

# In vitro toxicological responses in fish cells (RTgutGC) following exposure to chemicals with different modes of actions

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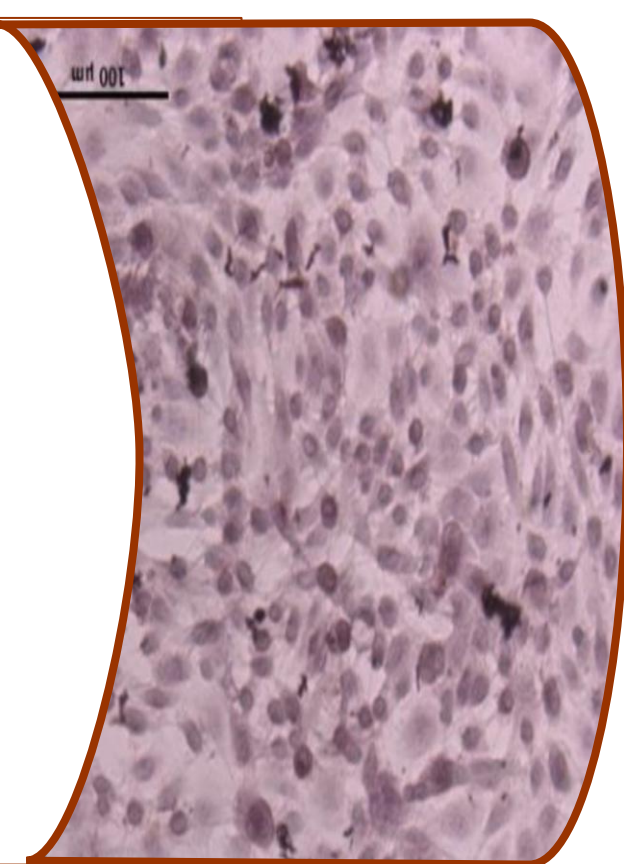
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## Aims

