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Screening of novel phytochemicals as secreted frizzled-related protein 4 inhibitors: An early stage biomarker of Type 2 diabetes

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Abstract: Diabetes is increasing at an alarming rate worldwide with high mortality and posing severe health and economic burden. Secreted frizzled related protein 4 (SFRP4) is released from adipose tissues to suppress insulin exocytosis from β-cells of pancreas and acts as a biomarker for early detection of T2D. In present study, the 3D structure of human SFRP4 was predicted using comparative modeling approach and evaluated through online bioinformatics tools. The best predicted model of SFRP4 was used as a receptor in molecular docking studies. Phytochemicals from already reported antidiabetic plants were docked and screened using molecular operating environment (MOE) software to target SFRP4. The ligands were optimized and a database was constructed in MOE. Out of 850 compounds from 150 antidiabetic plants taken from PubChem database, singrin was found to be the most potent one with root mean square deviation (RMSD) value of 1.39, S-score of -10.06 and by interactions to five amino acids of SFRP4 active pocket. Furthermore, boeravinone E, boeravinone D, wedelolactone, squamosamide and taxifolin also interacted strongly to SFRP4 by exhibiting RMSD values of 1.00, 0.94, 1.89, 1.37, 1.28 and S-scores of -12.45, -11.26, -9.25, -7.26, -10.66, respectively. These phytochemicals are proposed to act as potential medicine for delaying the onset and treating T2D by specifically targeting SFRP4.

Keywords: Type 2 diabetes, phytochemicals, obesity, SFRP4, molecular docking, MOE.

INTRODUCTION

Plant based compounds are employed as drugs in 75-80% population of the developing countries for treatment of different diseases (Mustafa *et al*., 2017; Sharif *et al*., 2017; Bukhari *et al*., 2019a). Diabetes mellitus is one of the most complex metabolic disorders with no permanent cure discovered so far. Restoration of normal metabolic functions in diabetic individuals is the major focus for current drug development procedures (Alam *et al*., 2018). Antidiabetic secondary metabolites are cost effective remedies with minimal side effects that not only restore normal blood glucose levels but also protect pancreatic β-cells from deleterious effects of diabetic environment (Martel *et al*., 2017; Sonkamble *et al*., 2019).

Secreted frizzled-related proteins (SFRPs) are secreted glycoproteins of about 300-400 amino acids long with signal peptide and characteristic cysteine rich domain at N-terminal. SFRPs bind to Wnt proteins and frizzled receptors to block both canonical and non-canonical Wnt signaling pathways. Five members of this group are identified in mammals named SFRP1-5 (Shi *et al*., 2007). These SFRPs are either up or down regulated in various diseases including cancer, obesity, diabetes and bones etc. thus inhibition of these proteins could be utilized in the treatment of various metabolism related disorders (Vincent and Postovit, 2017; Claudel *et al*., 2019).

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SFRP4 is the largest member of SFRP family characterized by presence of disulphide bridges formed from six cysteine residues at conserved positions. SFRP4 is a 346 amino acids long protein with molecular weight of 39.9 kDa having two distinct domains (Bukhari *et al*., 2019b). N-terminal signal peptide and a cysteine rich domain forming disulphide bridges homolog to the CRD domains of frizzled receptors have been found to inhibit angiogenesis and thus promoting inflammation (Longman *et al*., 2012). SFRP4 is also found to be overexpressed during adipocyte differentiation. Lean animals have reduced expression of SFRP4 as compared to obese animals (Bergmann and Sypniewska, 2014). In non-diabetic individuals serum concentration of SFRP4 shows significant correlation to decreased insulin sensitivity index and fasting blood glucose thus can act as novel therapeutic modality for the prevention and treatment of obesity and Type 2 diabetes (Bukhari *et al*., 2019b).

Though SFRP4 was nominated many years ago as a potential target for curing obesity and diabetes (Ehrlund *et al*., 2013; Bukhari *et al*., 2014) yet no drug has been reported so far to target/modulate SFRP4 over expression and improve health status of individuals. This study is the first of its kind to screen novel phytochemicals for their potential to modulate SFRP4 expression from already reported antidiabetic plants. These compounds could be studied *in vitro* and *in vivo* for their therapeutic role in curing diabetes via specifically targeting SFRP4 and

establishing normal blood glucose level by increasing release of insulin from β-cells.

MATERIALS AND METHODS

Various reported antidiabetic plant constituents were evaluated *in silico* with molecular operating environment (MOE) software for their potential to inhibit and/or modulate expression of SFRP4 by interacting with major structural amino acids.

