Academic Sciences

International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 4, Issue 3, 2012

Review Article

INSULIN LIKE ANTIGEN: SOURCES OTHER THAN PANCREAS

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Received: 09 May 2012, Revised and Accepted: 21 June 2012

ABSTRACT

Diabetes mellitus is the third largest cause of death after cardiovascular diseases and cancer. The failure to make insulin or insufficiency of insulin, is termed as diabetes mellitus. Insulin therapy is the only effective treatment of Type 1 diabetes mellitus. Insulin is a polypeptide key hormone which controls the level of the glucose in the blood and regulates the carbohydrate metabolism. In addition it also, metabolise fat and proteins and regulate expression of certain genes. Increased medicine's cost; their pharmacological adverse effects and tremendous increase in diabetic population became a driving force in shifting interest towards development of bio- resource based medicines. Insulin was originally discovered in mammals, but later, insulin-related peptides were also recognized in plants. Insulin like antigen from plant with function, structure and sequence identical to vertebrate insulin are called 'glucokinin'. Studies of our lab showing insulin-like antigens in *Spirulina platensis* S5 paved the path for in vivo study for anti-diabetic activity. Recently we reported ELISA based screening and western blot of Cyanobacterial strains using anti-human insulin antibody and showed that insulin like antigen was present in *Spirulina platensis* S5.[1,2] This paved the path for confirmation of its antidiabetic activity in-vivo.

Keyword: Diabetes mellitus, Insulin like antigen, Herbal medicines, and Sulphonylureas. biguanides.

INTRODUCTION

Diabetes mellitus is associated with a large variety of complications and a greater risk of all manifestations of atherosclerosis.[3] Once diabetes develops, it is a costly disease to manage because of its chronic nature and severity of complications.[4] Worldwide estimates of its prevalence are expected to rise from 2.8% (171 million people) in 2000 to 4.4% (366 million people) in 2030.[5] Approximately 0.7% of the world's population suffers from Type 1 diabetes mellitus; while about 90% of the diabetic world's population suffers from Type 2 diabetes mellitus.[6] Type 1 [insulin dependent Diabetes mellitus (IDDM) or juvenile onset of diabetes] is caused by genetic disposition, environmental exposure to virus, toxin, stress, autoimmune reaction where beta cells of pancreas are destroyed by its own immunological system. Type 2 [Non insulin dependent Diabetes mellitus (NIDDM) or adult onset diabetes] is caused by insulin resistance: unable to utilize insulin produced because of cell receptor defect, insufficient production of insulin in response to blood glucose, excess production of glucose from the liver, genetic predisposition and obesity. The third type of diabetes is gestational diabetes mellitus (GDM) and is caused by insulin resistance due to pregnancy.[7]

Insulin is the primary medication in the treatment of Type 1 diabetes mellitus by directly promoting glucose uptake into muscle and fat cells as regulator of glucose metabolism and is a single hope for diabetic patients. Besides, controlling blood glucose level, insulin also stimulates cell proliferation. Insulin and insulin- like growth factors (IGFs) stimulate a signal transduction pathway that targets the protein synthesis apparatus by phosphorylating the S6 ribosomal protein (rp) on the 40 S ribosomal subunit.[7,8] Maize embryonic axis gradually reinitiates protein synthesis, based on translation of stored mRNAs and translation was shown highly regulated.[9,10] However, two other related proteins, insulin like growth factor (IGF-I) and IGF-II are more potent cell proliferators.[11] Because of greater antigenicity and impurity of animal insulin (bovine and porcine), genetically engineered human insulin was introduced in 1982 by Eli Lilly and Company. Unfortunately, diabetic patients, who received recombinant insulin for long periods developed antibodies to insulin. Developing fruits of Cowpea plant contained proteins of similar mass and amino acid sequence as bovine insulin.[12] Its highest concentration was found in the empty pods and seed coats, and not in the embryo, suggesting its involvement in carbohydrate metabolism in facilitating glucose transport across membranes, similar to its role in animals. Therefore, the search for more effective and safer hypoglycaemic agent started as an important area of active research. Plant based products may be one of the alternative for production of natural insulin.

