water samples collected from Gujarat State, India. At the L-4 stage, multiple biological endpoints, which are measurable biological changes, were assessed. The results showed that ciprofloxacin exposure led to significant dose-dependent reductions in body length and developmental delays. Since severe toxicity was observed at 0.09 mg/mL, it was hypothesised that lower concentrations might reveal sub-lethal effects. Therefore, further investigations on feeding behaviour, lipid deposition, locomotion, and reproductive patterns were conducted using two reduced concentrations: 0.0009 mg/mL and 0.00009 mg/mL. At a concentration of 0.0009 mg/mL, ciprofloxacin significantly inhibited the pharyngeal pumping, reflecting reduced feeding capacity, and caused a decline in thrashing behaviour, indicating neuromuscular impairment. Furthermore, high and low concentrations of ciprofloxacin exposure resulted in altered lipid deposition and reduced reproductive output with a noticeable decline in brood size, reproductive age, and embryonic viability in *C. elegans*. Our findings suggest that environmental contamination of ciprofloxacin can have profound toxic effects even at very low concentrations. The study highlights the necessity of further research into the environmental risks of ciprofloxacin's toxic manifestations and the development of appropriate strategies and actions to foster a robust and healthy aquatic ecosystem.

Major Findings: Ciprofloxacin residues, detected in the ecosystem, caused developmental delays, neuromuscular impairment, and reproductive issues in *C. elegans*. Even at lower studied concentrations, ciprofloxacin disrupted lipid metabolism and feeding activity. These findings highlight the need for the mitigation of antibiotics (Ciprofloxacin) impacts on the environment.

Keywords: *Caenorhabditis elegans,* Ciprofloxacin, Environment, Pollution, Toxicity

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1. Introduction

Fluoroquinolones (FQ) are broad-spectrum antibiotics that effectively fight both gram-negative and gram-positive bacteria, including mycobacteria and anaerobes[1]. Fluoroquinolone antibiotics bioaccumulate in the dorsal-epaxial musculature of fish and cause potential human health risks[2]. Structural changes in fluoroquinolones, such as C-6 halogenation or N-1 substitutions, alter their toxicity in zebrafish. For instance, ciprofloxacin, enrofloxacin, and moxifloxacin show different effects on locomotion, lipid metabolism, and development, indicating structure-dependent toxicity[3]. Ciprofloxacin is a fluoroquinolone antibiotic used to treat bacterial infections, including bones, joints, abdomen, respiratory tract, skin, typhoid fever, and uterine infections. In the Azolla species (Mosquito fern), ciprofloxacin was shown to inhibit photosynthetic and nitrogenase activities, disrupt amino acid metabolism, and reduce cell proliferation. *In vivo*, ciprofloxacin induced clear electrocardiographic alterations in guinea pigs, demonstrating significant and dose-dependent cardiotoxicity. *In vivo* studies in guinea pigs revealed noticeable electrocardiographic changes following ciprofloxacin administration, indicating a significant and dose-related cardiotoxic effect[4]. Toxicological impacts of ciprofloxacin occur in different organisms through various mechanisms, including free radical generation, mitochondrial dysfunction, oxidative stress, apoptosis induction, and structural degradation of DNA[5]. It also caused elevation of cholestatic enzymes, such as Alkaline Phosphatase (ALP) and Gamma-Glutamyl Transferase (GGT), while hepatocellular injury was marked by a rapid increase in Alanine Aminotransferase (ALT) levels and risk of liver failure even after discontinuation[6]. Ciprofloxacin induces tendon disorders in people above 60 years old, especially those with a history of renal failure, diabetes, and musculoskeletal issues[7]. Furthermore, it is linked to several notable side effects, including psychosis, confusion, seizures, headaches, dizziness, and nausea.

