Molecular Docking Analysis of Novel Alpha-Glucosidase enzyme Inhibitors from Siddha Formulation *Pungampoo Chooranam* using Computer aided drug discovery

Dr. J. Nisha*1

*1P.G. Scholar, Post Graduate Department of Maruthuvam, Government Siddha Medical College, Arumbakkam, Chennai 600 106, Tamil Nadu, India.
E-mail: nis.evangeline@gmail.com

Abstract

Type II diabetes mellitus (T2DM) is a chronic metabolic disorder in which prevalence has been increasing steadily all over the world. As a result of this trend, it is fast becoming an epidemic in some countries of the world with the number of people affected expected to double in the next decade due to increase in ageing population, thereby adding to the already existing burden for healthcare providers, especially in poorly developed countries. Due to increased adverse event caused by conventional anti-diabetic agents researchers are at constant need of exploring alternate therapeutic strategy for clinical management of diabetes mellitus. Siddha system of traditional medicine through its valuable phytocomponents therapy provides highly beneficial effects in treating metabolic disorders such as diabetes since several centuries. Alpha-glucosidase inhibitors (AGIs) are drugs that inhibit the absorption of carbohydrates from the gut and may be used in the treatment of patients with type II diabetes or impaired glucose tolerance. There are some increasing evidence that AGIs are beneficial to prevent or delay mortality in type II diabetes. The main aim of the resent investigation is to screen the seven bioactive phytocomponents such as Beta Sitosterol, Glabrin, Kanjone, Pongol, Sterolin, Pinnantin, Quercetin, Isolonchocarpin present in the formulation *Pungampoo Chooranam* (PPC) against target protein Alpha-Glucosidase enzyme with PDB code 4J5T along with the standard acarbose using computational docking analysis. The results of the present investigation clearly shows that out of eight compounds screened the compound such as Beta sitosterol, Glabrin, Sterolin and Isolonchocarpin has tendency to binding with the most significant active site 428 PHE of the enzyme target responsible for biological activity, when compare to the standard Acarbose with the binding affinity towards 392 ASP, 709 TYR and 771 GLU. Hence from the results of the present investigation it was concluded that the bioactive phytocomponents present in the formulation *Pungampoo Chooranam* (PPC) has significant Alpha-glucosidase inhibition activity and there by promising anti-diabetic activity and may also be effective in clinical management of type II diabetes mellitus.

Keywords: Type II diabetes mellitus, Siddha system, Alpha-glucosidase inhibitors, *Pungampoo Chooranam*, Phytocomponents, Anti-diabetic activity.
1. Introduction

Diabetes mellitus (DM) is probably one of the oldest diseases known to man. It was first reported in Egyptian manuscript about 3000 years ago [1]. The distinction between type I and type II DM was clearly made [2]. Type II DM was first described as a component of metabolic syndrome in 1988 [3]. Type II diabetes mellitus is a heterogeneous syndrome characterized by abnormalities in carbohydrate and fat metabolism. The causes of type 2 diabetes are multifactorial and include both genetic and environmental elements that affect beta-cell function and tissue (muscle, liver, adipose tissue, pancreas) insulin sensitivity [4].

Type II DM results from interaction between genetic, environmental and behavioral risk factors [5,6]. People living with type II DM are more vulnerable to various forms of both short- and long-term complications, which often lead to their premature death. This tendency of increased morbidity and mortality is seen in patients with type II DM because of the commonness of this type of DM, its insidious onset and late recognition, especially in resource-poor developing countries.

Type II diabetes mellitus is a major health problem associated with excess morbidity and mortality. As the prevalence of this metabolic disorder is rapidly increasing and current treatment fails to stabilise the disease in most patients, prevention should be considered as a key objective in the near future. People who develop type II diabetes pass through a phase of impaired glucose tolerance (IGT). Defects in the action and/or secretion of insulin are the two major abnormalities leading to development of glucose intolerance. Any intervention in the impaired glucose tolerance phase that reduces resistance to insulin or protects the beta-cells, or both, should prevent or delay progression to diabetes. Acarbose, miglitol and voglibose act by competitively inhibiting the alpha-glucosidases, a group of key intestinal enzymes involved in the digestion of carbohydrates. They decrease both postprandial hyperglycaemia and hyperinsulinaemia, and thereby may improve sensitivity to insulin and release the stress on beta-cells [7].

