Antidiabetic effects of natural plant extracts via inhibition of carbohydrate hydrolysis enzymes with emphasis on pancreatic alpha amylase

Abstract

Importance of the field: The increasing prevalence of type 2 diabetes mellitus and the negative clinical outcomes observed with the commercially available anti-diabetic drugs have led to the investigation of new therapeutic approaches focused on controlling postprandrial glucose levels. The use of carbohydrate digestive enzyme inhibitors from natural resources could be a possible strategy to block dietary carbohydrate absorption with less adverse effects than synthetic drugs.

Areas covered in this review: This review covers the latest evidence regarding *in vitro* and *in vivo* studies in relation to pancreatic alpha-amylase inhibitors of plant origin, and presents bioactive compounds of phenolic nature that exhibit anti-amylase activity.

What the reader will gain: The state of the art of the search for new pancreatic alpha amylase inhibitors of plant origin for the treatment of type 2 diabetes mellitus.

Take home message: Pancreatic alpha-amylase inhibitors from traditional plant extracts are a promising tool for diabetes treatment. Many studies have confirmed the alpha-amylase inhibitory activity of plants and their bioactive compounds *in vitro*, but few studies corroborate these findings in rodents and very few in humans. Thus, despite some encouraging results, more research is required for developing a valuable anti-diabetic therapy using pancreatic alpha-amylase inhibitors of plant origin.

Keywords: alpha-amylase; flavonoids; proanthocyanidins; tannins; type 2 diabetes mellitus

1. Introduction

Diabetes mellitus is one of the world´s major diseases, with an estimation of 347 million adults affected in 2011 (1). Type 2 diabetes mellitus, by far the most common type, is a metabolic disorder of multiple etiology characterized by carbohydrate, lipid and protein metabolic disorders that includes defects in insulin secretion, almost always with a major contribution of insulin resistance (2). These abnormalities could lead to lesions such as retinopathy, nephropathy, neuropathy, and angiopathy (3). In this context, the inhibition of carbohydrate digestive enzymes is considered a therapeutic tool for the treatment of type 2 diabetes (4). The most important digestive enzyme is pancreatic alpha-amylase (EC 3.2.1.1), a calcium metalloenzyme that catalyzes the hydrolysis of the alpha-1,4 glycosidic linkages of the starch, amylose, amylopectin, glycogen, and various maltodextrins and is responsible of most of starch digestion in humans. A second enzyme named alpha-glucosidase or maltase (EC 3.2.1.20) catalyzes the final step of the digestive process of carbohydrates acting upon 1,4-alpha bonds and giving as a result glucose (4).

A positive correlation between human pancreatic alpha-amylase (HPA) activity and the increase in postprandial glucose levels has been shown, demonstrating the relevance of suppressing postprandial hyperglycemia (PPHG) in the treatment of type 2 diabetes (5). The ability of the alpha-amylase enzyme inhibitors to avoid dietary starch to be digested and absorbed in the organism has allowed to designateing these compounds as starch blockers. However, only a mild pancreatic alpha-amylase inhibition activity is recommended in order to prevent the abnormal bacterial fermentation of undigested carbohydrates in the colon as a result of an excessive inhibition of this enzyme, which results in flatulence and diarrhea (6). Currently, there are some antidiabetic drugs that act mainly by inhibiting carbohydrate digestion and absorption. Acarbose (BAY g 5421) was the first alpha-glucosidase inhibitor available for diabetes treatment. This inhibitor of microbial origin inhibits the activities of alpha-amylase, sucrase and maltase. Voglibose is a newer alpha-glucosidase inhibitor of bacterial origin that inhibits the activities of isomaltase, sucrase and maltase, whereas miglitol is a 1-deoxynojirimycin derivative that strongly inhibits glucoamylase, sucrase and isomaltase activities (7,

8). Although efficient in maintaining postprandial blood glucose levels under control in many patients, the administration of these drugs is often associated to serious gastrointestinal adverse effects (7). Moreover, the adverse clinical outcomes associated with the commercially available anti-hyperglycemic compounds are in one part responsible for the prevalent medication nonadherence that occurs in diabetic patients (9). The prominent side effects of such drugs have driven for seeking alternative therapies with less severe or no side effects. In this sense, herbal compounds appear to offer gentler means of managing metabolic disorders and, since ancient times, have been used in traditional medicine systems like Chinese, Indian ayurveda and Arabic unani (10). Therefore, evidence for a putative beneficial effect of a wide range of medicinal plants exists in relation to type 2 diabetes therapy (11). In addition to their effectiveness, herbal remedies seem to produce hardly adverse effects and present an economical alternative to the oral synthetic hypoglycemic agents. In 1990, World Health Organization (WHO) recommended that rigorous research on these plant beneficial effects should be performed (12). In this sense, the present review summarizes the studies concerning the alpha-amylase inhibitory activity of natural plants and their bioactive compounds that could be useful in the treatment of diabetes. For this purpose, a quasi-systematic review was conducted using Medline (PubMed) and ISI Web of Knowledge. Search terms included the combination of phrases like “amylase inhibitor”, “antidiabetic properties” “plant extracts” “pancreatic amylase” and all recent publications concerning pancreatic amylase (porcine and human) inhibitory activity of natural plant extracts were incorporated.

2. Antidiabetic plants used in traditional medicine

There is increasing interest to report evidences about the efficacy and safety of specific herbs and natural dietary supplements that have been used for treating diabetes in traditional medicine. In fact, many of the currently available drugs have been directly or indirectly derived from plants. Nevertheless, the evaluation of alpha-amylase inhibitory activity is not only limited to traditional herbs (13-16) or spices (17, 18), but also to diverse food extracts (19, 20).

Within the large number of plants have been used in traditional Chinese medicine for diabetes therapy (21), some of them have been approved by the Chinese health regulatory agency for their commercial use in China (22). That is the case of the total flavonoids from *Polygonatum odoratum*, that have been reported to exert an anti-diabetic effect in streptozotocin (STZ)-induced diabetic mice and alloxan-induced diabetic rats by inhibiting alpha-amylase activity (23).

