Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 13 (4), 2022; 925-932

Alpha amylase inhibitor as an Anti-diabetic potential from medicinal plants: A Review

Review Article

Lovepreet Kaur[1], Shabnam[1], Ashish Sutee[2], Rattandeep Singh[3*]

1. Research Scholar, 3. Associate Professor, School of Bioengineering and Biosciences,
2. Professor, School of Pharmaceutical Sciences,
Lovely Professional University, Phagwara, Punjab. India.

Abstract

Alpha amylases cleave the starch like molecules and led to increase in blood sugar level in diabetic patients then in order to manage it the inhibition action is carried out by their inhibitors. The α-amylase inhibitors catalyses the process of endo-hydrolysis of α (1→ 4) glycosidic linkage present in starch which ultimately led to decrease in the starch hydrolysis process. It also affects the carbohydrate absorption in the intestine. In 1945, the first alpha amylase inhibitor was reported from beans. It has already been isolated from various plant species namely, *Hibiscus sabdariffa.L, Geranium pratense L* and *Cedrus libani A. Rich* with inhibition percentage of 100, 43 and 31 respectively. The studies have already been reported on inhibitors and their interactions with human pancreatic alpha amylases are made .In-vitro and in vivo tests has already been done by various researchers which showed a significant drop in blood sugar level of a normoglycemic mice after an extract from the leaves of *Bersama abbyssinica* was tested for its potential effect as anti-diabetic.

Key Words: Diabetes, Alpha amylase inhibitors, Plants, Classification, In-vivo, In-vitro, In- silico approaches.

Introduction

Diabetes mellitus has a multiple biochemical impairment that is epidemic globally. It is caused due to disorder of pancreas which occurred due to insulin resistance, inadequate insulin secretion, or excessive glucagon secretion. It is an endocrinological disorder. About 3000 years ago diabetes was first reported in Egyptian manuscript and it is one of the oldest diseases. It is a long term impairment affecting the body regulation and causing high blood sugar level. In 1755, Dobson demonstrated the presence of the sugar in urine of a diabetic person (1). Later on, Joseph von mering and Oskar Minkowski reported the role of the pancreas in diabetes, they discovered that when the pancreas was removed from the dogs they developed the symptoms of diabetes and died. In 1910, it was determined by Sir Edward Sharpey-Schafer that from the lack of insulin diabetes occur. Type 1 diabetes and type 2 diabetes are two types of diabetes. The differentiation between two types was made clear in 1936. Later, in 1988 the metabolic component of Type 2 diabetes mellitus was discovered (2). It is a non-insulin dependent diabetes mellitus. In both developed and developing countries approximately 80% of people with diabetes are suffering from T2DM. The type 1 diabetes occurs due to auto-immune disorder and it led to demolition of pancreatic beta-cells (3). The type 2 diabetes is more common that is caused by the combine factors of dysfunction and resistance. Primarily, T2D is caused due to impairment of the regulation process of glucose, insulin resistance and pancreatic beta cells dysfunction. Both types of diabetes are lifelong serious conditions. The symptoms of diabetes include frequent urination, increase thrush, excessive hunger, fatigue, polyuria and glycosuria (4).

Globally, the prevalence of diabetes has increased leading to high mortality and morbidity rate. It has been observed that type 2 DM is more vulnerable as it can cause both short- and long-term complications, which often lead to premature death (5). Presently, the only method that is adopted to deal with diabetes is by lowering glucose level with various therapies. Such methods are best to treat people with T2DM (6). The classes of oral anti-diabetic drugs are involved in treatment of diabetes such as biguanides, sulfonylureas, meglitinide, thiazolidinedione (TZD), dipeptidyl peptidase 4 (DPP-4) inhibitors, sodium-glucose co-transporter (SGLT2) inhibitors and α-glucosidase inhibitors. Despite of their advantage of lowering the blood glucose level they offer various complications in return such as anaemia, skin issues, kidney and liver damage (7). Thus, the study has shifted for the determination of more plant based products to treat diabetes. The research has already been done on various plants for screening of their anti-diabetic potentials (8). Some of medicinal plants in which anti-diabetic potential has been reported are aloe, onion, cinnamon,

*** Corresponding Author:**
Rattan Deep Singh
Assistant Professor,
School of Bioengineering and Biosciences,
Lovely Professional University,
Phagwara, Punjab (India) India.
Email Id: drrattandeep@gmail.com

Croton cajucara, *Bidens pilosa*, fenugreek, *Allium sativum*, guava, nettle, coffee, soybean, *Gentiana olivier*, turmeric, walnut, and *Cassia auriculata* (9). Different bioactive compounds are present in plants such as lignans, alkaloids, flavonoids, saponins and carotenoids which provide a distinct characteristic to a plant for its treatment against diabetes. Medicinal plants also contain alpha amylase inhibitors (10). The class of enzymes that hydrolyse starch to yield low molecular weight dextrins and sugars is known as amylase inhibitors. Amylase inhibitors are also termed as starch blockers (11). They catalyse the hydrolysis of α-(1, 4)-D-glycosidic linkages of starch and other glucose polymer. The amylase inhibitor constitutes of a carbohydrate and nitrogen with an oligoamine unit (12).