Alpha-glucosidase inhibitors (AGIs) are commonly used oral hypoglycemic drugs, especially in the patient population from East Asia [9, 10, 11]. The guideline of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) recommended the use of AGIs as a potentially first-line agent or in combination with other antihyperglycemic drugs [12]. AGIs have proven similarly efficacious as other commonly used antidiabetes agents [13, 14, 15]. A recent large trial showed that acarbose is similar to metformin in terms of efficacy, and supports a viable choice for initial therapy in patients with newly diagnosed type II diabetes. Additionally, AGIs do not increase body weight, rarely cause hypoglycemia; and have minimal drug-drug interactions [16].

Drugs currently used for the treatment of diabetes include metformin, sulphonylureas, thiazolidinediones, meglinitides etc., whose long term usage is known to cause side effects like heart failure, myocardial infarction, anxiety, nervousness, seizures, palpitation, and depression. Hence there is a need for alternative therapies that can overcome the limitations of conventional anti-hyperglycemic medications. Siddha system of traditional medicine has novel preparation comprises of herbs which has tendency to limit the activity of crucial enzymes like DDP4. One such novel formulation is Pungampoo Chooranam (PPC) majorly comprises of the herb Pongamia pinnata and cow ghee which acts synergistically.

Pongamia pinnata (Fabaceae) is popularly known as Indian beech in English [17]. Commonly known by its vernacular names karanj (Hindi), honge/karajata (Kannada), pongai (Tamil). As per the literature the extract of stem bark of P. pinnata (L.) showed antihyperglycaemic activity in diabetic mice [18]. Further, reports available that concomitant administration of synthetic oral hypoglycemic drugs along with P. pinnata produced synergistic effect in diabetic mice [19]. The preliminary phytochemical analysis showed the presence of alkaloids, terpenoids, triterpenes, flavonoids, steroids, and volatile oils [20]. It has been identified that Cycloart-23-ene-3β, 25-diol (B2) isolated from the stem bark of P. pinnata possesses antidiabetic activity in diabetic animals [21]. Cycloart-23-ene-3β, 25-diol improved the abnormalities of diabetic conditions in diabetic mice due to increased glucagon-like peptide 1 (GLP-1) insulin secretion [22] and has a protective effect on vital organs like heart and kidney [23]. The main aim of the present investigation is to screen the anti-diabetic potential of phytocomponents such as Beta Sitosterol, Glabrin, Kanjone, Pongol, Sterolin, Pinnantin, Quercetin, Isolonchocarpin present in the formulation Pungampoo Chooranam (PPC) against target protein Alpha-Glucosidase enzyme with PDB code 4JST along with the standard acarbose using auto-dock computational docking analysis.
2. Materials and Methods

2.1. Source of raw drugs:

The herb is collected from southern zone of Tamil Nadu, and other required ingredient is procured from a well reputed indigenous drug shop from Parrys corner, Chennai, Tamil Nadu, India. Herb were authenticated by the Pharmacognosist, SCRI Chennai, Tamil Nadu, India.

2.2. Ingredients

The siddha formulation Pungampoo Chooranam (PPC) comprises of two main ingredients as listed below:

1. Pungam flowers (Pongamia pinnata)
2. Cow’s Ghee

2.3. Preparation [24]

The shade dried flowers of Pongamia pinnata were roasted slowly by adding little bit of cow’s ghee. Then it is powdered and sieved using cloth.