Other common effects include tendinitis, muscle pain, depression, and fatigue[8]. Another report by Bartoskova *et al.*[9] pointed out that ciprofloxacin can inhibit the growth of nematodes and zebrafish. It has also been found to interfere with the development and growth of aquatic species, including frogs, algae, and fish[10] and may induce genotoxic effects[11]. Another major concern with ciprofloxacin is its pseudo-persistence, a phenomenon where continuous emission and incomplete degradation result in prolonged environmental exposure. Its half-life varies widely, ranging from 2 hours to several years, depending on environmental conditions (reference)[12]. Prolonged and multigenerational exposure leads to continuous presence and heightened risks to vulnerable groups, including eggs, developing offspring, and pregnant or lactating organisms[13]. It contributes to antibiotic resistance and exerts toxic effects on a wide range of organisms[14]. These findings have raised growing concerns about the environmental hazards of ciprofloxacin and its risks to human health. Ciprofloxacin is now recognised as a significant environmental pollutant in aquatic systems due to its persistent presence and harmful effects even at low concentrations. Although the European Union Water Framework Directive includes water quality standards for many antibiotics, there is still a lack of comprehensive environmental standards data[15,16]. Ciprofloxacin and other antibiotics have standard limits set in the range of 0.0000005 mg/mL for the protection of aquatic organisms, according to the European Medicines Agency (EMA) and Water Framework Directive guidelines[17]. Ciprofloxacin has been detected in various environmental matrices, including water, sediment, and sludge, at concentrations that may pose ecological risks due to its established genotoxicity[18]. The study conducted in an urban sewage treatment plant detected ciprofloxacin at a concentration of 0.00000157 mg/mL, a level that should be evaluated against established environmental threshold limits[19], and raw wastewater from the hospital had 0.000007573mg/mL[20]. Sludges and effluents from hospitals were found to contain 0.00000148mg/mL of ciprofloxacin residues[21]. The predicted no-effect concentration (PNEC) of ciprofloxacin was found to be 0.00000047 mg/mL in algae, 0.00109 mg/mL in *Daphnia magna,* and 0.00006mg/mL in fish[22-24]. Limited awareness and insufficient infrastructure for the safe disposal of unused and expired antibiotics, such as landfill dumping and flushing, have led to the contamination of water bodies[25]. Healthcare facilities contribute to ciprofloxacin pollution when Wastewater Treatment Plants (WWTPs) fail to remove pharmaceutical residues[26] effectively. As a result, antibiotics like ciprofloxacin can enter nearby rivers and streams, leading to environmental contamination[27].

Given the existing knowledge about ciprofloxacin toxicity, the present study investigated its effects on growth, reproduction, locomotion, lipid deposition, and feeding behaviour in *C. elegans*. The nematodes

were continuously exposed to ciprofloxacin from the embryonic stage through to senescence to explore the underlying mechanisms of this widely used antibiotic's toxicity. The concentrations used in this study were based on environmental monitoring data collected from river water samples in Gujarat State, India (unpublished data).

2. Materials and Methods

2.1 Chemicals and Reagents

Ciprofloxacin (CIP) (CAS: 85721-33-1, C17H18FN3O3, >98%) and dimethyl sulfoxide (DMSO) (>99%, LC grade) were purchased from Sigma Aldrich, USA. NaCl (sodium chloride), $MgSO_4$ (magnesium sulfate), $C_{27}H_{46}O$ (cholesterol), $C_6H_{10}N_2O_2$ (peptone), $C_{14}H_{24}O_9$ (agar), KH_2PO_4 (potassium dihydrogen phosphate), $C_4H_4N_2O_2$ (uracil), $C_6H_{12}O_6$ (dextrose), NaOH (sodium hydroxide), NaClO (sodium hypochlorite solution), NaN_3 (sodium azide), and Na_2HPO_4 (sodium hydrogen phosphate) were procured from SRL Pvt. Ltd. and Merck Millipore, Mumbai, India. All chemicals used in the study were of analytical grade with a purity of ≥99%.

2.2 Nematode Culture

N2 wild-type of *C. elegans* was sourced from the Caenorhabditis Genetics Centre (CGC) and cultured on a sterile Nematode Growth Medium (NGM). The *Escherichia coli* OP50 strain was used as a food source. Nematodes were harvested and treated with a bleaching solution (0.45 M NaOH, 2% HOCl) to isolate the eggs. Synchronised embryos were exposed to ciprofloxacin for 48 hours until they reached the L4 stage, following established protocols[28,29].

2.3 Exposure Experiment

Ciprofloxacin was dissolved in 0.01% DMSO to prepare the desired stock solution, which was then diluted to working concentrations as previously described[30,31]. The stock solution of 0.09 mg/ml was maintained in the dark at 4°C. *C. elegans* were subjected to 0.09, 0.0009, and 0.00009 (mg/ml) for the dose-response study, and 0.0009 and 0.00009 (mg/ml) doses of ciprofloxacin were selected for further investigations. Embryos were placed onto ciprofloxacin-seeded plates and continuously exposed from the embryonic stage to the L4 stage, at which point most of the endpoints were examined.

