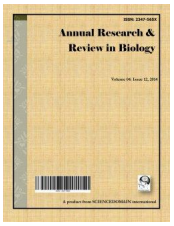

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Molecular Dynamics, Simulation of Protein-Protein Complex and their Role in Cytoprotective Process in Ethanol-induced Toxicity of HepG2 Cell Line

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Authors' contributions

This work was carried out in collaboration between both authors. Author VPK carried out the work, managed the literature searches and wrote the first draft of the manuscript. Author HPP designed the study, supervised, guided the entire work and corrected the manuscript. Both the authors read and approved the final manuscript.

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ABSTRACT

Aim: To examine the protein-protein interaction of *Wolbachia* Surface Protein (WSP of Uzifly) with six proteins involved in Ethanol-induced toxicity and the proteins involved in its cytoprotective process in HepG2 cell line (CYP2E1, Superoxide dismutase, Catalase, Death-associated protein kinase1, Alcohol dehydrogenases (Alpha/beta/gamma) and Cytochrome-C) and to study real time molecular dynamics.

Methodology: Modelled structure of WSP of Uzifly was retrieved from our laboratory archive. The proteins involved in the Ethanol-induced toxicity and the proteins involved in its cytoprotective process in HepG2 cell line were chosen based on the literature study. The six proteins like CYP2E1, Superoxide dismutase, Catalase, Death-associated protein kinase1, Alcohol dehydrogenases (Alpha/beta/gamma) and Cytochrome-C which are involved in the Ethanol-induced toxicity and the proteins involved in its cytoprotective process in HepG2 cell line were retrieved from PDB database

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with ID: PDB (3T3Z), PDB (2C9V), PDB (1DGG), PDB (2YAK), PDB (1U3W) and PDB (3NWV) respectively. Docking study was processed using ZDOCK and the best poses of protein were sorted using rDock. Finally, the atomic level interaction was studied for the best-scored protein-protein complex. The best complex was further subjected to molecular dynamics simulation to study its stability using standard dynamics cascade tool.

Results: From the results, it was observed that three proteins such as Cytochrome-C, CYP2E1 and Superoxide dismutase have more favourable shape complementarity for WSP binding to exhibit the cytoprotective process. However, the interaction analysis was done only for the top complex, Cytochrome-C-WSP. Time dependent parameter analysis of best complex Cytochrome-C-WSP showed that root-mean-square deviation (RMSD) values initially deviated but it was stabilized at the end of 1ns dynamics. The radius of gyration (Rg) during dynamics was within the limit.

Conclusion: This *insilico* study revealed that WSP has cytoprotective potential and therapeutical application.

Keywords: Protein-protein; Wolbachia Surface Protein (WSP); HepG2; ZDOCK; Cytochrome-C; cytoprotective.

1. INTRODUCTION

Liver is the major metabolic organ that inactivates and excretes toxins, secretes bile to absorb fats, breaks down proteins and is the site for drug metabolism [1]. High and long term consumption of alcohol plays an important role in the alcohol-related liver damage [2]. More than 55% of death was reported on alcohol abuse and the widespread of alcoholic liver disease relates with consumption of alcohol [3]. Europe is a leading country in alcoholic liver disease as their intake of alcohol is more [4]. According to the World Health Organization, the rate of mortality due to alcohol related disease is more in the developed world [5]. Hence, it is necessary to treat alcohol-based liver diseases either by drugs or natural compounds.

Furthermore, liver disease lead to hepatocyte apoptosis which is a complex biological process where more than one biochemical pathway will be activated [6]. Some of the pivotal proteins involved in the Ethanol-induced toxicity and the proteins involved in its cytoprotective process in HepG2 cell line are Cytochrome P450 2E1 (CYP2E1), Catalase, Superoxide dismutase (SOD), Cytochrome-C, Death associated protein kinase 1 (DAPK1) and Alcohol dehydrogenase (ADH) [7,8,9,10].

This current study focused to investigate the cytoprotective process in Ethanol-Induced toxicity of the HepG2 cell line by docking of WSP with the proteins involved in Ethanol-induced toxicity and the proteins involved in its cytoprotective process in HepG2 cell line. Therefore, the mode of binding of proteins was studied using computational chemistry and biology tools.

