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In Silico Studies On Membrane Protein Interaction With Alcohol Breakdown Products

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Abstract: Chronic heavy alcohol consumption disrupts normal organ function and causes structural damage in almost all body tissues. Thus, it is predicted that alcohol has a significant effect on red blood cells and other biochemical components of the blood stream. Studies assessing oxidative damage to erythrocyte membranes in alcoholic diabetics are closely related to associated biochemical and biophysical changes. The overwhelming body of evidence from human and animal studies clearly shows that chronic and heavy alcohol consumption causes structural damage and/or disrupts normal organ function in nearly all body tissues.

Index Terms – Alcohol, Acetaldehyde, Ethanol, membrane, *In silico*.

I. INTRODUCTION

Alcohol is known to readily enter the bloodstream and remain in a state of constant alcohol content for many years, with all cells and tissues being affected, particularly red blood cells and also affects biomembranes, because membranes are bilipid layers. The association of alcohol consumption with pancreatic diseases has been recognized for more than 100 years. The pancreas contains two functionally separate departments: the endocrine pancreas secretes insulin and glucagon, hormones that regulate blood sugar. As an exocrine gland, the pancreas produces zymogen precursors of digestive enzymes that are used to break down food in the intestine. Both departments can suffer the effects of chronic alcohol consumption (Paul *et al* ., 2021). In developed countries, chronic alcohol consumption is the second most common factor associated with acute pancreatitis. In up to 20% of cases, pancreatitis is associated with serious clinical complications with a mortality rate of up to 10%.(Wang *et al*., 2009). Both diabetes and excessive alcohol consumption are individually known to cause tissue damage and harm human health. Diabetes is a metabolic disease caused by a lack of insulin production or its action. According to recent estimates, around 285 million people worldwide (6.6%) suffer from diabetes, and by 2030 this number is expected to increase by 438 million people (7.8%) (Mohan, 2009 and King *et al*., 1998). The effects of diabetes are many. According to the International Diabetes Federation (IDF), there were approximately 425 million people with diabetes worldwide in 2017, and this number may increase to 629 million by 2045 (IDF, 2017). Globally 2/3rd of all deaths were estimated to occur due to non-communicable diseases especially CVDs, stroke, and DM. According to IDF, the Prevalence rate of

diabetes in India was 9.3%. Further the Indian diabetic population was 10.2% (578 million) by 2030 and it was predicted to grow up to 700 million by 2045 it could be on-going large-scale urbanization and increasing life expectancy (IDF, 2019).

By the late 1960s, only a few studies were published that reported the prevalence of DM. The prevalence of DM in Ahmedabad, Kolkata, Cuttack, Pune and Trivandrum was estimated to be 1.5% in rural areas and 2.1% in urban areas (Ahuja, 1979). The National Urban Diabetes Survey in the 1990s reported a prevalence of 9.3% in Mumbai and 16.6% in Hyderabad (Ramchandran et al., 2001). The prevalence of diabetes varies across countries, states and/or regions, with the prevalence of DM being higher in northern India than in southern India (Mote et al., 2016). The prevalence of DM has almost doubled (9–17%) in urban India over the past 20 years, but quadrupled (2–9%) in rural India (Gupta et al, 2012). The results of the ICMR-INDIBAD population-based cross-sectional study showed that the overall prevalence of DM in fifteen states was 7.3% (Andhra Pradesh, Bihar, Gujarat, Karnataka, Punjab, Assam, Mizoram, Arunachal Pradesh, Tripura, Manipur, Tamil Nadu , Chandigarh, Maharashtra, Jharkhand and Meghalaya) and the prevalence was lowest in Bihar (4.3%) and highest in Punjab (10.0%). The prevalence of diabetes in urban and rural areas of Andhra Pradesh is 12.6% and 6.3%; in Gujarat; Bihar, 10.8% and 6.3%; Gujarat, 9.8% and 5.2%; Karnataka, 11.01% and 5.6%; Punjab, 12.0% and 8.7%; Arunachal Pradesh, 5.8% and 4.9%; Assam, 12.4% and 4.4%; Manipur, 7.1% and 4.4%; Meghalaya, 8.9% and 3.5%; Mizoram 7.9% and 3.6% and Tripura 15.5% and 7.2%. In rural areas of all the above 15 states, people with higher socioeconomic status (SES) had a higher prevalence of DM, but in Chandigarh, Maharashtra and Tamil Nadu, people with lower SES had higher rates of DM. The overall prevalence of prediabetes in all 15 states studied was 10.3%, which was different from 6.0% in Mizoram and 14.7% in Tripura. The ratio of diabetes to pre-diabetes was lower in Northeast India (1:1.8) than in mainland India (1:1.2). Prevalence of DM was significantly higher in urban than rural areas in all age groups, but DM is lower in women than in men aged 35-65 years (Anjana et al., 2017).

Chronic excessive alcohol consumption is a major public health problem and one of the main causes of liver disease (Sherlock and Dooley, 2008; Bruha et al., 2012; WHO, 2018). Excessive alcohol consumption is associated with the development of alcoholic liver disease (ALD), a well-described spectrum of diseases ranging from fatty liver accumulation (steatosis) to cirrhosis and hepatocellular carcinoma (Miller et al., 2011; Ceni et al., 2014) .). . It is estimated that every third person in the world drinks alcohol (GBD 2016 Alcohol Collaborators, 2018). Although drinking patterns vary by country, the overall health burden of alcohol consumption is high and is among the top ten risk factors for death and disability-adjusted life years (DALYs). GBD 2016 Alcoholic Collaborators (2018).

