Plant Archives Vol. 21, No. 2, 2021 pp. 653-655 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no2.100

ISOLATION AND CHARACTERISATION OF AMYLASE INHIBITOR

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(Date of Receiving : 09-05-2021; Date of Acceptance : 18-08-2021)

ABSTRACT

Diabetes mellitus is a type of disorder of multiple aetiology which is related to the metabolism, symptoms of which included hyperglycaemia followed by the disturbance in the metabolism of carbohydrates, protein and fat. This abnormality may occur due to unusual insulin secretion or insulin action or both. The effect of diabetes mellitus includes long term damage, dysfunction and failure or various organs. α-amylase is saccharide degrading inherent in living body that controls the diabetes mellitus and keep the blood glucose level to its threshold level, without affecting to any normal body mechanism. Thus α-amylase performs an important role as a digestive enzyme for carbohydrate in the body and control of glucose in blood. In general, medicine is usually an intermixture of various chemical components & exert some side effect caused by prolong dosage. Plant derived extract contains some chemical substances i.e. secondary metabolites that have the ability to inhibits α-amylase present in saliva and are easily applied to food, drugs and medicine These inhibitors inhibit the activity of α -amylase.

Keywords : Metabolism disorder, amylase inhibitor, blood glucose level, hyperglycemia, GLUT proteins.

INTRODUCTION

Diabetes had reached today its toll in every part of the world and becoming a challenging problem for the mankind. According to the international Diabetes federation, during the last 20 years the total number of people with Diabetes worldwide raised from 30 million to 230 millions & expected to reach 350 million by 2025. Developing countries are the most sufferers of Diabetes. China and India now have the most Diabetes patients in the world (35.5 million, china-23.8 million). Diabetes is caused by a deficiency in secretion or action of insulin and destruction of insulin producing islet cells of pancreas. The increase in the number of diabetes patients is due to lifestyle, diet and genetics. People with Diabetes are at increased risk of cardiovascular, peripheral, muscular & cerebrovascular disease. In its most severe from ketoacidosis or non-ketotic hyper osmolar state may be developed and lead to stupor coma and in the absence of effective treatment may lead to death. The enzyme α-amylase present in the saliva is an important enzyme in the digestion system of carbohydrates and it plays a key role in the control of glucose in blood. It keeps the blood glucose level to its threshold level without affecting to any normal body mechanism. Hence efforts are being and to control the activity of enzymes. The objective of study is to investigate this feature of α-amylase inhibition with locally available plant extracts. The α -amylase inhibition activity of the plant extracts of *Gymnema syivester* (Gurmar), *Psidium guajava* (Guava), *Tamarindus indica* (Imli), *Mirabilis jalapa* (Mulberry) & *Morus nigra* (Gulbash) were calculated in aqueous & organic (ethanol) media. Diabetes is being studied through many perspectives like its types, symptoms, precautions and preventions. Here, the description of diabetes mellitus with these perspectives is made which is followed by the information in the sources used for generating ingredients for the α-amylase inhibitor.

Diabetes Mellitus

The term diabetes mellitus describe a metabolic disorder of multiple aetiology characterized by hyperglycemia with disturbance of carbohydrate, protein & fat metabolism resulting from defect in insulin secretion & insulin action or both.

Symptoms of diabetes mellitus

Diabetes mellitus may be present with characteristic symptoms such as thrust, polyurea, blurring of vision and weight loss. In its most severe from ketoacidosis or a non-ketotic hyper osmolar state may be developed & lead to stupor, coma & in absence of effective treatment leads to death.

Effects of Diabetes mellitus

The long term of Diabetes mellitus include progressive development of specific complication of retinopathy with potential blindness, nephropathy that may leads to renal failure & or nephropathy with risk of foot ulcers, amputation Charcot joint and features of autonomic dysfunction, including sexual dysfunction several pathogenetic progresses which destroy the beta cells of the pancreas with consequent insulin deficiency and others that result in resistant to insulin action. The abnormalities of carbohydrate, protein and fat

metabolism are due to deficient action of insulin or target tissue resulting from insensitivity of lack of insulin.

Normal Mechanism of Glucose Transport

GLUT is a family of membrane spanning proteins which facilitate the glucose entry into many cells. There are five such proteins with high degree of homology which are involved in concentration driven transport of glucose over cellular membranes.

Table 1 : Properties of glucose transpose proteins

Transporter	Tissue distribution	Special properties
GLUT 1	Most cells	High capacity relatively low km (1-2mM)
GLUT 2	Liver, beta cells, hypothalamus, small intestine	High capacity but low affinity part of "the glucose sensor" in β-cell carrier for glucose and fructose liver & intestine.
GLUT 3	Neurons, placenta, testes	Low Km (1mM) and high capacity
GLUT 4	Skeletal and cardiac muscle, fat	Activated by insulin. K= 5mM.
GULT 5	Mucosal surface in small intestine, sperm	Primarily fructose carrier in intestine

These transport proteins mediate facilitated transport, that is they can only transport glucose that is, they can only transport glucose (or fructose) from areas of high concentration to areas of lower concentration. The sugar is bound by the protein a flip-flop mechanism reverses the membrane direction of the sugar protein complex, the sugar is released & the protein flips around once more to initiate a new cycle transport activity dependent upon the sugar concentration and the number of transport proteins in the outer cell membrane. In principle the GLUT family can transport glucose both into & out of cells. In most tissues the internal glucose concentration is quite low. Transport can only processed from the extracellular areas into the cell.

In gluconeogenetic tissues (liver & kidney) intracellular glucose concentration can exceed blood glucose concentration in the post absorptive & fasting states. Export of glucose from liver & kidney occurs through GLUT 2.

