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A simple *in vitro* method to evaluate the toxicity of functional additives used in shrimp aquaculture

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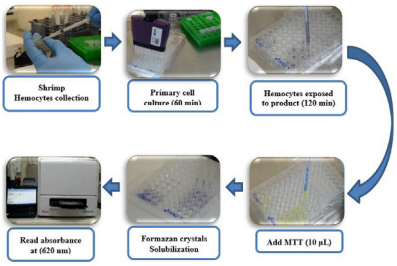
GRAPHICAL ABSTRACT



ABSTRACT

To mitigate the economic losses provoked by disease outbreaks, shrimp producers employ therapeutic additives. However, important issues such as the toxicity of these products on shrimp are not always considered. *In vivo* toxicity assays require a lot of time and large economic and physical resources. Here, we describe an *in vitro* procedure to evaluate the toxicity of functional additives, used in the production of shrimp *Penaeus vannamei*. This method adapted the cell viability assay based on the reduction of tetrazolium salts (MTT) to primary cell cultures of shrimp hemocytes.

- A simple and reliable tool that requires few physical and economic resources to evaluate in short time (6 h) the cytotoxic effect of therapeutic products and additives to be included in shrimp culture
- This inexpensive method requires only a modified Hank's balanced salt solution (HBSS) containing Ca^{2+} and Mg^{2+} to keep hemocytes metabolically active to successfully carry out the cytotoxicity assay
- This toxicity *in vitro* assay does not require exposure of the shrimp to compounds at toxic concentrations.

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Method details

Background

The expansive growth of the shrimp aquaculture industry is accompanied by the disease outbreaks. To mitigate the economic losses, shrimp producers employ therapeutic additives, such as antibiotics, immune modulators, organic acids, essential oil and antioxidants. However, important issues such as the toxicity of these products on shrimp are not always considered. While *in vivo* toxicity assays require considerable time and economic resources [1], the development of easy and robust *in vitro* protocols is highly relevant. The cell viability assay developed by Mosman [2], based on the reduction of tetrazolium salts (3-(4,5-dimethylthiazol-2-yl) −2,5-diphenyltetrazolium bromide) (MTT), is widely used to measure *in vitro* cytotoxic in eukaryotic cells [2,3]. To develop an *in vitro* toxicity test suited for the assessment of the toxicity of feed additives for shrimps, we adapted this Mosman protocol to primary cell cultures of shrimp hemocytes. This fast and inexpensive assay can be used by the shrimp industry to determine non-toxic therapeutic doses of functional additives as a pre-application process in *in vivo* trials, and shrimp farms.

Materials

Reagents

Acid chloride (1 N)
Ethanol (70% [v/v] in distilled water)
Calcium chloride solution ($Ca\ Cl_2$) (1 M; filtrated on a 0.22 μm filter)
Citric acid solution 1 N
Formaldehyde (4% [w/v] in distilled water)
Hanks balanced salt solution (HBSS 10x) (Gibco 14185-052)
Hepes solution (kept at 4 °C) (1 M; filtrated on a 0.22 μm filter)
Hydrochloric acid solution 1 N
Isopropanol (kept at 4 °C)
Magnesium chloride solution (Cl_2Mg) (1 M; filtrated on a 0.22 μm filter)
Milliq water (filtrated on a 0.22 μm filter)
3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)
Sodium chloride solution 2 M (kept at 4 °C)
Sodium citrate (5% and 10% [w/v] in distilled water. Adjusted pH 7) (kept at 4 °C)

Equipment

Combitips
Cotton swab
Membrane filters, white polycarbonate, type HTTP, 0.2-μm pore size, 47-mm diameter
Hematocytometer, Neubauer chamber
Micropipettors, 10 −, 100-, and 1000-μL, with corresponding tips
Microplate reader
Light microscope, phase contrast
Microplates (96-well) (Corning 3361)