Alcohol consumption plays an important role in various societies and is a common meal in many parts of the world. It accounts for 4–6% of total energy consumption in most countries (Block et al., 1985; Thomson et al., 1988; Christian et al., 1994). It is often claimed that it makes life more bearable in our stressful world by dampening painful emotions and promoting pleasant ones (Madan et al., 1987). Although alcohol use is generally considered an abuse (Hoek and Tarasachi et al., 1988), it also has many

beneficial effects when used in moderation. However, excessive alcohol consumption leads to addiction, toxicity and many unnecessary health disorders and social problems. Chronic alcohol consumption is known to affect every tissue in the body.

Excessive chronic alcohol consumption damages all organs and parts in humans and parts in humans and animals exclusively through ethanol. Evidence and accumulated literature strongly suggest that increased oxidative stress, depletion of antioxidant levels, excessive production of NO, disregulated cytokine metabolism and activity leading to TNF release are crucial in the development of alcohol damage. In addition, it is clear from previous studies that several plants are associated with alcohol-related diseases and injuries (resulting in multiple complications), requiring multi-target interventions.

Therefore, the aim of this study is to systematically investigate the changes in diabetics who consume alcohol, paying particular attention to the molecular interactions of ethanol and its compounds with red blood cell membrane proteins such as spectrins, ankyrin, Band-3 and protein. 4.1, 4.2. 4,9 and the activity of various enzymes that play an important role in oxidative and nitrosative stress, causing hemolysis.

MATERIALS & METHODS

Hardware Components : The aim of this work is to systematically study the changes in diabetics who consume alcohol, paying attention to the molecular interaction of ethanol and its compounds with erythrocyte membrane proteins such as spectrins, ankyrin, Band-3 and protein. 4.1, 4.2. 4,9 and the activity of various enzymes that play an important role in oxidative and nitrosative stress, causing hemolysis.

Software Components : Most of the softwares used were either Windows or Linux platform based which were well accepted and referred in various publications at high rated research journals. Academic license was obtained for the commercial software used in the present study by requesting the concerned suppliers. The online tools/software used in the present study was briefly detailed below.

ON LINE TOOLS

NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION (NCBI)

NCBI is part of the US National Library of Medicine (NLM), a branch of the NIH, located in Bethesda, Maryland and founded in 1983. NCBI maintains genome sequence data in GenBank and a biomedical article index in PubMed. Central and PubMed and other biotechnology related information. All of these databases are available online through the Entrez search engine (www.ncbi.nlm.nih.gov/).

PROTEIN DATA BANK (PDB)

The Protein Data Bank (PDB) is a repository of three-dimensional structural data for proteins and nucleic acids. These data, usually obtained by X-ray crystallography or NMR spectroscopy, have been collected by biologists and biochemists around the world, are published publicly, and are freely available. ATE's mission

is to maintain a single protein database archive of macromolecular structure data that is freely and publicly available to the global community (www.rcsb.org/pdb).

PDBSUM

PDBsum is an image database that provides a snapshot of the contents of each 3D structure stored in the Protein Data Bank (PDB). This server provides cracks and wrinkles on the surface of protein molecules stored in the protein database. Pdbsum can also provide ligand binding sites using Ligandplot plots in two dimensions (http://www.ebi.ac.uk /pdbsum/).

The active site amino acid residues of given protein was performed with the PDBSUM which mainly functions based on its alignment to the crystal structure templates from PDB. Based on high identity with active site residues from the crystal structure, the residues of active site of target protein have been established perfectly. The crystal structure was submitted to PDBSUM server which provides the catalytic sites and from that one can determine the conserved and catalytical residues in the active site of the built protein model by sequence alignment.

Molinspiration Server: The JME Molecular Editor is a Java applet that allows you to design/edit molecules and reactions (including generating substructure queries) and describe molecules directly on an HTML page. The editor can create a Dayligh SMILES or MDL mol file from the generated structures. The software was developed by Peter Ertl at Comenius University in Bratislava and later improved by Ciba-Geigy in Basel. Due to many requests, the applet was released to the public and became the standard for molecular structure entry on the web with more than 10,000 installations. In recognition of this generous gesture, Molinspiration offers this space to JME Home. Molinspiration can also help with JME installation and deployment. (http://www.molinspiration.com/docu/webme/).

PyMol is an advanced molecular graphics tool for Python; It facilitates 3D visualization of proteins, small molecules, density, surfaces and trajectories. Almost a quarter of all images of 3D protein structures published in the scientific literature are made with PyMOL. (http://www.delano-science.com/).

Visual molecular dynamics (VMD): It is a molecular modeling and visualization computer program. VMD is primarily developed as a tool for viewing and analyzing the results of molecular dynamics simulations, but it also includes tools for working with volumetric data, sequence data, and arbitrary graphics objects. (http://www.ks.uiuc.edu/Research/vmd/).

Hyperchem: Hyperchem is an advanced molecular modeling environment known for its quality, flexibility and ease of use. Combining 3D visualization and animation with quantum chemical calculations, molecular mechanics and dynamics, Hyperchem puts more molecular modeling tools at your fingertips than any other Windows program (http://www.hyper.com/).