Amylase is one of the oldest enzymes. In 1815, the foundation for the discovery of amylase was laid by Kirchhoff. From his experimentation he showed that gluten had the great capacity to convert the starch into sugar. In his experimentation he formed a starch paste from the four parts of water, two parts of starch, and malt (13). Later, the paste began to liquefy into sweet syrup which proved the capacity of gluten. In 1831, Erhard Friedrich Leuchs demonstrated the hydrolysis of starch by saliva that was due to the presence of an enzyme in saliva, "ptyalin", which is an amylase (14). With time the research starts widening and researchers started exploring the amylase more. Likewise, in 1833 Anselme Payen and Jean-Francois Persoz further describe and isolate another enzyme “diastase” which is basically, amylase in powder form. They isolated it from barley malt and represented that it as a heat labile in nature. It was the first enzyme to be discovered

Firstly, the alpha amylase inhibitor was reported in 1945 by Bowman. He did his investigation on beans and reported the presence of enzyme alpha amylase. Then, the research for the alpha amylase inhibitors intensified for years (15). In 1960-61, Stankovic and Markovic reported presence of alpha amylase inhibitor in acorns. Additionally, by 1978 Granum and Manjunath determined the enzyme in rye. In 1980 and 1981 by the four leading researchers Shivaraj, Pattabiraman and Blanco-Labra and Iturbechifias the study on alpha amylase inhibitor was done on millet and maize respectively. Due to great advantages of the alpha amylase inhibitors, they are widely under the field of interest by researchers.

Classification of alpha amylase inhibitors

There are various classes of alpha amylase inhibitors. Each class represents the inhibitors with distinct structural components. Some of the classes are described as follows

Lectin like alpha amylase inhibitors

The alpha amylase inhibitors of the lectin type comprises of 250 amino acid residues that results in formation of the five disulphide bonds. They are found in higher plants, cereals and legumes. They play a significant role in inhibition of the activity of salivary and pancreatic amylase in vitro and in vivo. Amylase inhibitors have potential in various fields, including crop protection as they are used as a source of defence strategy by plants (16). In pulses, three isoforms of alpha-amylase inhibitors are found mainly isoform 1, 2 and 3. Alpha-amylase inhibitor isoform 1 also known as (Alpha- AI1), similarly the isoform 2 and 3 of alpha amylase inhibitor is as (Alpha-A12) and (Alpha-AIL) respectively. The alpha amylase 1 is further divided into two categories namely Alpha amylase 1 – Pa1 and alpha amylase 1 – Pa2. Each has a distinct role in inhibition of the various alpha amylase enzymes present in case of storage pests. But these two isoforms do not act as inhibitory factors on the mammalian alpha amylases (17).

They are divided on the basis of their inhibitory activity as well as structure.

Table 1 - The list of various alpha amylase inhibitors falling under this category is as follows along with their inhibition response

S.No.	Type of alpha amylase	Inhibition action	Reference
1	Alpha amylase 1	Mammalian alpha amylase	(17)
2	Alpha amylase 1	Alpha amylase present in midgut of the *C. maculatus*	(18)
3	Alpha amylase 2	Alpha amylase present in the midgut of the *Z. subfasciatus*	(18)
4	Alpha amylase 1 – Pa1	Larval alpha amylases of *C.chinesis*	(17)
5	Alpha amylase 1 – Pa1	Larval amylase of *Tribolium molitor*	(19)
6	Alpha amylase 1 – Pa2	Larval amylase of *Tribolium confusum*	(19)

Cereal type alpha- amylase inhibitors

The alpha amylase inhibitors belonging to this class comprises of amino acid chains of approximately 110-160 residues. An intensive study has already been done on them and they have been purified from wheat flour. It has been noted that such inhibitors displays the molecular weight between 10- 60kDa. Basically, they act as different form of monomers such as hetero-oligomers and homodimers. These inhibitors differ from others due to their tertiary structure (20). There are 5 disulphide bonds that are formed due to presence of cysteine residues, additionally there is and up and down manner arrangement of the alpha helices which sets it apart from other classes. It is present in various cereals like wheat, ragi and maize seed. In case of wheat they are further comprises of two sub classes namely inhibitor 0.19 and 0.28.

The former has molecular weight of 24kDa while the latter is half of it. They inhibit various alpha amylases, for instance alpha amylase from human saliva, avian saliva and pig pancreas. Likewise, the bi-functional alpha amylase from ragi has potential to

inhibit amylase from porcine pancreatic amylase. It is formed of long chain of amino acids with approximately 122 residues unit. The binding site of the alpha amylase in this case is located in the N-terminal region (21).This type of alpha amylase inhibitor follows the mechanism of the competitive inhibition in which the active site prevents the binding of the enzyme to its substrate. For example, when p-nitrophenylaltoside substrate is used in case of the porcine pancreatic alpha amylase reaction mechanism, it acts as a competitive inhibition (17). About, 155 long amino acid residues are present of cereal type alpha amylase inhibitor in case of maize seeds. Such inhibitor when isolated from the maize seed can hinder the activity of the human trypsin enzyme. In addition to this, it also acts effectively on the alpha amylase of the insects.