In Japan and Korea there is also an ancient tradition in the use of herbal therapies for the treatment of diabetes , as has been demonstrated with the oral administration of the phlorotannins isolated from the brown alga *Ecklonia cava*, particularly dieckol, in streptozotocin-induced diabetic mice (24). Also different Malaysian and Indonesian plants traditionally used in the treatment of diabetes have been analyzed in relation to their inhibitory effect on amylase, with *Phyllanthus amarus* hexane extract (25) and *Phyllanthus urinaria* methanol extract (26) as the most promising.

In Ayurvedic Indian tradition, over 800 plants have been reported to be potential antidiabetic drug sources (27). Some of them exert an inhibitory action on pancreatic alpha-amylase, such as some of the 126 extracts isolated from 17 Indian medicinal plants that were analyzed by Ponnusamy *et al* (28), who concluded that isopropanol extracts from *Linum usitatisumum*, *Morus alba* and *Ocimum tenuiflorum* were the most potent (≥50% inhibition). When the same research group studied 91 extracts isolated from 11 Ayurvedic Indian medicinal plants, 10 of them (isolated from *Curcuma longa, Cinnamonum verum, Ficus bengalensis*, *Syzygium cumini*, *Bixa orellana*, *Murraya koenigii* and *Tribulus terrestris*) inhibited HPA (29). In other study that analyzed the effects of six ethnobotanical plants used to treat diabetes in Ayurveda, *Murraya koenigii* and *Ocimum tenuiflorum* were identified as the best inhibitors of pancreatic and glucosidase enzymes (30).

The traditional Arabic medicine is still practiced and may provide effective new compounds for treating diabetes and other diseases, as the 69 plant species that are reported in a review concerning diabetes treatment in Jordan (11). Some of these plants have been demonstrated to inhibit amylase and

glucosidase activities *in vitro* and *in vivo*, including *Geranium graveolens* and *Varthemia iphionoides* (31), *Pistacia atlantica*, *Rheum ribes* and *Sarcopoterium spinosum* (32).

In contrast to Chinese, Indian or Arabic living "great traditions", in other parts of the world the indigenous systems of medicine have become "little traditions "(33). Despite that, some of the plants used in Africa to treat diabetes are able to inhibit intestinal alpha-amylase and glucosidase activities, such as *Euclea undulata*, *Pteronia divaricata* and *Elaeodendron transvaalense* (34) or *Spondias mombin* (35).

3. Pancreatic alpha amylase inhibitory activity *in vitro*

In the following section the potential pancreatic alpha- amylase inhibitory activity of the most commonly used plants is mentioned. A summary of the plant extracts reported to display a pancreatic alpha-amylase inhibitory activity is provided in **tables 1** and **2**.

3.1. *Olea europaea* L. (Oleaceae)

Olea europaea L. (olive) is abundant in phenolic compounds, especially the leaves (36, 37) and the extra virgin olive oil (VOO) whose beneficial properties are attributed in part to the phenolic fraction (38). In fact, olive oil has been used for the treatment of diabetes since ancient times (37). Some of the phenolic compounds occurring in olives could act by inhibiting carbohydrate-hydrolyzing enzymes. In this sense, the *in vitro* pancreatic alpha-amylase inhibitory activity of olive leaves and their potential hypoglycemic effect were evaluated by Komaki *et al*. (39), showing that the hot water extract (OWE) and the ethanol extract (OEE) presented IC_{50} values against HPA of 70.2 mg/ml and 0.02 mg/ml, respectively. When different doses of olive leaf powder and different quantities of the isolated compounds were administered to GK/Jcl diabetic rats, a significant reduction in blood glucose levels was observed (39). Moreover, a significant decrease in glycemic values was achieved after cooked rice was given together with olive leaves to prediabetic volunteers, which was not observed in normoglycemic subjects. With the aim of identifying the major antidiabetic compounds, the OEE was

further studied and the IC_{50} values of luteolin glucosides (luteolin-7-*O*-β-glucoside and luteolin-4´-*O*-β-glucoside) and oleanolic acid were determined (39). In addition, the inhibitory activity of oleuropein´s aglycon moiety also showed a strong inhibitory capacity (IC_{50}= 0.03 mg/ml). In another trial conducted by Loizzo *et al* (40) pancreatic alpha-amylase inhibitory activity was detected in eight extracts from virgin olive oil. However, in all cases, the effects on alpha-amylase were weaker than on alpha-glucosidase enzyme.

3.2. *Castanea sativa* Mill. (Fagaceae)

Different health benefits have been attributed to *Castanea sativa* (chestnut) intake, including a preventive role in cardiovascular diseases and a reduction in the risk of type 2 diabetes mellitus and metabolic syndrome (41). The positive effects achieved with increased chestnut consumption could be associated to its high content in organic acids and phenolic compounds (42). Concerning the relevance of chestnut for diabetes treatment, chesnut astringent skin (CAS) extract has been studied as an amylase inhibitor (43). In this study, CAS was able to retard carbohydrate absorption and to reduce PPHG in diabetic rats (GK/jcl) by specifically inhibiting alpha-amylase activity. Moreover, it was suggested that polyphenols could be the main active compounds of the CAS extract. Thus in db/db mice, this research group observed that CAS extract not only delayed carbohydrate absorption and reduced PPHG by inhibiting the alpha-amylase enzyme, but also prevented the increase of body weight and fat mass (44).

3.3. *Allium* species: *sativum, cepa, Akaka* and *porrum* (Alliaceae)

The antidiabetic effects of *Allium* species (including garlic, *A. sativum* L. and onion, *A. cepa* L.) have been repeatedly studied *in vivo* by many authors (45). In this sense, Nickavar and Yousefian (15) reported alpha-amylase inhibitory potential of four *Allium* species (*A. akaka*, *A. sativum*, *A. porrum* and *A. cepa*), which showed IC_{50} values of 16.74 mg/ml, 17.95 mg/ml, 15.73 mg/ml and 16.36 mg/ml, respectively. In a recent study (46) the porcine pancreatic alpha- amylase (PPA) inhibitory activity of the ethyl alcohol extract (EOS) of a Korean onion skin was determined (IC_{50} of > 3mg/ml) and

compared to that of the major phenolic compound present in onion, quercetin, which showed an IC_{50} value of > 0.60 mg/ml. Nevertheless, both extracts were more effective in the inhibition of alpha-glucosidase, especially sucrase, and the inhibitory potential was further confirmed *in vivo*, suggesting the relevant contribution of *Allium* phenolic phytochemicals to achieve the anti-hyperglycemic effect.

