Report on Sertraline induced Acute Toxicity in Copepods (*Acartia tonsa*)

Environmental Monitoring & Risk Assessment

Syed Danish Ali

1. Introduction & Context:

The use of marine invertebrates as model organisms in toxicity testing has become a routine activity to monitor marine environment. This is considered as a valid method to evaluate the bioavailability of toxicants and their potential threats to marine organisms. The hazards linked to pharmaceutical residues like antidepressants are currently a major concern of ecotoxicology because they may have adverse effects on non-target aquatic organisms [1]. Our study assesses the ecotoxicity of a precription antidepressant: sertraline. The calanoid copepod Acartia tonsa was the animal in our bioassay and it is commonly used in ecotoxicology due to its widespread distribution and well-studied biology [2]. The marine planktonic calanoid copepod *Acartia tonsa* is a cosmopolitan, eurythermal, and euryhaline marine zooplanktonic organism common in subtropical and temperate latitudes and abundant in coastal and estuarine waters.

Moreover, this planktonic organism is distributed worldwide in marine and estuarine environments, being the most abundant species in the Atlantic and Pacific American coasts. In the Mediterranean and Baltic seas, *A. tonsa* was introduced by ship ballast waters in the 1980s [2] and has adapted well, since then, to euryhaline conditions, surviving sudden changes in salinities [3]. For these reasons, *A. tonsa* is widely used in ecotoxicology; bioassay protocols consider different end points, such as mortality, development, or fecundity in different life stages (eggs, nauplii, adults) after 48h short-term exposure (acute test). Over the last two decades, the presence of pharmaceutical compounds in aquatic environments has been a well-known fact that has forced the scientific community to consider this type of contamination as a potential issue that deserves attention [4]. Concern has been voiced due to the increasing prescription to treat mental disorders. A large proportion of the modern day population is medicated with antidepressants. The increasing consumption of drugs is likely to result in the presence of higher concentrations in the environment, and to a more likely occurrence of toxic effects on aquatic organisms [5].

Acute toxicity tests are routinely used to evaluate the quality of waters in coastal areas subjected to anthropogenic effects and to assess the pharmaceutical toxicity [6]. As these routine tests are simple, relatively inexpensive and rapid methods, they are used to compare the sensitivity of bioassays to chemical pollutants. The antidepressants we used as a contaminant were Sertraline STADA 50 mg film-coated tablets EFG. The active ingredient is sertraline (hydrochloride), and

each tablet contains 50 mg of sertraline, equivalent to 55.96 mg of sertraline hydrochloride. The endpoint of our experiment was an acute test (lethality) at 48h.

2. Experimental Design

The acute toxicity bioassay was designed to assess the lethal effects of sertraline on the copepod *Acartia tonsa*. The experimental setup included a range of sertraline concentrations and control groups to ensure the reliability and validity of the results. The primary objective of this study was to determine the lethal concentration (LC50) of sertraline for *Acartia tonsa* and to observe any dose-dependent lethality patterns. The test organism used in this study was the copepod *Acartia tonsa*. Copepods are a crucial component of the marine food web and serve as an important indicator species for assessing the environmental impact of contaminants.

2.1. Sampling

The copepods were sampled from a controlled laboratory culture maintained under standard conditions. Healthy, active animals were selected for the bioassay to ensure consistent and reliable results.

2.2. Culture Conditions

- Temperature: 22°C
- pH: 7.9
- Salinity: 33-35 PSU
- Light: Dark cycle of 18:6 hours
- Feed: Mix of tetraselmis & isochrysis
- Feed time: 2 days prior to experiment

2.3. Experimental Setup

The experiment consisted of three replicates for each concentration of sertraline, ranging from 100 to 1500 µg/L. A stock solution of sertraline hydrochloride was prepared by dissolving 50mg of sertraline hydrochloride, a constituent of the drug, in a solution of 0.01% dimethyl sulfoxide (DMSO). The use of DMSO is meant to efficiently dissolve sertraline in solution, but concentration was kept minimal avoid significant effect of the solvent on copepod mortality. The 0.01% DMSO solution was prepared by diluting 2M DMSO in filtered seawater.

To evaluate the acute toxicity of sertraline on Acartia tonsa, copepods were exposed to various concentrations of sertraline (0, 100, 300, 500, 700, 900, 1100, 1300, and 1500 mg/L) in 4.5 mL volumes per well. Initially, five copepods were placed into 5 mL wells containing a small amount of filtered seawater. Following careful selection and transfer of the copepods, specific volumes of the sertraline stock solution were added to each well and diluted to 4.5 mL, ensuring that the final concentration of sertraline corresponded to the desired treatment levels. The solvent control group contained the highest concentration of DMSO in the serial dilution. Three replicates

were done for each treatment. Surviving copepods were recorded every 24 hours over a 48-hour period, and percent mortality was calculated.

2.4. Concentration Levels

1. Control (seawater)
2. Control (DMSO+SW)
3. 100 µg/L
4. 300 µg/L
5. 500 µg/L
6. 700 µg/L
7. 1100 µg/L
8. 1300 µg/L
9. 1500 µg/L

2.5. Procedure

1. Each concentration and control group was distributed into three replicated test wells.
2. 5 copepods were introduced into each well. We attempted to have a homogenous distribution in each well by having only adult copepods but towards the end, we had a mix of nauplii and adults.
3. The wells were monitored upto 96h from exposure while we focussed on the result at 48h, and the number of dead copepods was recorded at the end of the exposure period.
4. The lethality endpoint was determined by counting the number of copepods every 24h that did not survive the exposure.

