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Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2011, 3(4):129-139

ISSN No: 0975-7384
CODEN(USA): JCPRC5

Molecular docking analysis of compounds present in *Trigonella foenum graceum* with angiotensin converting enzyme *insilico* analysis

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ABSTRACT

Angiotensin converting enzyme (ACE) catalyses the conversion of angiotensin I to angiotensin II a potent vasoconstrictor in a substrate concentration dependent manner and degrades bradykinin a potent vasodilator and other vasoactive peptides which leads to increase in blood pressure. Prolonged increase in blood pressure condition increases the risk of heart attacks, heart failure, and stroke or kidney failure. Naturally occurring proteins acts as angiotensin converting enzyme inhibitors. Inhibition of ACE by angiotensin converting enzyme inhibitors results in the decreased of formation of angiotensin II and decreased metabolism of bradykinin leading to systematic dilation o f the arteries and veins and a decrease in arterial blood pressure. The molecular docking analysis done indicates that the receptor of human angiotensin converting enzyme through an interaction with the chemical bonds .Herbal drugs were safe and milder with few or no side effects than the drugs currently used in the treatment lessening high blood pressure which can be better used for the development of new therapeutics to decrease the formation of angiotensin II and to decrease the activation of bradykinin. The target of this insilico analysis was to study the ability of the secondary metabolites of Trigonella foenum graecum(TFG) to serve as antagonist to angiotensin converting enzyme (ACE).

Key words: angiotensin converting enzyme, molecular docking, intermolecular bonding interactions, high blood pressure, diabetic nephropathy.

INTRODUCTION

Diabetes mellitus is a metabolic disorder which is emerging as a severe problem and is a disease involving chronic metabolic disorders of carbohydrate, protein and fat due to relative or absolute lack of insulin and various degrees of insulin resistance[1].It is currently affecting around 143

million people and it is projected that by 2030, the number of diabetes would be 4.835 million The list of drugs available for management of diabetes is short and drug treatments are not always satisfactory in maintaining euglycemia and avoiding late stage diabetic complications. Medicinal herbs with anti hyperglycemic activities are increasingly sought as an alternative approach by diabetic patients and health care professionals [2]. The holistic approach of herbs has accelerated the global efforts to harness and harvest medicinal plants having multiple beneficial effects .[3] Some of them have been evaluated and active principles isolated [3].Therefore, the discovery of more drugs which may have new modes of action is very pertinent. Traditional plant remedies have always provided sources of useful hypoglycemic agents and therefore, should continue to be investigated for possible drug alternatives. [1]

The increase in demand for the use of plant based medicines to treat diabetes may be due to the side effects associated with the use of orthodox drugs such as insulin and oral hypoglycemic agents Effective control of blood glucose level is a key step in preventing or reversing diabetic complications and improving the quality of life in both type 1 and type 2 diabetic patients [2]

Diabetic nephropathy (DN) is one of the most important micro vascular complications associated with type 2 diabetic patients and has emerged as a leading cause of the end stage renal disease in developed countries [4, 5]. Type II diabetes and diabetic nephropathy are clearly chronic progressive diseases that are associated with a combination of genetic, lifestyle and environmental factors [6]. DN is characterized by structural abnormalities of kidney including hypertrophy of both glomerular and tubular elements,

Increase in the thickness of glomerular basement membranes and progressive accumulation of extra cellular matrix components, eventually leading to proteinuria and renal failure [7]. Despite implementation of intensive glycemic and hypertensive control, DN remains an important clinical problem [4] and new therapeutic agents are needed for the treatment of this condition.

Angiotensin converting enzyme (ACE) is a circulating enzyme and an exopeptidase that participates in the renin-angiotensin system, (RAS) which mediates interstitial fluid and arterial vasoconstriction. ACE catalyses the conversion of angiotensin I to angiotensin II a potent vasoconstrictor in a substrate concentration dependent manner [8] and degrades bradykinin a potent vasodilator and other vasoactive peptides [9] ,these two actions make ACE inhibition a goal in the treatment of hypertension, cardiovascular complications, diabetic nephropathy and type 2 diabetes mellitus. Inhibition of ACE by ACE inhibitors results in the decreased formation of angiotensin II and decreased metabolism of bradykinin, leading to systematic dilation of the arteries and veins and a decrease in arterial blood pressure. In addition, inhibiting angiotensin II formation diminishes angiotensin II-mediated aldosterone secretion from the adrenal cortex, leading to a decrease in water and sodium reabsorption and a reduction in extra cellular volume. There are number of drugs like captopril,gimfibrozil, isovaleric acid quinapril, fluvastatin,lisinopril, benazepril, and fosinopril which are currently used as ACE inhibitors .

