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)

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In the modern world, the environment has been becoming contaminated enormously with many types of toxicants. As results human are facing the exposure of thousand types of chemicals every day. Among these contaminants heavy metals such as cadmium (Cd) are of great concerns worldwide due to their high toxicity against biological functions. Moreover, these metals can lead to pathophysiological status or diseased conditions at their low concentrations. Among the heavy metals Cd has picked up more attention for its ubiquitous characters depending on modern anthropogenic activities. Cd is also present in many foods, so Cd is easily taken into the human body. As Cd pollution is a global issue, many researchers have focused on that. Cd has longer biological half-life and bioaccumulative tendency in biological systems including humans. Although the pattern of toxicity exerted by Cd is very complex, researchers have already approached to unveil the mechanisms *in vivo* and *in vitro*, and achieved some success. 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. For the purpose, keeping both preventive measure and development of new therapeutic agent in mind, recently researchers have unveiled the ameliorating roles and their mechanisms of numerous anti-oxidative plant extracts and phytochemicals. 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 (Fig. 1) is a popular spice, and has historically been used for the remedy of many pathological consequences such as inflammatory, toxic, neurological, or respiratory tract disorders. These symptoms are also common in Cd toxicity manifestations. Moreover, the revalidation of these numerous therapeutic use has been achieved by demonstrating research works deciphering the therapeutic effects of ajwain extracts on disease model animals with molecular clarifications. Additionally, various ajwain extracts have been found as toxicity ameliorating agents against few organic and inorganic toxicants *in vivo* and *in vitro*.

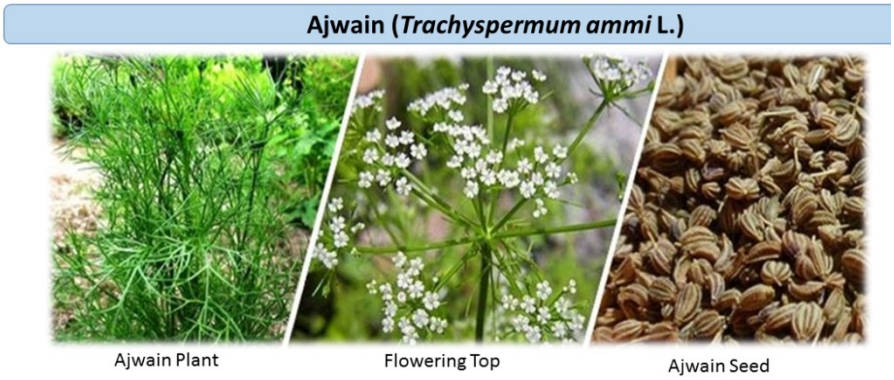

Fig. 1 **Ajwain (*Trachyspermum ammi* L.)** Image Source: Research Gate

In chapter 2, ethanolic extract of ajwain (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 decreases in intracellular reduced glutathione (GSH) levels and higher DNA fragmentations. 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 PC12 cells due to improvement of intrinsic pathway of apoptosis.

Like other ajwain extracts, 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.

Carvacrol (CVC) is a monoterpenoid phenol found in many *Labiateae* and *apiaceae* family plants. Numerous *in vivo* and *in vitro* studies have conducted on biological activities of CVC

including anti-inflammatory and anti-carcinogenic effects. Previous studies have already reported that CVC showed high antioxidative potential and also the protective effects against various toxicants *in vivo* and *in vitro*. In chapter 3, the effects of CVC against Cd-induced cytotoxicity and apoptosis in PC12 cells have been investigated. First, the non-toxic concentrations of CVC were 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 CVC. The simultaneous exposure of CVC with Cd reduced LDH level and increased the GSH levels and glutathione reductase expression. CVC also reduced Cd-induced DNA fragmentation. The rate of apoptosis in Cd exposed cells was considerably reduced by the co-exposure of CVC. Co-exposure of CVC also up-regulated the down-regulated expressions of mTOR, Akt, NF-κB, ERK-1 and NRF2 by Cd. Additionally, CVC reduced the cleavage of caspase 3, the cytosolic levels of cytochrome c, and expression of apoptosis inducing factor (AIF). CVC induced the MT expression in PC12 cells upon co-exposure with Cd. Overall, it was proposed that CVC 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 CVC. Therefore, it was suggested that both AE and CVC can act as anti-oxidative and anti-apoptotic agent in bioorganism (Fig. 2). 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.

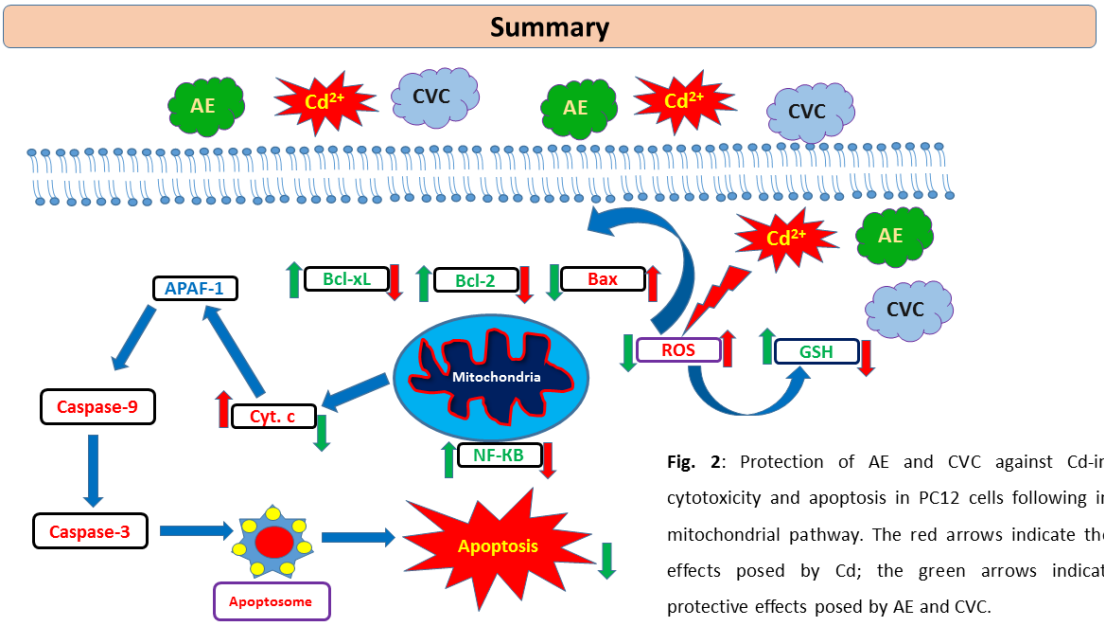

Fig. 2: Protection of AE and CVC against Cd-induced cytotoxicity and apoptosis in PC12 cells following intrinsic mitochondrial pathway. The red arrows indicate the toxic effects posed by Cd; the green arrows indicate the protective effects posed by AE and CVC.