Kunitz like alpha amylase inhibitors

With the presence of four cysteines, the kunitz like alpha amylase inhibitor contains 180 amino acids chain. They are abundantly present in cereals. Subtilisin inhibitor also known as barley alpha amylase inhibitor as it is present in barley is formed of a beta- trefoil protein. During the process of the seed germination of the barley, the soyabean trypsin inhibitor inhibits the isozyme 2 of the alpha amylase classification. The alpha amylase of rice has molecular weight of about 21 kDa (16). The kunitz like alpha amylase inhibitor is also found in legume such as flame tree i.e. *Delonix regia.* This legume alpha amylase inhibitor has a great potential to increase mortality of *Callosobruchus maculatus*. Additionally, it attacks on the larval stage of the insects thus affects their development. In contrasts to rice alpha amylase inhibitor this inhibitor can inhibit the alpha enzymes of the both mammalian as well as insects. They have an extended family with distinct molecular weights starting from 6 kDa to 24 kDa. Kunitz like inhibitor can also isolate from pigeon pea and wheat (15). The thermal stability of their protein is high and they work on a different mechanism. They form a dozen of hydrogen bonds and salt bridges results in high electrostatic forces in the active regions of the catalytic site thus, preventing the entry of the substrate.

Knot type alpha amylase inhibitors

They are one of the most smallest in size proteinaceous alpha amylase inhibitor. They are comprised of only 32 amino acid residues with only 3 disulphide linkages. It works on a complex mechanism to perform its inhibitory activity which is based on the water-mediated hydrogen bonds. In this mechanism, the alpha amylase inhibitory binds to the gap between the domains of the catalytic site and consequently forms a salt bridge. The knot type alpha amylase inhibitor inhibits the *Tenebrio molitor*, *Tribolium castaneum* and various other species (18). The primary souce of this inhibitor is amaranth. It was first discovered in 1994 by Chagolla and co-workers. As per studies, only four cysteine knot alpha amylase are discovered that contain two to four prolines.

Another, knot like alpha amylase inhibitor is found in plant *Wrightia religiosa.* These are shorter in length with only 30 amino acid residue chain and they are represented as Wr-Al1, Wr-Al2 and Wr-A13.

Thaumatin like alpha amylase inhibitors

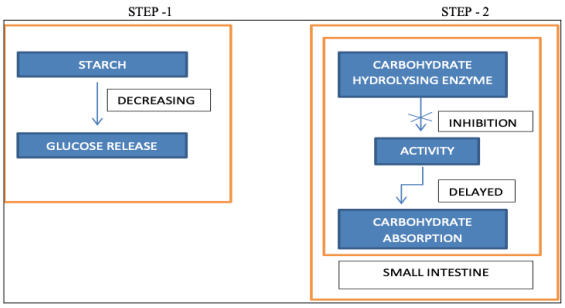
About 22 kDa protein is isolated from maize and barley and they are termed as zeamatin trypsin inhibitor. It has 133-227 amino acid long chain with molecular weight of 22 kDa. The mechanism of action is totally different as compare to the other types of the alpha amylase inhibitors (21). In this the thaumatin is induced with help of external resources i.e. abiotic stress such as temperature, pH, salt concentrations and light. It can inhibit various amylases like porcine pancreatic and insect alpha amylases. Few insects on which it works efficiently to inhibit the activity of the alpha amylase inhibitor are as follows *Tribolium castaneum*, *Neurospora crassa* and *Candida albicans* (22)

Gamma purothionin like alpha amylase inhibitors

It is formed of 47 amino acid residues and possesses the molecular weight of 5kDa. The gamma like alpha amylase has distinct features and mechanisms that set is apart from others. The mechanisms involve amendments in permeability of the membrane, protein synthesis inhibition and inhibition of the proteinase. The main sources from which gamma alpha amylase inhibitors can be isolated include sorghum, soyabean, papaya, barley and wheat. It inhibits the bacterial amylases and the insect alpha amylase (12).

Mechanisms of action of alpha- amylase inhibitor in treatment of diabetes

Figure 1- Representation of mechanism of alpha amylase inhibitors



Mechanism of action of alpha-amylase inhibitor

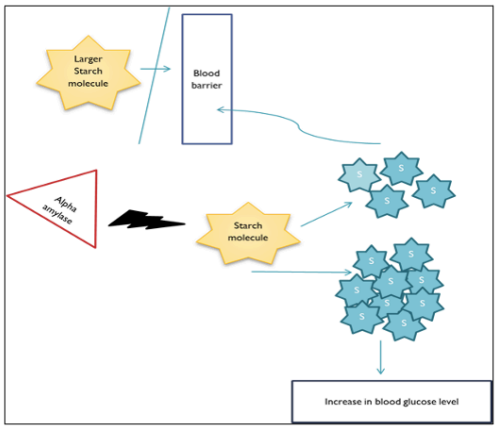
The inhibition action of α-amylase is carried out by α-amylase inhibitors by catalyses of the endohydrolysis of α (1→ 4) glycosidic linkage present in starch with help of an enzyme. Thus, causing decrease in the starch hydrolysis process. Furthermore, it affects the process of the carbohydrate absorption in the small intestine by inhibiting the activity of the carbohydrate hydrolysing enzymes. Thus, they are very helpful in management of the postprandial hyperglycaemia (PPHG) in diabetic patients (23).

