Experimental Procedure

Diabetes is one of the most prevalent disorders worldwide and its incidence continues to rise (Han et al., 2010). One of the major concerns with the diabetes epidemic is the expected increase in morbidity and mortality related to complications of the disease (Cefalu, 2007). Early diagnosis and aggressive treatment for diabetes, a rampantly growing disease can minimize the risk of microvascular and macrovascular complications (Leite et al., 2009). The experimental procedure pertaining to the study “Diagnostic approaches and interventional strategies of type 1, type 1.5 and type 2 diabetes” was conducted in three phases. The present study was carried out from May 2007 to December 2009 for a period of two years and six months.

PHASE 1

3.1 SCREENING AND DIFFERENTIAL DIAGNOSIS OF THE THREE TYPES OF DIABETES

During the study period 4,250 persons who visited three health care centres namely KR Hospital, Sneham Hospital and SPC Diabetes Care Centre in Coimbatore, Tamil Nadu were screened for diabetes.

3.1.1 Screening of diabetic patients

Blood sugar, according to “The World Health Organization” is a reliable parameter for a prompt and sound diagnosis of diabetes (Hill, 2005). Random blood glucose estimation was carried out in 4,250 persons as indicated in Appendix 1 (Trinder, 1969). Persons who were found to have a random blood glucose value > 200mg/dl were identified to have diabetes.

3.1.2 Selection of diabetic patients for differential diagnosis of type 1 and type 2 diabetes

Based on the inclusion criteria namely age above 20 and below 65 years, symptoms namely polyuria, polydypsia and unintentional weight loss, baseline random blood glucose > 200mg/dl and glycosylated hemoglobin
> 7.0%, diabetic patients were selected who were subjected for the differential diagnosis of type 1 and type 2 diabetes. Glycosylated hemoglobin and total hemoglobin were estimated by ion-exchange method using semi-auto analyzer microlab-300 (Trivelli et al., 1971 and Nathan et al., 1984) as explained in Appendix- 2.

3.1.3 Categorization of type 1 and type 2 diabetic patients

C-peptide level is a biomarker of β-cell function (Ko et al., 2009). C-peptide provides an indirect measure of the insulin secretory reserve (Sari and Balci, 2005). C-peptide level is most specific in distinguishing between type 1 and type 2 diabetes mellitus (Hu and Isaacson, 2009). In the present study, C-peptide analysis was carried out in patients using chemiluminescence immuno analyzer model- QQ-96 as indicated in Appendix 3 (Turkington et al., 1982). Based on the C-peptide value, diabetic patients were categorized as type 1 and 1250 were categorized as type 2.

3.1.4 Identification of suspected type 1.5 diabetic patients among type 2 diabetic patients

Type 1.5 diabetes presents intermediate features between type 1 diabetes and type 2 diabetes and this particularity has led to both mistaken diagnosis and inappropriate therapeutic management (Bermudez et al., 2010). Type 1.5 diabetes was found to occur in about 10% of the patients classified as type 2 diabetes and not initially requiring insulin (Agardh et al., 2005). Latent autoimmune diabetes in adults (LADA) risk score based on five clinical features- namely age at onset < 50 years, acute symptoms of hyperglycemia, body mass index (BMI) < 23 kg/m² (cut off value for South Asians), personal history of autoimmune disease and family history of autoimmune disease- has a higher sensitivity and specificity in detecting type 1.5 diabetes using autoantibody testing (Unger, 2008a). Early identification and use of insulin in type 1.5 diabetic patients could preserve endogenous insulin secretion and probably delay or prevent the decline of β-cell function (Romkens et al., 2006).
LADA risk score as indicated in Table 3 was applied to the type 2 diabetic patients. LADA risk score was calculated as per the method of Fourlanos et al. (2006). The Body Mass Index (BMI) cut off value for the designation of overweight as per the World Health Organization (WHO) Asia Pacific guidelines is $> 23 \text{ kg/m}^2$ (Mohan et al., 2007). Hence, in the present study, BMI of $< 23 \text{ kg/m}^2$ was incorporated in the method of Fourlanos et al. (2006) for calculating the LADA risk score. Based on the score, persons who had a score $\geq 2$ were suspected to belong to type 1.5 diabetes.

