IN VITRO STUDIES ON ALPHA GLUCOSIDASE AND ALPHA AMYLASE INHIBITORY ACTIVITIES OF *INDIGOFERA TIRUNELVELICA* AND *BASELLA ALBA*

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Abstract

Diabetes is a chronic, metabolic disorder characterized by Hyperglycaemia (elevated levels of blood glucose), which leads over time to serious damage to the heart, blood vessels, eyes etc. India is referred to as the "diabetes capital of the world" because. Around 77 million people are diagnosed with diabetes which makes it the second most affected country after China. Since antidiabetic drugs prescribed today have frequent side effects, research on plant-based alternatives is needed. This study aims to find out the antidiabetic activity of Indigofera tirunelvelica and Basella alba by evaluating their in vitro inhibitory enzyme activities. Treatment may involve inhibition of enzymes responsible for carbohydrate degradation, such as Alpha glucosidase and alpha amylase, in order to maintain blood sugar levels. For this experiment, the plant extract was made using methanol, chloroform, and distilled water. The activity of amylase and glucosidase was measured spectrophotometrically. Inhibitory potential was measured using regression equation. IC_{50} values were calculated for all the samples including Acarbose. The IC_{50} value of Acarbose(standard) was 13.91µg/ml. The IC_{50} value of Indigofera tirunelvelica (36.83µg/ml) was less compared to Basella alba (53.96µg/ml) This indicates a stronger inhibitory effect of Indigofera tirunelvelica as it has the nearest value to that of the standard (Acarbose). Significant inhibition was seen in Indigofera tirunelvelica compared to Basella alba which suggests that, these plants can contribute for future research in the treatment of diabetes.

Keywords: *Hyperglycaemia, alpha glucosidase, alpha amylase, IC_{50}.*

1. Introduction

Diabetes refers to a metabolic disorder where hyperglycaemia (elevated blood sugar levels) is chronic (American Diabetes Association, 2014). The condition happens because the body cannot make enough insulin (Type 1 diabetes) or does not use the insulin it does produce efficiently (Type 2 diabetes). It is a hormone secreted by the pancreas that helps control blood glucose levels (Rahman, M. S., *et al*., (2021). If left untreated, the disease can cause serious complications, including cardiovascular disease, kidney damage, neuropathy and problems with vision.

Type 1 Diabetes is an autoimmune disease in which the body's immune system attacks the insulin-producing beta cells in the pancreas leading to permanent damage (Szablewski, L. 2014). It is diagnosed in childhood/adolescence and is also called as Insulin-dependent Diabetes.

The more prevalent form, Type 2 Diabetes, results from insulin resistance — when the body's cells fail to respond appropriately to insulin or in simple words it can be said that the insulin produced by the body is not enough or not efficiently taken up by the body.

It is often associated with obesity, unhealthy diets, and lack of physical activity. It is also called as Noninsulin-dependent Diabetes.

In both forms of diabetes, the body is unable to regulate blood sugar levels, resulting in elevated blood glucose, also referred to as hyperglycaemia. Diabetes can affect individuals different depending on the type and each person, but some common symptoms of diabetes such as:

Increased urination (polyuria): Elevated blood sugar levels force the kidneys to filter and absorb excess sugar. It causes increased urination (Barlow, K. E. 1939).

Increased hunger (polyphagia): The body has high amounts of blood sugar, but it cannot utilize it effectively for energy, resulting in extreme feelings of hunger.

Fatigue: If the body is unable to utilize glucose in the right manner, it can lead to low energy levels, causing fatigue (Wlodek, D., & Gonzales, M. 2003).

Unexplained weight loss (in Type 1 diabetes): Weight can also be lost, even when food intake is normal or increased, as the body begins to use muscle and fat stores for energy.

Diabetes can also cause serious complications if it is not managed, including heart disease, kidney damage, nerve damage and blindness. Recognizing the symptoms sooner and managing the disease can help avoid or delay these complications. Blood sugar monitoring is an essential part of managing the condition, and so are regular medical checkups. Diabetes is successfully treated by medication as well as lifestyle changes such as exercise and diet. Certain drugs, for instance, targets the enzymes that break down complex carbohydrates in the gut, slowing the breakdown of the carbohydrate and blunting the absorption of glucose so that blood sugar levels don't spike after the meal.

