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Nitric oxide-scavenging and antioxidant effects of *Uraria crinita* root

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Abstract

The antioxidant activity and the nitric oxide-scavenging effect of methanol extracts (MEUR), and their ethyl acetate fraction (EAF), from *Uraria crinita* root, were investigated. The results of the comet assay indicated that both MEUR and EAF could inhibit DNA damage in macrophage induced by sodium nitroprusside at a concentration of 25–200 μg/ml. The antioxidant activity of the extracts was determined using the Trolox equivalent antioxidant capacity (TEAC) assay. The TEAC values of MEUR and EAF were 0.2 and 0.4, respectively, at a concentration of 200 μg/ml. The antioxidant activity and nitric oxide-scavenging effect of MEUR and EAF showed concentration-dependence. In addition, the results also showed a decreasing effect on nitric oxide production of lipopolysaccharide-induced RAW 264.7, for both extracts. One of the major antioxidative components was isolated from EAF and was identified as genistein, on the basis of UV-vis spectral, IR, MS and NMR analyses. Thus, the traditional edible plant, *Uraria crinita,* may have potential as an antioxidant and as a nitric oxide-scavenging agent.

Keywords: *Uraria crinita* extracts; DNA damage; Comet assay; NO scavenging; Antioxidant; Genistein

1. Introduction

In healthy organisms, production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) is approximately balanced by antioxidant defence systems. However, an organism can be suffering from so-called 'oxidative stress' while it is experiencing disturbance in the prooxidant–antioxidant balance in favour of the former, leading to potential damage (Halliwell & Gutteridge, 1999).

Recently, the concept of disease chemoprevention has been regarded as one of the most promising avenues for studying disease control. There is convincing epidemiological evidence that the consumption of fruits and vegetables can aid the prevention of degenerative processes, particularly by lowering incidence of and mortality rate for cancer and cardiovascular disease. The protection that fruits and vegetables provide against these diseases has been attributed to various antioxidant phytonutrients contained in these foods. Crude extracts of fruits, herbs, vegetables, cereals and other plant materials, rich in phenolics, are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food (Kähkönen et al., 1999; Rice-Evans, Miller, Bulwell, Bramley, & Pridham, 1995).

Recent studies have shown that reactive nitrogen intermediates, such as nitric oxide (NO), peroxynitrite ($ONOO^-$) and nitrogen dioxide (NO_2), also play an important role in the inflammatory process (Clancy & Abramson, 1995; Connor et al., 1995) and possibly in carcinogenesis (Tamir & Tannenbaum, 1996). A number of polyphenolic phytochemicals, such as resveratrol and quercetin (Kawada, Seki, & Kuroki, 1998), α-tocopherol (Arroyo, Hatch-Pigott, Mower, & Cooney, 1992) and catechins (Pannala, Rice-Evans, Halliwell, & Surinder, 1997) have been found to inhibit the RNS effect. Chan, Fong, Ho, and Huang (1997) found that epigallocatechin gallate (EGCG) could inhibit inducible nitric oxide synthase (iNOS) activity and its mRNA expression in LPS-activated macrophage. Kim, Murkani, Nakamura, and Oligashi (1998) in screening edible Japanese plants for nitric oxide generation inhibitory activities in RAW264.7 cells, also found some herb

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plants and vegetables expressing inhibitory activities. Thus, how to utilize these significant sources of natural antioxidants to prevent or improve ROS- or RNS-mediated injury, becomes very important.

Uraria crinita (UC), Leguminosae, is a traditional edible plant, and its root has been used for the treatment of swelling, coldness, ulcer and stomachalgia (Liu et al., 1995), with results indicating possible anti-inflammatory activities. This plant had been reported to be effective in inhibiting stress ulcers (Hsu & Liu, 1983). However, there are few reports on the nitric oxide-scavenging and antioxidant effects of *Uraria crinita* root extract. Therefore, we decided to evaluate the antioxidant activity and the nitric oxide-scavenging effect of methanol extracts (MEURs) and their ethyl acetate fraction (EAF) from *Uraria crinita* root. In general, plant phenolic compounds may act as antioxidants and often as characteristic components of herb plants; therefore, the antioxidants in *Uraria crinita* root were also investigated chemically.