3.4. *Salvia acetabulosa* L. (Lamiaceae)

Various species of the genus *Salvia* (sages) have been used as folk medicine to treat diabetes- induced hyperglycemia (47). The ability of nine plant species to inhibit carbohydrate digestive enzymes was analyzed by Loizzo *et al*. (48), who reported that the methanol extract of *S. acetabulosa* contained 80 mg/ml of phenolic compounds and strongly inhibited PPA (IC_{50} value of 91.2 µg/ml). A weaker activity was found in the n-hexane extract against the same enzyme (IC_{50}= 212.0 µg/ml).

3.5. *Ocimum basilicum* L. and *Ocimum tenuiflorum* L. (Lamiaceae)

Ocimum basilicum (basil) is a common culinary herb widely used in many traditional medicines. Health beneficial effects include bactericidal, anti-inflammatory, antioxidative, antiulcer, hypolipidemic, radiation protective and hypoglycemic (49, 50). In relation to the latter, different *in vitro* studies have analyzed the PPA inhibitory activity of *Ocimum basilicum* extracts. Thus, aqueous leaf extracts were reported to have a strong alpha-amylase inhibitory action with an IC_{50} value of 42.50 mg/ml (14). This inhibitory action was attributed to the high total polyphenol (146 mg catechin/g dry extract) and flavonoid (44 rutin/g dry extract) content of the extract. In another trial (17), the PPA inhibitory activity of aqueous and methanol extracts of different spices, including basil, was measured. *O. basilicum* exerted 20% inhibition (1 mg dry spice/100µl of aqueous extract), with aqueous extract being more effective than the methanolic one apparently due to its higher content in tannins. Furthermore, the isopropanol extract of *O. tenuiflorum* L., a species of *Ocimum* commonly used in Ayurveda, has been reported to produce a strong PPA inhibitory activity (53.4%) at a concentration of 0.0094 mg/ml. Alkaloids, tannins and flavonoids were identified in the extract as putative candidates for this effect (28).

3.6. *Theobroma cacao* L. and *Theobroma grandiflorum* L. (Malvaceae)

Theobroma cacao L. (Cocoa tree) is a polyphenol-rich food that contains monomeric flavanols ((-)-epicatechin and, in lower quantities, (+)-catechin, (+)- gallocatechin and (-)-epigallocatechin) which are found in a 10% of the total content and oligomeric and polymeric C4β-C8 linked B type procyanidins, as well as, anthocyanins and other flavanol glycosides, that could be found in a 90% of the total content (51).There are numerous studies describing the health benefits of cocoa in animal models, including antiobesity and antidiabetic actions (52, 53). The bioactive compounds occurring in cocoa have been reported to reduce blood glucose levels (54). In an effort to elucidate whether cocoa extracts and their procyanidins displayed an inhibitory action against carbohydrate digestive enzymes, three cocoa extracts (from regular, lavado, and Dutch-processed cocoa powder) were analyzed (55) and an inhibitory activity against alpha-amylase of 25, 20 and 10% was determined at a concentration of 200 μg/ml for each extract. The inhibitory potential of cocoa procyanidins was observed to be dependent on their degree of polymerization (DP), achieving higher inhibitory percentages (17-45.5%) with higher DPs (DP= 5-10) and lower inhibition (< 15%) with smaller DPs (DP < 5). In other study in which the *in vitro* alpha-amylase inhibitory activity of polyamide-purified phenolic extracts of different Brazilian plants were analyzed, *Theobroma grandiflorum*, commonly known as cupuaçu, exerted the most potent inhibition (2.5-9 times higher than other extracts) followed by cambuci fruit and pana frozen pulp (56).

3.7. *Curcuma longa* L. (Zingiberaceae)

The perennial herb *Curcuma longa*, commonly known as turmeric, is a medicinal plant widely cultivated in tropical regions of Asia. Most of the studies have been focused on its major active principle called curcumin and the yellow pigmented fraction of *C. longa*, which contains curcuminoids chemically related to curcumin (57). In this context, the antihyperglycemic effects produced by these compounds have been investigated (58) and the alpha-glucosidase inhibitory activity of curcuminoids and their analogs have been remarked (59) as well as the inhibitory potential of the water-soluble

protein fraction of turmeric rhizome (60). Isopropanol and acetone extracts of *Curcuma longa* L were among the eleven Ayurvedic Indian medicinal plants that were recently screened for their pancreatic alpha-amylase inhibitory activity (29), presenting an IC_{50} value of 0.16 µg/ml and 7.4 µg/ml, respectively. Podocarpic acid, curlone and cinnamic acid were considered as the major compounds within the isopropanol extract, whereas curlone, 3-cyano-7-hydroxyl-4-mehylcoumarin, and 5-amino-2-hydroxybenzoic acid were suggested as the major compounds in the acetone extract.

3.8. *Cinnamomum verum* Presl. and *Cinnamomum cassia* Nees (Lauraceae)

Cinnamon, ground from the bark of *Cinnamomum verum*, and *C. cassia* have been shown to present blood glucose lowering properties in animal models and diabetic patients (61). Different active constituents, such as the water-soluble polyphenol type-A polymers (62), cinnamaldehyde (63) and cinnamic acid (64) have been reported as candidates for these effects. The extracts of several *Cinnamomum* species have been reported to exert PPA inhibitory actions (65), including the leaves of *C. verum*, whose isopropanol and acetone extracts, containing 1,2,3,4-tetrahydro-1,1,6-trimethyl naphthalene, eugenol, and 4-acetoxycinnamic acid as major components, were observed to be the most efficient (IC_{50} value of 1.0 µg/ml) compounds (29).

