

Probing the mechanism of the anti-diabetic potential of a terpenoid from *Elephantopus scaber* L., an Indian ethnomedicinal plant in STZ diabetic rats- *In vivo* and *in silico* analysis

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Received 30 March 2016; revised 29 May 2018

Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. Since ancient times, traditional medicines all over the world have advocated the use of plants to treat diabetes. *Elephantopus scaber* L. assumes significance because it has shown by us to possess high hypoglycemic effect and justifies the traditional claims of being anti-diabetic drug candidate. The terpenoid isolated from *Elephantopus scaber* L. demonstrated promising antihyperglycemic property with an increase in insulin levels but the mode of action had not been investigated. Since increased insulin levels are associated with β -cells of the pancreas, histological studies were undertaken to evaluate the effect of the compound on β -cells protection in streptozotocin induced diabetic rats. Streptozotocin is known to cause damage to β -cells and hence the protective and curative role of terpenoid was evaluated on the restoration of β -cells. The regeneration of damaged β -cells on the administration of the terpenoid is evident from histological and ultra-structural investigations. In histological observations, islets of extract-treated diabetic rats are seen to have numerous cells with normal histoarchitecture. Ultrastructural studies revealed β -cells of compound-treated diabetic rats to contain granulated secretory vesicles. The plant may act independently or synergistically to regenerate the damaged endocrine pancreas and thereby stimulation of insulin secretion in β -cells as revealed by LM and TEM. To further confirm the mode of action of the compound, *in silico* docking with target proteins like PPAR γ , which plays a role in protecting β -cells from damage was undertaken. The docking analysis in the active sites of 2PRG was performed by Schrodinger program. The docking results showed good binding interactions of the ligand with the target at the very low energy level. In our *in silico* analysis, terpenoid isolated from *E. scaber* clearly demonstrated that it could improve the diabetic condition by increasing insulin secretion from remnant or regenerated pancreatic β -cells and could promote insulin sensitization and glucose uptake activities, which was a supporting evidence to the histological studies. Thus the terpenoid from *E. scaber* can be considered for developing into a potent antidiabetic drug.

Keywords: β -cell, Diabetes, Docking, *Elephantopus scaber* L., Glucose Streptozotocin, Terpenoid

Diabetes mellitus (DM) is a group of metabolic diseases, which is characterized by high blood sugar levels over a prolonged period¹. Globally, as of 2013, an estimated 382 million people have diabetes worldwide, with type 2 diabetes making up about 90% of the cases². The number of people with diabetes is expected to rise to 592 million by 2035. In the indigenous system of medicine (Ayurveda), a mention was made on a good number of plants for the cure of diabetes or 'Madhumeha' and some of them have been experimentally evaluated and the

active principles were isolated³⁻⁶. However, search for newer effective antidiabetic drugs continues. Earlier we have reported the antidiabetic activity of *Elephantopus scaber* L.⁷. The compound terpenoid was isolated from *E. scaber* previously at our institute and was reported to possess antidiabetic activity, with an increase in insulin levels⁸, yet its mode of action was unknown. Hence histological studies were undertaken to study the effect of terpenoid on β -cells of compound-treated diabetic rats. Bioinformatics tools have become very significant to support the results of experiments and it reveals the targets for different ligands. We made an attempt in using bioinformatics tools to evaluate whether the isolated

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terpenoid is a good ligand to the target protein related to diabetes such as PPAR γ . Activation of PPAR γ improves insulin resistance and therefore PPAR γ is an established molecular target for the treatment of type 2 diabetes⁹. The role of PPAR in combating diabetes has provided us the rationale to carry out structure-based drug design studies¹⁰. The Peroxisome Proliferator-Activated Receptor γ (PPAR- γ) has been the focus of intense research during the past decade because ligands for this receptor have emerged as potent insulin sensitizers used in the treatment of type 2 diabetes. Hence the present *in silico* study aimed at finding new selective human PPAR γ (hPPAR γ) modulators that are able to improve glucose homeostasis with reduced side effects compared with standard drugs. The above-mentioned target was subjected to *in silico* docking with a view to identifying whether terpenoid could be a good molecule to treat diabetes.