Dosage: 2 gm twice a day
Adjuvant: Warm water
Duration: 48 Days

2.4. Software’s required

Several docking tools were being used in recent times which work behind structure-based drug design strategies one among which is auto dock a componential software tools used to analyze the protein alpha-Glucosidase and to study the binding energy properties with the following lead component such as Beta Sitosterol, Glabrin, Kanjone, Pongol, Sterolin, Pinnantin, Quercetin and Isolonchocarpin along with standard Acarbose. Alpha-Glucosidase enzyme with PDB code 4J5T was obtained from protein data bank (www.pdb.org/pdb/). To get insight into intermolecular interactions, the molecular docking studies were done for the above mentioned phytoconstituents along with standard at the active site 3D space of enzyme of interest alpha-Glucosidase using auto dock – docking tool module.

2.5. Ligand preparation

The ligands such as Beta Sitosterol, Glabrin, Kanjone, Pongol, Sterolin, Pinnantin, Quercetin and Isolonchocarpin along with standard Acarbose built using Chemsketch and optimized using Docking server online web tool as shown in Figure 1 and 2 for docking studies by using Geometry optimization method MMFF94 and charge calculation was carried out based on Gasteiger method at pH 7 as shown in Table 1.

| S.No | Name of the Compounds | Molar weight g/mol | Molecular Formula | H Bond Donor | H Bond Acceptor | Rotatable bonds | Log P |
|------|-----------------------|--------------------|-------------------|--------------|-----------------|-----------------|-------|
| 1    | Beta Sitosterol        | 414.718 g/mol      | C_{29}H_{50}O     | 1            | 1               | 6               | 9.3   |
| 2    | Glabrin               | 175.184 g/mol      | C_{7}H_{13}NO_{4} | 3            | 5               | 1               | -3.5  |
| 3    | Kanjone               | 292.29 g/mol       | C_{18}H_{12}O_{4} | 0            | 4               | 2               | 3.6   |
| 4    | Pongol                | 292.29 g/mol       | C_{18}H_{12}O_{4} | 0            | 4               | 2               | 3.6   |
| 5    | Sterolin              | 576.859 g/mol      | C_{35}H_{60}O_{6} | 4            | 6               | 9               | 7.7   |
| 6    | Pinnantin             | 292.29 g/mol       | C_{18}H_{12}O_{4} | 0            | 4               | 2               | 3.6   |
| 7    | Quercetin             | 302.238 g/mol      | C_{15}H_{10}O_{7} | 5            | 7               | 1               | 1.5   |
| 8    | Isolonchocarpin       | 306.361 g/mol      | C_{20}H_{18}O_{3} | 0            | 3               | 1               | 3.8   |
| 9    | Acarbose              | 645.608 g/mol      | C_{25}H_{43}NO_{18} | 14          | 19              | 9               | -8.5  |
Fig 1: 2D Structure of lead 1. Beta Sitosterol 2. Glabrin 3. Kanjone 4. Pongol 5. Sterolin 6. Pinnantin 7. Quercetin 8. Isolonchocarpin and 9. Acarbose

Fig 2: 3D Structure of lead 1. Beta Sitosterol 2. Glabrin 3. Kanjone 4. Pongol 5. Sterolin 6. Pinnantin 7. Quercetin 8. Isolonchocarpin and 9. Acarbose

2.6. Active Site Prediction

Active site of enzyme was obtained by LIGSITE web server by using the automatic identification of pockets on protein surface given 3D coordinates of protein. The potential ligand binding sites in 4J5T target protein is identified using grid space of 1 and probe of radius 5.0 angstrom [25]. Ligand site prediction was performed by using online tool GHECOM and the respective pockets calculations [26,27].

2.7. Docking Methodology

Docking calculations were carried out using Docking Server [28, 29]. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged and rotatable bonds were defined. Docking calculations were carried out based on the binding free energy on the following compounds like Beta Sitosterol, Glabrin, Kanjone, Pongol, Sterolin, Pinnantin, Quercetin and Isolonchocarpin along with standard Acarbose and their binding affinity towards the target protein with PDB 4J5T as shown in figure 3. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of Auto Dock tools. Affinity (grid) maps of Å grid points and 0.375 Å spacing were generated using the Autogrid program. Auto Dock parameter set and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis and Wets local search method [30]. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking.
Each docking experiment was derived from 25-100 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied [31].