Insulin structure and function

Insulin is a small peptide hormone of about 6 KDa,[13]composed of the A chain with 21 amino acids and the B chain with 30 amino acids, produced in vivo in the pancreatic cells (Fig. 1). Both chains are held together by two disulphide bonds between the cystines in CysA7 and CysB7 positions, CysA20 and CysB19 resp. Another disulphide bond is internal (intrachain disulfide bond) (A6 a A11). During insulin synthesis proinsulin is converted to insulin by cleavage of the binding peptide (C-peptide), binding the A and B chains together.

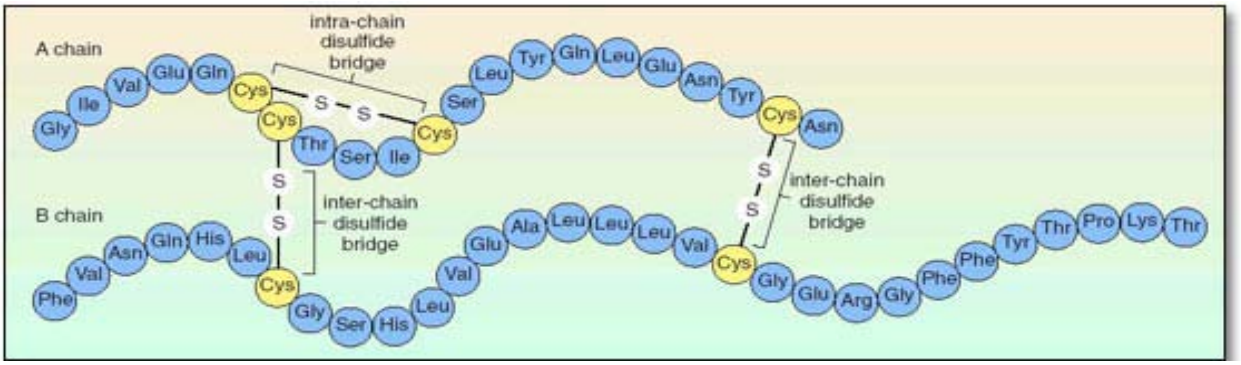

Fig. 1: Structure of insulin.

Insulin structure is highly conserved among vertebrates, which is evidenced by its high degree of homology. Porcine insulin has a single amino acid variation from the human variety (alanine substituing threonine in position B30), while bovine insulin has three amino acid variations (alanine instead of threonine in positions A8 and B30 and valine instead of leucine in position A10). The total of six insulin molecules, producing a hexamer, binds to two zinc atoms.

Spatial construction including polar and hydrophobic amino acids and relations between the individual amino acids within the chain, affect the molecule's stability and insulin binding to a receptor. Along with the normal insulin and proinsulin molecules, gene mutations with altered primary structures have also been described.[14] These include three types of variated insulin molecules PheB25Leu, PheB24Ser and ValA3Leu. Their biological activity is reduced, resulting in diabetes. Insulin gene mutations may impair release of insulin from the proinsulin molecule, which results in familiary hyperproinsulinemia. These variants include the HisB10Asp mutation. Proinsulin is less effective, has reduced clearance and accumulates in the circulation. The HisB10Asp insulin is released from proinsulin through proteolytic cleavage. Compared to other abnormal insulins, it exhibits increased affinity to the insulin receptor.

Insulin enhances glucose uptake by increasing the number of transporters in the plasma membrane of cells. Insulin is effective in restoring normoglycaemia, suppressing ketogenesis and in delaying or arresting diabetic complications. Insulin promotes the storage of the body's fuels, facilitating the transport of metabolites and ions (potassium) through cell membranes and stimulating the synthesis of glycogen from glucose, fats from lipids and proteins from amino acids. Insulin also profoundly affects lipid metabolism, increasing lipid synthesis in liver and fat cells, and attenuating fatty acid release from triglycerides in fat and muscle. Insulin resistance occurs when normal circulating concentrations of the hormone are insufficient to regulate these processes appropriately. Thus, by definition, insulin resistance is a defect in signal transduction.

