REVIEW ARTICLE

"Polypeptide-k" as phytoinsulin: How much and how far

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Abstract

Diabetes mellitus (DM) is a metabolic disorder characterized by high blood glucose levels, occurs due to insulin resistance or insulin deficiency. In 2015, 415 million people worldwide suffered from DM. There have been number of antidiabetic drugs and recombinant DNA insulin used for diabetes; however, these have certain limitations in terms of side effects and cost. Need of society and efforts of scientists led to discovery of phytoinsulins. A plethora of literature is available with reports of the presence of insulin-like hormones in plants. These include bacteria (*Escherichia coli*), protozoa (*Tetrahymena pyriformis*), fungi (*Neurospora crassa* and *Aspergillus fumigatus*), and plant (*Momordica charantia, Canavalia ensiformis, Vigna unguiculata, Bauhinia variegate,* and *Spirulina maxima*) that are used to treat DM. This theory of presence of phytoinsulins has been further strengthened by presence proteins associated with insulin signaling pathways in plants. Polypeptide-k (PPK), an isolated peptide from *M. charantia* has shown its therapeutic potential as antidiabetic drug. It has structural similarity with insulin moreover, safety and efficacy of PPK as antidiabetic drug has been proven through various preclinical and clinical studies. Phytoinsulins like PPK have potential to replace costly recombinant DNA insulin. However, more clinical studies are required to establish PPK and other phytoinsulins to establish as first-line therapy in the management of diabetes.

Key words: Antihyperglycemic protein, diabetes mellitus, marketed formulations, *Momordica charantia*, polypeptide-k

INTRODUCTION

Diabetes mellitus (DM) is considered as one of the global health emergencies of the 21st century. Many people are still unaware about its complications. About 50% of people remain undiagnosed with diabetes. This lack of awareness is one of the biggest barriers in management of DM. In 2015, 415 million people worldwide suffered from DM. This number has been predicted to be increased to 649 million by 2040. DM leads to death of 5 million people worldwide in 2015. When this number is compared in terms of mortality with mortality of malaria (0.6 million), tuberculosis (1.5 million) and acquired immunodeficiency syndrome (1.5 million), this number is several times more than these.[1] Number of children with type 1 DM (T1DM) was 5,42,000 worldwide in 2015. The prevalence of DM in South East Asia in 2015 was 8.5% with 78 million peoples suffering from DM. After China, India is the second largest country with higher prevalence of diabetes with 69.2 million peoples in 2015. There were about 1.2 million deaths estimated due to DM in South East Asia out of them about 1 million, were reported only in India. In 2040, the prevalence of people with diabetes was estimated to be 140 million in South East Asia. The prevalence of children with T1DM was 81,400 in 2015 rising with rate of 13,100 every year in South East Asia. Out of these 70,200 children with T1DM live only in India.[1]

DM is a metabolic disorder characterized by high blood glucose levels, occurs due to insulin resistance or insulin deficiency.[2,3] Diabetes was reported 3000 years ago, in Egyptian manuscript.[4] Type 2 DM (T2DM) is the most common form of diabetes characterized by hyperglycemia, insulin resistance, and relative insulin deficiency.[5]

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A person is diagnosed with diabetes when his fasting plasma glucose levels >126 mg/dl or postpariendal glucose levels >200 mg/dl or glycated hemoglobin (HbA1c) levels >6.5%.[3]

Uncontrolled diabetes may cause various acute and chronic complications such as retinopathy, cardiovascular disease, neuropathy, nephropathy, diabetic foot, and diabetes ketoacidosis.[3] Poor management of diabetes will cause early deaths and serious complications. However, a good management of diabetes and glucose levels with increase the quality of life and patient can live a long healthy life.[1] United Nation has kept the goal to reduce the mortality from non-communicable disease to one-third by 2030.[1]

Diabetes cannot be treated but can be managed with the help of insulin and oral antidiabetic drugs. As discussed, uncontrolled glucose levels may cause serious complications. Hence, management of diabetes is very important.[3] Since no or negligible insulin is produced in T1DM hence, administration of insulin is used for management of T1DM. In T2DM, both insulin and oral antidiabetic drugs can be used.[6,7] Subcutaneous administration of insulin to manage diabetes in one of the key treatment options but it has certain limitations such as painful delivery through subcutaneous route, lipid hypertrophy, and risk of hypoglycemia. Since insulin has to be administered regularly and the treatment period is long, such complications are very common to occur.[6,7] There have been number of antidiabetic drugs and recombinant DNA insulin used for diabetes; however, these also have limitations in terms of side effects and cost. Various oral antidiabetic drugs used are catagorized as sulfonylureas, gliptins, sodium/glucose cotransporter 2 (SGLT2) inhibitors, biguanides, α-glucosidase inhibitors, meglitinide analogs, and thiazolidinediones. Sulfonylureas usually cause hypoglycemia (especially with chlorpropamide and glibenclamide).[8,9] Different types of insulin and antihyperglycemics with mechanism of action is listed in Tables 1 and 2.[10]

Meglitinides may cause a range of side effects, most commonly hypoglycemia, visual disturbances, abdominal pain, diarrhea, constipation, nausea, and vomiting. Thiazolidinediones such as rosiglitazone and pioglitazone may cause edema, particularly in patient with hypertension and risks of other cardiovascular diseases. α-glucosidase inhibitors like acarbose causes abdominal discomfort associated with flatulence and diarrhea.[9] Gliptins and glucagon-like peptide-1 (GLP-1) analogs can cause pancreatitis while SGLT2 inhibitor can lead to urinary tract infections.[8] GLP-1 analogs administered by subcutaneous route which is painful.

