Introduction

Recently, one of the additions to this anti-diabetic armament of drugs is dipeptidyl peptidase-IV Inhibitors (DPP-IV) [1], DPP-IV Inhibitors increase incretin levels: Glucagon-like peptide-1 (GLP-1) and Glucagon inhibitory peptide (GIP), which inhibit glucagon release, which in turn increases insulin secretion, decreases gastric emptying, and decreases blood glucose levels [2,3]. A recent study has shown beneficial effects of DPP-IV Inhibitor on metabolic parameters in type 2 diabetic patients [4], DPP-IV Inhibitors have a large number of beneficial effects in the subgroup of metabolic syndrome with diabetes. Apart from glycemic control, they are known to lower blood pressure, are weight neutral, improve dyslipidemia, reduce inflammatory markers, diminish oxidative stress, improve endothelial function and reduce platelet aggregation in patients with type II diabetes [5]. Therefore, it is plausible to explore the therapeutic potential of DPP-IV Inhibitors in experimental diabetes with metabolic syndrome.

As a drug class, DPP-IV Inhibitors are widely used clinically because of their low risk of hypoglycemia, once daily dosing, pharmacological effects such as weight neutral and preservation of beta cell mass [6], However, in spite of their beneficial effects, they have limitations: high cost of therapy and unacceptable adverse effects such as pancreatitis, pancreatic cancer, angioedema and thyroid cancer. In this scenario, it would be desirable to identify novel DPP-IV Inhibitors from alternative sources that share the beneficial effects of the available synthetic DPP-IV Inhibitors and at the same time are cost effective. In this light, the DPP-IV Inhibitors obtained...
from natural source may be an alternative that needs to be 
explored, considering the rich biodiversity India is bestowed 
with. With these point in view, the present study was designed.

Asian countries such as India and China are already known 
for their contributions toward the usage of plant medicine 
in preventing and treating various diseases [7], Mangiferin, 
a major phytochemical in Mangifera indica has been reported 
to possess DPP-IV inhibitory activity. It belongs to family 
Anacardiaceae Mangiferin. Being a glucosylxanthone, it 
possesses strong antioxidant, anti-lipid peroxidation, 
immunomodulation, anti-diabetic cardiotoxic, hypotensive, 
which has a 

wound healing, anti-hyperlipidemic, anti-atherogenic 
and anti-degenerative properties [8], Mangiferin showed 
significant antiatherogenic and antiatherogenic activities 
as evidenced by significant alteration of lipid profile level and 
diminution of atherogenic index in diabetic rats [9], Although 
several benefits of Mangiferin have been reported by multiple 
pathways, there is no experimental evidence presently available 
in literature with regard to its DPP-IV inhibitory activity.

Berberine, an isoquinoline alkaloids originally isolated from 
the root of Berberis aristata belongs to family Berberidaceae. It 
has shown a wide array of pharmacological activities including 
antimicrobial, antitumor, anti-inflammation and antidiabetic 
activity [10], in 1988, the hypoglycemic effect of Berberine 
was found when Berberine was used to treat diarrhoea in 
diabetic patients in China. Since then, Berberine has been 
used as an anti-hyperglycemic agent also in a recent single-blind 
clinical observation, the study showed that Berberine 
supplementation was beneficial in correcting lipid metabolism 
disorders and reducing cardiovascular risk factors. Berberine 
has been reported in the several literatures to have beneficial 
effects in human type II diabetes. Experimentally, Berberine 
was found to inhibit human recombinant DPP-IV in vitro. The 
findings suggest that DPP-IV inhibition is, at least, one of the 
mechanisms that explain the anti-hyperglycemic activity of 

The DPP-IV Inhibitory activities of Berberine and Mangiferin 
were reported from the laboratory [12]. The results of in vitro 
and in vivo studies in the experimental model of diabetes 
co-existing with metabolic syndrome confirmed the DPP-IV 
Inhibitory activity of Berberine and Mangiferin. Serum DPP- 
IV levels were measured and was found to directly correlate 
to the antidiabetic efficacy of Berberine and Mangiferin in 
experimental rats [13], to further elucidate the binding sites 
and affinity of Mangiferin and Berberine for DPP-IV enzyme 
in silico docking studies were designed. Computational in silico 
studies were used to confirm the DPP-IV Inhibitory activity 
of Berberine and Mangiferin and identify the sites as well as 
amino acid residues on DPP-IV enzyme to which these natural 
DPP-IV Inhibitors binds.