2.4 Development and Growth Alteration

To assess the dose-response in *C. elegans*, NGM plates were seeded with OP50 and supplemented with different concentrations of ciprofloxacin. After that, the plates were incubated for 12 hours at 22°C. Each group was seeded with 200 µL of embryos. Nematodes were incubated at 22°C for two days. Worms were then collected and washed with M9 buffer. Each group received 20 µL of sodium azide to render the worms immobile. Nematodes in a volume of 20 µL were then placed on slides and covered with a cover slip. Each group›s 15 worm photos were obtained at a 10x magnification using a fluorescence microscope. Then, the body length of nematodes in each group was examined using freehand line tools in Image J software.

2.5 Pharyngeal Pumping

Nematode food intake was measured by the pharyngeal pumping process. Briefly, L4-stage worms were moved to microcentrifuge tubes after being washed with M9 buffer. Each plate was spotted with 15-20 µL of nematode suspension. After being given 30 min to become used to their new surroundings, the worms began to move around freely. Using a stereo-zoom microscope, pharyngeal pumping action was captured for ten seconds at 8x magnification. Feeding behaviour was observed and documented in at least 20 worms per group[32,33].

2.6 Nile Red Assay

Lipid levels in N2 wild-type worms treated with ciprofloxacin were assessed using Nile red staining. Acetone was used to make a 0.5 mg/ml Nile red solution, which was then kept at 4°C. 3 µl of the dye and 750 µl of OP50 mixed with the test chemicals were combined to create a working solution. Nematodes in the L4 stage were collected after 48 hours, cleaned, treated with 20 µl of sodium azide, and then placed on slides. Fluorescence microscopy with a rhodamine filter at 20x magnification was used to capture the images. Lipid content was quantified using Image J software, analysing at least 15 images per group based on Nile red fluorescence intensity. The boxes were adjusted to encompass the maximum area of the worm with fat deposition, and the background subtraction was applied to minimise the noise and ensure uniformity across all images. This approach ensured that only the Nile Red areas corresponding to lipid droplets were included in the analysis[34,35].

2.7 Locomotion Behaviour

The embryos were cultured for 48 h on NGM plates that had been seeded with ciprofloxacin. After collection and thorough washing with M9 buffer, 20-30 nematodes suspended in 20 µL were transferred to unseeded NGM plates. For a short time, the worms were allowed to become used to their new surroundings and wander around freely. Then, a 30-s video was captured for every group using Leica software and a stereo-zoom microscope. Over 20 worms per group were used, and the amount of thrashes was recorded every 10 s previously detailed by Buckingham and Sattelle[36].

2.8 1-Nonanol Assay

The 1-Nonanol assay was conducted to assess whether ciprofloxacin exposure induces neurotransmission defects in *C. elegans*. In this experiment, a worm picker was immersed in 200 µl of 1-Nonanol contained in a microcentrifuge tube. The worm picker was carefully positioned close to the snout of an actively moving worm under a stereo microscope. The time taken by the worm to react and migrate away from the 1-Nonanol was measured. In this investigation, a minimum of 20 nematodes from each group were evaluated as explained elsewhere[37].

2.9 Brood Size and Reproductive Age

Each experimental group's age-synchronised nematodes were moved to new plates. Worms were carefully transferred to fresh plates to make sure no embryos or L-1 offspring were left behind, and the number of progeny was noted. Daily transfers persisted until the egg production stopped, and the time to reproductive stoppage was also noted[38,39].

2.10 Embryonic Viability

Worms at the L-4 stage were moved onto freshly prepared plates, and eggs were counted the following day. The plates were left untouched for 1 day to allow sufficient time for embryos to hatch. The counts of hatched progeny and unhatched embryos were documented. This process was repeated for all experimental groups until the worms no longer produced embryos. Embryonic viability for each group was then determined as described in article[40].

