嘉南藥理科技大學專題研究計畫成果報告

枸杞、黃耆及當歸之抗氧化活性評估

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Antioxidant Activity of *Angelica sinensis*, *Lycium barbarum* and *Poria cocos*

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中文摘要

本研究係探討三種生藥包括當歸、枸杞及茯苓之抗氧化活性。結果顯示，所有抽出物之抗氧化活性均呈濃度效應。管外試驗發現三種生藥之熱水抽出物對氯化亞鐵-抗壞血酸所誘發之大鼠肝均質液所造成之脂質過氧化作用都有抑制效應。特別是枸杞之抽出物對大鼠肝均質液所形成之丙烯醛含量低($p<0.05$)，可能跟抗氧化傷害有關。在三種樣品間，枸杞抽出物之清除超氧陰離子能力最高且抗超氧陰離子產生之活性最強($p<0.05$) 。基於上述結果，發現枸杞抽出物被視為再膳食供應上具有潛力之抗氧化劑。

關鍵字: 當歸; 枸杞; 茯苓; 抗氧化活性; 脂質過氧化作用; 超氧陰離子

Abstract

The antioxidant activity of traditional Chinese medicines namely the root of *Angelica sinensis* (AS) (Oliv.) Dicls, the fruit of *Lycium barbarum* (LB) L and the sclerotium of *Poria cocos* (PC) (Schw.) Wolf was evaluated in this study. Results showed that all extracts displayed the antioxidant activity in a concentraction-dependent manner. Hot water extracts of three medicines exhibited a prominent inhibition effect on $FeCl_2$-ascorbic acid induced lipid peroxidation of rat liver homogenate *in vitro*. Specially, LB extracts produced significantly lower ($p<0.05$) malondialdehyde (MDA) formation of rat liver homogenate and might also protect against oxidative damage. Among three samples, LB extracts had the highest superoxide anions scavenging activity and the strongest anti-superoxide formation activity ($p<0.05$). Based on these results, the LB extracts regarded as a potential antioxidant agent in dietary supply.

Keywords: *Angelica sinensis*; *Lycium barbarum*; *Poria cocos*; antioxidant

activity; lipid peroxidation; superoxide anions

1. Introduction

Lipid peroxidation can lead to causes aging, coronary heart disease, stroke, diabetes mellitus, rheumatic disease, various liver disorders and carcinogenesis (Slater,1984; Wong, 1987). It has been reported that reactive oxygen species (ROS) such as $\bullet O_2^-$ (superoxide anion), $\bullet$ OH (hydroxyl radical), H_2O_2 (hydrogen peroxide) and $^1O_2$ (singlet oxygen), are not only one of the important classes of cellular injuries but also can initiate peroxidation of polyunsaturated fatty acids in biological membranes (Compori, 1985; Halliwell, 1997). The tissue injury caused by ROS may include DNA damage (Halliwell,1994;Halliwell,1997), protein damage, oxidation of important enzymes *etc* in the human body. Xanthine oxidase (XOD), a flavoprotein, converts hypoxanthine to xanthine and then it oxidizes xanthine to uric acid and to yield superoxide anion and hydrogen peroxide, and these radicals directly reduce ferri-chrome *c* to ferro- chrome *c* (Ho et al., 1999). Inhibition of superoxide anion formation by the enzymatic pathway would be benefical in ischaemia and edema (Hearse et al., 1986).

