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梅花鹿鹿筋胶原蛋白提取及生物学评价

Extraction and Biological Evaluation of Collagen Extracted From Sika Deer (Cervus Nippon) Hamstring

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中文摘要

梅花鹿鹿筋胶原蛋白提取及生物学评价

胶原蛋白是胶原的水解产物，具有稳定的三螺旋结构和相对较小的分子质量，降解速度较快、易于被人体吸收。近年来的相关研究证实，胶原蛋白具有多种生物学活性及药理学作用，被广泛应用于食品、化妆品、医药等领域。野生梅花鹿为国家一级保护动物，鹿乡镇大规模人工养殖梅花鹿，并开发其相关产业，梅花鹿的肌肉、筋腱、血液等可作为食品原材料使用，干燥鹿筋是中医学中一味被广泛使用的药材。本文主要以新鲜的梅花鹿鹿筋为原材料，提取胶原蛋白。

目的：以新鲜梅花鹿鹿筋为原料，建立胶原蛋白的提取工艺，并对其生物学活性进行评价，为新型胶原蛋白产品的开发提供实验依据。

方法：分别采用醋酸法和胃蛋白酶法提取梅花鹿鹿筋胶原蛋白，以醋酸浓度、浸提时间、料液比为主要因素，采用正交试验法优化梅花鹿鹿筋酸溶胶原蛋白（DASC）提取工艺，盐析法回收胶原蛋白，应用场发射环境电子显微镜（ESEM）观察梅花鹿鹿筋酸溶胶原蛋白及酶溶胶原蛋白（DPSC）的表面形貌；通过紫外光吸收、傅里叶红外光谱、SDS-PAGE 电泳及单宁酸沉淀试验鉴定所提取的梅花鹿鹿筋胶原蛋白；MTT 比色法检验梅花鹿鹿筋胶原蛋白浸提液对小鼠成纤维细胞 L929 的毒性影响，以兔子为实验对象进行皮肤致敏试验检验梅花鹿鹿筋胶原蛋白粗提取物对皮肤的刺激作用。

结果：通过正交试验优化出本实验条件下梅花鹿鹿筋酸溶胶原蛋白的最佳工艺参数为：醋酸浓度为 0.5mol/L、浸提时间为 36h、料液比为 1:40，盐析法胶原蛋白得率：DASC 为 7.49%，DPSC 为 7.9 %，简化盐析法的 DASC 得率为 7.4%，扫描电镜下观察到从梅花鹿鹿筋中获得的胶原蛋白粗提取物呈现褶皱的片状，片状胶原蛋白间相互连接，构成致密的蜂巢状多空孔结构，酸溶胶原蛋白主要呈现片状结构，纤维状的胶原束结构较少，酶溶胶原蛋白除片状结构外，还富含大量的胶原纤维束；紫外吸收法结果显示DASC及DPSC溶液皆在223nm附近存在最大吸收峰，初步判定所提取的产物是胶原蛋白。傅里叶红外光谱结

果显示 DASC、DPSC 及对照组（牛源酸溶胶原蛋白，CASC）都有 5 个特征峰，分别是酰胺 A、B 带以及酰胺 I、II、III 带，DASC 和 DPSC 特征吸收峰出现在 CASC 特征峰范围内，由此可见本文中梅花鹿鹿筋提取物具有胶原蛋白的三螺旋结构，SDS-PAGE 电泳图显示梅花鹿鹿筋酸溶胶原蛋白和酶溶胶原蛋白中的 α1、α2 链和 β 链完整无缺失，单宁酸试验说明梅花鹿鹿筋胶原蛋白的降解程度较低；DASC 浸提液组、DPSC 浸提液组分别与对照组比较，L929 细胞增殖情况差异无统计学意义，说明 DASC 浸提液、DPSC 浸提液对小鼠成纤维细胞 L929 增殖无影响，皮肤致敏试验结果显示，DASC 及 DPSC 与皮肤接触部位与对照组比较，DASC 组和 DPSC 组均未出现皮肤红斑、红肿甚至结痂形成，说明 DASC 和 DPSC 对皮肤无刺激作用。

