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Research Article

TARGET LEVEL ANALYSIS OF ANTIOXIDANT ACTIVITY OF COSTUNOLIDE AND EREMANTHIN ISOLATED FROM *COSTUS speciosus*

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ABSTRACT

Antioxidant properties of many medicinal plants have been widely recognized and some of them have been commercially exploited. Plant derived antioxidants play a very important role in alleviating problems related to oxidative stress. Costunolide and Eremanthin are two important compounds isolated from the root of C*ostus specious* and established its antioxidant activity. Administration of either Costunolide or Eremanthin for 60 days caused a significant increase in enzymatic activity of SOD, CAT and GPx in treated when compared to untreated diabetic rats. Till now, the mechanism behind this process is not known. Present study was aimed at assessing the target level analysis of antioxidant activity of these compounds through docking studies. 3D structure of Costunolide and Eremathin were docked with that of SOD, CAT and GPx by Discovery studio 2.1 version. Both Costunolide and Eremathin were bound only with SOD and not with CAT and GPx. The results show that both compounds possessed potential agonist characteristics that is capable of activating SOD. Among these two compounds Eremanthin showed better affinity towards SOD than Costunolide with docking score 71.871 and 58.27 respectively. Therefore, we can infer that Eremanthin and Costunolide have direct affinity towards SOD and hence these lead molecules activate SOD. Activated SOD follows further signalling cascades, such as activation of CAT and GPx to act as antioxidants. Further studies are needed to prove its mechanisms *in vitro* condition.

Keywords: Antioxidant, Costunolide, Eremanthin, SOD, CAT, GPx, docking.

INTRODUCTION

Diabetes mellitus (DM) is a serious complex chronic condition. It is a major source of ill-health worldwide. This metabolic disorder is characterized by hyperglycemia and disturbances of carbohydrate, protein and fat metabolisms, secondary to an absolute or relative lack of insulin [1]. Chronic oxidative stress due to hyperglycemia may, therefore, play an important role in progression of β-cell dysfunction in both types of diabetes. Studies have demonstrated that this can be inhibited by antioxidants [2].

Antioxidants are a group of substance which when present at low concentration in relation to oxidizable substances, significantly inhibit or delay oxidation processes. The O2 molecule is a free radical, as it has two impaired electrons that have the same spin quantum number. This spin restriction makes O2 prefer to accept its electrons one at a time, leading to the generation of the so called ROS (Reactive Oxygen Species), which can damage the cells. ROS are also produced continuously as byproducts of various metabolic pathways that are localized in different cellular compartments such as chloroplast, mitochondria and peroxisomes [3, 4].Oxidative stress is very common in diverse human disorders such as ageing, arthritis, cancer, AIDS, diabetis etc. Stress-induced ROS accumulation is counteracted by enzymatic antioxidant systems that include a variety of enzymatic scavengers, such as Superoxide dismutase (SOD), Glutathione peroxidase (GPX) and Catalase (CAT) [5]. Superoxide dismutase catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. This hydrogen peroxide will be decomposed as water and oxygen molecule, in presence of catalase and glutathione peroxidise (fig1).

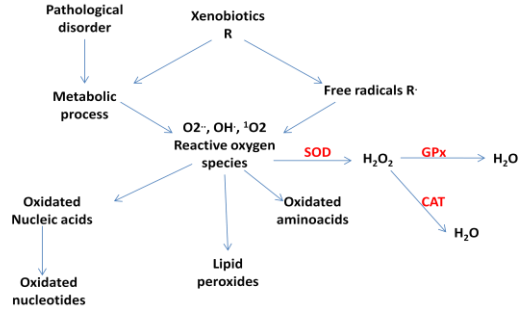

Fig.1: Pathway in which SOD, CAT and GPx are involved

Oxidative environment in cells is also created by the impairment in functioning of endogenous antioxidant enzymes namely superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). These enzymes are known to be inhibited in diabetes mellitus as a result of non-enzymatic glycosylation and oxidation.

Antioxidant compounds in food play an important role as a health-protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables.

Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with wide variety of physical and chemical properties. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources.

Antioxidant properties of many medicinal plants have been widely recognized and some of them have been commercially exploited. Plant derived antioxidants play a very important role in alleviating problems related to oxidative stress. The antioxidant property of Costunolide and Eremanthin isolated from *Costus speciosus* was established on streptozotocin-induced diabetic Wister rats. Administration of either Costunolide (20 mg/kg) or Eremanthin (20 mg/kg) for 60 days caused a significant increase in enzymatic activity of SOD, CAT and GPx, when compared with untreated rats [6]. To best of our knowledge, the receptor-level mechanism behind this process is no where mentioned. Present study was aimed at the analysis of receptor-level binding affinity of Costunolide and Eremanthin with SOD, CAT and GPx through molecular docking.