3.9. *Camellia sinensis* (L.) Kuntze (Theaceae)

There are a great deal of publications on green, oolong, and black tea, but also on catechins and (-)-epigallocatechin gallate (EGCG), the most abundant catechin in tea, in relation to their protective role against cancer, obesity, diabetes, and cardiovascular diseases (66). Regarding diabetes, conflicting data exist about hypoglycemic potential of different tea extracts (67). However, many authors have reported not only a blood glucose lowering and insulin sensitizing effect of tea plant extracts (68-70), but also of its bioactive compounds (71, 72) that have been evidenced to inhibit alpha-amylase enzyme activity.

3.10. *Vaccinium corymbosum* L. (Ericaceae) and other berries

A relationship between berry phenolic phytochemicals and health beneficial effects has been demonstrated (73). In this context, the antidiabetic effect of various members of *Vaccinium* species has been reported (74). In some studies, the alpha-amylase inhibitory activity of berry extracts has been proposed as a possible mode of action for the glycaemic control. Thus, fifteen blueberry cultivars of *Vaccinium corombosum* (75) have shown *in vitro* alpha-amylase inhibitory activities ranging from 91.8 to 103.3 %, which correlates well with the total phenolic content of the cultivars (r= 0.85). In other study, polyphenol-rich extracts of diverse berries were tested against alpha-amylase enzyme showing that raspberries (*Rubus idaeus*) and rowanberries (*Sorbus aucuparia*) were the most effective ones (IC_{50} values of 4.5 and 21.0 μg of gallic acid equivalent/ml). From the phytochemical analysis performed in raspberry extracts, it was concluded that low levels of anthocyanins did not substantially affect the amylase inhibitory activity, but high levels of ellagitannins (ET) did either brighten the amylase inhibitory activity. In contrast, the amylase inhibitory activity of rowanberry extracts was shown in the proanthocyanidins-rich fraction (76) supporting previous studies where proanthocyanidins (PAs) were described as potent alpha-amylase inhibitors (77). Similar to these findings, a study of McDougall *et al*., (78) described the strawberry and raspberry extracts as the most potent alpha-amylase inhibitors and pointed out to a mixture of ETs and ellagic acids as the major inhibitory compounds.

3.11. *Aloe vera* (L.) Burm.f. (Asphodeloideae)

Aloe vera has been used for many centuries due to its curative properties (79). Type 2 diabetes mellitus and hyperlipidemia, for instance, have been orally treated by the administration of this plant (80). However, reports regarding the *in vivo* antidiabetic effects of *A. vera* preparations are conflicting, with several studies demonstrating blood glucose lowering effects (81) but other investigations achieving different outcomes depending on the plant species, part of the plant (leaf pulp, leaf skin, leaf gel, leaf juice), mode of preparation and the diabetic model used (82). Nevertheless, the results obtained in two

nonrandomized clinical trials (n= 40 and n= 76) showed an improvement in fasting blood glucose levels after 6 weeks of treatment with aloe gel juice (83, 84). In a recent study that investigated 126 extract derived from 17 plants (28), *A. vera* extract, especially the cold water and the cyclohexane extracts, showed PPA inhibitory activity (23.3 and 15.8% of PPA inhibition at concentrations of 2.5 mg/ml and 2.4 mg/ml, respectively).

3.12. *Origanum vulgare* L. (Lamiaceae)

Origanum vulgare (oregano) has been used in traditional medicine systems of many countries, and its properties have been attributed to the presence of different phenolic compounds (85), especially rosmarinic (RA) and caffeic acids (86). Some of these polyphenols, such as flavonoids and proanthocyanidins, have been described to possess amylase inhibitory activity, providing a chance to control hyperglycemia in diabetes (87, 88). In this context, in a research work conducted by McCue and Shetty (89), the PPA inhibitory activity of lemon balm, oregano extracts (containing 50% and 7% of RA) and purified RA (97%) was analyzed. Curiously, the oregano-based extract (7% of RA) showed an alpha-amylase inhibitory activity similar to that of the lemon balm extract, which contained fairly higher content of RA. As a possible explanation, mechanistic synergies among different phenolic compounds were hypothesized. In other study performed by the same group, eight of the eleven clonal lines of oregano extracts analyzed were reported to display approximately 33% of PPA inhibition, observing a positive relationship between some of the oregano extracts and their phenolic content. However, RA, which was the major compound identified within all samples, was not reported to correlate well with the anti-amylase activity of seven of the tested extracts (19).

3.13. *Rosmarinus officinalis* L. (Lamiaceae)

Rosmarinus officinalis (rosemary) is a spice Mediterranean plant with strong antioxidant activity (90) that has been reported to possess antidiabetic properties (91). In this sense, rosemary clonal extracts have been demonstrated to exert alpha-glucosidase inhibitory activity (92), although no alpha-amylase inhibitory capacity was found in the same herb extracts. In contrast, in a recent study (17) that

analyzed the pancreatic alpha-amylase inhibitory activity of different spice extracts, rosemary aqueous extract exerted the most effective inhibitory activity (approximately 30%). In addition, the aqueous extracts of Lamiaceae species in this study (rosemary, sage and basil) were the richest in phenolic compounds.

3.14. *Moringa oleifera* (Moringaceae)

Moringa oleifera (Drumstick tree) is a tropical tree with many potential pharmacological actions including the treatment of diabetes mellitus (93). In fact, aqueous extract from *M. oleifera* leaves have been reported to reduce blood glucose levels in normolglycemic and diabetic rats (94). In a research work in which the PPA inhibitory activities of 11 medicinal plants were measured, *M. oleifera* showed a 16% of inhibitory activity (95).