Materials and Methods

Research method

Plant material, a method of extraction, isolation, and identification of the active compound, terpenoid procedures were published in our previous manuscript^{7,8}.

Treatment

Streptozotocin administered diabetic rats were treated with the compound for 30 days and were then sacrificed by decapitation and pancreas was dissected and removed for histological studies¹¹.

Acute toxicity studies

An acute oral toxicity study was performed as per the Organization for Economic Co-operation and Development (OECD) guidelines 420. Wistar albino rats of either sex (150-200 g) were used for the study and administered a limit dose of 2000 and 5000 mg/kg of the hydroalcoholic combined plant extract and animals were observed for mortality and clinical signs for the first hour, then hourly for 3 h and finally periodically until 48 h. All of the experimental animals were maintained under close observation for 14 days, and the number of rats that died within the study period was noted. Behavioural and neurological changes such as tremors, convulsions, salivation, diarrhoea, sleep, lacrimation and feed behaviour in drug-treated rats were observed for a sign of acute toxicity.

Ligand preparation

Chemical structure of the ligand (terpenoid) was obtained from spectral analysis and the three-

dimensional structure for the terpenoid was generated using Chem Draw Ultra 11. 2.2

Protein selection

Three-dimensional structures of PPAR γ (PDB ID: 2PRG), were retrieved from the Protein Data Bank (PDB), (<http://www.pdb.org>).

Protein and ligand structure preparation and molecular docking 3D crystallographical structure of target protein was obtained from protein data bank, before docking, the protein was prepared and optimized using Protein Preparation Wizard of Schrödinger¹² to rectify the assignment of bond orders, addition, and deletion of hydrogen atoms and remove water molecules. The refined and optimized protein structures were subjected to Site Map module of Schrödinger suite to identify the binding pocket of the ligand with standard grid cropping sitemaps at 3 Å from the nearest site point. The structures of terpenoid was drawn with aid of chem draw, both native ligand and terpenoid were fixed tautomeric and ionization states of ligands were generated in the pH range of 7.0 $\pm$ 2.0 using Epik during ligand preparation using Lig Prep wizard (Lig Prep, version 3.9, Schrödinger, LLC, New York, NY, 2016) of Schrödinger by assigning the bond orders and bond angles and then subjected to minimization using OPLS_2005 force fields.

After careful consideration of both ligand and protein structure preparation, they were subjected for molecular docking using Glide extra-precision (XP) mode in Schrödinger package. Both native and our ligands were forced to bind within the grid corresponding to the active sites of the target protein, to enclose it within 3 Å from the centric of these residues.

Docking analysis

The docking analysis of terpenoid was carried out by means of the Schrödinger tools. The molecular docking studies performed with the active sites of the 2PRG target protein by the Glide application of Schrödinger¹³.

Results and Discussion

Hematoxylin and eosin stained sections of the pancreas of untreated rats under a light microscope revealed that each islet of Langerhans is formed of numerous compactly arranged cells occurring as dense cords. The islets appeared lightly stained when compared with the surrounding acinar tissue. The Islet cells were round to ovoid with round vesicular nuclei

Table 1 — Comparing the docking score and properties of the standard drug and compound from *E. scaber*

Title	Docking score	Glide evdw	Glide energy	No of Bonds	Interactive residue	H bond length (Å)
Terpenoid	−8.397	−18.369	−20.639	2	HIE 449	2.31
					GLN 286	1.95
Rosiglitazone	−7.874	−49.183	−54.666	2	HIE 323	2.19
					SER 289	1.97

and pale pink cytoplasm. Capillaries were found in between the islet cells. Islets from the pancreas of diabetic control rats showed an entirely different picture in hematoxylin and eosin stained sections (Suppl. 1). Most of the cells in the islets possessed pyknotic nuclei, whereas some cells contained dark nuclei, and few cells at the periphery had round or ovoid nuclei, indicating the damaged condition of the cells. However, the treatment with the compound showed improvement in the architecture. Hematoxylin and eosin stained section of the pancreas of treated diabetic rats showed several islet cells to contain vesicular nuclei, although a few cells possessed shrunken nuclei. Capillaries were present in between the cells. A few cells possessed pyknotic nuclei.