MATERIALS AND METHODS

PDB

PDB (Protein Data Bank) is the single worldwide archive of structural data of biological macro molecules, established in Brookhaven National Laboratories. It contains structural information of the macromolecules determined by the X-ray crystallographic and NMR methods. 3D structure of SOD, CAT and GPX were taken from PDB, whose PDB ids are 1CB4, 2CAG, 2P31 respectively.

Discovery Studio

Structure of Costunolide and Eremanthin were drawn in Discovery studio 2.1 version. Hydrogen bonds were added and energy was minimized using CHARMm force field. Finally saved as .msv format. Further, docking studies also carried out using Discovery studio 2.1 version.

Docking

The protein atoms were typed using the CHARMm force field and saved as .msv format. The active site of the protein was first identified and defined using an eraser size 10.0 Aº. Then the ligands were docked into the active site using Libdock procedure. Libdock score, absolute energy were obtained from the study.

RESULTS

Fig.2 shows the ribbon diagram of SOD, CAT and GPx receptors, which were downloaded from PDB. Ball & stick model of Costunolode and Eremanthin are depicted in Fig.3. After docking, both compounds bound exactly at the active site of Superoxide dismutase, which was shown in Fig.4. A careful inspection of the binding pocket indicated that both the compounds at the Cu-Zn domain of SOD (Fig.5). A close view of hydrogen bond interaction has been viewed in Fig.6.Target information was given in Table 1. Docking details for both Eremanthin and Costunolide were tabulated in Table 2 and Table 3 respectively.

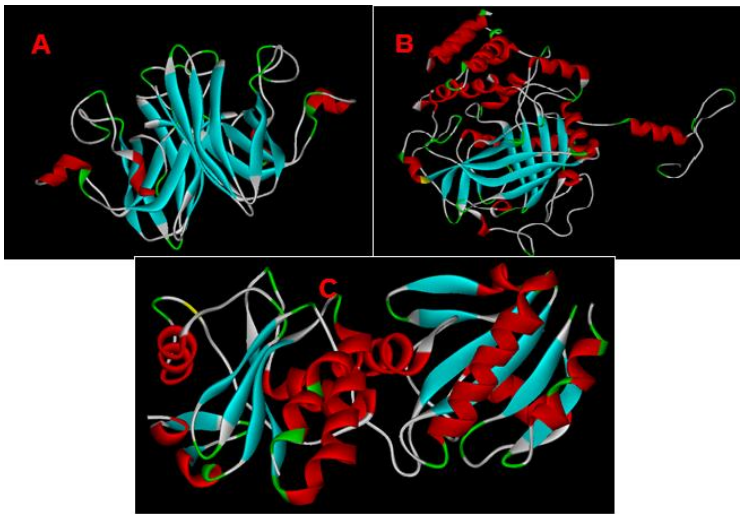

Fig.2. Secondary Structure Diagram of A) Superoxide dismutase (SOD), B) Catalase (CAT), C) Glutathione peroxidase (GPx).

