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Delineating the anti-cytotoxic and anti-genotoxic potentials of catechin hydrate against cadmium toxicity in human peripheral blood lymphocytes

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ABSTRACT

Catechins (flavan-3-ol) are a type of natural phenol and well-studied antioxidants. Catechin hydrate, also known as taxifolin; is non-mutagenic, low in toxicity compared to other immunomodulator antioxidants. We aimed to determine the potential of catechin hydrate to prevent the cyto-genotoxic effects of cadmium in lymphocytes; demonstrate the immuno-protective activity of catechin hydrate. Our previous study indicated that cadmium is apoptogenic. Lymphocytes were treated with catechin hydrate or cadmium and catechine hydrate combinations (range 0.1–100 μM) to determine their effects on cell viability. Lymphocytes treated with 100 μM catechin hydrate and 100 μM cadmium showed cell viability $70.65 \pm 6.92\%$ and $5.69 \pm 2.27\%$, respectively. In our previous study cadmium (10 and 20 μM) induced apoptosis in 31.8% and 44.4% of lymphocytes, respectively. However, the percentage of apoptotic cells after treatment with the combination of cadmium and catechin hydrate was not significantly different from that of catechin hydrate ($P > 0.05$). Only 7.3% and 10.5% of the lymphocytes were apoptotic after treatment with 10 μM cadmium + 10 μM catechin hydrate and 20 μM cadmium + 20 μM catechin hydrate, respectively. The anti-genotoxic and immuno-protective potential of catechin hydrate was also demonstrated by the non-significant expression of apoptosis-related genes after treatment with catechin hydrate.

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1. Introduction

Catechin is a polyphenolic flavonoid that has been isolated from a variety of natural sources, including tea leaves, grape seeds, and the wood and bark of trees such as acacia and mahogany. Catechin is a more potent antioxidant than

ascorbate or α -tocopherol in certain *in vitro* assays of lipid peroxidation. Catechin inhibits the free radical-induced oxidation of isolated LDL by AAPH (amidinopropane hydrochloride) (Alshatwi, 2010).

Catechin hydrate (CH) is a type of catechin and is also known as taxifolin (Makena et al., 2009) (Fig. 1). It is a flavanonol, a type of flavonoid that can be found mostly in the

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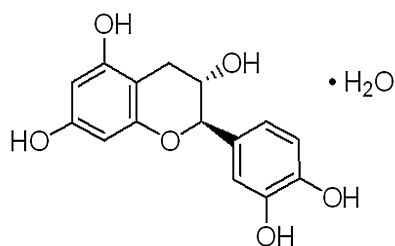


Fig. 1 – Molecular structure of catechin hydrate.

Siberian larch (*Larix sibirica*) and silymarin extract from milk thistle seeds (Pashinina et al., 1970; Hoffmann, 2003). It is also found in small quantities in red onions. CH is not mutagenic and has low toxicity compared to other flavonoids (Makena et al., 2009). It acts as a potential chemo-preventive agent by regulating genes via an ARE-dependent mechanism (Lee et al., 2007). Due to its high antioxidant activity, CH has been shown to inhibit breast cancer cell growth, cellular melanogenesis, and human cervical cancer in a dose-dependent manner (Alshatwi, 2010; Al-Hazzani and Alshatwi, 2011). CH acts as an immune enhancer and likely functions through a different immunomodulatory mechanism. Additionally, another study has suggested that the immune-enhancing properties of CH are also brought about by immune-enhancing TH1-type vaccine adjuvant properties (Yu and Vajdy, 2011).