Fig 3: Target protein Alpha-Glucosidase enzyme with PDB code 4J5T

3. Results

3.1. Dock scores

Inhibition potential of the lead molecules was evaluated by using core factor called Inhibitory constant contributes more to Inhibition constant and further it's directly proportional to binding energy. The results of the present investigation reveals that inhibition constant of the selected compounds ranges from (1.47 mM to 675.44 μM). Binding free energy of the study reveals the binding affinity and interaction of the lead with that of the target alpha glucosidase the results of the present study reveals that binding free energy of the leads varying from -4.33 to -7.97. The docking score with respect to Binding Free energy, Inhibition constant including Total Interaction Surface were listed in Table 2.

Table 2: Summary of the molecular docking studies of compounds against Alpha-Glucosidase enzyme with PDB code 4J5T

| S.No | Name of the Compounds | Binding Free energy Kcal/mol | Inhibition constant Ki mM(μM)(**nm) | Electrostatic energy Kcal/mol | Intermolecular energy Kcal/mol | Total Interaction Surface |
|------|-----------------------|------------------------------|-------------------------------------|------------------------------|-------------------------------|----------------------------|
| 1    | Beta Sitosterol        | -7.97                        | 1.43                                | -0.14                        | -9.6                          | 879.39                     |
| 2    | Glabrin               | -3.89                        | 1.47*                               | 0.08                         | -4.73                         | 425.99                     |
| 3    | Kanjone               | -5.78                        | 58.03                               | 0.11                         | -6.31                         | 667.71                     |
| 4    | Pongol                | -6.36                        | 21.7                                | -0.2                         | -6.95                         | 657.44                     |
| 5    | Sterolin              | -5.53                        | 88.43                               | -1.73                        | -6.51                         | 384.89                     |
| 6    | Pinnantin             | -5.68                        | 68.38                               | -0.11                        | -6.06                         | 623.74                     |
| 7    | Quercetin             | -6.59                        | 14.74                               | 0.06                         | -6.89                         | 603.99                     |
| 8    | Isolonchocarpin       | -6.65                        | 13.38                               | 0.07                         | -6.95                         | 604.54                     |
| 9    | Acarbose              | -4.33                        | 675.44                              | -0.45                        | -4.85                         | 562.76                     |
Fig 4: Possible ligand binding pockets on the surface of target Alpha-Glucosidase enzyme with PDB code 4JST. Pockets calculated by GHECOM.
1. Beta Sitosterol 2. Glabrin 3. Kanjone 4. Pongol 5. Sterolin 6. Pinnantin 7. Quercetin 8. Isolonchocarpin and 9. Acarbose

Table 3: Interaction of lead compounds with active site amino acid residue of Alpha-Glucosidase enzyme