3D structure prediction of human SFRP4

Three-dimensional structure of human SFRP4 protein was predicted using homology modeling based approach due to non-availability of SFRP4 crystal structure in protein data bank (PDB). Full-length amino acid sequence of human SFRP4 accession number CAG46532.1 was retrieved from protein database available at https://www.ncbi.nlm.nih.gov/protein. The 3D structure of SFRP4 protein was predicted using SWISS-MODEL server (Waterhouse *et al*., 2018) and visualized by UCSF Chimera workbench (Jabbir *et al*., 2019).

Structure evaluation

The predicted 3D structure was evaluated using online bioinformatics tools such as Ramachandran plot (Bukhari *et al*., 2018) and Z-score by proSA-web (Wiederstein & Sippl, 2007) to confirm backbone and the overall quality of the predicted model. SFRP4 structure was further confirmed by ERRAT (Colovos & Yeates, 1993).

Protein structure optimization for docking

The predicted 3D structure of SFRP4 was further optimized using molecular operating environment (MOE) software (Vilar *et al*., 2008). All water molecules were removed, hydrogen atoms were added, ionization state level achieved by 3D protonation. Molecular energy was also minimized by adjusting various parameters like force field: MMFF94x, gradient: 0.05 and chiral constraint: current geometry.

Ligand optimization and database construction

The chemical structures of phytochemical constituents from potential antidiabetic plants were retrieved from PubChem available at https://pubchem.ncbi.nlm.nih.gov /search/search.cgi to be opened in MOE software (Rahman *et al*., 2019). The ligands were also optimized by 3D protonation and energy minimization using force field MMFF94X and a gradient 0.05. The resulted optimized ligands were saved in .mdb format in MOE software.

Binding site prediction

The active or interacting site of SFRP4 was found by mining the already reported literature. The active site include glutamate 61, tyrosine 62, glutamate 63, leusine 64 near the N- terminal and leucine 111, methionine 112, tyrosine 115, histidine 117 and tyrosine at 119 position (Bukhari *et al*., 2014; Hassan *et al*., 2018).

Molecular Docking

Active site finder tool was used in MOE software to find residues in binding pocket. The database of ligands was then docked with SFRP4 protein using docking algorithm of MOE software. Various parameters being set for docking include Placement: Triangle matcher, rescoring function 1: London dG, Retain: 10, Refinement: Forcefield, Rescoring function 2: London dG and Retain: 10. The potential ligands were selected based upon the RMSD values and minimum S-score and further interaction studies like hydrogen bonding/π-π interactions.

RESULTS

Structure predicted and evaluation

The predicted 3D structure of human SFRP4 and its evaluation has been shown in fig. 1.

Docking analysis

About 850 phytochemicals from 150 potential antidiabetic plants were docked to target SFRP4 active pocket and the most suitable conformation was saved in a distinct database. Least value of S-score was used as the criteria for best conformation. Interactions of each ligand to SFRP4 were assessed acutely. Total of 38 phytochemicals showed interactions with SFRP4 by 1-5 amino acids in the active pocket. Top 6 interacting ligands with their RMSD value, S-score and interacting amino acids are given in table 1.

Binding interactions of protein and ligands

Out of 850 docked compounds 6 phytochemicals were shortlisted. Interactions between SFRP4 active site to the phytochemicals and complex of ligand protein interaction are shown in Fig 2. Singrin was found to be deeply bound to the binding pocket of SFRP4 and showed interaction with His117, Leu64, Glu63, Gln60 (fig. 2a). Additional interaction with Ala57 found outside the active pocket making it the most potent candidate for SFRP4 modulation.

Boeravinone E and Boeravinone D (figs. 2a and 2c) interacted strongly with Met112 while wedelolactone (fig. 2d) interacted strongly to Tyr61 in addition to His117 and Glu63 pointing importance of this residue also. Squamosamide (fig. 2e) showed interactions with His117, Gln60 and Ser118 while His48 and Leu46 were outside the active pocket. Ser118 was interacted by two compounds while Glu63 also interacted with two compounds. Ser122 in the binding site also interacted by taxifolin (fig. 2f).

