Therapeutic Stimulation of Glp-1 Protein by Implementing *in Silico* to *in Vitro* Approach for Type-2 Diabetes Treatment

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Abstract: Type 2 diabetes mellitus is a chronic disease in which there is high level of sugar (glucose) in blood caused by insufficient insulin secretary response. Glucagon-like peptide-1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP) are incretin hormones which account for higher insulin response after oral versus intravenous administration of glucose. These hormones have proposed a new mechanism for the treatment of type-2 diabetes but are easily degraded by DPP IV. Various synthetic drugs are available in the market as DPP IV inhibitors but, they have undesirable side effects. Therapeutic agents involving herbal plants with antidiabetic properties and beneficial effects have been identified to cure diabetes. The objective of present review is to ascertain the novel DPP IV inhibitors in the extract from specific part of a plant.

Key words: GLP-1 · GIP · DPP IV · Sitagliptin · Antidiabetic Drugs · Medicinal Plants · DPP IV Inhibitors

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a genetically diverse, polygenic disease with a multifaceted pattern of inheritance [1]. It can be portrayed by altered expression of many genes and their products in several tissue types [2, 3]. The disease may occur as a result of pancreatic β-cells impairment, leading to reduction in insulin secretion. Resistant insulin receptors to the functions of circulating insulin can also conduce in diabetes (ADA, 2010). Number of proteins was considered as a target to control the diabetes mellitus. Type II diabetes mellitus is a clinical syndrome due to relative or absolute deficiency of insulin or opposition to the cellular insulin execution, as a result hyperglycemia and glycosuria occurs.

An assemblage of lifestyle and genetic factors is the usual cause of its development [5, 6]. Diet and obesity can be individually controlled but, factors like increasing age, female gender and genetics cannot be [7]. A lack of sleep has been linked to type 2 diabetes and is considered to effect metabolically [8]. The nutritional status of a mother during fetal development may also play a role, with the mechanism of altered DNA methylation [9].

Presently available therapies for type II diabetes mellitus are inclusive of biguanides, oral insulin secretagogues, thiazolidinediones, sulfonylureas, repaglinide, insulin, nateglinide, alpha-glucosidase inhibitors, pramlintide and exenatide. Clinical trials have shown that oral hypoglycemic drugs are valuable in the treatment of patients with type II diabetes mellitus (NIDDM). Traditional drugs like sulphonylureas and biguanides are a cohesive source of treatment while certain new and potent drugs have gained availability now. Insulin is used as hypoglycemic agent in Type II diabetes mellitus [10]. The use of herbal medicines (medicinal plants or phytotherapy) has recently made headway all over the world for their efficacy in Type II diabetes mellitus.

Concept of Hormones and Incretin Mimetics: The disposition of absorbed glucose through the stimulation of insulin secretion from the endocrine pancreas is facilitated by gut hormones. The observation that enteral nutrition provided a more potent insulinotropic stimulus compared with isoglycaemic intravenous challenge led to the evolution of the incretin concept [11]. The gradual beginning of postprandial and fasting hyperglycemia is peculiar in Type 2 diabetes mellitus.

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The first incretin to be identified was glucose-dependent insulinotropic polypeptide (GIP), purified from porcine intestinal extracts and it had debile effects on gastric acid secretion but more potent insulinotropic actions in human beings [12].

- GIP is a 42-aminoacid hormone synthesised in duodenal and jejunal enteroendocrine K cells in the proximal small bowel. The cloning of the cDNAs and genes that encoded proglucagon led to the discovery of second incretin hormone, glucagon-like peptide-1 (GLP-1). GLP-1 subsists in two circulating equipotent forms of molecules, GLP-1(7-37) and GLP-1(7-36) amide, though after eating, GLP-1(7-36)amide is circulating profusely. Most GLP-1 is made in enteroendocrine L cells in the distal ileum and colon, but plasma levels of GLP-1, like GIP, also increase in postprandial period [13].
- Glucagon-like peptide 1 (GLP-1) is produced by the proglucagon gene in mucosal L-cells of the small intestine in response to nutrients[14]. It stimulates glucose-dependent insulin release from the pancreatic islets and the GLP-1 receptors on pancreatic β-cells mediate its insulinotropic activity. In addition to its insulin-related effects, it is thought to exert hypoglcemic effects by slowing gastric emptying[15], inhibiting inappropriate glucagon release[16], stimulating β-cell proliferation and differentiation[17] and improving satiety[18].