2. Materials and methods

2.1. Materials

Sodium nitroprusside (SNP), lipopolysaccharide (LPS: *Escherichia coli*, serotype O55:B5), sulfanilamide, *N*-(1-naphthyl)-ethylenediamine dihydrochloride, peroxidase, ethidium bromide, and 2,2′-Azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid; ABTS) were purchased from the Sigma Chemical Co. (St. Louis, MO). Dimethyl sulfoxide (DMSO) and Triton X-100 were obtained from E. Merk (Darmstadt, Germany). Agarose, low melting point agarose, Dulbecco's Modified Essential Medium (DMEM), and fetal calf serum were obtained from Gibco/BRL Life Technologies (Eggenstein, Germany). Trolox was obtained from the Aldrich Chemical Co. (Milwaukee, WI).

2.2. Sample preparation

Raw UC roots were purchased from a local market in Nantou, Taiwan. The roots of UC were cut into small pieces, dried under sunlight for 2 days, and ground into a fine powder in a mill (RT-08, Rong Tsong, Taichung, Taiwan). The powder (1.0 kg) was extracted with 10-l methanol and left to stand for 1 week at room temperature. The extract was filtered, and the residue was re-extracted under the same conditions. The combined filtrate was concentrated in a rotary evaporator at less than 50°C to obtain the MEURs. The MEURs were dispersed into water and then partitioned between ethyl acetate and water. The ethyl acetate layer was concentrated in a rotary evaporator at less than 50°C to obtain the EAF. Both MEUR and EAF used in this study were dissolved in DMSO.

2.3. Cell culture

The murine monocyte/macrophage cell line RAW 264.7 was obtained from the Culture Collection and Research Center (CCRC, Hsin Chu, Taiwan). RAW 264.7 cells were cultured in 25 or 75 cm^2 plastic flasks with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum and antibiotics (Gibco/BRL Life Technologies, Eggenstein, Germany). These cells were activated with 1 μg/ml LPS (*E. coli*, Serotype 0.55:B5) and cultured for 20 h at 37°C in an atmosphere of 5% CO_2.

2.4. Analysis of DNA damage (Comet assay)

Cells were incubated with or without SNP in the absence or presence of MEUR or EAF, individually, under various concentrations for 2 h at 37°C in a dark incubator, together with untreated control samples. Samples were then centrifuged at 800 rpm, and the RAW 264.7 cells were resuspended in low melting point agarose for comet analysis.

Single cell gel electrophoresis (COMET assay) was performed according to the procedure of Olive, Banath, and Durand (1990) with some modifications. Briefly, fully-frosted slides were covered with 0.5% normal melting agar as the first layer, with a mixture of cell suspension and 0.5% of low melting agar (LMA) as the second layer, and finally with 0.5% of LMA (without cell) as the third layer. After solidification at 4°C, all the slides were immersed in lysing buffer (2.5 M of NaCl, 100 mM of EDTA, pH 10, with freshly added 1% Triton X-100 and 10% DMSO) at 4°C for 1 h, and the slides were then placed in a horizontal electrophoresis tank. The tank was filled with freshly prepared electrophoresis solution (300 mM NaOH, 1 mM EDTA, pH 10, 4°C), and the slides were left in the solution for 20 min to allow DNA unwinding and expression of alkali-labile damage before performing electrophoresis. Electrophoresis was then conducted at 4°C for 20 min using 25 V and 300 mA. After electrophoresis, the slides were neutralized in neutralization buffer, stained with ethidium bromide, kept in a humidified airtight container and examined using a fluorescence microscope. Images of 100 randomly selected cells from each slide were analyzed. The degree of DNA damage was expressed as the tail moment value. Tail Moment = (Tail length × Tail DNA%) /100.

2.5. The nitrite oxide scavenging

The scavenging effect of MEUR or EAF on nitric oxide was measured according to the method of Marcocci, Maguire, Droy-Lefaiz, and Packer (1994). Four millilitres of extract solution at different concentrations were then added in the test tubes to 1 ml of sodium

nitroprusside solution (25 mM), and the tubes incubated at 37°C for 2 h. An aliquot (0.5 ml) of the ncubation solution was removed and diluted with 0.3 ml Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride was immediately read at 570 nm and referred to the absorbance of standard solutions of sodium nitrite salt treated in the same way with Griess reagent.

