Paracetamol Toxicity Assessment in Artemia salina

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***Abstract*—Background: Acetaminophen (paracetamol) is a widely used analgesic and antipyretic drug, increasingly detected in aquatic environments. Despite its therapeutic use, concerns exist regarding its hepatotoxic effects and ecological risks.**

Methods: The present study evaluated acetaminophen toxicity using *Artemia salina* as a model organism. Twenty-four-hour-old nauplii were exposed to serial dilutions of paracetamol (0.625–10 mg/mL) for 14 hours. Mortality was recorded hourly, and LC_{50} values were calculated using probit analysis in R. Time-dependent LC_{50} values were plotted to assess acute versus chronic toxicity.

Results: Mortality increased with both concentration and exposure duration. At 4 hours, LC_{50} was ~37,000 mg/L, indicating low acute toxicity. However, LC_{50} declined sharply between 6–8 hours (19,000–6,000 mg/L), stabilizing below 1,000 mg/L by 14 hours. This trend reflects toxicant accumulation and progressive physiological stress.

Conclusion: Acetaminophen exhibits strong time-dependent toxicity in *A. salina*. Short-term tests underestimate risk, whereas prolonged exposures reveal chronic toxicity even at low concentrations. These findings highlight the importance of incorporating time-dependent LC_{50} values into ecological risk assessments.

***Index Terms*—Acetaminophen, Paracetamol, *Artemia salina*, LC_{50}, Toxicity, Ecotoxicology, Bioaccumulation**

I. INTRODUCTION

Pharmaceuticals are among the most frequently detected emerging contaminants in aquatic environments. Continuous discharge from hospitals, households, and pharmaceutical industries introduces significant quantities of active pharmaceutical ingredients (APIs) into rivers, lakes, and coastal ecosystems. Unlike many conventional pollutants, pharmaceuticals are designed to be biologically active at low concentrations, raising concerns about their ecotoxicological effects on non-target organisms. Among these, acetaminophen (N-acetyl-para-aminophenol, APAP), commonly known as paracetamol, is one of the most widely consumed analgesic and antipyretic drugs worldwide [1,2].

Acetaminophen is available over-the-counter and is considered safe at therapeutic doses. Its widespread use has made it one of the most detected pharmaceuticals in wastewater, surface waters, and even groundwater. Once ingested, it is rapidly absorbed, with bioavailability reaching nearly 88%, and peak plasma concentrations occurring within 90 minutes [3]. While it is metabolized primarily in the liver through glucuronidation and sulfation, excessive doses overwhelm these pathways, leading to hepatotoxicity due to the accumulation of the toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI) [4]. Acute liver failure due to paracetamol overdose is a well-documented clinical emergency, and inter-individual variability in glutathione levels further influences susceptibility.

Beyond clinical concerns, emerging evidence suggests links between maternal paracetamol consumption and developmental effects in offspring, such as attention deficit hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) [2]. Such findings underline the dual significance of acetaminophen as both a medical necessity and a compound of toxicological concern.

From an environmental perspective, acetaminophen residues are increasingly reported in effluents of wastewater treatment plants and in surface water bodies, particularly in urbanized regions. Since pharmaceuticals are often incompletely removed during wastewater treatment, their persistence in aquatic environments raises questions about long-term ecological risks. Unlike acute toxicity studies that capture only immediate effects, chronic exposure may reveal more subtle but significant risks, including

bioaccumulation, metabolic disruption, and mortality in aquatic organisms.

To assess these risks, model organisms are essential. *Artemia salina* (brine shrimp) has emerged as a reliable test species for ecotoxicological research due to its ease of culture, short life cycle, cost-effectiveness, and sensitivity to a wide range of toxicants [5]. Nauplii can be easily hatched from dormant cysts under laboratory conditions, providing a consistent and reproducible bioassay system. Moreover, results from *Artemia* toxicity assays have shown strong correlations with mammalian models, making it a suitable alternative to vertebrate testing [3,4].

Although numerous studies have assessed the pharmacology and clinical toxicity of acetaminophen in humans, there is limited data on its ecotoxicological impacts on aquatic invertebrates under time-dependent conditions. Most studies focus on single-dose acute toxicity, which may underestimate the ecological risk posed by prolonged environmental exposure. In natural aquatic ecosystems, organisms are often subjected to chronic low-level exposures rather than acute high-dose events.