1.1 Pre-Diabetes

It is a term that applies to a state of glucose impairment in which glucose levels are above normal, but not high enough to be diagnosed as Diabetes (Hostalek, U. 2019). Other, two generally used terms for pre diabetes are 'Impaired fasting glucose (IFG)' or 'Impaired Glucose Tolerance (IGT)'. It is important to note that pre-diabetes is not a type of diabetes, still individuals with pre-diabetes are at a high risk for developing type 2 Diabetes (fig.1)

The diagnostic criteria for Pre-diabetes are as follows: Blood glucose levels between 140-200 mg/dL after eating (IGT) and Fasting blood glucose levels between 100-125 mg/dL (IFG).The diagnostic criteria for diabetes are as follows: Blood glucose levels above 200 mg/dL after eating, Fasting blood glucose levels above 126 mg/dL.

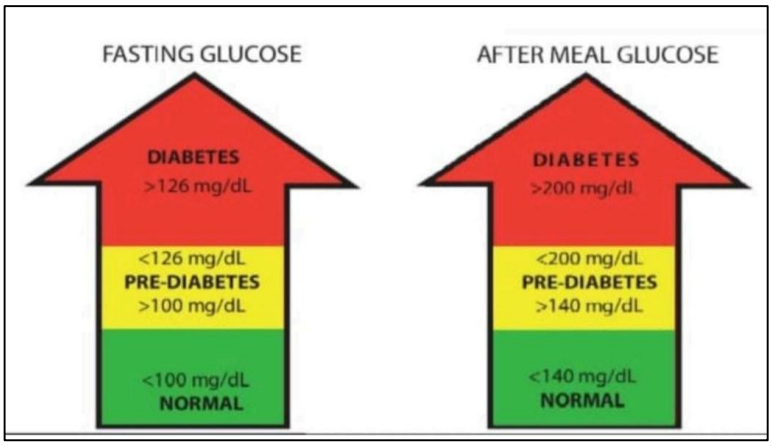

Figure 1: The Glucose Range for Diabetes, Pre-diabetes and Normal

In order to manage diabetes, it is very important to control the breakdown of carbohydrates into simple sugars as it determines the regulation of blood glucose concentration. Two catabolic enzymes involved in carbohydrate metabolism are alpha amylase and alpha glucosidase. Both of these enzymes catalyse hydrolysis of carbohydrates, but during different phases of the digestive process (Goto, T., Horita, M. et al 2012). Their functions can assist during diabetes management.

1.2 Alpha- Amylase

Alpha-amylase is an integral enzyme in the salivary glands as well as the pancreas that breaks down larger carbohydrate compounds such as starch into simpler, digestible sugars (Lapis, T. J., Penner, M. H., Balto, A. S., & Lim, J. 2017). Starch is a complex carbohydrate and metabolizes into glucose and simple sugars. Salivary amylase is the first enzyme to start carbohydrate digestion and is produced in the mouth, mixed in with saliva. Second phase of digestion involving complex carbohydrates is executed by pancreatic amylase, which is let out from the pancreas during small intestine digestion (Williams, J. A. 2019).

1.2.1 Alpha-Amylase

Alpha amylase's biological activity consists of hydrolysation of the alpha 1,4 glycosidic bonds of starch and glycogen and cleaving glucose units (Kasar, S. S., Maheshwari, V. L., & Pawar, P. K. 2022). The cleavage produces chains of glucose shorter than sugar molecules known as dextrin and maltose. It also converts starch into sugars that can be absorbed in the small intestine.

1.2.2 Alpha Amylase and Diabetes

In Type 2 Diabetes, carbohydrates inflow should be managed in their rate and speed to avert sharp elevations of glucose concentration in blood following ingestion. Carbohydrates do need to be broken down, but not at an excessively hasty pace. Use of alpha amylase inhibitors, such as acarbose, slows down the pace of enzymatic breakdown (DAMILOLA, O. M. 2015).

These drugs induce a delay in digestion of carbohydrates by inhibiting alpha amylase, which leads to gradual absorption of Glucose and consequently prevents sharp increments of blood Glucose levels.

1.3 Alpha- Glucosidase

Alpha-glucosidase is an enzyme that acts on carbohydrates and is primarily present in the small intestine (Bhatnagar, A., & Mishra, A. 2022). Patients with diabetes can also utilize it as a target site during glucose metabolism. Its primary utility is the transformation of di and oligosaccharides (short chains of glucose units) monosaccharides (glucose). Mono-sugars such as glucose can then be absorbed into the bloodstream.

