

Research Progresses of Plant Polysaccharides with α -Glucosidase Inhibition activities

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Abstract. Diabetes has become one of the major diseases threatening human health, and has become the main economic burden of the public health system. Alpha-glucosidase inhibitors are the first choice for the treatment of type II diabetes. However, the widely used AGI in clinical practice often has varying degrees of side effects. Plant polysaccharide is a kind of active substance that widely exists in plants and has great potential to alleviate diabetes. Screening of plant polysaccharides with hypoglycemic effects has attracted much attention due to efficient health care ability and good biocompatibility. This article focused on plant polysaccharides with α -glucosidase inhibitory activity, systematically elaborated on the hypoglycemic effect of plant polysaccharides, and provided a detailed review on the effects of extraction and purification methods on the physicochemical properties of plant polysaccharides and the activity of α -glucosidase inhibitors, as well as the researches on improving the AGI inhibitory abilities of plant polysaccharides through modification. This article aims to provide reference for the research and development of novel plant-based α -glucosidase inhibitors.

Keywords: Diabetes mellitus, α -glucosidase, plant polysaccharides, hypoglycemic effects, α -glucosidase inhibitors.

1. Introduction

Diabetes mellitus (DM), characterized by hyperglycemia, is a metabolic disorder caused by either insulin secretion lack, or/and impaired insulin action, of which type 2 diabetes accounts for 90%~95%. Nowadays, the global incidence of diabetes is increasing year by year. According to the data announced by the World Health Organization and the International Diabetes Federation at 2021, the number of DM reached 537 million and was expected to exceed 783 million (12.2%) in 2045, as for China the number will be 175 million in 2045 [1, 2].

Blood sugar management is essential to efficient to prevent and slow down the development of DM [3]. Alpha-glucosidases is the key enzyme to regulate postprandial blood glucose. Alpha-glucosidase inhibitor (AGI) can block the decomposition of linear or branched-chain oligosaccharides into glucose, so it is considered to be the most effective remedy to control postprandial hyperglycemia and diabetic complications in diabetes patients [4]. Nowadays, AGIs have become a kind of important hypoglycemic drugs in the clinical treatment of type 2 diabetes. However, the current clinically used AGIs often lead to some adverse side effects, such as flatulence and diarrhea, as well as a variety of complications. Therefore, many studies are devoted to obtaining natural AGIs with low side effects and high activity from natural plants [5].

Active component extracts from natural plants have been shown to effectively reduce blood glucose levels through a variety of mechanisms, including activating insulin receptors, regulating liver glucose release, stimulating insulin secretion and inhibiting carbohydrate digestion and glucose absorption [6]. Polysaccharides are widely found in plants, and more and more plant polysaccharides have been proved with antidiabetic activities. The hypoglycemic mechanism of plant polysaccharides include protecting pancreatic β -cells, enhancing insulin sensitivity, reducing starch digestion, increasing anti-inflammation, regulating lipid metabolism and intestinal microbiota, and so on [7]. Many plant polysaccharides have been proved to inhibiting α -glucosidase activity efficiently and being considered as an important source for screening AGIs. In this paper, the mechanism of plant polysaccharides on hypoglycemic function in recent years was reviewed, and the effects of extraction,

purification and modification methods on α -glucosidase inhibitory activity of plant polysaccharides were systematically summarized.

2. Alpha-glucosidase and α -glucosidase inhibitors

Alpha-glucosidases are a kind of key enzymes in the human body for hydrolyzing carbohydrates, which widely exist in microorganisms, plants and animals and are closely related to the level of blood glucose in patients with diabetes. They are located in the epithelial cells of the small intestine and participate in biochemical reactions in the body, hydrolyzing the non-reducing ends of disaccharides (maltose and sucrose) to produce absorbable monosaccharides (glucose and fructose) [8]. Therefore, α -glucosidase is very important for the metabolism and digestion of carbohydrates and is regarded as a key enzyme in regulating postprandial blood glucose in clinic and taken as one of the targets for the treatment of DM [9].

Alpha-glucosidase inhibitor has been used as the most effective drug to control postprandial hyperglycemia and diabetic complications in patients with diabetes since it can reduce postprandial blood glucose by inhibiting enzyme activity and consequently reducing carbohydrate absorption in the upper small intestine and delaying glucose absorption [10]. However, at present, the normally used AGIs in clinic, such as acarbose, voglibose and miglitol, generally have the problems like complex preparation process, high production cost, serious side effects. In particular, there is growing evidence that the side effects and toxicity seriously harm the health of patients, such as abdominal distension, diarrhea and other serious gastrointestinal discomfort symptoms [11]. Therefore, it is very important to constantly look for more efficient, low-cost, multi-target and low-side-effect AGIs.