(a) the ligand singrin with SFRP4 shows interaction with His117, Gln60, Glu63, Leu64, Ala57 while Tyr61, Glu62,

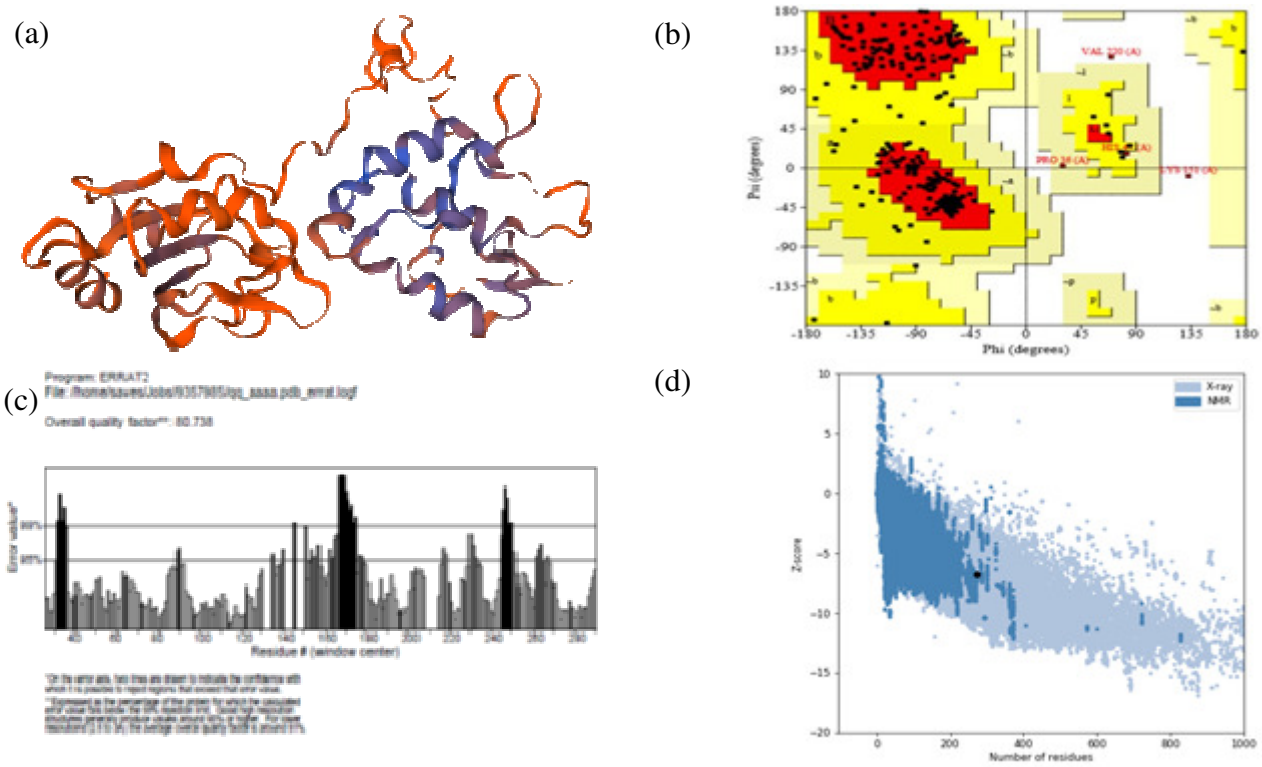

Fig 1: SFRP4 3D structure prediction and evaluation. (a) predicted 3D structure of SFRP4 protein, (b) Ramachandran plot of predicted SFRP4 representing 82.7% favored and 16.1% allowed regions, (c) SFRP4 structure evaluated by ERRAT showing 80.738% of amino acids are in ideal range, (d) Z-score predicted by proSA-Web is -6.72.

Met112, Tyr115, Pro120 and Ser118 are found in the environmental region, **(b)** boeravinone E shows interaction with amino acid His117, Met112, Gln60 and Ser118 while Glu63, Ala57 Tyr115, Pro120, Tyr61 and Leu64 are found in the environmental region, **(c)** boeravinone D interacted by His117, Met112 and Gln60 while Ser118, Ala57 Tyr115, Pro120, Tyr61 and Leu64 are in the environmental region, **(d)** wedelolactone interacted to His117, Tyr61 and Glu63 while Ala57, Pro120, Gln60, Met112, Ser122 and Ser118 are in the environmental region, **(e)** squamosamide interaction to His117, Gln60 and Ser118 of SFRP4 while His48, Leu46 are found in the environmental region, **(f)** taxifolin interacted to SFRP4 by amino acid His117, Ser122 and Gln60 and Tyr61, Glu63, Tyr81, Met112, Pro120 are found in the environmental region.