Transmission electron micrographs (TEM) of β -cells of untreated rats revealed that the β -cells contained abundant secretion granules. Each granule had a large clear space around it. Cytoplasmic organelles were also present. TEM of β -cells of diabetic control rats revealed the nucleus to be dark and shrunken and to possess an irregular outline. Numerous capillaries were observed in between the islet cells. A few cells showed pyknotic nuclei. TEM of treated diabetic rats showed the cytoplasm of the β -cell to contain numerous secretion granules and a few vacuoles. Capillaries were prominent. In contrast to β -cell, alpha cells contained extremely electron-opaque dense secretion granules. The core of the alpha cell granules showed two characteristic components, an extremely electron dense central spherical mass located asymmetrically with respect to the limiting membrane and a granular material of moderate electron density that filled the compartment between the electron opaque component and the limiting membrane. The cytoplasm possessed abundant mitochondria.

Streptozotocin diabetes results in degenerative and lytic changes in the islets of Langerhans of the pancreas. The islet is considerably reduced and shrunken, there is the destruction of some β -cells with central hyalinization, a few cells show pyknotic nuclei and the number of cells is lower¹⁴. Histopathological examination of the pancreas in streptozotocin-induced

Table 2 — Qikprep results of the standard drug and compound from *E. scaber*

Descriptors	Rosiglitazone	Terpenoid
Molecular weight	357.426	394.596
Donor Hydrogen Bond	1	0
Acceptor Hydrogen Bond	5.25	3
Human Oral Absorption	3	1
Rule of Five	0	1

diabetic rat treated with D-400 (a herbomineral formulation) revealed that the treatment restored the activity of the islets of Langerhans¹⁵. In the present study, oral intubation of terpenoid from *Elephantopus scaber* L. to STZ- induced diabetic rats brought about an improvement in the histoarchitecture of the islets in the pancreas of STZ- induced diabetic rats. As mentioned earlier, in the present study also, there was a complete loss of β -cell secretion granules in STZ-treated rats. Oral administration of the compound from *Elephantopus scaber* L. brought about an increase in insulin granules in STZ-induced diabetic rats.

The insulin result suggests that the hypoglycemic activity of the plants may be due to a sulfonylurea-like activity¹⁶, while histological studies clearly demonstrated the regenerated pancreatic β -cells in the extract treated, suggesting regeneration of β -cells¹⁷.

The molecular interaction of terpenoid and target protein was described by performing docking analysis by the Schrodinger program. It has been shown effective and observed experimentally the binding affinities in terms of lowest docking energy. The target protein structure of 2PRG was docked with terpenoid, which provided significant results by the least values of the binding energy comparable with known agonist Rosiglitazone (Table 2). Molecular docking results showed that the isolated terpenoid possess a greater binding affinity (glide score: −8.397 kcal/mol) as compared to Rosiglitazone (−7.874 kcal/mol) through two hydrogen bonds interaction by HIS 449 (2.31) and GLN 286 (1.95) residues of the 2PRG protein. The best possible binding affinities of the terpenoid at targeted protein's active sites are displayed and their corresponding energy values are listed in (Table 1). The processed target protein structure of were docked with isolated

terpenoid and standard drug Rosiglitazone which provided Glide Score that identified the best interacting and binding ligand in the respective protein's active site. The best possible binding modes of the terpenoid and Rosiglitazone at targeted proteins' active sites are displayed in (Figs. 1 & 2).

