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Uranium-induced Cytotoxicity in HEK 293T Cells is reduced by a *β*-glucan type Exopolysaccharide produced by *Enterobacter* sp. YG4

K. K. Nagaraj , A. B. Arun , P. D. Rekha*
Yenepoya Research Centre, Yenepoya University, Deralakatte - 575018, Mangalore, Karnataka, India.

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ABSTRACT

Uranium-induced nephrotoxicity is a major health concern and countering it is essential. In this regard; a β-glucan type exopolysaccharide produced by *Enterobacter* sp. YG4 was tested for its capacity to reduce the uranyl nitrate-induced cytotoxicity in Human embryonic kidney cell line (HEK 293T). This exopolysaccharide at 0.5 mg/mL concentration was able to remove 80.94% of uranyl ions from its aqueous solution. Experiments with HEK 293T cells showed that the exposure to 0.05 mg/mL uranyl nitrate resulted in 22.13% cytotoxicity and treatment with YG4 exopolysaccharide could reduce the cytotoxicity of uranyl nitrate significantly. These results highlight the potential of this exopolysaccharide in counteracting the nephrotoxic effects of uranium.

INTRODUCTION

Extensive use of heavy metals in agriculture and industrial applications has resulted in an increase of their environmental concentrations. Uranium is one such heavy metal, which is a common component of Earth's crust and its environmental concentration varies depending on the geographical features or anthropogenic activities. The kidney is the primary target organ of uranium overexposure because of its ability to filter, reabsorb and concentrate. Nephrotoxic outcomes in human populations have been reported from uranium-contaminated sites and it was observed that both glomerular and tubular functioning were affected (Kurttio *et al.*, 2002; Magdo *et al.*, 2007; Okaneku *et al.*, 2015). Uranium induced nephrotoxicity has been studied extensively in both *in vitro* and *in vivo* models. These studies show that the uranium can alter cellular nutrient/ion transport, oxidant-antioxidant balance and induce apoptosis thereby affecting glomerular and tubular functioning and structural integrity (Hao *et al.*, 2015; Vicente-Vicente *et al.*, 2010).

The countermeasure against uranium exposure involves the intravenous infusion of sodium bicarbonate and chelation therapy. However; blood alkalization is an inevitable outcome in sodium bicarbonate infusion. Apart from this; the inherent chemical nature of these countermeasures can put an additional burden on the already affected kidneys (Bergeron *et al.*, 2009; Durbin, 2008). In order to develop an effective and safer countermeasure against uranium-induced nephrotoxicity; we had tested a β-glucan type exopolysaccharide (EPS) produced by the bacterium *Enterobacter* sp. YG4 using albino Wistar rat model. This EPS was capable of reducing the uranium-induced decrease in renal functioning and histological damage (Nagaraj *et al.*, 2016). Continuing that study; we have conducted *in vitro* experiments to understand the mechanisms by which this EPS protects kidneys from the toxic effects of uranium.

MATERIALS AND METHODS

Extraction of EPS

The bacterium *Enterobacter* sp. YG4 was cultured in Yeast Extract Mannitol (YEM) broth for 72 h at 32°C in a shaking

*Corresponding Author
P. D. Rekha, Professor and Deputy Director, Yenepoya Research Centre, Yenepoya University, Deralakatte - 575018, Mangalore, Karnataka, India. E-mail: rekhapd @ hotmail.com

incubator (120 rpm). After incubation, the culture was centrifuged at 7000 rpm for 10 min and the cell-free supernatant was mixed with ice-cold ethanol (1:3) to precipitate EPS. The precipitated EPS (YG4 EPS) was collected by centrifugation (9000 rpm, 10 min), washed with distilled water, and reprecipitated with ice-cold ethanol. The EPS was dissolved in distilled water and dialyzed against distilled water for 24 h in a 12-kDa cut-off dialysis membrane (HiMedia, India). The dialyzed EPS was dried and stored for further use.

Removal of uranyl ions by YG4 EPS

The YG4 EPS solution (500 µL) was mixed with equal volume of uranyl nitrate solution so that the final concentration of the EPS and uranyl nitrate in the mixture was 0.5 mg/mL and 2 mg/mL respectively. This mixture was allowed to stand at room temperature for 15 min and centrifuged at 15000 rpm for 5 min. The uranyl ion concentration in the supernatant was determined by UV spectrophotometry. The aqueous solution of uranyl ion displays UV absorption spectrum with characteristic peaks at 415 nm, 413 nm, and 412 nm (Bell and Biggers, 1965). A standard curve generated using absorption at 415 nm was used to determine uranyl ion in the samples. Uranyl ion concentrations in the samples and blank (without EPS) were compared to determine the ability of EPS to remove the uranyl ions.

***In vitro* experiments**

Human embryonic kidney cell line HEK 293T (obtained from National Centre for Cell Science, India) was used for the experiment. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic solution and incubated in a humidified atmosphere with 5% CO_2 at 37°C.

