Elastin Structure Protein Non-Enzymatic Glycation Inhibition Studies by Use of Secondary Metabolites of *Withania somnifera*

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Abstract

NEGs (non-enzymatic glycation) accumulations particularly high in E.C.M., proteins are result in intra and inter molecular cross-linking and later has been hypotized to stiffening of these proteins and believed to play an important role in etiology of various NEGs related diseases (Basta *et al.,* 2004). The goal of this research asses the energy level of *W. somnifera* (Withaferin –A) in Elastin (hydrophobic) structural protein interaction that made glycation adducts with glucose. The results level of binding energy of proteins and phyto constituents are high in Elastin (-8.4). The glucose and protein binding energy Elastin (-3.9). The energy level of Elastin high that's clear indication that withaferin–A give best result of inhibitions was compared with glucose and protein binding energy. This preliminary finding gives an open window towards structure based drug design to finding noval inhibitory molecule of entities.

Introduction

NEG is result of sugar molecules such as glucose, fructose bonding to a protein or lipid molecule without controlling action of an enzyme. These are implicated in many age related diseases such as type–II (diabetic mellitus), cardiovascular disease (collagen, fibrinogen are damaged), Alzheimer diseases (amyloid protein product of the reaction progressing to AGEs), Cancer (acryl-amide and other product related), peripheral neuropathy (myelin is attached), and other sensory losses (de-myelination), and blindness (due to micro-vascular damage in the retina), this range of diseases is the result of very basic level at which glycation interfere with molecular and cellular functioning throughout the body (Vorum *et al.,* 1995). The long half-life proteins of the extracellular matrix (E.C.M.), typically Elastin (hydrophobic) (Basta *et al.,* 2004), accumulate reactive metabolites through uncontrolled non-enzymatic reactions such as glycation or atypical example of these non-enzymatic changes is the formation of advanced glycation end-products (AGEs), resulting from the reaction of carbohydrates with the free amino group of proteins (Rebecca *et al.,* 2008). The accumulation of AGEs and the resulting structural alterations cause tissue conventional properties (increased stiffness, reduced elasticity). *Withania somnifera* (Sanskrit: Ashwagandha, English: Winter cherry). *Withania somnifera*, commonly known as Ashwagandha, is a valued herb in Ayurvedic medicine. Roots, leaves and preparations of the plant are traditionally used as tonic, hypnotic, sedative and diuretic. *W. somnifera* mainly contains with anolides including withaferin-A which are specific to the Solanaceae family. With anolides are biologically active secondary metabolites present in roots and leaves of *W. somnifera*. The present study provides examples of the

anti-glycation activity of *W. somnifera,* phyto constituents Withaferin–A, which target the essential stages of glycation through-(i) inhibition of non-enzymatic glycation/ AGEs. To best of our knowledge, the receptor-level mechanism behind this process is nowhere mentioned. Present research was emphasis at the analysis of receptor-level binding affinity of Elastin with Withaferin–A and Glucose through molecular docking. *In-silico* studies would provide a clear understanding on the mode of action of the above mentioned Withaferin-A components against Structural E.C.M. protein can be used as inhibitory drug to reduce hyperglycemic related complications.

Materials and Methods

Structure retrieval

The protein data bank (PDB): The structure homologues for elastin and collagen protein Sequence query was retrieved in PDB.

The research collaboratory for structural bioinformatics (RCSB): The molecular shape of elastin was retrieved from RCSB PDB (PDB – www.rcsb.org/).

PDB sum: The PDB sum (PDB sum–www.ebi.ac.uk/pdbsum/), a pictorial database that provides an at-a-glance overview of the contents of each 3D structure deposited in the Protein Data Bank (PDB) was used to get the 3-D structure of elastin.

Structure visualization–RASMOL: RasMol (RasMol–www.rasmol.org/), a molecular graphics program intended for the visualization of proteins, used for structural visualization of elastin. Three dimensional structure of inhibitors-CHEMSKETCH: The Chemical

structure of Withaferin-A, (Chem Sketch–www.acdlabs.com/download/), a quite powerful chemical structure drawing program.

Docking: Docking was done with the PyRx software (pyrax–www.pyraxviana.com/), in which the result is being obtained on the basis of pose energy. Docking calculations attempt to place 'Ligand into Binding Sites'.

The binding affinity was expressed kcal/mol. The atoms that make up the Ligand like inhibitor, and the Binding Site on the protein where the inhibitors bind that Structure were drawn in Discovery studio 2.1 version.

Results and Discussion

The structural E.C.M. proteins (Elastin) were retrieved and analyzed and it was docked to Withaferin-A from *W. somnifera* (figure-1) and glucose that is responsible from non-enzymatic glycation.

The results and visualize data /figures are presented as follows:

Figure 2 shows the wireframe, ball stick, ball stick space-fill, ribbon, cartoon diagram of Elastin receptors, which were downloaded from PDB and visualize by use of RasMol software. The specification of proteins described n table no.1.the technical specification of ligands summarized n table no.2. Ball & stick, wireframe, sticks, dots and dots with space-fill model of Withaferin-A and Glucose are depicted in Figure 3, 4. After docking, both compounds bound exactly at the active site of Elastin, which was shown in Frame. A careful inspection of the binding pocket indicated that both the compounds at the Withaferin-A, glucose domain of E.C.M. proteins Figure 5 and detail docking results were presented in table no.3.

