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CNPq

USE OF BIOCHEMICAL, GENETICAL, HISTOLOGICAL AND PHYSIOLOGICAL BIOMARKERS IN THE ACUTE EXPOSURE TO THE CONTAMINANTS: COPPER, ATRAZINE AND PHENANTHRENE IN THE FISH *Rhamdia quelen*

Project INCT-TA
Federal University of Paraná
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Project Description

- Coordinator: Federal University of Rio Grande – UFRG
- Partner: Federal University of Paraná -UFPR

Laboratory participants:

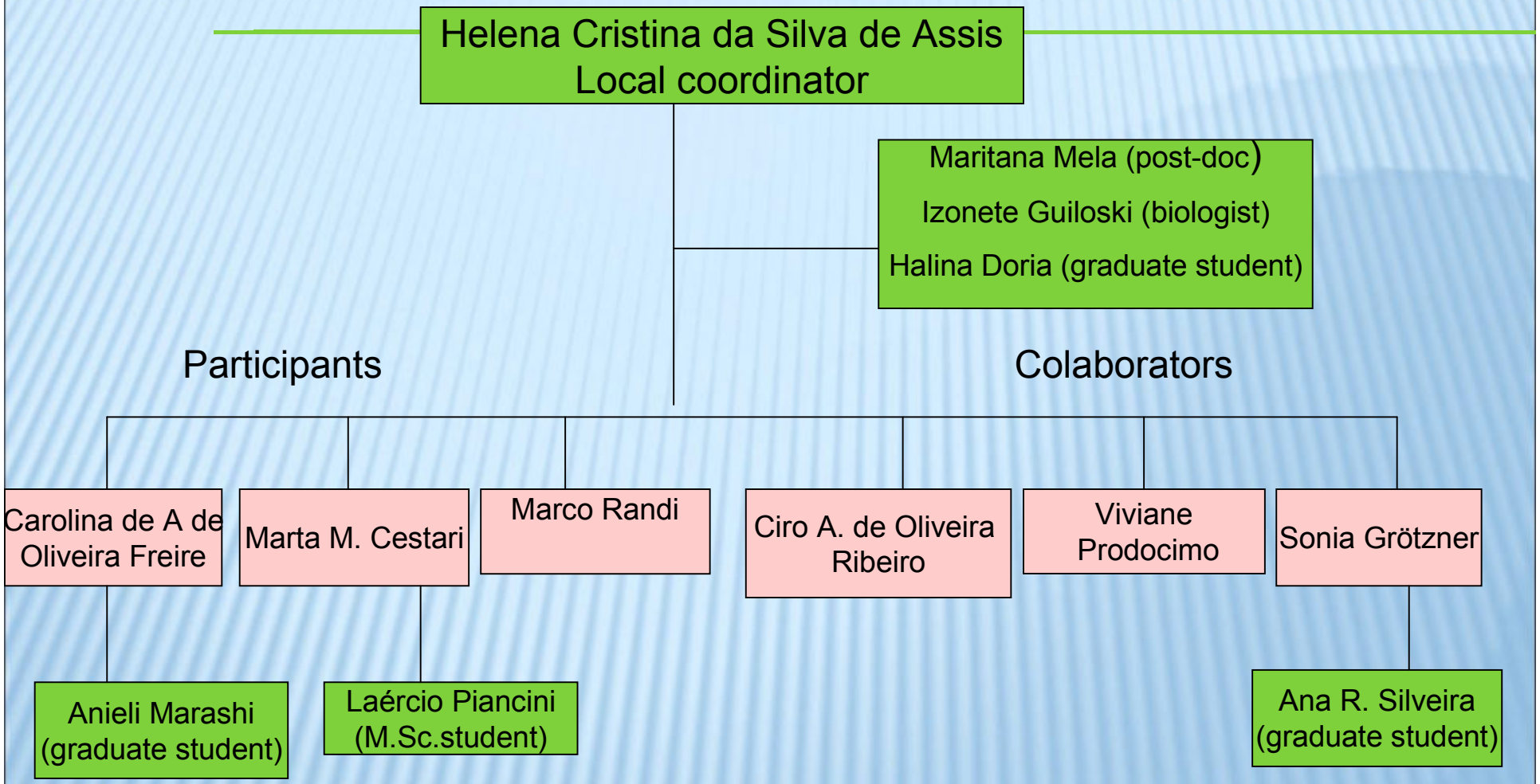
- **Laboratory of environmental toxicology;**
- **Laboratory of cell toxicology;**
- **Laboratory of comparative physiology and osmoregulation;**
- **Laboratoty of animal mutagenesis**