Thus, all the options available for management of diabetes will have their own limitations. These will cause a variety of side effects and also need to take regularly for the management of diabetes for a lifetime. Hence, there is a need for the development of safe and effective herbal alternative for effective management of diabetes.[11]

In contrast to allopathic treatments, the availability of herbal compounds from plant sources for the treatment of DM has provided a new era to rethink on indigenous remedies. The main advantage of these products relies on their safety as compared to allopathic drugs. Some of the herbal compounds used to treat DM are listed in Table 3.[12-155]

PHYTOINSULINS

Insulin is the major hormone that regulates glucose metabolism in body. Various roles of it are shown in Figure 1. Insulin is basically obtained from animal sources; however, there are various plants from which insulin type of peptides

Table 1: Types of insulin, their onset and duration of action, and mechanism of action[10]

Insulin type	Onset of action (h)	Duration of action (h)	Mechanism of action
Rapid acting			Insulin is anabolic hormone: Promotes synthesis of glycogen, lipids, and protein
Insulin lispro	0.2-0.3	3-5	
Insulin aspart	0.2-0.3	3-5	
Insulin glulisine	0.2-0.4	3-5	
Short acting			
Regular (soluble) insulin	0.5-1	2-3	
Intermediate acting			
Insulin zinc suspension or lente	1-2	20-24	
NPH or isophane insulin	1-2	20-24	
Long acting			
Insulin glargine	2-4	24	
Insulin detemir	1-4	20-24	

NPH: Neutral protamine hagedorn

Table 2: Types of antihyperglycemics with MOA[10]

Antidiabetic drug	Preparations	DOA (h)	Daily dose	Number of doses per day	MOA
Enhance insulin secretion					
Sulfonylureas					ATP sensitive K+channel blockers leading to depolarization of beta cells which results to release of insulin from stored granules
Tolbutamide	Rastinon (0.5 g tablets)	6-8	0.5-3 g	2-3	
Glibenclamide (glyburide)	Daonil, euglucon betanase (2.5, 5 mg tablets)	24	2.5-15 mg	1-2	
Glipizide	Glynase, glide minidiab (5 mg tablet)	12	5-20 mg	1-2	
Gliclazide	Diamicron (80 mg tablet), diazide (20,80 mg tablet), glizid (30,40,80 mg tablet)	12-24	40-240 mg	1-2	
Glimepiride	Amaryl, glypride, glimer (1,2 mg tablet)	24	1-6 mg	1-2	
Meglitinide/ phenylalanine analogues					
Repaglinide	Eurepa, raplin regan (0.5,1,2 mg tablet)	3-5	1-8 mg	3-4	ATP sensitive K+channel blockers with quick and short lasting insulinemic action
Nateglinide	Glinate, natelide (60,120 mg tablet)	2-4	180-480 mg	3-4	
DPP-4 inhibitors					
Sitagliptin	Januvia (100 mg tablet)	24	100 mg	1	DDP-4 inhibitors, which prevent degradation of GLP-1 which induces release of insulin
Vildagliptin	Galvus, jalra, zomelis (50 mg capsule)	12-24	50-100 mg	1-2	
Overcome insulin resistance					
Bisguanide					
Metformin	Glyciphage, glyomet (0.5, 0.85 g tablet, 0.5 g and 1.0 g SR tablets)	6-8	0.5-2.5 g	1-2	AMPK inhibitor, which mediate actions of biguanide
Thiazolidinedione					
Pioglitazone	Pionorm, Piorest, Piozone (15, 30 mg tablet)	24	15-45 mg	1	Selective agonists for the nuclear PPAR-α which enhances the transcription of several insulin responsive genes

DPP-4: Dipeptidyl peptidase 4, GLP-1: Glucagon like peptide-1, AMPK: AMP dependent protein kinase, PPAR-α: Peroxisome proliferator active receptor alpha, ATP: Adenosine tri phosphate

could be obtained which work in a similar way as that of insulin. This article basically focuses on phytoinsulins with more emphasis on polypeptide-k (PPK).

Early era of Phytoinsulins

Drugs of herbal origin could be one of the best alternatives for management of diabetes. Plants are important source of herbal medicine used for various ailments of human body. The use of number of medicinal plants in the treatment of diabetes has been mentioned in ayurvedic literature. A number of plants have been used for treatment of diabetes mentioned in Indian, Chinese, and Egyptian literature.[11] Phyto diab care database on herbal plant used in diabetes enlists 230 plants and 155 phytochemicals having medicinal activity against diabetes.[11]

Glucokinin, an insulin-like proteins is detected in various plants and microbes showing similar functions like animal insulin.[156] A number of medicinal plants such as *Allium cepa,*

