

## **Hydra as a model organism to decipher the toxic effects of copper oxide nanorod: Eco-toxicogenomics approach**

Murugadas Anbazhagan <sup>1,2</sup>, Zeeshan Mohammed <sup>1,2</sup>, Thamaraiselvi Kaliannan <sup>1</sup>, Ghaskadbi Surendra <sup>3</sup>, Akbarsha Mohammad Abdulkader\* <sup>2,4</sup>

<sup>1</sup> Department of Environmental Biotechnology, Bharathidasan University, Tiruchirappalli 620024, India

<sup>2</sup> Mahatma Gandhi - Doerenkamp Center for Alternatives to Use of Animals in Life Science Education, Bharathidasan University, Tiruchirappalli 620024, India

<sup>3</sup> Developmental Biology Group, MACS-Agharkar Research Institute, Pune 411004, India

<sup>4</sup> Department of Food Science and Nutrition, College of Food and Agriculture, King Saud University, Riyadh 11451, Kingdom of Saudi Arabia

### **\*Corresponding author**

Dr. M. A. Akbarsha

Director & Chair

Mahatma Gandhi-Doerenkamp Center, Bharathidasan University,

Tiruchirappalli – 620024, India,

Tel: +91-431-2407117, Mobile: +91-9790995854

[mgdcaua@yahoo.in](mailto:mgdcaua@yahoo.in), [akbarbdu@gmail.com](mailto:akbarbdu@gmail.com)

## **Video legends (Main article)**

**M1.** Feeding behavior of hydra

**M2.** Hydra's response to GSH (positive control)

**M3.** Alterations in feeding behavior of hydra exposed to 2.5  $\mu$ M CuO NR for 24 h

**M4.** Hydra's response to GSH after exposure to 2.5  $\mu$ M CuO NR for 24 h

## **Supplementary information**

### **Legends**

**Figure. S1.** Median score of hydra exposed to same mass concentration of CuSO<sub>4</sub> compared with CuO NR.

**Table S1.** Median score of hydra exposed to CuO NR. Significance between the control and the treatment groups were performed by adopting two way ANOVA with Dunnet's multiple comparison test (\*\*\*\*p < 0.001, \*\*p<0.05).

**Table S2.** Wilby's scale to measure toxicity end points.

**Table S3.** Annealing temperature of primers employed in the qRT-PCR analysis. The primers were adopted from Woo et al. (2012) and Ambrosone et al. (2012).

**Table S4.** List of forward and reverse primers employed in the qRT-PCR analysis.

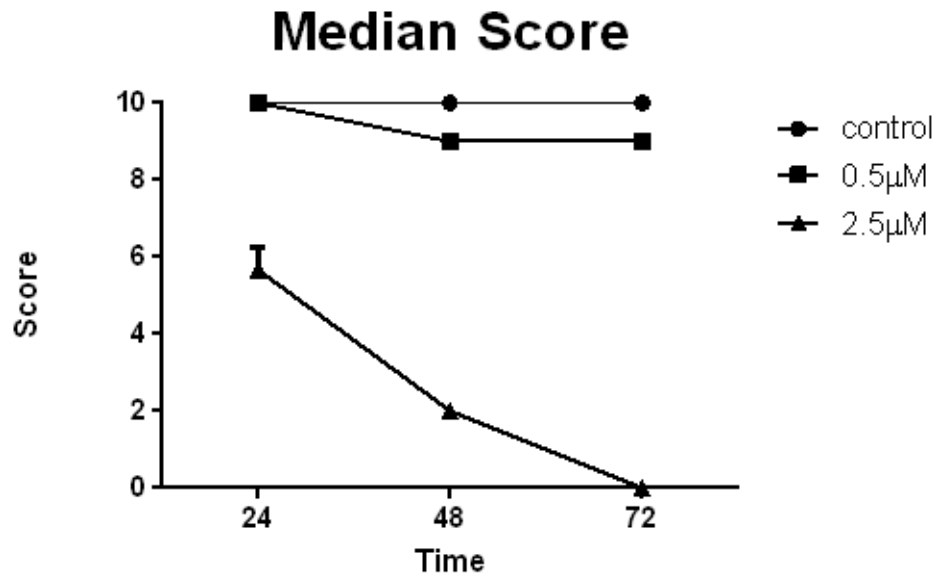


Figure. S1. Median score of hydra exposed to same mass concentration of  $\text{CuSO}_4$  compared with  $\text{CuO NR}$ .