3. Effect of extracts on NO production in LPS-activated RAW 264.7 cell lines

3.1. Nitrite assay

Cells were seeded in 96 well (8×10^4/200 μl), cultured for 2 days and then incubated with or without LPS in the absence or presence of MEUR or EAF, individually, at various concentrations for 20 h. The nitrite concentration in the supernatant was assessed, based on the Griess reaction, and determined through comparison with a sodium nitrite standard curve (Dirsch, Stuppner, & Vollmar, 1998).

3.2. Cell viability

Cell respiration, as an indicator of cell viability, was determined through the mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) to formazan. After the supernatants were removed from the plate for nitrite determination, the cells were incubated at 37°C with 0.5 mg/ml MTT for 45 min. The medium was aspirated, and the cells were solubilized in DMSO (250 μl) for at least 2 h in the dark. The extent of the reduction of MTT was quantified by measuring the absorbance at 550 nm.

4. Measurement of antioxidant ability by the $ABTS^+$ method

Measurement of antioxidant ability of MEUR or EAF was carried out as described by Miller, Rice-Evans, Davies, Gopinathan, and Milner (1993). Briefly, ABTS radical cation ($ABTS^+$) solution was diluted in PBS 5 mM to obtain an optical density at 734 nm of about 0.80 unit of absorbance. The solution was placed in a plastic cuvette, 50 μl of the antioxidant solutions were added, and the absorbance was read after exactly 1 min. A dose–response curve was plotted for trolox, and antioxidant ability was expressed as the trolox equivalent antioxidant capacity (TEAC).

4.1. Total phenolic content of MEUR and EAF

The phenolic content of MEUR or EAF was determined according to the method of Taga, Miller, and Pratt (1984) and calculated, using gallic acid as the standard. The extract (0.1 g) was dissolved in 5 ml of 0.3% HCl in methanol/water (60:40, v/v). The resulting solution (100 μl) was added to 2 ml of 2% Na_2CO_3. After 2 min, 50% Folin–Ciocalteau reagent (100 μl) was added to the mixture, which was then left for 30 min. Absorbance was measured at 750 nm using a spectrophotometer. Results were expressed as gallic acid equivalents.

5. Determination of the active compound in EAF

High-performance liquid chromatography (HPLC) analysis was performed with a Hitachi liquid chromatograph (Hitachi, Ltd., Tokyo, Japan), consisting of a Model L-6200 pump, a Rheodyne Model 7125 syringe-loading sample injector, and a diode array detector (Hitachi, L-7455 model) set at 260 nm. A LiChrospher RP-18 (250×4 mm, 5 μm) was used for analysis. The elution solvents were 50 mM ammonium dihydrogen phosphate, pH 2.6 (solvent A), 0.2 mM ortho-phosphoric acid, pH 1.5 (solvent B) and 20% of solvent A in 80% acetonitrile (solvent C). The solvent gradient elution program was used in the proportion of 100% A decreasing to 92%, and 0% C increasing to 8% after 10 min, to 0% A and increasing to 92% B after a further 5 min. Then, 92% B decreased to 20%, and 8% C increased to 80% after a further 65 min. The flow rate was 1 ml/min, and the injection volume was 10 μl. The same elution solvents were employed for purification of the major compound in EAF using a preparative Mightysil RP-18, 5 μm, 250×20 mm i.d. column (Kanto Chemical Co., Tokyo, Japan) and a flow rate of 5 ml/min. Fractions containing the major compound (where the typical retention time was 49.6 min in this study) were collected and concentrated in a rotary evaporator. Samples were further purified by means of preparative thin-layer chromatography (TLC, Merck silica plate 60 F254, 0.5 mm). The solvent system was chloroform/methanol (90/10, v/v).

5.1. Instrumental analysis of major compounds in EAF

UV-vis absorption spectra were recorded on a Hitachi U-3000 Ultraviolet Spectrophotometer. IR spectra were recorded on a Hitachi I-2001 Infrared Spectrophotometer with KBr. The fast atom bombardment mass spectra (FAB-MS) were recorded on a JEOL JMS-SX/SX 102A mass spectrometer with 1 N HCl-glycerol as the mounting matrix. ^1H NMR and ^{13}C NMR spectra were obtained by a Varian VXR-300S FT-NMR

spectrometer operating at 299.95 MHz for ^{1}H NMR and at 75.43 MHz for ^{13}C NMR in CD_3COCD_3 containing tetramethylsilane (TMS) as the internal standard.