### TABLE 3

**LADA RISK SCORE**

<table>
<thead>
<tr>
<th>Risk score parameters</th>
<th>Risk score value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset $&lt; 50$ years</td>
<td>1</td>
</tr>
<tr>
<td>Acute symptoms of hyperglycemia</td>
<td>1</td>
</tr>
<tr>
<td>Body mass index (BMI) $&lt; 23 \text{ kg/m}^2$ (cut off value for South Asians)</td>
<td>1</td>
</tr>
<tr>
<td>Personal history of autoimmune disease</td>
<td>1</td>
</tr>
<tr>
<td>Family history of autoimmune disease</td>
<td>1</td>
</tr>
</tbody>
</table>

**3.1.5 Confirmation of type 1.5 diabetic patients**

High titres of serum glutamic acid decarboxylase (GAD) antibodies act as a marker for type 1.5 diabetes (Fielding et al., 2007). Glutamic acid decarboxylase antibody (GADA) test was performed as given in Appendix 4 (Schmidli et al., 1995) for the suspected type 1.5 cases and those who patients were found to have positivity for GADA were identified as type 1.5 diabetic patients.

The schematic representation of the screening and categorization of the three types of diabetic patients in the present study is shown in Figure 9.
FIGURE 9
SELECTION OF THE PARTICIPANTS OF THE STUDY AND DIFFERENTIAL DIAGNOSIS OF THE THREE TYPES OF DIABETES

Persons screened for diabetes

Persons identified with diabetes

Participants selected based on selected inclusion criteria

C-peptide analysis

Patients identified as type 1 diabetes (C-peptide < 0.9ng/ml)

Patients identified as type 2 diabetes (C-peptide > 0.9 ng/ml)

Inclusion criteria
- Age above 20 years and below 65 years
- Polyuria, polydypsia and unintentional weight loss
- Baseline random blood glucose > 200mg/dl
- Glycosylated Hemoglobin >7.0%

Patients identified as type 1 diabetes

Patients identified as type 2 diabetes

LADA risk score

Patients suspected as LADA score ≥ 2

Patients with type 2 diabetes LADA score< 2

GADA positivity

Patients identified as type 1.5 diabetes

Patients identified as type 2 diabetes
PHASE 2

3.2 PROFILE OF THE DIABETIC PATIENTS AFTER CATEGORIZATION INTO TYPE 1, TYPE 1.5 AND TYPE 2

Type 1, type 1.5 and type 2 diabetic patients who were categorized and confirmed for their types in phase 1 were further grouped according to the sex and three age groups 20-35 years, 35-50 years and 50-65 years. Details of the patients namely name, age, sex, age at onset of diabetes, symptoms at onset (excessive thirst / frequent urination / unintentional weight loss > 10% within 2 months), family history of diabetes, personal history of autoimmune disease and also family history of autoimmune disease, lifestyle pattern (sedentary, active, exercise, diet, smoking, alcoholism) were recorded (Appendix 5).

3.2.1 Anthropometric measurement

Body mass index, central obesity, hypertension, family history of diabetes and lifestyle factors are the known risk factors of diabetes (Shi et al., 2006). BMI is one of the physical parameters of obesity (Riaz et al., 2009). Height was measured in cms using a metal stadiometer; weight was measured in kg using weighing scale; waist to hip ratio was measured using a measuring tape; blood pressure was measured using a sphygmomanometer and BMI was calculated by the formula (Barlow and Dietz, 1998) as given below:

\[
BMI = \frac{\text{Weight in kg}}{(\text{Height in meter})^2}
\]

