Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(12):860-865

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

In silico binding and interaction studies of aldose reductase with the active components of triphala, a traditional herbal formulation

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ABSTRACT

Aldose reductase is the key regulatory enzyme of the aldose reductase (AR)/polyol pathway which plays a major role in the development of diabetic retinopathy. Present study was an attempt to analyze the binding and interaction patterns of AR against 15 chosen active components of the herbal formulation Triphala in order to find a potent inhibitor of the enzyme. Molecular docking experiments were carried out using PatchDock online server. The protein ligand complexes thus obtained were analyzed on PyMol viewer. Phytochemicals such as ellagic acid, sennoside, ellagitannin, chebulinic acid, vitamin C and casuarinin showed significant interactions with AR. Of these ellagitannin was identified as a potent inhibitor of AR showing 18 hydrogen bond interactions.Therefore, this potential flavonoid could be used to prevent diabetic retinopathy and used as a potential therapeutic approach to diabetic retinopathy targeting AR.

Keywords: Diabetes, Aldose reductase, Triphala, active compounds, docking

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INTRODUCTION

Diabetes is a complex metabolic disorder where the affected individual is prone to both acute and chronic diabetic complications. The long term diabetic complications are mainly responsible for the morbidity and mortality in most cases of diabetes. These include vascular and non-vascular complications. Different molecular mechanisms have been proposed to be involved in the pathogenesis and progression of long-term diabetic complications. These include enhanced glucose flux through the polyol pathway, generation of reactive oxygen species (ROS), activation of protein kinase C (PKC) and formation of advanced glycation end products (AGEs) [1].

The polyol pathway plays a significant role in the development of microvascular complications [2]. Aldose reductase (AR) is the key regulatory enzyme of polyol pathway. AR catalyzes the reduction of glucose to sorbitol which is then acted upon by the enzyme sorbitol dehydrogenase (SDH) to yield fructose [3]. Several studies have demonstrated the increased activity of polyol pathway products which drive the utilization of NADPH and NAD^+ respectively. This affects the pyridine nucleotide flux and metabolism of glucose. Also, there is increased osmotic caused due to the accumulation of sorbitol leading to electrolyte imbalance causing swelling-up of cells and membrane damage [4].

Diabetic retinopathy is a complication of chronic hyperglycemia characterized by damaged blood vessels of the retina ultimately causing loss of vision. The pathogenesis of diabetic retinopathy involves increased activity of AR thereby causing derangement of the cellular membrane in ocular lens. This induces cataract formation. Furthermore,

the activation of nuclear factor-κB (NF-κB), up-regulation of vascular endothelial growth factor (VEGF) and increased oxidative stress causes changes in vascular permeability [5]. Also, the increased expression of AR causes activation of poly (ADP-ribose) polymerase (PARP) which acts as the underlying mechanism of diabetic retinopathy. The aforementioned mechanisms can be prevented by the use of aldose reductase inhibitors (ARIs) [6]. Studies involving screening and validation of several active compounds of herbal drugs and plant extracts in order to explore their efficacy as potent ARIs are being carried out by researchers [7, 8].

Triphala is a well-known Indian Ayurvedic herbal formulation that has been studied extensively for its antioxidant, anti-inflammatory, anti-hyperlipidemic and hepatoprotective properties [9-11]. Though studies in diabetic rats have shown that Triphala possesses anti-hyperglycaemic effect, the individual components of Triphala responsible for this effect and its underlying mechanisms are still unclear. Therefore studies targeting key regulatory proteins of pathways involved in the progression of diabetic complications need to be done.

Present study is an attempt to analyze the binding and interaction patterns of AR against the chosen active components of the herbal formulation Triphala in order to find a potent inhibitor of the enzyme that could prevent ocular complications of diabetes.

EXPERIMENTAL SECTION

2.1 Receptor preparation:

3D structure of the receptor protein aldose reductase (PDB id: 3P2V) was retrieved from Research Collaborator for Structural Bioinformatics (RCSB) protein databank (http://www.rcsb.org/pdb/home/home.do) (Figure 1). It was prepared for docking by removing water molecules and adding hydrogen atoms.