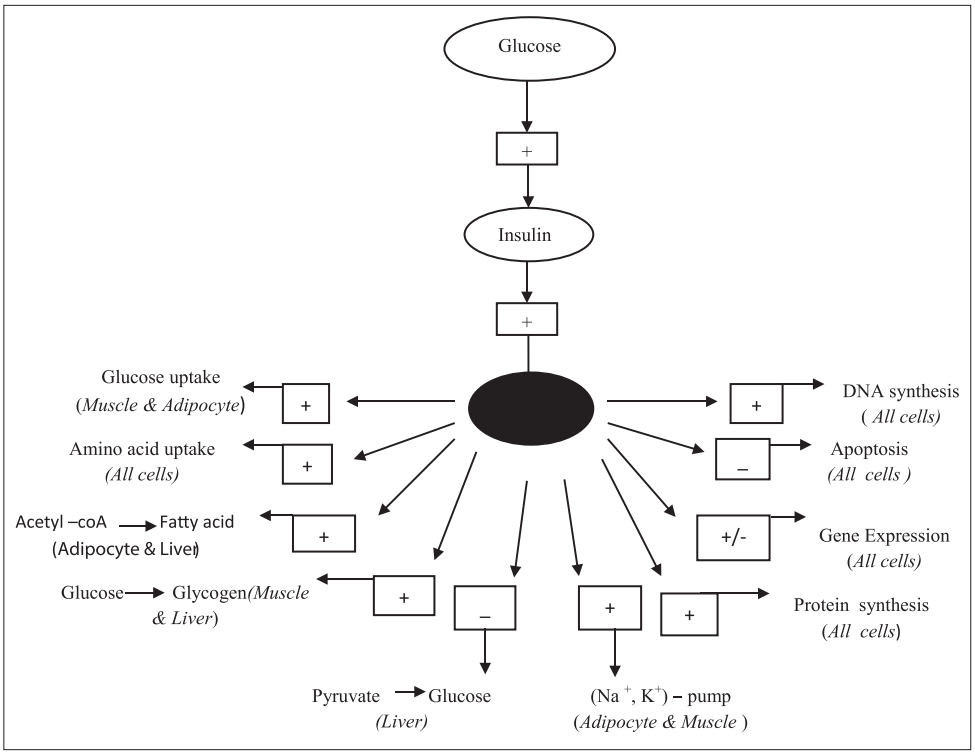

Figure 1: Physiological roles of insulin in different cells.[157] + Symbol reveals promotion; – Symbol reveals inhibition

Allium sativum, Aloe vera, Cajanus cajan, Coccinia indica, Caesalpinia bonducella, Ficus benghalensis, Gymnema sylvestre, Momordica charantia (MC), *Ocimum sanctum, Pterocarpus marsupium, Swertia chirayita, Syzygium cumini, Tinospora cordifolia, Trigonella foenum graecum, Mucuna pruriens, Murraya koenigii,* and *Brassica juncea* have antidiabetic potential.[157]

Pancreatic insulin was discovered by Banting in 1921-1922 after a collective effort led by Frederick Banting.[158] J.B. Collip and C.H. Best first published a paper on the presence of insulin-like substances in plant materials such as green tops of onions, lettuce leaves, green bean leaves, barley roots, and beetroots.[159] Discovery of insulin-like hormones in plants opened a new field in drug discovery in DM.[156]

Collip in 1923 published a paper on plant insulin. He extracted some phytoinsulins by several extraction processes including the process used for extraction of insulin from pancrease. When these plant extracts were tested on normal rabbits and pancreatectomized dogs, measurable decrease in glucose levels in blood was observed. He stated that "*The discovery of this hormone in tissues of the higher plants as well as in yeast opens up a new field of work in plant metabolism and affords another remarkable example of parallelism in certain physiological processes in the plant and animal kingdom."* In another passage he narrated, "*As the name insulin was given by the Toronto group to an extract of pancreas prepared according to a definite method elaborated by the writer, this somewhat analogous hormone derived from plant sources must be known by a more general term. Collip named it as glucokininm in order to differentiate insulin of plant origin from that of animals."*[159]

Best in 1923 reported presence of insulin-like materials in germinating potatoes and rice.[160] They mentioned in their manuscript that: "*In November, 1922, during the course of conversation with Dr. R. T. Woodyatt, in which the mechanism of the action of insulin was discussed, the idea presented itself that a hormone analogous to insulin might be present wherever glucose is metabolized, i.e., it might be present in plants*."[160] Best in 1924 also reported the presence of insulin-like materials in beetroot, which exerted the glucose lowering effect similar to insulin.[161-163] Even a step ahead, Best *et al*., in 1924, mentioned in their research that insulin may prove to be a constituent of every cell in which carbohydrate is metabolized.[162]

Elis and Eyster in 1924 showed action of insulin and glucokinin on maize germination. It was reported that growth process in plants involves metabolism of large quantity of starch into glucose. Similar process occurred in animals where glycogen mobilization occurred in liver. To carry out this work glucokinin was prepared from onion tops as well as from young maize seedlings and utilized inbred lines of maize. Authors reported both insulin and glucokinin promote the growth of maize seedlings.[164]

Late Era of Phytoinsulin (after 1970's)

After initial reports of plant insulin in this era, attention has not been provided to plant insulin. Till 1970, none of the scientists

