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Amylase inhibitors: Potential source of anti-diabetic drug discovery from medicinal plants

Kathirvel Alagesan[1]*, Prem Krishnan Raghupathi[2] and Sadasivam Sankarnarayanan[3]
1, Department of Biochemistry, University of Oulu, Oulu, Finland
2, Faculty of Life Sciences, University of Copenhagen, Denmark
3, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, Tamilnadu - India

Abstract

Diabetes mellitus is a metabolic disease characterized by hyperglycaemia resulting from either defect in insulin secretion, insulin action, or both. Though, there exists many therapeutics for the treatment of diabetes, plant based drugs are generally considered safe and are much effective. However, traditional usage of medicinal plants for the treatment of diabetes lacks scientific validation. Enzyme inhibitors have potential role in many areas of disease control and treatment and this review focuses on the plants which possess outstanding anti-diabetic property which could be possibly investigated further for the presence of alpha-amylase inhibitor.

Key-Words: Enzyme inhibitors - Diabetes – Medicinal plants

Introduction

Diabetes mellitus can be described as a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism due to defects in insulin secretion, insulin action, or both (Adolfo et al., 2008). According to International Diabetes Federation, around 194 million people live with diabetes and the epidemiological estimates that by 2025 there will be 333 million diabetes sufferers worldwide.

Types of diabetes

The three main types of diabetes are
- Type 1 diabetes
- Type 2 diabetes
- Gestational diabetes

Type I Diabetes

Type I diabetes is an autoimmune disease. An autoimmune disease results when the body's system for fighting infection (the immune system) turns against a part of the body. In diabetes, the immune system attacks and destroys the insulin-producing beta cells in the pancreas. The pancreas then produces little or no insulin. At present, scientists do not know exactly what causes the body's immune system to attack the beta cells, but they believe that autoimmune, genetic, and environmental factors, possibly viruses, are involved. It develops most often in children and young adults but can appear at any age.

Symptoms of type I diabetes usually develop over a short period, although beta cell destruction can begin years earlier. Symptoms may include increased thirst and urination, constant hunger, weight loss, blurred vision, and extreme fatigue. If not diagnosed and treated with insulin, a person with type I diabetes can lapse into a life-threatening diabetic coma, also known as diabetic ketoacidosis.

Type II diabetes

Type II diabetes mellitus is an increasingly common disorder of carbohydrate and lipid metabolism two important characteristics of this disease are insulin resistance, the failure of peripheral tissues; including liver, muscle, and adipose tissue, to respond to physiologic doses of insulin, and failure of pancreatic β-cells to properly secrete insulin in response to

* Corresponding Author
E-Mail: Kalagesa@paju.oulu.fi
kathir.alagesan@gmail.com
Telephone: +35 (8) 449650342

elevated blood glucose levels. Obesity is a significant risk factor for the development of type II diabetes mellitus. An extremely lean and lipoatrophic models have revealed a similar predisposition to developing diabetes. Although it may seem paradoxical that both increased adiposity and severely reduced fat mass cause diabetes, a common pathophysiologic process in fat may be responsible for the predisposition to develop hyperglycemia in both conditions (Nadler and Attie, 2001).

Broadhurst proposed the major causative factors for Non-Insulin Dependent-Diabetes Mellitus (NIDDM) involving obesity and over fatness; carbohydrate and fat over nutrition; lack of polyunsaturated fatty acids (PUFA) in plasma membranes and unbalanced triglyceride intake; chromium deficiency; and lack of soluble fiber and relevant beneficial phytochemicals (Broadhurst et al., 1997).

NIDDM is a complex disease that is currently thought to be influenced by more than a single gene or environmental factor. Although the relative contribution of genetic and environmental factors to the development of NIDDM differs among individuals, patients generally have two common metabolic abnormalities: insulin resistance and defects in glucose-stimulated insulin secretion, which lead to disease state.

Gestational Diabetes
Some women develop gestational diabetes late in pregnancy. Although this form of diabetes usually disappears after the birth of the baby, women who have had gestational diabetes have a 20 to 50% chance of developing type II diabetes within 5 to 10 years. Maintaining a reasonable body weight and being physically active may help prevent development of type 2 diabetes.

As with type 2 diabetes, gestational diabetes occurs more often in some ethnic groups and among women with a family history of diabetes. Gestational diabetes is caused by the hormones of pregnancy or a shortage of insulin. Women with gestational diabetes may not experience any symptoms [NIH, 2006, http://diabetes.niddk.nih.gov/dm/pubs/overview/index.aspx].