The known agonist Rosiglitazone of PPAR having a better affinity towards their binding packet, the residues in the binding packet of PPAR was already described by Nolte *et al.* 1998 by X-ray studies. An obtained interaction of Terpenoid was correlated with the previously reported study Mazumder *et al.*¹⁸, where the Rosiglitazone

showed hydrogen bond interaction with His-323 and Ser-289. Thiazolidinedione and several naturally occurring carboxylic acids showed strong interaction with the major binding residue of PPAR as follows His323, His449, and Tyr473¹⁹. This binding promotes the agonists effects against PPAR. They can strongly bond to the target protein and activated them even at micromolar concentrations. Also, human oral absorption rate of both isolated and available drug has predicted by Qikprep application. The terpenoid showed a lower level of absorption < 30%²⁰ compared with commercially available drug 99%²¹.

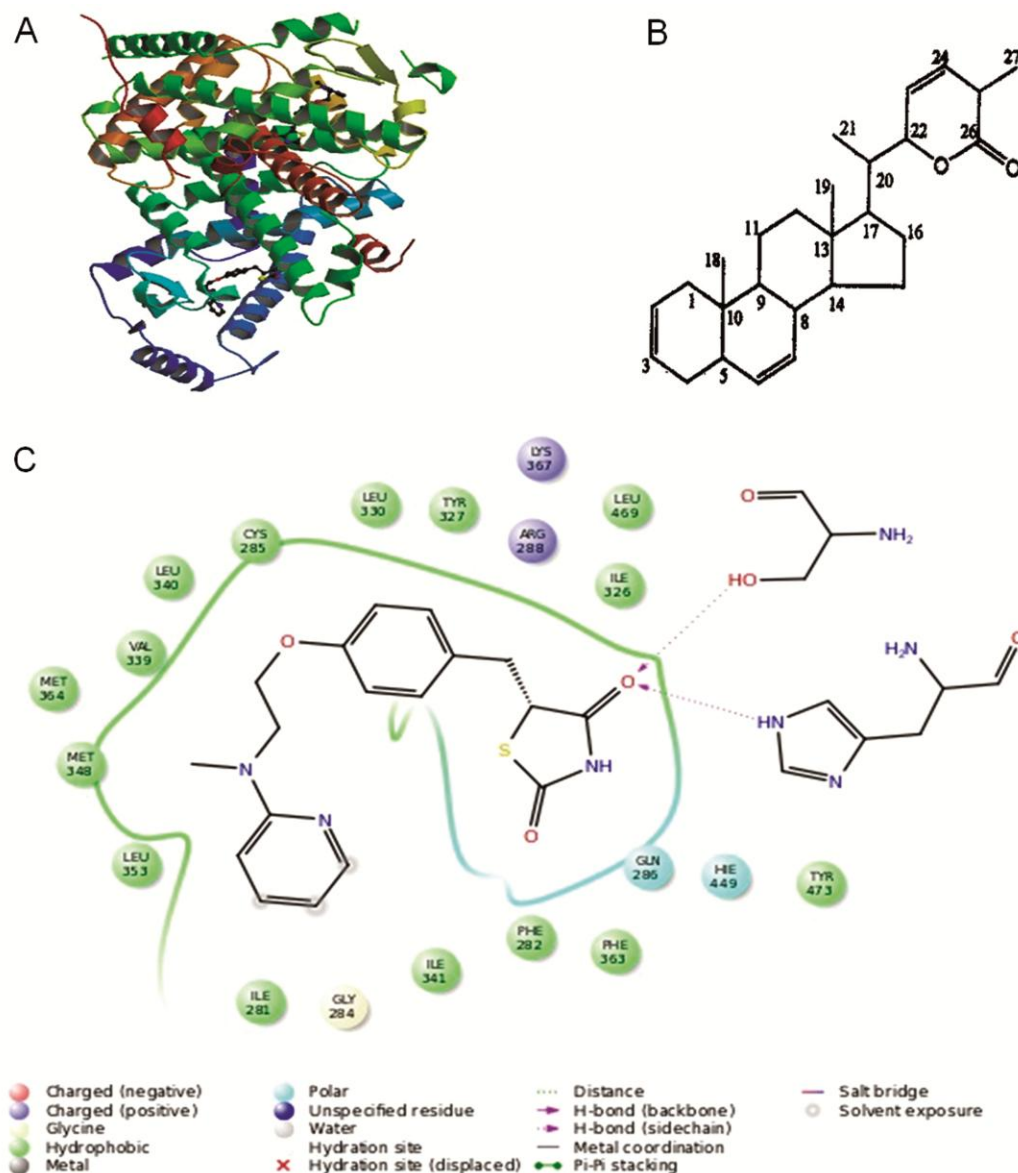


Fig. 1—(A) 3D structure of PPAR γ , PDB (2PRG), (B) Terpenoid from *E. Scaber* (C) & Interacting amino acids as predicted and enfolding of the compound in the active pocket

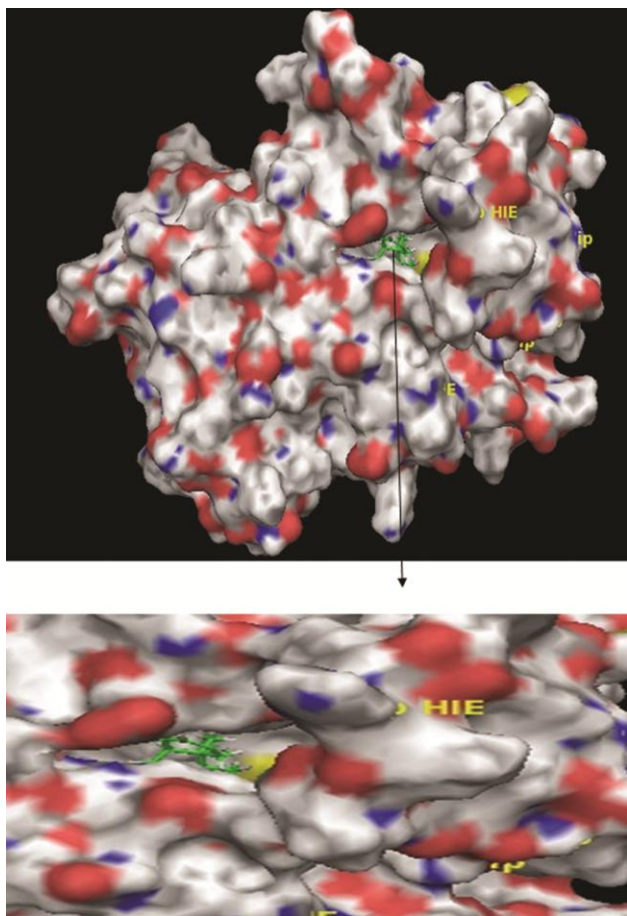


Fig. 2 — Interacting amino acids as predicted and enfolded of the compound, Terpenoid from *E. scaber* in the active pocket of PPAR γ , PDB (2PRG)

Acknowledgment

The authors acknowledge the support offered by BDU, DST-PURSE program (Sanction order No-SR/FT/LS-113/2009) for providing molecular docking facility.