Aquatox
GRUPO MULTIDISCIPLINAR EM TOXICOLOGIA AQUATICA

Aims of the Project

- Evaluate the biochemical, genetical, physiological, and morphological biomarkers in the species *Rhamdia quelen* exposed to the following contaminants:
- Atrazine - an organic compound, a widely used herbicide.
- Copper chlroride - industrial application
- Phenantrene - a polycyclic aromatic hydrocarbon composed of three fused benzene rings

Team work


Helena Cristina da Silva de Assis
Local coordinator
Maritana Mela (post-doc)
Izonete Guiloski (biologist)
Halina Doria (graduate student)
Participants
Colaborators
Carolina de A de Oliveira Freire
Marta M. Cestari
Marco Randi
Ciro A. de Oliveira Ribeiro
Viviane Prodocimo
Sonia Grötzner
Anieli Marashi (graduate student)
Laércio Piancini (M.Sc.student)
Ana R. Silveira (graduate student)

Helena Cristina da Silva de Assis

- **Responsability:** Local project coordinator
- **Research line:** Professor at the UFPR, ministers subjects as environmental toxicology and pharmacology. Supervisor in two post- graduation programs: Pharmacology and Ecology and Conservation. Experience in biomarkers of contamination, toxicology, and environmental pollutants.

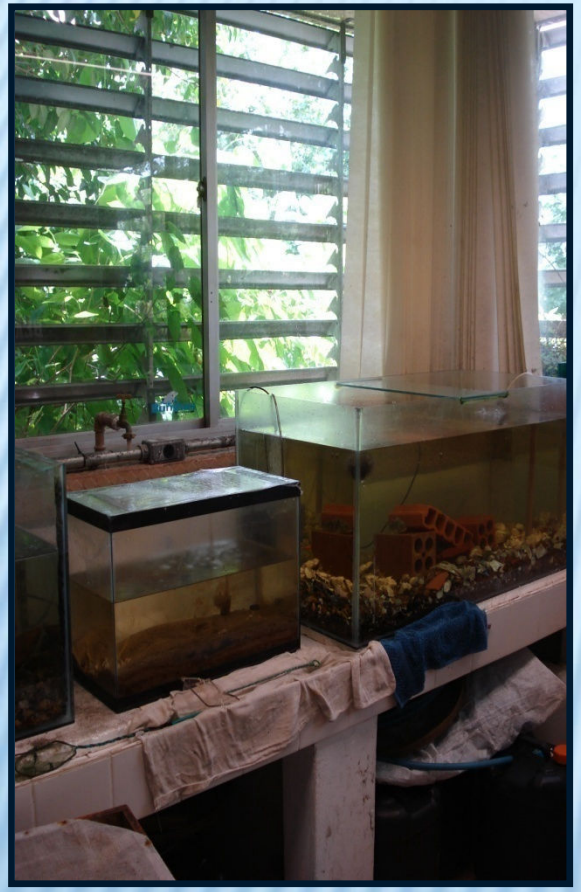
Infra-structure: espectrophotometer, centrifuge, homogenizer for tissues samples, freezer -80°C, microscope, microtome for light microsocpy, epifluorescence microscope

Laboratory of environmental toxicology

- Carolina Arruda de Oliveira Freire
- Viviane Prodocimo

  - **Responsability:** Physiological analysis
  - **Research line:** Professors at the UFPR. Experience in the field of comparative animal physiology, with emphasis on comparative physiology of osmoregulation, acting on the following topics: osmoregulation, gill and kidney function, effects of changes in salinity, osmoregulation as a biomarker of contamination in marine, estuarine and freshwater, stress associated with estuaries and intertidal, using mainly crustaceans, echinoderms and fish as models.

Laboratory of compartive physiology and osmoregulation



- Marco Randi
- Ciro Alberto de Oliveira Ribeiro

  - **Responsability:** Histopatological analysis
  - **Research line:** Professors at the Federal University of Parana. They have experience in the area of morphology, with emphasis on cytology and cell biology, acting on the following topics: cellular toxicology, ecotoxicology.