The insulin- sensitive glucose transporter GLUT4, is found bound to internal cellular membranes where it is inactive. GLUT4 is bound to golgi apparatus. GLUT4 brought to plasma membrane by an ATP requiring process. The transport protein molecules that arrive at the surface membrane contribute to glucose transport. Insulin shifts the balance between exocytosis & endocytosis such that the number of functional GLUT 4 molecules in the plasma membrane increases thereby activating glucose uptake.

α-Amylase- enzyme controlling blood glucose

Glucose is a major source of energy in human body, but unfortunately, free glucose is relatively rare in typical diet. Glucose is locked up in many larger forms, including lactose and sucrose, where two small sugars are connected together & long chain of glucose like starches & glycogen.

Attacking starch

α-Amylase begins the process of starch digestion. It takes starch chains and breaks them into smaller pieces with two or three glucose units. Two similar types of amylase are made in human body, one is secreted in saliva, where is starts to break down starch grains during chewing & the other is secreted by the pancreas, where it finishes it's job. Digestion begin in mouth where salivary α-Amylase is hydrolyses the internal glycosidic linkage of starch, producing short polysaccharide fragment or oligosaccharide. In stomach salivary α-Amylase is inactivated by low pH, but second form of α-Amylase secreted by pancreas in to small intestine, continues break down process pancreatic α-Amylase yield mainly maltose & maltotriose (the di & tri saccharides of α 1-4 glucose & oligosaccharide called limit dextrins, fragments of amylopectin containing α-1-6 branch point.

Amylase in Action:

Since amylase needs to perform its job in the unpleasant environment of the intestine, it is small, stable enzyme resistant to unfavourable conditions.

Types of Diabetes:

a. Type-1 Diabetes

b. Type-2 Diabetes

I. Type-1 Diabetes (Mellitus diabetes Type 1T1D, IDDM)

II. b.Type-2 Diabetes (diabetes mellitus type II, non insulin dependent diabetes (NIDDM), obesity related diabetes, or adult- onset diabetes.

Diabetes mellitus again sub-divided into

a. Insulin dependent (Type- I)

b. Diabetes in children.

c. Autoimmune diabetes mellitus (Beta cell destruction, usually leading absolute deficiency)

d. Genetic defect in insulin action

e. Drugs or chemical induced diabetes.

f. Infection (causes) β-cell distruciton.

Glucose Measurement in blood

For measurement of glucose in blood the o-toluidine method also remain in use but enzyme based method like hexokinase & glucose dehydrogenase method which are highly accurate & rapid device for measurement.

When glucose test in blood & urine is repeatedly positive after that the ketone body test should performed for confirmation of high level of diabetes. In this condition glycosuria the diabetic patient have an unacceptably several level of metabolic disturbance & indicates an urgent need for corrective action.

MATERIALS & METHODS

The plant resources (leaves & speeds) of *Gymnema sylvestres, Psidium guajava, Tamarindus indica, Mirabilis jalapa* & *Morus nigra* were collected. These leaves & seeds were healthy & disease free. These were dried in shed & powdered.

The power of dried leaves of each plant (25gm) was extracted in 300 ml solvent by using soxhlet apparatus separately. The solvent used for extraction 75% ethanol & water having boiling point 80-85°c & 100°C respectively.

Seeds of *Mirabilis jalapa* were crushed in mortar & pastel with hexane. Thus seeds were defatted, such obtained defatted cake was used for further extraction in distilled water. The extracts then were concentrated using rotary evaporator, under reduced pressure.

RESULTS & DISCUSSION

Quality assessment of traditional medicines (TM).

- To obtain standards of the secondary metabolites present in all above plants.
- Separation of biologically active components from extract.
- Structural activity, relationship (SAR) studies of active components.
- Clinical trials of molecules isolated.
- Parent components can be made more potent by chemical synthesis or drug designing.
- Formulation of herbal drug having anti diabetic activity.
- Characterisation by IR, HPCL, NMR, GLC.
- Antioxidants activity can be check by sulphur oxide dismutase (SOD); Nitrate.

Determination of α-Amylase inhibition activities

The α-Amylase inhibition shown by each plant extracts in aqueous & organic media were determined and calculated as percentage inhibition.

Table 2 : α-Amylase inhibitory activity of extracts in aqueous of extracts in aqueous & organic media

Plant	Solvent	% Inhibitory activity
Gymnema sylvestre	Ethanol/D.W.	97.5 %
Psidium guajava	Ethanol/D.W.	47.83 %
Tamarindus indica	Ethanol/D.W.	44.44 %
Mirabilis jalapa	Ethanol/D.W.	9.0 %
Morus nigra	Ethanol/D.W.	1.39 %

CONCLUSIONS

From experimental evidences & subsequent interpretation following points can be inferred.

1. The α-Amylase inhibition activities of aqueous and organic plant extracts for *Gymnema sylvestre, Tamarindus indica, Mirabilis jalapa, Psidium gujava Monus nigra* were determined.
2. *Gymnema sylvestre* was found to be having most inhibition activity while *Morus nigra* had the least contents of inhibitors.

Scope for future study

Quality assessment of traditional medicines (TM)

- To obtain standards of the secondary metabolites present in all above plants.
- Separation of biologically active components from extract.
- Structural activity, relationship (SAR) studies of active components.
- Clinical trials of molecules isolated.
- Parent components can be made more potent by chemical synthesis or drug designing.
- Formulation of herbal drug having anti diabetic activity.
- Characterization by IR, HPLC, NMR, GLC,
- Antioxidants activity can be check by sulphur oxide dismutase (SOD); Nitrate.

Acknowledgement

The author thanks Department of Biotechnology, Moolji Jaitha College, North Maharashtra University, Jalgaon, Maharashtra for providing necessary infrastructure and facilities to carry out the research work.

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