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Antioxidant and anti-inflammatory activities of aqueous extracts of *Schizonepeta tenuifolia* Briq.

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ABSTRACT

This study investigated the antioxidative and anti-inflammatory activities of aqueous extracts of *Schizonepeta tenuifolia* Briq. (STE). The results showed that STE displayed radical scavenging and reducing activity, as well as liposome protection activity. In addition, the implementation of an HPLC with a photodiode array detector helped to identify polyphenolic components including hesperidin, luteolin, and diosmetin. STE administration in the range of 125–500 mg/kg showed concentration dependent inhibition on carrageenan induced inflammatory response in mice. The anti-inflammatory effects of STE could be related to tissue NO and tumor necrosis factor a (TNF-α) suppression, and associated with the reduction of lipid peroxidation and an increase in antioxidant enzyme activities including catalase, superoxide dismutase, and glutathione peroxidase *in vivo*. Overall, the results showed that STE might serve as a natural inhibitor of oxidation and inflammation.

1. Introduction

Free radicals with unpaired electrons are generated under oxidative and nitrative stress (Rubbo et al., 2009). Oxidative and nitrative stress are not only derived from disequilibrium cellular metabolism, but also from pathological status such as inflammation (Kovacic et al., 2005). During inflammation, reactive oxygen (ROS) and nitrogen (RNS) species are produced by inflammatory cells and can oxidize biomolecules including lipids. At the same time, peroxynitrite can abstract a hydrogen atom from a polyunsaturated fatty acid and results in the production of nitrated lipids (Rubbo and Radi, 2008). Therefore, decreasing NO production under inflammatory conditions is an important step in decreasing the threats of oxidative and nitrative stress as well as the damage of inflammation.

The inflammation model of a carrageenan induced edema is usually used to assess the activity of natural products in resisting the pathological changes associated with acute inflammation. Carrageenan can induce acute inflammation which begins with infiltration of phagocytes and the burst of free radicals as well as the release of inflammatory mediators (Salvemini et al., 1996). However, previous studies have suggested that oxidative stress and tumor necrosis factor α (TNF-α), are the major mediators of inflammatory metabolism after the administration of carrageenan (Rocha et al., 2006). Recently, it appears that the important roles of enzymatic antioxidants protect organisms against oxidative stress in the process of inflammation (Valko et al., 2007). This has triggered studies focusing on the role of natural products in suppressing the production of oxidative stress and increasing enzymatic antioxidants in tissues.

Schizonepeta tenuifolia (Chinese name "Jing Jie") is of the Labiatae family of plants, commonly called the mint family, and is categorized as surface-relieving agents in the modern Chinese Materia Medica guides. This herb is an oriental medicinal plant that is widely used in China, Korea and Japan. It is a major herbal constituent included in treatments for the common cold with fever, ostitis media and other skin inflammations (Hsu et al., 1985). The dried above-ground parts of *S. tenuifolia* is often consumed in the East in sauce, beverage, beneficial tea or herbal medicine, with various quantities being added. Previous studies have suggested that *S. tenuifolia* Briq. display immunomodulatory and antioxidant effects (Kang et al., 2008; Yoon et al., 2007). Further, the differential regulation of *S. tenuifolia* Briq. on interferon (IFN)-γ, interleukin (IL)-4 and IL-2 may be due to its suppression of nuclear factor (NF)-κB, concomitant with its enhancement of nuclear factor of activated T cells (NFAT)c2 (Kang et al., 2010). Although *S. tenuifolia* Briq. shows various physiological effects, few studies have focused on its protective effects against carrageenan-induced inflammatory damage *in vivo*. However, natural antioxidants present in plants are not merely consumed by people to meet nutritional needs. They also exhibit protective capacity *in vivo*, helping to increase antioxidant capacity (Crozier et al., 2009). Consequently, the major objective of the present study is to determine the protective

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activities of the aqueous extract of *S. tenuifolia* Briq. (STE) against carrageenan induced inflammation and oxidative damage.

2. Materials and methods

2.1. Materials

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), thiobarbituric acid (TBA), λ-carrageenan (Carr) and indomethacin (Indo) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Dried samples of *S. tenuifolia* Briq. were obtained from Sun Ten Pharmaceutical Co., Ltd. in Taipei, Taiwan.