1.3.1 Action of Alpha-Glucosidase

Alpha-glucosidase is involved in the breakdown of oligosaccharides and disaccharides formed by the action of alpha - amylase. The enzyme acts on maltose, which is broken down into two glucose units. Glucose is the only monosaccharide that is absorbed by the small intestine. This step is crucial since glucose is the only monosaccharide that can be absorbed by the small intestine and thus enter the bloodstream.

1.3.2 Alpha Glucosidase and Diabetes

Alpha-glucosidase is the last enzyme acting on glucose metabolism in the body. It works on complex carbohydrates and is the last notably after everything is digested and the maximum quantity of glucose is released. In cases where an individual suffers diabetes, it will work at an optimized glucose absorption level, while if the individual does not have the condition, they risk suffering hyperglycaemia.

1.4 Inhibiting Enzymes for Type 2 Diabetes

Type 2 diabetes patients use alpha-glucosidase inhibitors for controlling blood glucose levels. Some hormones can achieve optimal glucose concentration on diabetes patients and a rapid increase in glucose for non-diabetes and an example is acarbose. These medications in blunt alpha-glucosidase work on increasing blood glucose boosting the disaccharides and oligo chains. Gaining control of hyperglycaemia removes exposed glucose to the bloodstream and upper banking carries it without delay.

Diabetes is Controlled in a Person through the Management of its Enzymes.

Both alpha-amylase and alpha-glucosidase, in addition to other enzymes, are involved in the digestion of starch and absorption of glucose in the body. In diabetes, especially in Type 2 diabetes, postprandial hyperglycaemia or excessive increase in blood sugar after meals needs to be controlled.

1.4.1 Alpha Amylase Inhibitors

This alpha-amylase technique helps to lessen the absorption of glucose by delaying the breakdown of multipolymer carbohydrates into simpler compounds.

1.4.2 Alpha Glucosidase Inhibitors

This type of inhibition ensures that there is a slower, more controlled conversion of oligo and di saccharides to glucose to prevent spikes from occurring in the blood glucoses levels, which in turn helps with blood glucose regulation, which in long-term, will significantly help in avoiding diabetes-related conditions**.**

In diabetes, the short-term effect of these enzyme inhibitor drug therapies is to decrease high blood glucose levels. Presently used synthetic enzyme inhibitors cause gastrointestinal side effects such as diarrhoea, flatulence, abdominal bloating, etc (Nair, S. S., Kavrekar, V., & Mishra, A. 2013). Therefore, natural alpha amylase and glucosidase inhibitors from the dietary plants can be used as an effective therapy for treating postprandial hyperglycaemia with minimal or no side effects.

In the study conducted, the inhibition potentials of methanolic, chloroform, and aqueous extracts of Indigofera tirunelvelica (fig.2.1) and Basella alba (fig.2.2) were evaluated against alpha amylase and alpha glucosidase enzymes.

1.5 Indigofera Tirunelvelica

Indigofera tirunelvelica (fig 2.1) is a medicinal plant from the Fabaceae family that is rich in phytochemicals such as flavonoids, tannins and alkaloids. It has been used for its antidiabetic, anti-inflammatory, and anti-oxidative properties. Some recent research concerning this property have shown its effects on carbohydrate-digesting enzymes, such as α-amylase and α-glucosidase, demonstrating anticancer mechanisms for arthritis and diabetes prevention, among others.

1.6 Basella Alba

Basella alba (Malabar spinach) (fig. 2.2) is a leafy vegetable of the Basellaceae family. Packed with vitamins, minerals, and antioxidants, it is also widely used for its nutritional and medicinal properties. It displayed moderate antidiabetic, antimicrobial, and anti-inflammatory activities. The plant's active constituents, including saponins and polyphenols, play a role in its inhibitory effects on enzymes, indicating its role in regulation of glucose metabolism.

Figure 2.1: ***Indigofera Tirunelvelica*** **Leaves Figure 2.2:** ***Basella Alba*** **Seeds**

2. Materials and Methodology

Indigofera tirunelvelica and *Basella alba* were selected for this study to evaluate their in vitro inhibitory activities against two very important enzymes: alpha-amylase and alpha-glucosidase. These two enzymes participate in the carbohydrate digestion process, and inhibition of these could be beneficial for controlling blood sugar levels in diabetes.