Materials and Methods

The crystal structure of human DPP IV (PDB Id: 2Q79) 
[14], was downloaded from Protein Databank [15], which has a 
resolution of 2.1 Å. This was considered as a receptor for docking 
studies. The ligand selected includes sitagliptin, vildagliptin, 
mangiferin and berberine. All of them were downloaded from 
Pubchem Database [16]. The later was obtained from Chemfaces 
(www.chemfaces.com). All these structures were retrieved in 
SDF file format which was further converted into 3D format 
using Frog v2.14 (Free On line druG conformation generation) 
[17]. In the Frog online server, the input was maintained as 
‘1D to 2D’ and the input drug description was ‘SDF’ file. In 
the calculation parameters the output format was maintained as 
‘PDB’. The minimize option was opted as ‘yes’. In the produce 
option we selected ‘single’. The total number of conformers 
generated was kept as 10. While rest of the options were left as 
default. The active site of DPPIV was retrieved through literature 
search and also predicted based on CASTp online server to 
identify pockets [18]. Now the receptor and the 3D generated 
ligands were considered for docking using Hex software 
8.0.0 [19], it is an interactive molecular graphics program 
for calculating and displaying feasible docking modes which 
uses spherical polar Fourier (SPF) correlations to accelerate 
the calculations. During docking, in the docking parameter 
settings, we opted for ‘shape and electro’ for correlation type. 
Sampling method was ‘range angles’. Post processing was 
OPLS minimization. The rest of the options were left default. 
Here the 2QT9 was loaded along with their inhibitor 4-aryl 
cyclohexylalanine in complex state. Now this was considered 
as the reference pose for the docking of DPPIV of Homo sapiens 
against all the available chemical compounds. Top ten docked 
poses were downloaded and considered for further analysis 
using Swiss pdb viewer and CHIMERA software [20].

Results

In this study, the crystal structure of DPP IV was considered 
as receptor which was docked against 14 chemical compounds. 
The available chemicals in 2D format were converted into 3D 
format and further minimized using Frog2 software. All these 
opimized chemical compounds were considered for docking 
against DPPIV based on the reference structure of DPPIV 
against 4-aryl cyclohexylalanine in complex state (2QT9). 
Basically, DPPIV is active in homo dimer form. The active site 
is a deep cleft in DPPIV which can be accessed via the opening 
of the propeller domain or through side opening formed 
at the interface of the β-propeller and hydrolase domains. 
Furthermore, the β propeller is a funnel shaped tunnel which 
extends to the active site (Figure 1a). All these chemical 
compounds were docked in the active site pocket (Figure 1b). 
In addition to this, the active sites were also predicted using 
CASTp software. This online server listed 198 pockets with 
default. The active site of DPPIV was retrieved through literature 
generated was kept as 100. While rest of the options were left as 

[PDB]. The minimize option was opted as ‘yes’. In the produce 
option we selected ‘single’. The total number of conformers 
generated was kept as 10. While rest of the options were left as 
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compounds were docked in the active site pocket (Figure 1b). 
In addition to this, the active sites were also predicted using 
CASTp software. This online server listed 198 pockets with 
area and volume. The first predicted pocket was the largest 
pocket with an area of 6086.4 Å² and a volume of 16471 Å³. The 
second largest pocket has an area of 574.3 Å² with a volume of 
171.8 Å³. Third largest pocket is observed proximal to the first 
largest pocket with an area 274.4Å² and a volume of 191.5 Å³ (Figure 2).

