Journal of Ethnopharmacology 151 (2014) 352–360

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Comparison of chemical compositions and osteoprotective effects of different sections of velvet antler

Sung-Hui Tseng [a,b], Chun-Hsien Sung [c], Lih-Geeng Chen [d], Ying-Jang Lai [e], Wei-Shun Chang [d], Hsin-Ching Sung [f], Ching-Chiung Wang [b,g,*]

[a] School of Medicine, College of Medicine, Taipei Medical University, 250 Wu-Hsing Street, Taipei City 11031, Taiwan
[b] Orthopedics Research Center, Taipei Medical University Hospital, 252 Wu-Hsing Street, Taipei City 11031, Taiwan
[c] Graduate Institute of Pharmacognosy, College of Pharmacy, Taipei Medical University, 250 Wu-Hsing Street, Taipei City 11031, Taiwan
[d] Department of Microbiology, Immunology and Biopharmaceuticals, College of Life Sciences, National Chiayi University, 300 Syuefu Rd., Chiayi City 60004, Taiwan
[e] Department of Food Science, National Quemoy University, 1 Daxue Rd., Jinning Township, Kinmen County 89250, Taiwan
[f] Department of Anatomy, Chang Gung University, 259 Wen-Hwa 1st Road, Kwei-Shan Township, Taoyuan County 33302, Taiwan
[g] School of Pharmacy, College of Pharmacy, Taipei Medical University, 250 Wu-Hsing Street, Taipei City 11031, Taiwan

ARTICLE INFO

Article history:
Received 21 June 2013
Received in revised form
17 October 2013
Accepted 23 October 2013
Available online 7 November 2013

Keywords:
Velvet antler
Ovariectomy
Micro-computerized tomography analysis
Osteocalcin
Alkaline phosphatase
MC3T3-E1 cells

ABSTRACT

Ethnopharmacological relevance: Velvet antlers (VA) have been claimed for centuries to have numerous medical benefits including strengthen bones. To investigate and compare the anti-osteoporotic activities from different sections of VA.

Materials and methods: Fresh VA prepared from farmed sika deers (*Cervus nippon*) was divided into upper (VAU), middle (VAM), and basal (VAB) sections. The chemical constituents and anti-osteoporotic effect of different sections from VA were evaluated using ovariectomized rats.

Results: Levels of water-soluble extracts, diluted alcoholic extract, amino acids, testosterone, insulin-like growth factor (IGF)-1and testosterone plus estradiol significantly differed among the different sections. Levels of these constituents were significantly higher in the upper section than in the basal section. Moreover, levels of testosterone and IGF-1 of the VAM were also significantly higher than those of the VAB. Calcium level increased downward from the tip with statistical significance. The strength of vertebrae increased in all VA-treated groups compared to the control, but only treatment with VAU and VAM increased the strength of the femur and the microarchitecure of the trabecular bone. Alkaline phosphatase levels of VAU- and VAM-treated groups significantly decreased, but osteocalcin did not significantly change. Moreover, VAU and VAM dose-dependently increased proliferation and mineralization of MC3T3-E1 cells.

Conclusion: Our study provides strong evidence for the regional differences in the effectiveness of velvet antler in treating osteoporosis. However, further studies are needed to elucidate the bioactive chemical constituents associated with the anti-osteoporotic effects of velvet antler.

1. Introduction

Osteoporosis is a common disease among aging populations. According to recently proposed clinical practice guidelines for managing osteoporosis, individuals with moderate to high risk of fracture should be offered anti-osteoporotic pharmacologic therapy in order to prevent osteoporosis-related fractures and the associated high health and economic costs (Papaioannou et al., 2010; Reginster, 2011). However, patients' adherence to osteoporosis medications was shown to be poor (Papaioannou et al., 2007; Iversen et al., 2011). Factors associated with non-adherence to osteoporosis therapies include adverse effects experienced by patients taking these medications (Papaioannou et al., 2007). Therefore, scientists are still searching for more-acceptable and

Abbreviations: α-MEM, α-modified minimal essential medium; ALP, alkaline phosphatase; BV/TV, bone volume over the total volume; DPD, deoxypyridinoline; ELISA, enzyme-linked immunosorbent assay; ES, ethinylestradiol; FBS, fetal bovine serum; GC, gas chromatography; HPLC, high-performance liquid chromatography; IGF, insulin-like growth factor; MS, mass spectroscopy; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; OVX, ovariectomy; RLX, raloxifen; SMI, structure model index; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; VA, velvet antlers; VAB, velvet antlers basal section; VAM, velvet antlers middle section; VAU, velvet antlers upper section

* Corresponding author at: School of Pharmacy, College of Pharmacy, Taipei Medical University, 250 Wu-Hsing Street, Taipei 11031, Taiwan.
Tel.: 886 2 27361661x6161; fax: 886 2 27329368.
E-mail addresses: m003089010@tmu.edu.tw (S.-H. Tseng), tim0603kimo@yahoo.com.tw (C.-H. Sung), lgchen@mail.ncyu.edu.tw (L.-G. Chen), lai@kmit.edu.tw (Y.-J. Lai), s0970602@mail.ncyu.edu.tw (W.-S. Chang), hcs@mail.cgu.edu.tw (H.-C. Sung), crystal@tmu.edu.tw (C.-C. Wang).