Activity of alpha amylase inhibitor in body

Starch like molecules cannot cross the blood brain barrier, they are cleaved by alpha-amylase cleaves the large starch molecules into smaller fragments of sugars to cross the blood brain barrier. The excessive

conversion of starch to sugars will ultimately increase the blood sugar level, and then the insulin will direct the cells to metabolize the excess sugar moieties and store it as an energy source i. e. glycogen (20). This cycle is routinely proceeding in healthy living system. But in some cases, due to excess activity of amylase enzyme and insulin deficiency or resistance to insulin, there is increase in level of blood glucose which might results in hyperglycaemia (17). Several studies on inhibition of amylase enzyme activity have already been reported to control hyperglycaemia.

Figure 2- Activity of alpha amylase inhibitor



Recent research on Medicinal plants in treatment of diabetes

Several medicinal plants possess alpha- amylase inhibitory activity and it has been reported that more than 500 plant species exhibit properties that help in treatment of diabetes. Studies have already been done and it has been observed that in case of rats when they were administered with aqueous extract prepared from leaves of the *P.S. cuminigua* a reduction in blood glucose level was noticed. Likewise, seeds of *Amaranthus caudatus* showed inhibition rate of approximately 80%.

As per reports various parts of different plant species were taken and studies have already been done in order to determine alpha amylase inhibition activity in plants. It has been observed that maximum inhibition percentage of enzyme alpha amylase was observed in case of flowering plant *Hibiscus sabdariffa* and in perennial legume i.e. *Cajanus cajan* that was about 100%. Following it the inhibition percentage was 93% in case rhizome of *Bergenia ciliate* (25). The alpha amylase inhibition percentage was 57 and 56 in case of extracts of bark and leaves of the *Balanitesa egyptiaca L* and *Murraya koenigii L*. Likewise, in case of annual herbaceous namely *Andrographis paniculate* the percentage of alpha amylase inhibition is 52%.

Table 2 - A list of different plant species with alpha amylase inhibition activity percentage.

S.No.	Plant	Plant type	Part	Alpha amylase Inhibition %age	Reference
1	*Hibiscus sabdariffa*	Flowering plant	Flower	100	(26)
2	*Cedrus libani A. Rich*	Pines	Cones	31	(25)
3	*Geranium pratense*	Flowering plant	Aerial part	43	(24)
4	*Andrographis paniculate*	Annual herbaceous	Leaf	52	(26)
5	*Balanitesa egyptiaca L*	Multipurpose tree	Bark	57	(47)
6	*Securidaca longepidunculata Fresen*	Deciduous shrubs	Root	35	(26)
7	*Pentaphylloides fruticosa*	Shrubs	Branch	31	(23)
8	*Cajanus cajan*	Perennial legume	Seed	100	(27)
9	*Murraya koenigii .*	Sub-tropical tree	Leaf	56	(24)
10	*Mitragyna inermis*	Shrub	Leaf	75	(39)
11	*Bergenia ciliata*	Perennial	Rhizome	93	(24)
12	*Ginkgo biloba*	Large trees	Leaf	70	(40)
13	*Paeonia anomala*	Perennial	Root	33	(27)

Further, it has been reviewed by various studies that the extracts from different medicinal plants possess the characteristics of alpha amylase enzyme inhibition. More than 500 plants exhibit such properties, out of them the most traditionally used plants for treatment of diabetes are *L. Syzygium cumini L.* (syn: *Eugenia jambolana* Lam) and *Psidium guajava L.* In addition to them reduction in blood glucose level has also been observed from the extracts of cumin seeds, *Amaranthus caudatus L.* seeds, *Melissa officinalis L., Taraxacum officinale and Tamarindus indica L* (28). Likewise, In India the Ayurvedic system of medicine reported that with no or least side effects the blood glucose level can be reduced with help of some medicinal plants (29). A study has already been done which reported that the *Mangifera indica L., Phyllanthus mader, Tenuflorum ocimum L.*, and *Linumusita tissimum L.* has potential effect on the alpha amylase activity which apparently results in lowering blood glucose level and a remedy to obesity.