3.2.2 Biochemical and hematological analysis

Pharmacologic treatment of diabetes aids to improve glycemic control, regulate blood pressure, reduce blood lipid concentrations and reduce the occurrence and progression of diabetes complications (Wolever et al., 2008). In all the three types of diabetic patients of both the sexes and three age groups, biochemical and hematological parameters were assessed as indicated in table 2 and table 3 at the time of diagnosis of diabetes as well as
after 6 months intervention with insulin for patients of type I diabetes, with Insulin + Voglibose / insulin + Metformin for patients of type 1.5 diabetes and with Glimepiride / Metformin / Voglibose / Pioglitazone / Sitagliptin + Metformin for patients of type 2 diabetes.

Changes in lifestyle and dietary habits, in conjunction with genetic susceptibility, have resulted in a remarkable increase in the incidence and prevalence of diabetes in the world. Maintaining blood glucose levels within the normal range is of utmost importance in the management of diabetes. Diet is one factor that can have a great impact upon stabilizing blood glucose levels in diabetic patients (Al-Khalifa et al., 2009).

Diet therapy is a type of treatment to understand abnormal metabolism of nutrients, to figure out and manage the relationship between food intakes and blood glucose levels. For effective blood glucose control, accurate nutrition information and active practice of diet therapy by a patient are required (Yun and Kim, 2009). Numerous studies with diabetic patients have shown the association of nutrition education with improving dietary behavior, nutritional knowledge and improving clinical outcomes such as lower blood glucose, HbA1c levels and lipid concentrations (Lim et al., 2009).

Foods with low glycemic indices include oats, barley, bulgur, beans, lentils, legumes, pasta, bread, apples, oranges, milk, yogurt and ice cream. Fiber, fructose, lactose, and fat are the dietary constituents that tend to lower glycemic response (Mayer-Davis et al., 2006).

Diet therapy is important in preventing diabetes, managing existing diabetes and preventing or at least slowing the rate of development of diabetes complications. It is, therefore, important at all levels of management of diabetes prevention. People with diabetes are encouraged to choose a variety of fiber-containing foods such as legumes, fiber-rich cereals (≥5 g fiber/serving), fruits, vegetables and whole grain products because they
provide vitamins, minerals and other substances important for good health. The primary goal with respect to dietary fat in individuals with diabetes is to limit saturated fatty acids, trans fatty acids and cholesterol intake so as to reduce the risk for cardiovascular disease (American Diabetes Association, 2008).

The three types of diabetic patients strictly followed the diet (Appendix 6) and lifestyle modification as per the counseling and recommendations rendered to them.

3.0 ml of blood was collected in two sets of heparinised vials separately. One set was used for the separation of plasma by centrifugation. Whole blood obtained in screw cap tubes were allowed to clot for one hour and serum was separated and used for selected biochemical analysis. Urine sample was collected in screw capped tubes containing 1g/L sodium azide to prevent contamination which was centrifuged prior to analysis and was used for the estimation of microalbumin.

Fasting blood glucose is the standard measure used to diagnose diabetes (Selvin et al., 2010). Assessment of postprandial blood glucose is also, a convenient and a reliable method in diabetes (Weerarathna and Dissanayake, 2006). HbA1c assay is the most widely accepted and reliable marker for assessing the glycemic status of diabetic patients (Abhyuday et al., 2009). HbA1c is used to evaluate the long-term glycemic control and the risk for the development of complications in patients with autoimmune diabetes and type 2 diabetes (Yoshiuchi et al., 2008). Hemoglobin concentration was found to be inversely related to endothelial function in diabetic patients with stage 1 to 2 chronic kidney disease (Sonmez et al., 2010).

Fasting blood glucose, postprandial blood glucose, glycosylated hemoglobin (HbA1c) and total hemoglobin were estimated in the three types of diabetic patients.
Assessment of various lipid fractions in diabetes is important in the prognosis of patients and in preventing the possibility of complications (Suryawanshi et al., 2006). Plasma triglycerides, total cholesterol and HDL cholesterol were determined according to the instruction manuals accompanying the diagnostic kits obtained from Qualigens Diagnostics, Mumbai, India as indicated in Appendix 7, 8 and 9 respectively.