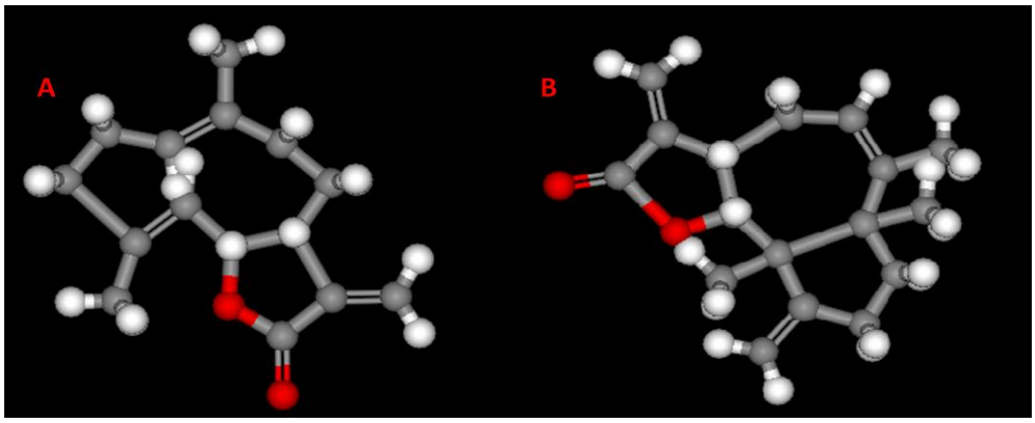

Fig.3. Ball & Stick Diagram of A) Costunolide B) Eremanthin

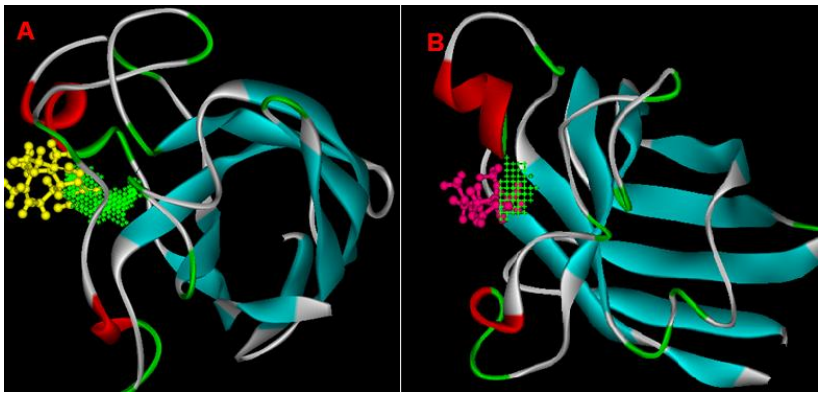

Fig.4. A) Eremanthin (yellow ball & stick diagram) and B) Costunolide (magenta ball & stick model)docked with the active site (green mesh) of SOD.

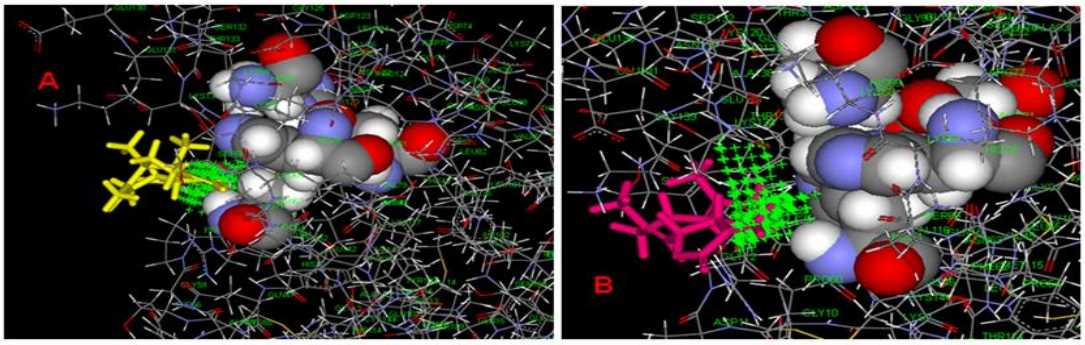

Fig.5:A) Eremanthin (yellow stick model) bound with the Zn-Cu domain (CPK model) of the receptor B) Costunolide (Magenta stick model) bound exactly with Zn-Cu domain of receptor.

Table.1: Target Information.

Target name	SOD	CAT	GPx
Pdb id	1CB4	2CAG	2P31
Aminoacid length	151aa	484aa	181aa
Binding site volume	X= 10.410,Y=87.880, Z=18.620 Radius=10Aº	X=58.380,Y=19.080 Z=18.3000 Radius=18 Aº	X=-5.830,Y=3.390 Z= 0.2000 Radius=10Aº

Table2:The receptor-ligand interaction details of Eremanthin.

Receptor	SOD	CAT	GPx
No. of pose after Dock	5	0	0
Energy	47.811	-	-
Libdock score	71.871	-	-
No. of hbonds	2	-	-
Aminoacid Involved	His61	-	-
Atoms of residue	HN	-	-
Atoms of Eremanthin	O16 O13	-	-
Bond length	2.497 1.9396	-	-
No. of other Interactions	8	-	-

Table 3:The receptor-ligand interaction details of Costunolide.

Receptor	SOD	CAT	GPx
No. of pose after Dock	1	0	0
Energy	35.177	-	-
Libdock score	58.27	-	-
No. of hbonds	0	-	-
No.of other interactions	12	-	-

DISCUSSION

Discovery of active compounds from natural products have gained enormous importance in the field of drug discovery. Demand for natural antioxidant has been increasing due to concerns about safety of synthetic antioxidants [6]. Drug discovery from plants involves a multidisciplinary approach combining botanical, ethnobotanical, phytochemical, and biological techniques. Drug discovery typically starts with an analysis of binding sites in target proteins, or an identification of structural motifs common to active compounds. According to [15], *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. According to [7], molecular docking continues to hold great promise in the field of computer based drug designing which screens small molecules by orienting and scoring them in the binding site of a protein. 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.

Oxidative stress, defined as an imbalance between oxidants and antioxidants, leads to any biochemical changes and acts as the causative factor for many diseases like diabetes, atherosclerosis, cardiovascular problems etc. Administration of either Costunolide (20 mg/kg day) or Eremanthin (20 mg/kg day) for 60 days caused a significant increase in enzymatic activities of SOD, CAT and GPx in the treated rats when compared to untreated diabetic rats. [6]. In order to find the mechanism of action behind this process, we have taken these receptors and compounds and carried out docking process.

