Food Chemistry 138 (2013) 751–756

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Anti-inflammatory effects of an aqueous extract of Welsh onion green leaves in mice

Bor-Sen Wang [a,b], Guan-Jhong Huang [c], Ya-Hui Lu [a], Lee-Wen Chang [b,*]

[a] Department of Applied Life Science & Health, Chia Nan University of Pharmacy and Science, 60, Erh-Jen Road, Sec.1, Jen-Te, Tainan 717, Taiwan, ROC
[b] Department of Food Science & Technology, Chia Nan University of Pharmacy and Science, 60, Erh-Jen Road, Sec.1, Jen-Te, Tainan 717, Taiwan, ROC
[c] Institute of Chinese Pharmaceutical Science, China Medical University, 91, Hsueh-Shih Road, Taichung 404, Taiwan, ROC

ARTICLE INFO

Article history:
Received 19 March 2012
Received in revised form 5 October 2012
Accepted 21 November 2012
Available online 5 December 2012

Keywords:
Welsh onion
Antioxidant
Anti-inflammatory
Carrageenan

ABSTRACT

The anti-inflammatory effects of an aqueous extract of Welsh onion green leaves (WOE) in mice was investigated. Administration of WOE, in the range of 0.25–1 g/kg, showed a concentration dependent inhibition on paw edema development after carrageenan treatment in mice. The anti-inflammatory effects of WOE were closely attributed to decreased levels of tissue NO and tumor necrosis factor-α (TNF-α). Further evidence for WOE's protection is shown in the reduction of lipid oxidation and the increase of antioxidant enzyme activities, including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX) *in vivo*. Further, WOE also decreased the number of acetic acid-induced writhing responses and formalin-induced pain in the late phase in mice. Overall, the results showed that WOE might serve as a natural source of anti-inflammatory compounds.

1. Introduction

Inflammation is recognised as a biological process in response to tissue injury. At the injury site, an increase in the permeability of the blood vessel wall followed by migration of immune cells can lead to edema formation during inflammation. Meanwhile, many other mechanisms such as the production of reactive nitrogen species (RNS) and proinflammatory cytokines are activated, which exacerbate the inflammatory damage. Numerous natural products rich in antioxidants display protective effects against 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. Carrageenans, which are highly flexible molecules, are a family of polysaccharides. Carrageenan can induce acute inflammation beginning with migration of phagocytes and the burst of free radicals as well as the release of inflammatory mediators (Salvemini et al., 1996). For example, 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, Fernandes, Quintao, Campos, & Calixto, 2006).

In addition, intracellular antioxidant mechanisms against these inflammatory stresses involve antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) in tissues. Recently, it has been shown that faulty cellular antioxidant systems cause organisms to develop a series of inflammatory and cancer-diseases (Valko, Rhodes, Moncol, Izakovic, & Mazur, 2006). On the other hand, the aggravating effects of inflammation in tissues are, in part, suspected to induce an oxidative stress status characterised by depletion of the activities of endogenous antioxidant enzymes (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.

Welsh onion (*Allium fistulosum* L., Alliaceae), an important vegetable used for flavouring in Asian dishes which is commonly called Ching-Tsung, has been reported to scavenge ROS (Wang et al., 2006), inhibit LDL oxidation (Wang, Chen, Liang, & Duh, 2005), modulate HDL receptors (Duh, Hsiao, & Wang, 2008) and lower blood pressure (Chen, Tsai, & Chen, 1999). Although Welsh onions show various physiological effects, few studies have focused on their protective effects against carrageenan-induced inflammatory damage *in vivo*. Consequently, the objective of the present study is to determine the protective effects of an aqueous extract of Welsh onion green leaves (WOE) against carrageenan induced inflammation and oxidative damage. Further, the analgesic effects of WOE were also evaluated.

2. Materials and methods

2.1. Materials

Thiobarbituric acid (TBA), λ-carrageenan (Carr), N-(1-naphthyl) ethylenediamide dihydrochloride, sulphanilamide, xanthine, xanthine oxidase, glutathione and indomethacin (Indo) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

* Corresponding author. Fax: +886 6 2667321.
E-mail address: leewen@mail.chna.edu.tw (L.-W. Chang).

0308-8146/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2012.11.106

2.2. Sample preparation

The green portion (500 g) of Welsh onion was squeezed and filtered by a food processor (National, Model MJ-C85; Tokyo, Japan; pore size of the filter: 0.2 mm), ten times, to obtain the Welsh onion green leave juice (200 g). After centrifugation at 10,000*g* for 30 min, the supernatant (170 g) was the aqueous extract of Welsh onion green leave (WOE). Furthermore, the extract was filtered, frozen at −80 °C, and then lyophilized for 24 h.