Discovery of insulin

Von Mering and Minkowski[15] first noted that removal of the pancreas led to the development of diabetes mellitus in dogs. Feeding pancreas has given harmful results in dogs.[16,17] After that, pancreatic extracts was intravenously given to depancreatised dogs and found transient reduction in blood glucose levels.[18,19,20] Schafer,[21], was first to speculate that the antidiabetic hormone, which he calls "insuline," was from the pancreatic islets. Banting and Best made remarkable progress; they had isolated material from pancreas extracts.[22] In 1922, a new series of injections began. Leonard Thompson (first patient took insulin) responded immediately. His glycosuria, ketonuria did disappeared and blood glucose dropped to normal. Nevertheless, the quality of the insulin administered at that time was far from the quality of today's products. Each vial of insulin had different effect because of difference in purity. After several years of laboratory work during the years 1963–1966, human insulin was chemically synthesized in Germany by Meienhofer et al[23], in China by Kung et al.[24] and in the United States by Katsoyannis et al,[25]. In 1975, fully synthetic insulin (CGP 12 831) was synthesized by Ciba-Geigy in Basel. In a clinical trial, 6 patients with diabetes were treated with this insulin for up to 2 weeks.[26] In 1978, scientists from the Biotechnology Corporation Genentech in San Francisco, California, succeeded in producing insulin with the same amino acid sequence as seen in humans using a genetically manipulated plasmid of *E. coli* bacteria. In 1980, recombinant DNA 'human' insulin was first tested on 17 non-diabetic volunteers in England. [27]

Insulin like antigen in plants

From the discovery of insulin, by Banting, Best, Macleod and Collip[22], insulin is a unique hormone and is present only in mammals and birds. But, Best and Scott [28], suggested that a hormone analogous to insulin must be present wherever glucose is metabolized, i.e., it might be present in plants; and later they reported insulin like materials from germinating potatoes and rice.

Collip[29], who had developed the method for the extraction of insulin from pancreas, also obtained an active extract from green wheat leaves and ordinary lawn grass. After obtaining insulin from plant extracts he performed experiments on normal rabbits and pancreatectomized dogs obtaining measurable decreases in the levels of blood glucose of the animals. He named the protein "glucokinin" instead of insulin because it did not originate from the islets of Langerhans in the pancreas. In the same year Best and Collip[28, 29] reported the presence of insulin-like substances in other plant materials like green tops of onions, lettuce leaves, green bean leaves, barley roots, beet-roots, yeast and others.

The beet-root extract oral doses to diabetic dogs[30] showed blood sugar-lowering effect as rapid as those of animal insulin doses subcutaneously.[31]

After a long gap, in 1970s Khanna and collaborators[32, 33] reported presence of insulin in plants and immediately patented a process for its production from the fruits of *Momordica charantia* (bitter gourd). The isolated product, which was diversely named v-insulin, polypeptide-p or p-insulin, showed hypoglycaemic activity but differed from insulin in immunoactivity.[34] Ng et al.[35] utilized the seeds and employed gel filtration and ionic exchange chromatography for isolation of active principle from *Momordica charantia* seeds and showed properties similar to animal insulin. Again, hypoglycaemic activity of *Momordica charantia* seeds was confirmed based on SDS-PAGE and amino acid composition.[36]

In addition, materials resembling insulin were described in spinach, rye and *Lemna gibba*, which were recognized by broad spectrum anti-pork and anti-chicken insulin antibodies and had molecular weights, chromatographic properties and biological activities similar to those of vertebrate insulin.[37] Insulin like proteins was isolated from the leaves or aerial parts of species of, mosses, whisk ferns, Selaginella, Equisetum, many gymnosperms and angiosperms including monocots and dicots using modified ELISA and Western Blotting.[38] They have also shown its presence in red alga *Gracilariopsis*, cyanobacterium *Spirulina maxima* and yeast (*Saccharomyces cerevisiae*).[39] Venâncio et al.[40] isolated insulin like proteins from seeds coat of *Vigna unguiculata* at 16 and18 days after pollination whose RP-HPLC analysis and amino acid sequence was identical to that of bovine insulin. Insulin potentiating activity was also reported in vitro from common spices such as cinnamon, clove, and bay leaves.[41]

Bauhinia variegate leaf insulin like protein showed partial sequence identity with bovine insulin that decreased blood glucose in alloxan induced diabetes in mice.[42] During immune-histochemical and immunocytochemical microscopic observation the protein was found associated with calcium containing crystals in vacuoles of chloroplast. They concluded that the calcium protect the insulin protein from proteolysis showed 1.2% accumulation of insulin within seeds. Transgenic Safflower, plant derived insulin developed by Semi Biosys insulin is analytically and physiologically similar to human insulin (EliLilly's Humulin). This insulin is cheaper, patient friendly plant sources. One acre of sunflower is expected to yield one Kg insulin, enough to supply 2500 patients for an entire year.