Table 3: Herbal compounds having antidiabetic property

Botanical name	Common name	Parts used	Extracts	Active C.C	Family	Result
Aegle marmelos[16-24]	Golden apple	Leaf, seed, fruit	Ethanolic, aqueous	Aegeline, coumarin, flavanoid, alkaloid	Rutaceae	Glucose, glycosylated hemoglobin, ↑C peptide, glucose tolerance, glycogen, insulin
Allium sativum[17,25-30]	Garlic	Root	Ethanolic	Diallyl disulfide oxide, ajoene, allyl propyl disulfide, S-allyl cysteine, S-allyl mercaptocysteine	Alliaceae	Glucose, lipid, ↑insulin, oxidative stress
Averrhoa bilimbi[17,24,33]	-	Leaf	Aqueous	-	Oxalidaceae	Glucose, lipid
Aloe vera[24,31,47]	Barbados aloe	Leaf	Ethanolic	Pseudoprototinosaponin, prototinosaponin	Liliaceae	Glycosylated hemoglobin
Amaranthus esculentus[12,57]	-	Whole plant	Oil fraction	-	Amaranthaceae	Gluose, insulin
Annona squamosal[12,58-61]	-	Leaf, fruit-pulp	Aqueous, ethanolic	-	Annonaceae	Glucose, lipid, lipid peroxidation
Areca catechu[85]	Betel nut	Fruit	-	Arecoline	Arecaceae	Glucose
Andrographis paniculata Nees[86-88]	Kalmegh, King of Bitters	Aerial parts	-	Andrographolide	Acanthaceae	Prevents glucose absorption from gut, Glucose
Aerva lanata[89]	Sunny Khur	Aerial parts	Alcoholic	-	Amaranthaceae	Glucose
Artemisia pallens[90]	Davana	Aerial parts	Methanolic	-	Compositae	Peripheral glucose utilization
Beta vulgaris[159]	Beetroot	Whole plant	-	Sugar beet pectin, polydextrose	Chenopodiaceae	-
Baccharis trimera[16,17,40-43]	-	Leaf	Aqueous	-	Myrtaceae	Glucose
Bryophyllum pinnatum[71]	-	Leaf	Alcoholic	-	Crassulaceae	Glucose
Bombax ceiba[91]	Silk cotton tree	Leaf	Isolated compound	Shamimin (a flavonol glucoside)	Bombacaceae	Glucose
Barleria lupulina[93,190]	-	Aerial parts	-	-	Acanthaceae	Glucose
Boerhavia diffusea[94-96]	Tar vine	Leaf	Aqueous	-	Nyctaginaceae	Plasma insulin and improves glucose tolerance
Canarium schweinfurthi[72]	-	Steam bark	Methanolic, methylene chloride	-	Burseraceae	Glucose
Chamaemelum nobile[16]	-	Leaf	Aqueous	-	Asteraceae	Glucose
Coscinium fenestratum[77]	-	Stem bark	Alcoholic	-	Menispermaceae	Glucose, glycosylated hemoglobin, glycogen, lipid, oxidative stress

(*Contd...*)

Table 3: (*Continued*)

Botanical name	Common name	Parts used	Extracts	Active C.C	Family	Result
Caesalpinia bonducella[98-100]	Chinese cinnamon	Seed	Ethanolic	-	Cesalpinaceae	Insulin from pancreatic cells
Capparis deciduas[101,102]	-	Fruit	Powdered	-	Capparidaceae	Glucose-6-phosphate dehydrogenase in kidney and heart
Citrullus colocynthis[103-105]	Bitter apple	Seed	Aqueous, glycosidic and saponin extract	-	Cucurbitaceae	Insulin
Casearia esculenta[106-108]	Carilla Fruit	Root	Aqueous	-	Flacourtiaceae	Glucose, glycosylated hemoglobin, liver hexokinase
Camellia sinensis[109-110]	Green tea	Leaf	Hot water extract	Epigallocatechin gallate	Theaceae	Glucose, insulin
Egyptian Morus alba[16,17,44-48]	-	Stem bark	Alcoholic	-	Moraceae	Glucose, lipid peroxidation, ↑insulin
Enicostemma littorale[111-115]	Chhota chirayata	Whole plant	Aqueous	-	Gentiaceae	Glycosylated haemoglobin, glucose-6- phosphate activity in liver, insulin
Eugenia jambolana[116-125]	Indian black berry	Fruit pulp, seed	-	Aqueous, ethanolic	Myrataceae	Glucose, lipid, glucose tolerance
Eugenia uniflora[157]	Pitanga	Leaf	Ethanolic	-	Myrataceae	Inhibit increase in plasma glucose level
Glycyrrhiza glabra[126]	licorice	Root	-	Glycyrrhizinic acid	Fabaceae	Glucose, abdominal fat
Gymnema montanum[127,128]	-	Leaf	Alcoholic	-	Asclepiadaceae	Glucose, glycosylated haemoglobin, ↑insulin
Hintonia standleyana[17,77-78]	-	Stem bark	methanolic	-	Rubiaceae	Glucose
Hypoxis hemerocallidea[79]	-	Fruit	Aqueous	-	Hypoxidaceae	Glucose
Hibiscus rosa sinensis[129,130]	China rose	Leaf, flower	Ethanolic	-	Malvaceae	Insulin, utilization of glucose
Ipomoea batatas[131,132]	Sweet potato	Storage roots	Isolated compound	Diacylated anthocyanin	Convolvulaceae	Insulin resistance and acts by maltase inhibition
Lepidium sativum[6,23-25]	-	Leaf	Aqueous	-	Brassicaceae	Glucose
Lycium barbarum[16,17,36-38]	Chirchita	Fruit	Crude polysaccharide extract	Polysaccharide	Solanaceae	Glucose, oxidative stress, GLUT-4, ↑insulin

(*Contd...*)

Table 3: (*Continued*)