On the other hand, GIP is involved in glucose metabolism by raising the secretion of insulin [19]. Both peptides have short half-lives (less than 2 minutes) because of their rapid degradation by DPP-IV. Therefore, inhibition of DPPIV prolongs the action of GLP-1 and GIP, which in turn, amends glucose homeostasis with a low risk of hypoglycemia [20].

Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) are incretin hormones, secreted from L-cells and K-cells of intestine in response to nutrient ingestion [21]. They are responsible for normal glucose tolerance postprandially. GLP-1 (7-36) is portrayed as the most potent insulinotropic hormone and is well thought-out of as a therapeutic means for the treatment of type 2 diabetes [22]. The continuous infusion of this peptide decreases plasma glucose and improves β-cells function. However, active GLP-1 (7-36) is converted into inactive GLP-1(9-36) by dipeptidyl peptidase IV [23]. The name incretin mimetics accentuates the glucoregulatory and metabolic effects rather than specific mechanism of action.

Incretin mimetics GLP-1 analogues that are resistant to degradation by DPP IV have been formulated. These include: Liraglutide (Denmark) which is in phase-2 trial, CJC-1131 (Canada) which has been put on hold and Exenatide which is the first incretin mimetic to be sanctioned by FDA for clinical use since it is not a GLP-1 analogue.

GLP-1 Receptor Signal Transduction Pathways in the Pancreatic B Cell: The importance of endogenous GIP and GLP-1 for glucose homoeostasis has been experimentally examined in physiological studies with receptor antagonists, or gene-knockout mice. Lowering of insulin secretion and increase in plasma glucose after glycaemic challenge in rodents [24] is related to acute antagonism of GIP or GLP-1 similarly, mice with inactivating mutations in the GIP or GLP-1 receptors also have defective glucose-stimulated insulin secretion and impaired glucose tolerance. GLP-1, but not GIP, is vital for control of fasting glycaemia, since acute antagonism or genetic disruption of GLP-1 action l contributes to enhanced levels of fasting glucose in rodents [25]. Recent studies and research with the antagonist expending show defective glucose-stimulated insulin synthesis, compact clearance of glucose, rise in the levels of glucagon and prompt gastric emptying after disruption of GLP-1 action [26] confirming GLP-1 as an essential need for glucose control in human beings.

Anti Diabetic Actions of GLP-1: Short-term intravenous infusions of GLP-1 (1-1•2 pmol kg^{-1} min^{-1}, leading to pharmacological plasma concentrations of total GLP-1 of 70-150 pmol/L and of intact biologically active GLP-1 of 10-20 pmol/L) lowers blood glucose in patients with type 2 diabetes through a transient glucose-dependent stimulation of insulin and suppression of glucagon secretion and gastric emptying.

The short-term control of hyperglycaemia can be sufficed by intravenous or subcutaneous GLP-1 infusions but, the long-term treatment of type 2 diabetes needs a more feasible approach to achieve sustained activation of GLP-1 receptors. The efficacy to inject GLP-1 receptor agonists (degradation-resistant peptides or larger proteins with more suitable pharmacokinetic properties) and DPP-4 inhibitors has been assessed in clinical trials [27].

Dipeptidyl peptidase IV (DPPIV, EC 3.4.14.5) is a unique serine protease, which removes N-terminal dipeptides from polypeptides and proteins, that include alanine or proline at the penultimate position [28, 29].