Fig.1 Plant herbarium image

Fig.2 3D Structure Diagram of Elastin
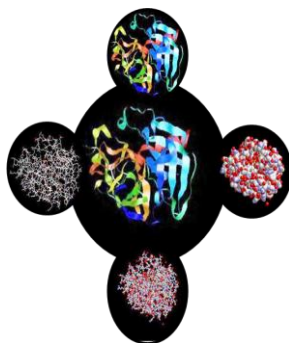

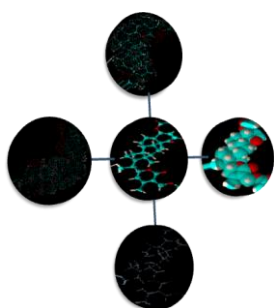**Fig.3** 3D Structure of Withaferin-A   **Fig.4** 3D Structure of Glucose
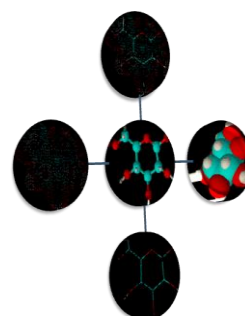

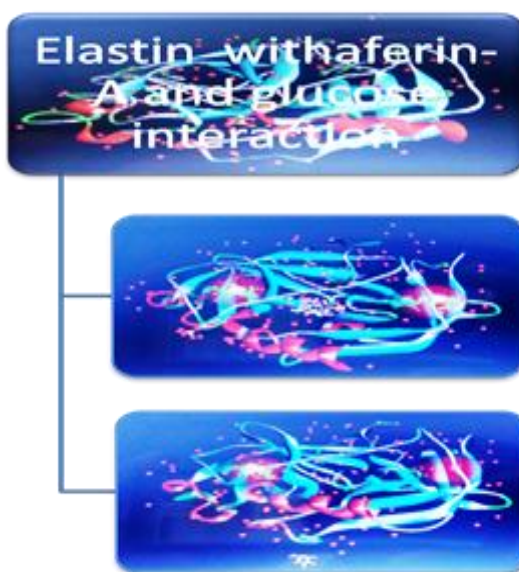**Fig.5** Glucose and Withaferin-A docked with the active site (sphere ball red color) of Elastin

Table.1 Target Information

S. no.	Specification	Elastin
1	PDB ID	2oii
2	Molecular size	51681.58
3	Solubility	Hydrophobic
4	Chain	1,2,3
5	Length	162
6	Amino acids	393
7	Type	Structural

Table.2 Properties of ligands acid obtained from *Chem-Sketch*

S. no.	Parameters	Withaferin-A	Glucose
1	PubChem ID	265237	5793
2	Chemical Name	5,6-epoxy-4,22,27-trihydroxy-1oxoergosta-2,24-dienoic acid delta-lactone	Glucose, (alpha-D)-Isomer; D-Glucose; D-Glucopyranose; Glucose; Glucopyranose; Grape sugar
3	Molecular formula	$C_{28}H_{38}O_6$	$C_6H_{12}O_6$
4	Molecular weight	470.606 mol/grams	180.156 mol/grams
5	Hydrogen bond donor count	2	5
6	Hydrogen Bond Acceptor Count	6	6
7	Rota table count	3	1
8	Topological Surface area	96.4 A^2	110 A^2
9	Mono-isotopic mass	470.267 g/mol	180.063 g/mol
10	Exact Mass	470.267 g/mol	180.063 g/mol
11	Melting point	-------	146*c

Table.3 The receptor-Ligand interaction details

S. no	Ligands	receptor	Centre	Number of Binding site(pose)	Bnding score (cal/mol)
1	Withaferin-A	Elastin	X:46.9380 Y:2.4375 Z:42.9441	7	-8.4
2	Glucose	Elastin	X:52.1256 Y:1.1728 Z:8.3721	8	-3.9

Structure based Drug discovery typically starts with an analysis of binding sites in target proteins, or an identification of structural motifs common to active compounds. *In silico* molecular docking is one of the most powerful techniques to discover novel ligands for receptors of known structure and thus play a key role in structure based drug design. The docking process involves the prediction of ligand confirmation and orientation (posing) within targeted binding site and their interaction energies were calculated using the scoring functions. An efficient inhibitor of non-enzymatic glycation should inhibit glucose-derived NEGs generation (Saxena, *et al.,* 1996). A large number and ever increasing number of synthetic and natural "AGE Inhibitors" have been reported in past. because the mechanisms of non-enzymatic glycation or AGEs formation involve complex sequential and parallel reactions that are poorly understood[4] had proposed a simple scheme that identifies common targets for AGEs inhibition, a guide and basis for rational design of new AGE inhibitors, an classification of existing AGE inhibitors.

AGE inhibitors can be classified in six categories or types (Kyselova *et al.,* 2004) (Type-A, B, C, D, E, and F). This group reduces AGE toxicity by breaking protein-AGE cross-linking. In order to find which type of inhibition mechanism of action behind this process, we have taken E.C.M. proteins as a receptor and compounds and carried out docking process. It was clear from Table -3 Withaferin-A and Glucose docked with Elastin and collagen. As a result of docking, different conformations were generated for both compounds with E.C.M. proteins. The score and pose (site of interactions) clearly indicate that Withaferin-A capping the proteins and prevent to glucose to bind non-specifically with elastin and collagen protein. From these docking studies, hence we conclude that binding of Withaferin-A may lead good inhibitor of NEGs activity and reduce its related complications. However, this mechanism of prediction requires further advance *in- silico* analysis.

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