Treatment for type ii diabetes
Present oral therapeutics available for the treatment of type II diabetes can be broadly classified into following categories based on their mode of action (Wadkar et al., 2008; Li et al., 2004)

- Sulfonylureas- improve insulin production.
- Metformin (Biguanide class)- regulates inappropriate release of glucose by liver and corrects the insulin resistance.
- Thiazolidinediones(glitazones)- attenuates insulin resistance.
- α-amylase inhibitor inhibits starch digestion in intestine.
- α-glucosidase inhibitor inhibits digestion of maltose and related oligosaccharides.

Amylase inhibitors – potential therapeutic target
Amylases (a-1,4-glucan-4-glucanohydrolase, EC 3.2.1.1) are a group of enzymes which catalyze the hydrolysis of the (a-1,4) glycosidic linkages in starch and various other oligosaccharides.(Heidari et al., 2005). Enzyme inhibitors can be a potential target in many areas of disease control and treatment, as enzymes catalyze the most important biochemical pathways. Controlled kinetics of carbohydrate digestion and monosaccharide absorption could be of great value in the avoidance of conditions such as diabetes, obesity, hyperlipoproteinaemia and hyperlipidaemia. In this aspect, amylase and glucosidase inhibitors are of particular important (Kim and Nho., 2004).

Main objective of this review is to give an insight into medicinal plants with outstanding anti-diabetic property which could be further pharmacologically exploited for the presence of low molecular weight amylase inhibitors.

Most of the commercially available amylase and glucosidase inhibitors are of microbial origin. Though these drugs help in maintaining constant level of glucose in blood by delaying the breakdown of starches, but however their usage have been limited due to their side-effects such as flatulence and diarrhea due to colonic fermentation of non-absorbed sugar. Small molecular weight inhibitors below 250, which can be absorbed appreciably from the gut into the bloodstream, are arousing great interest as therapeutic agents (Mahmoud Najafian et al., 2010 ; Kim & Nho., 2004 ; Mioko et al., 2001). When compared to the microbial counterparts, amylase inhibitors from medicinal plant are considerably safe and effective. Among 1200 plants which possess hypoglycemic property, only 30% of the anti-diabetic plants have been pharmacologically tested and investigated (Ayyanar et al., 2008).

Plant constituents with anti-diabetic activity
Dietary fibers, flavonois, alkaloids, saponins, amino acids and peptides isolated from medicinal plants have been reported for their hypoglycemic activity either by insulin release from pancreatic ß-cells, inhibition glucose absorption in gut or increased glucose utilization by the body (Li et al., 2004; Saravanan and

Leelavinothan, 2006; Modak et al., 2007). However the discussion has been limited to flavones and triterpenoids as most of the low molecular weight anti-diabetic compounds fall under these categories.

Flavones

The phenolic fractions of plants have long been recognized to inhibit carbohydrate hydrolyzing enzymes in mammals. Phenols such as quercetagetin, fisetin, and quercetin, belonging to the class of flavonoid, have been shown to be inhibit mammalian alpha-amylase with IC50s in the order of lM (Mahmoud Najafian et al., 2010). Likewise phenolic compounds derived from red cabbage, strawberries and raspberries have been reported to inhibit of α- amylase and α- glucosidase (Mc Dougall et al., 2005).

Flavonoids such as apigenin-7-O-glucoside have been reported to inhibited α-amylase activity. Luteolin, myricetin and quercetin flavonoids were reported as potent inhibitors of porcine pancreatic α-amylase with the IC50 values less than 500 µM1 (Karthic et al., 2008).

Mai & chuyen., (2007) have reported that polyphenols present in plants play an role in the regulation of carbohydrates hydrolyzing enzymes. Glycosides, flavanoids, tannins and alkaloids have been reported to possess hypoglycemic activities (Kumar et al., 2009). Inhibition of α-amylase by natural flavonoids and have reported that Quercetin has much more inhibitory activity when compared to that of apigenin (Tadera et al.,2006). Similar results has been observed by Torres-Piedra et al., (2010) who studied about the oral antidiabetic activity of flavonoids in diabetic rats and reported that quercetin being the most effective anti-diabetic agent.

Flavones isolated from Cirsium japonicum DC have been reported to possess anti-diabetic activity by regulating the activities of the enzymes involved in glucose metabolism thereby resulting in improved glucose and lipid homeostasis (Liao et al., 2010).

Triterpenoids

Pentacyclic triterpenoids, oleanolic acid, ursolic acid and lupeol have been shown to inhibit α-amylase by Hasenah Ali et al., (2006). Furthermore, they have reported that 2:1 mixture of oleanolic acid and ursolic acid exhibit maximum inhibitory activity towards α-amylase inhibitor with $IC_{50}=2.01\mu g/ml$ (4.41µM). Tan et al., (2008) reported that Cucurbitane triterpenoids characteristic constituent of bitter melon possess anti-diabetic activity.