All these listed compounds were docked against the reference 
docked pose of 4-aryl cyclohexylalanine inhibitor binding site from 2QT9 crystal structure. Berberine prefers the 

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binding site from 2QT9 crystal structure. Berberine prefers the
active site pocket of DPP-IV enzyme. Of these, in particular, Berberine binds very close to Glu205 and Glu206 (Figure 3). Compounds like Vildagliptin and Mangiferin prefers to bind near the interface region of the DPPIV as their biological active forms are homodimer (Figure 4a,b). Sitagliptin binds near the α/β hydrolase domain (Figure 5). It was observed that few of compounds preferred to bind within the active pocket, whereas others prefer to interact with interface region, β propeller and α/β hydrolase domains. However, as per CASTp prediction, in addition to the active site, the region of interface and the α/β hydrolase and β propeller domain combo is also considered as part of the largest active site pocket. Berberine and Vildagliptin prefers to bind within the active site pocket (the 1st largest pocket). Mangiferin prefers to bind in the second largest pocket and Sitagliptin prefers to bind with the third largest pocket.

**Discussion**

Several anti-diabetic drugs are available for the treatment of type 2 diabetes mellitus. A relatively new class of oral hypoglycemic agent, DPP-IV Inhibitors has recently been introduced. DPP-IV Inhibitors increase incretin levels {GLP-1 (glucose dependent insulinotropic polypeptide) and GIP (glucagon-like peptide-1)}, which in turn increases insulin secretion and decreases blood glucose levels. DPP-IV Inhibitors work by enhancing the sensitivity of β cells to glucose, and have also been shown to improve markers of β cell function. Since the introduction of DPP-IV Inhibitors in 2006, they are flourishing as monotherapy and also used in combination with commonly prescribed anti-diabetic agents [22]. However, in spite of potential multiple beneficial effects of DPP-IV Inhibitors, they do have certain limitations: high cost of therapy and unacceptable adverse effects. The marketed synthetic DPP-IV Inhibitors are expensive drugs when indicated for long term therapy for a chronic disease like diabetes and are also reported to cause unacceptable adverse effects like pancreatitis, upper respiratory infections, anaphylactic reactions and pancreatic cancer (411). In this scenario, research to explore DPP-IV Inhibitors from alternate sources is of paramount importance. The present study was developed to identify DPP-IV Inhibitors...
from indigenous sources which may work in concert with body’s own defense mechanism, have fewer adverse effects and would be more affordable.

Computational in silico studies were used to confirm the DPP-IV Inhibitory activity of Berberine and Mangiferin and identify the sites as well as amino acid residues on DPP-IV enzyme to which these natural DPP-IV Inhibitors binds. The active principles Berberine and Mangiferin were computationally designed and screened through in silico docking studies against crystal structure of DPP-IV. The in silico methods have been demonstrated to be useful in predicting the potential of proteins as precursors of peptides in various bioactivities, such as DPP-IV. Such in silico computational methodologies have been widely used for virtual ligand and target based screening and profiling to predict biological activity. They can also be used to explore the target structures for possible active sites, generate candidate molecules, check for their drug generated likeness, dock these molecules with the target, rank them based on variations on the structures according to their binding affinities and further optimize the molecules to improve binding characteristics [23].

The crystal structure of DPP-IV shows that the enzyme is a serine protease that specifically cleaves N-terminal dipeptides from polypeptides with Pro and Ala at the penultimate position. In DPP-IV, each monomer consists of an N-terminal \( \beta \)-propeller domain (Lys56-Asn497) and a C-terminal catalytic domain (Glu508-Pro766, together with segment Leu45-Val55). Catalytic domain and propeller domain together embrace an egg-shaped cavity of approximate dimensions 40Å \( \times 20Å \times 20Å \), which harbours the active centre [23].