3.15. *Phaseolus vulgaris* (Fabaceae)

Proteinaceous alpha-amylase inhibitors are especially abundant in common bean (*Phaseolus vulgaris*) (96). From the three isoforms of alpha-amylase inhibitor (alpha-A1, alpha-A2 and alpha-A3) that have been described in beans, the alpha-AI, which is found in the cotyledons and embryonic axes, are able to inhibit HPA (97). Several *in vitro* studies have demonstrated the amylase inhibitory activity of different compounds that, as phaseolamin (specific for animal alpha-amylases), have been isolated from white kidney beans (98). In this context, the use of kidney bean extracts as alpha-amylase inhibitors for obesity and diabetes treatment has been discussed in different reviews (99, 100) and a great body of research has gone into the use of some extracts, specifically Phase 2®, which is a water extract of *P. vulgaris* that is commercialized as a dietary supplement (97).

3.16. *Triticum aestivum* (Poaceae) and other cereals

Alpha- amylase inhibitors have been found in cereals including wheat (*Triticum aestivum)*, barley (*Hordeum vulgareum),* sorghum (*Sorghum bicolor)*, rye (*Secale cereal)* and rice (*Oriza sativa)* (101).

Wheat (*Triticum aestivum*) amylase inhibitors which were isolated and characterized by Maeda *et al.* (102), have been the most studied inhibitors within cereals (103). Interestingly, a higher inhibitory activity has been reported for wheat amylase inhibitor against HPA compared to the white bean amylase ones, which could be related to a larger carbohydrate malabsorption (104). Three families (60, 24 and 12 kDa) of wheat amylase inhibitors have been distinguished (105). The 0.19 amylase inhibitor, a member of the 24 kDa family, has been extensively studied as an inhibitor of PPA, human saliva and pancreas amylase enzyme (98, 106).

4. Phenolic phytochemicals

Phenolic compounds or polyphenols are secondary metabolites widespread in the plant kingdom and found in diverse quantities in usually consumed fruits, vegetables, beverages and grains (107). These numerous phenolic phytochemicals are classified depending on their ring structure and the number of carbon atoms that substitute the ring. Phenolic structure of these compounds could chemically differ from being simple molecules (e.g phenolic acids with a unique ring structure), presenting 2-3 phenolic rings (biphenyls and flavonoids), or being polymers of 12-16 phenolic groups, such as proanthocyanidins (PAs) (108).

Phenolic phytochemicals, apart from presenting several industrial applications, have been revealed to exert health beneficial effects, especially in chronic-oxidation-linked disorders such as cardiovascular disease, obesity and diabetes (109, 110). One of the mechanisms by which these compounds may produce a blood glucose lowering response is their inhibitory effect on carbohydrate digestive enzymes, particularly on alpha-glucosidases (especially sucrase and maltase) and pancreatic alpha-amylase (89, 111) (see **Table 3**). Although some studies have reported an interesting alpha-amylase inhibitory activity in addition to the most common alpha-glucosidase inhibitory effect (112, 113), it is more common to find natural plants possessing stronger inhibitory activity against alpha-glucosidase than on alpha-amylase, which could be attributed to the different phytochemicals present (72, 114-120). In this sense, several studies have found a direct correlation between the amount of phenolic

compounds in plant extracts and their capacity to inhibit carbohydrate digestive enzymes (19, 25, 89, 92, 112, 121). However, not always plant extracts with the highest phenolic content have been demonstrated to exert the strongest inhibitory activity on alpha-amylase (122), which points out to the importance of the nature of the different molecules and the interactions among them. Furthermore, several studies have confirmed the relevance of the extraction method, finding differences in the inhibitory yield of the tested extracts (4, 123-125).

4.1. Flavonoids

Flavonoids are the most common group of polyphenolic compounds. They are present in considerable quantities in common food products, spices, and beverages (107) and have been used since ancient times to treat a great variety of human diseases including diabetes (126). Their structure consists of two moieties: benzopyran (A and C rings) and phenyl (B ring) groups. Depending on the C ring type and to the linkage between the benzopyran and phenyl groups, six groups of flavonoids have been catergorized: flavones, flavonols, flavanones, isoflavones, flavanols (or flavan-3-ols), and anthocyanidins (127).

Some of the mechanisms by which these compounds exert their antidiabetic effects have been reported by Kim *et al.* (87). In this work, twenty-two flavonoids were evaluated for their inhibitory activity against yeast alpha-glucosidase and PPA *in vitro*. Among all of them, isoflavones (genistein and daidzein) and luteolin showed the lower IC_{50}. Moreover, luteolin was more efficient than acarbose in inhibiting alpha-amylase (IC_{50} of about 0.5mg/ml). In contrast, in another study conducted by Matsui *et al*. (128) different results were elicited, concluding that the flavonoids they tested did not have enough power to delay or inhibit the release of glucose in the gastrointestinal track. Tadera *et al.* (129) investigated the inhibitory activity of six groups of flavonoids on digestive enzymes and observed the relationship between the structures of the A, B and C rings with their inhibitory potential. Regarding the inhibition of PPA, luteolin, myricetin and quercetin were the most powerful compounds, showing IC_{50} values of 0.36, 0.38 and 0.50 mM, respectively. This study established a relationship between the

inhibitory activity and the increasing number of hydroxyl groups on the B ring (Flavonols: myrecetin > quercetin > kaempferol. Flavones: luteolin > apigenin). Nevertheless, differences between the amino acids that form the diverse alpha-amylases, porcine and human, should be considered and more studies are required to investigate the effects of flavonoids on human alpha-amylase and the mechanisms of action. In a different study, water and ethanol extracts of *Varthemia iphionoides* (Asteraceae) were tested against PPA by two different methods named 2-chloro-4-nitrophenil alpha-maltotrioside degradation (CNP-G_3) and iodo-starch. The extracts from aerial parts of the plant showed a pronounced inhibitory effect (>70%) with the first method, whereas a weaker inhibitory effect (14.8 and 21.2%) was achieved with the second one (130). Seven 3-methoxyflavones and an eudesmane sesquiterpene were isolated and their inhibitory capacity evaluated. The authors concluded that those compounds possessing more than three hydroxyl groups presented the highest inhibition against PPA. Therefore, the amount of hydroxyl groups contained in the flavonol structure was demonstrated to affect the inhibitory potential of these compounds (130).