The present study was designed to analyse the compound in TFG as inhibitors to ACE using molecular docking technique. The aim of this *insilico* analysis was to derive an intermolecular complex and tracing the bonding interactions between the ligand with the ACE. The results of this work may be of importance for the development of new therapeutics to decrease formation

of angiotensin II and to decrease the activation of bradykinin which in turn controls hypertension there by reducing the risk factors of heart attacks, heart failure, stroke, or diabetic nephropathy.

EXPERIMENTAL SECTION

Preparation of Ligands :

The hydro alcoholic extract of *Trigonella foenum graecum* was analyzed using GCMS technique, the compounds in it was identified using NIST. These secondary metabolites were used as ligands for this study. The 2 dimensional structures of these ligands were generated using ACD/ Chem. Sketch Tool. This software contains tools for 2D cleaning, 3D optimization and viewing. These data are saved as molecular format file (MDL MOL format).Molecular format converter tool.

(http://www.webqc.org/molecularformatsconverter.php) is used to convert this file into Protein Data Bank format and is used during docking analysis.

Retrieval of Target Protein Sequence:

The protein sequence for Angiotensin converting enzyme (ACE) was obtained from protein sequence database of UniProt (http://www.uniprot.org/uniprot/p30556). It was ascertained that the three dimensional structure of ACE was available in the PDB data base. The structure was visualized using RasMol Tool.

Domain Analysis:

The functional analysis of ACE was predicted using Pfam database (http://www.pfam.sanger.ac.uk/)

Active Site Prediction:

After obtaining 3D structure, the possible binding sites of ACE were searched using Q- Site Finder (http://www.modelling.leeds.ac.uk./qsitefinder/). Ten binding sites were obtained of which the first site is usually taken as the active site for docking analysis since it is the most conserved region.

Docking the inhibitors with the Active site of Angiotensin converting enzyme (ACE) :

Totally twenty four ligands were docked with ACE using the Lamarckin Genitic Algorithm (LGA) provided by the Auto Dock Program, Version 3.0 (http://www.autodock.scripps.edu/). Polar hydrogen was added to the receptor, kollaman charges were assigned and salvation parameters were added with"Addsol' option in AutoDock. For the inhibitors charges of the Gasteiger type were assigned. The internal degree of freedom and torsion were defined using the "Ligand Torsions" menu option of Auto Dock. The grid maps representing the protein were calculated using the "Auto Grid" option. The protein was centered on the geometric centre prior to docking. Docking stimulations were carried out with an initial population of 50 individuals and a maximum of 25,000 energy evaluations were used as the docking parameters for obtaining the final docking structures. In addition to returning the docked structure, Auto Dock also calculates an affinity contrast for each ligand -receptor configuration. The best ligand- receptor structure from the docked structures was chosen based on the lowest energy and minimal solvent accessibility of the ligand. The docking results were analyzed using we blab viewer tool.

Finding drug affinity using Lipinski Drug Filter:

The ligands used in the present study were subjected to Lipinski rule screening using the tool Lipinski Drug Filter of the Supercomputing Facility for Bioinformatics and Computational Biology (http://www.scfbioiitd.res.in/utlity/lipinskifilter.jsp) according to which prediction of high probability of success or failure is based on drug likeness for molecules complying with 2 or more of the rules namely – molecular weight less than 500 dalton , high lipophilicity(expressed as Log P less than 5), less than 5 hydrogen bond donors, less than 10 hydrogen bond acceptors and polar surface area should be between 0.0-150 Armstrong.

