学 位 論 文 内 容 の 要 旨

博士（環境科学）　　氏 名 Subrata Banik

学 位 論 文 題 名

Ajwain and its phenolic compound, carvacrol improve cadmium-induced apoptosis in PC12 cells
（Ajwainとそのフェノール化合物であるカルバクロールは、PC12細胞におけるカドミウム誘導アポトーシスを改善する）

In the modern world, the environment has been becoming contaminated enormously with many types of toxicants. Among contaminants heavy metals such as lead (Pb), cadmium (Cd) and mercury (Hg) are of great concerns worldwide due to their high toxicity against biological functions. Cd is present in many foods, so Cd is easily taken into the human body. The acute and chronic exposures to Cd can induce cellular, metabolic and physiological toxicities which cause diseases involving tissues and organ systems including lungs, liver, gastrointestinal tract, reproductive system and cardiovascular system. Although the pattern of toxicity exerted by Cd is very complex, researchers have already approached to unveil the mechanisms *in vivo* and *in vitro*. However, there is no therapy by using drug or chelation therapy against the toxicity of Cd. So, it will be required to explore and develop new anti-toxic agents against Cd. On the other hand, dietary interventions, traditional medicinal approaches or natural products for toxicity alleviation can act as better preventive measures against Cd toxicity without side effects. The objective of this research has been set to clarify the effects of ethanolic extract of ajwain (*Trachyspermumammi L.*) seed and its phenolic compound, carvacrol on Cd-induced cytotoxicity, oxidative stress and apoptosis. Moreover, the molecular mechanisms behind these alleviating roles have also been resolved.

Ajwain (AE) is a popular spice, and has historically been used for the remedy of many pathological consequences such as inflammatory, toxic, neurological, genital or respiratory tract disorders. These symptoms are also common in Cd toxicity manifestations. In chapter 2, ethanolic extract of AE has been investigated to clarify the ameliorating effects against Cd-induced cytotoxicity and apoptosis in PC12 cells. First, the non-toxic doses of AE were determined to observe effects of AE reduction of the toxicity against Cd (5 or 10 μM) upon simultaneous exposures for 24 h. The safe but most effective dose of AE (240 μg/mL) considerably improved viability of PC12 cells treated with both concentrations of Cd. From the results of cell viability and lactate dehydrogenase (LDH) activity in the media, it was indicated that AE reduced cytotoxicity and increased the cell

membrane integrity. Oxidative stress and apoptosis were observed in PC12 cells treated with Cd. These were proved by both increases in intracellular reduced glutathione (GSH) levels and ladder pattern of higher DNA fragmentation. AE significantly improved the cellular anti-oxidative status by increasing the GSH levels, and decreased the cytotoxicity by reducing Cd-induced DNA fragmentation. Additionally, the western blotting results revealed that AE down-regulated the Cd-induced increased expression of apoptotic protein Bax, and up-regulated suppressed expressions of anti-apoptotic proteins Bcl-2, Bcl-xL and NF-KB. Moreover, the Cd-induced release of cytochrome c from mitochondria into the cytosol was reduced by co-treatment with AE. The expressions of caspase-3 increased by Cd were also significantly decreased by AE. From all these findings it has been suggested that AE reduced Cd-induced cytotoxicity and apoptosis in P12 cells.

Like other AE extracts, this ethanolic extract of AE has already been phytochemically characterized in previously reported research. It was found that AE contains carvacrol (iso-thymol) as the major phenolic compound in highest concentration. Moreover, in this research, the highest intensity peak in LC-MS analysis profile of AE was shown as carvacrol. Therefore, next aim of the research was turned towards to clarify whether the improvement effects of AE on Cd-induced cytotoxicity and apoptosis was due to carvacrol.

Previous studies have already reported that carvacrol showed high antioxidative potential and also the protective effects against various toxicants *in vivo* and *in vitro*. In chapter 3, the effects of carvacrol against Cd-induced cytotoxicity and apoptosis in PC12 cells have been investigated. First, the non-toxic concentrations of carvacrol was found in between 0-200 μM in PC12 cells. However, upon co-exposure with Cd (10 μM) for 48 h the highest improvement in cell viability was found for 100 μM of carvacrol. The simultaneous exposure of carvacrol with Cd reduced LDH level and increased the GSH levels and glutathione reductase expression. Carvacrol also reduced Cd-induced DNA fragmentation. The rate of apoptosis in Cd exposed cells was considerably reduced by the co-exposure of carvacrol. Co-exposure of carvacrol also up-regulated the down-regulated expressions of mTOR, Akt, NF-κB, ERK-1 and NRF2 by Cd. Additionally, carvacrol reduced the cleavage of caspase 3, the cytosolic levels of cytochrome c, and expression of apoptosis inducing factor (AIF). Carvacrol induced the metallothionein expression in PC12 cells upon co-exposure with Cd. Overall, it was proposed that carvacrol reduced Cd-induced cytotoxicity, oxidative stress, and caspase-dependent and caspase-independent apoptosis in PC12 cells.

From the results, it was able to guess that improvement effect of AE on Cd-induced apoptosis was due to carvacrol. Finally, these two agents has been recommended as potential and safe therapeutic agents against metal toxicity and other toxicants in the biological system. Additionally, this study validated the ethno-pharmacological therapeutic uses of ajwain in various pathological conditions.