Plants with anti-diabetic potential

- *Psidium guajava* - The leaves of *P. guajava* have been shown to suppress/control the increase in blood sugar

levels during a glucose tolerance test in alloxan-induced diabetic rodents. Different parts of the *P. guajava* plant have been used to treat diabetes. In type 2 diabetes, methanolic concentrate of *P.guajava* leaves had a hypoglycemic effect (30). Watery concentrate of *P. guajava* leaves has been shown to significantly lower blood glucose levels. The dynamic constituents were flavonoid glycosides such as strictinin, isotrictinin, and pedunculagin, which are used in the clinical treatment of diabetes to improve insulin affectability. A 50,000-100,000 atomic load glycoprotein detached from the aq. Furthermore, leaf concentrate has anti-diabetic properties. (38).

- *Syzygium cumini* - In preliminary phytochemical screening, the presence of alkaloids, amino acids, steroids, and triterpenoids in thyl acetic acid derivation and methanol concentrates of S. cumini seed remove was discovered. *S. cumini* seed has been known to have various restorative properties aside from anti-diabetic activity, such as mitigating and pain relieving activity (31). *S.cumini* seed extract has anti-diabetic properties, and its component of activity is similar to that of Glibenclamide, a drug that stimulates insulin release from pancreatic cells. It was also discovered that Ethyl acetic acid derived from *S.cumini* seed and methanol concentrate of *S.cumini* seed have an anti-diabetic effect in STZ-induced diabetic rodents.
- *Costus speciosus*- Oral administration of rough concentrates of hexane, ethyl acetic acid derivation, and methanol of *C.speciosus* has been shown to significantly lower blood glucose levels in STZ-activated diabetic rodents. Furthermore, it was discovered that hexane rough concentrate is significantly more effective than ethyl acetic acid derivation and methanol extricates in decreasing serum glucose levels and normalizing other biochemical boundaries in diabetic rodents. (33).
- *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 (41). An experiment was conducted in which extracts from plants were administered for a month. Consequently, a decrease in plasma glucose level by 9.06% and 26.4% on 15 and 30 days was observed respectively. 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, anti-stress, antitumor and antiulcer. (34).

In silico approach for characterization of alpha amylase inhibitors

These approaches utilises the computational techniques for the analysis of the purified alpha amylase inhibitors. They work by figuring out the binding sites of the macromolecule. In order to attain the best conformation between the receptor and the ligand these approaches involves the use of the spatial energies. The molecular docking is one such method that ia used for the screening of the alpha amylase inhibitors for their effectiveness. Various studies have already been done for instance; the computational approach was used to study the inhibitory effect of the carotene on the alpha amylase activity (35). The molecular docking is done by using Auto Dock Vina. The studies on natural products have already been carried out and various inhibitors of the human pancreatic alpha amylase are reported. There crystal structures are also determined that showed a strong interaction between the human pancreatic alpha amylase and some derivatives like myricetin. Basically, the interaction is between the hydrogen bonds and the hydroxyl groups of the derivatives. Likewise, various interactions at molecular level can be revealed by molecular docking that can be helpful to bring out the ligand-receptor interactions (46). In past, an investigation was carried out for the determination of the alpha amylase inhibitors from the extracts of the medicinal plant namely; *Leucas ciliate* the solvent extracts were taken. Further, the ligands were obtained from the compounds and their receptor binding sites were determined using molecular docking studies in which PyRx software was used to determine the alpha amylase inhibition activity (36). Just like plants, an oral alpha amylase inhibitor has already been extracted from bacteria speice *Streptomyces dimorphogenes*. Post ingestion of sucrose this extract has shown very few effects on lowering blood glucose levels. The crystal structure has also been produced using GRASP which shows the complex formed between human pancreatic alpha amylase and inhibitor derived from trestatin. Additionally, structure formed reveals the binding of active sites of human pancreatic alpha amylase to trestatin inhibitor (37).

In vitro and in-vivo studies on alpha amylase inhibitors

α-amylase is one of the fundamental compounds in human body that is liable for the breakdown of starch to more basic sugars. Alpha-amylases hydrolyze complex polysaccharides to produce oligosaccharides and disaccharides which are then, at that point, hydrolyzed by α-glycosidase to monosaccharide that are ingested through the small digestion tracts into the hepatic gateway vein and led to increment of the postprandial glucose levels (42). In order to study the potential effects of the alpha amylase inhibitors for processing as an anti-diabetic agent various in-vitro studies have been performed. The studies on plant material have been carried out effectively and that has shown the inhibitory activity of various isolated compounds. One such research was carried out to determine the activities of the *O.basilicum* which is a culinary herb belonging to family Lamiaceace. It is also regarded as a traditional medicinal plant. The experiment done by researchers on *O.basilicum* revealed that its extract has potential inhibitory effect on the alpha glucosidase and amylase activity. It works in a dose dependent manner (43). Likewise, a study carried out on *Amaranthus spinosus reported that the drugs that can inhibit the carhohydrtae hydrolysing enzymes can also improve defective glucose functioning in body*

*by reducing the post prandial hyperglycaemia. In addition to this, the meathanol extract of the Amaranthus spinosus has a potential effect on the inhibition of the alpha amylase inhibitory activity. Just like others the leaves of the plant Bersama abyssinica that is an evergreen tree belonging to family Francoaceae also possess alpha amylase inhibitory activity and studies carried out on it in-vitro proved that concentration dependent inhibitions were observed (*44)

