Supplementary data

In-vitro toxicity, apoptosis and antimicrobial effects of phyto-mediated copper oxide nanoparticles

V. Gopinath *[a], S. Priyadarshini [a], A.R. Al-Maleki[a], M. Alagiri [b], Rosiyah Yahya [c], S. Saravanan [d], Jamuna Vadivelu [a].

[a] Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur - 50603, Malaysia.

[b] Center for Material science and Nanodevices, Department of Physics and Nanotechnology, SRM University, Kattankulathur – 603 203, Tamil Nadu, India.

[c] Department of Chemistry, University of Malaya, Kuala Lumpur, 50603, Malaysia.

[d] Department of Physiology and Pathophysiology, St-Boniface Hospital Albrechtsen Research Centre, University of Manitoba, Winnipeg, R2H A26, Canada.

Supplementary methods

Protein extraction of nanoparticles synthesizing *Tribulus terrestris* fruit extract

Approximately 5 mg of *T. terrestris* control and the extrct after exposed with $CuSO_4.5H_2O$ for 120 min were lysed and proteins were extracted using the ProteoSpin detergent-free total protein isolation kit (Norgen Biotek, Canada) with the Halt protease and phosphatase inhibitors cocktail (Thermo Scientific, Waltham, MA) included. The lysates were subsequently treated with 10 mM dithiothreitol (DTT; Bio-Rad, Hercules, CA) at 37° C for 10 min and alkylated with 55 mM iodoacetamide (IAA; Bio-Rad) for 30 min at room temperature. The proteins in the samples were digested with1:50 (trypsin: *protein*) of MS-grade Pierce trypsin protease (Thermo Scientific, Waltham, MA) at 37° C overnight. The samples were desalted using a Pierce C-18 spin column (Thermo Scientific, USA) and dried to completeness in a refrigerated CentriVap centrifugal vacuum concentrator (Labconco, Kansas City, MO) before mass spectrometry analysis.

LC-MS/MS analysis

The LC-MS/MS analysis of *Tribulus terrestris* proteins samples was performed using the nano capillary liquid chromatography (LC) column coupled to an LTQ Orbitrap mass spectrometer (Thermo Fisher Scientific, San Jose, CA) by an in-house-manufactured interface. Setting parameter were set as follows; Ion Transfer Tube Temperature was 275 °C, spray voltage were: static, positive Ions: 2000 V, negative ion: 600 V, pressure mode: Standard, Internal Mass Calibration: true, scan range (m/z): 400-1600 at a resolution of 60 k and AGC target: (4.0 × 105). Intensity threshold: (5.0 × 103). Dynamic exclusion (exclude after 3 times within 30 second), Mass tolerance (Low 10 and high 10).

Supplementary Results

Data analysis

Mass spectrometric data were processed and analysed using the Peaks software, version 7.5 (Bioinformatics Solutions Inc.) for MS/MS-based identification and *de novo* sequencing. For protein identification, MS/MS spectra were searched against the Uniprot/Swissprot *T. terrestris* accepting common variable modifications with maximum three variables PTM per peptide. The search parameters allowed for three trypsin missed cleavages per peptide, the precursor mass tolerance was set to 5 ppm, and fragment ion mass tolerance was set to 0.2 Da. To compare the observed LCMS data of *T. terrestris* control and copper exposed reaction mixture only one protein mutase (mut G) was matching with the Uniprot database. From the LCMS analysis report the plant protein mutase could be responsible for the biosynthesis and stabilization of copper oxide nanoparticles. The other observed peaks of (proteins/peptides) are not matching with the Uniprot/Swissprot database of *T. terrestris* which contains only 9 proteins. The above preliminary findings of nanoparticles synthesis mechanism will be further extensively studied in our following publication.

Note: The observed raw data file attached in separate file.

Fig. S1. The characteristics of CuONPs used in this study: (A) Zeta potential (B); Size distribution.