3. Hypoglycemic effect of plant polysaccharides

At present, a large number of plants with hypoglycemic effect have been proved to be an important source of AGIs, and have been widely concerned by researchers all over the world. For example, the active components from Chinese wolfberry, balsam pear, mulberry leaves, wolfberry, corn beard, yam, kelp, purslane, purple potato, etc., all have significant α -glucosidase inhibitory effects. Plant polysaccharides have aroused the interest of researches world widely because of their non-toxic, highly diversified structure, a variety of biological activities and functional properties [12]. For example, mulberry leaf polysaccharide can reduce the levels of free fatty acids and inflammatory mediators in diabetic rats, reduce the damage of oxidative stress, improve the mitochondrial function of islet cells and protect islet β -cells [13]. And in recent years, studies have shown that plant polysaccharides combined with other active substances could show stronger hypoglycemic effects [14]. Plant polysaccharides have become the important sources for screening natural α -glucosidase inhibitors. Pan obtained a homogeneous polysaccharide CIBP from *Glycyrrhiza inflata* Batalin, which was composed of galactose, arabinose and mannose. The α -glucosidase activity inhibition rate was positively correlated to the concentration of CIBP, which was 64.77% at 6mg/mL of CIBP [15]. Yang et al tested the inhibition of α -glucosidase and α -amylase of polysaccharides extracted from *Urtica fissa*. The results showed that *Urtica fissa* polysaccharides could reduce insulin resistance by affecting the glucose consumption of IR-HepG2 cells, and regulate glucose and lipid metabolism by increasing HK and competitive content, promoting glycogen synthesis and reducing TG content [16].

4. Effect of extraction methods on AGI activity of plant polysaccharides

Extraction is an essential operation to obtain polysaccharides from plants. Different extraction methods have potential and significant effects on the composition and structural characteristics of plant polysaccharides, and then affect their biological activities. On the other hand, polysaccharides from different plants have different composition and structures, correspondingly different biological activities. Various extraction strategies have been used in order to obtain plant polysaccharides with high biological activity. The traditional extraction methods of plant polysaccharides are mainly based

on different solvents. Hu et al isolated and purified four polysaccharides from mulberry leaves (MLP) by water extraction and alcohol precipitation. All the MPLs were composed by rhamnose, arabinose, xylose, mannose, glucose and galactose according to different ratios. While, only MLP-1 component could significantly inhibit α -glucosidase activity in a non-competitive manner, and the inhibition rates of the other three components on α -glucosidase were 0 [17]. Zheng et al proposed that the concentration of ethanol solution would significantly change the surface morphology of *Sargassum fusiforme* polysaccharides (SFPS) when extracted under different pH and precipitated with different concentrations of ethanol solution. Moreover, the inhibitory activity of α -glucosidase was dependent on the concentration of extracted pH and precipitated ethyl group [18].

In recent years, new extraction methods have been utilized for improving the extraction efficiency and α -glucosidase inhibitory activity of plant polysaccharides, such as enzyme extraction, ultrasonic-assisted extraction, microwave-assisted extraction, ultra-high pressure extraction, aqueous two-phase extraction, etc. Luo et al. reported that when extracted *Pleurotus ostreatus* polysaccharides by different methods, the addition of cellulase would result in the highest polysaccharides content, as well as strong inhibitory effect on the activities of α -amylase and α -glucosidase [19]. Meanwhile, the use of compound enzymes would further improve the extraction rate of plant polysaccharides. Zhang et al found that the compound enzyme composed of cellulase, pectinase and papain could make the yield of laminarin reach $18.19\% \pm 1.04\%$, and had obvious inhibitory effect on α -glucosidase. It was major because cellulase and pectinase could jointly decompose cellulose and pectin in the cell wall, while papain decomposes macromolecular proteins into water-soluble glycosides [20].

It was found that high temperature and different polar solvents destroyed more cell wall structure by ultrasonic extraction, resulting in the release of more conformational polysaccharides, including polysaccharides with triple helix structure, and these triple helix structures can give polysaccharides higher biological activity. Meanwhile, ultrasonic extraction of polysaccharides could promote intracellular components into the solvent, accelerate the mutual penetration and dissolution of cell components and solvents, so as to improve the solubility of plant polysaccharides in solvents [21]. The *Lycium barbarum* polysaccharides LBP-2 prepared by cold water extraction and boiling water reflux extraction contained triple helix structure and galacturonic acid, and the content of rhamnose was significantly higher than that of *Lycium barbarum* polysaccharides prepared by other extraction methods [22]. Xiao et al found that the yield of *Lycium barbarum* polysaccharides extracted by microwave extraction was higher than that by ultrasonic extraction, alkali extraction and hot water extraction [23]. Aqueous two-phase extraction technology had the advantages of fast mass transfer, short phase separation time, high selectivity and mild extraction conditions. This method has the advantage of removing a large number of soluble impurities and reducing the harm to human health. Therefore, it also widely used in the extraction of plant polysaccharides. Chu et al. studied the difficulty degree of adding 13 different inorganic salts to 15 types of ionic liquids to form a dual aqueous phase phenomenon. Then the selected ionic liquids that had the ability of easily forming excellent dual aqueous phase performance was used for the extraction of mulberry leaf polysaccharides and obtained high extraction effect [24].