Our prime objective and necessity is to develop alternative strategies that intend to prolong the antidiabetic activity of the hormone because the enzyme inactivates GLP-1 (7-36). One of the approaches to protract the half-life of GLP-1 is the application of DPP-IV inhibitors [21].

DPP IV inhibitors: Inhibition of DPPIV with vildagliptin improved the glycemic control of type 2 diabetes by enhancing the activity of GLP-1 and GIP with resultant improvement in β-cell function [30]. Over a period of time, DPP-IV inhibitors have shown improved efficacy in clinical studies [21]. Currently, the inhibitors of DPP-IV are under development and growth in preclinical and clinical studies as potential drugs for the treatment of type 2 diabetes [22, 23, 28]. The question remains as to the safety of inhibition of DPP-IV since DPP-IV is involved in the metabolism of a vast number of life-sustaining substrates (neuropeptides, chemokines, cytokines, etc.) [29]. A greater potential may lie in combinatorial treatment with other antidiabetic drugs.

The first DPP-IV inhibitor on the market was sitagliptin (by Merck and Co.) in 2006 [31], which was followed by the structurally similar vildagliptin (by Novartis) in 2007 [32] and saxagliptin (by Bristol-Myers Squibb and Astra Zeneca) in 2009 [33], linagliptin in 2011, allogliptin in 2013 and omarigliptin (by Merck) in 2014. The efficacy and safety profile of DPP-IV inhibitors have been promising and beneficial to date. DPP-IV inhibitors do not have an intrinsic risk of inducing or stimulating hypoglycemia and they are body-weight neutral in contrast to sulfonylureas and other antidiabetic drugs. Their tolerability profile is good [20].

The DPP-4 inhibitors are all orally available and are speedily ingested, with significant inhibition of plasma DPP-4 activity being seen within 5 min of administration. Oral biovailability is generally high in humans ˜87% for sitagliptin [34], 85% for vildagliptin[35] and ˜67% for saxagliptin [36], although somewhat lower for linagliptin (˜30%) [37].

Table 1: Some of the common drawbacks of DPP IV inhibitors

DPP IV inhibitors	Drawbacks
Sitagliptin	Upper respiratory tract infection,nasopharyngitis, and headache
Saxagliptin	Upper respiratory tract infection,urinary tract infection, andheadache
Vildagliptin	A small increase in the risk of angioedema has been observed, brings the problem of renal impairment
Linagliptin	Headache, joint pain, and sorethroat

The DPP-4 inhibitors are the first new therapeutic class of oral antihyperglycaemic drug for T2DM for many years. They were contrived for the treatment of the disease based on prior knowledge of the physiology of the incretin hormone GLP-1 and an understanding of the target (DPP-4), contrasting with the development of other antidiabetic agents whose blood glucose-lowering effects were initially discovered more by chance than by design without fully knowing the underlying mechanisms (e.g. metformin, sulphonylureas and glitazones).

Identification of the 3-dimensional/tertiary structure of the DPP-4 protein allowed the rational design of small molecule inhibitors which interact only with the catalytic site without interfering in any of the other functions of the DPP-4/CD26 molecule. This, together with the understanding of the role of GLP-1 in glucose homeostasis and its unique susceptibility to breakage by DPP-4, probably accounts for the noteworthy lack of adverse effects so far associated with the therapeutic use of the DPP-4 inhibitors.

The current review highlights the antidiabetic activity of DPP-4 inhibitors that increase GLP-1 serum concentration and the curative property of herbal plants.

Role of Medicinal Plants: It is worth noting that the investigations of the last years manifested the inhibition of DPP-IV and ADA activities by plant extracts [39-41].

Medical plants play an important role in the management of type 2 diabetes mellitus (T2DM) by delaying the development of diabetic complications and correcting metabolic abnormalities [42]. Traditional plant-based remedies have been and are being used by T2DM patients around the world (e.g., patients belonging to the Chinese [43], Indian [44] and Mexican [45] populations).