Silva et al,[39], found insulin like antigen from red alga *Gracilariopsis*, green alga *Acetabularia* and a photosynthetic prokaryote (Cyanobacterium) *Spirulina maximum* and by adopting ELISA. Reverse phase chromatography and N-terminal amino acid sequence of *S.maxima* protein showed homology with sequence of proinsulin. During cyanobacterial screening we found insulin like antigen in *Spirulina platensis* S5 in molecular weights, chromatographic behaviour, and immunological properties, isolated protein was similar to those of bovine insulin.[1,2] This paved the path for in-vivo studied for confirming its hypoglycaemic activity. Our findings with *S.platensis* S5 crude extract as well as purified insulin like antigen also confirmed their blood glucose lowering property (Anwer et al, communicated 2012).

Antihyperglycemic effect of *Spirulina* was also observed on streptozotocin induced diabetes in male albino wistar rats Layam and Reddy.[43] In a human clinical study involving 15 diabetic patients 2 g/day *Spirulina* supplementation showed, a considerable decrease

in the fasting blood sugar level after 21 days. [44] *Spirulina maxima* effect on Streptozotocin induced diabetic male wistar rats orally showed significant decrease in blood sugar level. At a dose of 15 mg/kg body weight a higher level of significance than the doses of 5 and 10 mg/kg body weight.[45]

Insulin like antigen in non-photosynthetic organisms

Glucokinin was also detected in prokaryotic bacteria (*Escherichia coli*) and eukaryotic organism protozoa (*Tetrahymena*) [46], fungi (*Neurospora crassa* and *Aspergillus fumigates Saccharomyces* cerevisae). [39, 47, 48]

Insulin like antigen was also reported from extra- pancreatic tissues of rodents and human brain. [49, 50, 51]

Amino acid sequence of insulin

Amino acid sequencing (MALDI-TOF) of *Spirulina S5* insulin like antigen when blasted with bovine insulin (N-terminal sequence) it showed 100% identity (3 residues) with one blast hit (Fig.2) with human insulin (N-terminal sequence) only 1% identity (3 residues) with one blast hit could be detected (Fig.3). Our result suggests only partial identity with bovine and human insulin. This also indicates that 3 residues (44 GER 46) which were matched with bovine and human insulin might be responsible for immunoactivity in ELISA and Western Blotting.

Insulin like protein extracted from *Bauhinia variegata leaves* (cow's foot) and *Vigna unguiculata* (cow pea) also showed only partial amino acid sequences identical with bovine insulin (Table.1).[13, 40] *Momordica charantia* (which is reported to have hypoglycaemic effect) insulin like protein, sequences was not identical with bovine insulin.[35, 52] Its amino acid composition had methionine residues unlike animal insulin. Our *Spirulina S5,* hypothetical protein also had methionine residue (Fig.4). Among the plant insulin protein only that insulin isolated from seed coat of *Canavalis ensiformis* (Jack bean) showed sequence equal to that of bovine insulin (α and β chain) (Table. 1).[53]

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
31331	unnamed protein product	10.8	10.8	2%	8.9	100%	

Fig. 2: *S. platensis* S5 insulin like antigen sequences producing significant alignments with bovine insulin.

>lcl|31331 unnamed protein product

Length=170

Score = 10.8 bits (16), Expect = 8.9, Method: Compositional matrix adjust.

Identities = 3/3 (100%), Positives = 3/3 (100%), Gaps = 0/3 (0%)

Query 44 GER 46

GER

Sbjct 126 GER 128

Accession	Description	Max score	Total score	Query coverage	E value	Links
AAA59179.1	insulin [Homo sapiens]	10.8	10.8	1%	7.6	G

Fig. 3: *S. platensis* S5 insulin like antigen sequences producing significant alignments with human insulin.

Insulin [Homo sapiens] Length=107

Score = 10.8 bits (16), Expect = 7.6, Method: Compositional matrix adjust.

Identities = 3/3 (100%), Positives = 3/3 (100%), Gaps = 0/3 (0%)

Query 126 GER 128

GER

Sbjct 44 GER 46

MKSKLLGGLCVLITAF**M**PVKALAQIGVPTTPSDFDIRYQRPDPQTGRVTPPRYYSLPDLSYPLRTLPAAFRYPI**M**YELGVNRNIRSDAVITDEVFIPNLPPIPAGITLD
NIPRVRAEYTRRVEEWGERVQECKRLDPLIIKTDTGNPVLINQRSAGRILLNANNISVCPR

Fig.4: *Spirulina platensis* S5 insulin like antigen sequences.