Sonia Regina Grötzner

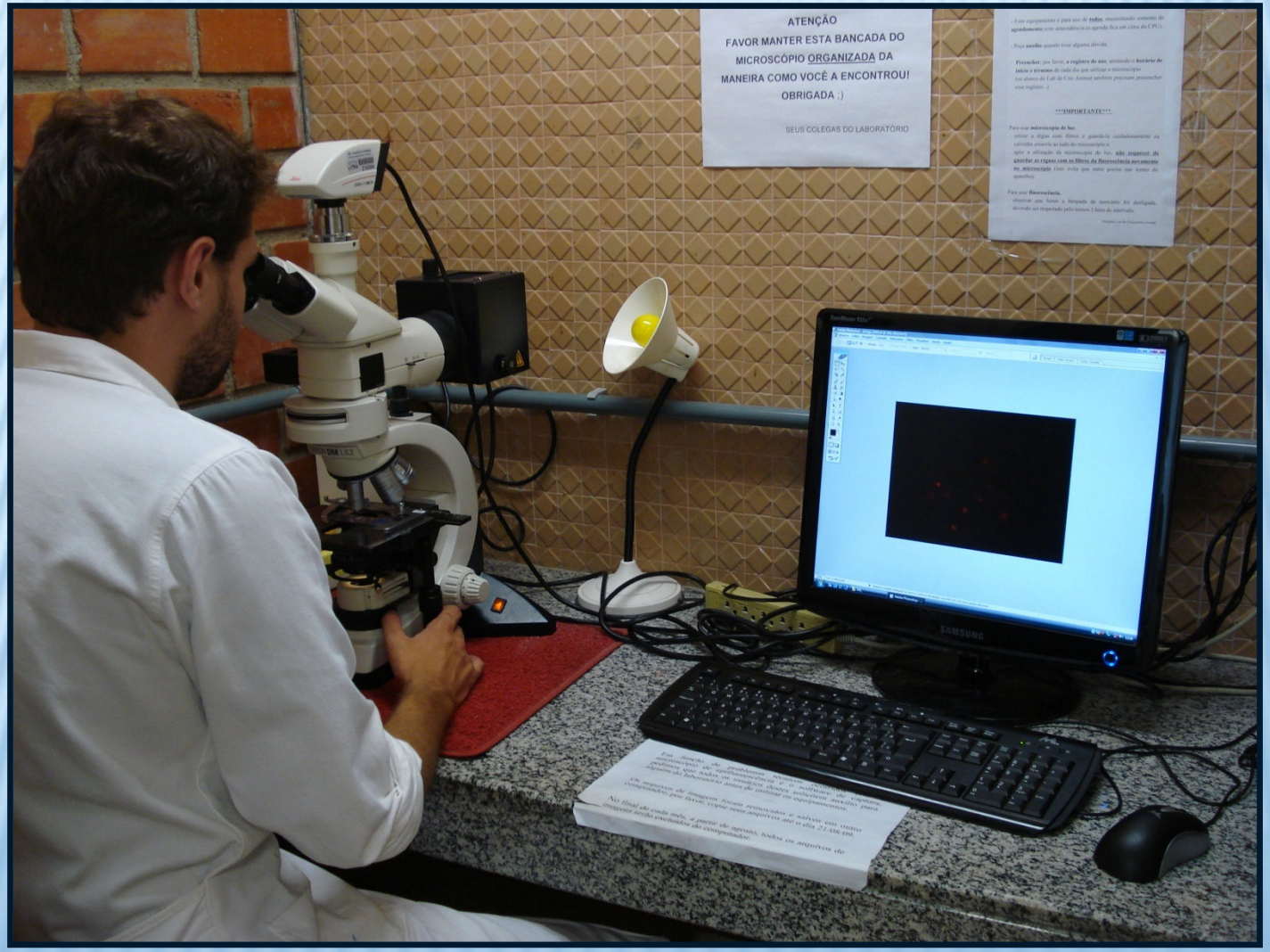
- **Responsability:** Histopatological analysis
- **Research line:** Professor at the UFPR, in the areas of histology, embryology and histological techniques. Experience in morphology and neurosciences, mainly in the study histophysiological the vertebrate retina and visual ecology.