结论：本文以新鲜梅花鹿鹿筋为原料，建立并优化梅花鹿鹿筋胶原蛋白的提取工艺，对所提取的胶原蛋白进行鉴定，通过细胞毒性试验和致敏试验证明梅花鹿鹿筋胶原蛋白无细胞毒性及致敏性。

关键词

胶原蛋白，提取，鉴定，生物活性，评价

Abstract

Extraction and Biological Evaluation of Collagen Extracted From Sika Deer Hamstring (Cervus Nippon)

Collagen is a hydrolyzate with a stable triple helix structure and relatively small molecular weight which is degraded faster and absorbed easier by the organism. It has been reported recently that collagen is widely used in the domain of food, cosmetics, pharmaceuticals and so on owing to its biochemical properties and pharmacological actions. The wild sika deer is the first class national protected animal, deer township develops large-scale artificial breeding of sika deer and the related industries such as sika deer's muscle, tendon and blood which can be used as a food material. Dried deer tendon is widely used medicinal herbs in traditional Chinese medicine. In this study, collagen was extracted from fresh tendon of sika deer.

[Objective] To establish collagen extraction process with fresh sika deer tendon and evaluate its biological activity and provide a scientific basis for the development of new collagen products.

[Methods] Acid soluble collagen (ASC) and pepsin soluble collagen (PSC) were extracted from sika deer hamstring (cervus nippon). Concentration of acetic acid, extraction time and the ratio of gardenia to liquor was regarded as the main factors. The extraction technology of acid-solubilized collagen from sika deer (DASC) was optimized by orthogonal experimental design. The collagens were reclaimed by salting out method and the surface topography of DASC and pepsin-solubilized collagen from sika deer (DPSC) was observed by environmental scanning electron microscopy (ESEM). The extracted collagens were identified by ultraviolet spectra, Fourier transform infrared spectrum (FTIR), SDS-PAGE and tannin test. L929 cell viability was detected by MTT assay when exposed to leach liquor of the collagen extracted from the sika deer hamstring (cervus nippon). Skin sensitization test was used to examine the stimulation of DASC and DPSC on the rabbit skin.

[Results] The results showed that the optimum extraction conditions as follows: concentration of acetic acid (0.5 mol/L), extraction time (36 h), the ratio of

gardenia to liquor (1:40). The yields of DASC and DPSC were 7.49% and 7.9% based on dry weight basis with salting out method. The yields of DASC were 7.4% based on dry weight basis with a simpler salting out method. DASC and DPSC took on a plicated and platelike structure under the ESEM, platelike structure was connected with each other and compact porous texture was formed. Much platelike structure existed in DASC. DPSC contained much platelike structure and collagenous fiber bundle. Both ASC and PSC from the sika deer hamstring (cervus nippon) have maximum absorptions near 223 nm, initially regarded the protein as collagen. FTIR spectra of DASC, DPSC and CASC exhibited the characteristic peaks of Amide I, II, III as well as amide A and B. Based on FTIR spectra, the acetic acid and pepsin did not disrupt the triple helical structure of collagen. It was found that the major constituents of both the ASC and PSC consisted of chains α1, α2 and β. Both DASC and DPSC showed a low degradation by tannin test. The cell proliferation of DASC leach liquor group and DPSC leach liquor group showed no significant difference compared with control group. Leach liquor of DASC and DPSC revealed no effect to L929 cells proliferation. Based on Skin sensitization test, no skin irritation was observed after treatment with by DASC and DPSC.

[Conclusion] Collagen extraction process was established with fresh sika deer tendon and the extracted collagen was identified. It showed no cytotoxicity to L929 cells and no skin irritation after treatment with by DASC and DPSC.

Keywords

collagen, extraction, identification, biological activity, evaluation

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