Table 1: Comparison of plant insulin sequences with bovine insulin sequences.

Species	Sequences
Bovine insulin (α-chain)	1 GIVEQCCASVCSLYQLENYCN 21
Bovine insulin (β-chain)	1 FVNQHLCGSHLVEALYLVCGERGFFYTPKA 30
C. ensiformis I-SC	1 GIVEQCCASVCSLYQLENYCN 21
C. ensiformis I-LC	1 FVNQHLCGSHLVEALYLVCGERGFFYTPKA 30
V. unguiculata I-SC	1 GIVEQXXASVXSLYQLENYXN 21
V. unguiculata I-LC	1 FVNQHLXGSHLVEALYLVXGERGFFYTPKA 30
B. variegata a	1 GIVEQ 5
B. variegata b	1 FVNQH 5
Spirulina platnsis S5	44 GER 46

CONCLUSION

The insulin like antigen reported in eubacteria, fungi, protoctista and plantae support wider distribution of insulin and their conserved nature during evolution in all organisms from unicellular bacteria to multicellular vertebrate. Presence of insulin like antigen in plant system shows parallelism between plant and animal physiology for glucose metabolism.

The success in isolation of insulin like antigen from plant sources their less toxic and less cross reactive effect and low cost provides an alternate/substitute to human/animal based insulin. Not only this use of transgenic photosynthetic prokaryotic and eukaryotic systems, may appear to be revolutionary in future.

Spirulina extract has ability to lower blood glucose level in mice and humans (Mani et al, 1998). Therefore, search for plant based antihyperglycemic medicines became indispensible.

Immuno-recognition with anti-bovine or human insulin antibodies in prokaryote and eukaryote it was suggested that the insulin-like peptide is present many organisms, other than animal pancreas.