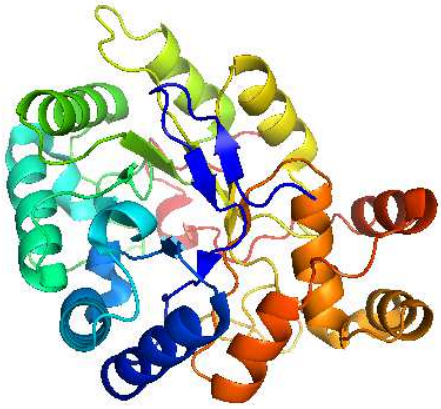


Figure 1: 3D structure of Aldose reductase (3P2V)

Table 1: Active components of Triphala chosen for molecular docking studies

Serial No.	Ligand	Compound Id.	Molecular Formula	Molecular Weight
1.	β- Sitosterol	222284	$C_{29}H_{50}O$	414.71
2.	Casurinin	157395	$C_{41}H_{28}O_{26}$	936.65
3.	Chebulagic acid	442674	$C_{41}H_{30}O_{27}$	954.66
4.	Chebulic acid	71308174	$C_{14}H_{12}O_{11}$	356.24
5.	Chebulinic Acid	452240	$C_{41}H_{32}O_{27}$	956.68
6.	Corilagin	75368	$C_{27}H_{22}O_{18}$	634.45
7.	Ellagic Acid	5281855	$C_{14}H_6O_8$	302.19
8.	Ellagitanin	71308261	$C_{116}H_{76}O_{74}$	2653.80
9.	Ethane Dioic Acid	971	$C_2H_2O_4$	90.03
10.	Ethyl Galate	13250	$C_9H_{10}O_5$	198.17
11.	Gallic Acid	370	$C_7H_6O_5$	170.12
12.	Glucogalin	124375	$C_{13}H_{16}O_{10}$	332.26
13.	Neo Chebulinic Acid	44584488	$C_{41}H_{34}O_{28}$	974.69
14.	Sennoside	73111	$C_{42}H_{38}O_{20}$	862.74
15.	Vitamin C	54670067	$C_6H_8O_6$	176.12

2.2 Active site prediction:
The prediction of ligand binding residues in 3P2V protein was carried out by submitting the pdb file of the protein on 3DLigandSite server (http://www.sbg.bio.ic.ac.uk/3dligandsite/). The server predicts the active site residues using homologous structures and conservation at CASP8 [12, 13].

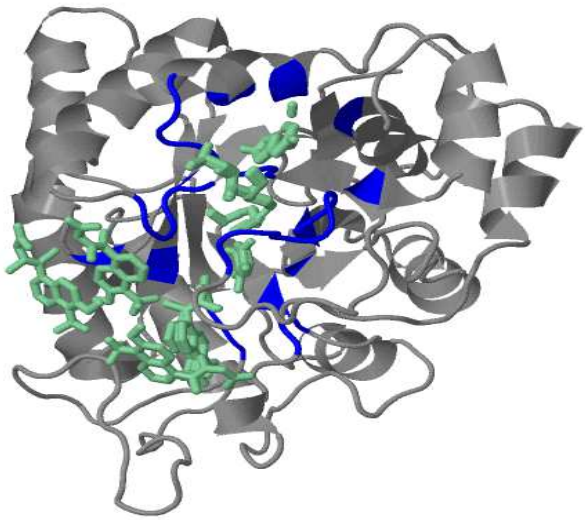
2.3 Ligand preparation:
About 15 active components of Triphala were chosen to be used as ligands in the present study (Table 1). The SMILES of these compounds were obtained from PubChem (http://pubchem.ncbi.nlm.nih.gov) and submitted on CORINA molecular networks (https://www.molecular-networks.com/online_demos/corina_demo) for generation of 3D structures of respective compound.

2.4 Molecular docking:
The docking experiments were carried out on PatchDock a molecular docking algorithm based on shape complementarity principles (http://bioinfo3d.cs.tau.ac.il/PatchDock/). The input files (receptor and ligand) for each experiment was submitted in PDB format. The email address of the user was submitted for each experiment through which the results were obtained. 20 best interactions were obtained for each experiment. PatchDock is an automatic server for molecular docking that works on shape complementarity principles. It is a geometry-based molecular docking algorithm [14].

2.5 Analysis of docked complexes:
The docked complexes were analyzed on PyMol molecular viewer. The interacting residues of 3P2V and interacting molecules of the ligands were labeled. The hydrogen bond lengths were also labeled.