2.11 Statistical Analysis

Statistical significance was assessed using a Student's t-test in GraphPad Prism 5, with significance levels defined as ($p<0.05$), ($p<0.005$), ($p<0.0001$), and ($p<0.0005$). Each

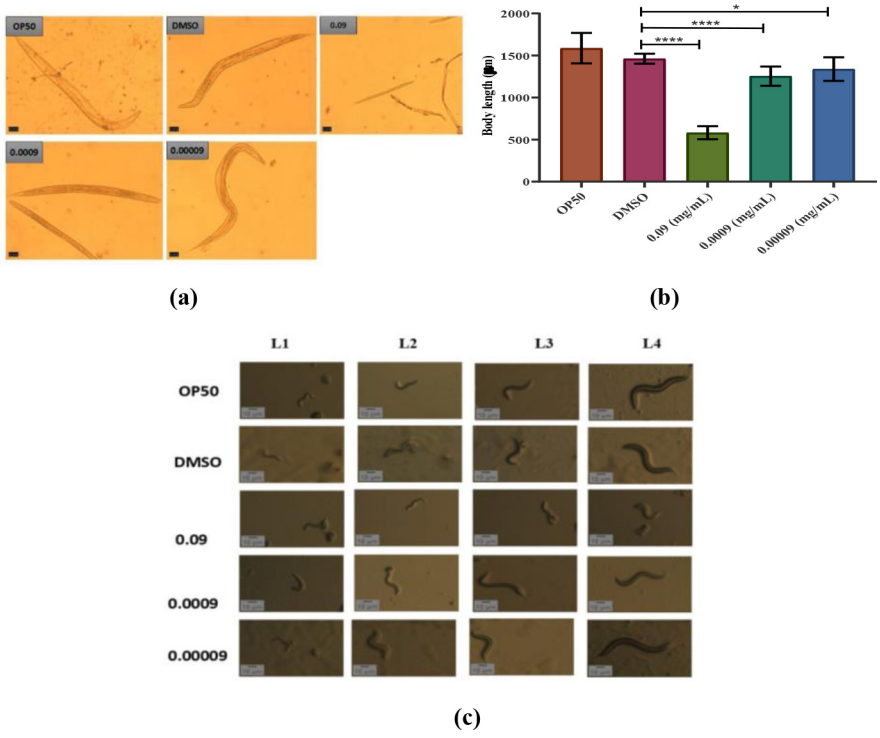


Figure 1. Developmental toxicity due to Ciprofloxacin in *C. elegans*.

experiment was performed at least twice to ensure the results were consistent and reliable.

3. Results

3.1 Developmental Toxicity due to Ciprofloxacin in *C. elegans*

Ciprofloxacin significantly affected the body length and overall morphology of *C. elegans*, causing a marked delay in developmental stages from embryo to L4, as depicted in Figure 1(a-c). Specifically, at a concentration of 0.09 mg/mL, the body length and general body shape were notably impacted, preventing the attainment of maximum body length. Since 0.09 mg/mL displayed extreme toxicity in terms of decline in the body length (growth) of *C. elegans*, two doses of 0.0009 mg/mL and 0.00009 mg/mL were taken for further investigation.

3.2 Feeding Behaviour of *C. elegans* When Treated with Ciprofloxacin

Ciprofloxacin may have disrupted the mitochondrial function and neuromuscular control at higher concentrations, leading to the observed impacts as shown in Figure 2. The altered feeding patterns suggest that ciprofloxacin impairs the neuromuscular function involved in pharyngeal pumping in *C. elegans.*

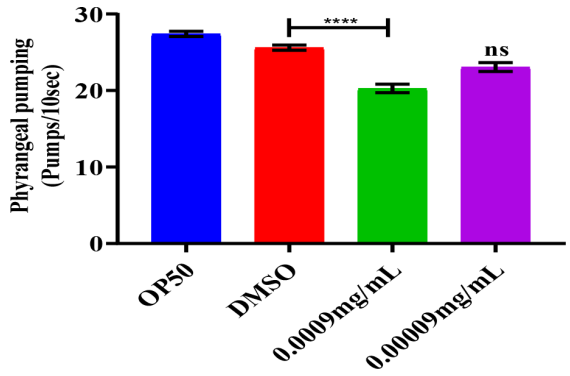


Figure 2. Feeding behaviour of *C. elegans* when treated with Ciprofloxacin.

