Antioxidant, Anti–acetylcholinesterase and Composition of Biochemical Components of Russian Deer Velvet Antler Extracts

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Abstract

Russian deer velvet antlers were divided into three parts and subjected to an extraction process using hot water at 100, 110, and 120°C or an extraction with 70% ethanol. Each extract was analyzed for its biochemical components, including uronic acid, sulfated-glycosaminoglycans (sulfated-GAGs), and sialic acid, and the antioxidant and anti-acetylcholinesterase activities were investigated. Different levels of uronic acid and sulfated-GAGs were observed in the extracts according to the water temperature used for the extraction, and contents decreased with increasing extraction temperature. The upper layer of each extract showed high amounts of uronic acid and sulfated-GAGs, followed by the middle and base layers. Ethanol extraction was more effective for recovering uronic acid than sulfated-GAGs. Sialic acid content was the highest in the 110°C extracts but was not observed in the ethanol extracts. Velvet antler extracts showed strong antioxidant activities against DPPH and hydrogen peroxide as well as strong reducing power in a dose-dependent manner. However, the antioxidant activities were different in each layer and according to the extraction method. Additionally, velvet antler extracts exhibited inhibitory activity against acetylcholinesterase, which is associated with Alzheimer’s disease, in a dose-dependent manner. These results suggest that velvet antler extracts are useful as a functional food ingredient and/or a pharmaceutical.

Key words: velvet antler, uronic acid, sulfated-glycosaminoglycans, sialic acid, antioxidant activity, anti-acetylcholinesterase

Introduction

Overproduction of free radicals and reactive oxygen species (ROS) is believed to be associated with cellular and tissue pathogenesis, which leads to several chronic diseases such as cancer, diabetes mellitus, and neurodegenerative and inflammatory diseases (Butterfield et al., 2002; Pryor and Ahn, 1982). It is also believed that ROS can oxidize biomacromolecules such as DNA, proteins, membrane lipids, and vital molecules. To prevent or slow down the oxidative stress induced by ROS, supplementation of antioxidants may be useful. Therefore, great interest has been focused on the development of natural antioxidants that are safe, non-toxic, and effective.

Acetylcholinesterase (AChE) is an oligomeric enzyme that attaches to the neuromuscular junction, which catalyzes the cleavage of neurotransmitter acetylcholine to choline and acetate. Based on the cholinergic hypothesis, loss of cholinergic function due to a deficiency in neurotransmitter acetylcholine is the only evident finding responsible for the cognitive characteristics of Alzheimer’s disease (AD). Therefore, AChE inhibitors are widely used in AD patients to inhibit AChE activity, thereby activating the central cholinergic system and alleviating cognitive deficits.

In Korea, unossified horn of Cervus elaphus (Cervidae) is generally referred to as ‘Nokyong’ and is one of the most popular Korean traditional medicines, the benefits of which are supported by extensive in vivo and in vitro studies (Kim, 1994; Suttie et al., 1994). Recently, the beneficial effects of C. elaphus with regard to its anti-inflammatory, anti-stress, anti-aging, and antioxidant activities were studied (Je et al., 2010; Sunwoo et al., 1997; Takikawa et al., 1972; Wang et al., 1988a; Wang et al., 1988b; Zhang et al., 1992). In our previous report, we...
reported the contents of biologically active materials such as uronic acid, sulfated-glycosaminoglycans (sulfated-GAGs), and sialic acid in New Zealand deer velvet antler extracts under various conditions as well as their antioxidant effects on various model systems (Je et al., 2010). These compounds are generally considered for quality evaluation of velvet antler, and in particular, sulfated-glycosaminoglycans had been shown to reduce pain in osteoarthritis patients (Paroli et al., 1991). However, there is very little information on Russian deer velvet antler in this regard.

In this study, Russian deer velvet antler was divided into three parts, which were then subjected to extraction using hot water at three different temperatures and also 70% ethanol solution. The biochemical compositions of the extracts were analyzed, and their antioxidant and anti-acetylcholinesterase activities were evaluated.

**Materials and Methods**

**Chemicals**

All chemicals, including 2,2-diphenyl-1-picrylhydrazyl (DPPH), ethylenediaminetetraacetic acid (EDTA), Folin-Ciocalteu’s phenol reagent, hydrogen peroxide, 2,2-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS), acetylcholinesterase (electric eel), acetylthiocholine, galacturonic acid, carbozole, 1,9-dimethylmethylene blue, chondroitin 4-sulfate, N-acetylneuraminic acid, periodic acid, peroxidase, and potassium ferricyanide, were purchased from Sigma Chemical Co. (USA). Other chemicals and reagents used were of analytical grade.

**Preparation of velvet antler extracts**

Russian red deer velvet antler (C. elaphus) was donated by Shin Hung Pharm. Co. (Yeosu, Korea). The antler was divided into three parts (upper (RU), middle (RM), and base part (RB)), with a 60 g portion being used for each experiment (Fig. 1). The first extraction process was conducted with hot water at 100°C for 1 h by autoclaving (MAC-601, Tokyo Rikakikai Co., Ltd., Japan) (designated as RU100, RM100, and RB100). After filtration, the residue was subjected to re-extraction at 110°C for 1 h, followed by filtration (designated as RU110, RM110, and RB110). Finally, the residue from the extraction at 110°C was subjected to re-extraction at 120°C for 2 h, repeated twice, and the supernatant was collected by filtering (RU120, RM120, and RB120). The justification for this is that water at 100°C may not be hot enough to completely extract all of the functional components. A 60 g portion of the antler was also subjected to extraction using 70% ethanol solution for 2 h, which was repeated three times (designated as RUE, RME, and RBE). All recovered extracts were lyophilized on a freeze dryer (FD8508, Ilshin Co., Ltd., Korea) for 5 d.

**Analysis of uronic acid, sulfated-GAGs, and silalic acid contents**

Uronic acid content was determined by the carbazole reaction. Briefly, a 50 µL serial dilution of standard or sample was placed in a 96-well plate, after which a 200 µL solution of 25 mM sodium tetraborate in sulfuric acid was added. The plate was heated for 10 min at 100°C in an oven. After cooling at room temperature for 15 min, 50 µL of 0.125% carbazole in absolute ethanol was carefully added. After heating at 100°C for 10 min in an oven and cooling at room temperature for 15 min, the plate was read using a microplate reader (ELx 808™, BioTek, VT, USA) at a wavelength of 550 nm (Cesaretti et al., 2003).

Sulfated-GAGs content was determined by the dimethylmethylen blue dye binding method (Farrandle et al., 1986). Briefly, the color reagent was prepared by dissolving 0.008 g of DMB in a solution containing 1.185 g of NaCl, 1.520 g of glycine, 0.47 mL of HCl (12 M), and 500 mL of distilled water. Each sample was mixed with 1 mL of color reagent, and the absorbance was read immediately at 525 nm.

Sialic acid content was determined by the method of Warren (1959) with slight modification. Briefly, samples were hydrolyzed in 0.1 N H₂SO₄ in a final volume of 1.0