嘉南藥理學院專題研究計畫成果報告

計畫名稱：食品中抗氧化劑可防止氧化劑造成 DNA 斷裂的快速檢驗分析法

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Abstract

The objective of this study was to investigate the antioxidant properties of seven different plant extracts, including flowers of four varieties of Haing Jyur (*Chrysanthemum morifolium* Ramat; FCMR), the seed of *Cassia occidentalis* L. (COL), raw seed and roasted seed of *Horderum vulgare* L.(RSHVL). In addition to their antioxidant activities against lipid autooxidation as published earlier, these seven extracts also reduced the DNA degradation caused by iron(II)-driven Fenton reaction. The inhibition of DNA damage may due to their strong ferrous ion chelation capability. In addition, it may also because they were all good H_2O_2 and superoxide scavengers. These findings support that these seven extracts, which have long been consumed regularly in Asia, possess antiperoxidant and antioxidant activities and can be used as health dietary supplements.

Keywords: antiperoxidant, seed, superoxide, Fenton reaction

Introduction

It has been well recognized that free radicals are formed in living organisms as a function of endogenous biochemical processes. Of particular relevance in aerobic systems are those reactive species derived from the metabolism of oxygen, which include hydrogen peroxide, singlet oxygen, superoxide radical, and hydroxyl radical, collectively known as reactive oxygen species (ROS) (Gutteridge, 1994). Over-production of ROS in cells have been involved in several diseases, including arthritis (Miesel et al., 1994), multiple scleorsis (Banki et al., 1994), diabetes (Giugliano et al., 1995), cataract (Spector et al., 1995), aging (Gutteridge, 1992) and cancer (Feig et al., 1994). Intracellular formation of ROS can produce extensive damage to all biological macromolecules in cell, including lipid peroxidation (Ramarathnam et al, 1995), protein fragmentation and structural change (Carmichael and Hipkiss, 1991), carbohydrate degradation (Cheeseman et al. 1988), and damage to genetic material.

Damage to DNA is perhaps one of the most crucial events in the cytotoxicity of ROS (Marnett and Burcham, 1993). DNA lesions resulting from exposure to ROS include modified bases, such as 8-hydroxydeoxyguanosine (8-OHdG) (Kasai et al., 1986), thymidine glycol (Hegi et al., 1989), 8-hydroxyadenine, cytosine glycol,

5-hydroxymethyluracil and so on, abasic sites, single and double strand breaks, and DNA-protein crosslinks (Blakely et al., 1990; Kawanishi et al., 1986; Kawanishi et al., 1989; Shi et al., 1994). There is growing evidence that transition metals, such as copper (II), iron (II), chromium (VI), cobalt (II), nickel (II), vanadium (III) etc., are able to interact with and alter DNA via Fenton-type reaction, which involves in the production of free radicals (Lloyd et al., 1997).

In Asia, some edible plants and herbs have long been considered as dietary supplements for preventing or curing chronic diseases and cancers (Pratt, 1992). In addition, the flower of *Chrysanthemum moriforlium* Ramat (FCMR), roasted seed of *Horderum vulgare* L.(RSHVL), and the seeds of *Cassia tora* L. (CTL) and *Cassia occidentalis* L. (COL), which are commonly called as Hang Chu, Chao Mai and Jue Ming Zi, are popular drinks in Taiwan. All of them have been reported to contain antioxidant activities against lipid autooxidation (Duh and Yen, 1997; Yen et al., 1998). Besides, plant hulls including those from rice (Asamarai et al., 1996; Ramarathnam et al., 1989), navy bean (Onyeneho and Hettiarachchy, 1991), peanut (Duh et al., 1992) and mung bean (Duh et al., 1997), have also been shown to contain some antioxidative components for preventing lipid autooxidation. Other plants, such as, chili pepper, ginger, green tea, pepper, sesame seeds, rosemary, clove, sage, oregano, thyme, and burdock (*Arctium lappa* Linne) are important part of the human diet and all contain substances for improving stability of fat (Fisher, 1992; Duh, 1998). It has been know that most natural antioxidants are phenolic in nature. The phenolic antioxidants not only inhibit the autooxidation of lipids, but sometimes, they also have the ability to retard lipid oxidation by inhibiting lipoxygenase activity (Ho, 1992)

In this report, we would like to extend our investigation on the antioxidant properties of these seven different plants extracts, including flowers of four varieties of Haing Jyur (*Chrysanthemum morifolium* Ramat; FCMR), burdock, , the seed of *Cassia occidentalis* L. (COL), and raw seed and roasted seed of *Horderum vulgare* L.(RSHVL). Our focus was on their properties of inhibition of iron(II)-dependent hydrogen peroxide-induced DNA damage. Our results showed that these natural materials possessed antioxidant activity for preventing both lipid peroxidation and DNA damage. This also provides a supporting evidence for some of these plants used as dietary supplement.

Materials and Methods

Materials

The flowers of four varieties of Haing Jyur (*Chrysanthemum morifolium* Ramat; FCMR), the seed of *Cassia occidentalis* L. (COL), as well as raw seed and roasted seed of *Horderum vulgare* L.(RSHVL) were purchased from local market in Tainan, Taiwan.