RESULTS AND DISCUSSION

The predicted active sites of 3P2V include the following residues: GLY-18, THR-19, TRP-20, LYS-21, ASP-43, TYR-48, LYS-77, HIS-110, TRP-111, SER-159, ASN-160, GLN-183, TYR-209, SER-210, PRO-211, LEU-212, GLY-213, SER-214, PRO-215, ASP-216, LEU-228, ALA-245, ILE-260, PRO-261, LYS-262, SER-263, VAL-264, THR-265, ARG-268, GLU-271, ASN-272, CYS-298. Figure 2 shows the predicted binding site of 3P2V.



Predicted binding site (blue) Other residues (grey)
Figure 2: Structural view of predicted ligand binding site

Figure 3 shows the scores and ACE for the docking experiments of AR with the chosen active compounds of Triphala. 3P2V-ellagitannin complex had a score of 9468 and ACE of -415.34. Ellagitannin showed significant interaction with 3P2V compared to the other 14 ligands. Ellagitannin-3P2V complex had 18 hydrogen bond interactions (Figure 4). Other compounds such as ellagic acid, sennoside, chebulinic acid and vitamin C also showed

significant interactions with 9, 8, 8, 8 hydrogen bonds respectively (Figure 5). The numbers of hydrogen bond interactions and the interacting residues for each docked complex is indicated in Table 2.