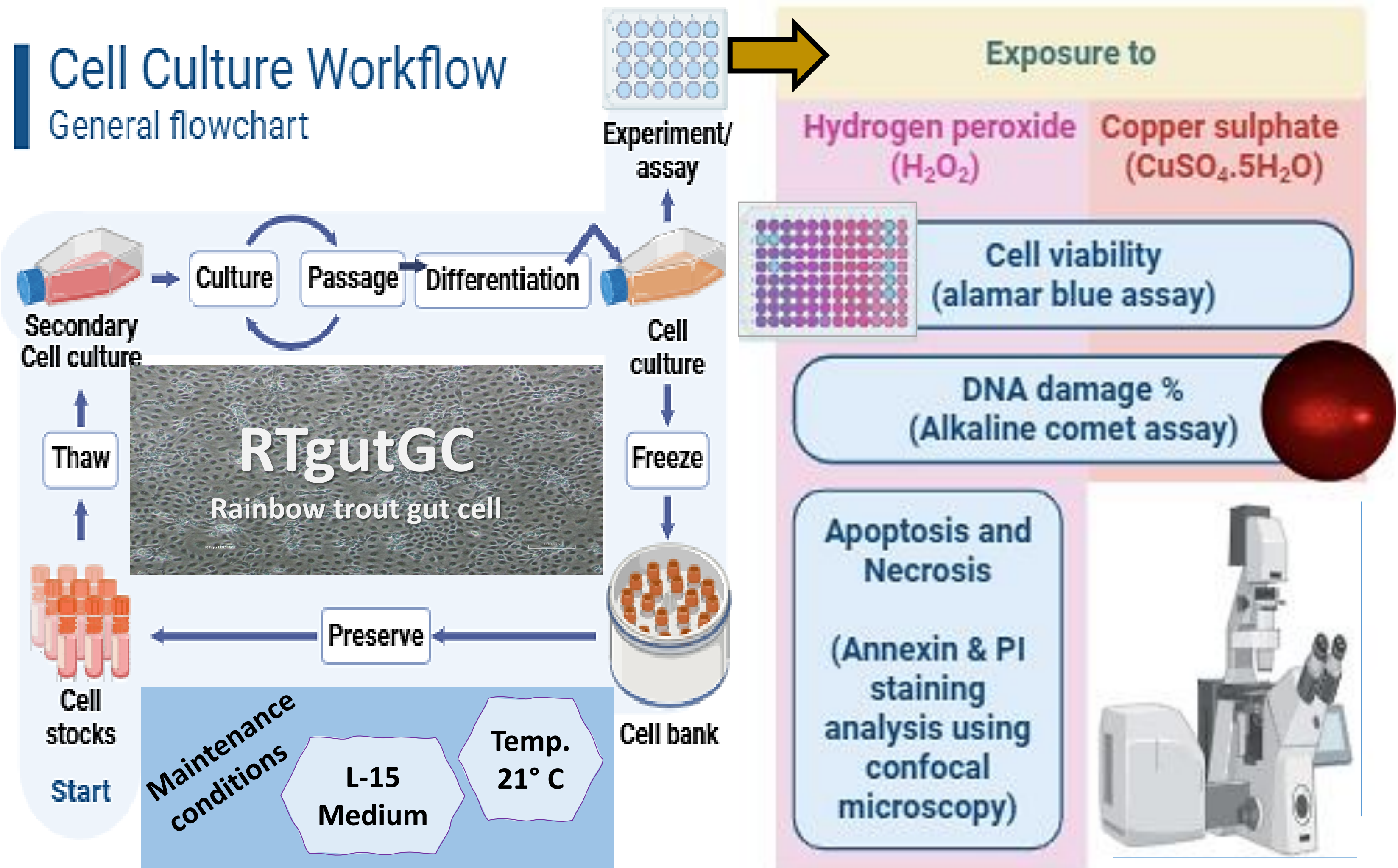
- Compare toxicological profiling of cells under diverse chemical stressors.
- Support New Approach Methodologies (NAMs) development for comparative stress responses.
- Assess biomarkers like cell viability, DNA damage, apoptosis, and necrosis.
- Generate baseline data for future comparison with human cell lines.

## Background

- In vitro* testing: ethical alternative to animal testing.
- Fish cell lines: ecologically relevant models for assessing aquatic toxicology.
- Human cell toxicity biomarkers well established; fish *in vitro* systems still need standardised approaches.
- Comparing fish and human cell responses can reveal conserved toxicological pathways.
- Support the development of NAMs and the 3Rs principle.