5.2. Statistical analysis

Data were analyzed using the Statistical Analysis System software package. Analyses of variance were performed using ANOVA procedures. Significant differences ($P<0.05$) between means were determined using Duncan's multiple ranged test.

6. Results

6.1. Effect of extracts on SNP-mediated macrophages DNA damage

The comet assay (single cell gel electrophoresis) is a quick, simple, sensitive, reliable and fairly inexpensive technique used to detect primary DNA damage in individual mammalian cells (Collin, Dobson, Dusinska, Kennedy, & Stetina, 1997). In all of the comet assays performed in this study, the cell viability generally exceeded 95% at the extract concentrations of 25–200 μg/ml. The effect of MEUR and EAF on SNP-mediated macrophage DNA damage is shown in Fig. 1. A positive increase in the tail moment value (ca. 35) was seen following 1 mM SNP treatment of macrophages for 2 h in comparison with the untreated control (0.37). However, in this study, the genotoxicity of SNP was significantly ($P<0.05$) inhibited by MEUR or EAF under all doses (25∼200 μg/ml), and EAF showed a better inhibitory effect than did MEUR.

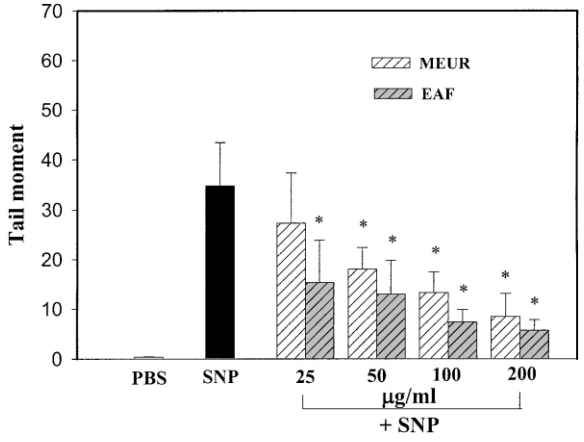

Fig. 1. DNA damage in RAW264.7 macrophages after treatment with 1 mM sodium nitroprusside (SNP) or a combination of SNP and the methanol extracts from *Uraria crinita* root (MEUR) and its ethyl acetate fraction (EAF). Data are mean±S.D. ($n=3$). Asterisks are significantly different by comparison with the control (SNP alone), $P<0.05$.

6.2. NO scavenging effect of MEUR or EAF

The compound SNP is known to decompose in aqueous solution at physiological pH, producing NO. Under aerobic conditions, NO reacts with oxygen to produce the stable products nitrate and nitrite, the quantities of which can be determined using Griess reagent (Marcocci et al., 1994). When solutions of 5 mM SNP in PBS were incubated at 25°C for 2 h, they generated time-dependent nitrite production, which was decreased by the presence of MEUR or EAF (Figs. 2 and 3) in a dose-dependent manner.

6.3. Effect of MEUR or EAF on NO production in LPS-activated macrophages

The effect of MEUR or EAF on NO production in macrophages is shown in Fig. 4. The result was expressed in nitrite levels in the culture medium as an index for

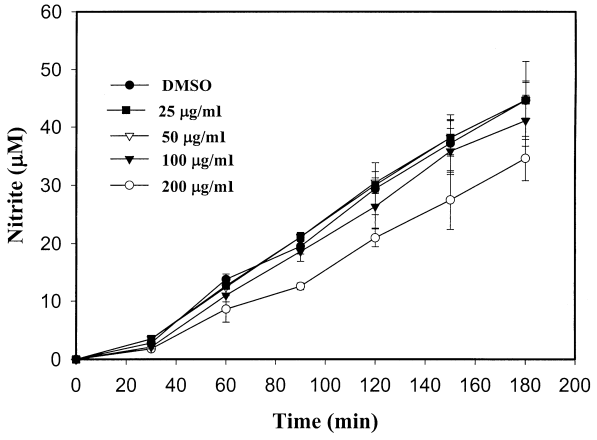

Fig. 2. Effect of methanol extracts of *Uraria crinita* root on the accumulation of nitrite upon decomposition of sodium nitroprusside (SNP; 5 mM). Incubation temperature was 25°C. Data are mean ±S.D. ($n=3$).