2.2. Sample preparation

All dried samples of *S. tenuifolia* Briq. were ground into fine powder. The powder (100 g) was extracted with water (1000 ml) at 100 °C for 60 min and then centrifuged at 10,000*g* for 20 min. The extract was filtered and the residue was re-extracted under the same conditions. The combined filtrate was then freeze-dried. The yield obtained was 23.6% (23.6 g). The final sample was named STE (aqueous extract of *S. tenuifolia* Briq.).

2.3. Determination of hesperidin, luteolin, and diosmetin by HPLC analysis

HPLC was performed with a Hitachi Liquid Chromatograph (Hitachi Ltd., Tokyo, Japan), consisting of two model L-7100 pumps, and one model L-7455 photodiode array detector. The samples were dissolved in 70% methanol and filtered through a 0.45 μm PVDF-filter. The injection volume was 10 μl and the separation temperature was 25 °C. The column was a Mightysil RP-18 GP (5 μm, 250 mm × 4.6 mm I.D.). The method involved the use of a binary gradient with mobile phases containing: (A) phosphoric acid in water (0.1%, *v*/*v*) and (B) H_2O/CH_3OH:20/80 (*v*/*v*). The solvent gradient elution program was as follows: 0–20 min, 100–60% A, 0–40% B; 20–35 min, 60–50% A, 40–50% B; 35–40 min, 50% A, 50% B; 40–65 min, 50–25% A, 50–75% B; and finally 65–75 min, 25–0% A, 75–100% B. The flow-rate was kept at 0.8 ml/min. A precolumn of μ-Bondapak™ C_{18} (Millipore, Milford, MA, USA) was attached to protect the analytical column.

2.4. Determination of total polyphenols

Total polyphenols were determined as gallic acid equivalents (Taga et al., 1984). Sodium carbonate (2 ml, 20% (*w*/*v*)) was added to different concentrations of STE solutions individually in a 10 ml volumetric flask. Then, 0.1 ml Folin–Ciocalteu reagent (50% (*v*/*v*)) was added and the volume was adjusted to 10 ml with H_2O. After incubation at 30 °C for 1 h, the absorbance at 750 nm was measured and compared to a gallic acid calibration curve.

2.5. Determination of DPPH radical inhibition

The effects of STE on the DPPH radical inhibition was estimated as previously described (Hatano et al., 1988). The samples were added to a methanolic solution (1 ml) of DPPH radical (the final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min; the absorbance of the resulting solution was then measured spectrophotometrically at 517 nm.

2.6. Determination of ABTS radical cation inhibition

As previously described, this assay determined the capacity of STE to scavenge the ABTS radical cation (Arnao et al., 2001). The ABTS radical cation was generated by reacting 1 mM ABTS with 0.5 mM hydrogen peroxide and 10 units/ml horseradish peroxidase in the dark at 30 °C for 2 h. After 1 ml of ABTS radical cation was added to samples, the absorbance was recorded at 734 nm after 10 min.

2.7. Determination of reducing activity

The reducing power of STE was determined as previously described (Oyaizu, 1986). Potassium ferricyanide (2.5 ml, 10 mg/ml) was added to samples in phosphate buffer (2.5 ml, 200 mM, pH 6.6) and the mixture was incubated at 50 °C for 20 min. Trichloroacetic acid (2.5 ml, 100 mg/ml) was added to the mixture, which was then centrifuged at 1,000*g* for 10 min. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 1.0 mg/ml), and then the absorbance was read at 700 nm. Higher absorbance of the reaction mixture indicated greater reducing activity.

2.8. Determination of liposome oxidation

Lecithin (500 mg) was sonicated in an ultrasonic cleaner (Branson 8210, Branson ultrasonic Corporation, Danbury, CT, USA) in phosphate buffer (50 ml, 10 mM, pH 7.4) for 2 h in an ice-cold water bath. The sonicated solution, $FeCl_3$, ascorbic acid and samples (0.2 ml) were mixed to produce a final concentration of 3.12 μM $FeCl_3$, and 125 μM of ascorbic acid and incubated for 1 h at 37 °C. The liposome oxidation was determined by the thiobarbituric acid (TBA) method (Tamura and Shibamoto, 1991). The absorbance of the sample was read at 532 nm against a blank, which contained all reagents except lecithin. A lower level of absorbance indicated stronger protective activity.