Natural antioxidants derived from plant products, such as herbs, legumes and spices etc. (Lin et al., 2001). Whenever the antioxidants are prevent, antioxidant enzyme activity and scavengers of the free radical will be induced to prevent the oxidative damage. The natural medicines, *Angelica sinensis* (Oliv.) Dicls (AS), *Lycium barbarum* (LB).and *Poria cocos* (Schw.) Wolf (PC) which are widely distributed in China. The root of *Angelica sinensis* (Oliv.) (Umbelliferae), is indicated against anemia, gynecic disorders, hypertension, headache, asthma and rheumatism *etc*. The fruit of *Lycium barbarum* (Solanaceae), is used as an antipyretic for hepatitis, antiinflammation, pneumonia, nephritis, diuretic, and antisenile agent. *Poria cocos* (Schw.) Wolf (Polyporaceae), is a saprophytic fungus. Its sclerotium, properly called “ Hoelen,” is a recognized Chinese medicine and is combined in many prescription as a diuretic, sedative, tonic, and antitumor (Hon *et al*., 1990; Lin *et al*., 1997). Hot water extracts of natural plants are often used to prepare Chinese medicated diets. However, it remains unclear if the agueous extracts possess antioxidant activity. The purposes of this study were to evaluate the antioxidant activity of the hot water extracts of three medicines.

2. Experimental

2.1. Plant

Samples of *A. sinensis*, *L. barbarum* and *P. cocos* were purchased from the Chyuan-Chang Chinese pharmacy in

Tainan. There were identified by Dr. J.J. Yang, Department of Pharmacy, Chia-Nan University of Pharmacy and Science, Tainan, Taiwan, ROC.

2.2. Chemicals

L-(+)-Ascorbic acid, thiobarbituric acid, xanthine, xanthine oxidase, cytochrome *c* were purchased from Sigma Chemical Co.(St. Louis, MO, USA). α -tocopherol and ferrous chloride were from Wako Pure Chemical Industries (Osaka, Japan). The other chemicals used were of reagent grade.

2.3. Preparation of extract

Each sample (100 g) was extracted with 1 l of boiling water for 1 hour. The extracts were filtered; the residue was re-extracted under the same conditions, and the combined filtrated were evaporated to dryness under vacuum and the yield of soluble constituents in AS, LB and PC were 15.63, 10.27 and 4.10% (w/w).

2.4. Animals

Male Wistar albino rats, 4-6 weeks old were obtained from the animal center, National Cheng Kung University, Tainan. They were housed in an air-conditioned room were temperature maintained at 22±3℃, and humidity at 55±5%. Animals were fed a standard laboratory diet and tap water ad libitum throughout the investigation. The rats were used for $FeCl_2$-ascorbic acid-induced lipid peroxidation study.

2.5. Antioxidant activity

2.5.1 $FeCl_2$-ascorbic acid stimulated lipid peroxidation in rat liver homogenate

The effect of crude extracts on rat liver homogenate induced with $FeCl_2$-ascorbic acid and lipid peroxidation was determined by the method of Kimuya *et al*. (1981) and Wong *et al*. (1987). A mixture containing 0.5 ml of liver homogenate, 0.1 ml of Tris-HCl buffer (pH=7.2), 0.05 ml of 0.1 mM ascorbic acid and 0.05 ml of 4 mM $FeCl_2$ and 0.05 ml of various concentrations of crude extracts, or α -tocopherol, were incubated for 1 hr at 37℃. After incubation, 0.9 ml of distilled water and 2 ml of 0.6% TBA were added and then shaken vigorously. The mixture was heated for 30 min in a boiling water bath (100℃). After cooling, 5 ml *n*-butanol was added and the mixture was shaken vigorously. The *n*-butanol layer was separated by centrifugation at 3000 rpm for 10 minutes. The absorbance of the supernatant was read at 532 nm against a blank, which contained all reagents expect liver homogenate.

2.5.2 Cytochrome c test

Enzyme formation of superoxide anions were assayed by the reduction of cytochrome *c* method described by McCord and Fridovich (1969). Fifteen

mg of samples were dissolved in 1 ml of distilled water and then diluted with distilled water to various concentrations (0.1 to 10.0 mg/ml), 0.07 units/ml of xanthine oxidase, 100 *u*M of xanthine and 50 *u*M of cytochrome *c* were added to these samples. They were then incubated for 3 min at the room temperature and read at 550 nm.