VLDL and LDL cholesterol were calculated using Friedwald formula

\[
\text{VLDL} = \frac{\text{TG}}{5} \\
\text{LDL} = \text{Total cholesterol} - (\text{HDL} + \text{VLDL}).
\]

The values were expressed as mg/dl plasma.

Lipoprotein A, a heterogeneous macromolecule consisting of apolipoprotein A acts as a marker for determining vascular or tissue injury and is an independent risk factor for the progression of diabetic nephropathy in diabetic patients (Nasri, 2008). Liver and kidney functions are highly altered in diabetics (Chandramohan et al., 2009).

High concentrations of high sensitive C-reactive protein (hs CRP), an inflammatory marker is associated with greater risks of cardiovascular disease (CVD) in persons with diabetes (Wolever et al., 2008). A quick and simple way to assess renal function in diabetics is to perform serum creatinine and blood urea test (Wagle, 2010). Elevated plasma levels of urea and creatinine are considered as significant markers of renal dysfunction (Kumara et al., 2007). Serum bilirubin level is associated with microalbuminuria and subclinical atherosclerosis in patients with diabetes (Fukui et al., 2008). Microproteinuria and albuminuria are important clinical markers of diabetic nephropathy (Kumar et al., 2007).

Lipoprotein A, apolipoprotein A1, apolipoprotein B, C-reactive protein, blood urea, serum creatinine, total protein, albumin, globulin, total bilirubin and urine microalbumin were estimated by the methods as indicated in Table 4.
TABLE 4
DETAILS OF ASSESSMENT OF SELECTED BLOOD AND URINE PARAMETERS

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Methods</th>
<th>References</th>
<th>Appendix No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipoprotein A</td>
<td>Turbidimetry</td>
<td>Albers et al. (1996)</td>
<td>10</td>
</tr>
<tr>
<td>Apolipoprotein A1</td>
<td>Turbidimetry</td>
<td>Evans et al. (1995)</td>
<td>11</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>Turbidimetry</td>
<td>Evans et al. (1995)</td>
<td>12</td>
</tr>
<tr>
<td>hs C-reactive protein</td>
<td>Turbidimetry</td>
<td>Price et al. (1987)</td>
<td>13</td>
</tr>
<tr>
<td>Blood Urea</td>
<td>Spectrophotometry</td>
<td>Chaney and Marbach (1962) and Searcy et al. (1967)</td>
<td>14</td>
</tr>
<tr>
<td>Total protein, albumin and globulin</td>
<td>-do-</td>
<td>Doumas et al. (1971) and Webster (1977)</td>
<td>15</td>
</tr>
<tr>
<td>Creatinine</td>
<td>-do-</td>
<td>Henry et al. (1974)</td>
<td>16</td>
</tr>
<tr>
<td>Urine microalbumin</td>
<td>Turbidimetry</td>
<td>Feldt-Rasmussen et al. (1994)</td>
<td>17</td>
</tr>
</tbody>
</table>

Increased concentrations of four liver enzymes namely serum gamma glutamyl transferase, serum aspartate transaminase, serum alanine transaminase and serum alkaline phosphatase are conventionally interpreted as markers of liver damage (Nannipieri et al., 2005). Increased activity of aspartate transaminase and alanine transaminase are of clinical importance in diabetes because elevated activity of aspartate transaminase is also suggestive of cardiac damage (Sundaram et al., 2009).