5. Effect of purification methods on the AGI activity of plant polysaccharide

The purification method can also significantly affect the biological characteristics of the prepared plant polysaccharides. Wen et al. used deproteinization, ultrafiltration, and column chromatography sequentially to purify the *Huidaba* polysaccharides that extracted by hot-water. Inhibitory activities of the purified *Huidouba* polysaccharides (HDBP-d, HDBP-1, and HDBP-2) on α -glucosidase were positively correlated with concentration and negatively correlated with temperature. All the polysaccharides could form HDBPS- α -glucosidase complex to achieve static fluorescence quenching. However, the inhibition types of the three polysaccharides were different [25]. Peng et al. obtained crude apricot kernel extraction (CAE) by hot water extraction, then deproteinized to obtain crude polysaccharide CAP, and further purified on DEAE-52 and SephadexG-100 columns finally to obtain

neutral polysaccharide (AP-1). The inhibition rate of different polysaccharides on α -glucosidase expressed as acarbose > AP-1 > CAP > CAE. The relatively high AGI activity of AP-1 was owing to the higher content of α -(1-4) glycosidic bonds and it has the same inhibition rate as acarbose at 5 mg/mL concentration [26].

6. Modification to improve the AGI activity of plant polysaccharides

Through the molecular modification of polysaccharides, not only different structural types of polysaccharide derivatives can be obtained, but also the physicochemical properties and biological functions of polysaccharides may be changed. At present, the reported modification methods of polysaccharides include carboxymethylation, phosphorylation, sulfation, acetylation and selenylation. Carboxymethylation and acetylation of jujube polysaccharides decreased the inhibitory activity of jujube polysaccharides on α -amylase, but enhanced their inhibitory activity on α -glucosidase, especially carboxymethylation modification. Zhao et al. reported that the selenized *Ribes nigrum* L. polysaccharides (PRCP) had significantly higher inhibitory activities on α -amylase and α -glucosidase than those of natural polysaccharides. They proposed that it may be because the selenization modification would lead to the decrease of molecular weight and the increase of uronic acid content of PRCP. In addition, the presence of selenium after selenization modification was beneficial to the binding of polysaccharides to the active sites of α -amylase and α -glucosidase. The PRCPs showed competitive inhibition on α -amylase and α -glucosidase before and after selenization, and could form non-fluorescent carnosic acid glycosidase complexes with α -amylase / α -glucosidase. It also caused the conformational transformation of α -amylase / α -glucosidase, which made the microenvironment around the binding site changed from hydrophilic to hydrophobic [27]. Chen et al. modified *Grifola frondosa* polysaccharides to obtain S-GFP, which showed stronger inhibitory effect on α -glucosidase than acarbose, and the type of inhibition was non-competitive inhibition. The results of inhibition mechanism showed that S-GFP quenched the endogenous fluorescence of α -glucosidase and changed the skeleton structure of the protein. Circular dichroism (CD) analysis showed that S-GFP changed the conformation of α -glucosidase, increased the content of α -helix structure, and destroyed the hydrogen bonding system of the enzyme [28]. Hu et al conducted the selenization modification of *Huaishan yam* polysaccharide (CYP) to obtain the selenium-polysaccharide from *Huaishan yam* (Se-CYP). The α -glucosidase inhibition rate of Se-CYP was higher than that of CYP at the same concentration, because selenium existed in Se-CYP could promote the α -glucosidase inhibitory activity. In addition, polysaccharide functional groups and trace elements could form a stable complex, thus enhancing its activity [29]. Song et al chelated garlic polysaccharides (GP) with Cr (III) and successfully prepared GP-Cr (III). The GP-Cr (III) complex significantly increased the inhibitory effect on α -glucosidase and showed a more significant blood glucose control effect than GP in the form of selenium ester [30].

7. Conclusion and prospects

The high incidence rate of diabetes has aroused widespread concern all over the world, and has rapidly become one of the most common and high-cost chronic diseases. Alpha-glucosidase, as the main target enzyme for the prevention and treatment of T2DM, has been widely used in clinical practice. However, its application is limited due to the potential serious side effects. Screening α -glucosidase inhibitors with high efficiency and low side effects from natural plants has been a hot research topic. More and more researches have confirmed the role of plant polysaccharides in the treatment of diabetes. However, the problems of low efficiency, high cost of separation and purification, and lack of efficacy research have hindered the rapid development of plant polysaccharides in the development of therapeutic drugs for diabetes. Therefore, there are still significant challenges in the clinical application of plant polysaccharides as natural α -glucosidase inhibitors in terms of large-scale production, pharmacological researches and clinic application. In

the future, more in-depth researches should be conducted on developing new extraction, separation, purification, and concentration methods, thoroughly investigating the structure-activity relationship between the composition and structure with AGI efficacy of plant polysaccharides, meanwhile, conducting more reasonable and comprehensive evaluation of the hypoglycemic effect of polysaccharides.

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