DISCUSSION

The plant based compounds attracted a great deal of attention for curing diabetes and other diseases due to their cost effectiveness and safety with least gastrointestinal, renal or hypertensive side effects (Rahman *et al*., 2019; Mustafa *et al*., 2016). The activity and affinity of drug candidates to target proteins is predicted through their binding orientations by using molecular docking algorithms (Seeliger and de Groot, 2010). The 3D structure of human SFRP4 was modeled and evaluated using online bioinformatics tools. The evaluation of the predicted 3D model is an important step to show how accurate and suitable the 3D model is for intended applications (Mustafa *et al*., 2018). In this study phytochemicals from different potential antidiabetic plants were docked with SFRP4 to elucidate their modulatory role a key first step in identifying potential candidates to rare protein targets.

After getting nominated as early biomarker a great deal of attention was diverted to identify drug candidates to target SFRP4 but no compounds are reported so far. Molecular docking was also done previously (Bukhari *et al*., 2014) and the same active pocket was implemented in current study to dock phytochemicals against SFRP4. Strongly interacting compounds displayed unique binding patterns as observed previously (Bukhari *et al*., 2014; Hassan *et al*., 2018). All compounds were confined in the active pocket and exhibited different binding orientations. In addition aromatic residues of SFRP4 and phytochemical compounds exhibited no π-π stacking. These results are in accordance with Hassan *et al*. (2018) who reported anti-cancer compounds to target SFRP4 for treatment of ovarian carcinoma.

Singrin, a glucosinolate found extensively in seeds of *Brassica juncea* was found to be the most potent compound based upon root mean squared deviation

Table 1: Docking interactions details of top 6 phytochemicals with SFRP4 protein active pocket by using MOE software

Sr. No.	Ligands	RMSD Value	S-score	Interacting residues in active pocket	Residues in surrounding region
01	Singrin	1.3904	-10.0577	His117, Leu64, Glu63, Gln60, Ala57	Tyr61, Glu62, Met112, Tyr115, Ser118, Pro120
02	Boeravinone E	1.0011	-12.4470	His117, Met112, Gln60, Ser118	Glu63, Ala57 Tyr115, Pro120, Tyr61, Leu64
03	Boeravinone D	0.9372	-11.2610	His117, Met112, Gln60	Ser118, Ala57 Tyr115, Pro120, Tyr61, Glu63, Leu64
04	Wedelolactone	1.8884	-9.2450	His117, Tyr61, Glu63	Ala57, Pro120, Gln60, Met112, Ser122, Ser118
05	Squamosamide	1.3732	-7.2637	His117, Gln60, Ser118	His48, Leu46
06	Taxifolin	1.2819	-10.6637	His117, Ser122, Gln60	Tyr61, Glu63, Tyr81, Met112, Pro120

