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Pomegranate leaf attenuates lipid absorption in the small intestine in hyperlipidemic mice by inhibiting lipase activity

YU Xuan[1Δ], WANG Xin-Pei[1Δ], LEI Fan[2], JIANG Jing-Fei[1], LI Jun[3], XING Dong-Ming[1*], DU Li-Jun[1*]

[1] *Laboratory of Molecular Pharmacology and Pharmaceutical Sciences, School of Life Sciences, Tsinghua University, Beijing 100084, China;*
[2] *School of Pharmaceutical Sciences, Tsinghua University, Beijing 100084, China;*
[3] *State Key Laboratory of Innovative Drugs and Efficient Energy-saving Pharmaceutical Equipment, Jiangxi University of Chinese Medicine, Nancang 330006, China;*

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[ABSTRACT] Pomegranate leaf (PGL) has a definite role in regulating lipid metabolism. However, pharmacokinetic results show the main active ingredient, ellagic acid, in PGL has lower oral bioavailability, suggesting that the lipid-lowering effect of PGL may act through inhibiting lipid absorption in the small intestine. Our results demonstrated that pomegranate leaf and its main active ingredients (i.e., ellagic acid, gallic acid, pyrogallic acid and tannic acid) were capable of inhibiting pancreatic lipase activity *in vitro*. In computational molecular docking, the four ingredients had good affinity for pancreatic lipase. Acute lipid overload experiments showed that a large dosage of PGL significantly reduced serum total cholesterol (TG) and triglycerides (TC) levels in addition to inhibiting intestinal lipase activity, which demonstrated that PGL could inhibit lipase activity and reduce the absorption of lipids. We also found that PGL could reverse the reduced tight-junction protein expression due to intestinal lipid overload, promote Occludin and Claudin4 expression in the small intestine, and enhance the intestinal mucosal barrier. In conclusion, we demonstrated that PGL can inhibit lipid absorption and reduce blood TG and TC by targeting pancreatic lipase, promoting tight-junction protein expression and thereby preventing intestinal mucosa damage from an overload of lipids in the intestine.

[KEY WORDS] Pomegranate leaf; Lipase activity; Hyperlipidemia; Ellagic acid; Pyrogallic acid

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Introduction

Hyperlipidemia, a form of dyslipidemia, increases the risk of cardiovascular disease [1]. Common causes include diabetes mellitus and medication, but acquired hyperlipidemia can also be closely associated with excessive energy intake, which is called postprandial hyperlipidemia [2]. Whole fruit or the peels of pomegranates (*Punica granatum* L.) have been-reported to have anti-hyperlipidemia, antioxidant [3-4], and antibacterial [5] effects and to provide protection for the gastro-intestinal system from barium chloride [6] and for the liver from injury due to alcohol and chemicals [7-8]; they have also been linked to the chemoprevention of tumorigenesis [10] and anticoagulant and antiplatelet [9] activity, among other roles. A recent study has shown that pomegranate fruits have neuroprotective effects against Alzheimer's disease mediated by ellagitannin-gut-microbial metabolites [11]. The juice, peels, fruit, seeds, and leaves of pomegranates can benefit human health [12]. Phenolic compounds are the active ingredients in pomegranates [13].

Pomegranate peel has been used as a traditional Chinese medicine for centuries to treat acidosis, hemorrhage, diarrhea, helminthiasis, and microbial infections [14-15]. Additionally, Pomegranate leaves (PGL) can modulate lipid metabolism,

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[*Corresponding authors] Tel: 86-10-62796270, E-mail: lijundu@mail.tsinghua.edu.cn (DU Li-Jun); Tel: 86-10-62796270, E-mail: pharm@mail.tsinghua.edu.cn (XING Dong-Ming)
[Δ]These authors contributed to the work equally.
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decrease levels of TC and TG in the serum of hyperlipidemia animals after a long-term, high-fat diet, and inhibit weigh increases in mice [16]. However, the pharmacokinetic results of pomegranate show that the major active ingredient, ellagic acid, has poor absorption [17], which implies that there may be an absorption target during pomegranate administration. In the presnted study, we investigated the effects of PGL on intestinal lipase activity in acute hyperlipidemic mice to look for a potential active target of PGL in lipid metabolism.