Modelled structure of WSP of Uzifly was retrieved from our laboratory archive. Through the literature study, the list of proteins involved in the Ethanol-induced toxicity and the proteins involved in its cytoprotective process in HepG2 cell line was collected and screened based on the role and functions in the pathway. Further, the structures which are available in the structural databases were taken to avoid the process of homology modelling and validation. Finally, the top six proteins were taken for protein-protein docking study and was docked with WSP using ZDOCK tool. ZDOCK uses Fast Fourier transform method for docking proteins [11]. More than one cluster was generated during docking, but we primarily focused only on the top large cluster docked sites because it was comparable to biological binding process. Docking using ZDOCK was followed by rDock [12] to sort the clusters based on energy and to choose the best-docked pose. The best complex was further subjected to molecular dynamics simulation to study its stability using standard dynamics cascade tool. Time dependent parameter analysis of best complex showed that root-mean-square deviation (RMSD) values were initially deviated but it was stabilized at the end of 1ns dynamics. Radius of gyration (Rg) during dynamics were within the limit. Thus, this study helped to understand the affinity, shape complementarity and stability of two proteins and its biological role. Another main advantage of this study is that it was possible to completely understand the atomic level interaction of amino acid.

2. MATERIALS AND METHODS

2.1 Structure Retrieval

The protein structures were retrieved from PDB database (https://www.rcsb.org/), a source of 3D structure of proteins, nucleic acids and drug molecules. In this study, the structure of proteins involved in the Ethanol-induced toxicity and the proteins involved in its cytoprotective process in HepG2 cell line were retrieved which includes CYP2E1 of PDB (3T3Z), Superoxide dismutase of PDB (2C9V), Catalase of PDB (1DGG), Death-associated protein kinase1 of PDB (2YAK), Alcohol dehydrogenases (Alpha/ beta/ gamma) of PDB (1U3W) and Cytochrome-C of PDB (3NWV). The retrieved protein structures were used for further proceedings.

2.2 Receptor Protein Preparation

The crystal structure of proteins was prepared prior to docking, in order to optimize each atom in the structure by removing the alternative conformers of amino acids, deleting identical chains, deleting water, ligand or drug molecule that bound to the native structure of the proteins. This was followed by applying the suitable CHARMM (Chemistry at HARvard Macromolecular Mechanics) force filed to each structure. At last, processed structures were finally prepared using prepare protein protocol of discovery studio to build loops. The output of prepared proteins were used for further proceedings.

2.3 Ligand Protein Preparation

Previously modelled and structurally validated small molecular weight surface protein of *Wolbachia* that is *Wolbachia* Surface Protein (WSP) of Uzifly was retrieved from our laboratory archive. The obtained structure was prepared like that of receptor protein preparation and was used for protein-protein docking.

2.4 ZDOCK and rDock of Proteins

Protein-protein rigid docking was carried out using ZDOCK protocol of macromolecules in Discovery studio suite. The high Dalton protein expressed on the hepatocyte was taken as a receptor and WSP of Uzifly as a ligand. Euler angle sampling of 6 degrees was optioned to get more accurate rotation and prediction of the sample with 54,000 poses. ZDOCK is a protein-protein docking program which uses the Fast Fourier transform (FFT) algorithm to explore the translational and rotational space of a protein-protein system [11]. The output of large sampling clusters formed each protein-WSP complex and was refined using rDock [12].

2.5 Molecular Dynamics and Simulation

The best protein-WSP complex was taken for simulation studies, CHARMM (Chemistry at HARvard Macromolecular Mechanics) and CFF (Consistent Force Field) were applied to top protein-WSP complex system. Subsequently, minimization was done in two steps of 500 cycles of steepest descent (SD) and conjugate gradient (CG) to eliminate steric strain. A minimized complex was subjected to three steps of cascaded dynamics and simulation process. During the heating/cooling step, the system was gradually simulated from 50 k to 300 k and then equilibrated to few steps to ensure that all the degrees of freedom of atoms were evenly disturbed. Finally, NVT production with automatic electrostatic method was done. The entire system was introduced with leapfrog verlet, a dynamics integrator with SHAKE constraint. The time dependent parameters results were analyzed by means of the Radius of gyration (Rg) and the root-mean-square deviation (RMSD) [13].