RESULTS

The structures of the ligands were drawn using ACD/ChemSketck tool and converted into PDB format using Molecular converter Tool. Table - 1 show the compounds used as ligands for this study. The sequence of Angiotensin converting enzyme retrieved from swiss - Prot database (swiss – Prot ID: P12821) The 3D structure of ACE (PDB ID: 2C6F) was downloaded from PDB database and visualized using RasMol tool is shown in figure - 1.The functional region of ACE predicted from Pfam was in

Table 1: Total ionic chromatogram (GC–MS) of ethanol extract of *Trigonella foenum graecum* obtained with 70 eV using a Elite-1 fused silica capillary column with He Gas as the carrier

S. No.	Name of the compound	Molecular Formula
1.	Aziridine, 1,2,3-trimethyl-, trans-	$C_5H_{11}N$
2.	2-Propen-1-amine, N-ethyl-	$C_5H_{11}N$
3.	1-Azabicyclo[2.2.2]octane, 4-methyl-	$C_8H_{15}N$
4.	á-D-Glucopyranoside, methyl	$C_7H_{14}O_6$
5.	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
6.	Dibutyl phthalate	$C_{16}H_{22}O_4$
7.	Heptanoic acid, 2-ethyl-	$C_9H_{18}O_2$
8.	Hexane, 3-bromo-	$C_6H_{13}Br$
9.	1-Dodecyne	$C_{12}H_{22}$
10.	Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-	$C_{10}H_{16}O$
11.	Piperidine, 1,1'-methylenebis-	$C_{11}H_{22}N_2$
12.	1-Octanol, 2-nitro-	$C_8H_{17}NO_3$
13.	Pentanal, 2-methyl-	$C_6H_{12}O$
14.	Didodecyl phthalate	$C_{32}H_{54}O_4$
15.	1-Tridecyne	$C_{13}H_{24}$
16.	Squalene	$C_{30}H_{50}$
17.	9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester	$C_{25}H_{38}O_2$

Identified as peptidase M2 (region 31- 630) a single domain region, which belongs to peptidase M2 family. The possible active sites of ACE were identified using Q-site finder, which produced ten binding sites of this site 1, is considered highly conserved and was chosen as the most

favorable site for docking analysis. Figure - 2 shows the ACE protein with the active site and its residues. Out of the seventeen ligands which were Auto Docked with the active site of ACE. Only 7 compounds were able to form hydrogen bonds with ACE receptor.

Figure-1 Three Dimensional Structure of Angiotensin-converting enzyme

3D Structure of Angiotensin-converting enzyme in Cartoon model Visualized using Rasmol (pink color indicates Helices, Yellow color indicates Strands and White and blue color indicates turns)

Docking Score and Number of Hydrogen Bonds formed between *Angiotensin- converting enzymes (ACE)* With Various Inhibitors