The present study addresses this gap by investigating the concentration- and time-dependent toxicity of acetaminophen on *Artemia salina***.** Mortality rates were recorded at hourly intervals for 14 hours, and LC_{50} values were determined using probit analysis. By plotting LC_{50} against exposure time, this study aims to illustrate how prolonged exposure amplifies toxicity, thereby providing insights into the ecological risks of acetaminophen contamination in aquatic systems.

II. MATERIALS AND METHODS

Test Organism Preparation

Commercially available *Artemia salina* cysts were obtained from an authorized supplier. The cysts were hydrated and hatched in 5% NaCl saline solution under constant aeration for 24 hours. Illumination and aeration were maintained to optimize hatching efficiency. After 24 hours, actively swimming nauplii were collected and used as the test organisms. These freshly hatched nauplii were chosen due to their uniformity in age, high sensitivity to toxicants, and wide applicability in ecotoxicological assays.

Preparation of Test Solutions

A paracetamol (acetaminophen) infusion with a stock concentration of 10 mg/mL was used as the test toxicant. From this stock, serial dilutions were prepared in 5% NaCl saline solution to obtain the following test concentrations: 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, and 0.625 mg/mL. A control group consisting of only 5% NaCl solution, without paracetamol, was maintained under identical conditions to account for natural mortality. All solutions were freshly prepared immediately prior to the experiment to avoid degradation of the compound.

Experimental Design

Toxicity assays were carried out in sterile 24-well microplates. Each well contained 30 *Artemia salina* nauplii in 2 mL of the corresponding test solution. Separate wells were designated for each concentration and for the control. The experiments were performed in triplicate to ensure reproducibility and statistical validity. Test conditions were maintained at room temperature with continuous light exposure to simulate optimal hatching and survival conditions.

Mortality Assessment

The nauplii were observed at 1-hour intervals for a total exposure duration of 14 hours. Mortality was determined by the absence of swimming activity and lack of response to gentle stimulation. The number of dead organisms was recorded for each concentration at every observation point. Mortality rates were corrected against the control group to eliminate background deaths unrelated to toxicant exposure.

Data Analysis

Corrected mortality data were used to estimate the median lethal concentration (LC_{50}) values at each time point. LC_{50} values were calculated using probit analysis, a standard statistical method for quantifying dose-response relationships. Statistical computations and curve fitting were performed using R software. The temporal relationship between LC_{50} (mg/L) and exposure duration (hours) was plotted, and an exponential decay model was fitted to describe the trend in toxicity over time.