Molecular docking : Molecular docking technique is the famous in structural bioinformatics to solve the problems in protein and ligand interaction studies. A molecule is characterized by a pair (A;B),in which A represents a collection of atoms, and B represents a collection of bonds between pairs of atoms. Information used for kinematic and energy computations is associated with each of the atoms and bonds. Each atom carries standard information, such as its Waals radius. Three pieces of information are associated with each bond: (i) the bond length is the distance between atom center; (ii)the bond angle, is the angle between two consecutive bonds; (iii) whether the bond is rotatable or not. Since bond lengths and angles do not affect significantly the shape of a molecule, it is common practice to consider them fixed. Thus the degrees of freedom of the molecule arise from the rotatable bonds. The three dimensional embedding of a molecule defined when we assign values to its rotatable bonds is called the conformation of the molecule. Ligands typically have 3 to15rotatable bonds, while receptors have 1,000 to 2,000 rotatable bonds. The dimension of the combined searched space makes the docking problem computationally intractable. One key aspect of molecular modelling is calculating the energy of conformations and interactions. This energy can be calculated with a wide range of methods are ranging from quantum mechanics to purely empirical energy functions. The accuracy of these functions is usually proportional to its computational expense and choosing the correct energy calculation method is highly dependent on the application. Computation times for different methods can range from a few milliseconds on a workstation to several days on a massively parallel supercomputer. In the context of docking, energy evaluations are usually carried out with the help of a scoring function and developing these is a major challenge facing structure based drug design. Scoring functions are a critical part of the structure based drug design process. No matter how efficient and accurate the geometric modelling of the binding process is, without good scoring functions it is impossible to obtain correct solutions. The two main characteristics of a good scoring function are selectivity and efficiency. Selectivity enables the function to distinguish between correctly and incorrectly docked structures and efficiency enables the docking program to run in a reasonable amount of time.

AutoDock : The program AutoDock was developed to provide an automated procedure for predictingtheinteractionofligandswithbiomacromoleculartargets.The motivation for development of AutoDock software arises from problems in the design of bioactive compounds, and in particular the field of computer aided drug design. Progress in bio molecular x-ray and NMR crystallography continues to provide a number of important protein and nucleic acid Structures. These structures could be targets for bioactive agents in the control of animal and plant diseases, or simply key to understanding of a fundamental aspect of biology. The precise interaction of such agents or candidate molecules is important in the development process. Indeed, AutoDock can be a valuable tool in the x-ray structure determination process itself: given the electron density for a ligand, AutoDock can help to narrow the conformational possibilities and help identify a good structure, in any docking scheme two conflicting requirements must be balanced: the desire for a robust and accurate procedure, and the desire to keep the computational demands at a reasonable level.

Preparation of files for AUTODOCK

The advanced molecular docking program AutoDock 4.2 which uses a powerful Lamarckian genetic algorithm (LGA) method for conformational search and docking, was applied for the automated molecular docking simulations. Briefly, the LGA described therelationship between the antagonists and receptors by the translation, orientation, and conformation of the antagonists. These so-called 'state variables' were the ligands' genotype, and the intramolecular energies were the antagonists' phenotype. The environmental adaptation of the phenotype was reverse transcribed into its genotype and became heritable traits. Each docking cycle or generation, consisted of regimen of fitness evaluation, crossover, mutation, and selection. The docked structures of the ligands were generated after a reasonable number of evaluations. The whole docking scheme could be stated as follows. First, the receptor molecules were checked for polar hydrogen and assigned for partial atomic charges, the PDBQT file was created, and the atomic salvation parameters were also assigned for the macromolecules. Meanwhile, all of the torsion angles of the antagonists that would be explored during molecular docking stage were defied. Therefore, it allowed the conformation search for ligands during molecular docking process.

Identification of protein target

The functional and structural characteristics of RBC membrane proteins such as α-Spectrin, β- Spectrin, Ankyrin, Band-3, Protein-4.1, Protein-4.2 and Protein-4.9 (1-0) were established with various Bioinformatics tools. The 3D (Three-dimensional) structures of these proteins were retrieved from RCSB protein databank with molecular information are as shown below. The α-Spectrin (PDBID: 3NGP, resolution factor: 1.08 Å.), β- Spectrin (PDBID: 3F57, resolution factor: 2.90 Å), Ankyrin (PDBID:4D80, resolution factor: 3.60 Å), Band-3 (PDBID: 1HYN, resolution factor: 2.60 Å), Protein-4.1 (PDBID: 4P1T, resolution factor: 2.90 Å), Protein-4.2 (PDBID: 7V0Q, resolution factor: 2.50 Å) and Protein-4.9 (PDBID: 1QZP, resolution factor: 2.20 Å). This serves as a useful resource to establish pharmacophore analysis of structure based proteins. Using the CASTp calculations analysis, the computational model of active site amino acids were predicted with on line protocols. A graphical database called PDB sum provided overview of the information for each 3D structures of various erythrocyte membrane proteins along with active site amino acids present in every proposed membrane protein (https://pwww.rcsb.pdb.org).

Molecular Interaction studies using Docking Tool:

The compounds such as Ethanol, Acetate and Acetaldehyde ligand molecules were retrieved from PubChem compound database (https://pubchem.ncbi.nlm.nih.gov/). By using professional molinspiration (http://www.molinspiration.com/cgi-bin/properties) and Hyperchem 7.5. the drug-likeness properties along with the pharmacophore and biological activity against different proposed erythrocyte membrane proteins has been calculated.