These studies help in drug development because they provide the knowledge about the drug's characteristics, including drug interactions in living system, its biochemical response and adverse effects. The in vivo methods also aids in providing an assessment on how safe and efficient a drug is, which is under experimentation for clinical trials. To determine the safety various investigations have been carried out on rats (45). According to a study which is conducted on animals the citrus extracts not only shown results in decreasing glucose uptake but also bring out the movement of glucose in hepatic organ. Another study reported that peel of *Citrus limetta* can potentially reduce blood glucose level in diabetic rats (48). The extract from the leaves of the *Bersama abbyssinica* were tested to check potential alpha amylase inhibition activity. This test was performed on normoglycemic mice and the results showed a significant decrease in blood glucose level of the mice as compare to the others.

References

1. Akshatha JV, SantoshKumar HS, Prakash HS, Nalini MS. In silico docking studies of α-amylase inhibitors from the anti-diabetic plant *Leucas ciliata* Benth. and an endophyte, *Streptomyces longisporoflavus*. 3 Biotech. 2021;11(2); 51.
2. Wang, H. H., C. L. Chen, T. L. Jeng, and J. M. Sung. Comparisons of α-Amylase Inhibitors from Seeds of Common Bean Mutants Extracted through Three Phase Partitioning." *Food Chemistry,*2011;128(4);1066–71.
3. Abeykoon, A. M. C. N., H. M. T. Herath, and M. A. Jayasinghe. "In-Vitro Analysis of Trypsin and Alpha - Amylase Inhibitory Activities in Selected Legume Varieties in Sri Lanka." *Asian Food Science Journal*. 2021; 20(1);1–9.
4. Singhal, Poonam, Geetanjali Kaushik, and Pulkit Mathur., "Antidiabetic Potential of Commonly Consumed Legumes: A Review." *Critical Reviews in Food Science and Nutrition*, 2014; 54(5); 655–72.
5. Prashant Aggarwal and Ritika Gupta., Alpha-Amylase Inhibition Can Treat Diabetes Mellitus. Research and reviews journal of medical and health sciences.2016; 5(4); 1-2.
6. Alqahtani AS, Hidayathulla S, Rehman MT, ElGamal AA, Al-Massarani S, Razmovski-Naumovski V, Alqahtani MS, El Dib RA, AlAjmi MF. Alpha-Amylase and Alpha-Glucosidase Enzyme Inhibition and Antioxidant Potential of 3-Oxolupenal and Katononic Acid Isolated from *Nuxia oppositifolia*. Biomolecules. 2019 Dec 30;10(1); 61.
7. Sales PM, Souza PM, Simeoni LA, Silveira D. α-Amylase inhibitors: a review of raw material and isolated compounds from plant source. J Pharm Pharm Sci. 2012; 15(1);141-183.
8. Karthic K, Kirthiram KS, Sadasivam S, Thayumanavan B. Identification of alpha amylase inhibitors from Syzygium cumini Linn seeds. Indian J Exp Biol. 2008 Sep; 46(9);677-680.
9. Ashok Kumar BS, Lakshman K, Nandeesh R, Arun Kumar PA, Manoj B, Kumar V, Sheshadri Shekar D. In vitro alpha-amylase inhibition and in vivo antioxidant potential of Amaranthus spinosus in alloxan-induced oxidative stress in diabetic rats. Saudi J Biol Sci. 2011 Jan;18(1):1-5.
10. Acelya, Turkmen, AK Ezgi Seyhan, and Ayfer Ozab , "Research of Knowledge and Attitudes of Patients with Diabetic Foot Ulcer Regarding Foot Care." International Journal of Diabetes and Clinical Research. 2021; 8(2).
11. Borzoui E, Nouri-Ganbalani G, Naseri B. In Vitro and In Vivo Effects of α-Amylase Inhibitor From Avena sativa Seeds on Life History and Physiological Characteristics of Sitotroga cerealella (Lepidoptera: Gelechiidae). J Insect Sci. 2017 Nov 1; 17(6); 108.
12. Chelladurai, Gladis Raja Malar, and Chellaram Chinnachamy. "Alpha Amylase and Alpha Glucosidase Inhibitory Effects of Aqueous Stem Extract of Salacia Oblonga and Its GC-MS Analysis." Brazilian Journal of Pharmaceutical Sciences. 2018; 54(1).
13. Kifle ZD, Enyew EF. Evaluation of In Vivo Antidiabetic, In Vitro α-Amylase Inhibitory, and In Vitro Antioxidant Activity of Leaves Crude Extract and Solvent Fractions of *Bersama abyssinica* Fresen (Melianthaceae). J Evid Based Integr Med. 2020;25.
14. Neeraj Choudhary, Pranav Kumar Prabhakar, Gopal L.Kathik, Subba Rao Chamakuri, Devesh Tewari, Ashish Suttee. Evaluation of Acute Toxicity, in-Vitro, in-Vivo Antidiabetic Potential of the Flavonoid Fraction of the Plant Chenopodium Album L." Pharmacognosy Journal, 2021; 13(3); 765–779.
15. De Gouveia NM, Alves FV, Furtado FB, Scherer DL, Mundim AV, Espindola FS. An in vitro and in vivo study of the α-amylase activity of phaseolamin. J Med Food. 2014 Aug;17(8);915-20.
16. 16. Fatemeh Ghorbani. Masoud sadeghi, Abbas aghaei, Arezou ghahghaei, Study of α-Amylase Inhibitors among Different Bean Cultivars and Evaluation of their Effectiveness Compared with a Commercial Product using In Vitro/In Vivo Experimental Systems. Journal of Research in Medical and Dental Science. 2018; 6(1); 381-383.
17. Gladis Raja Malar Chelladurai, Chellaram Chinnachamy, Alpha amylase and alpha glucosidase inhibitory effects of aqueous stem extract of Salacia oblonga and its GC-MS analysis. Brazillian Journal of Pharmaceutical Sciences. 2018; 3-5.