References

- Global status report on noncommunicable diseases 2014, Geneva. *World Health Organization*, 2012.
- World Health Organization, Global Health Estimates: Deaths by Cause, Age, Sex and Country, 2000-2012, Geneva, *WHO*, http://www.who.int/diabetes/action_online/basics/en/, 2014.
- Chopra RN, Chopra IC, Handa KL & Kapur LD, Indigenous drugs of India, (2nd Ed., Dhar and Sons, Calcutta, India) (1958) 816.
- Rao BK, Giri R, Kesavulu MM & Apparao C, Herbal medicine in the management of diabetes mellitus. *Manphar Vaidhya Patrika* I, (1997) 33.
- Liu IM, Liou SS, Lan TW, Hsu FL & Cheng JT, Myricetin as the active principle of *Abelmoschus moschatus* to lower plasma glucose in streptozotocin-induced diabetic rats. *Planta Med*, 71 (2005) 617.
- Subash Babu P, Prabuseenivasan S, Ignacimuthu S. Cinnamaldehyde--a potential antidiabetic agent. *Phytomedicine*, 14 (2007) 15.
- Jasmine R & Daisy P, Effect of crude extract and fractions from *Elephantopus scaber* L. on hyperglycemic in streptozotocin – diabetic rats. *Int J Biol Chem*, 1 (2007) 111.
- Daisy P, Jasmine R, Ignacimuthu S & Murugan E, A novel steroid from *Elephantopus scaber* L. an ethnomedicinal plant with antidiabetic activity. *Phytomedicine*, 16 (2009) 252.
- Staels B & Fruchart JC, Therapeutic roles of peroxisome proliferator-activated receptor agonists. *Diabetes*, 54 (2005) 2460.
- Tove O, Svensson S & Selen G, A New Class of Peroxisome Proliferator-activated Receptor Agonists with a Novel Binding Epitope Shows Antidiabetic Effects. *J Biol Chem*, 279 (2004) 41124.
- Jasmine R & Daisy P, Regeneration of the β -cells in the islets of langerhans by *Eugenia jambolana* in streptozotocin-diabetic rats. *Pharmacologyonline*, 3 (2011) 283.
- Szkudelski T, The mechanism of alloxan and streptozotocin action in β -cells of the rat pancreas. *Physiol Res*, 50 (2001) 537.
- Sastry GM, Adzhigirey M, Day T, Annabhimoju R & Sherman W, Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. *J Comput Aided Mol Des*, 27 (2013) 221.
- Kumar TD & Velmurugan D, Molecular docking studies on inhibition of human kynurenine aminotransferase-1 for treating schizophrenia disorder. *Star Bio Info*, 01 (2013).
- Ghosh S & Suryawanshi SA, Effect of *Vincarosea* extracts in treatment of alloxan diabetes in male albino rats. *Indian J Exp Biol*, 39 (2001) 748.
- Abdel-Zaher AO, Salim SY, Assaf MH & Abdel-Hady RH, Antidiabetic activity and toxicity of *Zizyphusspina-christi* leaves. *J Ethnopharmacol*, 101 (2005) 129.
- Palanichamy S, Nagarajan S & Devasagayam M, Effect of *Cassia alata* leaf extract on hyperglycemic rats. *J Ethnopharmacol*, 22 (1988) 81.
- Nagappa AN, Thakurdesai PA, Rao NV & Singh J, Antidiabetic activity of *Terminalia Catappa* Linn. fruits. *J Ethnopharmacol*, 88 (2003) 45.
- Mazumder M, Ponnai P, Das U, Gourinath S, Khan HA, Yang J & Sakthar MK, Investigations on Binding Pattern of Kinase Inhibitors with PPAR γ : Molecular Docking, Molecular Dynamic Simulations, and Free Energy Calculation Studies. *PPAR Research*, 2017, 1.
- Rizos CV, Elisaf MS, Mikhailidis DP & Liberopoulos EN, "How safe is the use of thiazolidinediones in clinical practice?" *Expert Opin Drug Saf*, 8 (2009) 15.
- Singla AK, Garg A & Aggarwal D, Paclitaxel and its formulations. *Int J Pharm*, 235 (2002) 179.
- Cox PJ, Ryan DA, Hollis FJ, Harris AM, Miller AK, Vousden M & Cowley H, Absorption, Disposition, and Metabolism of Rosiglitazone, a Potent Thiazolidinedione Insulin Sensitizer, in Humans. *DMD*, 28 (2000) 772.