

Protective Role of Dietary Antioxidants in Oxidative Stress

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Oxygen radical and free radicals are normal by-products in biological systems during numerous physiological and pathophysiological processes. Oxidant by-products of normal metabolism cause extensive damage to DNA, proteins, and lipids. This oxidative damage appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction. Antioxidants, which can neutralize free radicals, may be of central importance in the prevention of these diseases. The antioxidative and free radical scavenging properties of polyphenolic compounds in several plant extracts have recently been reported, suggesting possible protective role of polyphenolic compounds. Thus, the search for antioxidants in edible plants has drawn much attention.

The herb *Mesona procumbens* Hemsl., called Hsian-tsao in China, is consumed as an herb drink and jelly-type dessert in the Orient. It is also used as herbal remedy in folk medicine in China against heat-shock,

hypertension, diabetes, and muscle and joint pains. Most research on this product has focused on its gelation property and proximate composition.

The result from the investigation on the antioxidative activities showed that the water extracts of Hsian-tsao exhibited strong activities against linoleic acid peroxidation and correlated with its polyphenol contents. The water extracts of Hsian-tsao showed positive concentration-dependent scavenging effect on DPPH radical, superoxide anion, hydrogen peroxide, nitric oxide and peroxy radical. Furthermore, the water extracts of Hsian-tsao exhibited the inhibitory effect on oxidative damage to biomolecules. The extracts also showed

inhibitory effects, both on the lipid peroxidation of ghost membrane and Chang liver cells induced by hydrogen peroxide. No toxicity was found in the water extracts of Hsian-tsao towards Chang liver cells. The water extracts of Hsian-tsao could inhibit oxidative DNA damage induced by hydrogen peroxide and exhibit protective effect against oxidative damage in Chang liver cells and intracellular reactive oxygen species (ROS).

Du-zhong (*Eucommia ulmoides* Oliv.) tea (the aqueous extract of leaves) is commonly used in Japan and Taiwan for treatment of hypertension and is thought to be a functional food. Investigation also showed that leaf extract of Du-zhong may have recuperative effects from hypercholesterolemia and fatty liver. Du-zhong (leaf) tea had a suppressing effect on mutagenicity and chromosome aberration following mutagen treatment. The effect on oxidative damage in biomolecules and free radical-/ or ROS-/ scavenging effects of water extracts of Du-zhong (WEDZ) were investigated. The WEDZ was prepared from leaves, raw cortex, and roasted cortex. All of these WEDZ inhibited the oxidation of deoxyribose induced by Fe^{3+} -EDTA/ H_2O_2 /ascorbic acid, in a concentration-dependent manner. At a concentration of 1.14 mg/ml, the inhibitory effect of the extracts of leaves, roasted cortex, and raw cortex was 85.2%, 68.0% and 49.3%, respectively. The extract of leaves inhibited the strand breaking of DNA induced by the Fenton reaction. WEDZ also inhibited the oxidation of 2'-dG to 8-OH-2'-dG induced by Fe^{3+} -EDTA/ H_2O_2 /ascorbic acid. The leaf extract of Du-zhong had inhibitory effect on oxidative damage in biomolecules. Therefore, drinking of Du-zhong tea (leaf) over a long period of time may have anticancer potential. The WEDZ also showed remarkable activity as a ROS-scavenger, and the scavenging effect was concentration dependent. Also scavenging

activity of WEDZ on ROS was closely correlated to protocatechuic acid (PCA) content. The content of PCA in Du-zhong measured by HPLC followed the order: leaves > roasted cortex > raw cortex. The extract of Du-zhong possibly act as a prophylactic agent to prevent from free radical-related diseases.

Jue-ming-zi”, the seed of *Cassia tora* L., has been used as a laxative and a tonic Chinese herb for several centuries. The recent investigation showed that Jue-ming-zi, had physiological functions as an antiseptic, diuretic, diarrheal, antioxidant, and antimutagen. The effects of water extracts from *Cassia tora* L. (WECT) treated with different degrees of roasting on benzo[*a*]pyrene (B[*a*]P)-induced DNA damage in human hepatoma cell line HepG2 were investigated via the comet assay WECT alone, showed neither cytotoxic nor genotoxic effect toward HepG2 cells. B[*a*]P-induced DNA damage in HepG2 cells could be reduced by WECT in a dose-dependent manner. The inhibitory effects of WECT on DNA damage were in the order unroasted roasted at 150 roasted at 250. Ethoxyresorufin-*O*-dealkylase activity of HepG2 cells was effectively inhibited by WECT, and a similar trend of inhibition in the same order was observed. The activity of NADPH cytochrome P-450 reductase was also decreased by the unroasted samples and by 150 μ J-roasted samples (50 % and 38 %, respectively). The contents of anthraquinones (AQs) in WECT, including chrysophanol, emodin, and rhein, were decreased with increasing roasting temperature. Each of these AQs also demonstrated significant antigenotoxic activity in the comet assay. The inhibitory effects of chrysophanol, emodin, and rhein on B[*a*]P-mediated DNA damage in HepG2 cells were 78, 86, and 71 %, respectively, at 100M. These findings suggested that decreased antigenotoxicity of the roasted samples might be due to a reduction in their AQs content.