3. RESULTS AND DISCUSSION

3.1 Analyzing Cluster of Top Poses

The ZDOCK resulted in 54,000 poses in 2000 clusters of 10 Å cutoff distance and 6 Å ligand interface to the cluster center. Each protein docked complex showed different counts in top largest clusters (Fig. 1). The least poses on clusters were observed in complex of CYP2E1-WSP and Catalase-WSP with pose numbers of 12 and 14 respectively. On the other hand, a large number of clusters were seen in Alcohol dehydrogenases-WSP (24 poses) and Cytochrome-C-WSP (23 poses), while other two complex Superoxide dismutase-WSP (17 poses) and Death-associated protein kinase1-WSP (16 poses) clusters were intermediate between small and large clusters of poses. In largest cluster poses, receptor and ligand have more probability to intact each other and share its interface for strong binding, but in case of least poses, there is only minimum chance of contact of receptor-ligand. However, ZDOCK score is directly proportional to the binding of complex rather than on poses. From the obtained results it was

observed that Superoxide dismutase-WSP and Alcohol dehydrogenases-WSP have maximum dock score of 18.54 and 18.04 respectively. While, CYP2E1-WSP and Death-associated protein kinase1-WSP showed the dock score as 16.52 and 16.04, whereas, Catalase-WSP and Cytochrome-C-WSP have least dock score of 13.78 and 13.18 respectively (Table 1.).

3.2 Refining Docked Poses and Interaction Analysis

The top 50 poses were retrieved and processed for refine docking using rDock to screen poses based on energy such as electrostatic and desolvation energy. The top poses graphical representation is shown in the Fig. 2. in which the least negative of the complex implies the best binding complex. Among six different complexes (Table 1), Cytochrome-C-WSP complex with E_RDock value of -34.034 is leading complex, followed by Superoxide dismutase-WSP with energy value of -29.098.

The binding interaction analysis of Cytochrome-C-WSP is tabulated in the Table 2 From the interaction table (Table 2), the various types of interaction between the two different proteins can be observed. The types of bond includes Salt Bridge; Attractive Charge, Attractive Charge, Conventional Hydrogen Bond, Carbon Hydrogen Bond, Alkyl and Pi-Alkyl. In this study, the atomic level interaction and the role of each amino acid that is responsible for the cytoprotective process in ethanol-induced toxicity of HepG2 cell line treated with WSP can be understood.

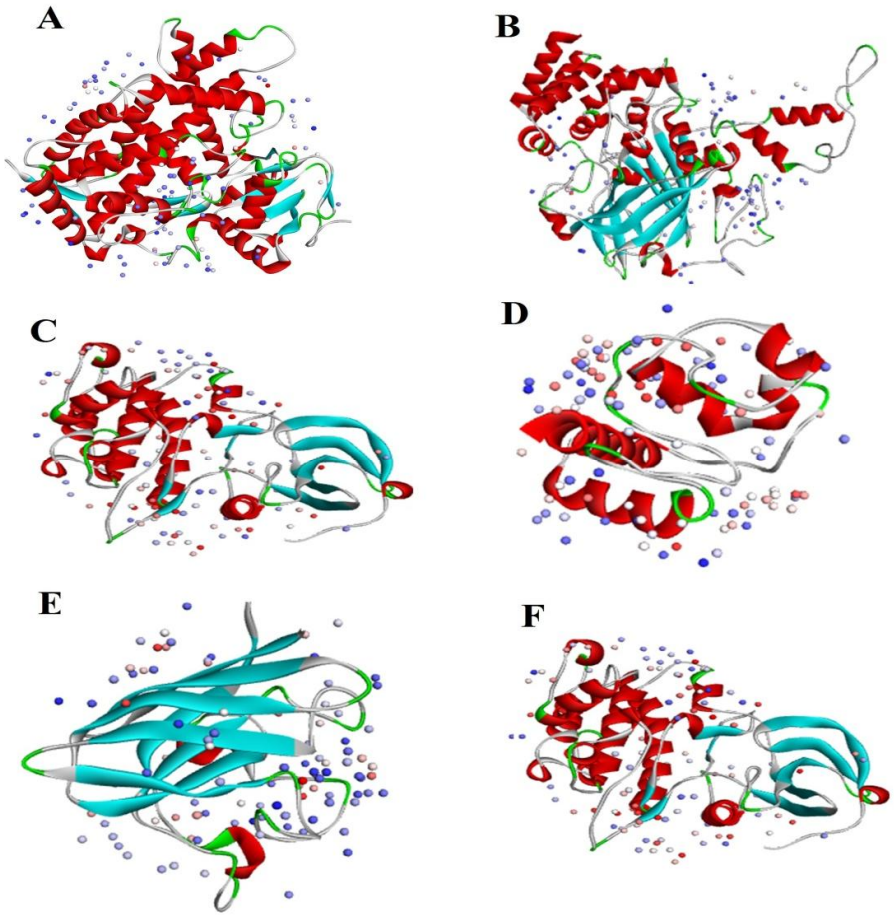

Fig. 1. Clusters of protein-protein complex A: CYP2E1-WSP, B: Catalase-WSP, C: Alcohol dehydrogenases-WSP, D: Cytochrome-C-WSP, E: Superoxide dismutase- WSP, F: Death-associated protein kinase1-WSP

