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Original Article

Comparison of anti-inflammatory effect and protein profile between the water extracts from Formosan sambar deer and red deer

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ABSTRACT

Velvet antler (VA), the unossified antler from members of the family Cervidae, has been used in traditional Chinese medicines and health foods for over 2000 years in enhancement of kidney function and treatment or prevention of cardiovascular, immunological and gynaecological disease. The aim of this study was to investigate the anti-inflammatory effect of velvet antler water extracts from Formosan sambar deer (*Rusa unicolor swinhoei*, SVAE) and red deer (*Cervus elaphus*, RVAE). Results indicated that both SVAE and RVAE significantly reduced the pro-inflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) productions in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells at concentrations above 200 $\mu g\ mL^{-1}$. SVAE seems to demonstrate a better anti-inflammatory effect than that of RVAE *in vitro*. Both SVAE and RAVE also enhanced the anti-inflammatory cytokine IL-10 production in LPS-stimulated RAW 264.7 cells. The results of MTT assay indicated that SVAE and RVAE did not exhibit any cytotoxicity in LPS-stimulated RAW 264.7 cells. Two-dimensional (2D) gel electrophoresis analysis revealed that the levels of 6 specific proteins were different between these two velvet antlers samples. Furthermore, the storage period was the major factor affecting the anti-inflammatory activity of SAVE. In this study, we demonstrated the difference of anti-inflammatory effect and the protein profile between SVAE and RVAE. SVAE showed better anti-inflammatory potential than RVAE. In the future, the anti-inflammatory active components and their related mechanisms should be further investigated.

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1. Introduction

Inflammatory response plays a crucial role in body's defense mechanisms that protect the host from invading pathogens and biochemicals [1]. However, dysregulation of inflammatory response caused many disorders such as atherosclerosis, Alzheimer's disease, ischaemic heart and brain diseases, cancer, Grohn's disease, colitis, obesity, metabolic syndrome, asthma, and type 1 & 2 diabetes [2,3]. Using anti-inflammatory drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) and pro-inflammatory cytokine inhibitor that ameliorating inflammatory response is one of the most common treatment for inflammatory-related disease [4]. Unfortunately, NSAIDs has side effects, including gastrointestinal tract injury, renal damage and heart problems, high blood pressure, swelling, and rashes. Due to the side effects of current NSAIDs, researchers have focused on finding the new source of less toxic anti-inflammatory agents.

Recently, natural products from animals and plants seem to be a possible source for developing the potential pharmaceutical agent and food supplement. Velvet antler (VA) is one of the most famous animal-divided medicine material, which is the unossified tissue that isolated from deer or elk (members of family Cervidae). The Chinese medical classics, *Shen Nong Ben Cao Jing* and *Compendium of Materia Medica*, recorded that VA possessed beneficial effect on kidney function, body strengthening, and anti-aging. In addition, VA has been used as a traditional Chinese medicine for enhancement of kidney function and treatment or prevention of cardiovascular, immunological and gynaecological disease for centuries in East Asia. Many previous reports and clinical observations have convincingly demonstrated that VA and its extracts can alleviate the symptoms of rheumatoid arthritis, osteoporosis and osteoarthritis, promote dermal cell proliferation and angiogenesis, and treat heart failure [5–10]. Although VA and its extracts have showed various beneficial effects *in vitro* and *in vivo*, no previous studies have distinguished the difference of anti-inflammatory effect among VA from different species or different extraction methods.

Sui et al. [7] reported that various components including mineral elements, amino acids, proteins and peptides, saccharides, lipids and polyamines are the major substrates that contributed the bio-activities to VA and VA extracts. Among these components, amino acid, polypeptides, and proteins are the most abundant components in VA, and also have been reported with excellent bio-activities. Fox example, the polypeptides from VA possess anti-osteoporosis effects in both of the *in vitro* osteoarthritic rabbit chondrocytes model and the *in vivo* retinoic acid-induced osteoporotic rat model [11,12]. In addition, the polypeptides from VA also exhibited anti-heart failure, anti-fatigue, and wound healing effects [13–15].

In Taiwan, there is an indigenous subspecies of deer, which inhabit at low to middle elevation forest, named Formosan sambar deer (*Rusa unicolor swinhoei*). The farming deer industry began in the 1960's in Taiwan, thus the use of products from Formosan sambar deer have attracted many attentions, especially in VA and its pharmaceutical components [5,6]. However, there is another popular VA source from red deer (*Cervus elaphus*). No previous study evaluated the differences between Taiwan indigenous deer species and other species in the bio-activities and the components. The influence of storage conditions on its anti-inflammatory effect also remains unclearly. Therefore, the aim of this study was to assess the anti-inflammatory effects of different VA extracts through RAW 264.7 cell model, and to identify the major components of each sample using 2D SDS-PAGE electrophoresis (2DE) and LCMS^2. The effect of storage conditions on the anti-inflammatory effect of the VA extracts was also evaluated. The final aim of this study was to develop a potential anti-inflammatory agent from VA extracts.

2. Material and methods

2.1. Chemicals and reagents

Lipopolysaccharide (LPS), dimethyl sulfoxide (DMSO), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), trichloroacetic acid (TCA), and bovine serum albumin (BSA) were purchased from Sigma–Aldrich (St. Louis, MO, USA). DMEM (Dulbecco's Modified Eagle Medium) medium, fetal bovine serum (FBS), and other cell culture reagents were obtained from Corning (Tewksbury, MA, USA). Urea, Dithiothreitol (DTT), and triton X-100 were purchased from Merck (Darmstadt, Germany). All of the other chemicals and solvents used in this research were analytical grade.

2.2. Cell culture

RAW 264.7 cell line was purchased from Bioresource Collection and Research Center (BCRC, Taiwan). Cells were cultured in DMEM containing 10% FBS and 1% antibiotic antimycotic in a 37 °C humidified incubator containing 5% CO_2. Cell subculture was prepared by scraping 2 to 3 times per week.

2.3. Morphology and chemical composition analysis of velvet antler

The morphology of velvet antler from Formosan sambar deer and red deer were observed using microscopy (SG-EX30, SAGE Vision, Taiwan). Chemical composition analysis, including moisture, ash, crude protein, and crude fat contents were analyzed according to Chinese National Standards (CNS) 5033, 5034, 5035, and 5036, respectively.

2.4. Preparation and extraction of velvet antler

The 70–75 days' VA samples of Formosan sambar deer were obtained from Kaohsiung Animal Propagation Station, Taiwan Live Stock Research Institute (Pintong, Taiwan). The 60–65 days' VA samples of red deer were purchased from Feng Ying Deer Ranch (Tainan, Taiwan). After harvested and sliced, the VA samples were stored in a −80 °C freezer until analyzed. The frozen VA samples were lyophilized by a freeze dryer (Kingmech Co. Ltd., Taipei, Taiwan) and then ground into a fine powder (VA powder) by a pulverizing machine. The VA power extracted by soaking with cold water (50 g L^{-1}) at 4 °C for 24 h, then collected and freeze-dried the supernatant to obtain the VA water extract. For the storage test, the lyophilized slides

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