Another recent study (56) has reported that, in order to attain higher pancreatic alpha-amylase inhibitory activity, not only the amount of flavonoids is important, but also their molecular nature. In this study, a potent PPA inhibitory activity was found for the polyamide-purified phenolic extracts of *Campomanesia phaea* (Myrtaceae), *Theobroma grandiflorum* (Sterculiaceae) and *Annona crassifolia* (Annonaceae), which showed IC_{50} values of 1.0, 1.1 and 1.3 mg of sample dried weight/ml of reaction, respectively. In addition, rutin, quercetin, chlorogenic acid and catechins, occurring in these plants, were investigated with the purpose of characterizing their inhibitory potential against PPA. Quercetin was reported as the most potent compound (IC_{50}= 0.9 μM) while chlorogenic acid was the least efficient (IC_{50}= 3.9 μM) (56).

It is worth mentioning that the presence of phenolic compounds in *Camellia sinensis* (Theaceae) fractions, but particularly flavonoids, confers this herb the highest inhibitory potential against alpha-amylase when compared to other plants like *Trigonella foenum-graecum* (Leguminosae) and *Urtica dioica* (Urticaceae) (70). Fruits and leaves of *Schizandra chinensis* (Schisandraceae) contain high

levels of anthocyanins, which could be related with its favorable health properties (131). Thus, when water extracts of Omija pulp/skin and seed extracts were evaluated against PPA activity, both extracts were able to inhibit the enzyme (74% and 2%, respectively) at a concentration of 1mg/ml (132).

Catechins are a group of flavonoids belonging to the flavan-3-ol class that are present in fruits, vegetables and wine but especially in tea and cocoa (133, 134). They are able to reduce dietary carbohydrate bioavailability via reduction of glucose uptake, by inhibiting intestinal glucose transporters (135) or pancreatic alpha-amylase, as observed for the ethyl acetate fraction of the Nepalese medicinal herb *Bergenia ciliata* (Saxifragaceae) (136). This study identified (-)-3-*O*-galloylepicatechin and (-)-3-*O*-galloylcatechin (with IC_{50} values of 739 and 401 μM, respectively) as the compounds responsible for this inhibition.

4.2. Tannins: Proanthocyanidins and hydrolysable tannins

Hydrolysable tannins (gallotannins and ellagitannins) are a group of phenolic metabolites of relatively high molecular weight that, besides antioxidant properties, could present the ability to strongly complex with carbohydrates and proteins (137). These compounds are common in Grossulariaceae and Rosaceae plants (i.e., berries) and some of them are able to inhibit alpha-amylase enzyme activity, including casuarictin, tellimagranadin I and II, and rugosin A and D, all of them isolated from *Rosa gallica* petals (138). In a study performed by Li *et al*. (139), six ellagitannins designated as rubusuaviins A-F, and seven tannins called pedunculagin, 1(β)-O-galloyl pedunculagin, strictinin, sanguiin H-5, lambertianin A, sanguiin H-6 and 1-desgalloyl sanguiin H-6, all of them isolated from the dried leaves of *Rubus suavissimus* (Rosaceae), were able to inhibit alpha-amylase activity. The authors concluded that the potential of ETs to inhibit the alpha-amylase enzyme depended on the type of galloyl group (alpha-galloyl, β-galloyl or free hydroxyl group).

PAs, from fruits and berries, legume seeds, cocoa, wine and tea, are polymer chains of flavan-3-ols whose elementary units are linked by C-C and occasionally C-O-C bonds (140). Several beneficial properties have been assigned to these compounds, including a preventive role in some kinds of

cancer, cardiovascular diseases and diabetes development (141). Persimmon is one of the foods richer in PAs, especially in its peel (142). In this context, Lee *et al.* (143) tested PA polymers and oligomers from persimmon peel against alpha-amylase from *Bacillus licheniformis* and alpha-glucosidase from *Saccharomyces cerevisiae*. In this study, the inhibitory activity against alpha-amylase was linearly correlated with the DP, with polymers exerting stronger inhibitory activity against alpha-amylase than oligomers, whereas the opposite result was observed for alpha-glucosidase. Similarly, in a more recent study (144), the inhibitory action on PPA activity was higher when the polymerization degree of the oligomeric procyanidin fractions was also higher. So, the authors hypothesized that the most polymerized compounds were more reactive towards alpha-amylase due to the existence of more interaction sites. In this sense, in another research where polyphenols quantity and composition in persimmon leaves and persimmon leaf tea was determined, a varying polyphenol content and activity was found during the growth of the leaves, concluding that the best harvesting time for polyphenol-rich leaves with higher alpha-amylase inhibitory activity was June. In this study, PAs were reported as the main components responsible for the alpha-amylase inhibitory activity of persimmon leaves (145).

5. Pancreatic alpha amylase inhibitory activity of natural plant extracts *in vivo*

Several *in vivo* experiments have demonstrated the anti-hyperglycemic or hypoglycemic effects of different natural plant extracts that acted through various mechanisms (132, 146-148). Specific studies carried out in animal models have supported the hypothesis that pancreatic alpha-amylase enzyme inhibition of plant extracts might be the most promising mechanism for the observed antidiabetic effect. In these studies, the amylase inhibitory activity of plant extracts was firstly confirmed by *in vitro* bioassays and a verification of these findings was obtained in the subsequent *in vivo* tests. Some of these animal studies were focused on analyzing the reduction in blood glucose levels, after an oral starch tolerance test (OSTT) in which a decline in starch digestion and absorption was expected (31, 32, 44, 149). In some other studies, in contrast, an oral sucrose or glucose tolerance test was performed (150-152). Nevertheless, the *in vitro* amylase inhibitory activities of some extracts were not potent enough to produce an *in vivo* response (153).