Laboratory of cell toxicology

Marta Margarete Cestari

- **Responsability:** Genetic analysis
- **Research line:** Professor at the UFPR in the Department of Genetics. Experience in the field of genetics, with emphasis on fish cytogenetics and environmental mutagenesis using fish as bioindicators.

Laboratory of animal mutagenesis



Experimental Design - Fish

- *Rhamdia quelen (*weight 30 ± 2g; length 14 ± 2 cm*)* obtained from EPAGRI (Company of Agricultural Research and Extension of Santa Catarina) were mantained in glass aquaria (60-l of capacity);
- The fish were fed with a dry commercial diet;
- The water was oxygened by aeration and aquarium temperature was maintained at 25 ± 1°C with photoperiod of 12h light and 12h darkness.

Rhamdia quelen

Experimental Design – Cooper chloride exposure

- After acclimatization for 30 days fish were divided in groups (n=20).
- A preliminarly experiment was carried out with the highest concentration (20 µg/L). The fish were submitted to acute 96-hours exposure.
- One group as control and the other three as exposed groups for three differents concentrations of $CuCl_2$ – 2 µg/L, 10 µg/L and 20 µg/L.
- The concentration of 2 µg/L used in this study was authorized for Conama (Resolution 357, March 17, 2005)

Sampling

Biochemical Analysis

- Measurement of activities of **CAT, GST,** and **GSH** and **LPO**, liver pools (from 2 individuals) were sampled and frozen at −80 °C
- **GSH (Glutathione)** was measured at 340 nm on the basis of the method described by Sedlak and Lindsay (1968)
- **CAT (Catalase)** activity was measured at 240 nm on the basis of the method described by Aebi (1984)
- **GST (Glutathione S-transferase)** activity was measured at 340 nm by the method described by Keen et al. (1976)
- The analysis of **LPO (Lipid peroxidation)** was carried out using the ferrous oxidation–xyleno assay at 570 nm (Jiang et al., 1992)

Biochemical Analysis

- A fragment of axial muscle and the brain were also collected to evaluate the **AChE** activity (acetylcholinesterase)
- The AChE activity was measured spectrophotometrically at 405 nm according to the method of Ellman et al. (1961), modified for use in the microplate assay by Silva de Assis (1998)
- **Protein concentration** was determined using Bradford's method (1976) with bovine serum albumin as the standard