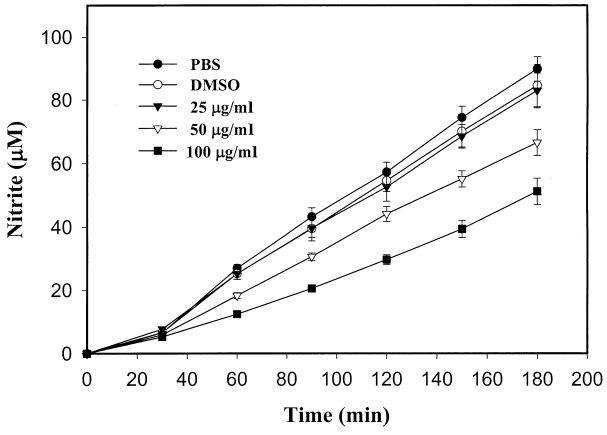

Fig. 3. Effect of the ethyl acetate fraction from methanol extracts of *Uraria crinita* root on the accumulation of nitrite upon decomposition of sodium nitroprusside (SNP; 5 mM). Incubation temperature was 25°C. Data are mean±S.D. ($n=3$).

NO synthesis from these cells using Griess reagent since NO is reactive in oxygenated aqueous solution and decomposes to nitrite. As the data show, unstimulated macrophages (PBS control), after 20 h in the culture, produced the lowest levels of nitrite (3.60±0.14). A major increase in nitrite was observed after treatment with LPS (1 μg/ml), and this effect was significantly inhibited by MEUR or EAF in a dose-dependent manner. When these resting cells were incubated with MEUR or EAF alone, amount of nitrite in the medium stayed at a level similar to that of unstimulated macrophages (data not shown).

6.4. Antioxidant ability of MEUR or EAF (the $ABTS^{\cdot+}$ method)

The antioxidant efficiency of MEUR or EAF was expressed in terms of TEAC (trolox equivalent antioxidant activity) according to Miller et al. (1993). As shown in Table 1, the TEAC values of MEUR and EAF with a concentration of 200 μg extracts/ml were 0.2 and 0.4, respectively.

6.5. Total phenolic content of MEUR and EAF

It is well known that plant phenolic extracts act as free radical scavengers and as antioxidants (Sato, Ramarathnam, Suzuki, Ohkubo, Takeuchi, & Ochi, 1996). Therefore, the amount of total phenolic compounds in MEUR or EAF was determined in this study. As expected, EAF had more than twice the total phenolic compound content of MEUR (Table 1), which indicated that ethyl acetate had a concentrating effect on polyphenolic compounds, owing to its solvent polarity.

6.6. Isolation of antioxidants from EAF

In order to identify the compound, that was responsible for antioxidant activity in MEUR or EAF, the phenolic compound profile was also investigated by means of HPLC. The results showed that there typically was a major peak (compound I) in the HPLC profile of EAF (Fig. 5B) but a relative minor peak in MEUR profile (Fig. 5A) at retention time of 49.65 min. Compound I was collected by means of preparative HPLC and further purified using preparative TLC, as described in the method section. The UV absorption of compound

Table 1
TEAC values and total polyphenolic compound contents of the methanol extracts of *Uraria crinita* root (MEUR) and its ethyl acetate fraction (EAF)[a]

Sample	TEAC (mM)[b]	Total phenolic compound (mg/g extracts)[c]
MEUR	0.16±0.01	87.4±2.4
EAF	0.44±0.03	193±1.3

[a] Values are means ±S.D. of three replicate analyses.
[b] TEAC is the millimolar concentration of a Trolox solution having the antioxidant capacity equivalent to 200 μg/ml solution of the extracts under investigation.
[c] As gallic acid equivalents.

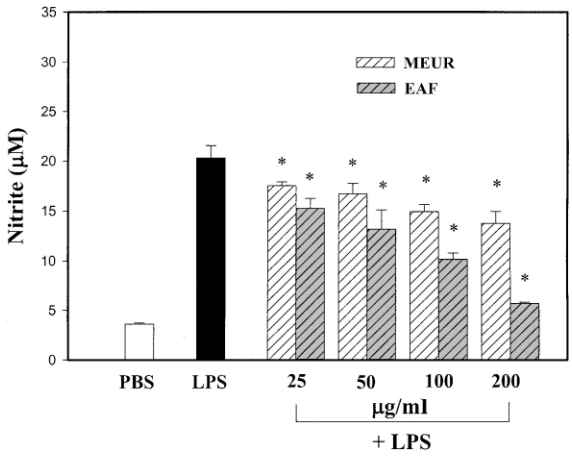

Fig. 4. Inhibition of nitrite accumulation in cell culture supernatants of 1 μg/ml LPS-activated RAW 264.7 macrophages by methanol extracts of *Uraria crinita* root (MEUR) and its ethyl acetate fraction (EAF) in the indicated concentration (μg/ml). Bars represent mean±S.D. ($n=3$). Asterisk are significantly different by comparison with the control (LPS alone), $P<0.05$.