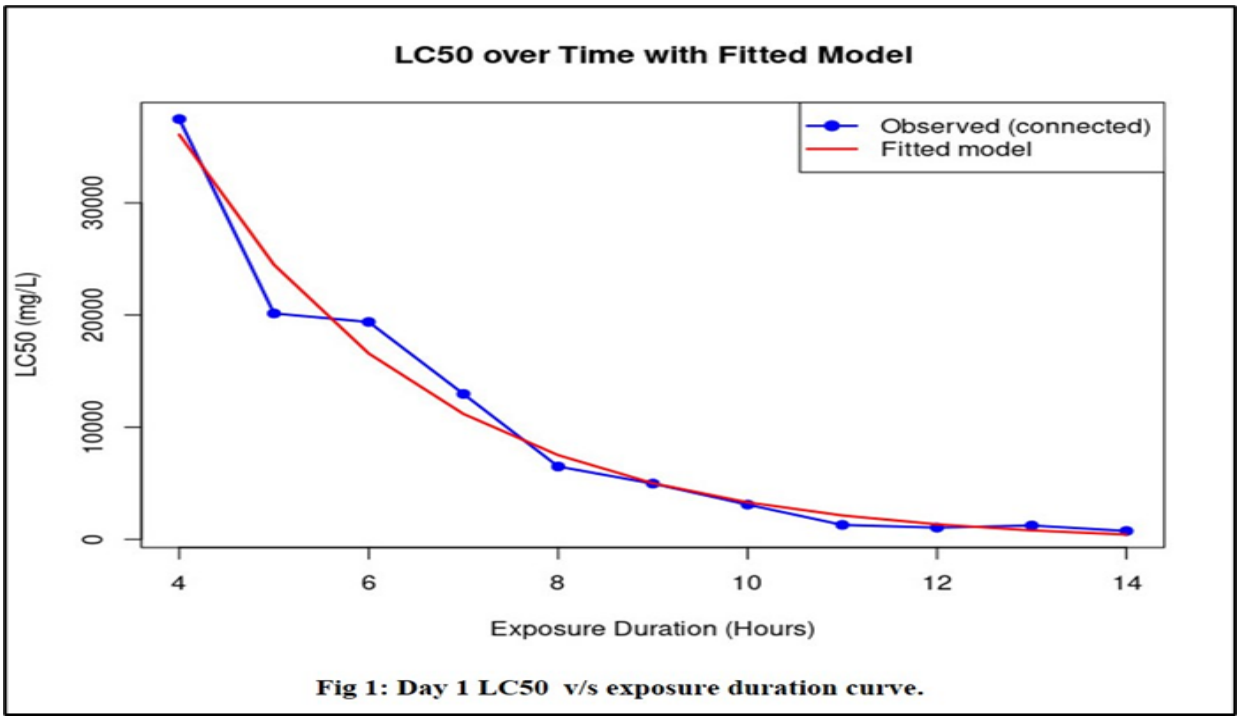

Fig 1: Day 1 LC50 v/s exposure duration curve.

III. RESULTS

Mortality data were corrected by subtracting natural deaths observed in the control population, ensuring that only treatment-related effects were considered. Hourly mortality rates of *Artemia salina* were recorded across varying concentrations of acetaminophen (0.625, 1.25, 2.5, 5, and 10 mg/mL) over a 14-hour exposure period. The experiment was conducted in three independent sets, with each set observed daily for 14 hours.

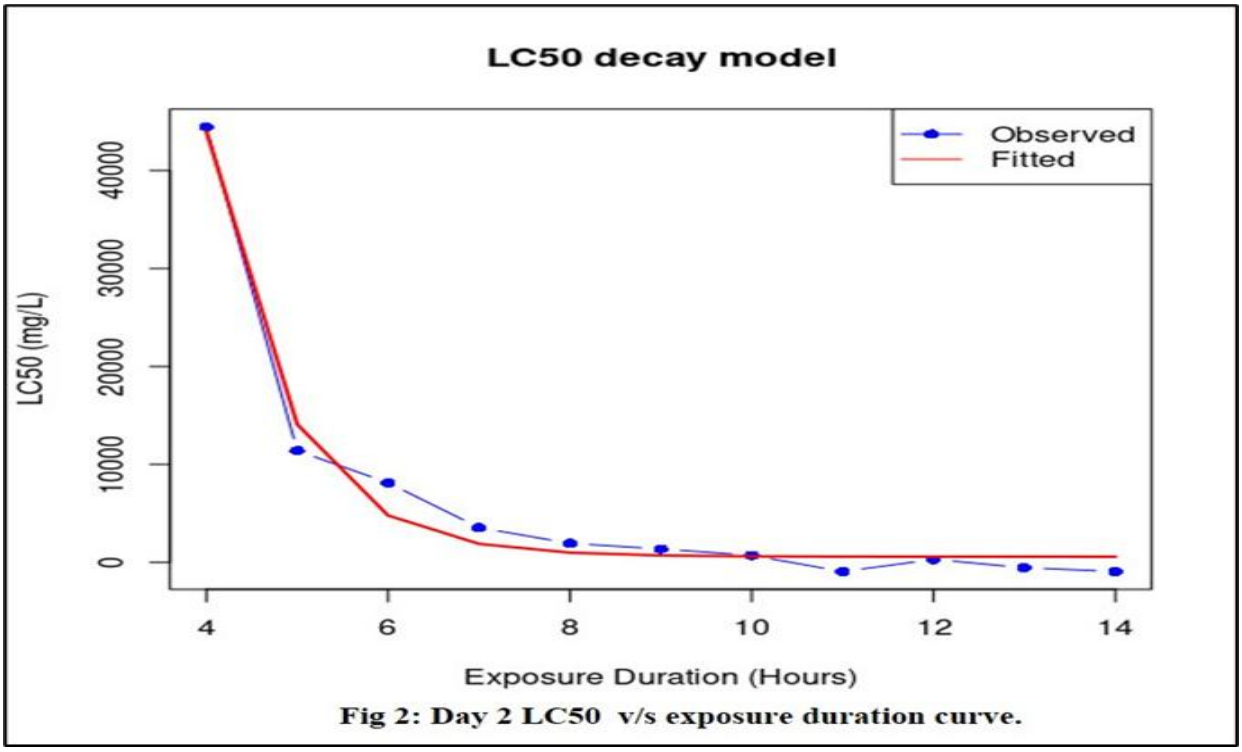

Fig 2: Day 2 LC50 v/s exposure duration curve.

In **Set 1**, the highest mortality (30%) was observed at 5 mg/mL at the 14th hour.
In **Set 2**, the maximum mortality (28.57%) occurred at both 5 mg/mL and 10 mg/mL at the 14th hour.
In **Set 3**, the peak mortality (28.57%) was recorded at 10 mg/mL at the 10th hour.