18. Muhammad Nawaz, Muhammad Taha, Faiza Quershi, Nisar Ullah, Manikandan Selvaraj, Sumaira Shahzad, Sridevi Chigurupati, Abdul Waheed and Fadiah Ammar Almutairi, Structural Elucidation, Molecular Docking, α-Amylase and α-Glucosidase Inhibition Studies of 5-Amino-Nicotinic Acid Derivatives." BMC Chemistry. 2020; 14(1).
19. Imad I. Hamdan & Fatma U. Afifi. Screening of Jordanian Flora for α-Amylase Inhibitory Activity, Pharmaceutical Biology. 2008; 46:1011; 746-750.
20. Hargrove JL, Greenspan P, Hartle DK, Dowd C. Inhibition of aromatase and α-amylase by flavonoids and proanthocyanidins from Sorghum bicolor bran extracts. J Med Food. 2011 Jul-Aug; 14(7-8); 799-807.
21. Holman RR, Coleman RL, Chan JCN, Chiasson JL, Feng H, Ge J, Gerstein HC, Gray R, Huo Y, Lang Z, McMurray JJ, Rydén L, Schröder S, Sun Y, Theodorakis MJ, Tendera M, Tucker L, Tuomilehto J, Wei Y, Yang W, Wang D, Hu D, Pan C; ACE Study Group. Effects of acarbose on cardiovascular and diabetes outcomes in patients with coronary heart disease and impaired glucose tolerance (ACE): a randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinology. 2017 Nov; 5(11):877-886.
22. Ishnava KB and Motisariya DM, In-vitro study on alpha amylase inhibitory activity of selected ethonobotanical plant extracts and its herbal formulations. International Journal of pharmacognosy and chinese medicine. 2018; 2(3), 1-4.
23. Zhao BT, Le DD, Nguyen PH, Ali MY, Choi JS, Min BS, Shin HM, Rhee HI, Woo MH. PTP1B, α-glucosidase, and DPP-IV inhibitory effects for chromene derivatives from the leaves of Smilax china L. Chemico Biological Interaction. 2016; 253; 27-37.
24. Rahul L Gajbhiye, Anand Ganapathy and Parasuraman Jaisankar. A Review of A-Glucosidase and A-Amylase Inhibitors for Type 2 Diabetes Isolated From Some Important Indian Medicinal Plant. Annals of Clinical Pharmacology and Therapeutics. 2018; 1(1); 1-7.
25. Kajaria D, Ranjana, Tripathi J, Tripathi YB, Tiwari S. In-vitro α amylase and glycosidase inhibitory effect of ethanolic extract of antiasthmatic drug - Shirishadi. J Adv Pharm Technol Res. 2013; 4(4); 206-209.
26. 26. M.N. PierO, G.M. Nzaro and J.M. Njagi. Diabetes Mellitus – a Devastating Metabolic Disorder. Asian Journal of Biomedical and Pharmaceutical Sciences, 2015; 14(40); 1–7.
27. Drulyte D, Orlien V. The Effect of Processing on Digestion of Legume Proteins. Foods. 2019 Jun 24; 8(6):224.
28. Arumugam Madeswaran, Kuppusamy Asokkumar, Muthuswamy Umamaheswari, Thirumalaisamy Sivashanmugam, Varadharajan Subhadradevi., "In silico approach for alpha-amylase inhibitory activity of diosmetin and galangin, International Journal of Phytopharmacy. 2014; 4(5); 124-126.
29. Kazeem MI, Adamson JO, Ogunwande IA. Modes of inhibition of α -amylase and α -glucosidase by aqueous extract of Morinda lucida Benth leaf. Biomed Res Int. 2013.
30. Moradi, B., Abbaszadeh, S., Shahsavari, S., Alizadeh, M., & Beyranvand, F. The most useful medicinal herbs to treat diabetes. Biomedical Research and Therapy. 2018; 5(8); 2538-2551.
31. Uddin N, Hasan MR, Hossain MM, Sarker A, Hasan AH, Islam AF, Chowdhury MM, Rana MS. In vitro α-amylase inhibitory activity and in vivo hypoglycemic effect of methanol extract of Citrus macroptera Montr. fruit. Asian Pac J Trop Biomed. 2014 Jun;4(6):473-479.
32. Olokoba AB, Obateru OA, Olokoba LB. Type 2 diabetes mellitus: a review of current trends. Oman Med J. 2012 Jul;27(4); 269-273.
33. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. Plants (Basel). 2017;6(4);42.
34. Sales PM, Souza PM, Simeoni LA, Silveira D. α-Amylase inhibitors: a review of raw material and isolated compounds from plant source. Journal of pharmacy and pharmaceutical sciences. 2012;15(1);141-83.
35. Prateeksha, B. S. Paliya, R. Bajpai, V.Jadaun, J.Kumar, S.Kumar, D.K. Upreti, B.R. Singh, S. Nayaka, Y.Joshi and Brahma N.Singh. The genus Usnea: a potent phytomedicine with multifarious ethnobotany, phytochemistry and pharmacology. RSC Advances. 2016; 6(26);21672–21696.
36. Sadikot SM, Nigam A, Das S, Bajaj S, Zargar AH, Prasannakumar KM, Sosale A, Munichoodappa C, Seshiah V, Singh SK, Jamal A, Sai K, Sadasivrao Y, Murthy SS, Hazra DK, Jain S, Mukherjee S, Bandyopadhay S, Sinha NK, Mishra R, Dora M, Jena B, Patra P, Goenka K; DiabetesIndia. The burden of diabetes and impaired fasting glucose in India using the ADA 1997 criteria: prevalence of diabetes in India study (PODIS). Diabetes Res Clin Pract. 2004 Dec; 66(3):293-300.
37. Saiedullah M., Insulin Sensitivity or Resistance in Type 2 Diabetes Mellitus with Obesity. Diabetes Case Report. 2016; 1(2).
38. Mrinal Samtiya, Rotimi E. Aluko, and Tejpal Dhewa. Plant Food Anti-Nutritional Factors and Their Reduction Strategies: An Overview. Food Production, Processing and Nutrition, 2020, 2(1).
39. 39. He Li, Haochun Zhou, Jian Zhang, Xiaohang Fu, Zhiwei Ying & Xinqi Liu. Proteinaceous α-amylase inhibitors: purification, detection methods, types and mechanisms, International Journal of Food Properties. 2021; 24(1); 277-290.
40. Shukia R, Sharma SB, Puri D, Prabhu KM, Murthy PS. Medicinal plants for treatment of diabetes mellitus. Indian J Clin Biochem. 2000 Aug; 15(Suppl 1):169-77.

