A Mixture of Ethanol Extracts of Persimmon Leaf and Citrus junos Sieb Improves Blood Coagulation Parameters and Ameliorates Lipid Metabolism Disturbances Caused by Diet-Induced Obesity in C57BL/6J Mice

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This study investigated the effects of a flavonoid-rich ethanol extract of persimmon leaf (PL), an ethanol extract of Citrus junos Sieb (CJS), and a PL–CJS mixture (MPC) on mice fed a high-fat diet (HFD). We sought to elucidate the mechanisms of biological activity of these substances using measurements of blood coagulation indices and lipid metabolism parameters. C57BL/6j mice were fed a HFD with PL (0.5% (w/w)), CJS (0.1% (w/w)), or MPC (PL 0.5%, CJS 0.1% (w/w)) for 10 weeks. In comparison with data obtained for mice in the untreated HFD group, consumption of MPC remarkably prolonged the activated partial thromboplastin time (aPTT) and prothrombin time (PT), whereas exposure to PL prolonged aPTT only. Lower levels of plasma total cholesterol, hepatic cholesterol, and erythrocyte thiobarbituric acid-reactive substances, hepatic HMG-CoA reductase, and decreased SREBP-1c gene expression were observed in mice that received PL and MPC supplements compared with the respective values detected in the untreated HFD animals. Our results indicate that PL and MPC may have beneficial effects on blood circulation and lipid metabolism in obese mice.

Keywords: Obesity, persimmon leaf, Citrus junos Sieb, blood circulation, lipid metabolism

Introduction

Hemostasis maintains the integrity of the blood vessel system by regulating blood coagulation and platelet activation [6, 9, 29]. During hemostasis, three stages of the response to a blood vessel damage can be recognized. The first stage is the vascular spasm when blood vessels constrict to reduce blood loss. During the second stage, platelets adhere together to form a temporary seal, a platelet plug, to cover the break in the vessel wall. The third and final stage is coagulation or blood clotting [32, 50], which involves formation of a thrombus that prevents any further loss of blood from damaged tissues, blood vessels, or organs [32].

In general, regulation of blood coagulation occurs via extrinsic and intrinsic pathways and involves the fibrinolytic system [9]. Activation of the extrinsic pathway takes place upon extravascular plasma factor VIIa binding to the tissue factor [9]. Activation of the intrinsic pathway involves plasma coagulation factors XIIa, XIa, IXa, and VIIIa [6]. Finally, the two pathways jointly induce formation of factor Xa that catalyzes cleavage of prothrombin yielding active thrombin (FIla), which in turn facilitates transformation of soluble fibrinogen to insoluble fibrin [6, 9].
When an endothelial cell is damaged, activated platelets are aggregated at the site of the vascular injury [34]. Initially, the inner vascular wall promotes the release of α-granules containing protein mediators such as von Willebrand factor and glycoprotein Ib/V/IX in response to collagen [8]. Then, various agents, including adenosine diphosphate (ADP), thromboxane A₂ (TXA₂), and serotonin are released from storage granules in the platelets [8, 34]. In addition, soluble P-selectin (sP-selectin), an adhesion molecule that is upregulated upon platelet activation, is also secreted to regulate platelet-to-platelet cohesion [2, 37].

However, obesity and excessive adipose tissue can result in multiple thrombi due to elevated concentrations of the plasma coagulation factor and upregulated platelet adhesion and activation [35, 45]. Furthermore, obesity can induce reactive oxygen species (ROS) generation as a consequence of oxidative stress and effects of inflammatory mediators, such as macrophage chemoattractant protein-1 (MCP-1) and plasminogen activator inhibitor-1 (PAI-1), and thereby recruit monocytes/macrophages to move toward the damaged endothelial cells [10, 45]. In particular, LDL, oxidized and accumulated by migrated macrophages in the intima of the blood vessel, turns these cells into foam cells, which in turn contribute to the arteriosclerosis process, exacerbate cytotoxicity, and generally manifest pro-inflammatory and pro-coagulant properties [14, 16]. These processes, which take place in narrow arterial walls, disrupt blood flow and cause blood circulation disorders [11, 14, 16].

Persimmon (Diospyros kaki) leaves and Citrus junos Sieb are widely cultivated in East Asia and used in herbal teas and traditional medicine in South Korea, Japan, and China. The persimmon leaf is rich in polyphenols, tannins, phenols, organic acids, chlorophyll, vitamin C, and caffeine. It is commonly used for brewing tea in Asia and is colloquially known as a Chinese herbal medicine. It has been used in traditional medicine in South Korea, Japan, and China. The persimmon leaf is rich in polyphenols, tannins, phenols, organic acids, chlorophyll, vitamin C, and caffeine. It is commonly used for brewing tea in Asia and is colloquially known as a Chinese herbal medicine. It has been used in traditional medicine in South Korea, Japan, and China.

According to these measurements, the dried ethanol extract of the persimmon leaf contained 1.5 mg/g of gallic acid, 1.1 mg/g of catechin, and 0.8 mg/g of epicatechin. The PL yield was 19.6% (3.92 kg/20 kg) after drying. The yield of the CJS was 40% (8.0 kg/20 kg) after drying. The yield of the CJS was 40% (8.0 kg/20 kg) after drying.

Preparation of CJS

Citrus junos Sieb. ex Tanaka was harvested in Goheung County in Korea and the seeds were removed. The fruit flesh and peel were treated with 50% ethanol and then concentrated under reduced pressure. The yield of the CJS was 40% (8.0 kg/20.0 kg) of the total polyphenols.

Preparation of PL

Persimmon (Diospyros kaki Thunb.) leaves were harvested in Cheongdo County in Korea. The leaf constituents were extracted with 50% ethanol and then concentrated under reduced pressure. The PL yield was 19.6% (3.92 kg/20.0 kg) after drying. The concentrations of three major flavonoids (catechin, epicatechin, and gallic acid) in the dried ethanol extract of the leaf were determined by high-performance liquid chromatography (HPLC) using an Agilent 1100 series system (Agilent Technologies, Palo Alto, CA, USA) with an Xbridge C18 column (4.6 × 150 mm, film thickness 5 µm; Waters Corp., Milford, MA, USA) and a two-mobile-phase gradient elution system. Mobile phase A contained water with 2% acetic acid (v/v) and mobile phase B contained acetonitrile. The flow rate was 0.8 ml/min with an injection volume of 10 µl, while ultraviolet detection was performed at 280 nm. According to these measurements, the dried ethanol extract of the leaf contained 1.5 mg/g of gallic acid, 1.1 mg/g of catechin, and 119 mg/g of total polyphenols.

Materials and Methods

Preparation of PL

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