3.3 Thrashing and 1-Nonanol Response Influenced by Ciprofloxacin in *C. elegans*

There was a significant decline in the thrashing frequency of *C. elegans* when exposed to both the highest (0.0009 mg/mL) and lowest (0.00009 mg/mL) concentrations of ciprofloxacin, as depicted in Figure 3a**.** During the 1-Nonanol assay to assess *C. elegans* chemosensitivity following ciprofloxacin exposure, no notable alterations were observed in the nematodes' sensitivity to 1-Nonanol. This observation remained consistent across both the highest and lowest environmentally relevant concentrations of ciprofloxacin, as depicted in Figure 3b.

3.4 The Impact of Ciprofloxacin on Lipid Deposition in *C. elegans*

It was found that a low concentration of ciprofloxacin influenced the significant increase in lipid content as seen in Figure 4 (a, b). However, a high studied concentration of ciprofloxacin did not cause any significant changes in lipid content in nematodes.

3.5 Effect of Ciprofloxacin on the Reproductive Fitness of Nematode

The number of brood sizes significantly declined at the highest concentration of 0.0009 mg/mL of ciprofloxacin, and the reproductive age of *C.elegans* was significantly reduced to a maximum of four days as seen in Figure 5 (a-c). Still at the lowest concentration, the reproductive age of *C. elegans*

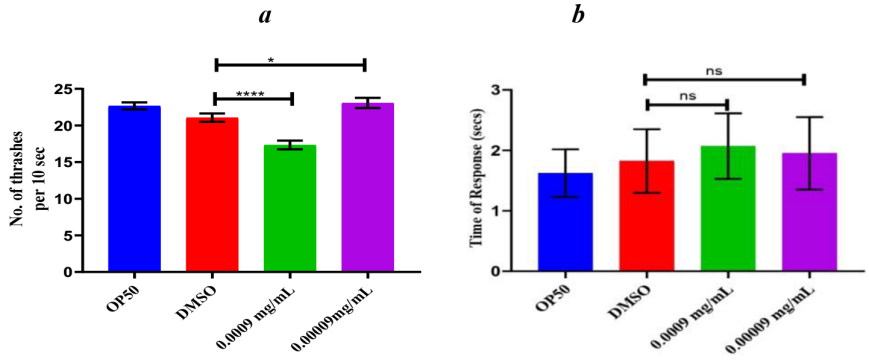


Figure 3. Thrashing and 1-Nonanol response influenced by Ciprofloxacin in *C. elegans*