It was clear from Fig.1 that in the free radical scavenging cascade SOD comes in the first position. Its activation will further activate CAT and GPx. Here, docking studies demonstrated that Costunolide and Eremanthin docked only with SOD, and not with CAT and GPx. As a result of docking, different conformations were generated for both compounds with SOD. But only for the top ranked docked complex, the scores were copied from the table browser view of Discovery studio for binding affinity analysis. To correlate the biological activity of the receptor and site-directed docking of ligands, here we used Libdock score (which is PLP like score (steric

and H-bonding intermolecular functions, Higher PLP scores indicate stronger receptor-ligand binding)) [8, 9, 10]. The docked results further more explains that the ligands were fit with a specific optimal orientation exactly to the active site cavities of the receptor. In this study, docking score of Eremanthin and Costunolide were 71.871 and 58.27 respectively. A higher score indicates a stronger receptor-ligand binding affinity. The scoring functions have been used to estimate ligand-binding affinity to screen out active and inactive compounds during the process of virtual screening [11]. When biological activities were compared the scoring functions, Eremanthin showed good binding affinity to the receptor SOD than Costunolide. Previous work has also shown that a correlation does exist between binding affinities and dock scores [12].

To ensure that the ligand orientation obtained from the docking studies were likely to represent valid and reasonable binding modes of the inhibitors, the libdock program parameters had to be first validated for the crystal structure's active site. Protein utilities and health protocol of Discovery studio was used to find out the active sites in the structure and it was found that the active site contains amino acids such as His 48, His 46, SO41,His 61, His 63 and His 120. Docking results showed that libdock determined the optimal orientation of the docked inhibitor, exactly to the active site. It is also reported that His 46, His 48 and his 120 are important residues for the activity of Superoxide dismutase to function as an antioxidant [13]. In Fig.5 the space-fill portion represented the functional part of the receptor which was known as Cu-Zn domain. Both Eremanthin (Fig.5A) and Costunolide (Fig.5B) bound at the Cu-Zn domain of the receptor.[14] stated that the most suitable method of evaluating the accuracy of a docking procedure is to determine how closely the lowest energy poses predicted by the docking score. By binding these two active phytocompounds with SOD receptor, the energy values were found to be minimum in Costunolide (35.177) and maximum in Eremanthin (47.811).

We analyzed the hydrogen bond interaction of the receptor with Costunolide and Eremanthin. A close view of the binding interactions of the receptor with compounds was shown in fig.6. As shown in fig.6A, there are two hydrogen bonds (shown in green dotted lines) formed between the receptor and Eremanthin. The residue involved in forming hydrogen bonds with the compound was His61. Costunolide did not form any hydrogen bond interaction with the receptor. The detailed atoms, which forming the hydrogen bonds are given Table 2, which may provide useful information for in- depth understanding in binding mechanism of the compound to the active site of the protein. Hydrogen bond formation also makes important contributions to the interaction between ligand and the receptor.

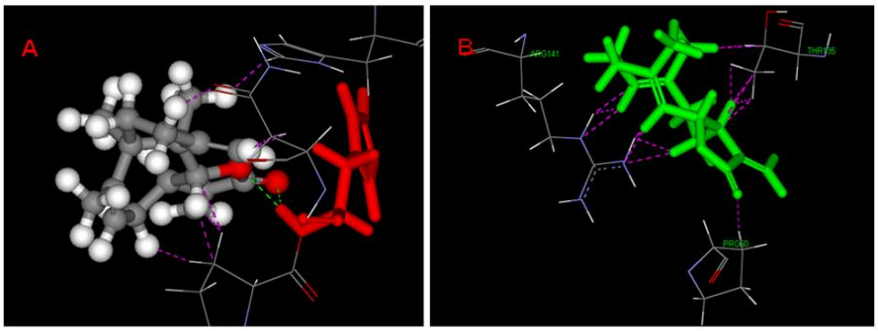

Fig.6. A) A close view of interaction of Hbonds (green dotted lines) between Eremanthin and B) Costunolide with the residues of the receptor.

CONCLUSION

The protein- ligand interaction plays a significant role in structural based drug designing. Based on dock score values it was predicted that both Costunolide and Eremanthin have good binding affinity towards SOD and also among the two compounds, Eremanthin showed better activity. From these docking studies, hence we conclude that binding of Eremanthin and Costunolide to the Cu-Zn domain of the SOD receptor may lead to increase its activity and reduce oxidative stress. However this mechanism of prediction requires further *in- vitro* analysis.

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