ACUTE TOXICITY OF GENERIC AND IN VITRO REFERENCE TOXICAMOL USING THE ARTEMIA SALINE TOXICITY BIOINDICATOR LEACH

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Abstract: This study aims to analyze, through an experimental study with a quantitative and descriptive approach, the toxicity and identification of the 50% Lethal Concentration (LC50) of the reference and generic paracetamol drops, both commercially available and produced in Brazil, comparing them with each other. The lethality test was performed on Artemia salina according to the methodology described by Meyer (1982), with some modifications. When performing the toxicity test on Artemia salina, for generic paracetamol, the LC50 obtained after 24 hours was 998.3 ± 0.2µg/mL. The reference paracetamol presented LC50 of 612.2 ± 1.3µg/mL. Based on the analysis of this research, it was found that the reference paracetamol presented signs of toxicity in nauplii of the microcrustacean Artemia salina, presenting a LC50 ≤1000µg/mL. Generic paracetamol showed a LC50 ≤1000µg/mL with a range above 1000 ug/mL indicating non-toxicity for this sample.

Keywords: Toxicity. Paracetamol. Artemia salina

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INTRODUCTION

Paracetamol or acetaminophene is a drug considered safe and effective, with analgesic and antipyretic properties, with poor anti-inflammatory action, to relieve light to moderate pain, being effective for all age groups (BRAYNER, SILVAEALMEIDA, 2018). It is a low -cost and low -cost drug (Mezarobba and Bitencourt, 2018).

Because it is a medicine that does not require medical prescription there is a high index of indiscriminate use. Despite being considered safe and effective in its therapeutic doses, but when consumed at doses higher than the recommendation it can cause hepatotoxicity. (Ribeiro et al. 2023).

Paracetamol -induced poisoning happens more frequently in young people 15 to 24 years old, individuals who make abuse of alcohol and individuals aged 40 years of 40 years occur at high risk, the hepatic failure and may be fatal, and may be fatal. (SILVA JUNIOR et al; 2019).

There are several pharmaceutical presentations sold, among them: tablets, oral solution, suppositories and injectables (RIBEIRO et al, 2023). Due to ease and broad use in Brazil, it is important to evaluate the pharmacological patterns of acetomiphene. The variability of laboratories, forms of presentation and classification of the drug (reference, generic, similar and manipulated), suggests a diversity in the quality standard of this, and it is necessary to evaluate such criteria to avoid possible health damage (Teixeira, 2016).

In Brazil, according to Law 9,787 of February 10, 1999, the drugs considered “reference” are innovative products whose effectiveness, safety and quality were scientifically proven with the competent federal agency, at the time of registration. Upon protection period of the reference product patent, generic and similar drugs can be produced that, similar to the reference, follow the principles of bioequivalence and bioavailability to obtain the same therapeutic effect for later records and commercial use (. (Brazil, 1999; .Lopes; Costa, 2016, Garcez et al., 2019).

Soline artemia is a species of microcrustacean of the order years, found in Salgadas waters (Carvalho et al., 2008). It is used as a living food for fish, and its eggs are found in aquarizers stores and

is widely used in toxicological studies due to low cost and easy cultivation. Several works have been trying to correlate toxicity over A. saline with antifungal, viruscid, antimicrobial, tripanosomicide and parasiticide activities. Lethality tests are done in toxicological tests and the median lethal concentration (Cl50) indicates death in half a sample (BEDNARCZUK, 2010).

Thus, this work aimed to evaluate the toxicity of the acetaminopus drops: generic and reference, in A. saline model.

METHODOLOGY

This work resulted from an experimental study, with quantitative and descriptive approach, which evaluated toxicity and identified the lethal concentration 50% (CL50) of acetaminophen (oral solution) reference and generic, both commercially available and produced in Brazil, comparing them each other.

A. saline eggs were placed to hatch in an artificial marine water solution (NaCl 77.23%, MGSO4 9.62%, MGCl 7.13%, CACL23.32%, KCl 2.1%and NAHCO3 0, 59%) and after 48 hours, ten nauplies of saline artemia were transferred with a micropipette to testing tubes containing artificial marine water and reference and generic paracetamol samples that were diluted in serial concentrations (125µg/mL, 250 µg/ml, 500 µg/ml, 750 µg/ml and 1250 µg/ml).

The lethality test was held in Artemia Salina, cultivated at the Food Analysis/Sanitation Analysis Laboratory of the Federal Institute of Piauí-IFPI/CTZS, according to the methodology described by Meyer (1982), with some modifications.

The specimens were distributed in three specimens for each concentration of brand and generic acetaminophen, as well as negative control with artificial marine water and positive control with 0.0006 mg/ml of copper sulfate (COSO4).

The tubes were left at room temperature for 24 hours. After this period, the number of surviving cassaways was held. They were observed near a light source and considered alive all those

who had any kind of movement when subjected to a slight stirring.

The number of living naupli in relation to the increase in the concentration of extracts and fractions was used to calculate the values of the CL50. Thus, given the values of the CL50 obtained through this method, substances tested in different concentrations are classified as toxic when the Cl50 is less than 1000 µg/ml and non -toxic when the Cl50 is greater than 1000 µg/ml.

SUBMITTED AND DISCUSSION

The results were expressed CL50 obtained by the statistical method of nonlinear regression followed by the 95% confidence interval (GraphPadPrism 6.0®).

Table 1 - Lethal Concentration 50% (Cl50) of the generic acetaminophen and the reference paracetamol after 24 hours of exposure by the toxicity test with A. saline

SAMPLES	Average Lethal Concentration (CL_{50}) Mg/mL	Interval with 95% Confidence (µg/mL)
Paracetamol Generic	998.3 ± 0.2	845.3 - 1179
Paracetamol Of reference	612,2 ± 1.3	547.5 – 684.5

A comparison between generic and reference paracetamol performed by Lima (2019) against A. Salina found a CL50 for the generic acetaminopus of 265.377 µg/ml with confidence interval within 258,223 to 272,060 µg/mL and for the reference paracetamol of 252.578 µg/mL with a break within 249,600 to 255.619 µg/mL that were distant from the CL50 found for the generic paracetamol and reference in the present study that were 998.3 ± 0.2 µg/ml and 612.2 ± 1 , 3 µg/ml, respectively.

Garcez et al. (2018) performed a toxicity bioassay with A. saline using the same method with generic and reference dipyrone and obtained a CL50 for generic dipyrone of 986.9 µg/ mL and 654.1 µg/mL for reference dipyrone . Results close to those obtained in this study.

Conclusions

The reference paracetamol showed indications of toxicity in microcrustacean naúplies artemia saline with a cl50 ≤1000µg/ml. The generic acetaminophen showed a cl50 ≤1000µg/ml with an interval above 1000 Ug/ml indicating atxicity for this sample.

Thus, it is possible to carry out other deeper studies, comparing the generic acetaminophen with the reference.

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