Botanical name	Common name	Parts used	Extracts	Active C.C	Family	Result
Leonotis leonurus[17,62-64]	-	Leaf	Aqueous	-	Lamiaceae	Glucose
Lantana camara[133]	-	Leaf	Leaf juice	-	Verbenaceae	Glucose
Momordica charantia[16,17,49-58]	Bitter melon	Whole plant	methanolic, aqueous, chloroformic	Charantin, momordicin, galactosebinding lectin non-bitter, diosgenin, cholesterol, lanosterol, β-sitosterol, cucurbitacin glycoside	Cucurbitaceae	Glucose, glycosylated hemoglobin, oxidative stress, glycogen, lipid peroxidation
Momordica cymbalaria[16,17,49-58]	Kaarali-kanda	Fruit	Aqueous	Steroidal glycoside or phenolics	Cucurbitaceae	-
Mangifera indica[16,17,60,61]	Mango tree	Fruit, leaf, stem bark	Ethanolic	Mangiferin, phenolics, flavonoid	Anacardiaceae	Glucose
Malmea depressa[24,67-70]	-	Root	Aqueous, ethanolic, n-butanol fraction	-	Annonaceae	Glucose
Memecylon umbellatum[134]	-	Leaf	Alcholic	-	Melastomataceae	Glucose
Mucuna pruriens[135,136]	Velvet bean	Seed, whole plant	Powdered seeds, alcoholic extract of plant	Trace elements manganese, zinc etc	Leguminosae	Insulin, glucose
Psidium guajava[16,17,40-43]	Guava	Leaf, fruit	Aqueous, methanolic	Terpen, flavonoid, strictinin, isostrictinin, pedunculagin, polysaccharide	Myrtaceae	Glucose
Phyllanthus emblica; Phyllanthus acidus[16,17,59]	Indian gooseberry	Fruit	Aqueous	Tannin	Euphorbiaceae	-
Piper betle[80,81]	Pan	Leaf	Aqueous	-	Piperaceae	Glucose, glycosylated hemoglobin
Picrorhiza kurroa[136]	-	Leaf, bark, root, rhizomes	Alcholic extract	-	Scrophulariaceae	Glucose levels in serum, serum lipid peroxides
Phyllanthus amarus[137,138]	-	Leaf	Methanolic extract	-	Euphorbiaceae	Glucose, glycosylated hemoglobin
Pterocarpus marsupium[139,140]	Vijaysar	Bark and heart wood	Isolated compounds	Epicatechin, marsupsin, pterosupin, pterostilbene	Fabaceae	Glucose
Pterocarpus santalinus[154]	-	Bark	Aqueous	-	Leguminosae	Glucose

(*Contd...*)

Table 3: (*Continued*)

Botanical name	Common name	Parts used	Extracts	Active C.C	Family	Result
Retama raetam[12-17]	-	Whole plant	Aqueous	-	Fabaceae	Glucose
Raphanus sativus[17,33-35]	-	Whole plant	Aqueus	-	Brassicaceae	Glucose, lipid, ↓insulin
Syzygium cordatum[16,17,40-43]	-	Leaf	Aqueous	-	Myrtaceae	↓Glucose, ↑hepatic glycogen
Salvia officinalis[17,62-64]	-	Leaf	Aqueous	-	Lamiaceae	↓Glucose↓gluconeogenesis
Scoparia dulcis[68,82]	-	Whole plant	Aqueous	-	Scrophulariaceae	↓Glucose, ↓lipid, ↓oxidative stress, ↑insulin
Strobilanthes crispus[17,49]	-	Leaf	Aqueous	-	Acanthaceae	↓Glucose
Salacia reticulate[142,143]	Salacia	Leaf	Aqueous decoction	Salacinol (alpha–glucosidase inhibitor)	Celastaceae	↓Glucose, inhibits alpha–glucosidase activity
Salacia oblonga[144,145]	-	Root	Aqueous methanolic extract	-	Celastaceae	↓Glucose, inhibits alpha–glucosidase activity
Swertia chiravita[146]	Indian gentian	Hexane fraction of plant	Isolated compound	Swerchirin	Gentianaceae	↑Insulin secretion from islets of langerhans
Scoparia dulcis[147,148]	Broomweed	Leaf	Aqueous	-	Scrophulariaceae	↓Glucose, ↓glycosylated hemoglobin, ↑plasma insulin, ↑plasma anti-oxidants
Syzygium alternifolium[149]	-	Seed	Aqueous, ethanolic, hexane	-	Myrtaceae	↓Glucose
Sida cordifolia[150]	-	Root	Methanolic	-	Malvaceae	↓Glucose
Terminalia chebula[72,83,84,154]	Chebulic myrobalan	Fruit, seed	chloroform, aqueous	Shikimic, gallic, triacontanoic, palmitic acid, β-sitosterol, daucosterol	Combretaceae	↓Glucose
Terminalia superba[16,72,83,84]	-	Stem bark	methanolic, methylene chloride	-	Combretaceae	↓Glucose
Terminalia pallid[151]	-	Fruit	Ethanolic	-	Combretaceae	↓Glucose
Terminalia arjuna[152]	Arjuna	Stem bark	Ethanolic	-	Combretaceae	↓Glucose-6-phosphatase, ↓fructose-1,6diphosphatase, ↓aldolase, ↑phosphoglucoisomerase, ↑hexokinase

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Table 3: (*Continued*)

Botanical name	Common name	Parts used	Extracts	Active C.C	Family	Result
Tinospora cordifolia[153]	-	Leaf	Hexane	-	Menispermaceae	↓Glucose
Trigonella foenum graecum[154]	Fenugreek	Seed	Isolated compound from fibers, proteins and saponins from seeds	Trigonelline	Fabaceae	↓Glucose
Ziziphus spinachristi[16,84]	Christ thorn	Leaf	n-butanol fraction, hydroalcoholic	Christinin-A, fatty acid	Rhamnaceae	↓Glucose

GLUT-4: Glucose transporter type 4

or research groups started research on phytoinsulins. But phytoinsulins, again came into picture when in 1970 Pusha Khanna reported the presence of insulin in plants.[165] Khanna, in 1976, patented a process for production of plant insulin from MC.[166] Phytoinsulin isolated from MC was called as polypeptide-p (PPP) or p insulin. This PPP was reported to have hypoglycemic activity.[167]

Ng *et al.,* in 1986, further worked on plant insulin and extracted it utilizing the seeds with acid ethanol procedure from several tissues. They also concluded that the seeds of MC contain insulin-like molecules.[168] Collir *et al.*, in 1987, published results on the isolation of proteins from etiolated rye, leaves of spinach and *Lemna giba*, which showed properties similar to animal insulin. However, the sequence analysis was not performed on plant insulin and did not contributed further to the field.[169]

Various peptide hormones whose actions are similar to insulin and the insulin-like growth factors are also present in plants.