2.3. Animals

Male imprinting control region (ICR) mice (18–25 g) 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 55 ± 5% with 12 h dark-light cycle for at least 2 weeks before the experiment. 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.

2.4. Determination of carrageenan (Carr) induced edema

Carr-induced hind paw edema model was used for the determination of anti-inflammatory activity (Sheu et al., 2009). After a two-week adaptation period, twenty-five 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 the 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) WOE-treated groups: WOE was administered orally at a dose of 0.25, 0.5, and 1 g/kg for 2 h before the injection of 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* minus *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 Carr-induced edema paw tissues were dissected. The tissues were rinsed in ice-cold normal saline, and immediately placed in cold normal saline one times their volume and homogenised at 4 °C. Then the homogenate was centrifuged at 12,000*g* for 5 min. The supernatant of hind paw tissue was stored at −20 °C for malondialdehyde (MDA) assays and the antioxidant enzymes assays. In addition, blood was withdrawn for NO and TNF-α assay.

2.5. Determination of tissue lipid oxidation

Tissue lipid oxidation was evaluated by the thiobarbituric acid reactive substances (TRARS) method (Tamura & Shibamoto, 1991). Briefly, lipid oxidation product was reacted with thiobarbituric acid in the acidic high temperature and a red-complex TBARS was formed. The absorbance of TBARS was determined at 532 nm.

2.6. Determination of nitric oxide in serum

Serum samples were diluted four times with distilled water and deproteinized by adding zinc sulphate (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 colour development at room temperature, the absorbance was measured and compared to a sodium nitrite calibration curve.

2.7. Determination of tumor necrosis factor-α (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.

2.8. Determination of antioxidant enzyme activity in paw tissue

Total superoxide dismutase (SOD) activity was determined by the inhibition of cytochrome c reduction (Flohe & 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 & Browne, 1994). In brief, the reduction of 10 mM of H_2O_2 in 20 mM phosphate buffer (pH 7.0) was monitored by measuring the absorbance at 240 nm. The activity was calculated using a molar absorption coefficient. Total glutathione peroxidase (GPX) activity was determined as previously described (Flohe & Gunzler, 1984). The enzyme solution was added to a mixture containing hydrogen peroxide and glutathione in 0.1 mM of Tris buffer (pH 7.2), and the absorbance at 340 nm was measured. Activity was evaluated from a calibration curve, and the enzyme activity was defined as nanomoles of NADPH oxidised per milligram of protein per minute. The protein concentration of the tissue was determined by the Bradford dye-binding assay (Bradford, 1976).

2.9. Acetic acid-induced writhing response

Mice (18–25 g) were randomly assigned as mentioned above. (1) Acetic acid alone group: mice were intraperitoneal injection of 1% acetic acid (10 ml/kg). (2) Positive indomethacin (Indo) group: Indo (10 mg/kg) was injected intraperitoneally 25 min before the injection of acetic acid. (3–5) WOE-treated groups: WOE was administered orally at a dose of 0.25, 0.5, and 1 g/kg for 55 min before the injection of acetic acid. Five minutes after the intraperitoneal injection of acetic acid, the number of writhing during the following 10 min was recorded (Sheu et al., 2009).

2.10. Formalin test

The antinociceptive activity of the samples was determined using the formalin test (Sheu et al., 2009). Mice (18–25 g) were randomly assigned as mentioned above. (1) Formalin alone group: twenty microliters of 5% formalin was injected into the dorsal surface of the right hind-paw. (2) Positive indomethacin (Indo) group: Indo (10 mg/kg) was injected intraperitoneally 30 min before the injection of formalin. (3–5) WOE-treated groups: WOE was administered orally at a dose of 0.25, 0.5, and 1 g/kg for 60 min before the injection of formalin. The mice were observed for 30 min after the injection of formalin, and the amount of time spent licking the injected hind paw was recorded. The first 5 min post formalin injection was referred to as the early phase and the period between 15 and 40 min as the late phase. The total time licking or biting the injured paw (pain behaviour) was measured with a stop watch. The activity was recorded at 5 min intervals.

2.11. Statistical analysis

Data are expressed as mean ± SE. Statistical evaluation was carried out by one-way analysis of variance (ANOVA followed by