Plants with anti-diabetic property

Psidium guajava

The leaves of *P. guajava* have been known to inhibit/control the increase in sugar levels in plasma during glucose tolerance test in alloxan induced diabetic rats. Various parts of *P. guajava* have been used for the treatment of diabetes. Methanolic extract of P. guajava leaves showed hypoglycaemic effect in type 2 diabetes (Rai et al., 2007).

Aqueous extract of *P. guajava* leaves is known to possess good effect to lowering the blood glucose. Active constituents were found to be flavonoid glycosides such as strictinin, isotrictinin and pedunculagin, which are being used in clinical treatment of diabetes to improve insulin sensitivity. Glycoprotein with a molecular weight of 50,000-100,000 isolated from the aq. Extract of leaves also possesses anti-diabetic activity (Li et al., 2004).

Syzygium cumini

Presence of alkaloids, amino acids, steroids and triterpenoids in thyl acetate and methanol extracts of *S. cumini* seed extract revealed in initial phytochemical screening. *S. cumini* seed have been known to possess various medicinal properties apart from being anti-diabetic activity such as anti-inflammatory and analgesic activities (Kumar et al., 2009).

Mycaminose, isolated from *S.cumini* seed extract possess anti-diabetic property whose mechanism of action is much more similar to that of Glibenclamide - drug which stimulate insulin secretion from pancreatic β cells. It was also found that Ethyl acetate and methanol extract of *S.cumini* seed possess antidiabetic effect in STZ-induced diabetic rats. Further comprehensive chemical and pharmacological investigations are needed to elucidate the exact mechanism of the observed hypoglycemic effect due to *S.cumini* seed (Kumar et al., 2008).

Betulinic acid and 3,5,7,4`-tetrahydroxy flavanone isolated from *S. cumini* have been reported to inhibit alpha-amylase (Karthic et al.,2008) .

Andrographis lineata

Shade dried leaf powder taken orally along with cow's milk or goat's milk has been used traditionally to treat diabetes, however there is no proper scientific evidence to support its hypoglycemic activity. Further investigation may result in the development of good oral therapeutics for diabetes. Dosage: 2 teaspoon of powder is taken twice a day after food for 2-3 months (Ayyanar et al., 2008).

Abrus precatorius

Chloroform – methanol extract of *A. precatorious* Linn seed have been known to reduce blood glucose levels in diabetic rats and hypoglycemic activity exhibited by the extract is to a little extent more potent than chlorpropamide - class of sulfonylurea drug used for the treatment of diabetes. The possible mechanism for the reported blood glucose reduction by the extract

may be attributed towards the binding of fat soluble extract to the receptor sites especially the peroxisome proliferator, which plays a major role in the regulation of glucose metabolism. Further investigations are needed to elucidate the lead component(s) present in this extract (Monago & Alumanan., 2005).

Costus speciosus
Oral administration of crude extracts of hexane, ethyl acetate and methanol of *C.speciosus* have known to reduce blood glucose level in STZ induced diabetic rats significantly. Further it was found that, hexane crude extract is much more effective in decreasing the serum glucose level and normalizing the other biochemical parameters in diabetic rats and being more potent than ethyl acetate and methanol extracts. Evidences from other researchers suggested that the rhizome of *C.Speciosus* could be of greater help in protection and alleviation of complications associated with diabetes (Daisy et al., 2008).

Nelumbo nucifera
Oral administration of the ethanolic extract of Nelumbo nucifera rhizomes have been reported to reduce blood glucose level in healthy, glucose-fed hyperglycemic and streptozotocin- induced diabetic rats when compared to control rats (Bnouham et al., 2006 ; Li et al., 2004).

Catharanthus roseus
Ethanolic extract of *C. roseus* have been known to lower the blood glucose level in alloxan induced hypoglycemic rats. The leaves seem to have a promising value for the development of potent phytomedicine for diabetes and have shown to reduce total cholesterol and triglyceride levels. Possible mechanism by which the extracts lowered blood glucose levels in diabetic rats may be due to increase glycogenesis, inhibiting gluconeogenesis in the liver, or inhibiting the absorption of glucose from the intestine. However, further comprehensive pharmacological investigations are needed to elucidate the exact mechanism of the antihyperglycemic and antihyperlipidemic effect of *C.roseus* (Akhtar et al., 2007).

Ethanolic extract of leaf and flower have been reported to decreases blood glucose levels. Recent studies have shown that aqueous extract decreases blood glucose levels about 20% in diabetic rats; whereas dichlromethane and methanol extract decreases blood glucose levels to 49%-58% significantly better than that of controls (Eric yarnell).

Punica granatum
Administration of the aqueous-ethanolic (50%, v/v) extract of *P.granatum* flowers have been shown to decrease the glycaemia in normo-glycemic, glucose-fed hyperglycaemic and alloxan induced diabetic rats significantly (Bnouham et al., 2006).