3. Methods

3.1. Lethality Assessment

Lethality was assessed by observing the physical state of the copepods at the end of the 48-hour exposure period. Copepods were considered dead if they did not exhibit any movement, even when gently prodded or by introducing bubbles with the pipette.

3.2. Statistical Analysis

Various statistical analyses were employed to evaluate the acute toxicity of Sertraline (SRT) on Acartia tonsa. Percent mortalities for each concentration were calculated and analyzed using ANOVA to compare mortalities between different concentrations, followed by Dunnett's test for multiple comparisons with the control. Time-dependent mortality comparisons were conducted using a Bonferroni-adjusted paired t-test. Probit analysis was performed to calculate the median lethal concentration (LC50) values. The statistical analyses were carried out using R for ANOVA and t-tests, while SPSS was utilized for Probit analysis.

3.3. Quality Control

To ensure the reliability of the results, we followed quality control measures such as the use of gloves and clean glassware and equipment to avoid contamination.

4. Results

The study assessed the acute toxicity of Sertraline (SRT) on Acartia tonsa. The primary findings of our experiment are as follows:

1. **Mortality Rates**:
   - *24-Hour Exposure:* At 24 hours, significant mortality was observed at concentrations of 300 µg/L and above compared to the control group. The median lethal concentration (LC50) was calculated to be 529.8 µg/L, with a 95% confidence interval of 462.1 to 596.6 µg/L (**Fig.1**).
   - 48-Hour Exposure: After 48 hours, significant mortality occurred at low concentrations itself, at 100 µg/L and higher. The LC50 value for this exposure was 179.6 µg/L, with a 95% confidence interval of 109.3 to 238.8 µg/L (**Fig.1**).
2. **Time-Dependent Increase in Mortality**:
   - Mortality generally increased with exposure time from 24 to 48 hours (**Fig.1 & 2**). This increase was statistically significant at lower concentrations (100, 300, and 500 µg/L) but not significant at higher concentrations (700-1100 µg/L).

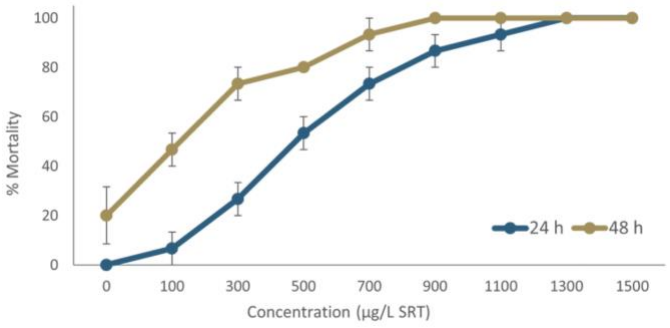

Fig.1: Mortality of Acartia tonsa exposed to varying concentrations of SRT over 24 and 48 hours.

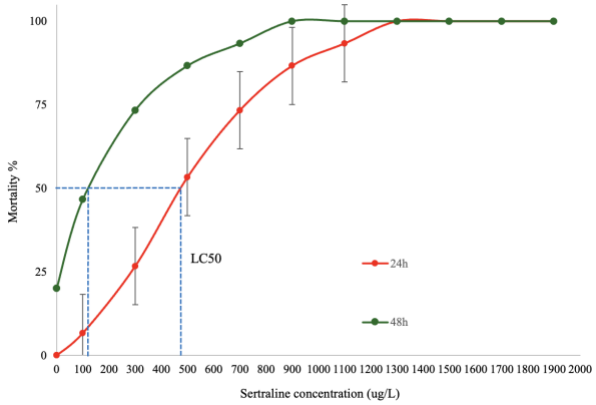

Fig.2: LC50 observation from mortality% graphs.

5. Discussion

The findings of this study demonstrate that Sertraline (SRT) induces significant acute toxicity in the marine copepod Acartia tonsa at relatively low concentrations and short exposure times. The significant mortality observed at SRT concentrations of 300 µg/L and above after 24 hours, and 100 µg/L and above after 48 hours, suggests that SRT can pose a substantial risk to marine invertebrates even at low environmental concentrations. The increase in mortality rates with longer exposure times indicates that the toxicity of SRT is both dose-dependent and time-dependent. The

LC50 values of 529.8 µg/L for 24-hour exposure and 179.6 µg/L for 48-hour exposure highlight the increasing lethality of SRT with prolonged exposure.

The results were consistent with studies on other freshwater crustaceans such as Daphnia magna, Ceriodaphnia dubia, and Thamnocephalus platyurus, which showed similar LC50 values at comparable exposure times; reinforcing the concern over the presence of pharmaceuticals in aquatic environments. The detection of toxic effects at low concentrations emphasizes the need for regular monitoring and assessment of pharmaceutical contaminants to safeguard aquatic ecosystems.

Furthermore, the experiment proves that we need to further explore the long-term effects of chronic exposure to SSRIs and the potential interactions between multiple pharmaceuticals (cocktail effect) on aquatic organisms. Such studies are essential for developing comprehensive strategies to mitigate the impact of pharmaceutical pollution on marine life. In conclusion, this study underscores the acute toxic effects of Sertraline on Acartia tonsa and calls for increased regulatory measures and monitoring of SSRIs in aquatic environments to protect marine biodiversity.

References:

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