## Materials & Methods



## Results

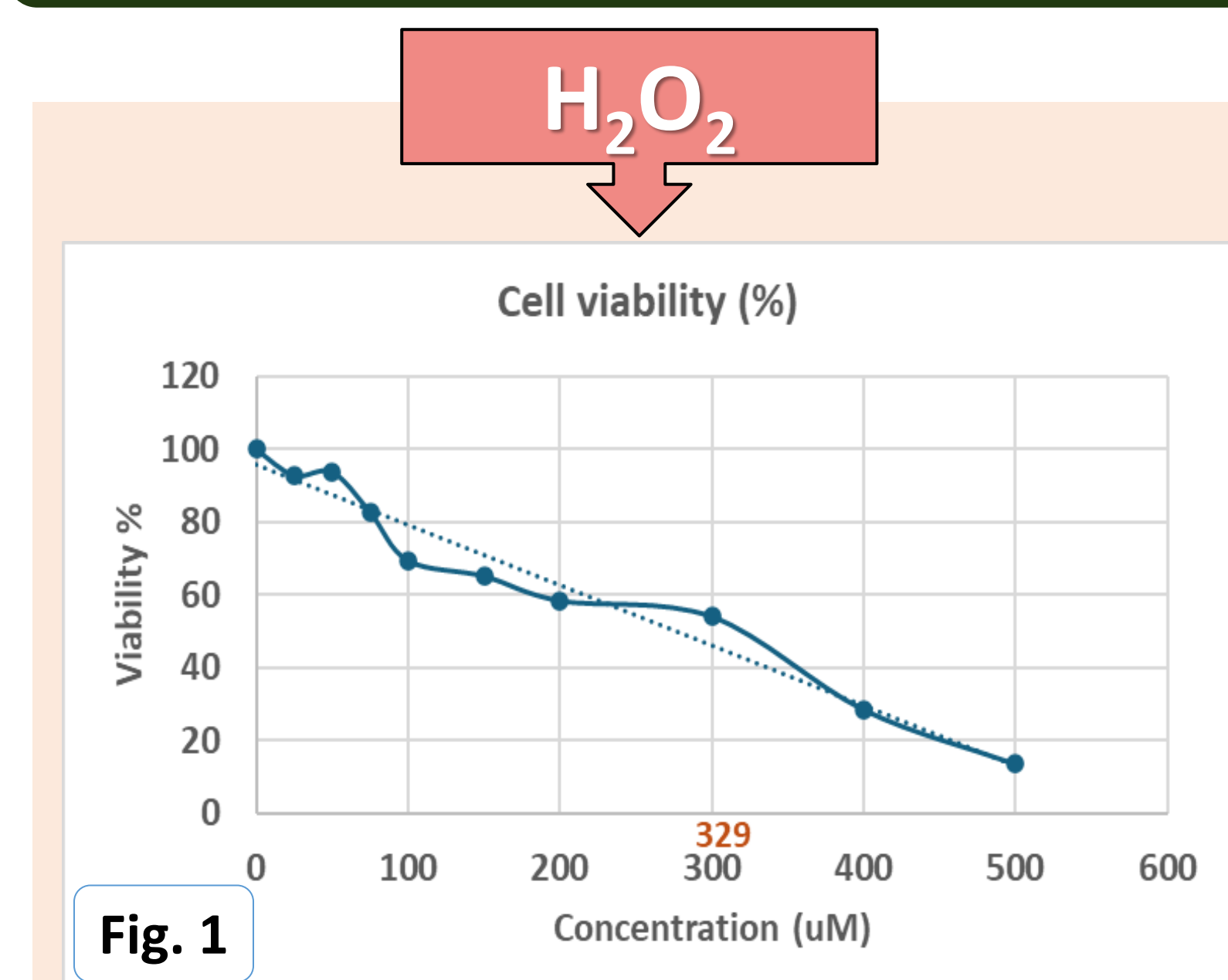


Fig. 1

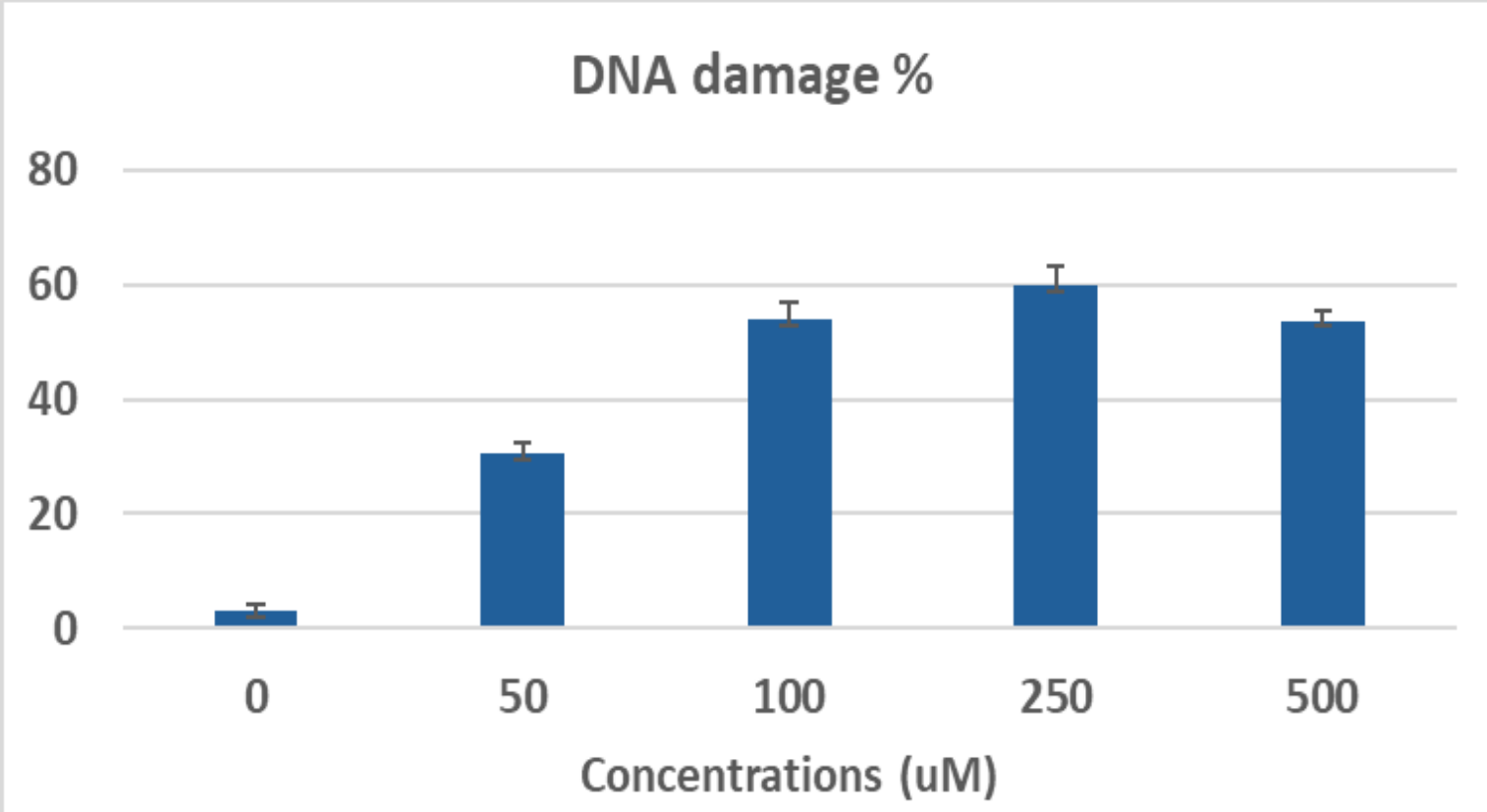


Fig. 3

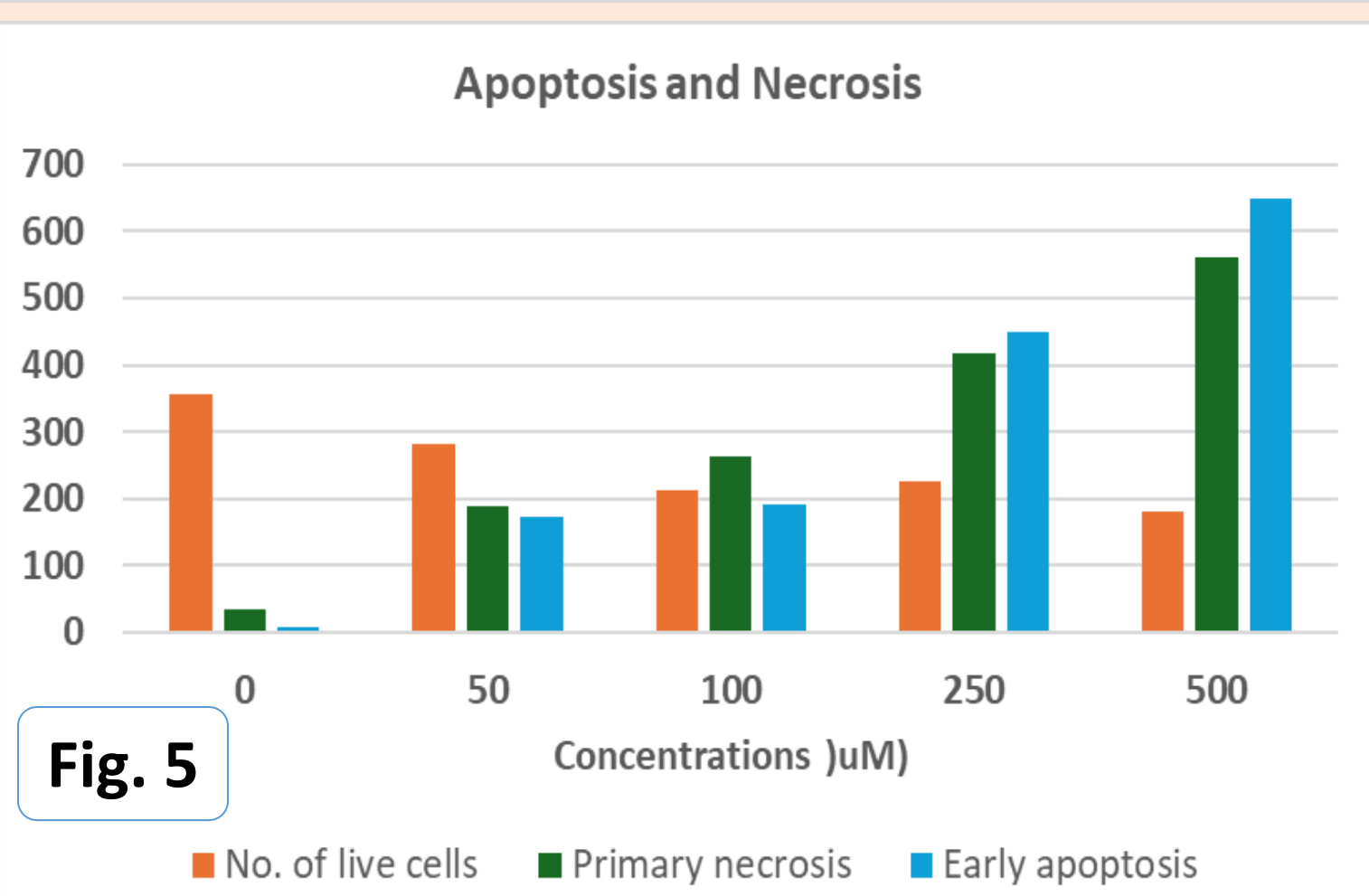


Fig. 5

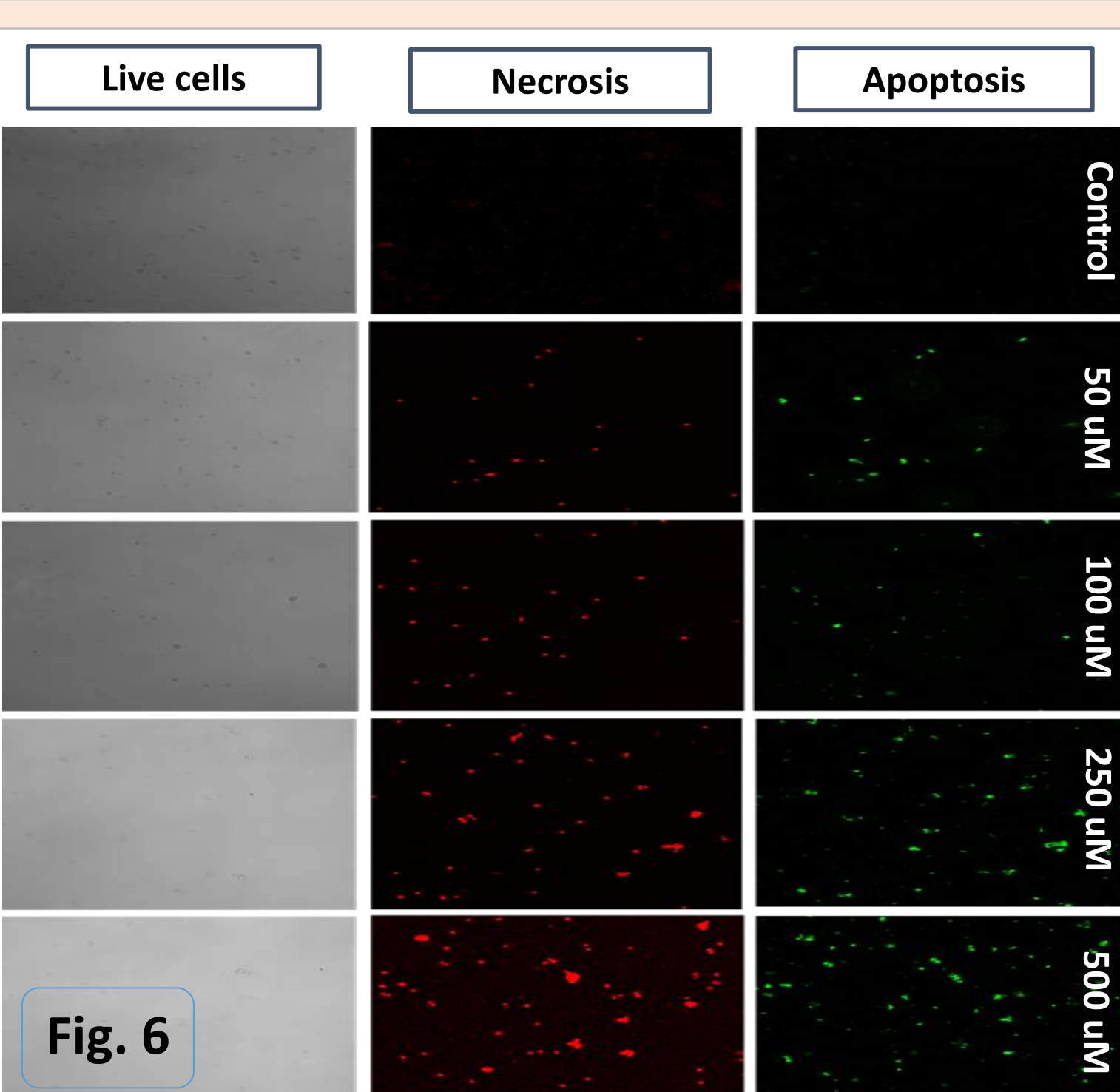


Fig. 6

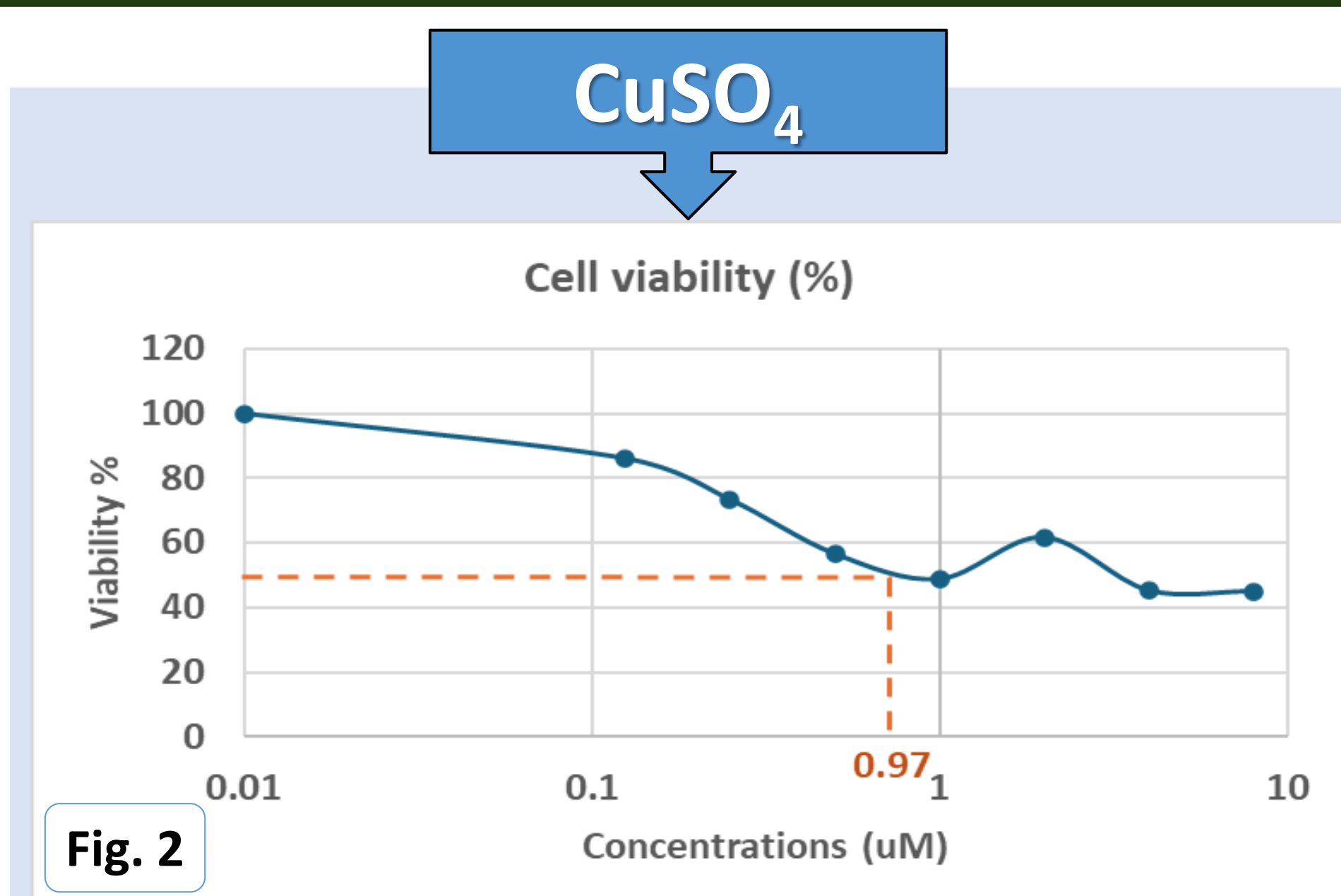


Fig. 2

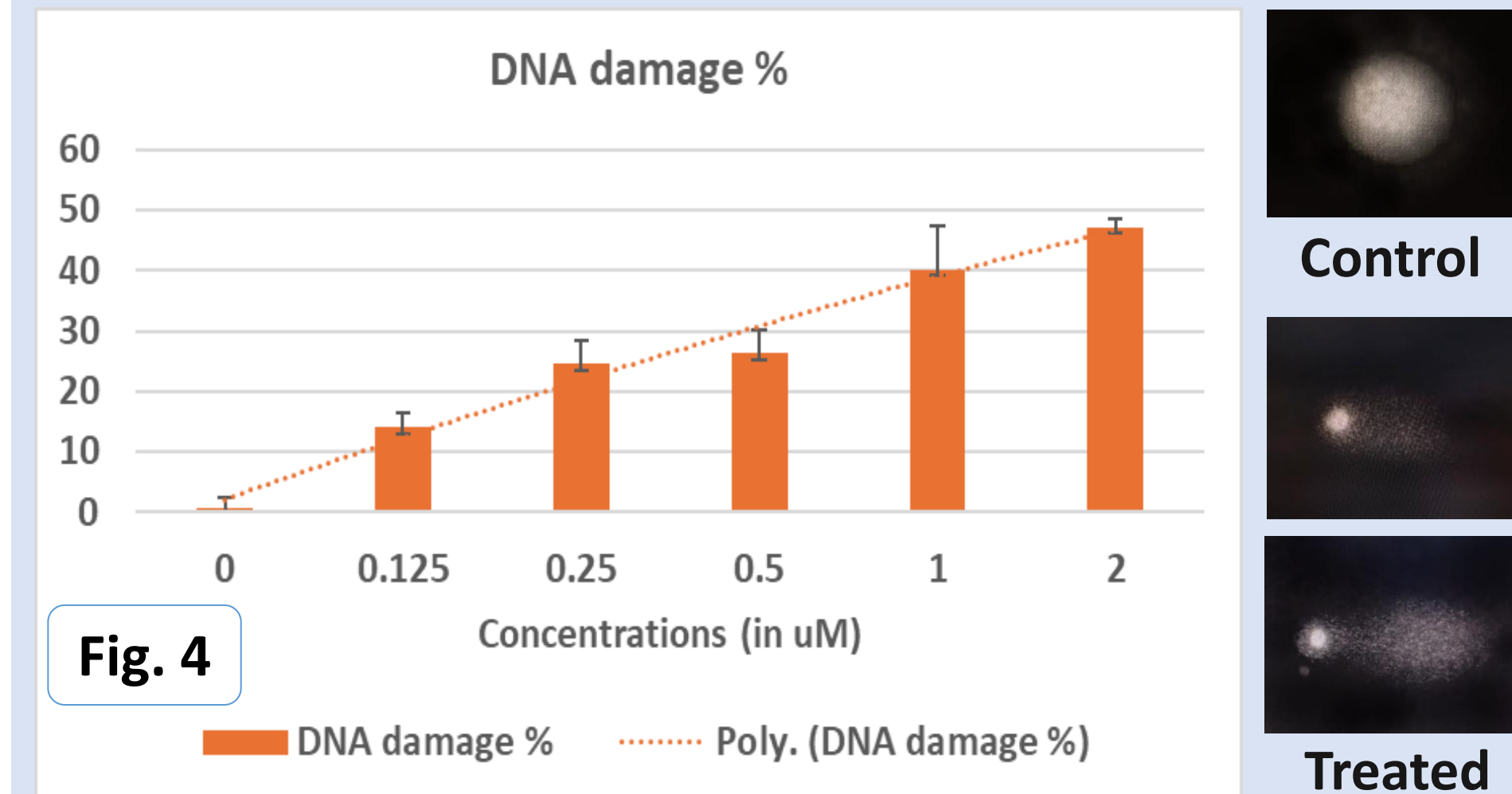


