Journal of Ethnopharmacology 122 (2009) 227–233

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Suppressive effects of wild bitter gourd (*Momordica charantia* Linn. var. *abbreviata* ser.) fruit extracts on inflammatory responses in RAW 264.7 macrophages

Chong-Kuei Lii[a,1], Haw-Wen Chen[a,1], Wen-Tzu Yun[b], Kai-Li Liu[b,*]

[a] Department of Nutrition, China Medical University, No. 91, Hsueh-Shih Road, Taichung 40402, Taiwan, ROC
[b] Department of Nutrition, Chung Shan Medical University, No. 110, Sec. 1, Jianguo N. Road, Taichung 40203, Taiwan, ROC

ARTICLE INFO

Article history:
Received 16 July 2007
Received in revised form
21 September 2008
Accepted 13 January 2009
Available online 7 February 2009

Keywords:
Wild bitter gourd
Momordica charantia Linn. var. *abbreviata* ser.
Inducible nitric oxide synthase
Nitric oxide
NF-κB

ABSTRACT

Aim of the study: Bitter gourd (*Momordica charantia*) is used to treat various diseases including inflammation. A wild species of bitter gourd, *Momordica charantia Linn. var. abbreviata ser.* (WBG), is considered to be more potent in disease prevention than is bitter gourd; however, little is known about the biological and physiological characteristics of WBG.
Materials and methods: The present study investigated the anti-inflammatory effect of WBG on lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages.
Results: Among the hot water, 95% ethanol, and ethyl acetate extracts of WBG, the ethanol extract showed the greatest reduction of LPS-induced nitric oxide (NO) and prostaglandin E_2 (PGE_2) production and inducible nitric oxide synthase (iNOS) and pro-interleukin-1β expression. LPS-induced cyclooxygenase-2 expression was not affected by WBG extracts. Compared with WBG, extracts from bitter gourd showed a lesser inhibition of LPS-induced events. Electrophoretic mobility shift assay further showed that both the hot water and the ethanol extracts of WBG inhibited NF-κB activation. Although information is lacking on the bioactive components of WBG, the phenolic compound contents of each extract significantly paralleled its anti-inflammatory ability ($r = 0.74$, 0.88 and 0.65 for NO, PGE_2 and iNOS expression, respectively, $P < 0.05$).
Conclusions: These results suggest that WBG is beneficial for reducing LPS-induced inflammatory responses by modulating NF-κB activation.

1. Introduction

Considerable effort has been made over the past decade to evaluate the importance of traditional drugs of plant origin in various diseases (Blumenthal, 1992). *Momordica charantia*, which is commonly known as bitter gourd (melon) or kerala, is widely planted in tropical areas and is usually consumed as a vegetable. Bitter gourd has also been frequently used as a medicinal herb in Asia, Africa, and South America because of its anti-diabetic, anthelmintic, abortifacient, anti-bacterial, anti-viral, and chemopreventive functions (Basch et al., 2003; Grover and Yadav, 2004). Regarding its anti-inflammatory activity, bitter gourd significantly decreases prostaglandin E_2 (PGE_2), interleukin (IL)-7 and tumor necrosis factor-α and increases transforming growth factor-β and IL-10 secretion in RAW 264.7 macrophages, Caco-2 cells and THP-1 cells (Huang and Wu, 2002; Manabe et al., 2003; Kobori et al., 2006). In in vivo studies, feeding mice the fruit of bitter gourd effectively enhanced T helper 2 hormonal responses (Manabe et al., 2003) and T helper 1 cellular immunity (Ike et al., 2005). Recently, several phytochemicals with the health benefits of bitter gourd have been isolated and studied (Husain et al., 1994; Xie et al., 1994, 1998; Murakami et al., 2001). Charantins, a mixture of steroidal saponins that are abundant in the fruit of bitter gourd, have been proposed to contribute to the hypoglycemic and antihyperglycemic activity of bitter gourd (Harinantenaina et al., 2006; Krawinkel and Keding, 2006). Additionally, bitter gourd is a good source of phenolic compounds, including gallic acid, gentisic acid (2,5-dihydroxyl benzoic acid), catechins, chlorogenic acid, and epicatechin (Holmes et al., 1985; Horax et al., 2005). Gentisic acid has been proven as an active metabolite of salicylic acid and may account for the anti-inflammatory property of salicylic acid by inhibiting cyclooxygenase-2 (COX-2) mRNA expression and activity as well as PGE_2 production (Holmes et al., 1985; Hinz et al., 2000).

