Study on the effect and mechanism of active ingredients of propolis against oxidative damage of cardiomyocytes

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Oxidative damage to cardiomyocytes is one of the important causes of abnormal structure and function of cardiovascular system. Finding and developing natural substances that protect myocardial cells from oxidative damage is a prerequisite for the prevention and treatment of cardiovascular diseases. Propolis is a natural active substance with multiple biological activities, such as antioxidant, antibacterial, anti-inflammatory, anti-tumor, etc.

In this paper, active substances with strong anti-oxidative damage to cardiomyocytes were isolated and screened from propolis, and the mechanism of anti-oxidation was studied to provide a material basis and theoretical basis for the prevention and treatment of cardiovascular diseases.

In this paper, ethanol extraction, chromatographic separation, preparative high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS) were used to separate, purify and identify 6 compounds from propolis. The survival rate of H9c2 cardiomyocytes measured by the CCK-8 method was determined to be 700μmol / L and 4h as H2O2 oxidative damage conditions, and six kinds of propolis were screened for active substances with anti-oxidative damage to cardiomyocytes, including three kinds of coffee The esters of caffeic acid benzyl ester, caffeic acid ethyl phenate (CAPE) and caffeic acid cinnamyl ester have higher antioxidant activity than the two flavonoids chrysin, brevisporin and a phenolic acid3,4 -Dimethoxycinnamic acid (DMCA). Using 1μmol / L, 5μmol / L and 10μmol / L as the three pretreatment concentrations, the mechanism of anti-oxidative damage of cardiomyocytes by benzyl caffeate, CAPE and cinnamyl caffeate was studied from the biochemical and molecular levels. Related kits measured the activity of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA), and the ion ratio / imaging system determined the intracellular free calcium ion concentration ([Ca2 +] i); Annexin V-FITC / PI double staining method combined with flow cytometry to measure the apoptosis rate and semi-quantitative PCR (RT-PCR) method to determine Bcl-2, Bax and caspase 3 (Caspase-3) mRNA expression level.

Results: (1) The MDA content of H9c2 cardiomyocytes induced by H2O2 was significantly increased, the SOD activity and GSH-Px activity were significantly reduced, and it was significantly different from the normal group of cells (P0.01). After pretreatment with 1, 5 and 10 μmpl / L benzyl caffeate, CAPE, cinnamyl caffeate and 5 μmol / L positive control quercetin for 12 hours, the cardiomyocytes were treated with oxidative damage. As a result, all concentrations of test products and positive controls reduced the MDA content to different degrees, increased the activity of SOD and GSH-Px, and showed a dose-dependent effect. (2)

After oxidative injury of H9c2 cardiomyocytes, [Ca2 +] i is significantly higher than that in the normal group (P0.01). After pretreatment with benzyl caffeate, CAPE and cinnamyl caffeate, [Ca2 +] i showed a downward trend, and as the concentration of the test product increased, the greater the decrease. Among them, [Ca2 +] i was only about 2/3 of the injured group after pretreatment with high concentration of each substance. (3) Apoptosis of H9c2 cardiomyocytes was induced by H202 oxidative damage, the mRNA expression level of Bcl-2 in the damaged cells decreased, and the mRNA expression levels of Bax and Caspase-3 increased. After the intervention of benzyl caffeate, CAPE and cinnamyl caffeate, the mRNA expression of Bcl-2 was up-regulated, and the mRNA expression of Bax and Caspase-3 was down-regulated, indicating that benzyl caffeate, CAPE and cinnamyl caffeate can effectively inhibit the induction of oxidative damage Apoptosis. The caffeic acid esters of bee propolis (benzyl caffeate, CAPE and cinnamate of caffeic acid) and flavonoids (alkaline, brevispin) have the effect of protecting oxidatively damaged cardiomyocytes, and the mechanism of action may be through protection Antioxidant enzymes, inhibition of lipid peroxidation, reduction of [Ca2 +] i and improvement of apoptosis are achieved. Its anti-apoptotic effect may be achieved by regulating the expression levels of genes related to apoptosis, such as up-regulating the mRNA expression level of Bcl-2 and down-regulating the mRNA expression of Bax and Caspase-3.

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