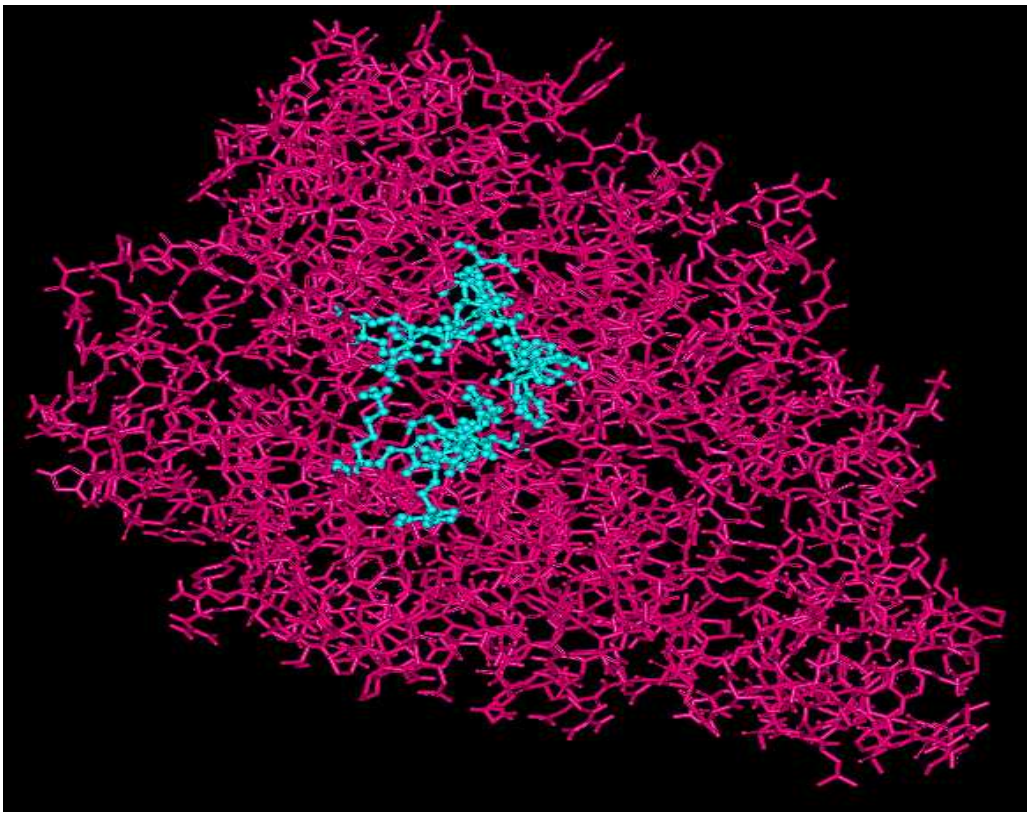
Table-2

Ligand Name	Docking score Kcal/mol	No. of Hydrogen bonds formed
3-O-Methyl-d-glucose	-7.52	6
9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester	-15.07	2
Dibutyl phthalate	-10.63	3
Didodecyl phthalate	-14.66	2
á-D-Glucopyranoside, methyl	-7.16	4
Pentanal, 2-methyl-	-5.41	2
Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-	-6.47	2

The final docked conformation obtained for the different inhibitors were evaluated based on the number of hydrogen bonds formed and their docking scores (Table -2). Some of the docked configurations (ACE complex with the ligands) are shown in figure 3,4,5,6,7,8,9. The Key interacting sites of ACE are HN O,of Asp(Aspartic acid) 140; HN,N, of Leu (Leucine)139; OD1, ND2, HD22 of Asn(Asparagine)46; O, HD22, ND2 of Asn(Asparagine)122; O of Ala (Alanine)125; O of Tyr(Tyrosine)122; OG of Ser(Serine)123; HN,N,ND1 of His

(Histadine)331; N,SGlys (lysine) 330. The result of Lipinski's rule suggests the analyzed compounds as best therapeutic drug (Table -3). In the present study all the seven compounds which are able to form hydrogen bonds with the active site of ACE satisfied more than two rules predicting high probability of success to show drug likeliness. Didoceyl phthalate satisfied three rules .Other ligands satisfied all the five Lipinski rules.

Figure-2 Q-Site Finder

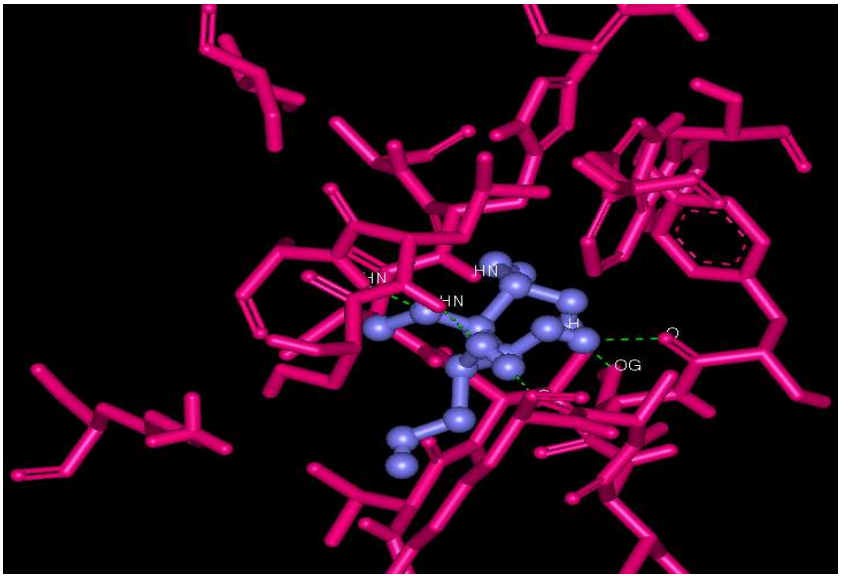


Angiotensin converting enzyme showing its active sites in blue color and pink color indicates protein TYR122,SER123,THR124,ALA125,LYS126,SER138,LEU139,ASP140,THR144,TRP163,TRP257,LYS321,GLY325,ARG326,GLU327,VAL328,VAL329,CYS330,HIS331,ALA332,PHE490