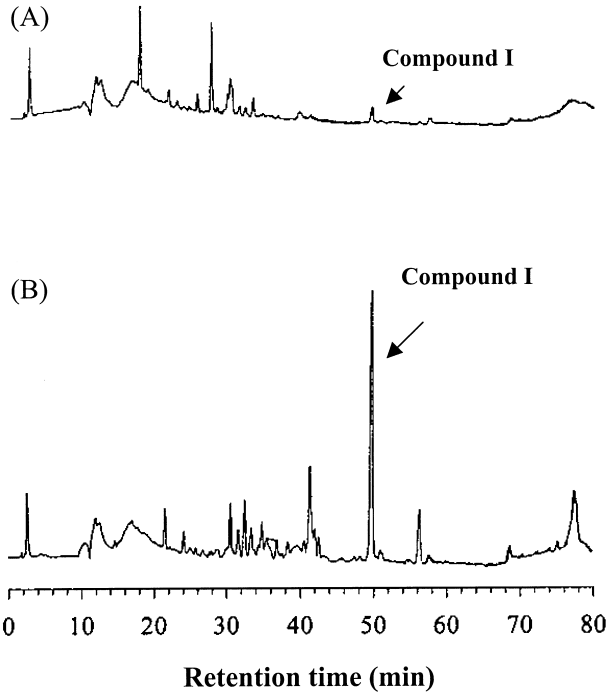

Fig. 5. Typical HPLC profiles of methanol extracts (A) and its ethyl acetate fraction (B) from *Uraria crinita* root. Diode array detector set at 260 nm.

I showed spectra characteristic of flavonoids: λ_{max} in methanol 260 nm. The IR spectrum showed absorptions at 3420, 3320 and 1655 cm^{-1}. The FAB-MS of compound I gave an M + 1 ion peak at 271, suggesting a molecular weight of 270. The ^{1}H NMR spectrum showed the following chemical shifts: δ 6.28 (1H, s, H-6), 6.42 (1H, s, H-8), 6.91 (2H, d, $J = 8.4$ Hz, H-3′, H-5′), 7.45 (2H, d, $J = 8.4$ Hz, H-2′, H-6′), 8.17 (1H, s, H-2) and 13.03 (1H, s, H-5). The ^{13}C NMR spectrum revealed 15 peaks with the following chemical shifts: δ 93.70 (C-8), 99.82 (C-6), 106.09 (C-10), 115.91 (C-5′), 116.12 (C-3′), 123.02 (C-1′), 123.98 (C-3), 129.00 (C-6′), 131.34 (C-2′), 154.25 (C-2), 158.38 (C-9), 159.02 (C-4), 163.87 (C-5), 165.07 (C-7) and 181.59 (C-4). Therefore, compound I was identified as genistein on the basis of these data. The spectral properties obtained from compound I were also consistent with those reported in the literature (Chang, Nair, Santell, & Helferich, 1994).

7. Discussion

Since ROS and RNS have been shown to generate mutations and cause DNA damage (Salgo, Bermudez, Squadrito, & Pryor, 1995a; Salgo, Stone, Squadrito, Battista, & Pryor, 1995b; Yu & Anderson, 1997), it might be beneficial to human health if consumed foods could scavenge ROS and RNS. Our results show that in SNP-mediated macrophages, DNA damage is inhibited by MEUR or EAF (Fig. 1), and that this decreasing effect may be attributed to free radical scavenging abilities (Table 1) and directly to the NO scavenging abilities of MEUR or EAF (Figs. 2 and 3). Morita, Arisawa, Naguse, Hsu, and Chen (1977) studied the chemical constituents of leaves of *Uraria crinita* and found that they contained several polyphenols, such as vitexin, isovitexin and orientin. Among them, isovitexin has been shown to be a potential antioxidant in rice hulls (Ramarathnam, Osawa, Namiki, & Kawakishi, 1989). Plant phenolic compounds may act as antioxidants or as agents of other mechanisms contributing to anticarcinogenic action (Salsh, Miller, Paganga, & Tijburg, 1995). However, the total phenolic compound content, measured using the Folin–Ciocalteu procedure, does not give a full picture of the quantity or quality of the phenolic constituents in the extracts. In addition, the antioxidant activities of phenolic compounds are dependent on their structures (Satue-Gracia, Heinonen, & Frankel, 1997). However, correlations could be found in this study between the total phenolic content and TEAC value and NO scavenging ability of the extracts. The results are in agreement with those reported (Tsushida, Suzuki, & Kurogi, 1994). Since several phenolic compounds express antioxidant activity, we may infer that the antioxidant activity of MEUR or EAF comes from these phenolic compounds.