The most commonly studied hypoglycemic plants are *Opuntia streptacantha, Trigonella foenum-graecum, Momordica charantia, Ficus bengalensis, Polygala senega*and *Gymnema sylvestre* [38]. However, it is apparent that additional research needs to be undertaken on these and other medicinal plants with hypoglycemic effects because the compounds which are bioactive and their modes of action still remain unclear in most cases.

Regarding the stimulation of insulin secretion, one target of interest for the anti diabetic action of these extracts is the serine protease dipeptidyl peptidase-IV (DPP-IV; EC 3.4.14.5) as the DPP-IV inhibition has been shown to be an appropriate treatment for T2DM [20]. DPP-IV specifically removes N-terminal dipeptides from substrates containing proline or alanine as the second

residue, altering them into inactive or antagonistic species. Incretins like glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are most important substrates of DPP-IV, which stimulates insulin secretion [50].

Medicinal Plants Ascertained for the GLP-1 Stimulation till Date: In 2010, *Mangifera indica* leaves have been estimated by the scientists Shivanna Yogisha and Koteshwara Anandarao Raveesha for presence of antidiabetic properties using glucose-induced hyperglycemia, normoglycaemic and streptozotocin (STZ) induced diabetic mice. DPP-IV inhibitory assay (*in-vitro*) was done to test the activity of methanolic extract of *M.indica* leaves. The study confirmed that *M. indica* methanolic extract inhibited DPP-IV mediated degradation of GLP-1 *in-vitro*.

In 2011, the crude bark extract of tree turmeric (*Berberis aristata*) was tested by Rituparna Chakrabarti, et al., using Diprotin A as the standard inhibitor of DPP-IV which is an effective inhibitor. The scientists used DPP-IV assay of Diprotin and *B. aristata* which helped in isolation of berberine, a natural DPP-IV inhibitor from the bark of *B. aristata*. This phytochemical exhibited effective inhibition against DPP-IV enzyme and explained the role of *B.aristata* in regulating diabetes.

In 2012, *Inonotus obliquus* (a medicinal mushroom) and whose previous studies have demonstrated that its mycelium powers possess significant antihyperglycemic effects in a mouse model of diabetic disease induced by alloxan was analysed. The study conducted by scientists in School of Pharmaceutical Science, Jiangnan University aimed to identify the active ingredients of *I. obliquus* mycelium powders. They applied bioassay-guided fractionation approach and explored the mechanism of action of the active ingredients by using inhibitory assay model of DPP-4 (an important enzyme as a new therapeutic target for diabetes). It was depicted that the chloroform extract of mycelium was potential inhibitory against DPP-4. Bioactivity guided fractionation led to the identification of 19 compounds using UPLC-Q-TOF-MS. Molecular docking between the compounds and DPP-4 disclosed that 5, 8, 9, 14, 15 compounds may be the active components responsible for the DPP-4 inhibitory activity.

In 2013, the anti diabetic effect of *Nelumbo nucifera* (family Nymphaeaceae) was tested in streptozotocin induced diabetic rats. The scientists Nallani Venkata Rama Rao, A.Narendra Babu and M.Sathish Kumar confirmed that the results obtained from methanolic extract of Nelumbo nucifera leaf and stem had antidiabetic property

due to presence of alkaloids, amino acids, saponins, glycosides, vitamins, etc.

In 2014, Anand-Krishna Singh, Rameshwar Jatwa and Jaya Joshi evaluated *Ocimum sanctum* and *Momordica charantia* for their cytoprotective potential and presence of DPP IV inhibition activity. The use of different antioxidant activity assays such as *in vitro* DPP IV activity; ferric reducing potential and reducing power in *O. sanctum* and *M. charantia* extracts clearly exhibited that the leaf extract of *O. sanctum* and fruit extract of *M. charantia* contains novel DPP-IV inhibitors with cytoprotective potential.

CONCLUSION

The adverse effects related with DPP IV inhibitors lead to an increased risk for infection and headache. DPP IV inhibitors as monotherapy may not be sufficiently active in patients with poor and long time disease.