Abbreviations: COX-2, cyclooxygenase-2; EMSA, electrophoretic mobility shift assay; GAE, gallic acid equivalents; iNOS, inducible nitric oxide synthase; IL, interleukin; LPS, lipopolysaccharide; MTT, 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide; NO, nitric oxide; PPAR, peroxisome proliferator-activated receptor; PMSF, phenylmethylsulfonyl fluoride; PBS, phosphate-buffered saline; PGE_2, prostaglandin E_2; WBG, wild bitter gourd.

* Corresponding author. Tel.: +886 4 2473 0022x11748; fax: +886 4 2324 8192.
E-mail address: kaililiu@csmu.edu.tw (K.-L. Liu).

[1] These two authors contributed equally to this work.

0378-8741/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2009.01.028

A wild species of *Momordica charantia*, wild bitter gourd (*Momordica charantia* Linn. var. *abbreviata* ser., or WBG), is native to tropical areas of Asia. WBG grows in the eastern and southern regions of Taiwan and is consumed not only as a vegetable but also as a folklore medicine for disease prevention by local residents. A recent study showed that the ethyl acetate extract of WBG significantly activates not only peroxisome proliferator-activated receptor (PPAR)-α in a manner comparable with that of bitter gourd but also PPAR-γ in the PPAR-γ-GAL4 (USA)$_4$-AP reporter system (Chao and Huang, 2003). These data suggest that the disease-preventing properties of WBG may be due to regulation of PPAR activation, which is linked with lipid metabolism, anti-diabetic, and anti-inflammatory effects.

Inflammatory reactions are not only the response of living tissues to injury and infection but also are relevant to disease developments, such as asthma, multiple sclerosis, colitis, inflammatory bowel disease, and atherosclerosis (de Boer et al., 2000; Holtzman et al., 2002; Bruck, 2005; Rychly and Nebe, 2006). NF-κB acts as a key regulator of the host immune and inflammatory responses by increasing the expression of genes encoding cytokines, chemokines, growth factors, cell adhesion molecules, and several acute phase proteins (Baeuerle and Henkel, 1994; Chen et al., 1999). Among those NF-κB targeting genes, inducible nitric oxide synthase (iNOS), COX-2, and IL-1β serve as important mediators in the inflammatory responses. IL-1β produced by stimulated leukocytes is converted from inactive pro-IL-1β protein and serves as a fundamental contributor to local and systemic inflammation (Stylianou and Saklatvala, 1998). Nitric oxide (NO) and PGE_2 are pleiotropic mediators produced at inflammatory sites respectively by iNOS and COX-2 which are induced in response to cytokines, bacterial endotoxins, growth factors and phorbol esters (Kleinert et al., 2003; Smith et al., 2000). Because chronic inflammation initiates and furthers the pathogenesis of many chronic diseases, dietary plants that inhibit the expression of IL-1β, iNOS, and COX-2 have attracted much attention in the functional food industry.

Although WBG is believed to have lots of health benefits in Taiwanese people, limited evidence of the biological activity and molecular working mechanisms of WBG is available. Based on Chinese medicine, foods are classified into 'heating' or 'cooling'. Foods traditionally regarded as 'cooling' are shown to decrease lipopolysaccharide (LPS)-induced PGE_2 production in vitro (Anderson, 1980; Huang and Wu, 2002). WBG is regarded as 'cooling' food by Taiwanese people and its anti-inflammatory effect is worth studying. In the present study, we examined the effects of WBG on modulating LPS-induced pro-inflammatory events in RAW 264.7 macrophages. In addition, the molecular basis of the anti-inflammatory effects of WBG was investigated.