REFERENCES

1. Khursheed S, Anwer R, Zutshi S, Fatma T. Screening of photosynthetic O_2 evolving prokaryotes for insulin like antigen. J. Phycol. 2012; (48), 243–245.
2. Anwer R, Khursheed S, Fatma T. Detection of immunoactive insulin in *Spirulina.* J Appl Phycol. 2012; DOI 10.1007/s10811-011-9757-1 (In Press).
3. Khuwaja AK, Rafique G, White F, Azam SI. Macrovascular complications and their associated factors among persons with type 2 diabetes in Karachi, Pakistan-a multi center study. J Pak Med Assoc. 2004; (54):60-66.
4. Khuwaja AK, Fatmi Z, Soomro WB, Khuwaja NK. Risk factors for cardiovascular disease in school children- -a pilot study. J Pak Med Assoc. 2003; (53):396-40.
5. 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. 27. 2004; 1047–53.
6. Winter J, Neubauer P, Glockshuber R, Rudolph R. Increased production of human proinsulin in the periplasmic space of Escherichia coli by fusion to DsbA. J. Biotechnol. 2001; (84),175–185.
7. Sangeetha M K, Vasanthi H R. Plant kingdom claims for Insulin!!! Sri Ramachandra Journal of Medicine, 2009; 1(1) 23-31.
8. Chung J, Grammer T C, Lemon K P, Kazlauskas A, Blenis J. PDGF and insulin-dependent pp70s6k activation mediated by phosphatidylinositol-3-OH kinase. Nature (London). 1994; (370), 71-74.
9. Fumagalli S, Thomas G. S6 rp Phosphorylation and signal transduction. In Translational Control of Gene Expression, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (Hershey, J. W. B., Mathews, M. B. And Sonenberg, N., eds.). 2000 ; 695-717.
10. Bewley J.D, Black M. Seeds. In Physiology of Development and Germination (Bewley, J. D. and Black, M., eds.). 1994; 147-148, Second Edition, Plenum Press, New York and London.
11. Sanchez de Jimenez E, Aguilar R. Protein synthesis patterns: relevance of old and new messenger RNA in germinating maize embryos. Plant Physiol. 1984; (75), 231-234.
12. Jones JJ, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 1995; (16):3-34.
13. Venancio TM, Oliveira AE, Silva LB, Machado OL, Fernandes KV, Xavier-Filho J. A protein with amino acid sequence homology to bovine insulin is present in the legume *Vigna unguiculata* (cowpea). Braz J Med Biol Res. 2003; (36): 1167-1173.
14. Harfenist EJ, Craig LC. The molecular weight of insulin. *Journal of the American Chemical Society*, 1952; (74): 3087-3089.
15. Rhodes Ch J, Shoelson S, Halban P A. Insulin biosynthesis, processing, and chemistry.In: Joslin's Diabetes mellitus..In: Joslin's Diabetes mellitus. Eds.: Kahn, C. Lippincott Williams & Wilkins, Philadelphia. 2005; 66- 82.
16. Von Mering J, Minkowski O. Diabetes mellitus nach pankreas exterpation. Arch Exp Path Pharmacol (Leipzig) .1890; 26:371.
17. Sandmeyer W: ZBiol 1895; 31: 12-8. In Banting F G, Best C H, Collip J B, Campbell W R, Fletcher A A. Pancreatic extracts in the treatment of diabetes mellitus. Preliminary report. Can. Med. Assoc. J. 1922; (12): 141–146.
18. Pfluger-PJIugers Arch., 1907; 118, 265-321. In Banting F G, Best C H, Collip J B, Campbell W R, Fletcher A A. Pancreatic extracts in the treatment of diabetes mellitus. Preliminary report. Can. Med. Assoc. J. 1922; (12): 141–146.
19. Murlin-Jour. Biol. Chem., 1913; XV, 365. In Banting F G, Best C H, Collip J B, Campbell W R, Fletcher A A. Pancreatic extracts in the treatment of diabetes mellitus. Preliminary report. Can. Med. Assoc. J. 1922; (12): 141–146.
20. Kleiner-Jour. Biol. Chem., 1919; XL, 153. In Banting F G, Best C H, Collip J B, Campbell W R, Fletcher A A. Pancreatic extracts in the treatment of diabetes mellitus. Preliminary report. Can. Med. Assoc. J. 1922; (12): 141–146.
21. Paulesco-Comp. rend. de Soc. de biol., 1921. In Banting F G, Best C H, Collip J B, Campbell W R, Fletcher A A. Pancreatic extracts in the treatment of diabetes mellitus. Preliminary report. Can. Med. Assoc. J. 1922; (12): 141–146
22. Schafer EA. An introduction to the study of internal secretion. In: The Endocrine Organs. London: 1916 ; Longmans,Gree
23. Kung YT, Da YC, Huang WT. Total synthesis of crystalline insulin. Sci Sin. 1966; (15):544.
24. Meienhofer J, Schnabel E, Bremer H. Synthese der Insulinketten und ihre Kombination zu insulactiven Präparaten. Z Naturforsch 1963; (18):1120.
25. Katsoyannis PG, Tometsko A, Zalut C. Insulin peptides XII. Human insulin generation by Combination of synthetic A and B chains. J Am Chem Soc. 1966; (88):186.
26. Teuscher A. The biological effect of purely synthetic human insulin in patients with diabetes mellitus. Schweiz Med Wochenschr. 1979; (109):743–747.
27. Keen H, Glynne A, Pickup JC. Human insulin produced by recombinant DNA technology: safety and hypoglycaemic potency in healthy men. Lancet. 1980; 398-401.
28. Banting F G, Best C H, Collip J B, Campbell W R, Fletcher A A. Pancreatic extracts in the treatment of diabetes mellitus. Preliminary report. Can. Med. Assoc. J. 1922; (12): 141–146.
29. Best, MA. Scott. Possible sources of insulin. J Metab Res.1923;(3): 177-179.
30. Collip JB Glucokinin. A new hormone present in plant tissue. Preliminary paper. J. Biol. Chem. 1923; (56):513-543.
31. Best CH. Recent work on insulin**.** Endocrinology 1924; (1): 617-629.
32. Rychlik, w. Compt. Rend. Soc. Biol., 1934; 116, 1135.
33. Khanna P, Nag TN, Jain SC, Mohan S Extraction of insulin from a plant source. 3rd International Congress on Plant Tissue & Cell Cultures. 1974; 21-26th July, Leicester,UK.
34. Khanna P, Nag TN, Chandrajaia S, Mohan SV. Process for isolation of insulin from plant source. 1976; Patent No. U.S. 3: 945-988.
35. Khanna P, Jain SC, Panagariya A, Dixit VP. Hypoglycemic activity of polypeptide-p from a plant source. J Nat Prod 1981; (44): 648-655.
36. Ng TB, Wong CM, Li WW, Yeung HW. Insulin-like molecules in *Momordica charantia* seeds. J. Ethnopharmacol. 1986; (15):107-117.
37. Sheng Q, Yao H, Xu H, Ling X, He T. Isolation of plant insulin from *Momordica charantia* seeds by gel filtration and RP-HPLC. Zhong Yao Cai. 2004; (27): 414-416.
38. Collier E, Watkinson A, Cleland CF, Roth J. Partial purification and characterization of an insulin-like material from spinach and Lemna gibba G3. J Biol Chem. 1987; (262): 6238-47.
39. Silva LB, Santos SS, Azevedo CR, Cruz MA, Venancio TM, Cavalcante CP. The leaves of green plants as well as a cyanobacterium,a red alga, and fungi contain insulin- like antigens. Braz J Med Biol Res. 2002; (35): 297-303.
40. Venancio TM, Oliveira AEA, Silva LB, Machado OLT, Fernandes KVS, Xavier Filho J. A protein with sequence homology to bovine insulin is found in the legume Vigna unguiculata (cowpea). Braz. J. Med. Biol. 2003; (36): 1167-1173.
41. Richard R. Rubin, Paul Ciechanowski, MPH, Leonard E. Egede, Elizabeth H.B. Lin, MPH, Patrick J. Lustman.Recognizing and Treating Depression in Patients with Diabetes. 2004,(4):119–125.