Functions of Phytoinsulins and Similarity with Insulin Pathways

Plant insulin possess similar properties like animal insulin. It possesses various metabolic activities like animal insulin. They help in glucose transportation by various metabolic activities. Eyster and Elis showed similar activities in maize plant.[164]

A protein isolated from MC when given orally showed reduction in blood glucose levels at the predetermined points, i.e., 30, 60, 90, 150 and 180 min in a glucose infusion study on Sprange-Dawley rats. In another study on the preventive model of streptozotocin-induced diabetes showed, the fraction isolated from MC supported its suitability in treating Type I autoimmune diabetes.[166,167,170] Carboxymethyl cellulose (CMC) purified substances of spinach and *L. giba* were comparable with labeled insulin for binding to insulin receptors on IM-9 lymphocytes in a dose-dependent manner.[169] An extract of *Bauhinia variegate* showed similar properties as that of bovine insulin was studied for its hypoglycemic activity in Swiss albino mice. Intravenous injection of crude protein extract and eluted protein fractions showed a significant decrease in blood glucose levels as similar to that promoted by commercial swine insulin.[171] A number of reports published that suggested the existence of plant proteins with functions, localization, and sequences of the corresponding gene or protein, that are similar to proteins which are members of the insulin pathways. Table 4[172-179] enlists various plant proteins having similar mechanism of action as that of insulin signaling pathway in Figure 2.[180,181]

Insulin receptor is a member of family of ligand-activated receptor tyrosine kinase that includes receptors for many growth factors. Intracellular portion of receptor consist of tyrosine kinase activity.[182] Many plants consist of number

Table 4: Plant species having sequence homology of proteins of insulin signaling pathway

Proteins of insulin signaling pathway	Plant source	References
RTK	*Arabidopsis thaliana*	173
IRS protein	*Arabidopsis thaliana*-LSD 1 gene	174
GLUT-4	Sugar beet	175
PI3K	Soybean from its cDNA	176,177
Hexokinase	Plant hexokinase which is involved in sugar sensing process	178
MAPK pathway	Rice associated with insulin involved in promotion of its cellular growth	179
TOR	A potential component of the PI3K pathway in *Drosophila*	180

RTK: Receptor tyrosine kinase, GLUT-4: Glucose transporter type 4, PI3K: Phosphoinositide 3-kinase, TOR: Target of Rapamycin, IRS protein: Insulin receptor substrate proteins

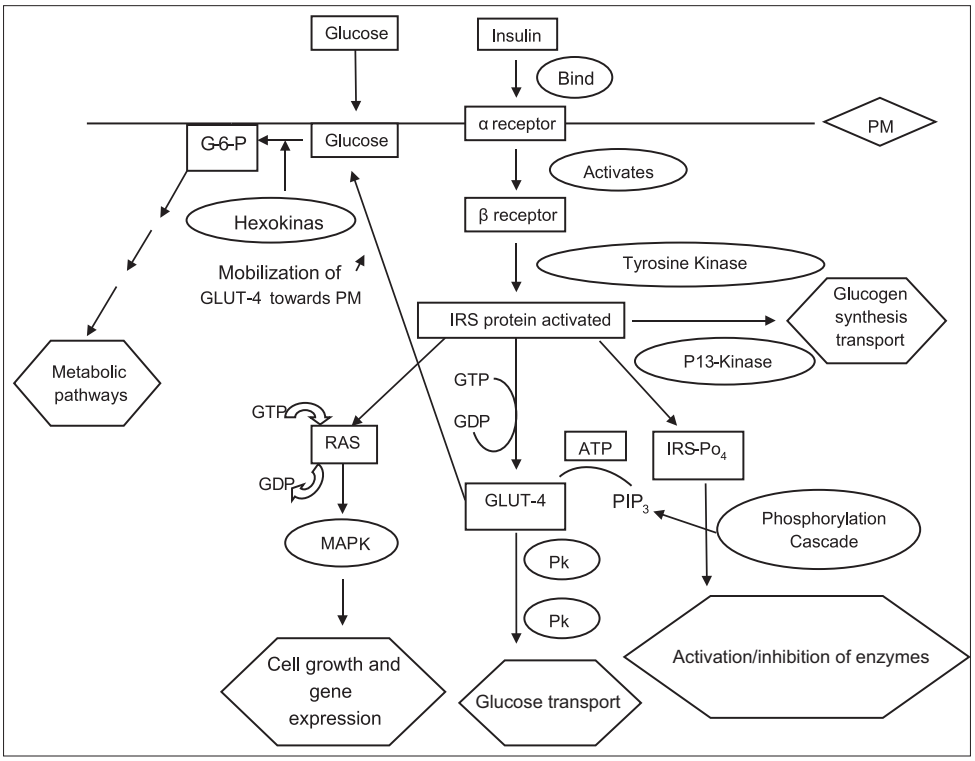

Figure 2: Insulin signaling pathway showing protein playing crucial role in signal transduction. ATP: Adenosine tri phosphate, GTP: Guanosine tri phosphate, GDP: Guanosine di phosphate, G-6-P: Glucose 6 phosphate, PIP_3: Phosphatidyl inositol triphosphate, PM: Plasma membrane, P13 kinase: Phosphatidylinositol kinases K, IRS protein: Insulin receptor substrate proteins, GLUT-4: Insulin dependent glucose transporter, PkC: Protein kinase c, PkB: Protein kinase b, RAS: Regulator of cell division and differention

of glucose/hexose transporters.[183] One such transporter was cloned from sugar beet showed high sequence homology with a mammalian glucose transporter.[174,184] Hong and Verma showed homology of cloned phosphatidylinositol 3-kinase (PI3K) cDNA from soybean to mammalian PI3K. PI3K activation is one of important steps in insulin stimulation of glucose transport.[176,182]