The potential of *Phaseolus vulgaris* to inhibit the pancreatic alpha-amylase enzyme has been extensively studied in rodents (154) , Thus, the administration of *P. vulgaris* water extract produced a significant antihyperglycemic effect (dose of 200 mg/kg body weight) in STZ-induced diabetic rats after 30 and 45 days of treatment. Similar results were obtained with the administration for 22 days of a pancreatic alpha-amylase inhibitor isolated from *P. vulgaris* in non-diabetic (ND) and type 2 diabetic (neonatal diabetes models n0- STZ and n5- STZ) Wistar rats (155). In addition to the reduction of blood glucose levels, a significant decrease in body weight gain was observed in ND rats. These results confirmed a previous work performed in ND Wistar rats (154) in which the authors did not observe signs of malabsorption, diarrhea or an increase in stools. Regarding the use of wheat (*Triticum aestivum*) amylase inhibitors for diabetes and obesity treatment, the blood glucose lowering capacity has been also demonstrated *in vivo* in mongrel dogs (104).

Different bioactive constituents isolated from natural plant extracts have also been found to produce antihyperglycemic effects *in vivo*. Kobayashi *et al*. (156) reported the capacity of scirpusin B, an active component from the ethyl acetate extract of *Callistemon rigidus* (Myrtaceae), to suppress hyperglycemia in glycogen-loaded ddY mice. Also, the administration of andrographolide (10 mg/kg), the major active principle of *Andrographis paniculata* (Acanthaceae), significantly reduced postpandrial blood glucose levels after an OSTT (118). The *in vitro* capacity to inhibit pancreatic alpha-amylase enzyme of total flavonoids from *Polygonatum odoratum* was demonstrated by Shu *et al.* (23), who also observed a significant hypoglycemic effect at doses of 100 and 200 mg/kg in alloxan-induced diabetic rats. The alpha-amylase inhibitory effect of tea catechins has been widely studied (71, 129, 157, 158). Also tea polysaccharides have shown anti-amylase properties, as reported by Quan *et al*. (159), who studied two water-soluble polysaccharide fractions (TFP-1 and TFP-2) from *Camellia sinensis* flower. According to these authors, TFP-2 not only exerted *in vitro* alpha-amylase inhibitory activity but also produced a marked hypoglycemic effect when administered (75, 150 and 300 mg/kg/body weight) for 3 weeks to alloxan-induced diabetic mice.

Despite the interesting results of some of these studies (see **table 4**), very few *in vivo* studies have been carried out in order to corroborate the amylase inhibitory effects observed in *in vitro* assays. Therefore, further *in vivo* studies in different models of hyperglycemia are required.

6. Pancreatic alpha amylase inhibitory activity of natural plant extracts in humans

Clinical trials testing hypoglycemic effects of plant extract are not abundant in the literature (160), with the exception of wheat and bean alpha-amylase inhibitors. With respect to amylase inhibitors from wheat (*Triticum aestivum*), reductions in peak levels of postprandial glucose have been found in humans with minimal carbohydrate malabsorption signs (161, 162). Concerning the latter, a randomized, double blind, placebo-controlled trial was conducted in forty overweight and obese subjects that received Phase 2, an aqueous extract of white kidney bean, for twelve weeks (163). The treated group was dosed with two tablets of the product after the three main meals, each one containing 200 mg Phase 2, 200 mg inulin and 50 mg of *Garcinia cambogia* extract. At the end of the study a significant reduction in weight and fat mass were demonstrated. However, other human placebo-controlled trial observed a small but not statistically significant reduction in body fat and weight in body weight and fat after administering 1500 mg of Phase 2 with lunch and dinner for eight weeks (164). When the same research group administered 1000 mg Phase 2 twice a day for four weeks, but accompanied by a program of dietary modification, exercise and behavioral intervention, a significant reduction in body weight and waist girth was achieved (165). In other study, in which 445 mg of Phase 2 were administered to overweight subjects once a day for 30 days together with a meal rich in complex carbohydrates, a decrease in body weight, fat mass, and waist and hip circumferences was observed (166). When two doses (2 g and 2.9 g) were tested in eight healthy subjects that received a meal of 650 calories, a significant anti-hyperglycemic response was observed with the higher dose but not with the lower (167), unfortunately, in some cases, accompanied by diarrhea. More recently, two random double-blind, crossover human pilot studies analyzed the inhibition of starch absorption by administering two doses of Phase 2 (0.75 g and 1.5 g) in normoglycemic volunteers (168). A dose-

dependent response was found, with the highest dose causing a 66% inhibition while the lowest dose provoked a maximum inhibition of 41%.

The anti-obesity effect of the formula "Phaseolamin^{TM} 1600 diet", which is composed of the alpha-amylase inhibitor Phaseolamin (750mg), clove (a seasoning that increases body temperature, 200 mg), and three amino acids (lysine, arginine and alanine, 20 mg of each), was investigated in 10 subjects with a body mass index (BMI) between 23 and 30 kg/m^2. It was administered twice per day for eight weeks, 30 minutes before lunch and dinner, and at the end of the treatment, significant reductions in body weight, fat mass, waist circumference, BMI, and energy intake were observed (169).

The effectiveness of the pancreatic alpha-amylase inhibitory activity of other natural plant extracts has been evidenced in humans after *in vitro* or *in vivo* animal models. Thus, Tsujita *et al.*, (43) reported that the carbohydrate-hydrolyzing enzyme present in chestnut astringent skin (CAS) from *Castanea sativa* produced no adverse effects at doses of 2000 mg/kg body weight/d *in vivo*. When eleven healthy Japanese volunteers received two different doses of CAS extract (300 mg and 600 mg) together with 200 g of boiled rice after an overnight fasting, a dose-dependent reduction in plasma glucose levels (11 and 23%, respectively) was observed. In a similar study with *Olea europea* leaves, the glycemic response of 14 adult healthy volunteers who were previously classified as normal and borderline group for diabetes was studied. For this purpose two consecutive experiments were carried out; in the first one, the volunteers ate 300g of cooked rice after 12 hours of fasting whereas in the second one 1g of olive leaves (*Olea europea* L.) was added to the same amount of cooked rice (39). A significant reduction in blood glucose levels was observed in the borderline group 30 and 60 minutes after the administration of the olive leaf extract.