Selected serum enzymes namely, serum aspartate transaminase, serum alanine transaminase, serum gamma glutamyl transferase and serum alkaline phosphatase were estimated in the present study, in the three types of diabetic patients as explained in Table 5.
**TABLE 5**

**DETAILS OF ESTIMATION OF SELECTED SERUM ENZYME ACTIVITIES**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Methods</th>
<th>References</th>
<th>Appendix No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum aspartate transaminase</td>
<td>-do-</td>
<td>Reitman and Frankel (1957)</td>
<td>18</td>
</tr>
<tr>
<td>Serum alanine transaminase</td>
<td>-do-</td>
<td>Reitman and Frankel (1957)</td>
<td>19</td>
</tr>
<tr>
<td>Serum gamma glutamyl transferase</td>
<td>Spectrophotometry</td>
<td>Persijn and van der Slik (1976)</td>
<td>20</td>
</tr>
<tr>
<td>Serum alkaline phosphatase</td>
<td>-do-</td>
<td>King and Armstrong (1934)</td>
<td>21</td>
</tr>
</tbody>
</table>

### 3.2.3 Assessment of microvascular and macrovascular complications

Diabetic patients are at an increased risk of developing specific complications including: neuropathy, retinopathy, nephropathy and atherosclerosis (Wagle, 2010). The incidence of neuropathy increases with duration of diabetes and is accelerated by poor sugar control (Ahmed et al., 2010). Diabetic retinopathy is one of the most important complications of diabetes mellitus. The development of appropriate screening strategies for the identification and treatment of patients is important (Lueder and Silvestein, 2005). Diabetic nephropathy is the leading cause of end-stage renal disease (ESRD) worldwide, and it is estimated that approximately 20% of type 2 diabetic patients reach ESRD during their lifetime (Unnikrishnan et al., 2007).

The albumin-to-creatinine ratio (ACR) is closely linked to cardiometabolic risk factors, vascular disease and insulin resistance (Friedman et al., 2008). High risk for heart disease results from both high incidence of coronary artery disease and hypertension in the diabetics (Lopaschuk, 2009). Cerebrovascular reactivity impairment was reported as a marker of cerebral microangiopathy in long-term diabetes (Kozera et al., 2009). Peripheral vascular disease is more common among diabetics and is a
A sub-study consisting of six patients from each type of diabetes (one male and one female from each age group 20-35 years, 35-50 years and 50-65 years) with complications were screened for the presence of complications as given in Table 6.

<table>
<thead>
<tr>
<th>Complications</th>
<th>Parameters assessed</th>
<th>Instrument / Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic neuropathy</td>
<td>-</td>
<td>Biothesiometry (vibratory threshold reading) using digital sensitometer-VPT DSL dhansai</td>
</tr>
<tr>
<td>Diabetic retinopathy</td>
<td>-</td>
<td>Fundoscopy using Heinke opthalmoscope</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>Albumin: Creatinine ratio</td>
<td>Ratio calculated from albumin and creatinine values obtained as per the procedures detailed in appendices 15 and 16 respectively.</td>
</tr>
<tr>
<td></td>
<td>Urine microalbumin</td>
<td>As per the procedure detailed in appendix 17</td>
</tr>
<tr>
<td>Cardiovascular complications</td>
<td>-</td>
<td>Electrocardiogram using full automatic electro cardiograph machine- M3708 Of Philips Medical Systems</td>
</tr>
<tr>
<td>Cerebrovascular complications</td>
<td>-</td>
<td>Carotid doppler study echo cardiogram ultrasound machine; logic 3 of GE medical systems</td>
</tr>
<tr>
<td>Peripheral vascular complications</td>
<td>-</td>
<td>Doppler study echo cardiogram ultrasound machine; logic 3 of GE medical systems</td>
</tr>
</tbody>
</table>
PHASE 3

3.3 In silico DOCKING OF DRUGS FOR TYPE 1.5 DIABETES

Molecular docking methods are commonly used for predicting binding modes to proteins and energies of ligands (Bikadi and Hazai, 2009). Prevention and treatment of autoimmune diabetes should be achieved by intervening with the autoimmune process (Eldor et al., 2009). The heat shock protein (Hsp90) plays a role in glutamic acid decarboxylase (GAD) antigen presentation by Major Histocompatability Complex (MHC) class II molecules (Houlihan and Blum, 2008). Hsp90 chaperones contain an N-terminal adenosine triphosphate (ATP) binding site that has been effectively targeted by competitive inhibitors (Pearl, 2005). Inhibition of the Hsp90 ATP binding site leads to the targeted degradation of client proteins via the ubiquitin proteasome pathway (Chu et al., 2006).