Across all sets, the highest mortality consistently occurred at 5 and 10 mg/mL concentrations, indicating that elevated acetaminophen levels are acutely harmful to *Artemia* populations. Mortality at lower concentrations (0.625–2.5 mg/mL) remained minimal during the initial hours but progressively increased with time. The lowest mortality was observed at 0.625 mg/mL, suggesting that hepatotoxicity at sub-lethal doses may occur but is insufficient to cause significant mortality during short exposures. However, with prolonged exposure, mortality rates increased across all concentrations, reflecting the cumulative toxic effect.

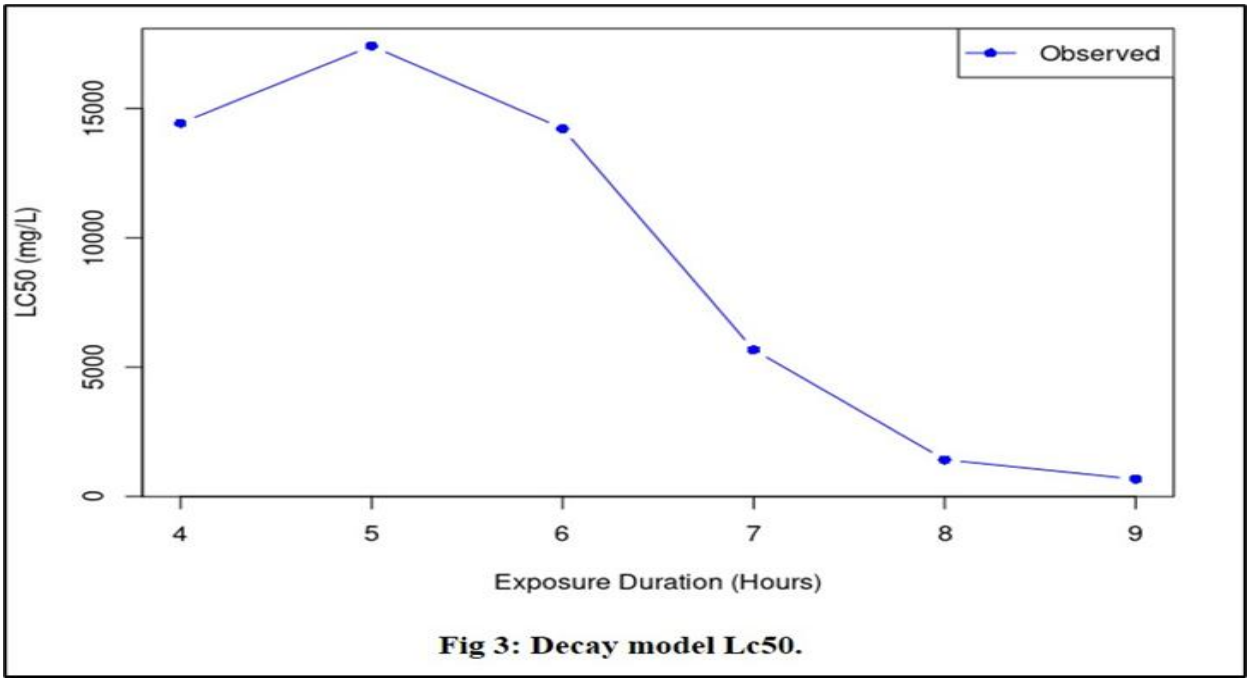

Fig 3: Decay model Lc50.

These results highlight the influence of both concentration and exposure time on acetaminophen toxicity. The increasing mortality over time suggests that toxicant accumulation surpasses elimination capacity, consistent with acetaminophen's pharmacokinetics in higher organisms. Acetaminophen exhibits low plasma protein binding (10–25%) and distributes extensively throughout body tissues (excluding fat), with most metabolites excreted in urine and less than 5% appearing as free acetaminophen [7]. Over 90% of a therapeutic dose is eliminated within 24 hours [8]; however, sustained exposure may disrupt this clearance, enhancing toxicity in aquatic species.

The LC_{50} values were determined using probit analysis, and LC_{50} (mg/L) versus exposure duration (hours) was plotted. The curve demonstrated a pronounced time-dependent decline. At 4 hours, LC_{50} was extremely high (~37,000 mg/L), indicating low acute toxicity. Between 6–8 hours, LC_{50} dropped steeply (~19,000–6,000 mg/L), reflecting rapid toxicant uptake and physiological stress accumulation. By 10–14 hours, LC_{50} stabilized at <1,000 mg/L, revealing significant chronic toxicity potential.