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http://dx.doi.org/10.1016/j.jep.2013.10.060

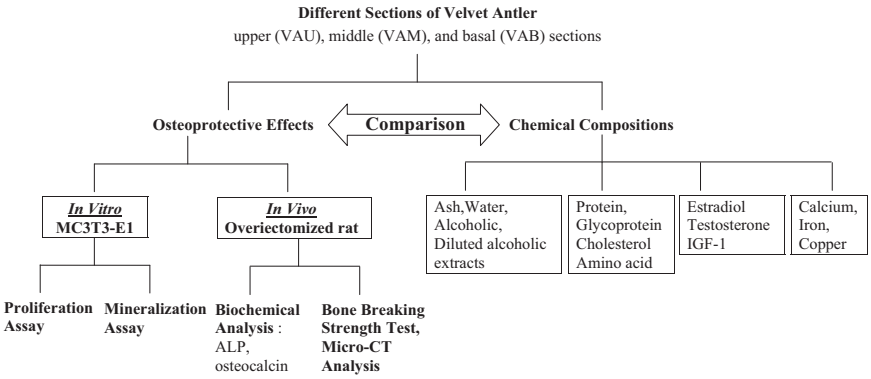

Fig. 1. Flow chart of the experimental design.

safer natural agents to prevent and treat osteoporosis and fragile fractures (Zhang et al., 2009).

Antler extracts have been claimed for centuries to have numerous medical benefits. As a tonic of yang and qi acting through the kidney meridian, Velvet antler (VA), the immature antlers of male deer, is traditionally used in Chinese medicine to replenish the vital essence, strengthen bones, promote virility, nourish the blood, and promote both male and female sexual functions (Kawtikwar et al., 2010). Although the science behind these claims remains lacking, VA-related products showed potential positive effects on modern ailments such as those associated with aging, infection, and immune dysfunction through *in vivo* or *in vitro* studies. However, the mechanisms of actions and the bioactive compounds responsible are mostly not clear (Shao et al., 2012; Dai et al., 2011; Mikler et al., 2004; Kuo et al., 2012; Zhang et al., 2011; Shi et al., 2010).

Concerning benefits of VA on bone health, several preclinical studies have demonstrated that antler related products were effective in alleviating ovariectomy-induced osteoporosis in animals (Li et al., 2010; Yang et al., 2010, Meng et al., 2009). The underlying mechanisms of the effect include facilitation of proliferation and mineralization of osteoblasts (Lee et al., 2011). On the other hand, there are also reports about the inhibitory effect of antler related products on osteoclast differentiation (Choi et al., 2013). Chemical analyses of VA revealed that there are regional differences in chemical composition: the contents of proteins and lipids decrease downward from the tip to the base, while those of ash, calcium, and collagen increase (Jeon et al., 2009). Traditionally, the market values of antlers are downgraded with increasing degree of calcification. However, which parts of VA are suitable for preventing and managing osteoporosis had not been clarified. The modes of action of VA and the ingredients active against osteoporosis also require further elucidation.

The objective of this study was to investigate and compare the anti-osteoporotic activity of VA from different sections using *in vitro* and *in vivo* models in order to elucidate the beneficial effects on the bone mass and bone quality of different sections of VA as a potential therapeutic agent worthy of future development for protecting women from morbidity related to postmenopausal osteoporosis. In accordance with this aim, we designed an experimental flow chart as shown in Fig. 1.

2. Materials and methods

2.1. Materials

Eight VAs were obtained from farmed sika deers (*Cervus nippon*) 75 days after casting kindly provided by the Min-Sheng Deer Farm (Kinmen County, Taiwan, ROC). The antler removal

Fig. 2. Fresh velvet antler (VA) was divided into upper (VAU), middle (VAM) and basal sections (VAB).

procedure was done in accordance with the procedures announced by Council of Agriculture, Executive Yuan, ROC. Briefly, the animals were first minimally restrained. The base of VA was tightened by a tourniquet to alleviate the pain and blood loss of deer. Immediate antler removal was done after local sterilization. Hemostatic agent was applied to the wound immediately after removal. All procedures were under the supervision of veterinarian experienced in velvet antler removal. VAs were then divided into tip, upper (VAU), middle (VAM), and basal sections (VAB) (Fig. 2). The criteria used for dividing the antler into four sections is defined as following: tip section, top 3 cm of each antler beam; upper section, the part of the main beam, without the tip portion, to the 2nd division of a deer's antlers from its head; middle section, the upper half of the remaining main beam; base section, the lower half of the remaining main beam. Sample from upper, middle and base section was sliced with a bone slicer, dried in oven with temperature maintained at 60 °C, and ground into powder. Powdered VA of various sections was immersed in 60% ethanol for 1 month. The extracts were then filtered and freeze-dried. Respective yields of various sections, from upper to basal, were 11.45%, 7.68%, and 5.45%.

2.2. Determination of total ash

Sample powder from upper, middle and base section (2 g) was placed in a porcelain crucible, put in a muffle furnace, and dried at 550 °C for more than 12 h until a constant weight was reached. The ash weight was determined, and the sample was suitably diluted with deionized water and assayed for calcium, iron, and copper by atom absorption spectroscopy.

2.3. Determination of the water content

Sample powder from upper, middle and base section (10 g) was placed in a weighing bottle, kept in a muffle furnace, and dried at 105 °C for more than 5 h until a constant weight was reached. The weight loss represented the water content of the VA.