Extraction

The flowers of four varieties of Haing Jyur (*Chrysanthemum morifolium* Ramat; FCMR), burdock the seed of *Cassia occidentalis* L. (COL), as well as raw seed and roasted seed of *Horderum vulgare* L.(RSHVL) (20g each) were extracted with 600ml boiling water for 10 mins, and the filtrate was evaporated in a vacuum below 70^{o}C on a rotary evaporator.

Fenton Reaction for DNA Damage

Reactions were performed in 10 ml of 10 mM Tris-HCl buffer (pH 7.8) containing 200ng pUC18 double-stranded supercoiled DNA, 1mM hydrogen peroxide,

50mM ferric chloride, 100mM ascorbic acid and various amount of extracts from different plants. The reaction mixtures were incubated at 37^{o}C for 30 mins and then stopped by adding 1 ml of 0.5M EDTA. The samples were then separated on 1 % agarose gel electrophoresis. The resulting gel was then captured and analyzed by ImageMaster VDS and ImageMaster 1D Elite software (Amersham Pharmacia Biotech, Sweden).

Results

Previously we have demonstrated that seven different plants extracts, including flowers of four varieties of Haing Jyur (*Chrysanthemum morifolium* Ramat; FCMR), burdock, he seed of *Cassia occidentalis* L. (COL), as well as raw seed and roasted seed of *Horderum vulgare* L.(RSHVL) all contained antioxidative and reduction activities as summarized in table 1. Here we further studied their properties on inhibition of iron(II)-dependent hydrogen peroxide-induced DNA damage and to study the possible mechanisms.

Inhibition of DNA peroxidation

Water extracts of flowers of four varieties of Haing Jyur (*Chrysanthemum morifolium* Ramat; FCMR), burdock, the seed of *Cassia occidentalis* L. (COL), as well as raw seed and roasted seed of *Horderum vulgare* L.(RSHVL) were used in a typical Fenton reaction to determine the inhibition ability for DNA damage as described in Materials and Methods. As shown in figure 1, hydrogen peroxide and ascorbate *per se* induced DNA strand breakage thus part of supercoiled DNA was transformed to relaxed circular form (lane 2, 3). When Fenton reaction was performed by adding both hydrogen peroxide and ascorbate, most of DNA was degraded due to extensive peroxidation (lane 4). In contrast, in the presence of water extracts of flowers of Haing Jyur (*Chrysanthemum morifolium* Ramat; FCMR), at the concentration of 10 mg/ml, 5 mg/ml and 2 mg/ml, part of the plasmid remained supercoiled forms indicating the protection effect against peroxidation (lanes 5-7). The relative intensities of supercoiled DNA in the three lanes were 15% no matter what the concentration was. The same effects were observed in the rest of six extracts, and as low as 1 mg/ml, the protection effect is significant (Table 2). This indicated that the extracts may contain both anti-oxidativeand pro-oxidative compunds thus when the concentration increase, the protection effect did not significantly changed. On the other hand, the inhibitory effect of DNA damage by vitamin E was dose-dependent. Less than 10% protection was observed when vitamin E concentration was 1 mg/ml, while 40% protection can be reached when concentration was escalated to 10 mg/ml (lanes 3 and 4 of figure 2). The other two common antioxidant used as food additives: BHA and BHT while showed no effect against DNA peroxidation (data not shown)..

The induction of DNA damage by metal such as lead, zinc, iron, copper, nickel,, is dose- and time-dependent [Kobayashi, Ueda, Komano and Yang et al] . It has also been known that the DNA damage can be classified into two classes, one being direct DNA-strand cleavage, and the other being base modification labile to hot piperidine. The former reaction occurred unambiguously at any nucleotide while the latter was base specific[Kobayashi, Ueda, Komano]. One of the oxidative DNA lesions 8-hydroxydeoxyguanosine (8-OHdG) has been reported to be an important biomarker relevant to carcinogenesis because this can mispair with adenine during DNA replication and subsequently lead to base transversions. Transition metal ions such as

iron, copper can catalyze the reduction of H_2O_2 to $O_2^{\cdot -}$ (superoxide anion), thereby resulting in $^{\cdot}OH$ (hydrogen radical) and $^1O_2$ (singlet oxygen). Their induction can thus be inhibited by radical, H_2O_2, singlet oxygen, and hydrogen radical scavengers as well as metal chelators

It has been shown that ascorbate is a ROS scavengers, in the presence of flavonoids, a synchronic effect was observed. However in the presence of metal, ascorbate can also induce ROS[master thesis]. In our experiment, we found that ascorbate *per se* (in the absence of H_2O_2 and metal) can induce DNA damage as well. In addition, in the presence of H_2O_2 but absence of metal, the induction of DNA damage by ascorbate is also dose-dependent (Figure 3). Thus, in our analytic system, ascorbate may not only serve as an reducing agent for converting Fe^{+3} to Fe^{+2}, thus catalyzing Fenton reaction, but also directly induce ROS prodution. This seven extracts tested are all strong Fe+2, $^{\cdot}OH$ (hydrogen radical), and H_2O_2 scavengers [], this can explain why they can inhibit Fenton reaction-driven DNA damage.