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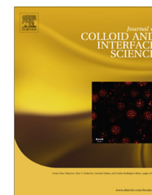

Antioxidative and cytoprotective response elicited by molybdenum nanoparticles in human cells

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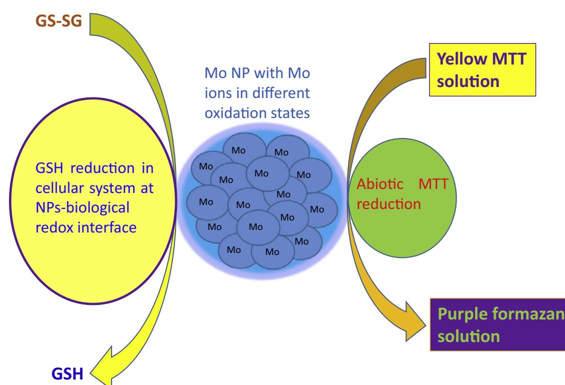
HIGHLIGHTS

- *In vitro* data suggest Mo NPs to be non-toxic in MCF-7 and HT-1080 cells.
- GSH depletion caused by oxidants was significantly replenished by Mo NPs.
- Mo NPs significantly protected two cells from death induced by H_2O_2 and ZnO (NPs).
- Study suggest Mo NPs to be placed with rare antioxidant NPs like those of fullerenes.

GRAPHICAL ABSTRACT

In biological systems, as part of redox enzymes activity, Mo functions by shuttling itself between three different oxidation states. Chemically, Mo has been key factor in reduction of chemicals at the surfaces of Mo based NPs of sulfides or oxides. In the present study, Mo NPs reduced GS-SG and MTT respectively under biological and chemical systems.



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ABSTRACT

Nanotechnology based therapeutics can offer an alternative platform in a wide variety of biomedical applications. Here we report novel cytotoxicity preventive potential of molybdenum nanoparticles (Mo NPs) in human breast (MCF-7) and fibrosarcoma (HT-1080) cells compromised with oxidant exposure. Physicochemical properties such as size, crystallinity, purity and band gap (an optical characteristic) of Mo NPs were characterized respectively by field emission transmission electron microscopy (FETEM), X-ray diffraction (XRD), energy dispersive spectrum (EDS) and UV–vis absorption spectroscopy. The average size of crystalline Mo NPs was found to be 35 nm with a band gap of 1.4 eV. Potential cytotoxicity of Mo NPs was evaluated by a battery of cell viability and oxidative stress parameters. Cell viability and oxidative stress data suggested Mo NPs to be reasonably non-cytotoxic. Cytotoxic preventive and GSH restoring potential of Mo NPs was determined against cytotoxicity and oxidative stress induced by H_2O_2 (and ZnO NPs) in two cells. Mo NPs significantly increased GSH level in MCF-7 and HT-1080 cells, an activity that was comparable to antioxidant N-acetyl cysteine (NAC). GSH level was increased 1.56 times in MCF-7 cells and 1.25 times in HT-1080 cells by 100 μg/ml of Mo NPs relative to control cells

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in 24 h. End-point data clearly suggest that Mo NPs significantly protected cells against cytotoxicity induced by H_2O_2 and ZnO (NPs) ($p < 0.05$). Our study warrants further investigation about Mo NPs that could be exploited in myriads of nanotechnology applications.

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1. Introduction

Currently, nanoparticles (NPs) are being engineered to be used in a multitude of therapy and diagnostics [1,2]. NPs, with a diameter of less than 100 nm, acquire catalytic functions as compared with their bulk counterparts. In recent times, NPs with pharmacological potential has gained lots of attention [3,4]. In essence, nanoparticle therapeutics *per se* can offer an alternative platform in a wide variety of biomedical applications. Therefore, synthesis and engineering of relatively safe and non-immunogenic NPs is highly desirable. Molybdenum (Mo) exists in three oxidation states (+4, +5 and +6) meaning it can readily participate in redox reactions [5]. The trace element Mo is essential for nearly all organisms and forms the catalytic center of a large variety of enzymes such as nitrogenase, nitrate reductases, sulfite oxidase and xanthine oxidoreductases [6]. Within enzyme, Mo shuttles between three oxidation states (+4, +5 and +6), thereby catalyzing two-electron reduction–oxidation reactions [7]. Mo is bioavailable as molybdate (MoO_4^{2-}). Once molybdate enters the cell, it is subsequently incorporated by complex biosynthetic machineries into metal cofactors [8] participating in biological redox reactions. With these well documented bio-catalytic functions of Mo (element), we motivated to explore response of Mo nanoparticles (NPs) in human cells.