2.1 Sources and Preparation of Plant extracts

Fresh leaves of t*irunelvelica* and *Basella alba* were procured from a local source. The plants were washed to remove dirt, dried, and grounded into a fine powder. The bioactive compounds were extracted from the powdered plant material. Extraction was carried out using methanol, chloroform and aqueous solution.

2.2 Enzyme Sources

Two enzymes were used for the enzyme inhibition assays; alpha-amylase and alpha-glucosidase. Alpha-amylase was either from porcine pancreas or human saliva and alpha-glucosidase was from Saccharomyces cerevisiae (yeast) or sweet potato.

2.3 Determination of alpha amylase inhibitory activity

Alpha amylase inhibition was first determined using a starch substrate to evaluate plant extracts for enzyme inhibition. Starch was incubated with the plant extract and alpha amylase at 37°C for 30 minutes. Phosphate buffer is used to maintain the pH of the solution. Stop the reaction and determine starch hydrolysis by measuring the colour change of DNS reagent and record absorbance at 540 nm. Inhibition percentage (IC50 value) was calculated by comparing enzyme activity in the presence and absence of plant extract.

2.4 Determination of alpha glucosidase inhibitory activity

A different method was employed for alpha-glucosidase inhibition. The extracts of the plants were incubated with p-nitrophenyl-α-D-glucopyranoside (pNPG) as substrate and alpha-glucosidase enzyme at 37°C. Phosphate buffer was added to the solution to maintain the pH. After incubation, the reaction was allowed to be stopped by adding Sodium carbonate (Na2CO3) and the absorbance was measured at 440 nm. This assay determines the capability of the plant extract in the inhibition of glucose release from the substrate.

Concentration-Response Curve: Varied concentrations of the plant extracts were tested (50 µg/ml, 100 µg/ml, 200 µg/ml) to determine IC_{50}, which is the concentration required to inhibit 50% of enzyme activity. Control groups (without the plant extract) were included to account for baseline enzyme activity. Less the IC_{50}, value more will be the inhibition.

The percentage of enzyme inhibition will be calculated using the following formula:

$$\text{Inhibition } \% = \left(\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right) \times 100$$

The enzyme activity was calculated as: Blank value – Sample / blank value x 100

IC_{50} values are calculated using the formula: (regression analysis)

$Y = mx + c$

IC_{50} (X) = Y – c /m (or) Y + c /m

Where,

X = unknown concentration, Y = 50, C = intercept, m = Slope

The IC_{50} values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non-linear regression analysis from the mean inhibitory values. Acarbose was used in studies as a positive control or reference substance for enzyme inhibition. All tests were performed in triplicate.

3. Results and Discussion

The current investigation assesses the inhibitory activity of *Indigofera tirunelvelica* and *Basella alba* on Starch digesting enzymes- alpha-amylase and alpha-glucosidase using varying solvent extracts. The bioactivity (enzyme inhibition) of the extracts were determined and compared to the standard Acarbose. Results showed an inhibition of the enzyme depending on the solvent extract used.

Alpha-glucosidase showed significant inhibition when treated with methanolic extracts from both Indigofera tirunelvelica and Basella alba as demonstrated in Figures 3.1 and 3.4 respectively. The chloroform and aqueous extracts failed to show any inhibitory action on this enzyme. Alpha-amylase inhibition was only detected in *Indigofera tirunelvelica's* aqueous extract according to Figure 3.2. Both methanolic and aqueous extracts of *Basella alba* demonstrated inhibitory effects on alpha-amylase activity as shown in Figure 3.5.

The IC_{50} values of all extracts were measured alongside the standard inhibitor Acarbose as shown in Figure 3.3. Lower IC_{50} values indicate stronger enzyme inhibition. Acarbose showed an IC_{50} value of 13.91µg/ml which served as the baseline for comparison. *Indigofera tirunelvelica* demonstrated superior inhibitory activity against alpha-glucosidase by displaying a lower IC_{50} value of 36.83µg/ml in comparison to *Basella alba's* IC_{50} value of 53.96µg/ml. *Indigofera tirunelvelica* demonstrates as a superior natural alpha-glucosidase inhibitor because its IC_{50} value approaches that of Acarbose.