42. Azevedo CR, Maciel1 FM, Silval LB, Ferreira1 ATS, Cunha M, Machado OLT. Isolation and intracellular localization of insulin-like proteins from leaves of Bauhinia variegate. Braz J Med Biol Res 2006; 39(11):1435-1444.
43. Layam A, Reddy C. L. K. Antidiabetic property of *Spirulina*. Diabeto. Croa. 2007; (35:29–33.
44. Mani S, Iyer U, Subramanian S. Studies on the effect of *Spirulina* supplementation in control of diabetes mellitus. In: Subramanian G, et al., eds. Cyanobacterial Biotechnology. USA:Science Publishers Inc. 1998; 301-304.
45. Pandey J P, Tiwari A, Mishra G, Mishra R M. Role of *Spirulina maxima* in the Control of Blood Glucose Levels and Body Weight in Streptozotocin induced Diabetic Male Wistar rats. J. Algal Biomass Utln. 2011, 2 (4): 35– 37.
46. LeRoith D, Shiloach J, Heffron R, Rubinovitz C, Tanenbaum R, Roth J. Insulin-related material in microbes: similarities and differences from mammalian insulins.Can. J. Biochem. Cell. Biol. 1985; (63):839-849.
47. Le Roith D, Shiloach J, Roth J, Lesniak MA. Evolutionary origins of vertebrate hormones: substances similar to mammalian insulins are native to unicellular eukaryotes. Proc Natl Acad Sci U S A. 1980; (77): 6184-6188.
48. Muthukumar G, Lenard J. A preproinsulin-like pseudogene from Neurospora crassa. Mol. Cel.Endocrinol. 1991; 82:275-283.
49. Havrankova, H., Schmechel, D., Roth, J. & Browstein, M. J. (1978) Proc. Nati. Acad. Sci. USA 75, 5737-5741.
50. Rosenzweig, J. L., Havrankova, J., Lesniak, M. A., Brownstein, M. J. & Roth, J. Proc. Natl. Acad. Sci. USA. 1980; (77), 572-576.
51. Havrankova, J., Brownstein, M. J. & Roth, J. J. Clin. Invest. 1979; (64), 636-642.
52. Singh J, Adeghate E, Cummings E, Giannikipolous C, Sharma A K, Ahmed. Beneficial effects and mechanism of action of *Momordica charantia* juice in the treatment of streptozotocin-induced diabetes mellitus in rat. Molecular and Cellular Biochemistry 5266034 February 23, 2004; 13:29.
53. Oliveira AEA, Machado OLT, Gomes VM, Xavier-Neto J, Pereira AC, Vieira JGH, Jackbean seed coat contains a protein with complete sequence homology to bovine insulin. Protein Pept Lett. 1999; (6): 15-21.