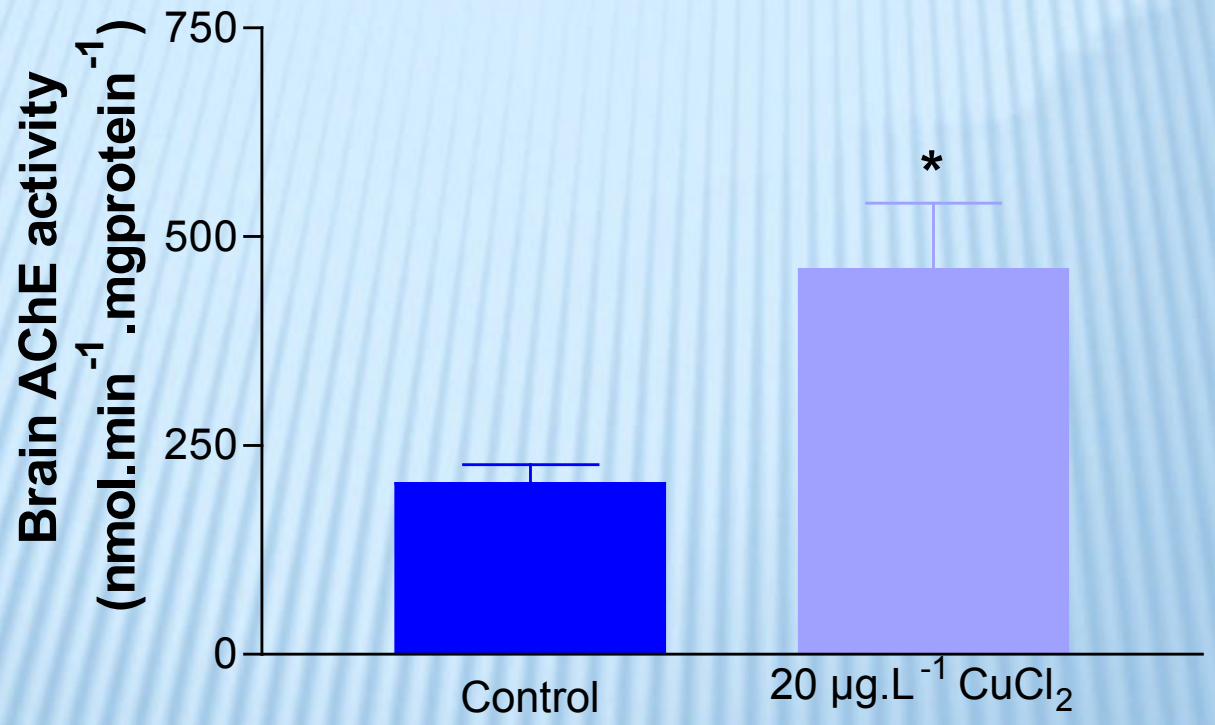
Statistical Analysis

The results were expressed as mean ± standard error of the mean

For statistical comparisons of control and copper groups (20 µg/L) were used Student´s t- test ($P < 0.05$)

Biochemical Analysis - Preliminary findings

Brain AChE activity - (control X 20 µg/L Cu)

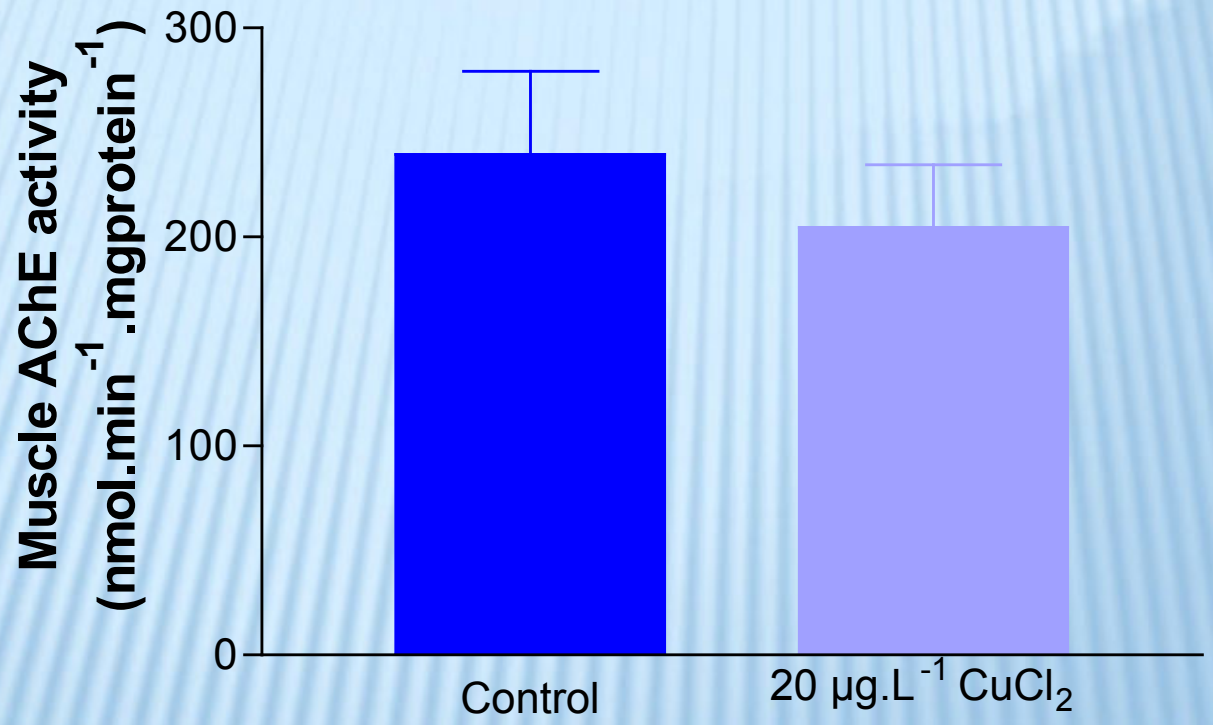


Control and copper-exposured groups were statistically different

Results are expressed as mean values ± SEM

Biochemical Analysis - Preliminary findings

Muscle AChE activity - (control X 20 µg/L Cu)