Table 1. ZDOCK score and rDock score of six different protein-protein complex

Protein-protein complex	Top Poses	ZDOCK score	E_vdw1	E_elec1	E_vdw2	E_elec2	E_sol	E_Rdock
CYP2E1-WSP	Pose12	16.52	464.899	-3.3114	-74.145	-4.3712	-21.3	-25.234
Superoxide dismutase-WSP	Pose8	18.54	40.2918	-6.1728	-118.01	-23.564	-8.7	-29.908
Catalase-WSP	Pose37	13.78	-83.943	-0.2868	-80.633	-33.867	15.1	-15.381
Death-associated protein kinase1- WSP	Pose5	16.04	-108.35	-5.3801	-99.001	-31.12	10.4	-17.608
Alcohol dehydrogenases- WSP	Pose4	18.04	-112.2	-2.1281	-117.95	-21.087	3.5	-15.479
Cytochrome-C–WSP	Pose24	13.18	-65.351	-1.0656	-88.51	-25.816	-10.8	-34.034

**E_vdw1 = Energy of Vanderwaals 1, E_elec1 = Energy of electrostatic 1, E_vdw2 = Energy of Vanderwaals 2, E_elec2 = Energy of electrostatic 2, E_sol = Energy of solvation, E_RDOCK = Energy of RDOCK*