To overcome the drawbacks related with the synthetic drugs (DPP IV inhibitors), herbal supplements have gained popularity. Therapeutic plants are mainly utilized for their cost effectiveness and beneficial effects. Due to the contribution of numerous substantial components, the herbal medicines market has evolved at an expressive rate internationally. There is a belief that herbal medicines might be of effective benefit in the treatment of certain diseases where conventional therapies and medicines have proven to be inadequate. The propensity of plants towards self-medication; quality improvement and certainty of being safe in herbal medicines and high cost of synthetic medicines has opened gateway throughout the world.

GLP-1⁻based therapy would be a complementary approach to diabetes management for various reasons. It does not cause hypoglycemia. No weight gain occurs that can be seen with insulin or sulfonylureas and may in fact facilitate loss of weight. It can be applied as a bridge to insulin therapy or to reduce insulin requirements of insulin resistant patients in order to avoid weight gain. Aggrandizement of GLP-1 and GIP protein concentration by inhibition of DPP IV is a unique technique in a way that it has been shown to reduce the rise in glucagon. It also promotes β-cell rescue and practically halt diabetes progression. But, the technique is mainly used along with the orally available drugs that act as DPP IV inhibitors.

Although scientists have immensely studied the cure and treatment of type 2 diabetes by the use of extracts of medicinal plant parts throughout the world to resurrect the insulin level in body but, still the stimulation level

could be improved to a higher percentage. This could be improved by internalization of plant extract (stem-bark of *Mangifera indica*) to augment GLP-1 factor in humans without the involvement of synthetic drug inhibitors for DPP IV. This would serve as a novel aspect in the experiment to be fared.

- ABBREVIATIONS
- DPP IV: Dipeptidyl peptidase IV
- ADA: American Diabetes Association
- NIDDM: Non Insulin-Dependent Diabetes Mellitus
- CJC-1131: ConjuChem
- FDA: Food and Drug Administration
- pmol kg^{-1} min^{-1}: picomole per kilogram per minute
- EC: Enzyme Commission
- N-terminal: amino terminal, NH$_2$-terminus
- Co.: Company
- CD26: Cluster of Differentiation
- UPLC-Q-TOF-MS: Ultra-performance Liquid Chromatography Quadrupole Time Of Flight Mass Spectrometry

REFERENCES

1. LeRoith, D., 2002. β-cell dysfunction and insulin resistance in type 2 diabetes: role of metabolic and genetic abnormalities. The American journal of medicine, 113(6): 3-11.

2. Gerich, J.E., 1998. The genetic basis of type 2 diabetes mellitus: impaired insulin secretion versus impaired insulin sensitivity. Endocrine reviews, 19(4): 491-503.

3. Gloyn, A.L., 2003. The search for type 2 diabetes genes. Ageing research reviews, 2(2): 111-27.

4. Akram, M., 2013. Diabetes Mellitus Type II: Treatment Strategies and Options: A Review. Journal of Diabetes and Metabolism.

5. Ripsin, C.M., H. Kang and R.J. Urban, 2009. Management of blood glucose in type 2 diabetes mellitus. Am Fam Physician, 79(1): 29-36.

6. Risérus, U., W.C. Willett and F.B. Hu, 2009. Dietary fats and prevention of type 2 diabetes. Progress in lipid research, 48(1): 44-51.

7. A Book: S. Melmed, K.S. Polonsky, P.R. Larsen and H.M. Kronenberg, 2011. Williams textbook of endocrinology: Expert consult. Elsevier Health Sciences.

8. Touma, C. and S. Pannain, 2011. Does lack of sleep cause diabetes? Cleveland Clinic journal of medicine, 78(8): 549-58.

9. Christian, P. and C.P. Stewart, 2010. Maternal micronutrient deficiency, fetal development and the risk of chronic disease. The Journal of nutrition, 140(3): 437-45.

10. Hu, G., J. Rico-Sanz, T. Lakka and J. Tuomilehto, 2006. Exercise, genetics and prevention of type 2 diabetes. Essays Biochem., 42: 177-92.