2.9. Animals

Male ICR mice (6–8 weeks old) were obtained from the BioLASCO Taiwan Co., Ltd. The animals were kept in plexiglass cages at a constant temperature of 22 ± 1 °C, relative humidity of 55 ± 5% with a 12 h dark–light cycle. They were given food and water *ad libitum*. All experimental procedures were performed according to the NIH Guide for the Care and Use of Laboratory Animals. This study was approved by the ethics committee of the Institutional Animal Care and Use Committee (IACUC) of China Medical University.

2.10. Determination of carrageenan (Carr) induced paw edema

The Carr-induced hind paw edema model was used for determination of anti-inflammatory activity (Sheu et al., 2009). The route of administration and range of dose were selected as in a previous study (Kang et al., 2010). After a 2 week adaptation period, 25 mice (18–25 g) were randomly divided into five groups ($n = 5$ in each group). (1) Carr alone group: mice were injected with 1% Carr (50 μl) in the plantar side of right hind paws. (2) Positive indomethacin (Indo) control group: Indo (10 mg/kg) was injected intraperitoneally 90 min before the injection of Carr. (3–5) STE-treated groups: STE was administered orally at a dose of 125, 250, and 500 mg/kg for 2 h before the injection Carr. Paw volume was measured after Carr injection at 1, 2, 3, 4 and 5 h intervals using a plethysmometer (model 7159, Ugo Basile, Varese, Italy). The degree of swelling induced was evaluated by $a - b$, where a was the volume of the right hind paw after Carr treatment and b was the volume of the right hind paw before Carr treatment.

After 5 h, the animals were sacrificed and the right hind paw tissue and liver tissue were dissected. The right hind paw tissue was rinsed in ice-cold normal saline, and immediately placed in cold normal saline four times their volume and homogenized at 4 °C. Then the homogenate was centrifuged at 12,000*g* for 5 min. The supernatant was obtained for tissue lipid peroxidation assays. On the other hand, the whole liver tissue was rinsed in ice-cold normal saline, and immediately placed in cold normal saline one time their volume and homogenized at 4 °C. Then the homogenate was centrifuged at 12,000*g* for 5 min. The supernatant was obtained for the antioxidant enzymes activity assays. Also, blood was withdrawn for serum NO and TNF-α assay.

2.11. Determination of lipid peroxidation in edema paws

The hind paw tissue lipid oxidation was evaluated by the thiobarbituric acid (TBA) method. Briefly, lipid degradation products reacted with thiobarbituric acid in the acidic high temperature and formed red-complex TBARS. The absorbance of TBARS was determined at 532 nm.

2.12. Determination of nitric oxide in serum

Serum samples were diluted four times with distilled water and deproteinized by adding zinc sulfate (300 mg/ml) to a final concentration of 15 mg/ml. After centrifugation at 10,000*g* for 5 min at room temperature, 100 μl of supernatant was applied to a microplate, followed by 100 μl of Griess reagent (0.1% *N*-(1-naphthyl) ethylenediamide dihydrochloride, 1% sulphanilamide in 5% phosphoric acid). After 10 min of color development at room temperature, the absorbance was measured and compared to a sodium nitrite calibration curve.

2.13. Determination of tumor necrosis factor (TNF-α) in serum

Serum TNF-α were determined using a commercial ELISA kit (Biosource International, Inc., Camarillo, CA) according to the instructions of the manufacturer. TNF-α was calculated from a standard curve.

2.14. Determination of antioxidant enzyme activity in liver

Total superoxide dismutase (SOD) activity was determined by the inhibition of cytochrome C reduction (Flohe and Otting, 1984). The reduction of cytochrome C was mediated by superoxide anions generated by the xanthine/xanthine oxidase system and monitored at 550 nm. One unit of SOD was defined as the amount of enzyme required to inhibit the rate of cytochrome C reduction by 50%. Total catalase (CAT) activity was measured as previously described (Armstrong and Browne,