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Author(s)	Md., Shiblur Rahaman
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学 位 論 文 内 容 の 要 旨

博士（環境科学）　　氏 名 Md. Shiblur Rahaman

学 位 論 文 題 名

Alleviation of arsenic-induced toxicity by curcumin and D-pinitol, the main components of the herbs commonly used in Bangladesh, and their mechanism

（バングラデシュで日常的に用いられているハーブの主成分であるクルクミンと D-ピニトールによるヒ素誘発毒性の緩和とそのメカニズム）

Arsenic toxicity and arsenic-related health consequences are major public health concerns and a global problem affecting countries on all five continents. Arsenic contamination in the aquatic environment due to natural sources and anthropogenic activities is posing a dreadful threat to human health. Both acute and chronic arsenic exposure through drinking water and foods can induce cellular, metabolic and physiological toxicities. So far there is no particular cost-effective treatment available for arsenic-related diseases. Though chelation therapy is a well-known treatment for arsenic-related diseases; however, it is costly and showed several undesirable side effects. In the last few decades, the interest in the nutritional field has gone a step forward, searching for novel natural bioactive compounds with the capacity to reduce the risk of non-communicable diseases. As hypothesis for the thesis, natural dietary supplements and a balanced diet can be a cost-effective and safe therapeutic approach against arsenic toxicity. In the present research, natural dietary bioactive compounds; D-pinitol and curcumin have been used to detoxify arsenic toxicity in PC12 cells which has been well known as model cell line for fundamental molecular and toxicological study. D-pinitol is a natural dietary bioactive compound that has antioxidant properties and drawn great attention due to its diverse biological activities and therapeutic potential against many human ailments.

In chapter 2, the ameliorative effects of D-pinitol on arsenic-induced toxicity have been investigated in PC12 cells. Co-exposure of D-pinitol (1, 5 and 50 μM) with arsenic (5 μM) increases cell viability, and decreases DNA damage and protects PC12 cells from arsenic-induced cytotoxicity by increasing glutathione (GSH) level and glutathione reductase (GR). Protein expression of western blot analysis revealed that co-exposure of D-pinitol and arsenic significantly decreased arsenic-induced autophagy which further suppressed apoptosis through up-regulation of survival factors; mTOR, p-mTOR, Akt, p-Akt, NF-κB, Nrf2, ERK1, GR, and Bcl-x, and down-regulation of death factors; p53, Bax, cytochrome c, and LC3, although arsenic regulated those factors negatively. From the results, D-pinitol protects PC12 cells from arsenic-induced

cytotoxicity.

Similarly, in Chapter 3, the protective effects of curcumin on arsenic-induced toxicity have been investigated using PC12 cells. Arsenic (10 μM) treatment in PC12 cells for 24 h induced cytotoxicity by decreasing cell viability and intracellular GSH level and DNA fragmentation in PC12 cells. Also, arsenic caused apoptotic cell death in PC12 cells, which were confirmed from flow cytometry results. In addition, arsenic (10 μM) treatment significantly down-regulated the survival factors; mTOR, Akt, Nrf2, ERK1, Bcl-x and Xiap, up-regulated the death factors; ULK, LC3, p53, Bax, cytochrome c, caspase 9 and cleaved caspase 3, and eventually caused autophagic and apoptotic cell death. However, curcumin (2.5 μM) pretreatment (1 h) with arsenic (10 μM) protects PC12 cells from arsenic-induced cytotoxicity by increasing cell viability, GSH level and boosting the antioxidant defense system, and limiting DNA damage. Furthermore, pretreatment of curcumin with arsenic expressively alleviated arsenic-induced toxicity and cell death by reversing the expressions of protein mTOR, Akt, Nrf2, ERK1, Bcl-x, Xiap, ULK, LC3, p53, Bax, cytochrome c, caspase 9 and cleaved caspase 3. These findings indicated that curcumin showed antioxidant properties through the Nrf2 antioxidant signaling pathway and reduces arsenic-induced toxicity in PC12 cells via modulating autophagy/apoptosis.

Both dietary compounds, curcumin and D-pinitol are often consumed together; however, there is no information at all whether co-exposure of curcumin and D-pinitol has an additive, synergistic, or no effect on arsenic-induced toxicity. Thus, in chapter 4, we hypothesized that the combination treatment of curcumin and D-pinitol might have synergistic or additive protective effects against arsenic toxicity. As results, pretreatment of curcumin or D-pinitol, or their combined pretreatment with arsenic increases cell viability, decreases DNA damage and protects PC12 cells from arsenic-induced cytotoxicity by increasing GSH level and antioxidant defense. Protein expression of western blot analyses showed that combined pretreatment of curcumin and D-pinitol with arsenic significantly inhibited arsenic-induced cell death through up-regulation of survival factors; mTOR, Akt, Nrf2, ERK1, Bcl2, Bcl-x, and XIAP and down-regulation of death factors; p53, Bax, cytosolic cytochrome c, caspase 9 and cleaved caspase 3. These findings indicated that curcumin and D-pinitol showed antioxidant properties and protects PC12 cells from arsenic-induced cytotoxicity. However, the protective effect of combined treatment with curcumin and D-pinitol was not so strong as expected.

Exposures of arsenic possess serious health hazards and lack of a balanced diet could exaggerate the toxic effects. In short, a balanced diet and proper dietary intake of natural bioactive compounds promise to reduce the arsenic toxicity. The present study suggested that both natural dietary compounds; curcumin and D-pinitol have beneficial role against arsenic toxicity in vitro and the needs for future animal experiments were shown for practical application.