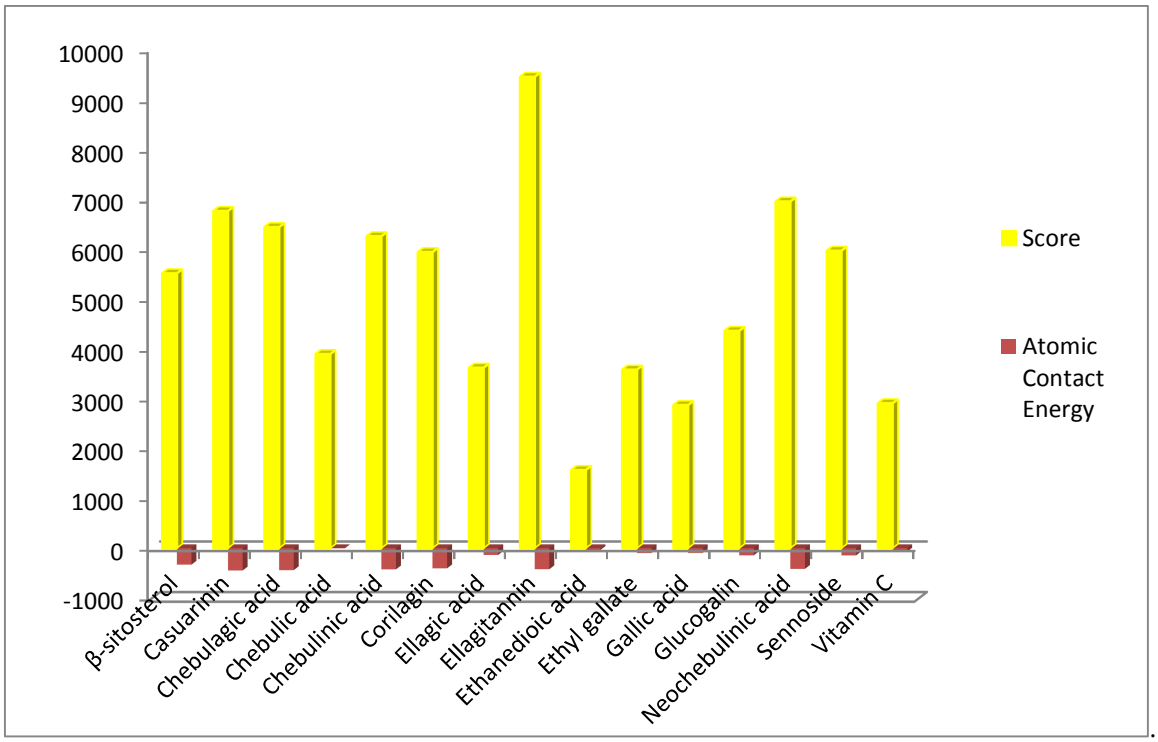


Figure 3: PatchDock scores and ACE for the docked complexes

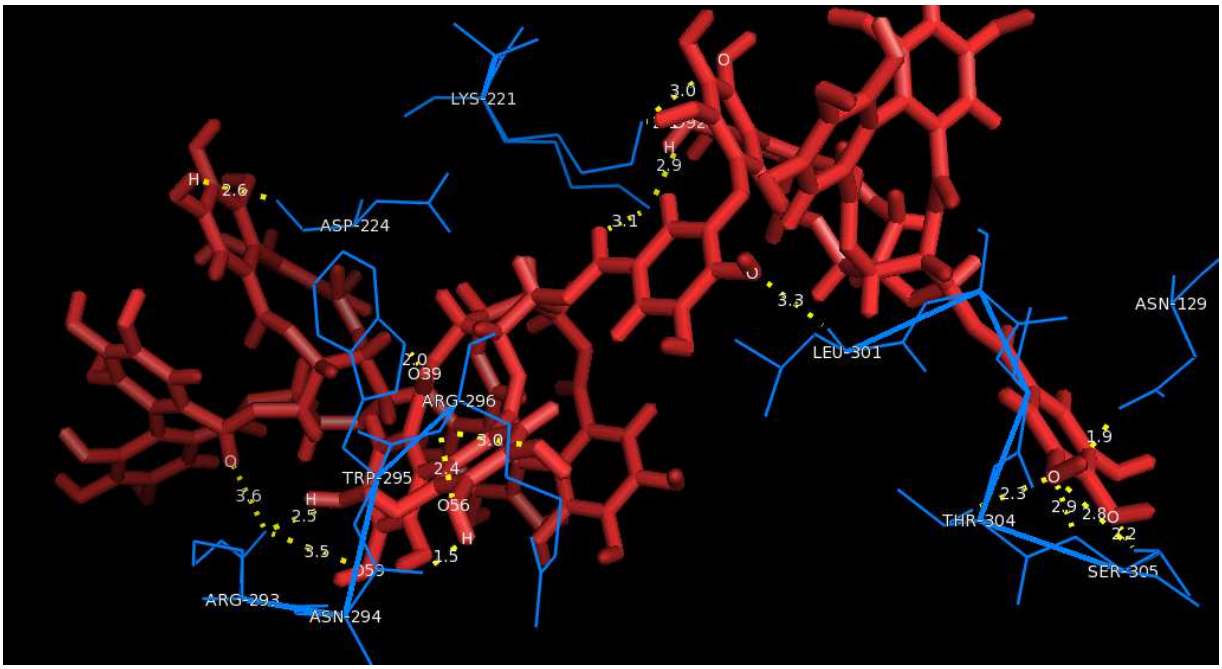


Figure 4: 3P2V-ellagitannin docked complex

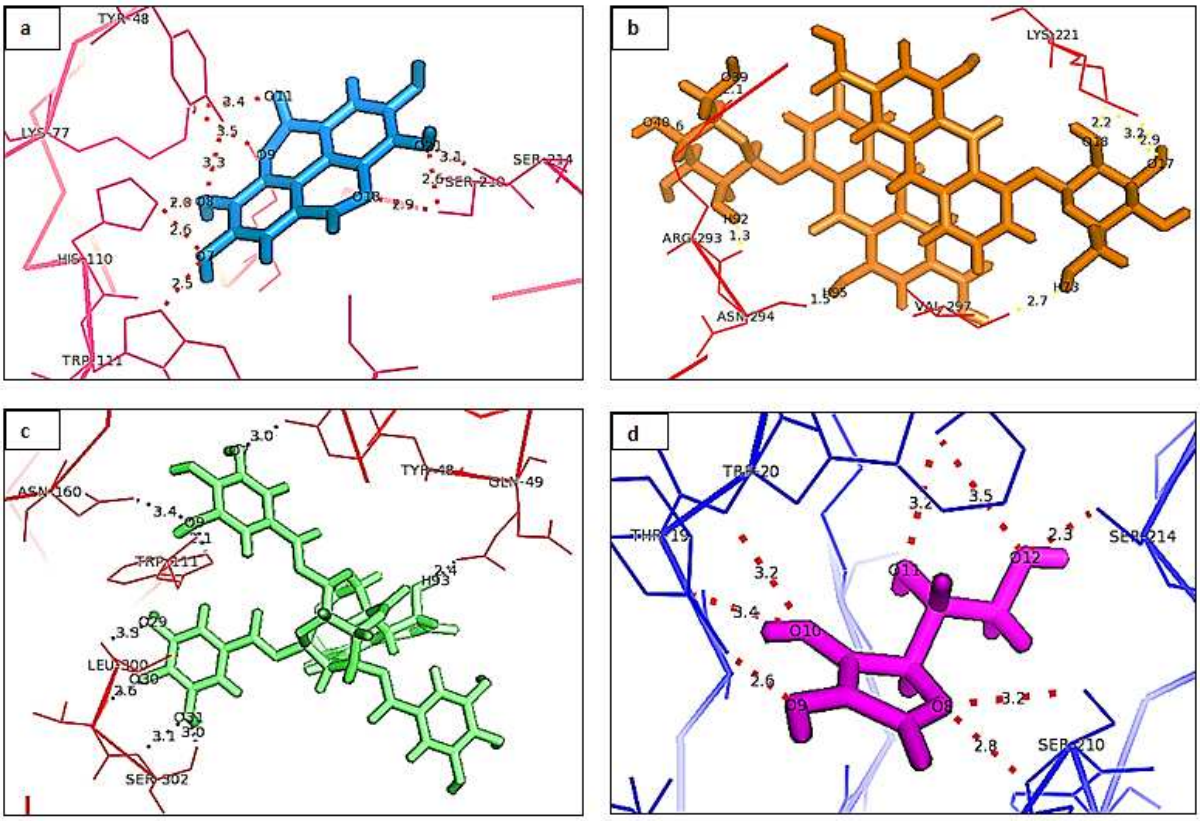


Figure 5: AR in complex with (a) ellagic acid (b) sennoside (c) chebulinic acid and (d) vitamin C

Table 2: Interacting residues and hydrogen bond numbers of docked complexes

Receptor	Ligand	No. of hydrogen bond interactions	Interacting residues
3P2V	β-sitosterol	Nil	-
	Casuarinin	8	TRP-20, HIS-110, PHE-122, LEU-301, SER-202
	Chebulagic acid	7	TRP-20, VAL-47, TYR-48, HIS-110, TRP-111, SER-302
	Chebulic acid	4	LYS-262, THR-265, ARG-268
	Chebulinic acid	8	TYR-48, GLN-49, TRP-111, ASN-160, LEU-300, SER-302
	Corilagin	5	TYR-48, HIS-110, TRP-111, SER-210, ILE-260
	Ellagic acid	9	TYR-48, LYS-77, HIS-110, TRP-111, SER-210, SER-214
	Ellagitannin	18	ASN-129, LYS-221, ASP-224, ARG-293, ASN-294, TRP-295, ARG-296, LEU-301, THR 304, SER-305
	Ethanedioic acid	5	LYS-21, SER-210, SER-214, ILE-260
	Ethyl gallate	3	HIS-110, TRP-111, ASN-160
	Gallic acid	3	TYR-48, LYS-262, SER-210
	Glucogalin	3	SER-159, ASN-160
	Neochebulinic acid	6	ASN-129, LYS-221, ARG-296, LUE-301, SER-302, THR-304
	Sennoside	8	LYS-221, ARG-293, ASN-294, VAL-297
	Vitamin C	8	THR-19, TRP-20, SER-214, SER-210

The identification of ellagitannin as a potent inhibitor of AR by molecular docking in current study further implies significance of this antioxidant compound in preventing glucose flux via polyol pathway. Ellagitannin from *Potentilla recta* has been shown to possess *in vitro* antioxidant and anti-inflammatory activities [15]. Virtual screening of few plant active compounds has shown the identification of ellagitannin as one of the significant inhibitors of AR [16]. These hydrolysable tannins are also being studied for their ability to regulate blood glucose levels [17].

Virtual screening of compounds by molecular docking has become essential in drug discovery process as it cuts down time and expenditure involved in preliminary *in vitro* and *in vivo* methods. Compounds found to possess significant inhibitory potential by *in silico* studies could be tested further by appropriate gene expression studies. Current study has revealed that ellagitannin is a potent inhibitor of the enzyme AR among the chosen active components of Triphala. Inhibition of AR is a promising therapeutic approach to control the development of diabetic complications and hence identification of potent ARIs would help in the management of diabetes.

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