Materials and Methods

Animals

Male ICR mice, weighing 20–22 g, were purchased from Vital River Laboratories (Beijing, China). The animals were housed in temperature- and humidity-controlled rooms, kept on a 12 h/12 h light/dark cycle and provided with unrestricted amounts of rodent chow and drinkable water. All experimental procedures were approved by the IACUC (Institutional Animal Care and Use Committee) of Tsinghua University (Approval ID: 15-DLJ1). The laboratory animal facility was accredited by AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care International).

Chemicals

An extract of PGL (Pomegranate leaves) (*Punica granatum* L.), containing 8.3% ellagic acid (EA), 1.49% gallic acid (GA), 0.79% pyrogallic acid (PGA), and 18.27% tannic acid (TA), according to an HPLC assay, and ellagic acid (purity of 98%) were prepared by Dr. XIANG Lan at the Laboratory of Molecular Pharmacology and Pharmaceutical Sciences, Tsinghua University, Beijing, China. The chemical structures and CID numbers in PubChem at GeneBank for EA, GA, PGA and TA are shown in Fig. 1. Tannic acid was purchased from the Yuehai Chemical Plant (Zhejiang, Yuehai, China). Gallic acid was purchased from the China Pharmaceutical Company (Beijing, China). Pyrogallic acid was purchased from the Zunyi Second Chemical Plant (Guizhou, Zunyi, China). Triglycerides (TG), total cholesterol (TC), glucose and lipase assay kits were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Porcine pancreatic lipase (PPL, type II >100 U·mg^{-1}) and orlistat (> 98%) were purchased from Sigma Aldrich (Shanghai, China).

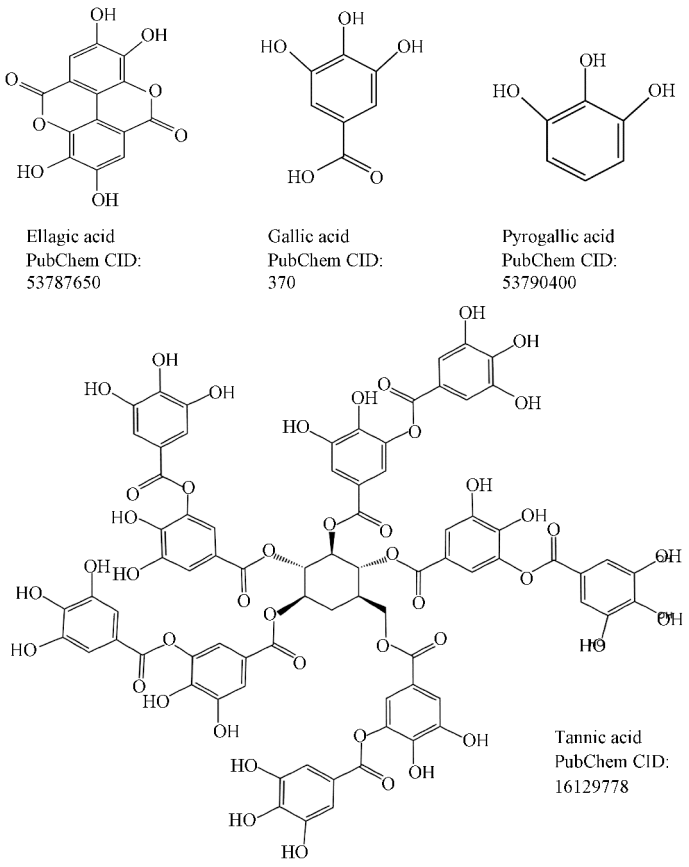

Fig. 1 Chemical structures of ellagic acid, gallic acid, pyrogallic acid and tannic acid in the extracts of pomegranate leaves

The inhibition of lipase activity in vitro

Lipase activity was determined according the instructions of the Lipase Activity Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The lipase activity index is shown as U/mg protein.

To determine lipase inhibitory activity, TA, GA, PGA, EA, and orlistat at different concentrations were pre-incubated with PPL for 10 min. After pre-incubation, a lipase substrate