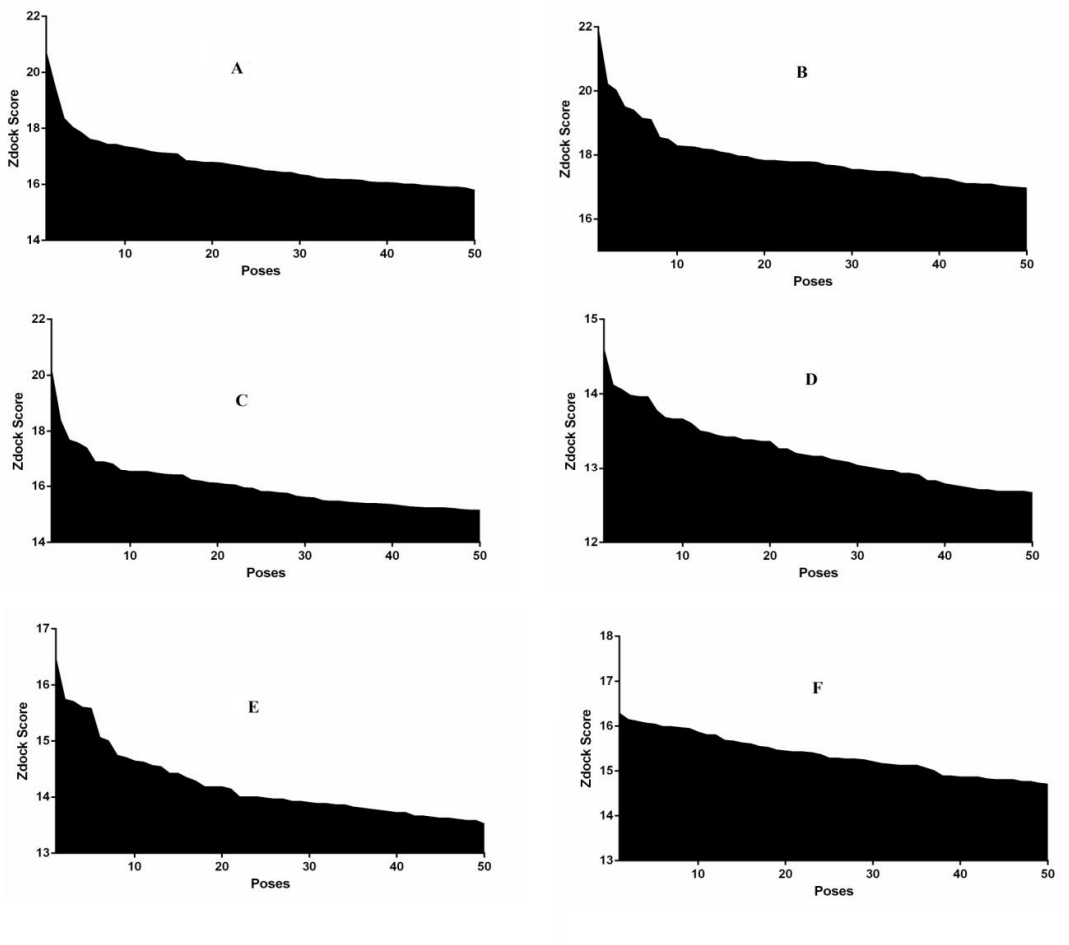

Fig. 2. Top 50 poses of protein-protein complex A: CYP2E1-WSP, B: Catalase-WSP, C: Alcohol dehydrogenases-WSP, D: Cytochrome-C-WSP, E: Superoxide dismutase- WSP, F: Death-associated protein kinase1-WSP

Table 2. Atomic level interaction of cytochrome-C-WSP complex

Protein-protein interaction	Distance in Å	Types of bond
A:LYS22:HZ1 - :GLU25:OE2	2.06718	Salt Bridge; Attractive Charge
A:LYS22:NZ - :ASP23:OD2	5.15691	Attractive Charge
A:LYS53:NZ - :GLU88:OE2	5.41421	Attractive Charge
A:LYS22:HZ2 - :ASP23:O	2.05613	Conventional Hydrogen Bond
A:GLY23:HN - :VAL50:O	2.73122	Conventional Hydrogen Bond
A:LYS53:HZ3 - :SER87:OG	2.55686	Conventional Hydrogen Bond
:ALA52:HN - A:ASN31:OD1	2.63241	Conventional Hydrogen Bond
:ALA52:HN - A:HIS33:O	2.16576	Conventional Hydrogen Bond
:SER87:HG - A:SER41:O	1.85915	Conventional Hydrogen Bond
:GLU88:CA - A:TYR48:O	3.57864	Carbon Hydrogen Bond
A:PRO44 - :PRO86	4.0154	Alkyl
:ALA52 - A:LEU32	5.04238	Alkyl
:ALA52 - A:LEU35	5.15636	Alkyl
A:HIS26 - :VAL50	4.37259	Pi-Alkyl
A:HIS33 - :ALA51	5.2077	Pi-Alkyl
:PHE53 - A:ARG38	4.77959	Pi-Alkyl

Table 3. Energy parameters of protein-protein complex of cytochrome-C-WSP complex

Name	Stage	Initial potential energy (kcal/mol)	Total energy (kcal/mol)	Potential energy (kcal/mol)	Kinetic energy (kcal/mol)	Temperature (K)	Vander waals energy (kcal/mol)	Electrostatic energy (kcal/mol)
Cytochrome-C- WSP	Mini1*	99074.86		-13199.50			-2049.1	-12999.09
	Mini2*	-13199.5		-16456.47			-1914.7	-16586.24
	Heat*	-16456.4	-10759.6	-13968.49	3208.87	303.10	-1592.8	-17051.46
	Equili*	-13968.4	-11449.1	-14688.98	3239.28	305.9	-1633.0	-17811.21
	Prod*	-14688.9	-12146.3	-15361.41	3215.07	303.6	-1635.0	-18514.58