11. Elrick, H., L. Stimmler, C. Hlad Jr and Y. Arai, 1964. Plasma Insulin Response to Oral and Intravenous Glucose Administration 1. The Journal of Clinical Endocrinology and Metabolism, 24(10): 1076-82.

12. Dupre, J., S. Ross, D. Watson and J. Brown, 1973. Stimulation of insulin secretion by gastric inhibitory polypeptide in man. 1. The Journal of Clinical Endocrinology and Metabolism, 37(5): 826-8.

13. Drucker, D.J. and M.A. Nauck, 2006. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. The Lancet, 368(9548): 1696-705.

14. Nielsen, L.L., A.A. Young and D.G. Parkes, 2004. Pharmacology of exenatide (synthetic exendin-4): a potential therapeutic for improved glycemic control of type 2 diabetes. Regulatory peptides, 117(2): 77-88.

15. Nauck, M.A., U. Niedereichholz, R. Ettler, J.J. Holst, C. Ørskov, R. Ritzel and Wolff H. Schmiegel, 1997. Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. American Journal of Physiology-Endocrinology And Metabolism, 273(5): E981-E8.

16. Ørskov, C., J. Holst and O. Nielsen, 1988. Effect of truncated glucagon-like peptide-1 [proglucagon-(78-107) amide] on endocrine secretion from pig pancreas, antrum and nonantral stomach. Endocrinology, 123(4): 2009-13.

17. Stoffers, D.A., T.J. Kieffer, M.A. Hussain, D.J. Drucker, S. Bonner-Weir, J.F. Habener and J.M. Egan, 2000. Insulinotropic glucagon-like peptide 1 agonists stimulate expression of homeodomain protein IDX-1 and increase islet size in mouse pancreas. Diabetes, 49(5): 741-8.

18. Zhou, J., X. Wang, M.A. Pineyro and J.M. Egan, 1999. Glucagon-like peptide 1 and exendin-4 convert pancreatic AR42J cells into glucagon-and insulin-producing cells. Diabetes, 48(12): 2358-66.

19. Meier, J.J., M.A. Nauck, W.E. Schmidt and B. Gallwitz, 2002. Gastric inhibitory polypeptide: the neglected incretin revisited. Regulatory peptides, 107(1): 1-13.

20. Havale, S.H. and M. Pal, 2009. Medicinal chemistry approaches to the inhibition of dipeptidyl peptidase-4 for the treatment of type 2 diabetes. Bioorganic and medicinal chemistry, 17(5): 1783-802.

21. Mest, H.J. and R. Mentlein, 2005. Dipeptidyl peptidase inhibitors as new drugs for the treatment of type 2 diabetes. Diabetologia, 48(4): 616-20.

22. Pederson, R.A., H.A. White, D. Schlenzig, R.P. Pauly, C.H. McIntosh and H.U. Demuth, 1998. Improved glucose tolerance in Zucker fatty rats by oral administration of the dipeptidyl peptidase IV inhibitor isoleucine thiazolidide. Diabetes, 47(8): 1253-8.

23. Reimer, M.K., J. Holst and B. Ahrén, 2002. Long-term inhibition of dipeptidyl peptidase IV improves glucose tolerance and preserves islet function in mice. European Journal of Endocrinology, 146(5): 717-27.

24. Procter, A.M., J.A. Phillips III and D.N. Cooper, 1998. The molecular genetics of growth hormone deficiency. Human genetics, 103(3): 255-72.

25. Jain, R., U. Kabadi and M. Kabadi, 2008. Is β-cell failure in type 2 diabetes mellitus reversible? International journal of diabetes in developing countries, 28(1): 1.

26. Kirkham, S., R. Akilen, S. Sharma and A. Tsiami., 2009. The potential of cinnamon to reduce blood glucose levels in patients with type 2 diabetes and insulin resistance. Diabetes, obesity and metabolism, 11(12): 1100-13.