**TABLES**

| <b>Concentration<br/>(<math>\mu</math>M)</b> | <b>Exposure time (hr)</b> |           |           |
|--|---------------------------|-----------|-----------|
|  | <b>24</b>                 | <b>48</b> | <b>72</b> |
| Untreated                                    | 10                        | 10        | 10        |
| 0.5  | 10                        | 10        | 10        |
| 1  | 10                        | 10        | 10        |
| 1.5  | 10                        | 9**       | 7****     |
| 2  | 10                        | 7****     | 5****     |
| 2.5  | 10                        | 6****     | 4****     |
| 3  | 10                        | 5****     | 4****     |
| 3.5  | 10                        | 4****     | 4****     |

**Table S1. Median score of hydra exposed to CuO NR**

| Key for assessing progressive toxic effects in hydra polyps, Wilby's scale (1988) |   |
|---|---|
| Score   | Morphology of polyp                           |
| 10  | Extended tentacles; body reactive             |
| 9   | Partially contracted; slow reactions          |
| 8   | Clubbed tentacles; body slightly contracted   |
| 7   | Shortened tentacles; body slightly contracted |
| 6   | Tentacles and body shortened                  |
| 5   | Totally contracted; tentacles visible         |
| 4   | Totally contracted; no visible tentacles      |
| 3   | Expanded; tentacles visible                   |
| 2   | Expanded; no visible tentacles                |
| 1   | Dead but intact                               |
| 0   | Disintegrated                                 |

**Table S2. Wilby's scale to measure toxicity end points**

| <b>Primer</b>   | <b>Annealing temperature</b> |
|-----------------|------------------------------|
| FoxO            | 48 °C                        |
| Hsp70.          | 52 °C                        |
| Bcl-2 like 4    | 52 °C                        |
| Catalase (CAT)  | 48 °C                        |
| G6PD            | 48 °C                        |
| GPx             | 52 °C                        |
| GR              | 48 °C                        |
| GST             | 52 °C                        |
| SOD             | 52 °C                        |
| TUB- $\alpha$ 1 | Both 48 °C and 52 °C         |

**Table S3. Annealing temperature of primers employed in the qRT-PCR analysis. The primer designs were adopted from Woo et al., (2012) and Ambrosone et al., (2012)**

|                   |                  |                              |                            |     |
|-------------------|------------------|------------------------------|----------------------------|-----|
| FoxO              | XP_002167754     | GGATTTCCGATGCAAG<br>TACG     | TAAGTCTGCTTGGC<br>GAACG    | 109 |
| Hsp70.1           | XP_002159813     | CGACGTATTCAGACA<br>ATCAACC   | CAATTTGAGGAAC<br>ACCTCTTGG | 136 |
| Bcl-2 like<br>4   | XP_002167578     | AACAAGGTGGATGGG<br>ATGG      | ATAAGTAATGCGC<br>CCACACC   | 147 |
| Catalase<br>(CAT) | CN631284         | GCTCCAAACTACTTCC<br>CTAACAG  | GCTCATCTATCGCT<br>TCATTT   | 298 |
| G6PD              | DT614664         | GCATTGCCACCATCTG<br>TATTCA   | GCAAACCTTAGCA<br>CCATTAT   | 230 |
| GPx               | DQ286040         | TCGATATCTGGAACCA<br>ATGACAAA | CGAGGCGCCCACT<br>ATGACTT   | 209 |
| GR                | XM_00215997<br>9 | GAGGAGCGTATTTTGG<br>GTAT     | GTTTAACCTCAGCA<br>ACCAGT   | 209 |
| GST               | XM_00215396<br>8 | CGAGGCAGCTAAGTT<br>AAAGT     | ACTTAAGGTAATG<br>GGGGATG   | 246 |
| SOD               | XM_00215747<br>1 | TCAGTTTGGGGATTAT<br>TCAGGTG  | TCCAGCATTTCCGG<br>TAGTTTTG | 280 |
| TUB- $\alpha$ 1   | CV660826         | TTGATGAAATACGCAC<br>AGGAACA  | CCACCAAAGGAAT<br>GAAAAAT   | 205 |

**Table S4. List of forward and reverse primers employed in the qRT-PCR analysis.**