41. Sultana R, Alashi AM, Islam K, Saifullah M, Haque CE, Aluko RE. Inhibitory Activities of Polyphenolic Extracts of Bangladeshi Vegetables against α-Amylase, α-Glucosidase, Pancreatic Lipase, Renin, and Angiotensin-Converting Enzyme. Foods. 2020 Jun 29;9(7):844.
42. Syed Bilal Shah, Lubna Sartaj, Fawad Ali, Syed Izhar Ali Shah, Muhammad Tahir Khan, "Plant Extracts Are the Potential Inhibitors of α-Amylase: A Review." MOJ Bioequivalence & Bioavailability. 2018; 5(5).
43. Ulbricht C, Bryan JK, Conquer J, Costa D, Stock T, Tanguay-Colucci S, Weissner W. An evidence-based systematic review of amylase inhibitors by the Natural Standard Research Collaboration. J Diet Suppl. 2010 Mar;7(1):78-95.
44. Agatonovic-Kustrin S, Morton DW. HPTLC - Bioautographic methods for selective detection of the antioxidant and α-amylase inhibitory activity in plant extracts. MethodsX. 2018; 20(5); 797-802.
45. Rawand S.Abu Soud, Hamdan Lmad I, and Fatma U Afifi., Scientia Pharmaceutical. Alpha Amylase Inhibitory Activity of Some Plant Extracts with Hyporrlvcemic Activity, (Sci. Pharm.), 2004; *72*(1), 25-33.
46. V Mering, J., and O. Minkowski., "Diabetes Mellitus Nach Pankreas exstirpation." Archiv für Experimentelle Pathologie und Pharmakologie, 1890, 26(5–6); 371–87.
47. Virginie Nahoum, Genevieve Roux, Veronique Anton, Pierre Rouge, Antoine puigserver, Hilmar Bischoff, Bernard Henrissat and Francoise Payan, " Crystal structure of human pancreatic alpha amylase in complex with carbohydrate and proteinaceous inhibitors, Biochemistry Journal. 2000; 201-205.
48. Wild, S., Roglic, G., Green, A., Sicree, R., & King, H.. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes care*, 2004, 27(5), 1047–1053.

*****