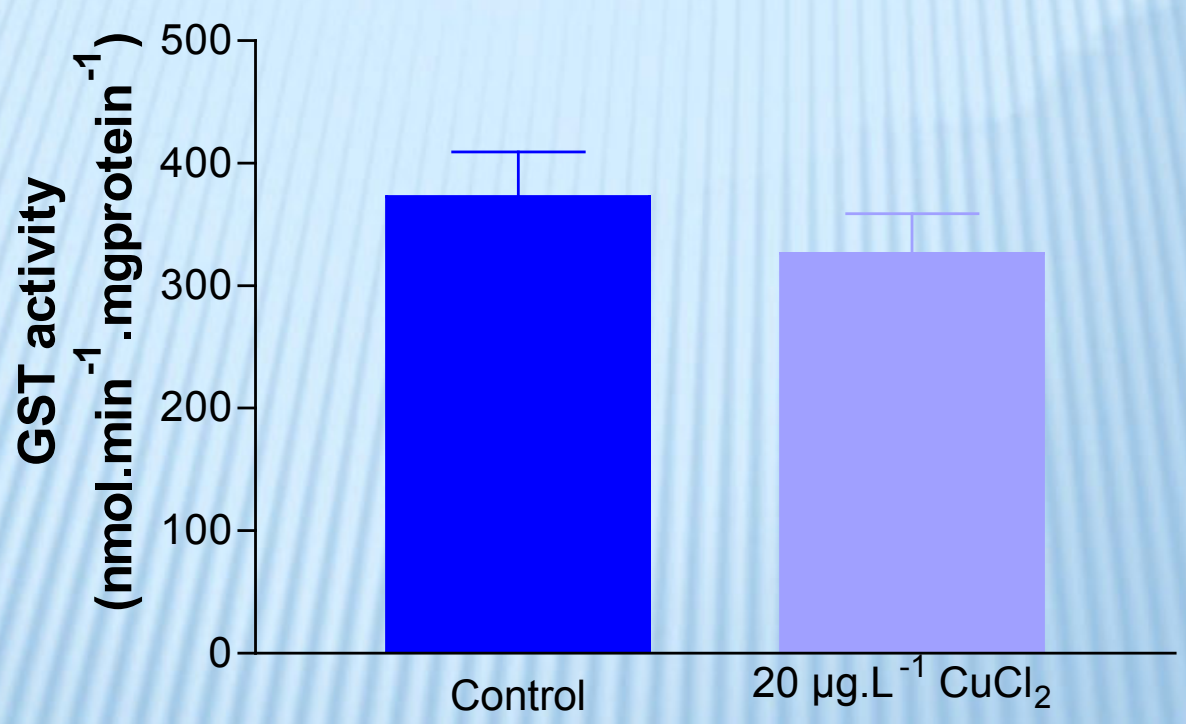


Control and copper-exposed groups were not different

Results are expressed as mean values ± SEM

Biochemical Analysis - Preliminary findings

GST activity - (control X 20 µg/L Cu)