Traditionally male abortive flowers of *P.granatum* have been used for the treatments of diabetes. Seed extracts of *P.granatum* has also been reported to possess anti-diabetic activity and it has been hypothesized that ursolic acid may be the active constituent (Li et al., 2004).

Butea frondosa
Butea frondosa (Syn: monosperma) is traditionally used for the treatment of diabetes in Indian medicine. Aqueous extract of bark have been known to reduce glucose levels significantly in normal and alloxan induced diabetic mice. Preliminary phytochemical screening revealed the presence of saponin glycosides, tannins and proteins; further extensive research can bring out an outstanding medicine for diabetes (Deore et al., 2008).

Ocimum sanctum
Aqueous extract *O.sanctum* leaves have been showed to reduce blood glucose level in control and alloxan induced diabetic rats to a greater extent and also uronic acid, total amino acid, total cholesterol, triglyceride and total lipid concentration were reduced significantly. Decrease in plasma glucose level by 9.06 and 26.4% on 15 and 30 days of the experiment was observed when plant extract were for administrated orally for 30 days. Moreover, this plant is known to possess a broad range of activity against in controlling bacterial, fungal growth and to a greater extent antiviral property, antistress, antitumor, antiulce

Conclusion
As point out earlier in this review, only about 30% of the plants having anti-diabetic property have been pharmacologically tested and investigated. Moreover, out of the plants investigated, it is necessary to find out the lead compound responsible for the reported activity. Drug discovery from plant has always been challenging as it involves a multi- displiniary research which begins with with a botanist, ethnobotanist, ethnopharmacologist, or plant ecologist who collects and identifies the plants of interest, latter phytochemists involved in the preparation of plant extract and screening them for phytochemicals, and isolating and characterizing the active compound. Overview of developing plant based drug has is shown in *Fig 1*.

Natural products, in general, are typically isolated in very small quantities which are not sufficient enough for lead optimization or clinical trials. Therefore it is necessary to carryout collaborative research so that lead compounds are developed either by synthetic or

semi-synthesic process. Methodological approach and use of modern approaches like computer aided drug discovery, creation of natural product libraries can help to overcome the challenges and in easy development of plant based drug which are safe and much more effective than their counterparts.

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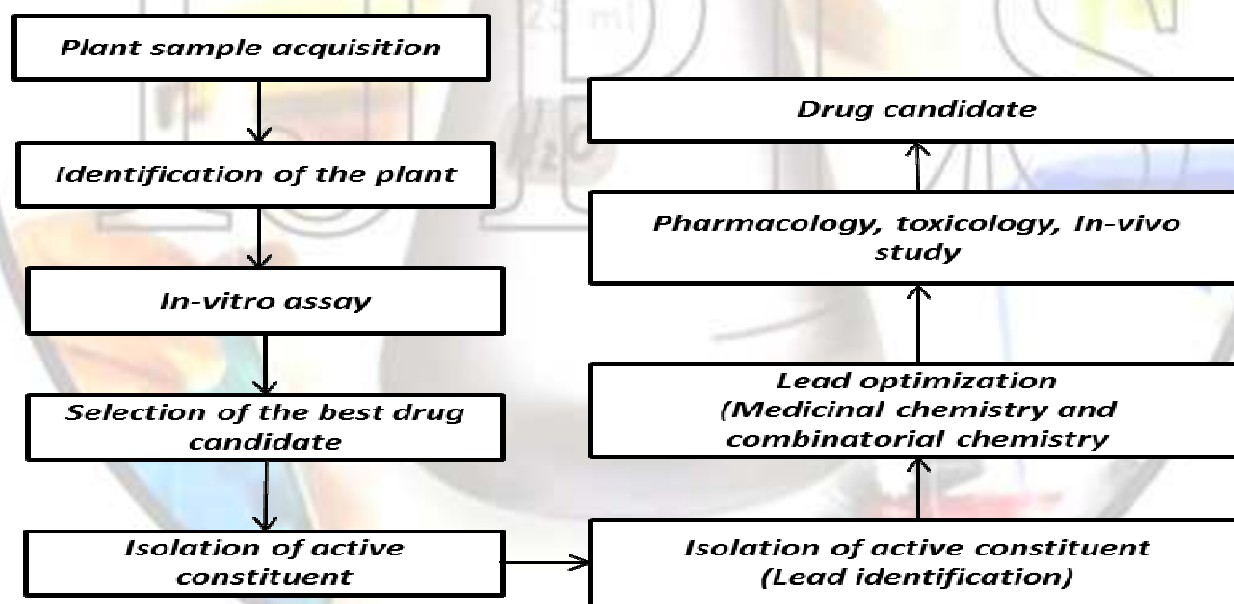
Fig 1: Overview of Drug discovery from plant source