The results demonstrate Indigofera tirunelvelica and Basella alba's potential as enzyme inhibitors which could help manage postprandial hyperglycemia. Researchers must conduct additional studies to extract and identify the active phytochemicals that produce the observed biological effects.

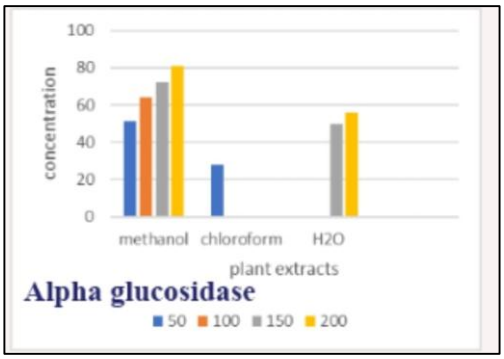

Fig.3.1: Inhibitory Activity of Tirunelvelica against Alpha Glucosidase

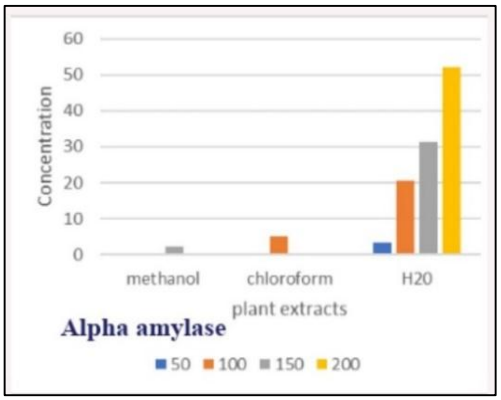

Fig.3.2: Inhibitory Activity of Tirunelvelica against Alpha Amylase

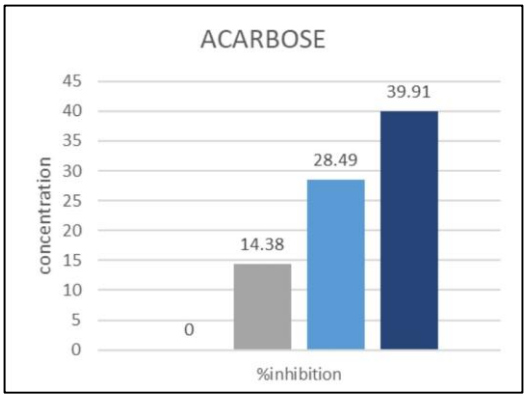

Fig.3.3: Inhibitory Activity of Acarbose

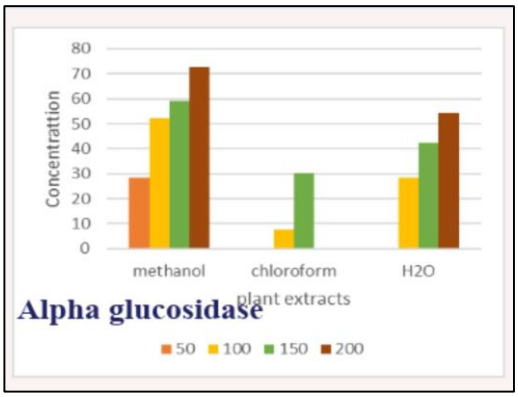

Fig.3.4: Inhibitory Activity of Alba against Alpha Glucosidase

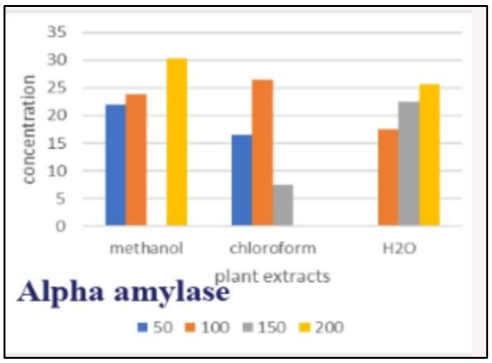

Fig.3.5: Inhibitory Activity of Alba against Alpha Amylase

4. Conclusion

The results of this study indicate that both *Indigofera tirunelvelica* and *Basella alba* possess significant alpha-amylase and alpha-glucosidase inhibitory activities in vitro, which suggests their potential as natural agents for managing blood glucose levels in type 2 diabetes. *Indigofera tirunelvelica* displayed more potent inhibitory effects compared to *Basella alba*, with both plants exhibiting dose-dependent inhibition of the enzymes. The identified phytochemical compounds, including flavonoids and phenolics (Kanchana, S. P., & John, A. A. 2020), may play a role in the observed activity, but additional research is needed to determine the exact bioactive compounds involved. This study contributes to the discovery of natural therapeutic agents by providing a scientific basis for traditional use of these plants in diabetes management. However, additional research, including *in vivo* studies and clinical trials, is required to validate their effectiveness and safety for diabetic patients. This study also supports the, potential application of these plants in managing postprandial hyperglycemia and diabetes.