Geldanamycin, a natural product isolated from the bacteria Streptomyces hygroscopicus, was the first identified Hsp90 inhibitor. In recent years, molecular chaperones such as the 90 Kilodalton (kDa) heat-shock proteins (Hsp90) have surfaced as promising targets for drug discovery (Amolins and Blagg, 2009). Radicicol is the most potent natural product inhibitor of Hsp90 and its mechanism of action is similar to that of geldanamycin, in that it binds to the N-terminal ATP binding pocket of Hsp90 (Wang et al., 2006). Radanamycin is a new class of chimeric analogue composed by Shen and co-workers, which is a combination of the pro-inhibitory properties of geldanamycin and radicicol (Hadden et al., 2006).

Discriminating “drug-like” molecules from non-drug like molecules is one of the prime focuses of current research in the field of computer aided drug design. Drug-likeliness can be deduced as a delicate balance among molecular properties affecting pharmaco-dynamics and pharmacokinetics of molecules which ultimately affects their absorption, distribution, metabolism, and excretion in human body like a drug. Molecular properties include
molecular weight, electronic distribution, hydrophobicity, hydrogen bond donors/acceptors potential, solubility, viscosity, excess volume and other related properties (Mishra et al., 2009).

In the present study, an attempt was made to dock four ligands – two natural inhibitors of Hsp90 namely geldanamycin and radicicol, one chimeric inhibitor namely radanamycin and one plant compound namely mangiferin with the target protein Hsp90 and to find the best ligand based on the docking score of Grid based Ligand Docking with Energetics (GLIDE), which is a docking software of Schrodinger (Maestro, version 7.5, Schrodinger, 2006). Drug likeliness was found using Lipinski drug filter and Molsoft tool.

Docking of ligands and finding of drug likeliness were carried out according to the following steps:

**Step 1. Protein preparation**

Hsp90 N-terminal domain complexed with 1-4-[(2R)-1-(5-chloro-2,4-dihydroxybenzoyl) pyrrolidin-2-yl]benzyl]-3,3-difluoroppyrrolidinium (PDB ID: 3HEK) was selected for the study. The structure coordinates of the protein was downloaded from the Protein Data Bank database (www.pdb.org). Protein preparation was done using protein preparation Wizard of Schrodinger software, which performs the final stages of the preparation of proteins for use in Glide. The protein was treated to add missing hydrogen, assign proper bond orders and to delete water compounds that were more than 5Å from the heterogeneous groups. Water molecules were removed initially to avoid unwanted steric interactions. The H-bonds were optimized using sample orientations. All the polar hydrogens were displayed. Finally, the protein structure was minimized to the default Root Mean Square Deviation (RMSD) value of 0.30 Å.

The ligand molecules in the complex receptor protein were picked. APPLICATIONS option was selected from the Maestro window and protein
preparation option was clicked. After protein preparation and refinement of the target protein complex, protein preparation jobs were finished. The refinement component performs a restrained impact minimization of the co-crystallized complex, which reorients side chains, hydroxyl groups and alleviates potential steric clashes. The output of the refined protein was obtained as PV. mae file, which is a pose viewer file in the maestro window.

**Step 2. Receptor grid generation**

The co-crystallized ligand of the receptor was differentiated from the active site of receptor. The atoms were scaled by van der Waals radii of 1.0 Å with the partial atomic charge less than 0.25 defaults. The active site was defined as an enclosing box at the centroid of the workspace ligand as selected in the receptor folder. The ligands similar in size to the workspace ligand were allowed to dock into the active site. No constraints either positional, H-bonding or hydrophobic were defined.