From the HPLC analysis profile of the phenolic compounds of MEUR or EAF, we found that there was a unique peak in the chromatograph of EAF. After isolation and instrumental analysis, this compound was identified as genistein, an isoflavone compound, well known as a potential antioxidant in soybean and its related products (Jha, Von Kecklinghausen, & Zilliken 1985; Pratt, Di Pieteo, Porter, & Giffee, 1981). Genistein, was also shown to inhibit the activity of protein tyrosine kinases and to modulate cell proliferation (Akiyama et al., 1987). Recently, genistein has been shown to inhibit oxidative modification of isolated LDL by means of copper and peroxyl radical (Kerry & Abbey, 1998). In addition, genistein plays a anticarcinogenic role by preventing oxidative DNA damage induced by UV light and the Fenton reaction (Wei, Cai, & Ronald, 1996).

Murine macrophages could be stimulated by cytokines, such as interleukin-1β, interferon-γ or endotoxin (LPS), produced in large amounts of nitric oxide in vitro. Determination of cellular NO release is interesting since cytokine or endotoxin was found to induce overproduction of NO and its metabolites, in particular the deleterious molecule peroxynitrite (Blough & Zafiriou, 1985). Both seem to contribute to numerous pathological conditions associated primarily with inflammatory disorders (Clancy & Abramson, 1995). Therefore, murine macrophage-like cell lines, e.g. RAW 264.7 and J744, are suitable cell models for performing in vitro studies on the iNOS system. The Griess assay used to determine the NO level in LPS-treated macrophages has been found to be a suitable method for bio-guided fractionation of potentially anti-inflammatory plant extracts (Dirsch et al., 1998). Since *Uraria crinita* is used for anti-inflammation and anti-ulcer treatments, the effects of MEUR and EAF on NO production of LPS-activated macrophages were also investigated. From the results, the inhibitory effect on the production of NO of LPS-induced RAW 264.7 cell lines by MEUR and EAF (Fig. 4), supports its folk medicine use for anti-inflammation or anti-ulcer treatments. Kobuchi, Droy-Lefaix, Christen, and Packer (1997) showed that *Ginkgo biloba* extract (Egb 761) not only directly acts as a NO scavenger, but also inhibits NO production in LPS/IFN-γ-activated macrophages by concomitant inhibition of induction of iNOS mRNA and the enzyme activity of iNOS. Epigallocatechin gallate (EGCG), a natural product from green tea, also causes similar effects (Chan et al., 1997). According to the results of recent studies conducted in our laboratory (Sheu, Lai, & Yen, 2001) and by others (Kim, Cheon, Kim, Kim, & Kim, 1999), inhibition of NO production in LPS-activated macrophages by isoflavones, including genistein, is similar to that in Egb 761 or EGCG. Therefore, genistein might be the active component at least partially responsible for the nitric oxide-scavenging and antioxidant abilities of MEUR and EAF.

8. Conclusion

Our results have demonstrated that both the methanol extracts and their ethyl acetate fraction from *Uraria crinita* root showed nitric oxide-scavenging and antioxidant abilities, which indicates that *Uraria crinita* root contains certain substances which are potential antioxidants. In this study, we first isolated genistein from *Uraria crinita* and suggested that genistein might be responsible for the free-radical-scavenging ability of EAF. Since reactive oxygen and nitrogen species are thought to be associated with chronic infection and inflammation diseases, the inhibitory effect on SNP-mediated macrophage DNA damage and on the NO production level of LPS-activated RAW 264.7, by both MEUR and EAF, may partially explain the helpful effects of *Uraria crinita*.

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