In brief, these clinical trials highlight the potential of these natural extracts to exert an anti-diabetic effect possibly by inhibiting the HPA. Nevertheless, more human trials are needed to demonstrate the safety and the antidiabetic properties of alpha-amylase inhibitors obtained from other natural plant extracts.

7. Side effects of natural alpha-amylase inhibitors

Several anti-diabetic drugs, such as acarbose, miglitol, voglibose, sitagliptin, nojirimycin and 1-deoxynojirimycin, target different glucosidases, especially sucrase, maltase and alpha-amylase, and produce favourable effects on glycemic values after food intake (170). Although their safety and tolerability has been widely evaluated due to the common clinical use of these drugs, their lack of specificity has been seen to produce several gastrointestinal side effects like abdominal cramping, flatulence and diarrhea (171-174). Natural alpha-glucosidase and alpha-amylase inhibitors are being investigated as new candidates to control hyperglycemia in diabetic patients, but few data are available regarding the negative effects they might produce. Most of the studies have been carried out with alpha-amylase inhibitors of bean origin. Thus, different acute and subchronic toxicity studies have been developed in animal models regarding consumption of Phase 2 with no side effects being reported (175, 176) and safety studies in humans have also been carried out. For instance, the safety of "Phaseolamin^{TM} 1600 diet", Phase 2 and Suco-Block$^{®}$ consumption was investigated and no significant side effects were found (163, 164, 169). In summary, it could be stated that the principle advantage of carbohydrate digestive enzyme inhibitors of plant origin consists in not causing severe side effects and may also be beneficial in weight reduction in individuals consuming large amounts of starch (168, 177).

8. Conclusions

Inhibition of carbohydrate hydrolyzing enzymes is emerging as a useful tool for type 2 diabetes treatment. Therefore this review has been focused on summarizing the existing data on pancreatic alpha- amylase inhibitory actions of natural plant extracts and related bioactive compounds. Pancreatic amylase enzyme inhibitors retard carbohydrates digestion and absorption in the diet reducing postprandial blood glucose levels. Several *in vitro* studies have confirmed the inhibitory potential of traditional plants and in some cases the bioactive compounds, which presumably are responsible of this mechanism of action, have been identified. However, studies conducted in animal models are few and

even less abundant are the studies performed in human subjects. Further investigations will provide useful information to set doses of compounds and natural extracts that will produce the seeking health benefits with no adverse effects.

9. Expert opinion

Significant progress has been achieved with the latest findings on the existence of different plant extracts with pancreatic alpha-amylase inhibitory activity that could lead to a slow rise of the postprandial blood glucose levels becoming an interesting therapeutic target for diabetes treatment. Moreover, clinical studies reporting a successful weight loss accompanied with a fat mass reduction, have suggested the possible antiobesity effect derived from this specific inhibition. However, although many plant extracts are able to inhibit HPA *in vitro,* their effect in diabetic animal models has been poorly investigated, even with less studies being conducted in humans. On the other hand, most of the reported data demonstrate the blood glucose lowering effect of natural plant extracts but the precise bioactive compounds underlying the mechanism have not been clearly identified. Thus, on one hand, the biggest goal in this field is the requirement of stronger evidence from preclinical studies regarding the response of diabetic patients to the natural pancreatic amylase inhibitors and the overall benefits obtained from this treatment. On the other hand, further research is required in order to assess the nature, isolation, purification and analysis of individual compounds responsible for the observed effects and information about potential synergistic effects of these compounds with other metabolites and different antidiabetic therapies that should be determined, with the aim of establishing the effective and safe doses in each case.

A huge challenge lies in developing new approaches for treating diabetes. Alternative therapies should be safer and produce any or hardly negative effects, while should give rise to efficient results in reducing glycemic values in diabetic patients. In addition, this approach will be economically less expensive than the currently available products, which is an important feature since diabetes is becoming a serious problem not only in industrialized countries but also in many developing countries.

Pancreatic alpha- amylase inhibitors might be used for the design of novel functional foods with blood glucose lowering potential, which could be useful as a complement of other andiabetic drugs, but could be also used for the industrial synthesis of analogous molecules to the existing antidiabetic ones.

Therefore, inhibition of pancreatic alpha- amylase obtained from natural resources seems to be a promising strategy for diabetes. Nevertheless, there is still a long way to go and research work should be focused mainly on the isolation of the principle active compounds and more clinical studies are essential to draw concise conclusions regarding the safety and efficacy of acute and long- term administration of the extracts and/or their bioactive compounds in type 2 diabetic patients.

Article highlights

- The control of postprandial blood glucose levels in diabetic patients is considered of relevance in the treatment of Type 2 Diabetes Mellitus. A mild inhibition of the pancreatic alpha-amylase is emerging as a potential therapeutic target.
- Studies reporting the antidiabetic effect of many herbs used in ancient traditions are extent.
- Potential of commonly used plant extracts to inhibit pancreatic alpha- amylase (porcine and human) has been demonstrated by *in vitro* studies.
- Phenolic phytochemicals, secondary metabolites found in plants, such as different flavonoids and proanthocyanidins have been reported to display a pancreatic alpha- amylase inhibitory activity.
- Few studies corroborate the amylase inhibitory potential of plant extracts and their bioactive compounds *in vivo*. Amylase inhibitors isolated from *Phaseolus vulgaris* have been the most extensively studied, finding positive blood glucose lowering effects in animal models.
- With the exception of human studies reporting the pancreatic alpha- amylase inhibitory activity from wheat and bean species, there are no many clinical trials analyzing the effects of natural

plant extracts and bioactive compounds previously demonstrated to exert an amylase inhibitory action.

- Safety and tolerability of natural amylase inhibitors have been demonstrated in various studies, and no significant adverse effects have been found till now.

10. Aknowledgments

The authors thank Línea Especial from University of Navarra (Spain) for financial support. U. Etxeberria and A. L. de la Garza hold pre- doctoral grants from Asociación de Amigos from University of Navarra.The authors declare that there are no conflicts of interest.

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