Receptor grid generation was done by the following steps:

1. The output file (PV. mae file) of the prepared protein (complex structure) was imported in Maestro
2. Ligands in the target protein were picked
3. Site points were generated
4. Hydrophobic cells were analyzed and generated
5. Start menu was clicked and the jobs were allowed to run
6. After finishing of the jobs, the grid was generated, the cells were grown in the hydrophobic sites and the output file of the grid generated protein was obtained as PV. mae file

**Step 3. Preparation of the ligands**

The ligand discovery can be regarded as a simple matching problem to identify a small molecule with the appropriate shape and charge properties to
bind effectively to a target protein. The ligands were built using Maestro build panel. The ligand molecule used for the present study namely geldanamycin, radicicol, radanamycin and mangiferin were prepared using LigPrep module which uses Merck Molecular Force Field (MMFF) and gave the corresponding low energy 3D conformers of the ligands. After selecting LigPrep option from the Maestro window, the small molecules (ligands) were imported, start menu was clicked and the jobs were allowed to run. After finishing of the jobs, the prepared ligands were obtained as ligand outfile.

The structure of geldanamycin and radicicol with the identification number DB02424 and DB03758 respectively were downloaded from the drug bank database (www.drugbank.org). The structure of radanamycin and mangiferin with the identification number CID11962160 and CID5281647 respectively were downloaded from the PubChem compound database (www.pubchem.org).

**Step 4. Molecular docking**

Ligand docking was performed using Optimized Potential for Liquid Simulations-all atom (OPLSAA) force field. Flexible docking was performed using the Standard Precision (SP) feature of Glide module. The Van der Waals radii were scaled using a default scaling factor of 0.80 and default partial cutoff charge of 0.15 to decrease the penalties for close contacts. Receptor Van der Waals scaling makes the protein site “roomier” by moving back the surface of non Polar Regions of the protein and ligand. This kind of adjustments emulate to some extent the effect of breathing motion to the protein site, it is a kind of giving breathing to the receptor, this approach softens the active site region of the receptor making it flexible.

The receptor protein after generation of grid obtained as PV. mae file was docked with the ligands obtained as ligand outfile by selecting docking option from the Maestro window. After finishing of the job the docked molecules were obtained as PV. mae file.
Step 5. Viewing docked results

Docked results were viewed using pose-viewer. The H-bonds and Van der Waals contacts (good, bad and ugly) to the receptor were visualized using default settings to analyze the binding modes of the ligands to receptor.

Step 6. Finding the drug likeliness of the ligands using Lipinski Drug Filter

All the four ligands namely geldanamycin, radicicol, radanamycin and mangiferin were analysed for drug likeliness using the tool Lipinski drug filter of the Supercomputing Facility for Bioinformatics and Computational Biology (http://www.scfbio-iitd.res.in/utility/LipinskiFilters.jsp).

Step 7. Comparing the drug likeliness and non likeliness of mangiferin using Molsoft tool

The drug likeliness and drug non likeliness of the plant compound mangiferin was compared using the tool Molsoft. (http://www.rdchemicals.com/chemistry-software/molsoft.html).

3.4 STATISTICAL ANALYSIS

Two way Analysis of variance with statistical significance at 5 percent and/or 1 percent was performed to compare the biochemical parameters among the three types of diabetic patients- type 1, 1.5 and 2 between men and women and between three selected age groups, at diagnosis and after interventions.

Correlation analysis was performed between fasting blood glucose and HbA1c, fasting blood glucose and C-peptide, HbA1c and C-peptide, apolipoprotein A1 and HDL-cholesterol and between apolipoprotein B and LDL-cholesterol and statistical significance of correlation co-efficient at 5 per cent level was assessed. Chi-square analysis was performed to assess the association between the battery of lipid profile namely total...
cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, apolipoprotein A1 and apolipoprotein B and incidence of heart disease at 5 percent level of significance.