Despite this disbelief, a number of reviews have recently appeared presenting a body of circumstantial evidence that suggests that the presence of insulin in plants will be recognized.[185]

MC AND ITS PHYTOINSULINS

MC

MC also called as bitter gourd is a medicinal plant belonging to the family Cucurbitaceae.[186] It is well known for its use as traditional medicine in developing countries such as "Brazil, China, Columbia, Cuba, Ghana, Haiti, India Mexico, Malaya, New Zealand, Nicaragua, Panama and Peru"[187] MC has antimicrobial, antihelminthic, anticancerous, antimutagenic, antitumorous, abortifacient, antifertility, and antidiabetic properties.[188] It is used for treatment of diabetes, expulsion

of intestinal gas, promotion of menstruation, treatment of measles, hepatitis, and feverish conditions.[186]

"In Brazil MC is used for treatment of tumours, wounds, rheumatoid arthritis, malaria, vaginal discharge, inflammation, menstrual problems, diabetes, fevers, and worms. It is also used to induce abortions, as an aphrodisiac, treatment of vaginitis, hemorrhoids, scabies, itchy rashes, eczema, leprosy and other skin problems. In Peruvian herbal medicine, the leaf or aerial parts of the plant are used to treat measles, malaria, and all types of inflammation. In Nicaragua, the leaf is commonly used for stomach pain, diabetes, fevers, colds, coughs, headaches, malaria, skin complaints, menstrual disorders, aches and pains, hypertension, infections, and as an aid in childbirth."[186] In India, it is well known for antidiabetic and laxative properties. It is also used for treatment of dysmenorrhea, eczema, emmenagogue, galactagogue, gout, jaundice, kidney stones, leucorrhea, leprosy, piles, pneumonia, scabies, and rheumatism."[186]

MC contains biologically active chemicals that include glycosides, saponins, alkaloids, fixed oils, triterpenes, proteins, and steroids.[187] Extracts of MC fruit (250 mg/kg) within 2 weeks of treatment achieved euglycemic activity.[187]

Antidiabetic activity has been shown by various animal and human studies.[189-199] MC has many pytoconstituents which act as antidiabetic and antilipidemic. A polyherbal preparations containing MC showed significant reduction in blood glucose, glycosylated hemoglobin, increase in plasma insulin and total hemoglobin in animals.[190] MC has shown its effectiveness in complications of diabetes such as nephropathy and neuropathy.[190] With the time and search of newer and safer antidiabetic agent, scientists have been able to find the active constituents responsible for antidiabetic activity of MC. The two isolated peptides having similarity with insulin and called as phyto insulin of MC that are PPP and PPK.[166,167,200]

PPP

PPP is a polypeptide extracted from MC with structural similarity with bovine insulin. It differs from bovine insulin in respect of extra amino acid methionine.[195] Three dimensional structure of PPP shows that it has 2 chains of aminoacids linked together by sulfide bonds. PPP was shown to be stable at 4°C. The electrophoretic pattern also resembles that of bovine insulin. "Infrared spectrum of p-insulin is superimposable on that of standard zinc crystalline insulin."[199,201]

Efficacy of PPP has been reported in animals as well as humans. Antidiabetic activity of PPP has been reported in gerbils and male langurs. For this study, 0.5 units per kg body weight dose of PPP was given subcutaneously to one group and normal saline to another group. Significant glucose lowering capacity of PPP was observed in both gerbils and langurs.[199-201]

In a clinical trial conducted on 9 patients, reduction of 45.8% ± 13.6% blood glucose level was observed on single subcutaneous injection of PPP. The decrease in blood glucose level started in 30-60 min and maximum effect was observed at 4-8 h in juvenile patients and at 12 h in adults suffering from type 2 diabetes.[199]

Another study was conducted on 19 human subjects (type 1 diabetes mellitus: 11 subjects, type 2 diabetes: 8 subjects). PPP had significantly reduced blood glucose levels in both type 1 and type 2 diabetic patients. The results of polypeptide P were found similar to that of neutral protamine hagedorn insulin in term of action. However, no side effects were observed with PPP.[199-201]

Despite such a good similarity with animal insulin, in terms of structure and actions, PPP was unable to make its place in market. It may be due to ignorance of pharmaceutical companies toward this phytoinsulin over drugs of synthetic origin or, due to the challenge of cultivation and extraction. Till date, no formulation of this protein is marketed in any of the countries. Another reason for this is due to its use by subcutaneous route and its unstability via oral route. One more such protein was isolated from MC which overcomes these drawbacks of PPP.