Adlay (soft-shelled job’s tears,” *Coix lachryma-jobi* L. var. *ma-yuen* Stapf) is a grass crop that has long been used in traditional Chinese medicine and as a nourishing food. The pharmacological activities of the seed adlay were used in China for treatment of warts, chapped skin, rheumatism, and neuralgia, and also used as an anti-inflammatory or antihelminthic agent. Adlay also pointed out to have stomachic, diuretic, antiphlogistic, anodynic, antispasmodic, and antitumor effects. It is widely planted in Taiwan,

China, and Japan, and it is considered to be a supplement healthy food.

The anti-tumor effect of adlay processing food (APF) on animals was investigated. The experimental diets containing amount of APF were fed to ICR male mice during a subcutaneous injection of Sarcoma-180 tumor cells. The accessory anti-tumor effect was determined by the tumor weight, transplantation speed of tumor cells, and mean survival time. The tumor weights of the test group fed with the diets containing APF levels of 9%, 18% and 36% were significantly lighter than the tumor weights of the control group. In the test group (APF 18%), tumor cell transfer was undiscovered after 60 feeding days. However, 25% of the mice in the control group on day 45 and 50% on day 60 were discovered to have tumor cells transferred to the lungs. The average survival time of the test group (106.5 days) was significantly longer than the control group (77 days). The results indicate that APF has an accessory anti-tumor effect, and the recommended daily intake is approximately 2.85 g/kg for cancer patients.

The investigations on the antioxidative effects of methanolic extracts from different parts of adlay seed and their antiproliferative activity in malignant human cells have been conducted. The methanolic extracts from the hull (AHM), testa (ATM), bran (ABM), and polished adlay (PAM) were prepared. AHM exhibited greater capacity to scavenge superoxide anion radicals in the PMS-NADH system than ATM, ABM, or PAM. The scavenging capacities of AHM and ATM on hydrogen peroxides were about 20% at a dose of 250 μ g/mL. By using the method of deoxyribose degradation to assess damage caused by hydroxyl radicals, AHM was found to inhibit damage in deoxyribose at a higher concentration. The inhibitory effect on enzymatic oxidation of xanthine to uric acid was found to follow the order: AHM > ATM > ABM > PAM. Exposing human histolytic lymphoma U937 monocytic cells to tert-butyl hydroperoxide, AHM protected the cells against the cytotoxicity. In addition, AHM exhibited antiproliferative activity against human histolytic lymphoma U937 monocytic cells in a dose-dependent manner. The antiproliferative properties of AHM appeared to be attributable to its induction of apoptotic cell death as determined by flow cytometry. These results showed that AHM displayed multiple antioxidant effects and

induced apoptosis of malignant human cells.

Betel quid, a natural masticatory in south-eastern Asia, is composed of various components in different countries or areas. Taiwanese betel quid includes an entire fresh green areca fruit (containing the husk), *Piper betle* (leaf or inflorescence) and slaked lime paste. The slaked lime, handled as pasty form, is either white (white lime paste), with no additives, or brown (red lime paste) due to the addition of catechum, an extract of *Acacia catechu*. An average of 14-23 betel quids are chewed per day by a Taiwanese chewer, relatively higher compared with the amount consumed by chewers in India or the Philippines.

Betel quid chewing appears to be closely associated with an elevated risk of pre-invasive lesions such as leukoplakia or submucous fibrosis and oral cancer. The habit alone or in combination with tobacco smoking has been condemned as the major aetiology of oral cancer in some south Asian countries.

Areca fruit contains some alkaloids, of which arecoline is the major one. N-nitrosoguvacoline (NG), one of the N-nitrosation products of arecoline, is the only one N-nitrosamine found in Taiwanese chewing saliva. The mutagenic studies in Ames *Salmonella* microsome test showed that crude alkaloid extracts of areca fruit and arecoline were active in *Salmonella typhimurium* TA100, and NG was weakly active in TA98 and TA100. The activities in both arecoline and NG decreased further in the presence of rat liver S9 mix. Nitrite was significantly consumed during the N-nitrosation of arecoline and at acidic condition (pH3), whereas the formation of NG was favored at neutral condition (pH7). Crude phenolic extracts

of leaf and inflorescence of *Piper betel* inhibited the formation of NG by blocking the nitrite. However, a high amount of crude phenolic extracts of areca fruit enhanced the formation of NG.

In the genotoxic study, the Ames *Salmonella* microsome test showed that an aqueous extract of betel quid did not induce mutagenicity in *salmonella typhimurium* strains TA98 and TA100. Mammalian cell studies (Chinese hamster ovary K1 cell; CHO-K1 cell) revealed that only higher concentrations (100 and 1000 µg/ml) of aqueous extract increased the frequencies of sister-chromatid exchange (SCE) in the absence of S9. Animal (male Sprague-Dawley rat) studies showed that low-dose feeding (0.53g dry aqueous extract/kg diet) significantly increased the activities of glutathione (GSH) peroxidase and cytoplasmic glutathione S-transferase (cGST) of liver, whereas high-dose feeding (26.5g dry aqueous extract/kg diet) lowered the contents of GSH and total glutathione. The effect of an aqueous extract of betel quid on the oxidation of 2'-deoxyguanosine (2'-dG) to 8-hydroxy-2'-deoxyguanosine (8-OH-dG) revealed that this aqueous extract may act as a pro-oxidant at lower dosage and may be Fe-dependent in the model system. However, the aqueous extract of betel quid showed antioxidant activity at higher doses by the scavenging effect of the hydroxyl radicals.

Through the introduction to antioxidant physiological functions of Taiwan edible plants, the cooperative studies on the subject should be established among the Asian universities. It is also our goal to promote extensive cooperation and research scientist development. These studies will improve human health from the benefit of