2. Materials and methods

2.1. Materials

The mouse macrophage-like cell line RAW 264.7 was purchased from the Food Industry Research and Development Institute (Hsinchu, Taiwan), and fetal bovine serum was from Biowest LLC (Miami, FL). RPMI 1640 medium and media supplements for cell culture were obtained from Invitrogen Corporation (Carlsbad, CA). LPS and Folin-Ciocalteu phenol reagent were from Sigma Chemical Company (St. Louis, MO). Gallic acid and gentisic acid were obtained from Wako Chemical Company (Osaka, Japan). Antibodies to iNOS and COX-2 were from Santa Cruz Biotechnology (Santa Cruz, CA), and the antibody to pro-IL-1β was from CytoLab Ltd. (Rehovot, Israel). The biotin-labeled and unlabeled double-stranded NF-κB consensus oligonucleotides and a mutant double-stranded NF-κB oligonucleotide for electrophoretic mobility shift assay (EMSA) were synthesized by MDBio Inc. (Taipei, Taiwan). All other chemicals were of the highest quality available.

2.2. Preparation of extractions

WBG and bitter gourd were purchased from local markets at Haulian and Taichung, Taiwan, respectively, and were recognized by Dr. Lee-Yan Sheen (Graduate Institute of Food Science and Technology, National Taiwan University, Taipei, Taiwan). Voucher specimens were kept in our laboratory, Department of Nutrition, Chung Shan Medical University, Taichung, Taiwan, for further reference.

The fruits of WBG and bitter gourd were washed and seeds were removed from the pulps. Pulps were put into a −70 °C freezer for 12 h then freeze-dried (FD4, Heto Lab Equipment, Denmark) for at least 12 h. The dried fruits were ground to fine power by an electrical food grinder and were then stored at −20 °C for further use.

Successive extraction of food material was performed according to the method described by Huang and Wu (2002). The powdered freeze-dried food material was boiled in Milli-Q water (25 mL/g powder) for 30 min and then centrifuged at 1000 × *g* for 5 min. The resultant supernatants were filtered through Whatman #1 filter paper and freeze-dried. The hot water extract was weighed to measure the extraction yield and was stored at −20 °C.

Additionally, the powdered freeze-dried fruits of WBG and bitter gourd were extracted sequentially with water, 95% ethanol, and ethyl acetate. Briefly, freeze-dried samples were first extracted with Milli-Q water (15 mL/g powder) for 1 h at room temperature with constant blending and were then centrifuged at 1000 × *g* for 5 min and filtered through Whatman #1 filter paper. The water extraction step was repeated two more times. The residue was then extracted by 95% ethanol (15 mL/g powder), and the ethanol extract was concentrated at 37 °C under reduced pressure and was then freeze-dried. Finally, the residue was extracted by ethyl acetate (15 mL/g powder), and the extract was first concentrated at 37 °C under reduced pressure and then dissolved in ether. The ether in the ethyl acetate extract was evaporated by nitrogen. The extracts of water, 95% ethanol, and ethyl acetate were weighed to measure the extraction yield and were stored at −20 °C until analyzed.

For cell treatments, the hot water and water extracts were dissolved in phosphate-buffered saline (PBS), and the ethanol and ethyl acetate extracts were dissolved in dimethyl sulfoxide.

2.3. Cell culture

The RAW 264.7 macrophages of passages 8–13 were maintained in RPMI-1640 medium supplemented with 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% (v/v) heat-inactivated fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO_2. Cells were plated at a density of 8×10^5 per 30-mm culture dish or 3×10^5 per 24-well culture dish and were incubated until 90% confluence was reached. For the determination of cell viability, nitrite and PGE_2 concentrations, as well as protein level of iNOS, the cells were treated with various concentrations of WBG and bitter gourd extracts in the presence of 1 μg/mL LPS for 6–24 h as indicated. Moreover, for the determination of protein levels of COX-2 and IL-1β, cells were treated with various concentrations of WBG extracts in the presence of 1 μg/mL LPS for 24 h and 6 h respectively. For EMSA, the cultures were pre-incubated with various concentrations of hot water and ethanol extracts of WBG for 4 h and were then treated with 1 μg/mL LPS for an additional 30 min. The final concentration of dimethyl sulfoxide in the medium was 0.1% (v/v).