PPK

PPK is a protein isolated from dried seeds and fruits of MC. PPK has structural and functional similarity with insulin. Work "k" is used for Karela which is Hindi name for MC.[167,200]

The most abundant amino acids are glutamic acid (17.08%), aspartic acid (9.71%), arginine (9.50%), and glycine (8.90%)."[159] PPK possess a challenge in formulation development because of its limited solubility. Its solubility is reported in 10% v/v formic acid and ammonia buffer pH 9.5. It has reported stability of 18 months at room temperature.[167,200] PPK has also shown to possess α-glucosidase inhibitory properties, which is similar to Voglibose. An *in vitro* study showed 79.18% inhibition of α-glucosidase and 35.58% inhibition of α-amylase with PPK. The significant inhibition of α-glucosidase and α-amylase revealed the potential of PPK as an antihyperglycemic agent.[202]

Extraction of PPK was reported in patent by Khanna *et al*. (2004).[200] Dried seeds from ripen fruits were used for extraction of PPK. These seeds were crushed to fine powder and kept in mixture of hexane and acetone in ratio of 3:1 for de-oiling. This mixture was then filtered and powder was dried and dispersed in water, pH was adjusted to 9.5 with ammonium hydroxide. Supernatant was collected and pH was adjusted to 3 with dilutesulfuric acid. Formed precipitates were filtered. These were further washed with water and acetone mixture to remove impurities. This dried precipitates were PPK.[200,203,204]

Table 5: Description of Polypeptide-k formulations[204,210-215]

Brand name	Manufacture	Dosage form	Route of administration
The prime sugard with PPK	Biomagna, Malaysia	Powder in sachet	Oral
Diabegard (buccal tablet) – polypeptide-k	Biomagna, Malaysia	Tablets	Sublingual route
Organic spirulina atta noodle	Organic Noodle Kitchen, Malaysia	Noodles	Oral

Various studies are available on safety and efficacy of PPK. PPK is reported to be safe even at higher doses in animals. A study published by Hakim *et al*., in 2011, showed safety of PPK in rats. Rats were divided into three groups with 10 rats in each group. After acclimatizing rats for 7 days, on 8th day rats were given oral dose of - 1000 mg/kg (group 1), 500 mg/kg (group 2) and 0 mg/kg (group 3). No signs of toxicity were observed even after 72 h of dosing. At the end of 72 h rats were sacrificed, organs were isolated and weighed. Weight of liver in control group, Group 2 and Group 3 was found to be 4.35, 4.12 and 4.42 g/100 g body weight, respectively. The weight of kidney in control group, Group 2 and Group 3 was found to be 6.70, 6.92 and 6.59 g/100 g body weight, respectively. Weight of heart in control group, group 2 and group 3 was found to be was found to be 3.20, 3.15 and 3.35 g/100 g body weight, respectively. Weight of lungs in control, Group 2 and Group 3 was found to be 5.89, 5.71 and 5.74 g/100 g body weight, respectively. Thus, statistically similar ($P < 0.05$) organ weight was observed in all the groups. Moreover, histopathological examination of liver, heart, kidney and lungs disclosed normal histopathology. The biochemical analysis such as liver function test and kidney function test revealed no change in the level of blood urea nitrogen and creatinine. There was no change observed in hematological and histological parameters of rats. It was concluded in the study that the doses of up to 100mg/kg did not have any adverse effects on rats.[205]

PPK is found to have antidiabetic activity in various human studies. Khanna reported that PPK has the ability to rejuvenate pancreas and activate insulin. It route of delivery was confirmed (i.e., Polymerase enzyme-DNA-tRNA-mRNA and then to pancreas), which leads to rejuvenation of pancreatic cells.[206] Several case studies reported 12-70 mg of PPK powder has antihyperglycemic activity. PPK showed not only antihyperglycemic activity but laos reduces, blood pressure and lipids. It also reduces nephropathy and neuropathy.[167,200,207]

A study on 18 healthy human volunteers was conducted to show hypoglycemic activity of PPK supplemented soft buns. Each bun consists of 2 mg of PPK. Same buns were used in both test and control except PPK was added in buns of test group. Significant decrease in glucose was observed with PPK supplemented buns as compared to control buns at 90, 150, and 210 min." Blood glucose level with PPK supplemented soft roll bun was further dropped to - 0.9 mmol/L after 210 min while for control soft roll bun, a slight drop of - 0.2 mmol/L in blood glucose level was observed. PPK supplemented soft roll caused decreased in sugar level as compared to control soft roll in healthy adults.[208] A significant reduction in blood glucose levels (50%) and 42% reduction in glycated hemoglobin HbA1c was observed.[209] A combination of PPK along with spirulina buccal tablet was claimed to have better antidiabetic activity than PPK alone in a patent.[210] PPK is a single small polypeptide, rejuvenates pancreas, and activate insulin. Activated insulin will effectively metabolize blood sugar to energy for daily use. Since, PPK has the ability to provide number of active insulin, its long term usage rejuvenates pancreas. When taken orally, PPK reduces level of triglycerides and cholesterol and thus benefits people with cardiovascular and weight problems.[211-212] There are only three preparations of PPK available in market- Diabegard®, Sugard®, Organic spirulina Atta noodle. Diabegard is available in the form of tablets whereas Sugard comes in powdered form. Both products are marketed by Bio-magna, Malaysia.[227-229] The various marketed preparations of PPK are shown in Table 5.

CONCLUSION

It is well-known fact that search for better medicine has been turned out toward natural products, particularly of plant origin. However, their successful appearance in the market is cumbersome and doubtful. The major impediments that hinder their appearance include quality in terms of stability; their method of quantification and physiochemical properties that restrict their formulation development into desired dosage form. PPK is one of the best examples to understand these bottlenecks. Despite having wide availability, ease of cultivation and simple process of extraction, its use as antidiabetic is less popular due to the poor aqueous solubility and gastric degradation. In our recent research, an attempt has been made to prepare PPK for successful oral delivery and development of self nanoemulsifying delivery systems; however, this research is still under its juvenile stage until *in vivo* tests will not be carried out. Nevertheless, a thoughtful process could be adopted for the development of other alternative delivery systems to make the product reach behind the bedside of patients.

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