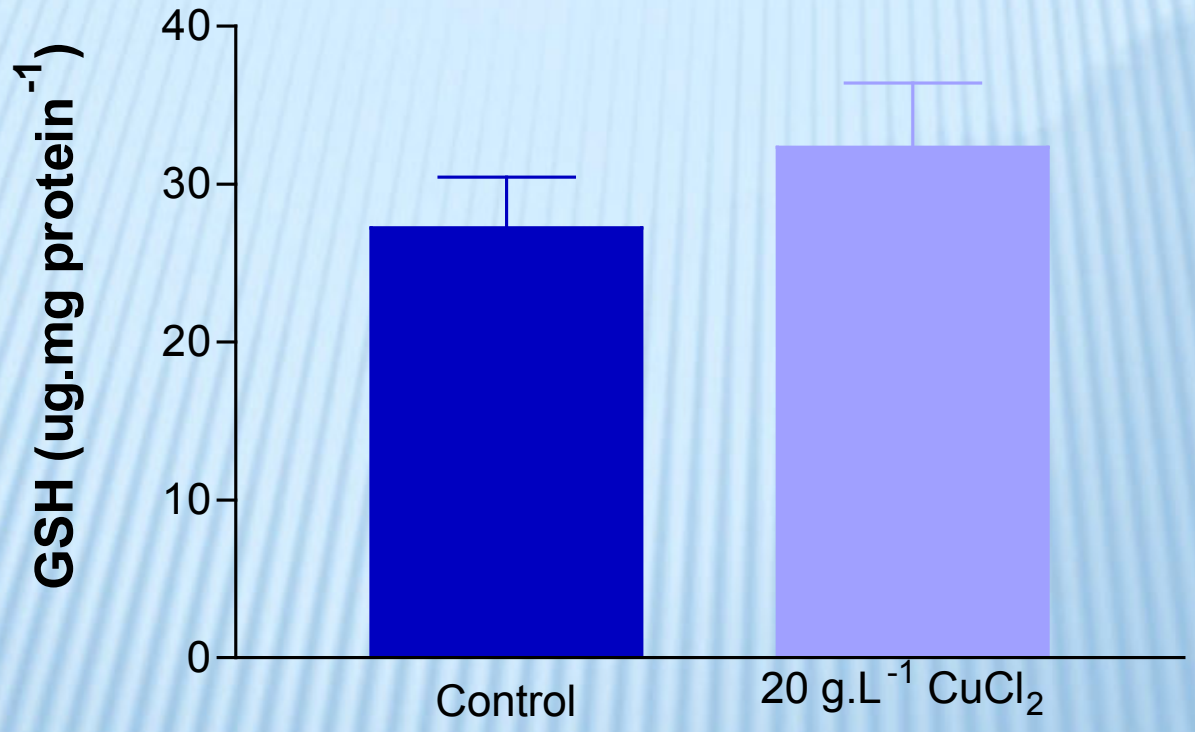


Control and copper-exposed groups were not different

Results are expressed as mean values ± SEM

Biochemical Analysis - Preliminary findings

GSH - (control X 20 µg/L Cu)