AutoDock Vina (Eberhardt,, 2021 and Trott, et al., 2010) is an efficient and open-source program for protein–ligand docking and claims to improve the average accuracy of the binding mode predictions compared to the earlier version AutoDock 4.2., which is improved in terms of speed and accuracy of docking with a new scoring function, efficiently optimized for multithreading. Therefore, the AutoDock Vina was used for virtual screening. The grid box was set with a grid volume of 8, 14, and 14 for X-axis, Y-axis, and Z-axis respectively with the grid center values of 3.194, 43.143, 69.977 respectively for X, Y, and Z centres. The results were tabulated based on the binding energy and physical contacts of the sorted ligands. The interactions were visualized in 2D using LigPlot V 1.4.5. The ligand–protein interactions were further visualized in 3D with PyMol.

Results and Discussion :

Absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of lead molecules/compounds play vital roles in every stage of drug discovery and development. Molinspiration supports for calculation of important molecular properties (logP, polar surface area, number of hydrogen bond donors and acceptors and others), as well as prediction of bioactivity score of ethanol, acetate and acetaldehyde for the most important drug targets (GPCR ligands, kinase inhibitors, ion channel modulators, nuclear receptors) (Table 1).

Molinspiration is a web-based tool used to predict the bioactivity score of ethanol, acetate and acetaldehyde against regular human receptors such as GPCRs, ion channels, kinases, nuclear receptors, proteases and enzymes (Table 2).

Table.1. ADMET properties for selected break down compounds of Alcohol

Sl.no	Ligand	LogP	TPSA	n atoms	MW	n ON	n OHNH	n robt
1.	Ethanol	0.06	20.25	2	46.07	1	1	0
2.	Acetate	-2.70	40.13	4	59.04	2	0	0
3.	Acetaldehyde	0.51	17.07	3	44.05	1	0	0

mi LogP (Octanol–water dissolution)180–500 kDa, hydrogen bond acceptor (nON)>1–10; hydrogen bond donor (nOHNH)>1–5, Number of Rotatable Bonds (n robt)>1–12; Volume (Vol)>180–500

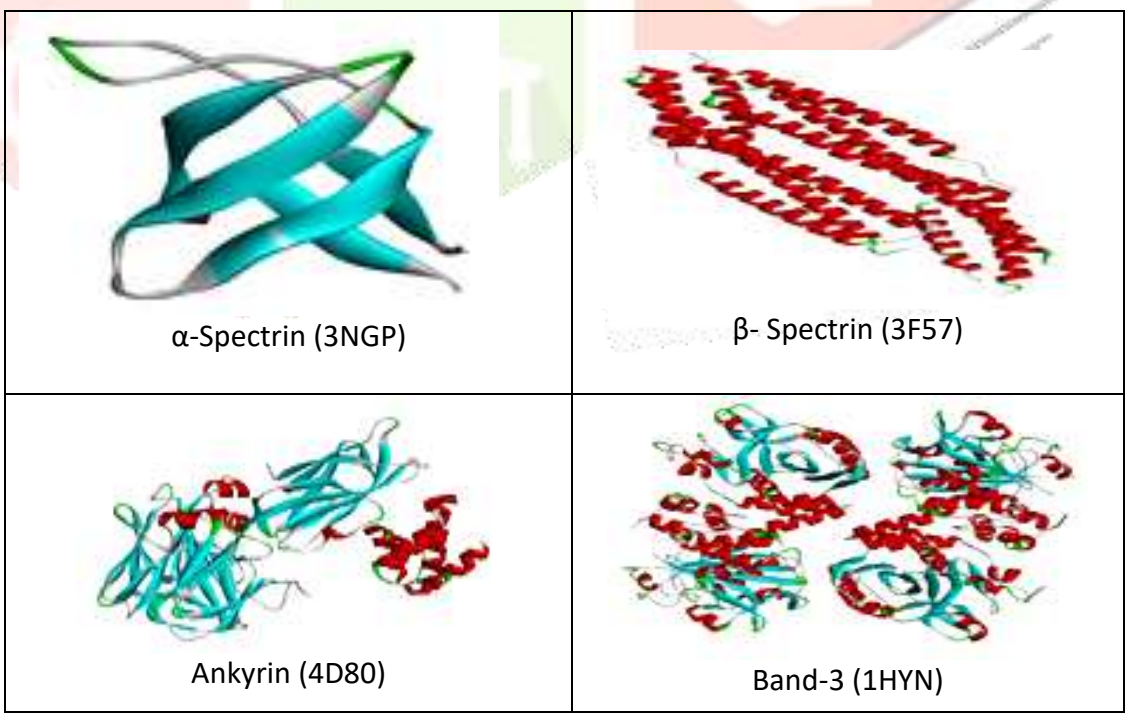
Table.2. Bioactivity score for selected break down compounds of Alcohol

Sl.no	Ligand	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1.	Ethanol	-3.81	-3.79	-3.84	-3.72	3.77	3.76
2.	Acetate	-3.80	-3.77	-3.82	3.79	-3.78	-3.77
3.	Acet-aldehyde	-3.85	-3.77	-3.87	-3.69	-3.27	-3.72

These bioactivity scores for organic molecules can be interpreted as active (when the bioactivity score > 0), moderately active (when the bioactivity score lies between −5.0 and 0.0), and inactive (when the bioactivity score < −5.0).