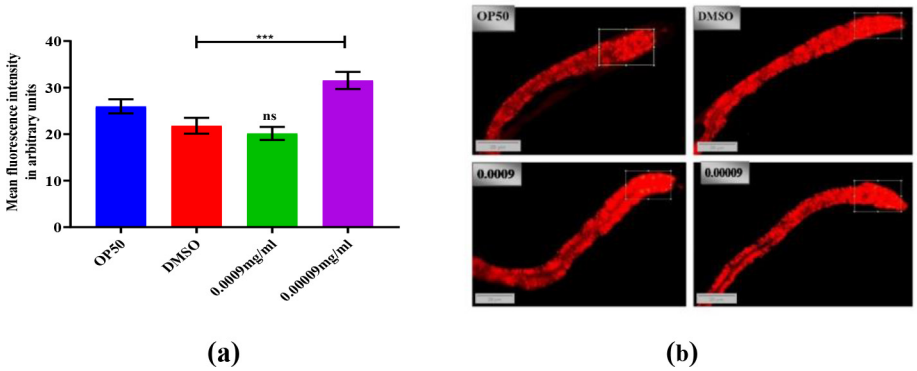


Figure 4. The impact of Ciprofloxacin on lipid deposition in *C. elegans*

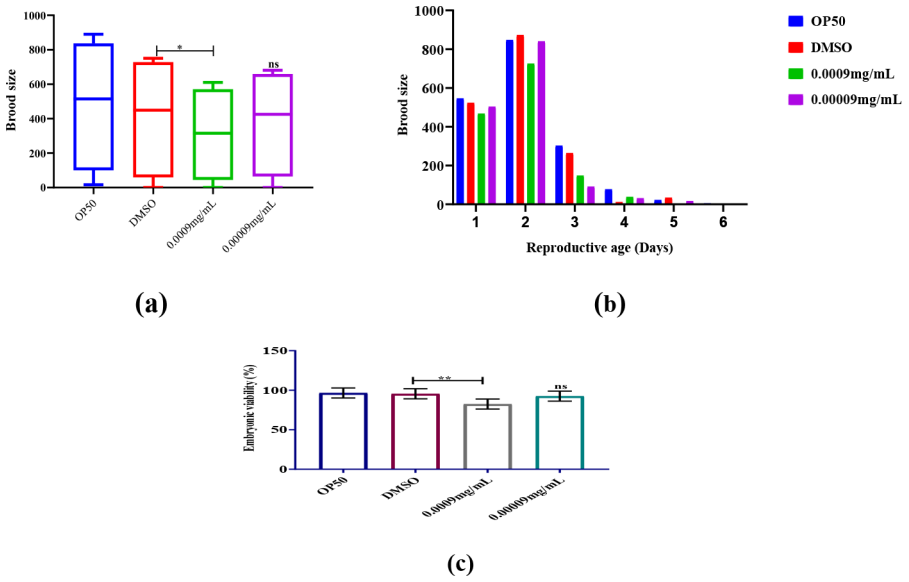


Figure 5. Effect of Ciprofloxacin on the reproductive fitness of nematode.

was extended to 5 days as depicted in Figure 5b. The ability of *C. elegans* to hatch was observed, and it was shown that a significant proportion of laid eggs (embryos) were unable to hatch into offspring at the maximum concentration of ciprofloxacin, as depicted in Figure 5c. This might have been caused by ciprofloxacin inhibiting DNA gyrase and topoisomerase IV, which might have induced DNA damage, which significantly led to abnormalities in cell proliferation, meiosis, and embryonic development, thus affecting the brood sizes, embryonic viability, and declined reproductive capability in N2 *C. elegans*.

4. Discussion

Fluoroquinolone-induced neurotoxicity has been shown to cause non-feeding behaviour in model organisms such as *C. elegans*, leading to reduced food intake and impaired swimming activity[41]. In the present work, *C. elegans*' pharyngeal pumping slowed, suggesting that food intake may have been hampered when exposed to ciprofloxacin. This may be due to the disruption of rhythmic pharyngeal contractions in *C. elegans* by hyperpolarising the nervous system and reducing the pumping in the corpus, isthmus, and terminal bulb by ciprofloxacin[42,43]. Furthermore, ciprofloxacin has been reported to cause decreased appetite and reduced body size in *Rhinella arenarum* larvae, as well as reduced exploratory behaviour in *Danio rerio*[44]. Insights from *C. elegans* may help to predict ciprofloxacin impacts on human gut motility[45] and neurotoxicity, which could signal potential risks for human neurological side effects[46].

In humans, ciprofloxacin has been associated with gastrointestinal side effects, including abdominal pain, vomiting, and diarrhea[47]. Changes in nutrient absorption in *C. elegans* due to imbalances in gut microbiota by ciprofloxacin may reflect similar disruptions in human gut microbiota, potentially leading to dysbiosis[48]. This antibiotic has been associated with neurotoxic effects, leading to complications like dizziness and peripheral neuropathy[49]. Ciprofloxacin can result in metabolic disturbance, including hyperglycemia and hypoglycemia, specifically in diabetic patients. This condition might lead to increased thirst but decreased food intake, high-sugar food cravings, and erratic feeding patterns[50].

Moreover, the locomotion activity of *C. elegans* decreased, but there were no significant changes in the response to 1-nonanol chemical stimuli. Locomotion in *C. elegans* may have been impaired through neuromuscular disruption, plus low energy production through mitochondrial damage[51], and calcium signalling imbalances induced by ciprofloxacin[52]. Ciprofloxacin and enrofloxacin have been reported to cause muscle spasms and impair movement in fish, which ultimately may result from cardiac failure[53]. Ciprofloxacin has been involved in the disruption of cholinesterase in muscular tissues, which might lead to the impairment of neurotransmission[54] and reduced motor activity in *Daphnia magna*[55]. Birds administered a dose of ciprofloxacin above 5mg/kg per body weight led to severe gastrointestinal symptoms, optimal weight loss, and leg weakness; the animals were unable to move[56]. Ciprofloxacin exposure to *Oreochromis niloticus* significantly increased the level of cholinesterase activity but returned to normal on the 14th day of exposure[57]. In another case, ciprofloxacin exposure to *C. elegans* compromised the chemosensory neurons, and this alteration in behaviour indicates disruptions in neural functions[58]. This provides valuable insights into the drug's potential sensory side effects in humans[59]. Impaired chemotaxis in *C. elegans* by ciprofloxacin could correlate with sensory disturbance in humans, like anosmia and ageusia[60]. This sensory deficit can cause unhealthy conditions like hyperalgesia or hypoalgesia, and these alterations can complicate pain management in patients[61]. Drugs can impair motor integration in humans, which may result in coordination issues, delayed reflexes, and an increased risk of accidents[62].