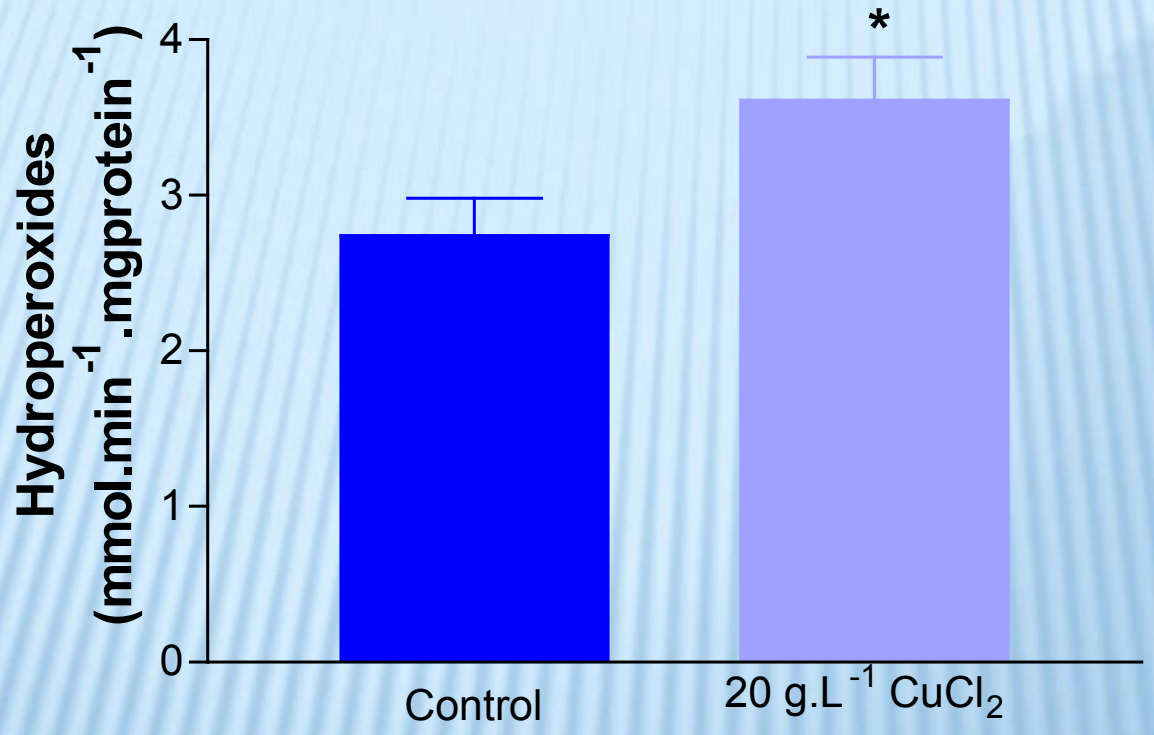


Control and copper-exposed groups were not different

Results are expressed as mean values ± SEM

Biochemical Analysis - Preliminary findings

Hidroperoxides - (control X 20 µg/L Cu)

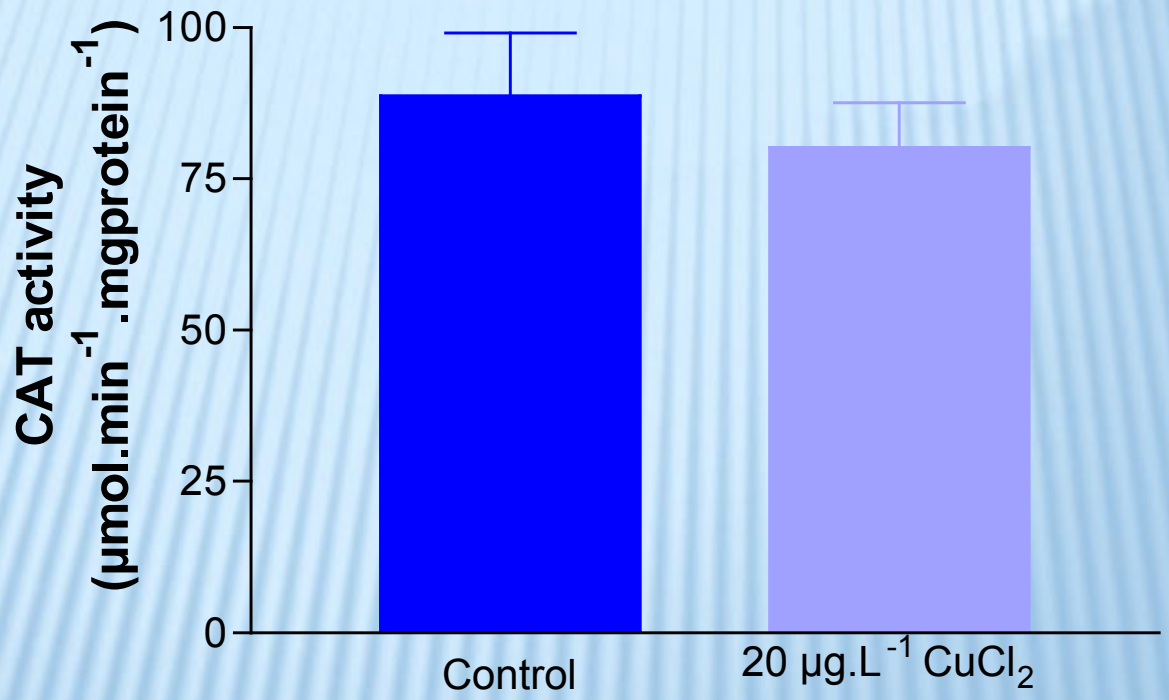


Control and copper-exposed groups were statistically different

Results are expressed as mean values ± SEM

Biochemical Analysis - Preliminary findings

CAT activity - (control X 20 µg/L Cu)



Control and copper-exposed groups were not different

Results are expressed as mean values ± SEM

Histological analysis

- **Light microscopy procedure**
  - Samples analysed: liver, retina and sensory organs
  - Samples preserved in Alfac fixative solution for 16 hours; dehydrated in a graded ethanol baths and embedded in Paraplast plus resin (Sigma).
  - Sliced sections (5 μm) stained in hematoxilin/eosin;
  - Observed in Zeiss Axiophot photomicroscope

Histological analysis

Scanning electron microscopy

- Samples analysed: Gills, retina and sensory organs
- Samples were fixed in 3% glutaraldehyde dissolved in 0.1 M cacodylate buffer
- Samples were dehydrated and critically point dried using liquid Co_2
- Dried samples were coated with gold.