The 3-D structures of various erythrocytes membrane proteins which play major role in maintenance of integrity of RBC membrane, were down loaded from PDB data bank with on line tools and depicted below Figure.1. These 3D structures of various RBC membrane proteins were subjected to docking studies to establish the molecular interaction with Ethanol, Acetate and Acetaldehyde by using AutoDock-V.3.0.

Figure.1. 3D structure of some erythrocyte membrane proteins selected for molecular interaction with Ethanol, Acetate and Acetaldehyde.



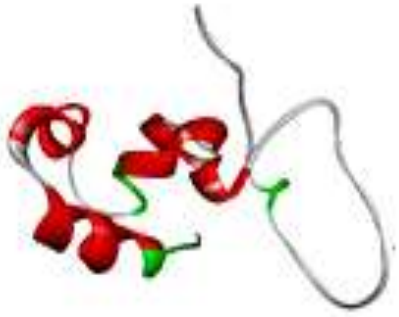
Active site amino acids are computationally predicted for all the erythrocyte membrane proteins proposed in the present study. Distal residues are biochemically important in enzyme activity (Table 3). Local arrays of active residues can be used to predict biochemical function. Interactions between residues are important to consider in protein design.

Table :3. Active site amino acids of erythrocyte membrane proteins (α-Spectrin, β- Spectrin, Ankyrin, Band-3, Protein-4.1, Protein-4.2, Protein-4.9, NaK Atpase, Mg Atpase, Ca Atpase)

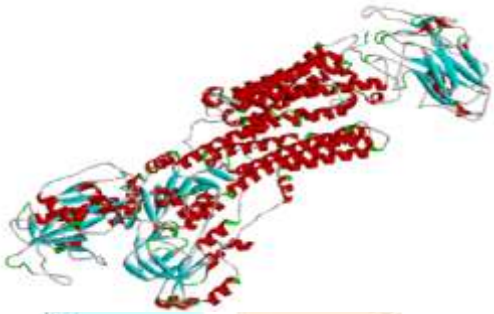
Sl. No	Proteins	PDB ID	Interference surface area (A^0)	Active amino acids
1.	α-Spectrin	3NGP	582	Arg233, Tyr278, Tue189, Ile276, Lue189.
2.	β- Spectrin	3F57	923	Thr1726, Ser1715, Glu1755, Arg1733, Arg1737.
3.	Ankyrin	4D80	963	Thr1188, Gln1164, Met1316, Arg1265.
4.	Band-3	1HYN	2545	Asp277, Ala273, Ile276, Ser106.
5.	Protein-4.1	4P1T	695	Arg1349, Lys1455, Arg1349, Arg1628, His1345.
6.	Protein-4.2	7V0Q	658	Arg563, Val368.
7.	Protein-4.9	1QZP	405	Arg66, Arg40.
8.	NaK Atpase	4XE5	842	Asp885, Pro123, Glu122, Asp889, Ile320, Asp126.
9.	Mg Atpase	3GWI	898	Arg529, Gln528
10.	Ca Atpase	1SU4	562	Gly210, Ile170, Leu230, Glu165, Leu230.

For establishing the molecular interactions of ethanol, acetate and acetaldehyde with various erythrocyte membrane proteins, AutoDock tool was used for virtual screening with grid box and grid volume set with standard conditions as described in material methods. The results were tabulated based on the binding energy and physical contacts of the sorted ligands. The interactions at molecular level involving active site amino acids of various erythrocyte membrane proteins with ethanol, acetate and acetaldehyde (bond angles, bond distance etc.,) were visualized with LigPlot V 1.4.5. and PyMol. and summarised in pictorial form as shown below.

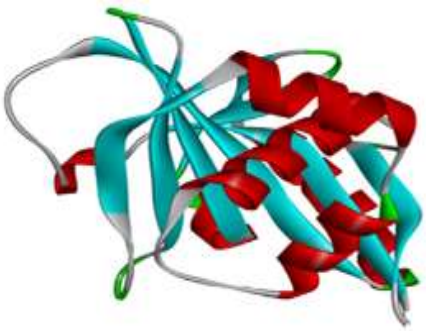
Protein-4.2 (7V0Q)



Protein-4.9 (1QZP)



NaK Atpase (4XE5)



Mg Atpase (3GWI)

Figure 1. Molecular Binding interaction of α-Spectrin with Ethanol, Acetate and Acetaldehyde