Furthermore, Ciprofloxacin's diverse impact on *C. elegans* behaviours could be linked to the various neurological and cellular mechanism that underpins each reaction. Sensory neurons like ASH and ADL appear to be less vulnerable to ciprofloxacin poisoning than motor and pharyngeal neurons[63,64]. This absence of significant effect implies that sensory circuits engaged in 1-Nonanol response are either more robust, and have defensive mechanisms such as reduced drug permeability or strong detoxifying capacity[65]. Alternatively, feeding and thrashing behaviour, which relies on intensive neuromuscular coordination, may be more susceptible to ciprofloxacin toxicity[66]. Ciprofloxacin's interference with mitochondrial function, oxidative stress, and ion homeostasis could disproportionately disrupt these behaviours, impairing neurotransmitter release and muscle contraction[67].

The lowest concentration of ciprofloxacin was associated with lipid deposition in *C. elegans*, which may have been influenced by microbiota[68] and interference with lipid metabolic pathways[69]. As a broad-spectrum antibiotic, ciprofloxacin may disrupt the transient gut microbiota in *C. elegans*, which are acquired from the environment, and influence lipid metabolism, as *C. elegans* does not have a complex gut microbiota of its own, as explained elsewhere[70]. Enrofloxacin was reported to disrupt the accumulation of lipids in the *Alosa sapidissima* by affecting the metabolic pathway[71]. A study done on zebrafish suggested a link between the structure of floroquinoles and the induction of neurotoxicity[72]. Ciprofloxacin was found to decrease lipid peroxidation in *Lemna gibba* by enhancing the activity of the antioxidant catalase enzyme[73]. Observing lipid accumulation in *C. elegans* may indicate the need for lipid profile monitoring in patients taking ciprofloxacin. Elevated levels of cholesterol and triglycerides have been associated with dyslipidemia in humans due to exposure to antibiotics[74], which leads to cardiovascular diseases like heart attack, stroke, and atherosclerosis[75]. Altered fat distribution leads to increased visceral fat or ectopic fat deposition, risk factors for metabolic diseases[76]. Furthermore, changes in body composition along with high body weight may result from disturbances in glucose and lipid metabolism[77].

Ciprofloxacin was observed to decrease brood size, shorten the reproductive age, and decrease embryonic viability at its highest studied concentration in *C. elegans*. The observed reproductive patterns in *C. elegans* may have been influenced by impairment of the Gonadotropin-releasing hormone[78] and glycopeptide-releasing hormone receptors[79]. Ciprofloxacin may result in a significant decline in metabolism and reproductive ability in *C. elegans*.

Norfloxacin was shown to decrease the hatching rate of embryos and increase deformity and mortality in *Danio rerio*[80]. Ciprofloxacin led to a decline in the number of *Lumbriculus variegatus,* suggesting its potential ecotoxicity, and it also highlighted the spread of resistance even at its low concentration[81]. The growth and brood size of *L. minor* species were significantly affected[82] by the increasing concentration of ciprofloxacin. Administered to pregnant female rats during organogenesis, ciprofloxacin reduced the number of fetuses[83] and increased fetal impairments[84]. Male zebrafish exposed to 0.0000156 mg/mL of ciprofloxacin experienced damage to their reproductive system, reduced spermatogonia weight, and reduced growth level[85]. Exposure to 12.5 mg/mL of ciprofloxacin in *Daphnia magna* resulted in inhibited newborn growth production and sublethal effects on the timing of first egg-bearing and overall reproduction[86]. Ciprofloxacin exposure in *C. elegans* can provide critical insights into the drug's potential impact on human reproductive health. Reduced fertility and fecundity could manifest decreased sperm count, sperm motility[87] and impaired ovulation and egg utility in females[88]. Reduced libido, menstrual irregularities, and decreased gamete quality have been observed due to antibiotic exposure[89]. Ciprofloxacin impairs gametogenesis and increases the risk of infertility in humans[90].