27. Kim, J.K., Y-J. Kim, J.J. Fillmore, Y. Chen, I. Moore, J. Lee, Minsheng Yuan, Zhi Wei Li, Michael Karin, Pascale Perret, Steven E. Shoelson and Gerald I. Shulman, 2001. Prevention of fat-induced insulin resistance by salicylate. Journal of Clinical Investigation, 108(3): 437-46.

28. Larsen, M.O., B. Rolin, U. Ribel, M. Wilken, C.F. Deacon, O. Svendsen, Carsten F. Gotfredsen and Richard David Carr, 2003. Valine pyrrolidide preserves intact glucose-dependent insulinotropic peptide and improves abnormal glucose tolerance in minipigs with reduced β-cell mass. Journal of Diabetes Research, 4(2): 93-105.

29. Wiedeman, P.E. and J. Trevillyan, 2003. Dipeptidyl peptidase IV inhibitors for the treatment of impaired glucose tolerance and type 2 diabetes. Current opinion in investigational drugs (London, England: 2000), 4(4): 412-20.

30. Holmes, D., 2006. Twelve-week Monotherapy with the DPP-4 Inhibitor Vildagliptin Improves Glycemic Control in Subjects with Type 2 Diabetes. Horm Metab Res., 38: 423-8.

31. Kim, D., L. Wang, M. Beconi, G.J. Eiermann, M.H. Fisher, H. He, Gerard J. Hickey, Jennifer E. Kowalchick, Barbara Leiting, Kathryn Lyons, Frank Marsilio, Margaret E. McCann, Reshma A. Patel, Aleksandr Petrov, Giovanna Scapin, Sangita B. Patel, Ranabir Sinha Roy, Joseph K. Wu, Matthew J. Wyvratt, Bei B. Zhang, Lan Zhu, Nancy A. Thornberry and Ann E. Weber, 2005. (2 R)-4-Oxo-4-[3-(trifluoromethyl)-5, 6-dihydro [1, 2, 4] triazolo [4, 3-a] pyrazin-7 (8 H)-yl]-1-(2, 4, 5-trifluorophenyl) butan-2-amine: a potent, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. Journal of medicinal chemistry, 48(1): 141-51.

32. Villhauer, E.B., J.A. Brinkman, G.B. Naderi, B.F. Burkey, B.E. Dunning, K. Prasad, Bonnie L. Mangold, Mary E. Rusell and Thomas E. Hughes, 2003. 1-[[(3-hydroxy-1-adamantyl) amino] acetyl]-2-cyano-(S)-pyrrolidine: a potent, selective and orally bioavailable dipeptidyl peptidase IV inhibitor with antihyperglycemic properties. Journal of medicinal chemistry, 46(13): 2774-89.

33. Augeri, D.J., J.A. Robl, D.A. Betebenner, D.R. Magnin, A. Khanna, J.G. Robertson, Aiying Wang, L.M. Simpkins, Prakash Taunk, Qi Huang, Song-Ping Han, Michael Cap, Li Xin, Li Tao, Effie Tozzo, G.E. Welzel, Donald M. Egan, Shu Y. Chang, Scott A. Biller, Mark S. Kirby, Rex A. Parker and Lawrence G. Hamann, 2005. Discovery and preclinical profile of Saxagliptin (BMS-477118): a highly potent, long-acting, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. Journal of medicinal chemistry, 48(15): 5025-37.

34. Herman, G., P. Stein, N. Thornberry and J.Wagner, 2007. Dipeptidyl peptidase-4 inhibitors for the treatment of type 2 diabetes: focus on sitagliptin. Clinical Pharmacology and Therapeutics, 81(5): 761-7.

35. He, Y-L., B.M. Sadler, R. Sabo, S. Balez, Y. Wang, J. Campestrini, Aziz Laurent and Dan Howard, 2007. The absolute oral bioavailability and population-based pharmacokinetic modelling of a novel dipeptidylpeptidase-IV inhibitor, vildagliptin, in healthy volunteers. Clinical pharmacokinetics, 46(9): 787-802.