Potential biological response of Mo NPs was explored in human breast (MCF-7) and fibrosarcoma (HT-1080) cells. Physicochemical properties such as size, crystallinity, purity and band gap (an optical characteristic) of Mo NPs were characterized by field emission transmission electron microscopy (FETEM), X-ray diffraction (XRD), energy dispersive spectrum (EDS) and UV–vis absorption spectroscopy respectively. Potential cytotoxicity of Mo NPs was determined by their effects on cell viability and oxidative stress. Cell viability was determined by tetrazolium bromide (MTT) and neutral red uptake (NRU). Oxidative stress was determined by lactate dehydrogenase (LDH) release, lipid peroxidation (LPO), reactive oxygen species (ROS) generation and mitochondrial outer membrane potential (MOMP) induction in the two cells due to Mo NPs treatment for 24 h. Inhibitory concentrations 50 (IC50s) of ZnO NPs have been used as a positive control in the present test of Mo NPs, because NPs of ZnO is well known for its toxicity as well as for nanoparticle-cell contact [9]. Toxic concentrations of H_2O_2 was used for inducing cytotoxicity and oxidative stress in the two cells. Then, ameliorating effects of Mo NPs in H_2O_2 treated cells was compared with N-acetyl cysteine (2 mM NAC) a well-known antioxidant) as carried out by studies in similar context [10,11].

2. Materials and methods

2.1. Chemicals and reagents

Fetal bovine serum, penicillin–streptomycin was purchased from Invitrogen Co. (Carlsbad, CA, USA). DMEM, DMEM F-12, Neutral red dye, MTT [3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide], JC-1, N-acetyl cysteine, BSO, GSH), o-phthalaldehyde (OPT), 2,7-dichlorofluorescin diacetate (DCFH-DA), were obtained from Sigma–Aldrich (Sigma–Aldrich, MO, USA). Ultrapure deionized-water was prepared using a Milli-Q system (Millipore, Bedford, MA, USA). All other chemicals used were of reagent grade.

2.2. Molybdenum NPs

NPs of molybdenum was commercially obtained. As per the information provided by supplier, Mo NPs TEM size was below 100 nm and 99.8% pure based on trace metals analysis. Color of nanopowder was greyish black.

2.3. Physico-chemical characterization of Mo NPs

Physicochemical properties such as size, crystallinity and optical properties of Mo NPs were characterized by field emission transmission electron microscopy (FETEM), X-ray diffraction (XRD), and UV–vis absorption spectroscopy respectively.

2.3.1. Transmission electron microscopy of Mo NPs

Morphology of Mo NPs was determined by field emission transmission electron microscopy (FETEM) (JEM-2100F, JEOL Inc.) at an accelerating voltage of 200 kV [12]. Normal and high resolution (HR) TEM images was taken. Suspension of ultra-sonicated Mo NPs was placed onto a carbon-coated copper grid, air dried and observed with FETEM. Purity of Mo NPs was determined by energy dispersive spectrum (EDS) analysis.

2.3.2. X-ray diffraction of Mo NPs

The phase characteristics of Mo NPs were examined by powder X-ray diffraction. The XRD pattern of Mo NPs was acquired at room temperature with the help of PANalytical X'Pert X-ray diffractometer equipped with a Ni filter using Cu Ka (k = 1.54056 Å) radiations as an X-ray source.

2.3.3. Agglomeration and zeta-potential of Mo NPs

Hydrodynamic size of Mo NPs in complete cell culture medium and distilled water was determined by dynamic light scattering (DLS) (Nano-ZetaSizer-HT, Malvern Instruments, Malvern, UK) as described by Murdock et al. [13].

2.3.4. Band gap energy of Mo NPs

UV–vis absorption spectroscopy is one of the most widely used simple and sensitive techniques for the observation of nanoparticles synthesis and characterization. Pure ethanol was taken as a reference. The UV–vis (Thermo scientific evolution 60S) analysis was carried out in the wavelength range of 300–900 nm using a double beam UV–vis–NIR spectrophotometer with a resolution of 0.5 nm.

2.4. Cell culture

Human breast cancer (MCF-7) and fibrosarcoma (HT-1080) cells (ATCC) were maintained in DMEM and DMEM-12 medium respectively with high glucose supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin, at 37 °C in a humidified, 5% CO_2 incubator. The cells were passaged for every 3–4 days before reaching confluence level.

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