HEK 293T cells were loaded in 96 well plates at 5000 cells/well density. Cells were exposed to 0.05 mg/mL uranyl nitrate dissolved in DMEM and YG4 EPS (0.05, 0.10, 0.20, and 0.40 mg/mL) dissolved in DMEM and incubated for 24 h. After the incubation period; cell viability was determined by methylthiazol tetrazolium (MTT) assay. Cells not exposed to uranyl nitrate or YG4 EPS were taken as control and used for comparison.

RESULTS AND DISCUSSIONS

Removal of uranyl ions by YG4 EPS

Uranium compounds dissociate into uranyl ions [$(UO_2)^{2+}$] in the body fluids. These ions react with biomolecules in the kidneys and lead to kidney injury. Therefore, the property of a compound to bind with and remove uranyl ions may be useful in reducing uranium-induced nephrotoxicity. In our experiments; we observed that the YG4 EPS at 0.5 mg/mL concentration could remove 80.94 ± 1.56% uranyl ions from its aqueous solution. The interaction between EPS molecules and metal ions has been ascribed to electrostatic interactions and coordination bond formation (Gupta and Diwan, 2017; Mohite *et al.*, 2017). This property of removing uranyl ions can play an important role in the reduction of uranium-induced nephrotoxicity by either decreasing the absorption and distribution of uranyl ions or increasing the rate of their excretion.

The activity of YG4 EPS against uranyl nitrate-induced cytotoxicity in HEK 293T cells

The YG4 EPS did not exhibit cytotoxicity towards HEK 293T cells at concentrations of 0.05, 0.10, 0.20, and 0.40 mg/mL for 24 h (Figure 1).

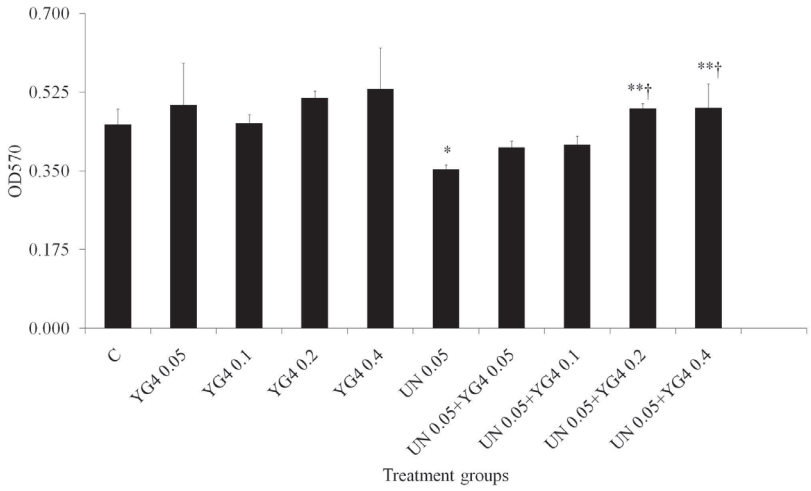

Fig. 1: The cytotoxicity of 0.05 mg/mL uranyl nitrate on HEK 293T cells and activity of YG4 EPS (YG4) against it at 0.05, 0.10, 0.20, and 0.40 mg/mL concentrations as determined by MTT assay. Data presented as mean ± SD (n = 3). Bar labeled *significantly different from Control (C) at $p < 0.05$ and bars labeled **†significantly different at $p < 0.01$ from uranyl nitrate (UN) treatment at 0.05 mg/mL.

The uranyl nitrate exposure at 0.05 mg/mL concentration for 24 h in HEK 293T cells induced 22.13% cytotoxicity compared to control (Figure 1). Such cytotoxicity has been ascribed to uranium-induced oxidative stress or apoptosis resulting in cellular damage (Hao *et al.*, 2012; Thiébault *et al.*, 2007). The treatment with β-glucan type YG4 EPS completely prevented the cytotoxic effects induced by uranyl nitrate in HEK 293T cells. The treatment with YG4 EPS at 0.20 and 0.40 mg/mL concentrations against 0.05 mg/mL uranyl nitrate resulted in significant cell proliferation by 38.24% and 38.90% respectively, compared to the uranyl

nitrate group. The treatments at 0.05 and 0.10 mg/mL also induced proliferation by 13.81% and 15.53% respectively. However; this proliferation was not statistically significant. The capacity of β-glucans to reduce cytotoxic effects under *in vitro* conditions has been reported earlier and such activity has been attributed to their ability to neutralize reactive oxygen species, protect cells against oxidative stress and prevent apoptotic events (Kao *et al.*, 2012; Oliveira *et al.*, 2007; Pourahmad *et al.*, 2011). Our previous report (Nagaraj *et al.*, 2016) had also shown that this β-glucan type YG4 EPS possesses antioxidant activities and is capable of reducing uranium-induced nephrotoxicity in albino Wistar rats.

CONCLUSION

The β-glucan type EPS produced by *Enterobacter* sp. YG4 is capable of reducing uranium-induced cytotoxicity in HEK 293T cells. This ability can be attributed to its capacity to remove uranyl ions and its antioxidant activities. Such activities can play an important role in reducing the uranium-induced nephrotoxicity *in vivo*. Further experiments to comprehensively understand such activity are in progress.

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CONFLICT OF INTERESTS

There are no conflicts of interest.

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