2.5.3 Xanthine oxidase inhibition test

Xanthine oxidase activity was estimated by the formation of uric acid from xanthine (Chang et al., 1994). Fifteen mg of samples were dissolved in 1 ml distilled water, and then diluted with 50 mM KH_2PO_4 buffer (pH=7.8) to various concentrations (0.1 to 10.0 mg/ml). After 100 uM of xanthine in phosphate buffer and 20ul of xanthine oxidase (0.4 units) were added, samples were incubated for 3 min at room temperature. Superoxide formation was counted by measuring uric acid production by spectrophotometry at 295 nm.

2.6. Statistical analysis

Statistical analysis involved use of the Statistical Analysis System (SAS) software package. The data were indicated as the mean±S.D. IC_{50} value of each sample was calculated. Analysis of variance was performed by one-way analysis of variance (ANOVA) procedures. Significant differences between the means were determined by Duncan's Multiple Range tests.

3. Results and discussion

Anti-lipid peroxidation activity

The rat liver homogenate was induced with $FeCl_2$-ascorbic acid for nonenzymatic lipid peroxidation. MDA (malondialdehyde) is very reactive and takes part in cross-linking with DNA and proteins, and it also damages liver cells.

The inhibitory effect of all extracts were compared with α-tocopherol on malondialdehyde (MDA) production in rats liver homogenate, induced by $FeCl_2$-ascorbic acid *in vitro* , were shown in Table 1. The inhibition of MDA formation of rats liver homogenate in lipid peroxidation increased with increasing concentration of three samples. All of the water extracts at concentration of 0.5-5.0 mg/ml displayed anti-lipid peroxidation activities, and the inhibition rates were in the range of 20.33-70.05%. These results indicate that three medicines exhibited remarkable antioxidant activity in inhibiting peroxidation of rats liver homogenate *in vitro*. The significant differences (P<0.05) were found between the different concentration of hot water extracts in various medicine. The fruits of *L.*

barbarum had the most effective activity, followed by the roots of *A. sinensis* and the sclerotia of *P. cocos*. The natural medicines may also protect against damage to cell membranes because they reduce the level of lipid peroxides.

Free radical scavenger activity

Enzymatic formation of superoxide anions was estimated by reduction of cytochrome *c*. Scavenging effects of different concentration of all extracts on superoxide anions, were shown in Table 2. The scavenging effect of three extracts on the superoxide anions increased with increasing concentration of samples. All extracts at concentration of 0.1-10.0 mg/ml showed antioxidant activities, and the scavenging rates were in the range of 28.83-82.17% (P<0.05). In the cytochrome *c* test, the IC_{50} values of ranged from 0.95~2.63 mg/ml, with the LB extracts of showed the highest scavenging effect on superoxide anions. Xanthine oxidase converts hypoxanthine to xanthine and then xanthine to uric acid in the presence of molecular oxygen to yield superoxide anion and hydrogen peroxide and these radicals directly reduce ferri-cytochrome *c* to ferro-cytochrome *c*(Ho *et al*., 1999).

Anti-superoxide anions formation

All extracts had the capability of forming anti-superoxide in a concentration-dependent manner (Table 3). Three extracts at 1-10 mg/ml inhibited the superoxide formation and the rates were from 38.03-88.49%, displaying anti-superoxide formation activity. The above results indicate that the extent of anti-superoxide formation activity followed the order of LB extracts > PC extracts > AS extracts. Xanthine oxidase-derived superoxide anion has been linked to post-ischaemic tissue injury and edema as well as changes in vascular permeability (Mc Cord and Fridovich 1968; Hearse *et al*., 1986). Inhibition of superoxide anion regeneration by the enzymatic pathway would be beneficial in ischaemia and edema.

4.Conclusions

This can be concluded from Table 1 and Table 2 that the extracts of LB had the strongest antioxidant action not only in the rat homogenate model system but also in the cytochrome *c* test. These results clearly reveal that the fruits of LB show significant antioxidant activity (P<0.05), a scavenging effect on superoxide anions. The extracts of LB have exhibited anti-superoxide formation activity. Thereforce, LB as a potential antioxidant to retard the aging process, caused by ROS, may advance healthful functions and possible prevention some diseases. This observation suggests that LB can be

prepared into beverages or Chinese medicated diets and be consumed in daily life.