The crystal structure of DPP-IV was considered as receptor which was docked against the Berberine, Mangiferin, Sitagliptin and Vildagliptin. All these optimized chemical compounds were considered for docking against DPP-IV based on the reference structure of DPP-IV against 4-aryl cyclohexylalanine in complex state (2QT9). Basically, DPPIV is active in homodimer form. The active site is a deep cleft in DPPIV which can be accessed via the opening of the propeller domain or through side opening formed at the interface of the \( \beta \)-propeller and hydrolyase domains. Furthermore, the \( \beta \) propeller is a funnel shaped tunnel which extends to the active site. The propeller domain gets well packed against the hydrolyase domain, and the catalytic triad (Ser630, H740, and D708) which is at the interface of the two domains [23]. The active site cavity is well guarded by residues like W659, Y631, V547, P550, W629, Y752, F357, Y666, E205, E206, Y662, N710, R125, V656, S630 and V711 [24]. All these chemical compounds were docked in the active site pocket. In addition to this, the active sites were also predicted using CASTp software. This online server listed 198 pockets with area and volume. The first predicted pocket was the largest pocket with an area of 6086.4 Å\(^2\) and a volume of 16271 Å\(^3\). The second largest pocket has an area of 574.3 Å\(^2\) with a volume of 1171.8 Å\(^3\). Third largest pocket is observed proximal to the first largest pocket with an area 274.4Å\(^2\) and a volume of 191.5 Å\(^3\).

Berberine and Mangiferin showed significant inhibition of DPP-IV enzyme. Berberine binds to the active site pocket, very close to Arg 356 and ser 209 of DPP-IV receptor. Mangiferin binds near the interface region near Tyr 238 of the DPP-IV receptor. Vildagliptin preferred to bind to Asp739 amino acid near the interface region of the DPP-IV as their biological active forms are homodimers and Sitagliptin binds near the \( \alpha/\beta \) hydrolase domain. It was observed that few of the ligands preferred to bind within the active pocket, whereas others prefer to interact with interface region, \( \beta \) propeller and \( \alpha/\beta \) hydrolase domains. However, as per CAST prediction, in addition to the active site, the region of interface and the \( \alpha/\beta \) hydrolase and \( \beta \) propeller domain combo are also considered as part of the largest active site pocket. The synthetic DPP IV inhibitors: Sitagliptin binds to amino acids: Glu452 and Vildagliptin to Asp739. Berberine and Mangiferin bind to the amino acid residues: Ser 209, Arg356 and Tyr 238. The binding energy is inversely proportional to the DPP-IV inhibitory activity. Based on the binding energy results, it was found all the ligands (Berberine, Mangiferin and Vildagliptin) studied had superior DPP-IV binding affinity as compared to Sitagliptin.

Previous in vitro and in vivo experimental studies from the laboratory demonstrated the DPP-IV Inhibitory activities of Berberine and Mangiferin. Results demonstrated that DPP-IV inhibition is one of the mechanisms attributing to the therapeutic efficacy of Berberine and Mangiferin in experimental diabetes with metabolic syndrome [12]. In the present study the DPP-IV inhibitory activity of Berberine and Mangiferin has been delineated using in silico docking studies against crystal structure of DPP-IV and compared to the synthetic DPP-IV Inhibitors. In silico results emphasize the potential of developing Berberine and Mangiferin as natural alternative to synthetic DPP-IV Inhibitors (Table 1).

### Conclusion

Vildagliptin, Mangiferin prefers to bind near the interface region of the DPP-IV as their biological active forms are homodimer. Berberine prefers to bind to the active site pocket of DPP-IV enzyme. Berberine binds very close to Glu205 and Glu206. Sitagliptin binds near the \( \alpha/\beta \) hydrolase domain of DPP-IV enzyme. Berberine, Mangiferin and Vildagliptin prefer to bind within the active site pocket (the 1st largest pocket) of DPP-IV enzyme whereas Sitagliptin prefers to bind in the second largest pocket of DPP-IV enzyme.

### Acknowledgement

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### Table 1: Details of chemical compounds with their IC50 value, binding energy and their interacting residues against DPPIV.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Chemical compound</th>
<th>IC50</th>
<th>Binding energy</th>
<th>Amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sitagliptin</td>
<td>18nm</td>
<td>-139.91</td>
<td>Glu452</td>
</tr>
<tr>
<td>2</td>
<td>Vildagliptin</td>
<td>3nm</td>
<td>-237.57</td>
<td>Asp739</td>
</tr>
<tr>
<td>3</td>
<td>Mangiferin</td>
<td></td>
<td>-296.76</td>
<td>Tyr238</td>
</tr>
<tr>
<td>4</td>
<td>Berberine</td>
<td>13.3nm</td>
<td>-229.45</td>
<td>Ser209,Arg356</td>
</tr>
</tbody>
</table>
References