| Name of the Compounds | Amino Acid Interaction |
|-----------------------|------------------------|
| Beta Sitosterol        | 424 TRP 428 PHE 487 THR 587 TRP 598 SER 600 MET 617 ASP 621 TRP 624 VAL 627 ARG 689 TYR 690 VAL 693 LEU 695 ILE 710 TRP 715 TRP 771 GLU 789 TRP |
| Glabrin               | 424 TRP 428 PHE 487 PRO 600 MET 617 ASP 621 TRP 624 VAL 627 ARG 689 TYR 690 VAL 693 LEU 751 ILE |
| Kanjone               | 492 LEU 495 SER 496 GLU 624 VAL 627 ARG 697 LEU 698 LYS 699 LEU 754 LEU 757 ASP 758 ALA |
| Pongol                | 492 LEU 495 SER 496 GLU 624 VAL 627 ARG 697 LEU 698 LYS 699 LEU 754 LEU 757 ASP 758 ALA |
| Sterolin              | 424 TRP 428 PHE 489 THR 492 LEU 600 MET 621 TRP 624 VAL 627 ARG 689 TYR 693 LEU 696 LEU 698 LYS 751 ILE 754 LEU |
| Pinnantin             | 343 TYR 372 PRO 374 GLU 414 SER 417 GLU 418 MET 420 GLU |
| Quercetin             | 424 TRP 487 PRO 587 TRP 598 SER 600 MET 605 ARG 617 ASP 621 TRP 689 TYR 690 VAL 692 VAL 693 LEU 696 ALA 751 ILE 754 LEU |
| Isolonchocarpin       | 424 TRP 428 PHE 489 THR 598 SER 600 MET 605 ARG 617 ASP 621 TRP 689 TYR 692 VAL 693 LEU 696 ALA 751 ILE |
| Acarbose              | 380 PRO 385 PHE 389 PHE 392 ASP 444 PHE 709 TYR 710 TRP 715 TRP 771 GLU 789 TRP |
The result of binding interactions of the ligand with Alpha-glucosidase has revealed that out of eight compounds docked against PDB code 4J5T. The lead such as Beta Sitosterol, Gabrin, Sterolin and Isolonchocarpin has tendency to binding with the most significant active site 428 PHE of the enzyme target responsible for biological activity, when compare to the standard Acarbose with the binding affinity towards 392 ASP, 709 TYR and 771 GLU. The lead such as Kanjone, Pongol, Pinnantin, Quercetin has no binding affinity towards the selected target. As shown in table 3 and fig 4.

4. Discussion

Type II DM is characterized by insulin insensitivity as a result of insulin resistance, declining insulin production, and eventual pancreatic beta-cell failure [32]. This leads to a decrease in glucose transport into the liver, muscle cells, and fat cells. There is an increase in the breakdown of fat with hyperglycemia. The involvement of impaired alpha-cell function has recently been recognized in the pathophysiology of type II DM [33].

Tests for screening and diagnosis of DM are readily available. The test recommended for screening is the same as that for making diagnosis, with the result that a positive screen is equivalent to a diagnosis of pre-diabetes or DM [34]. Although about 25% of patients with type II DM already have microvascular complications at the time of diagnosis suggesting that they have had the disease for more than 5 years at the time of diagnosis [35]. It is still based on the American Diabetic Association (ADA) guidelines of 1997 or World Health Organization (WHO) National diabetic group criteria of 2006, which is for a single raised glucose reading with symptoms (polyuria, polydipsia, polyphagia and weight loss), otherwise raised values on two occasions, of either fasting plasma glucose (FPG) ≥7.0 mmol/L (126 mg/dL) or with an oral glucose tolerance test (OGTT), two hours after the oral dose a plasma glucose ≥11.1 mmol/L (200 mg/dL) [36].

Acarbose, Voglibose and Miglitol have not widely been used to treat type II DM individuals but are likely to be safe and effective. These agents are most effective for postprandial hyperglycemia and should be avoided in patients with significant renal impairment. Their use is usually limited due to high rates of side-effects such as diarrhoea and flatulence [37]. Voglibose, which is the newest of the drugs, has been shown in a study to significantly improve glucose tolerance, in terms of delayed disease progression and in the number of patients who achieved normoglycemia [38].

Alpha-glucosidase inhibitors seem to be the most effective in reducing post-prandial hyperglycemia. We conducted a review analyzing the clinical efficacy and safety of α-glucosidase inhibitors, both alone and in combination with other anti-diabetic drugs, with respect to glycemic control, inflammation and atherosclerosis. α-Glucosidase inhibitors proved to be effective and safe both in monotherapy and as an addition to other anti-diabetic drugs [39,40]. The result of binding interactions of the ligand with Alpha-glucosidase has revealed that out of eight compounds docked against PDB code 4J5T. The lead such as Beta Sitosterol, Gabrin, Sterolin and Isolonchocarpin has tendency to binding with the most significant active site 428 PHE of the enzyme target responsible for biological activity, when compare to the standard Acarbose with the binding affinity towards 392 ASP, 709 TYR and 771 GLU. The lead such as Kanjone, Pongol, Pinnantin, Quercetin has no binding affinity towards the selected target.