Fig. 4

## Results-

- Fig. 1 Concentration-dependent ↓ in cell viability; LC50 ≈ 329 μM.
- Fig. 2 Concentration-dependent ↓ in cell viability; LC50 ≈ 0.97 μM.
- Fig. 3 Concentration-dependent ↑ in DNA damage (%)
- Fig. 4 DNA damage (%) ↑ with ↑ in concentration.
- Fig. 5 ↑ in apoptotic and necrotic cells with ↑ in exposure.
- Fig. 6 Microscopy images of concentration-dependent ↑ in necrotic and apoptotic cells.

## Discussions

RTgutGC showed concentration-dependent responses to both H<sub>2</sub>O<sub>2</sub> and CuSO<sub>4</sub> exposure.

CuSO<sub>4</sub> showed higher cytotoxicity, with a much lower LC50 value. Reduced cell viability suggests disruption of cellular metabolism and membrane integrity.

CuSO<sub>4</sub> produced stronger genotoxic effects at lower doses. DNA damage was indicative of oxidative DNA strand breaks and ROS-mediated toxicity.

H<sub>2</sub>O<sub>2</sub> induced apoptosis at moderate concentrations, indicating controlled oxidative stress-induced cell death. Promoted greater necrosis at higher concentrations, suggesting more cellular injury.

Both toxicants activated oxidative stress, although CuSO<sub>4</sub> demonstrated higher overall potency across biomarkers.

## Conclusions

- RTgutGC: robust *in vitro* model for ecotoxicology assessment.
- Cells respond to both oxidative agent and metal exposure.
- The study supports comparative ecotoxicology and future NAM development.
- The work contributes to more ethical and predictive hazard assessment in the future.

## Acknowledgements



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