Table-3 Lipinski's Rule results

S. NO	Compound name	M. wt.	Donors	Acceptors	Polar surface area	Log p
1.	**3-O-Methyl-D-GLUCOSE**	194.1	4	6	107.22	-2.81
2.	**9,12- octadecadienoic Acid [2,2]-phenyl Methyl Ester**	370.3	0	2	26.30	8.21
3.	**Didoceyl pthalate**	502.3	0	4	52.60	11.80
4.	**Dibutyl pthalate**	278.2	0	4	52.60	4.59
5.	**a-D-Gluco pyranoside, methyl**	194.1	4	6	99.38	-2.04
6.	**Pentanal, 2- Methyl-**	100.1	0	1	17.07	2.02
7.	**Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-**	152.1	0	1	17.07	2.43

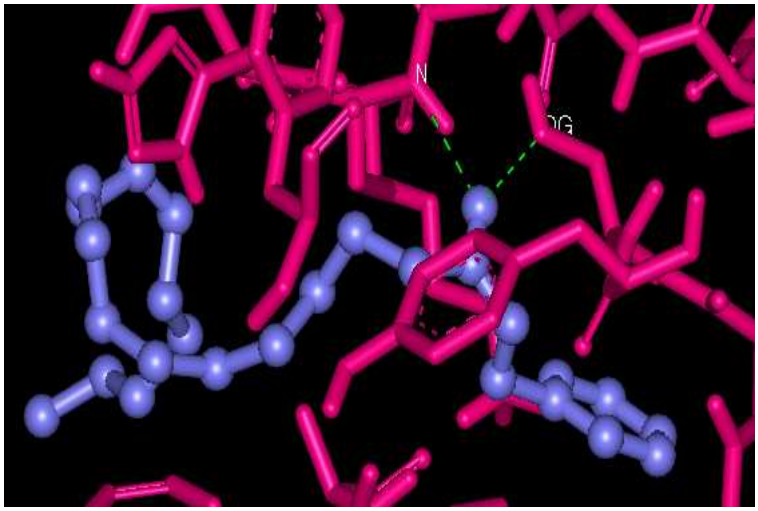
Figure-3 ACE interaction with 3-O-Methyl-d-glucose



ACE		3-O-Methyl-d-glucose	Distance Å
RESIDUE	ATOM	ATOM	
ASP140	HN	O	2.28
LEU139	HN	O	1.90
ALA125	O	H	1.81
TYR122	O	H	1.90
SER123	OG	H	2.18
HIS331	HN	O	2.45

Pink Color indicates Stick model of protein active sites and blue color indicates Ball and stick model of ligand and dotted green line indicates hydrogen bond

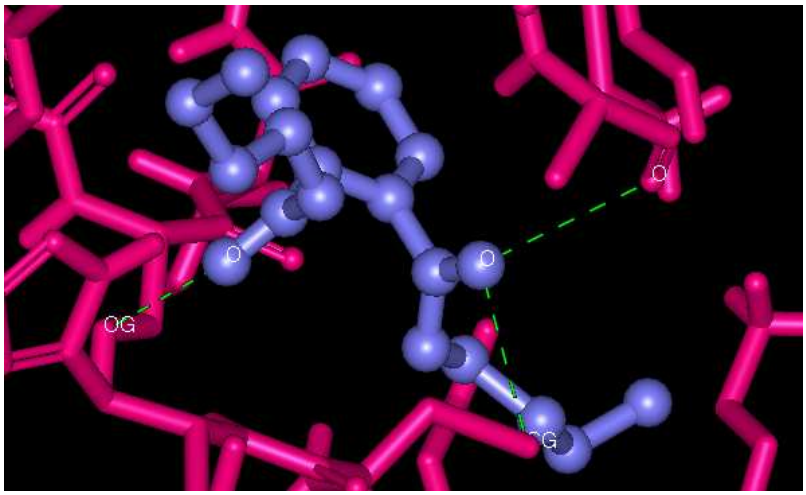
Figure-4 ACE interactions with 9, 12-Octadecadienoic acid (Z, Z)-, phenylmethyl ester



ACE		9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester	Distance Å
RESIDUE	ATOM	ATOM	
CYS330	N	O	2.06
SER123	OG	O	3.09

Pink Color indicates Stick model of protein active sites and blue color indicates Ball and stick model of ligand and dotted green line indicates hydrogen bond