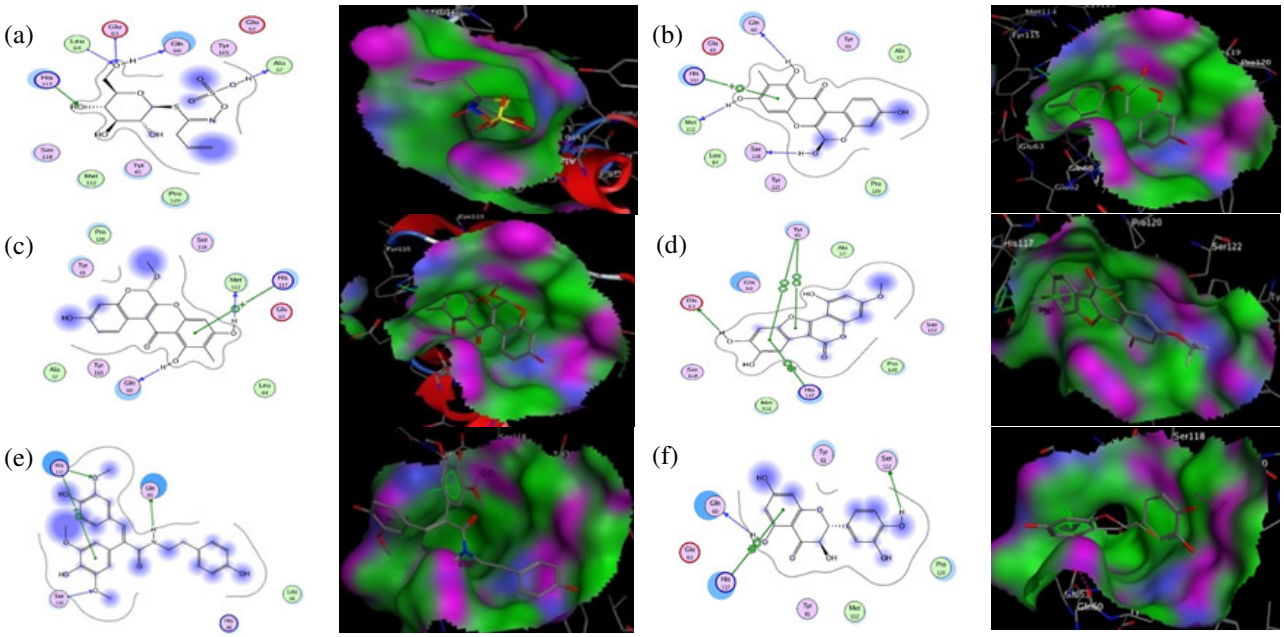

Fig 2: Docking results of various compounds with SFRP4 active pocket by using MOE software showing ligand interaction and hydrophobic interactions.

(RMSD) value 1.3904 and S-score -10.0577 with highest number of interactions to amino acids in active pocket. Singrin is also reported to be a strong hypoglycemic agent (Bhushan *et al*., 2010) and is an important phytochemical of *B. juncea* with strong ability to increase insulin level (Thirumalai *et al*., 2011) in accordance with our results supporting its additional role to modulate SFRP4 and resulting in hypoglycemia indirectly. Hydrophobic interactions commonly found are also exhibited as shown by four compounds previously (Hassan *et al*., 2018). It is postulated that increased release of insulin is due to its ability to inhibit or modulate activity of SFRP4 that needs to be elucidated and confirmed *in vitro* and *in vivo*.

Boeravinone E and boeravinone D belong to ratinoid family of phytochemicals and found extensively in *Boerhaavia diffusa* with strong therapeutic potential (Riaz *et al*., 2014). Both compounds were found to be deeply embedded in the active pocket and interacted with Glu63 in the surrounding region which is in accordance with the results of Hassan *et al*. (2018). *In vivo* studies showed that leaf extracts reduce blood glucose level dose dependently in streptozotocin induced diabetic rats by redundant pancreatic mechanism and renewal of pancreatic β-cells (Nalamolu *et al*., 2004). It is also postulated by present study to exert its hypoglycemic potential by enhancing the release of insulin possibly by altering SFRP4 expression.

Wedelolactone is an active constituent of *Wedelia calendulacea* and reported to be a strong hypoglycemic agent. In experimental animal models it reduced the levels of various inflammatory mediators including CRP, TNFα and IL-6 (Kumar *et al*., 2018) and is found in this study to reduce level of SFRP4 co-expressed with these inflammatory markers. Wedelolactone showed S-score -9.2450 and RMSD value of 1.8884 with strong interactions to three amino acids in active pocket including Glu63 in accordance to the previous results of Hassan *et al*. (2018). Squamosamide is found extensively in *Annona glabra* and its derivative has been reported to be highly potent against diabetes. It has strong hypoglycemic and anti-inflammatory activity by enhancing Akt-FOXO1 pathway thereby protects β-cells of pancreas from glucotoxicity (Kong *et al*., 2015). Interestingly, Glu63 is not interacted by squamosamide in contrast to previously reported results (Hassan *et al*., 2018).

All the compounds have close values of RMSD. His117 and Gln60 were found to be the major amino acids interacting with most of these ligands. All 6 phytochemicals showed strong interactions with His117 while 5 out of top 6 phytochemicals showed interactions with Gln60 and Glu63 in accordance to results by Bukhari *et al*. (2014) and Hassan *et al*. (2018). These interaction studies reflect the importance of these amino acids in SFRP4 active pocket. Further, chances of side effects of these compounds are minimum as they are assessed already *in vitro* and/or *in vivo* models.

CONCLUSION

Protein docking technique is applied as a preliminary step to shortlist phytochemicals that would be used as SFRP4 modulators to treat obesity induced diabetes. Six phytochemicals were found strongly interacting with SFRP4 and could be used as potential medicine. Singrin is found to be the most potent compound to interact with SFRP4 based upon S-score and RMSD value. Boeravinone E, boeravinone D, wedelolactone, squamosamide and taxifolin also showed strong interactions thus could be tested if they reduce levels of SFRP4 and for their recommendation as potential medicine. After confirmation of their innovative SFRP4 inhibitory or modulatory roles, mutational studies could also be designed to elucidate the role of these residues in the interaction properties of SFRP4.

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