5. Conclusion

Type II DM is a metabolic disease that can be prevented through lifestyle modification, diet control, and control of overweight and obesity. Education of the populace is still key to the control of this emerging epidemic. Novel drugs are being developed, yet no cure is available in sight for the disease, despite new insight into the pathophysiology of the disease. Management should be tailored to improve the quality of life of individuals with type II DM.

According to the literature the most active amino acid residue involved in the alpha glucosidase enzymatic action are Asp568, Tyr709, Glu771, Asp392 and Arg428. The results of the present investigation clearly shows that out of eight compound screened Insilco the lead such as Beta Sitosterol, Gabrin, Sterolin and Isolonchocarpin has tendency to binding with these significant active site and hence from this it was concluded that the bioactive phytocomponents present in the formulation Pungampoo Chooranam (PPC) possess promising anti-diabetic activity and may also be effective in clinical management of type II diabetes.

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References

1. Ahmed AM. History of diabetes mellitus. Saudi Med J 2002; 23:373-378.
2. Angel Lopez-Cuenca. Comparison between type-2 and type-1 myocardial infarction: clinical features, treatment strategies and outcomes. J Geriatr Cardiol. 2016; 13: 15–22.
3. Pattak M. New weapons to combat an ancient disease: treating diabetes. FASEB J. 2002; 16:1853.
4. Scheen AJ. Pathophysiology of type 2 diabetes. Acta Clin Belg. 2003; 58(6):335-41.
5. Robbins and Cotran Pathologic basis of disease (7th ed) 2005. Philadelphia, Saunders; 1156-1226.
6. Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus: present and future perspectives. Nature reviews endocrinology. 2011.
7. Genetic basis of type 1 and type 2 diabetes, obesity, and their complications. Advances and emerging opportunities in diabetes research: a Strategic Planning report of the DMICC. 2011.
8. Scheen AJ. Is there a role for alpha-glucosidase inhibitors in the prevention of type 2 diabetes mellitus. Drugs. 2003; 63:933-51.
9. Lebovitz HE. Alpha-Glucosidase inhibitors. Endocrinol Metab Clin North Am. 1997; 26:539-51.
10. Yang W. Acarbose compared with metformin as initial therapy in patients with newly diagnosed type 2 diabetes: an open-label, non-inferiority randomised trial. The Lancet Diabetes & Endocrinology. 2014; 2: 46–55.
11. Li Y. Acarbose monotherapy and weight loss in Eastern and Western populations with hyperglycaemia: an ethnicity-specific meta-analysis. International journal of clinical practice.2014; 68: 1318–1332.
12. Stephen P. & Clissold C. E. Acarbose. Drugs. 1988;25 : 214–243.
13. Silvio E. Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care.2015; 38: 140–149.
14. Chiasson JL. Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. The Lancet.2002; 359: 2072–2077.
15. Chiasson JL. Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. JAMA. 2003; 290: 486–494.
16. Hanefeld M. Cardiovascular benefits and safety profile of acarbose therapy in prediabetes and established type 2 diabetes. Cardiovasc Diabetol.2007; 6: 20.
17. van de Laar F. A. et al. . Alpha-glucosidase inhibitors for patients with type 2 diabetes: results from a Cochrane systematic review and meta-analysis. Diabetes Care.2005; 28: 154–163.
18. Badole SL, Bodhankar L. Antihyperglycemic activity of Pongamia pinnata stem bark in diabetic mice. Pharmaceutical Biology. 2008;46:900–905.