Cadmium (Cd) is a highly toxic chemical that possesses a long biological half-life and primarily targets the lungs, liver, kidney, immune system, cardiovascular system, and reproductive system (Fowler, 2009). Cd has been listed in the Priority List of Chemicals by the U.S. Environmental Protection Agency. Moreover, in 1992, in a report the International Agency for Research on Cancer has classified Cd as a human carcinogen (IARC, 1992). Cd produces malignant tumors in the testes, prostate, and lungs of experimental animals (IARC, 1992; Waalkes et al., 1992). Cd is not a strong mutagen, but it acts as a promoter through mitogenic effects on gene expression (Beyersmann and Hechtenberg, 1997). Cd is found in abundance in the environment, particularly at specific work places and in food and water. Therefore, human exposure to Cd is essentially unavoidable. Higher levels of Cd have been detected in the urine and in various organs of exposed individuals (Yassin and Martonik, 2004). The toxicological responses to Cd exposure are primarily kidney damage, respiratory diseases, and neurological disorders (Waalkes et al., 1992). In addition, Cd has been reported to induce apoptosis in isolated T lymphocytes (El Azzouzi et al., 1994) and cultured LLC-PK1 cells (Matsuoka and Call, 1995); it can also lead to apoptotic cell damage in canine proximal tubules (Hamada et al., 1994) and rat testicular tissue (Xu et al., 1996). Most observations of Cd-mediated cell death are consistent with the caspase-dependent intrinsic pathway of apoptosis. Several investigators have reported the release of cytochrome c and the activation of caspase-9 in cell lines treated with Cd (Kondoh et al., 2002; Watjen et al., 2002). However, there have also been reports of Cd-induced caspase-independent cell death. For example, in Cd-treated MRC-5 cells, apoptosis-like nuclear changes were mediated by AIF (Shih et al., 2003). In addition, our earlier findings suggested that the CD95/Fas

complex and a transcription-independent function of p53 were involved in Cd-induced apoptosis in human peripheral blood lymphocytes (Al-Assaf et al., 2013).

Finding an antidote against Cd has been a matter of scientific interest for quite some time. Aged garlic extract (AGE) and diallyl disulfide (DADS) have been found to have beneficial effects against Cd-induced toxicity in 1321N1 and HEK293 cells. This protection appears to be mediated via the induction of cytoprotective enzymes in a transcription factor (Nrf2)-dependent manner (Lawal and Ellis, 2011). *Hibiscus sabdariffa* L. petal aqueous extract can also protect rats against Cd-induced liver, prostate, and testis lipoperoxidation (Asagba et al., 2007). Moreover, some terpene compounds have also been reported to have protective roles in HepG2 cells against Cd toxicity (Miura et al., 1999).

Despite these examples, no study has been performed on the potential immuno-protective behavior of a compound against Cd toxicity in human lymphocytes. Our earlier study showed that Cd induced cell death in human peripheral blood lymphocytes (hPBLs) via apoptosis. Hence, this study was undertaken with the purpose of extending that study and investigating the immunoprotective role of CH in human lymphocytes.

2. Materials and methods

All the chemicals, reagents and kits used were of research grade.

2.1. Preparation of peripheral blood lymphocytes (PBLs)

hPBLs were separated from whole blood from healthy donors using the Ficoll-Hypaque gradient centrifugation technique (Histopaque®-1077, Sigma-Aldrich, St. Louis, MO, USA). The hPBLs were prepared under sterile conditions in an RPMI-1640 (Life Technology-Invitrogen, California, USA) medium containing 10% fetal calf serum (Manassas, VA, USA). Their viability, as determined by the trypan blue exclusion test (Trypan Blue Solution, Life Technologies – Invitrogen, California, USA), was more than 98%, and their concentration was finally adjusted to 5×10^5 cells/mL.

2.2. Cell viability assay

The cell viability assay was performed using the 3-(4,5-dimethyl-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction method as described earlier (Liu et al., 1997) with slight modifications. Briefly, the lymphocytes were at a concentration of 1×10^5 cells/mL and were seeded in a 96-well plate. After treatment of the lymphocytes with CH and CH + Cd (Cd chloride CdCl₂) (Sigma-Aldrich, St. Louis, MO, USA), combinations in a concentration range of 0.1–100 μM for 24 h, 10 μL of MTT (5 mg/mL, Sigma-Aldrich, USA) was added to the wells for 4 h, and the lymphocytes were incubated again. The plate was centrifuged at $1200 \times g$ for 10 min, and 100 μL of dimethylsulfoxide (DMSO) was added after removing the supernatant to dissolve the formed formazan. After gentle shaking for five minutes, the absorbance was read at 570 nm in a microplate

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