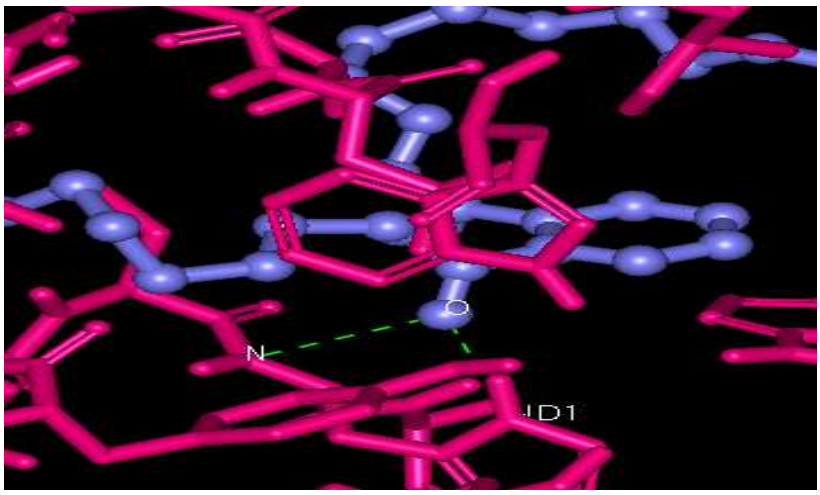
Figure-5 ACE interaction with Dibutyl phthalate



ACE		Dibutyl phthalate	Distance Å
RESIDUE	ATOM	ATOM	
SER123	OG	O	3.06
CYS330	SG	O	3.30
ASP140	O	O	3.05

Pink Color indicates Stick model of protein active sites and blue color indicates Ball and stick model of ligand and dotted green line indicates hydrogen bond

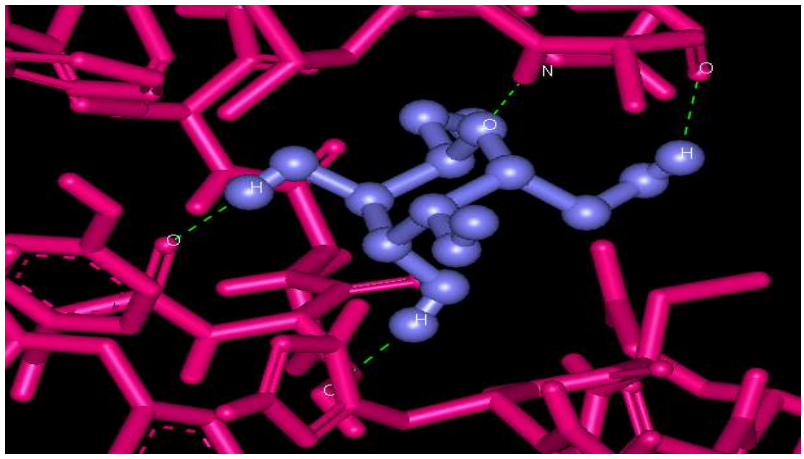
Figure-6 ACE interaction with Didodecyl phthalate



ACE		Didodecyl phthalate	Distance Å
RESIDUE	ATOM	ATOM	
HIS331	ND1	O	2.13
HIS331	N	O	2.50

Pink Color indicates Stick model of protein active sites and blue color indicates Ball and stick model of ligand and dotted green line indicates hydrogen bond

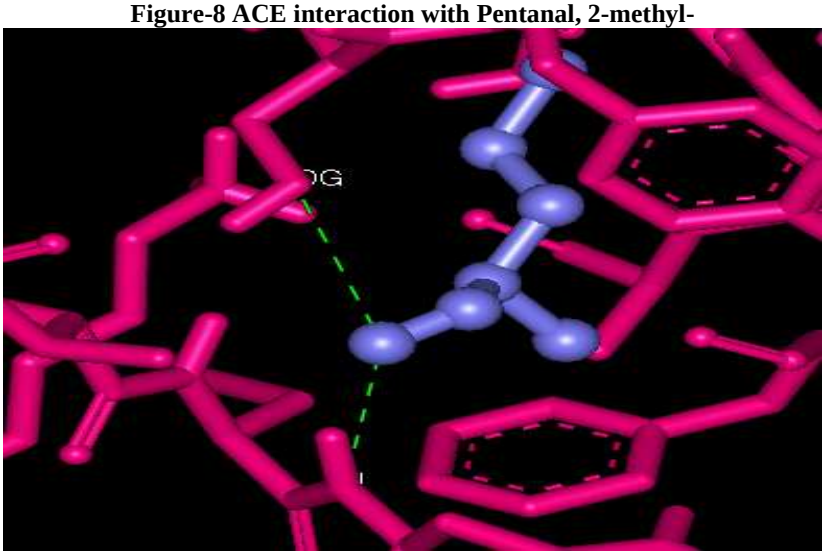
Figure-7 ACE interaction with á-D-Glucopyranoside, methyl