19. Badole SL, Bodhankar L. Investigation of antihyperglycaemic activity of aqueous and petroleum ether extract of stem bark of Pongamia pinnata on serum glucose level in diabetic mice. Journal of Ethnopharmacology. 2009; 123:115–120.
20. Badole SL, Bodhankar SL. Concomitant administration of petroleum ether extract of the stem bark of Pongamia pinnata (L) Pierre with synthetic oral hypoglycaemic drugs in alloxan-induced diabetic mice. European Journal of Integrative Medicine. 2009; 1:73–79.
21. Badole SL, Bodhankar SL. Antihyperglycaemic activity of cycloart-23-ene-3β, 25-diol isolated from stem bark of Pongamia pinnata in alloxan induced diabetic mice. Research Journal of Phytochemistry. 2009;3: 18–24.
22. Badole SL, Bodhankar SL. Antidiabetic activity of cycloart-23-ene-3β, 25-diol (B2) isolated from Pongamia pinnata (L. Pierre) in streptozotocin-nicotinamide induced diabetic mice. European Journal of Pharmacology. 2010; 632:103–109.
23. Badole SL, Bagul PP, Mahamuni SP. Oral L-glutamine increases active GLP-1 (7-36) amide secretion and improves glycemic control in streptozotocin-nicotinamide induced diabetic rats. Chemico-Biological Interactions. 2013; 203:530–541.
24. Badole SL, Bodhankar SL, Raut CG. Protective effect of cycloart-23-ene-3β, 25-diol (B2) isolated from Pongamia pinnata L. Pierre on vital organs in streptozotocin-nicotinamide induced diabetic mice. Asian Pacific Journal of Tropical Biomedicine. 2011;1: 186–190.
25. Boga Munivar Vaithiyam 700.B. Rathina Nayakkar & Sons.2012. Seiyul no: 199, 200.
26. Bingding H, Michael S. Schroeder “LIGSITE csc: Predicting protein binding sites using the Connolly surface and degree of conservation. BMC structural Biology.2006; 6:01-11.
27. Kawabata T. Detection of multi-scale pockets on protein surfaces using mathematical morphology. Proteins.2010; 78:1195-1121.
28. Kawabata T. Detection of pockets on protein surfaces using small and large probe spheres to find putative ligand binding sites. Proteins.2007; 68:516-529.
29. Bikadi Z, Hazai E. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of Auto Dock. J. Cheminf.2009; 1:15.
30. Halgren TA. Molecular force field. I. Basis, form, scope, parametrization, and performance of MMFF94.J Comput Chem.1998; 17:490-519.
31. Morris GM. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem. 1998;19:1639-1662.
32. Solis FJ. Minimization by Random Search Techniques. Mathematics of Operations Research. 1981; 6:19-30.
33. Kahn CR. Banting Lecture. Insulin action, diabetogenes, and the cause of type II diabetes. Diabetes 1994;43:1066-1084.
34. Robertson RP. Antagonist: diabetes and insulin resistance—philosophy, science, and the multiplier hypothesis. J Lab Clin Med 1995; 125:560-564.
35. Fujibara K. Pathophysiology of type 2 diabetes and the role of incretin hormones and beta-cell dysfunction. JAAPA 2007; 3-8.
36. Garcia-Roves PM. Mitochondrial pathophysiology and type 2 diabetes mellitus. Arch Physiol Biochem 2011; 117:177-187.
37. Cox EM, Elelman D. Test for screening and diagnosis of type 2 diabetes. Clin Diabetes 2009; 4:132-138.
38. Chiniwala N, Jabbour S. Management of diabetes mellitus in the elderly. Curr Opin Endocrinol Diabetes Obes 2011;18:148-152
39. Kawamori R, Tajima N, Iwamoto Y, Kashiwagi A, Shimamoto K, Kaku K, Voglibose Ph-3 Study Group Voglibose for prevention of type 2 diabetes mellitus: a randomised, double-blind trial in Japanese individuals with impaired glucose tolerance. Lancet. 2009; 373:1607-1614.
40. Giuseppe Derosa. α-Glucosidase inhibitors and their use in clinical practice. Arch Med Sci. 2012; 8: 899–906.