Overall, the results establish that acetaminophen toxicity is both concentration- and time-dependent. Short-term exposures underestimate toxicity, while prolonged exposure markedly increases lethality even at lower concentrations.

IV. DISCUSSION

The results of this study reveal a clear time-dependent trend in acetaminophen toxicity toward *Artemia salina*. On Days 2 and 3, the organisms initially exhibited apparent resistance to the toxicant during the early hours (4–5 h). However, between 6–8 h, a sharp

decline in LC_{50} was observed, suggesting that the organism's detoxification or defense mechanisms were progressively overwhelmed. This indicates that with increasing exposure, the rate of toxicant accumulation within the organism surpasses its elimination capacity, resulting in accelerated mortality across the population.

From 10–15 h, the LC_{50} values stabilized, yet mortality occurred even at relatively low concentrations (as low as 1 mg/L). This trend demonstrates that longer exposure durations substantially reduce the threshold concentration required to induce 50% lethality. In other words, while higher concentrations are lethal in short exposures, even sub-lethal concentrations become fatal over extended timeframes. The exponential decay model fitted to the data aligns well with this observation, validating that mortality is a joint function of both exposure concentration and duration. The monotonic decline in LC_{50}, characterized by a steep initial drop followed by an asymptotic plateau, is consistent with the classical behavior described by exponential decay or log-linear models [9].

LC_{50} calculations were performed using probit analysis, and the fitted exponential decay curve confirmed that the time-dependent decrease in LC_{50} is statistically robust. Such patterns are well-documented in toxicology, reflecting the principle that prolonged exposure allows toxicants to accumulate internally, thereby lowering the external concentration required for lethality. This highlights a critical limitation of acute bioassays: short-term assessments may significantly underestimate the risk posed by contaminants. For instance, the LC_{50} estimated at 4 h was much higher than that observed at 12 h, underscoring the importance of incorporating exposure duration in risk assessment protocols [10].

The ecological implications of these findings are substantial. In natural aquatic systems, organisms are often chronically exposed to low concentrations of pharmaceuticals and other toxicants discharged from industrial, agricultural, and municipal sources. Bioaccumulation—the progressive increase in toxicant concentration within an organism relative to its environment [11]—further exacerbates this risk, often leading to physiological damage, organ dysfunction, and mortality. Our results support the notion that contaminants persisting in aquatic habitats, even at concentrations traditionally considered safe, may become increasingly hazardous over time. This is particularly concerning for pharmaceuticals such as acetaminophen, which are continually introduced into aquatic environments and may exert subtle but significant effects on non-target species.

Therefore, it is imperative that ecotoxicological studies account for both acute and chronic exposures. Long-term toxicity testing not only provides more accurate estimates of ecological hazard but also better reflects real-world environmental conditions. The present findings demonstrate that LC_{50} values decline to near 1 mg/L with prolonged exposure, emphasizing the heightened sensitivity of organisms over time. These results underscore the need to integrate time-dependent toxicity models into regulatory frameworks to avoid underestimating the ecological risks posed by pharmaceutical contaminants [12].

V. CONCLUSION

The present study demonstrates that acetaminophen exhibits concentration- and time-dependent toxicity in *Artemia salina*. Mortality increased proportionally with both higher concentrations and longer exposure durations, highlighting the significance of these two parameters in determining toxicological outcomes. The use of *Artemia salina* as a model organism proved effective due to its ease of culture, rapid hatching from durable cysts, cost-efficiency, and tolerance to laboratory conditions. Importantly, previous studies have established a strong correlation between toxicity outcomes in *Artemia* and mammalian models, thereby supporting its relevance as a reliable alternative in ecotoxicological assays.

The observed decline in LC_{50} values with increasing exposure duration reflects a cumulative toxic effect, where even lower concentrations of acetaminophen become lethal over time. Such patterns are consistent with reports on aquatic organisms exposed to pesticides, hydrocarbons, and heavy metals, where extended exposure significantly amplifies toxicity. These findings emphasize the importance of incorporating time-dependent analyses, rather than relying solely on acute endpoints, when evaluating the ecological risk of pharmaceutical contaminants.

In conclusion, the results underscore that both exposure concentration and duration are critical determinants of acetaminophen toxicity. Further studies involving molecular, biochemical, and

comparative physiological approaches are warranted to strengthen the link between these ecotoxicological observations and potential human health implications.

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