ACE		á-D-Glucopyranoside, methyl	Distance Å
RESIDUE	ATOM	ATOM	
ASP140	O	H	1.92
ASP140	HN	O	1.80
TYR122	O	H	1.97
SER123	OG	H	2.11

Pink Color indicates Stick model of protein active sites and blue color indicates Ball and stick model of ligand and dotted green line indicates hydrogen bond

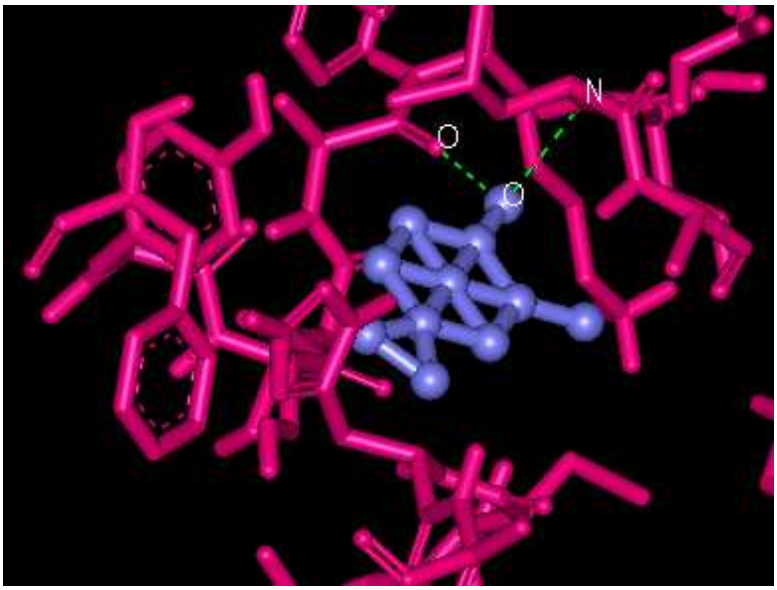
Figure-8 ACE interaction with Pentanal, 2-methyl-



ACE		Pentanal, 2-methyl-	Distance Å
RESIDUE	ATOM	ATOM	
SER123	OG	O	3.08
HIS331	N	O	2.05

Pink Color indicates Stick model of protein active sites and blue color indicates Ball and stick model of ligand and dotted green line indicates hydrogen bond

Figure-9: ACE interaction with Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-



ACE		Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-	Distance Å
RESIDUE	ATOM	ATOM	
LEU139	N	O	2.21
ALA125	O	O	2.56

Pink Color indicates Stick model of protein active sites and blue color indicates Ball and stick model of ligand and dotted green line indicates hydrogen bond .

DISSCUSION

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Based on the docking score lead compounds are selected .The present study shows that the *Trigonella foenum graceum* have more docking score , when compared with that of standard drugs like captopril, gemfibrozil, quinapril,. isovaleric acid ,fluvastatin. Captopril docking score -6.07037Kcal/mol quinapril, docking score -6.13296Kcal/mol. Gemfibrozil docking score -5.75246 Kcal/mol, isovaleric acid -7.14431Kcal/mol fluvastatin-. -6.82398 Kcal/mol.

Docking was performed with the screened molecules with ACE protein where docked results were compared with that of the existing drugs and the variations were noted which showed that designed drugs are having good affinity than the existing drugs of which is 9,12 –octa decardienoic acid -15.07 ,Dido decyl phaalate -14.66 Kcal/mol , Dibutyl phathalate -10.63

Kcal/mol ,3-o methyl D-glucose docking score -7.52, Kcal/mol Kcal/mol, a' D- glucopyranoside -7.16 Kcal/mol , bicyclo -6.47 Kcal/mol ,Pentanal -5.41 Kcal/mol having more affinity when to compared to other drugs.

CONCLUSION

The receptor-ligand interactions play a significant role in molecular docking and drug designing. The receptor of human ACE interacts with the compound of *Trigonella foenum graecum* ligand effectively, which may be used in the development of new therapeutics to decrease the formation of Angiotensin –II and the activation of bradykinin.

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