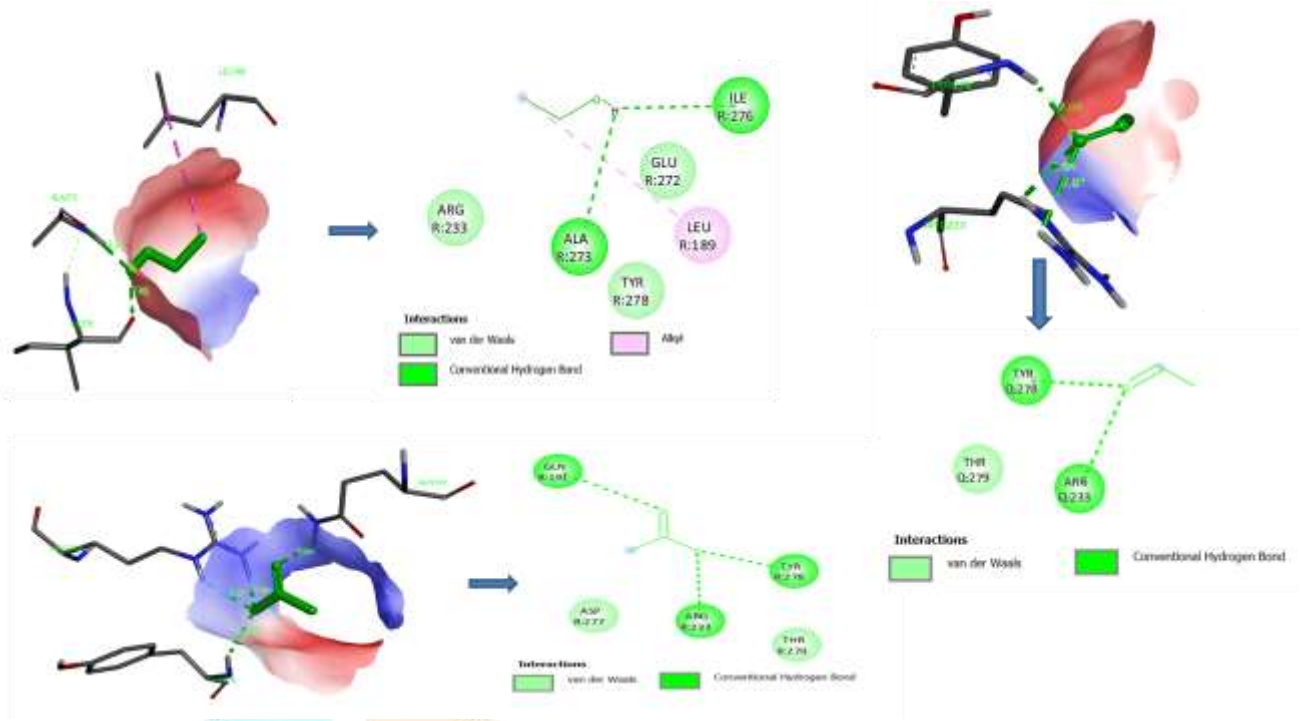


Figure 2. Molecular Binding interaction of β- Spectrin with Ethanol, Acetate and Acetaldehyde

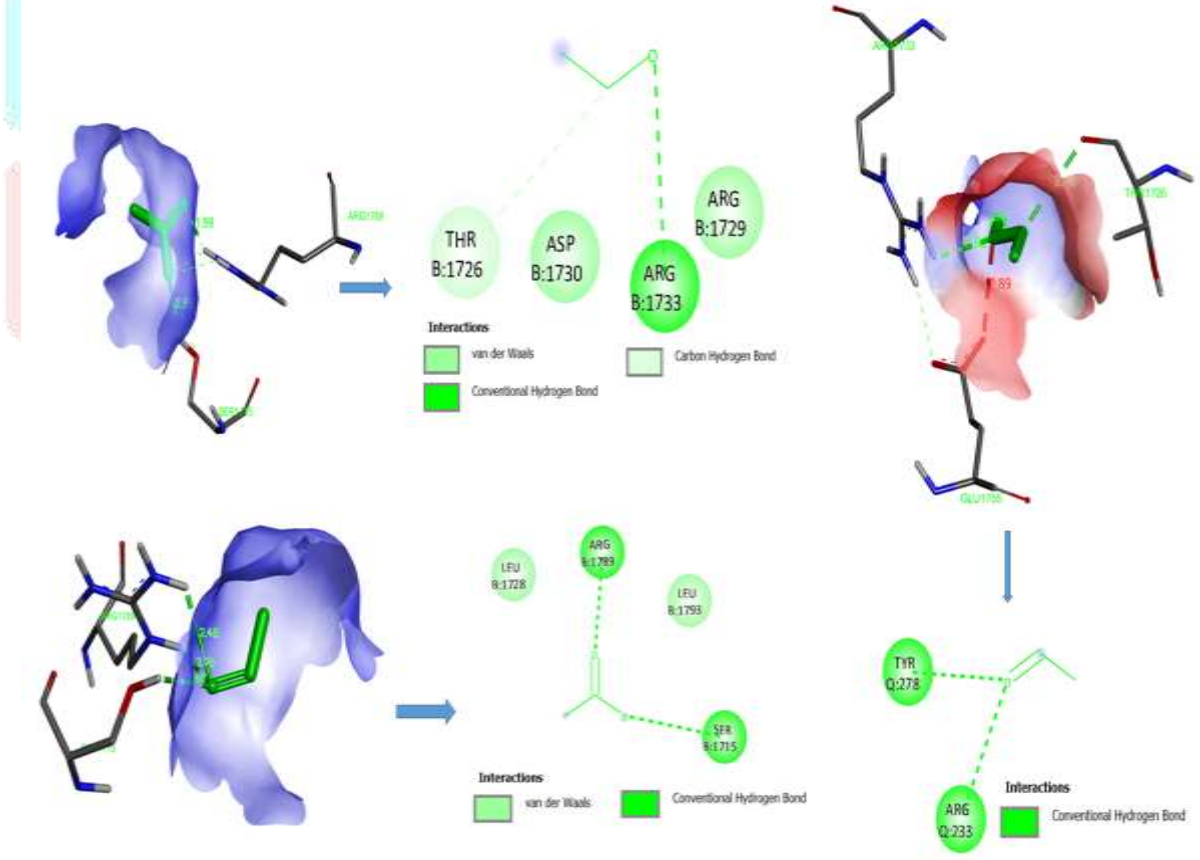


Figure 3. Molecular Binding interaction of Ankyrin with Ethanol, Acetate and Acetaldehyde