References

1. American Diabetes Association. (2014). Diagnosis and classification of diabetes mellitus. Diabetes Care, 37(Supplement_1), S81-S90.
2. Barlow, K. E. (1939). Diabetes and kidney failure. British Medical Journal, 2(4110), 765.
3. Bhatnagar, A., & Mishra, A. (2022). α-Glucosidase inhibitors for diabetes/blood sugar regulation. In Natural products as enzyme inhibitors: An industrial perspective (pp. 269-283). Singapore: Springer Nature Singapore.
4. Damilola, O. M. (2015). Influence of gallic acid on α-amylase and α-glucosidase inhibitory and antioxidant properties of acarbose (Doctoral dissertation, Department of Biochemistry, Faculty of Science, Federal University Oye-Ekiti).
5. Engelgau, M. M., Thompson, T. J., Smith, P. J., Herman, W. H., Aubert, R. E., Gunter, E. W., ... & Ali, M. A. (1995). Screening for diabetes mellitus in adults: the utility of random capillary blood glucose measurements. Diabetes Care, 18(4), 463-466.
6. Goto, T., Horita, M., Nagai, H., Nagatomo, A., Nishida, N., Matsuura, Y., & Nagaoka, S. (2012). Tiliroside, a glycosidic flavonoid, inhibits carbohydrate digestion and glucose absorption in the gastrointestinal tract. Molecular Nutrition & Food Research, 56(3), 435-445.
7. Hostalek, U. (2019). Global epidemiology of prediabetes-present and future perspectives. Clinical Diabetes and Endocrinology, 5(1), 5.
8. Kanchana, S. P., & John, A. A. (2020). Pharmacogenetic and phytochemical studies of Indigofera tirunelvelica Sanjappa. Research Journal of Pharmacy and Technology, 13(2), 923-927.
9. Kasar, S. S., Maheshwari, V. L., & Pawar, P. K.* (2022). Bioactive α-amylase inhibitors: Sources, mechanism of action, biochemical characterization, and applications. Natural Products as Enzyme Inhibitors: An Industrial Perspective, 59-104.
10. Lapis, T. J., Penner, M. H., Balto, A. S., & Lim, J. (2017). Oral digestion and perception of starch: Effects of cooking, tasting time, and salivary α-amylase activity. Chemical Senses, 42(8), 635-645.
11. Nair, S. S., Kavrekar, V., & Mishra, A. (2013). In vitro studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. European Journal of Experimental Biology, 3(1), 128-132.

12. Rahman, M. S., Hossain, K. S., Das, S., Kundu, S., Adegoke, E. O., Rahman, M. A., ... & Pang, M. G. (2021). Role of insulin in health and disease: an update. International Journal of Molecular Sciences, 22(12), 6403.
13. Sharma, V., Mishra, A., & Rani, J. (2024). Pathophysiology, nutrition, and clinical outcomes in gestational diabetes mellitus: A comprehensive review.
14. Smith, D. L., Orlandella, R. M., Allison, D. B., & Norian, L. A. (2021). Diabetes medications as potential calorie restriction mimetics—a focus on the alpha-glucosidase inhibitor acarbose. Gero Science, 43, 1123-1133.
15. Szablewski, L. (2014). Role of immune system in type 1 diabetes mellitus pathogenesis. International Immunopharmacology, 22(1), 182-191.
16. Wang, X., Guo, L., Zheng, L., Zhao, W., & Li, L. (2024). Natural sweetener glycyrrhizin acid monoglucuronide improves glucose homeostasis in healthy mice. Journal of Agricultural and Food Chemistry, 72(7), 3483-3494.
17. Williams, J. A. (2019). Amylase. Pancreapedia: The Exocrine Pancreas Knowledge Base.
18. Wlodek, D., & Gonzales, M.* (2003). Decreased energy levels can cause and sustain obesity. Journal of Theoretical Biology, 225(1), 33-44.