36. Fura, A., A. Khanna, V. Vyas, B. Koplowitz, S.Y. Chang, C. Caporuscio, David W. Boulton, Lisa J. Christopher, Kristina D. Chadwick, Lawrence G. Hamann, W.G. Humphreys and Mark Kirby, 2009. Pharmacokinetics of the dipeptidyl peptidase 4 inhibitor saxagliptin in rats, dogs and monkeys and clinical projections. Drug Metabolism and Disposition, 37(6): 1164-71.

37. Dittberner, S., V. Duval, A. Staab, I. Troconiz, U. Graefe-Mody and U. Jaehde, 2007 editors. Determination of the absolute bioavailability of BI 1356, a substance with nonlinear pharmacokinetics, using a population pharmacokinetic modelling approach (abstract 1110). Poster presented at 16th Annual Meeting of the Population Approach Group in Europe, København, Denmark.

38. Bnouham, M., A. Ziyyat, H. Mekhfi, A. Tahri and A. Legssyer, 2006. Medicinal plants with potential antidiabetic activity-A review of ten years of herbal medicine research (1990-2000). International Journal of Diabetes and Metabolism, 14(1): 1.

39. Koch, H., W. Jäger, Groh Ua and G. Plank, 1991. In vitro inhibition of adenosine deaminase by flavonoids and related compounds. New insight into the mechanism of action of plant phenolics. Methods and findings in experimental and clinical pharmacology, 14(6): 413-7.

40. Melzig, M., 1996. Inhibition of adenosine deaminase activity of aortic endothelial cells by selected flavonoids. Planta medica, 62(01): 20-1.

41. Lendeckel, U., M. Arndt, C. Wolke, D. Reinhold, T. Kähne and S. Ansorge, 2002. Inhibition of human leukocyte function, alanyl aminopeptidase (APN, CD13) and dipeptidylpeptidase IV (DP IV, CD26) enzymatic activities by aqueous extracts of *Cistus incanus* L. *ssp. incanus*. Journal of ethnopharmacology, 79(2): 221-7.

42. Soumyanath, A., 2005. Traditional medicines for modern times: antidiabetic plants. CRC Press.

43. Yin, J., H. Zhang and J. Ye, 2008. Traditional Chinese medicine in treatment of metabolic syndrome. Endocrine, metabolic and immune disorders drug targets, 8(2): 99.

44. Modak, M., P. Dixit, J. Londhe, S. Ghaskadbi and T.P.A. Devasagayam, 2007. Indian herbs and herbal drugs used for the treatment of diabetes. Journal of clinical biochemistry and nutrition, 40(3): 163.

45. Andrade-Cetto A. and M. Heinrich, 2005. Mexican plants with hypoglycaemic effect used in the treatment of diabetes. Journal of ethnopharmacology, 99(3): 325-48.

46. Haque, N., U. Salma, T. Nurunnabi, M. Uddin, M. Jahangir, S. Islam and M. Kamruzzaman, 2011. Management of type 2 diabetes mellitus by lifestyle, diet and medicinal plants. Pakistan journal of biological sciences: PJBS, 14(1): 13-24.

47. Malviya, N., S. Jain and S. Malviya, 2010. Antidiabetic potential of medicinal plants. Acta Pol Pharm, 67(2): 113-8.

48. Prabhakar, P.K. and M. Doble, 2008. A target based therapeutic approach towards diabetes mellitus using medicinal plants. Current diabetes reviews, 4(4): 1291-308.

49. Qi, L.W., E.H.. Liu, C. Chu, Y.B. Peng, H.X. Cai and P. Li, 2010. Anti-diabetic agents from natural products-an update from 2004 to 2009. Current topics in medicinal chemistry, 10(4): 434-57.

50. Mentlein, R., B. GALLWITZ and W.E. SchmidT, 1993. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1 (7-36) amide, peptide histidine methionine and is responsible for their degradation in human serum. European Journal of Biochemistry, 214(3): 829-35.