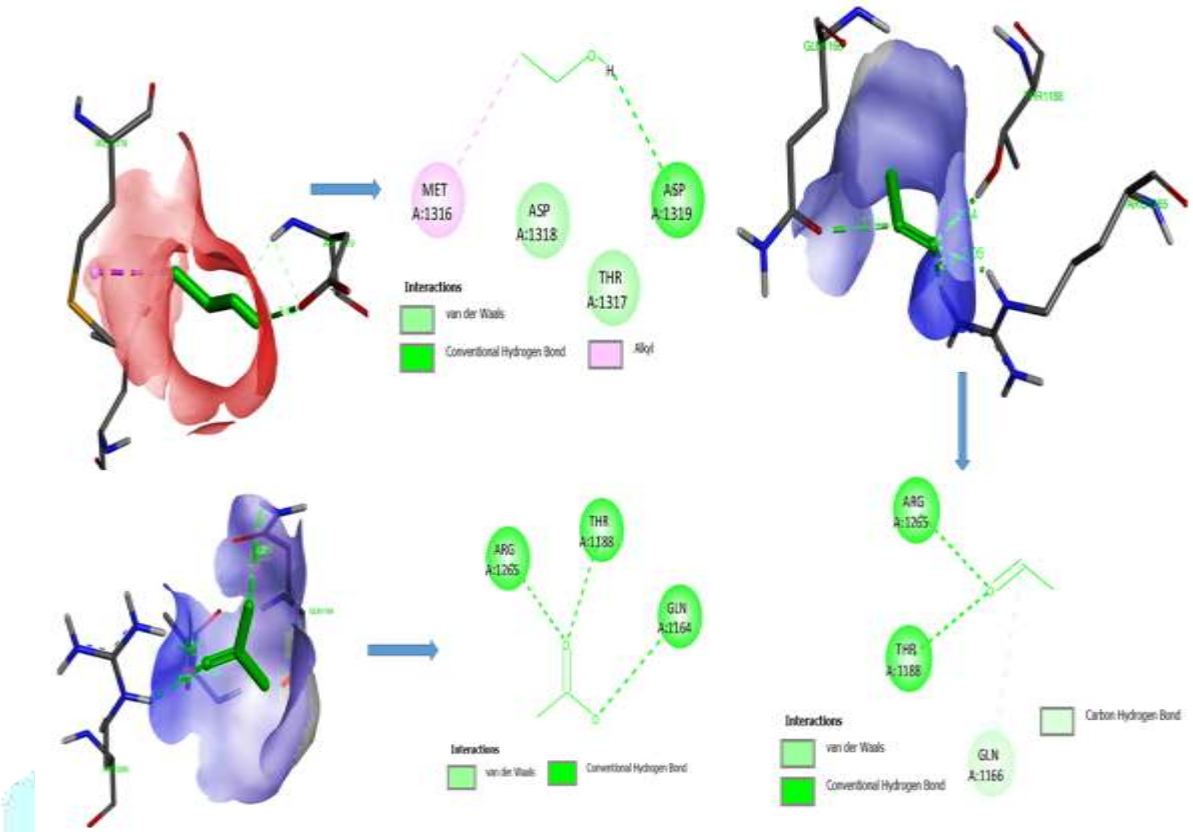


Figure 4. Molecular Binding interaction of Band-3 with Ethanol, Acetate and Acetaldehyde