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Table 1

Inhibitory effects of different concentrations of hot water extracts of *Angelica sinensis* (AS), *Lycium barbarum* (LB), *Poria cocos* (PC) andα-tocopherol on MDA production in rat liver homogenate, induced by $FeCl_2$-ascorbic acid *in vitro*

Samples	Concentration (mg/ml)	MDA[1] (*u*m /mg protein)	Inhibition rate[2] (%)
$FeCl_2$-AA	-	43.0±0.20	-
Normal (Control)	-	13.0±0.10	-
$FeCl_2$-AA+samples			
AS (root)	0.5	36.9±0.90^{c}	20.33
LB (fruit)	0.5	36.3±0.02^{b}	22.00
PC (sclerotium)	0.5	34.1±0.37^{a}	29.67
$FeCl_2$-AA+samples			
AS (root)	1.0	29.4±0.80^{b}	45.00
LB (fruit)	1.0	28.5±0.70^{a}	48.00
PC (sclerotium)	1.0	28.7±0.53^{a}	48.00
$FeCl_2$-AA+samples			
AS (root)	5.0	26.0±0.10^{c}	56.70
LB (fruit)	5.0	22.1±0.30^{b}	70.05
PC (sclerotium)	5.0	26.4±0.90^{d}	55.33
α-tocopherol	5.0	12.5±0.01^{a}	100.00

1.MDA Data were presented as the means±S.D. (n=5).

2.The inhibitory rates within a column with the different superscript letters were significantly different at $P<0.05$.

Table 2

Superoxide scavenger activities of different concentrations of hot water extracts of *Angelica sinensis* (AS), *Lycium barbarum* (LB), *Poria cocos* (PC) andα-tocopherol in the cytochrome *c* test[1]

Samples	Concentration (mg/ml)	Scavenging effect[2] (%)
AS (root)	0.1	28.83[c]
LB (fruit)	0.1	35.14[a]
PC (sclerotium)	0.1	30.02[b]
AS (root)	1.0	46.67[d]
LB (fruit)	1.0	50.20[b]
PC (sclerotium)	1.0	48.33[c]
α-tocopherol	1.0	82.00[a]
AS (root)	5.0	62.59[c]
LB (fruit)	5.0	66.56[b]
PC (sclerotium)	5.0	70.22[a]
AS (root)	10.0	75.38[b]
LB (fruit)	10.0	82.17[a]
PC (sclerotium)	10.0	75.63[b]

1.Data were presented as the percentage scavenging of free radicals. (n=3).

2.Values within a column with the different superscript letters were significantly different at $P<0.05$.

Table 3

Antioxidant activities of different concentrations of hot water extracts of *Angelica sinensis* (AS), *Lycium barbarum* (LB), *Poria cocos* (PC) and α-tocopherol in the xanthine oxidase inhibition test[1]

Samples	Concentration (mg/ml)	Inhibition rate[2] (%)
AS (root)	0.1	38.03[b]
LB (fruit)	0.1	40.68[a]
PC (sclerotium)	0.1	38.48[b]
AS (root)	1.0	47.56[c]
LB (fruit)	1.0	57.78[b]
PC (sclerotium)	1.0	41.79[d]
α-tocopherol	1.0	84.39[a]
AS (root)	5.0	60.80[c]
LB (fruit)	5.0	70.65[a]
PC (sclerotium)	5.0	67.65[b]
AS (root)	10.0	86.24[b]
LB (fruit)	10.0	88.49[a]
PC (sclerotium)	10.0	81.41[c]

1.Data were presented as the percentage of inhibition by the superoxide formation (n=3).

2.Values within a column with the different superscript letters were significantly different at $P<0.05$.

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