Ciprofloxacin may induce developmental abnormalities and increase the risk of congenital defects[91]. It may interfere with DNA replication and cellular metabolism, leading to developmental delays, reduced pharyngeal pumping, altered lipid storage, and impaired reproduction. Pregnant women taking ciprofloxacin experienced adverse outcomes, including miscarriages, birth defects, and preterm births[92]. In *C. elegans*, changes in body morphology suggest broader toxic effects, aligning with findings in humans showing developmental toxicity, growth retardation, and malformations following prenatal exposure. Embryonic exposure is critical, as ciprofloxacin may reach infants during pregnancy or through breastfeeding[93].

R. arenarum larvae exposed to <0.001 mg/mL ciprofloxacin for 96 h showed stunted growth and development[44]. In *C. elegans*, mitochondria and chloroplasts may have contributed to reduced growth[94]. Fluoroquinolones also hindered algal development by inhibiting enzymes. Enrofloxacin caused leaf yellowing by affecting the chloroplast-specific DNA gyrase[2]. *Daphnia magna* exposed to enrofloxacin for 12 days exhibited reduced development, growth, and survival[95].

Ciprofloxacin has been associated with multiple organ toxicities. It may cause cardiotoxicity, including prolonged QT and QTc intervals and extended action potential duration, due to its ability to block cardiac voltage-gated potassium channels[97]. Hepatotoxic effects include hepatocellular necrosis, mixed inflammatory infiltrates, and eosinophil accumulation in liver tissue have also been reported[4]. Ciprofloxacin-induced neurotoxicity has also been reported, manifesting as abnormal postures and body shapes resulting from nerve impairment[49,98]. These neurotoxic effects are likely driven by ciprofloxacin's interference with DNA integrity, mitochondrial function, and protein synthesis, ultimately reducing cell division and overall growth[96].

Ciprofloxacin is well known for its mechanisms of action by interfering with the DNA gyrase and topoisomerase IV enzymes critical for DNA replication and repair. These effects are not limited to nuclear DNA and can extend to mitochondrial DNA, given its similar topology and reliance on similar enzymatic processes. Furthermore, different studies have shown the effects of ciprofloxacin on mitochondrial DNA as detailed by Fromenty[99]. Other findings showed that levofloxacin suppressed the growth in *M. flos-aquae* at a 0.000001 mg/mL concentration[100]. *Raphidocelis subcapitata* exposed to (0.0000001-0.0005) mg/mL of ciprofloxacin led to a reduced growth rate and decreased cell density[10]. Our findings suggest altered behavioural and physiological functioning of *C. elegans* subjected to ciprofloxacin from an embryonic stage, as antibiotics may have disrupted essential cellular processes during this critical period of growth. Furthermore, lipid deposition and reproductive potential in *C. elegans* significantly declined at the studied concentrations. Present findings also reveal that ciprofloxacin has the potential to cause damage to living organisms even at low concentrations. Further investigation to understand its detailed toxicity in living organisms and to develop strategies to mitigate its environmental impacts is highly recommended.

To mitigate its environmental impact, it is recommended to strengthen wastewater treatment technologies, such as advanced oxidation processes, activated carbon filtration, and membrane bioreactors, to remove ciprofloxacin residues effectively. In addition, stricter regulations and routine monitoring of antibiotic discharges from pharmaceutical industries, hospitals, and agricultural runoff should be enforced. Promoting rational antibiotic use through public health awareness

and prescription control is also crucial in reducing unnecessary environmental contamination. Moreover, the development of eco-friendly and biodegradable antibiotic alternatives should be encouraged to lower ecological risk. Continued research and environmental surveillance are essential to track antibiotic residues and assess long-term impacts on ecosystem health.

5. Conclusion

This study demonstrates that ciprofloxacin contamination in the environment can exert significant toxicological effects on *C. elegans*, causing disruptions in growth, locomotion, chemical stimulus response, feeding behaviour, reproductive capacity, and lipid metabolism. The findings in this study underscore the need for further research to understand the environmental hazards caused by ciprofloxacin. They also highlight the importance of developing strategies to mitigate these risks, including stronger enforcement of environmental protection laws and legal actions against violators.

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7. Authors' Contributions to the Manuscript

EK - Investigation, methodology, writing original draft preparation, writing review and editing, and formal analysis. AN - Conceptualisation, visualisation, supervision, resources, validation. RA - Conceptualisation, writing, review and editing, data curation, supervision, validation.

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