**Mini1 = Minimization1, *Mini2 = Minimization2, *Heat = Heating, *Equili = Equilibration, *Prod = Production*

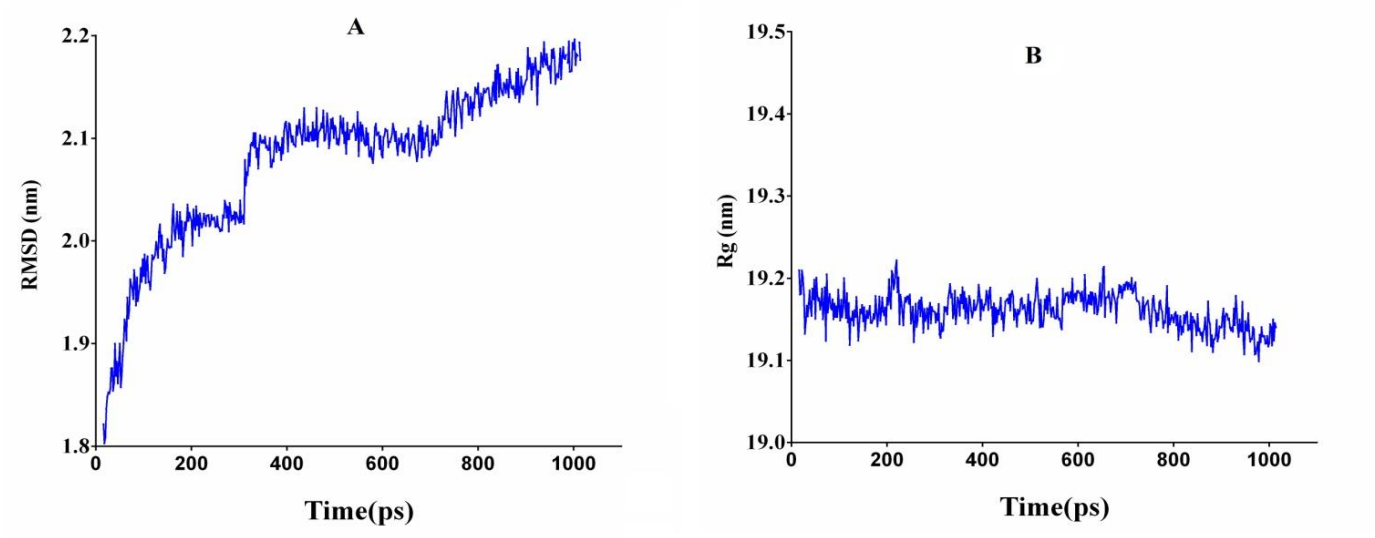

Fig. 3. Time dependent analysis A: Root-mean-square deviation (RMSD), B: Radius of gyration (Rg) of Cytochrome-C-WSP complex

3.3 Biological Significance

Liver disease induces hepatocyte apoptosis which is a complex biological process where various biochemical pathway will be activated [6]. A previous study by some researchers stated that the apoptosis process is more favourable through protein Cytochrome-C [14]. Another study on Ethanol-induced apoptosis in HepG2 cell line clearly revealed that the expression of CYP2E1 in the absence of bcl-2 will induce apoptosis [15]. CYP2E1 is also a powerful producer of reactive oxygen species [16]. Thus, binding of WSP to Cytochrome-C, CYP2E1 and Superoxide dismutase may reduce the Ethanol-induced toxicity and apoptosis in HepG2 cell line. From the rDock results it was clearly understood that WSP has more shape complementarity to bind with Cytochrome-C, CYP2E1 and Superoxide dismutase, thereby it may reduce the cell damage and protects the liver from injury. Hence, from the docking study, it was possible to understand the cytoprotective and therapeutic role of WSP.

3.4 Energy Parameter Analysis

The rDock top complex Cytochrome-C-WSP was subjected for 1ns molecular dynamics and simulation in five different stages. Initially, before simulation the potential energy of the complex was calculated and found to be unstable (99074.86 kcal/mol)**.** When the same complex was made to undergo for 500 steps minimization 1 and 2 with steepest decent, the conjugate gradient increased the stability of the complex. Details of energy of the complex in each step of dynamics is tabulated in Table 3. Finally, the production with NVT resulted in the more stable biological complex.

3.5 Dynamics Time Dependent Parameter Analysis

In molecular dynamics and simulation, each step in the process is time dependent because the energy of the complex may vary with respect to time. Crucial parameters of time dependent analysis of protein-protein or protein-ligand complex like root-mean-square deviation (RMSD) and radius of gyration (Rg) were determined. Fig. 3. A depicts root-mean-square deviation of the initial structure to various conformation, at the beginning of the dynamics the deviations were <1.9 nm and it started to elevate till 400 ps. After that, the conformations attained stable dynamics, not much difference in divergence were observed at the end of the 1ns dynamics. The compactness and protein folding during dynamics process was calculated using a radius of gyration. From the Fig. 3.B it is observed that folding of protein-protein complex structure was not changed over time and the deviations were within 19.1 to 19.2 nm.

4. CONCLUSION

The Protein-protein docking studies of WSP with proteins involved in Ethanol-induced toxicity and the proteins involved in its cytoprotective process in HepG2 cells showed that among docking with six different proteins, Cytochrome-C binding activity is more favourable for WSP to form a more stable complex with better ZDOCK and rDock score. From the results of molecular interaction, the binding action of Proteins and the cytoprotective role of WSP against Ethanol-induced toxicity of HepG2 cells were understood. This preliminary *insilico* study provides the atomic level details of binding of two proteins. Thus WSP can be used as therapeutic protein to cure Ethanol/Alcohol based liver diseases and can be an alternative to chemical drugs having side effects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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