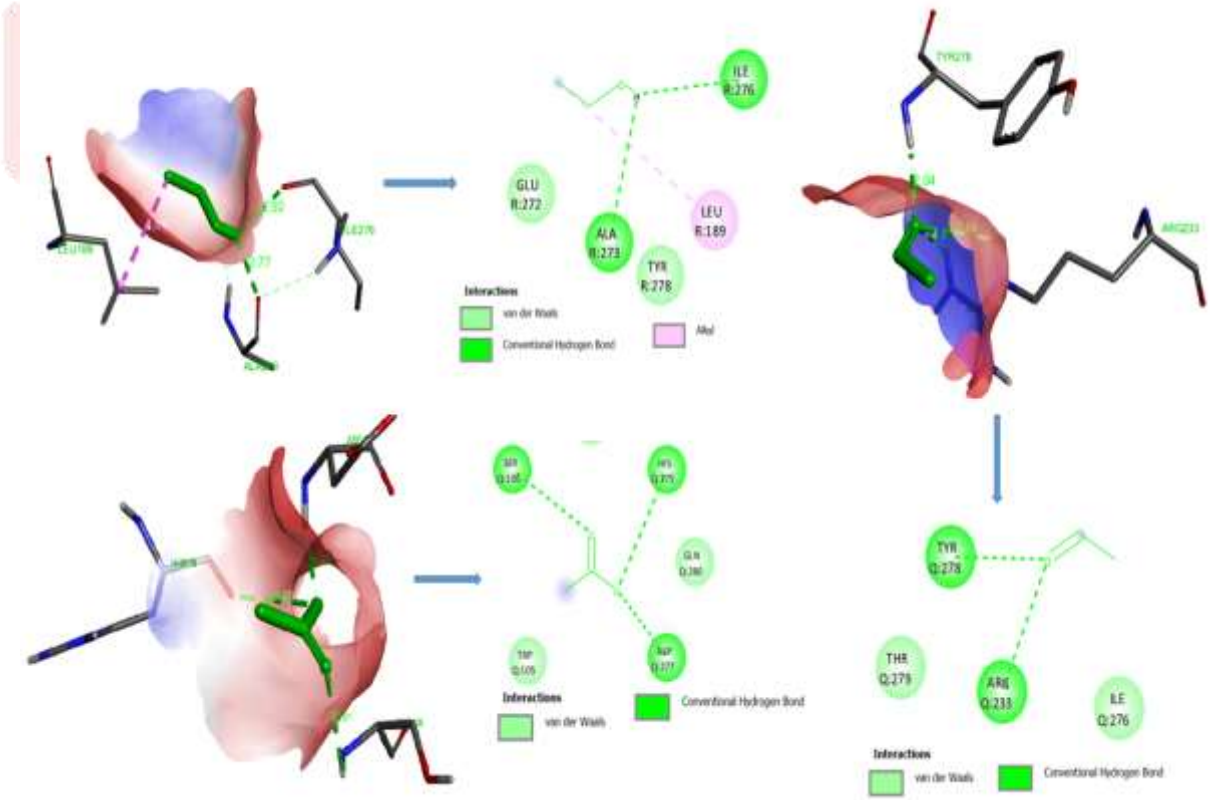


Figure 5. Molecular Binding interaction of Protein-4.1 with Ethanol, Acetate and Acetaldehyde

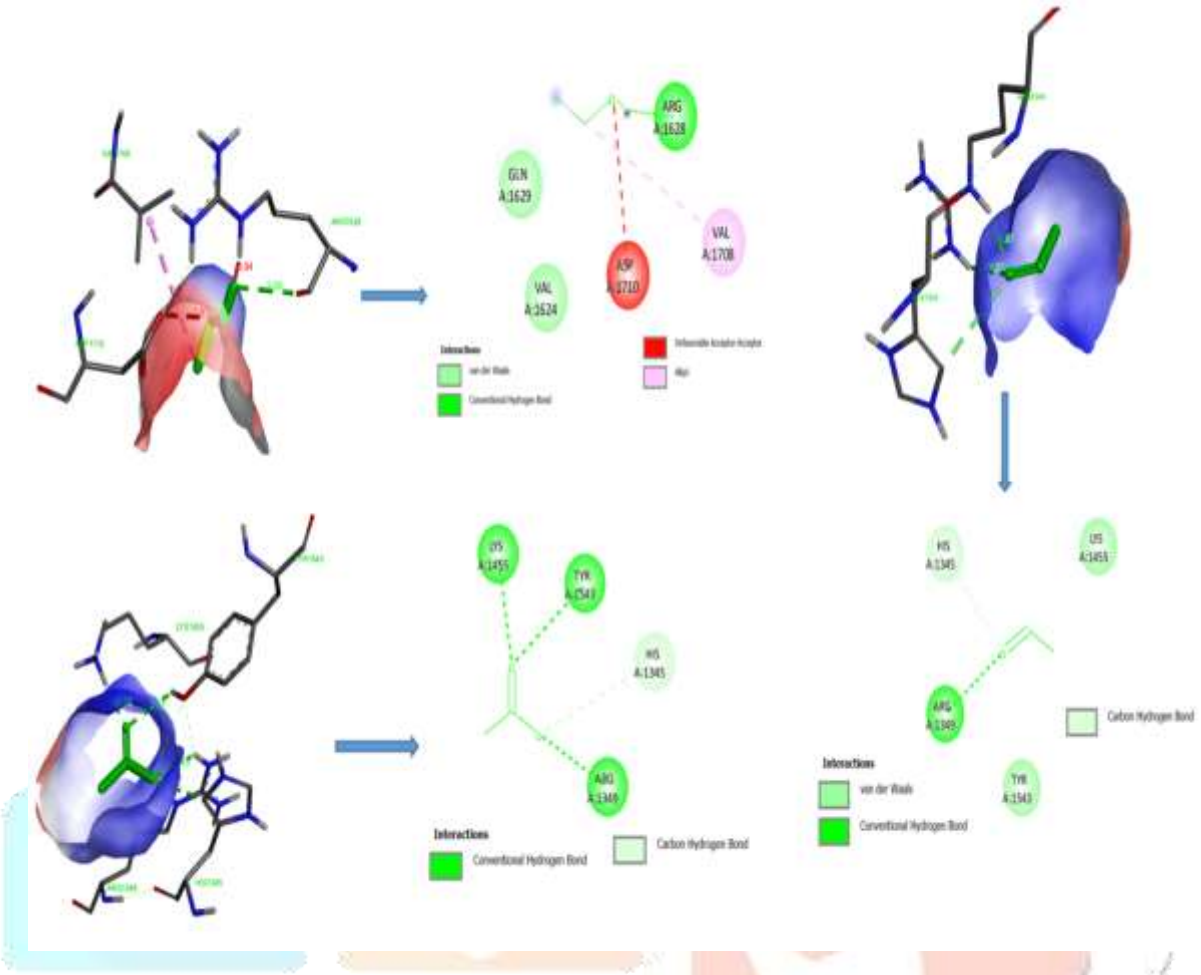


Figure 6. Molecular Binding interaction of Protein-4.2 with Ethanol, Acetate and Acetaldehyde

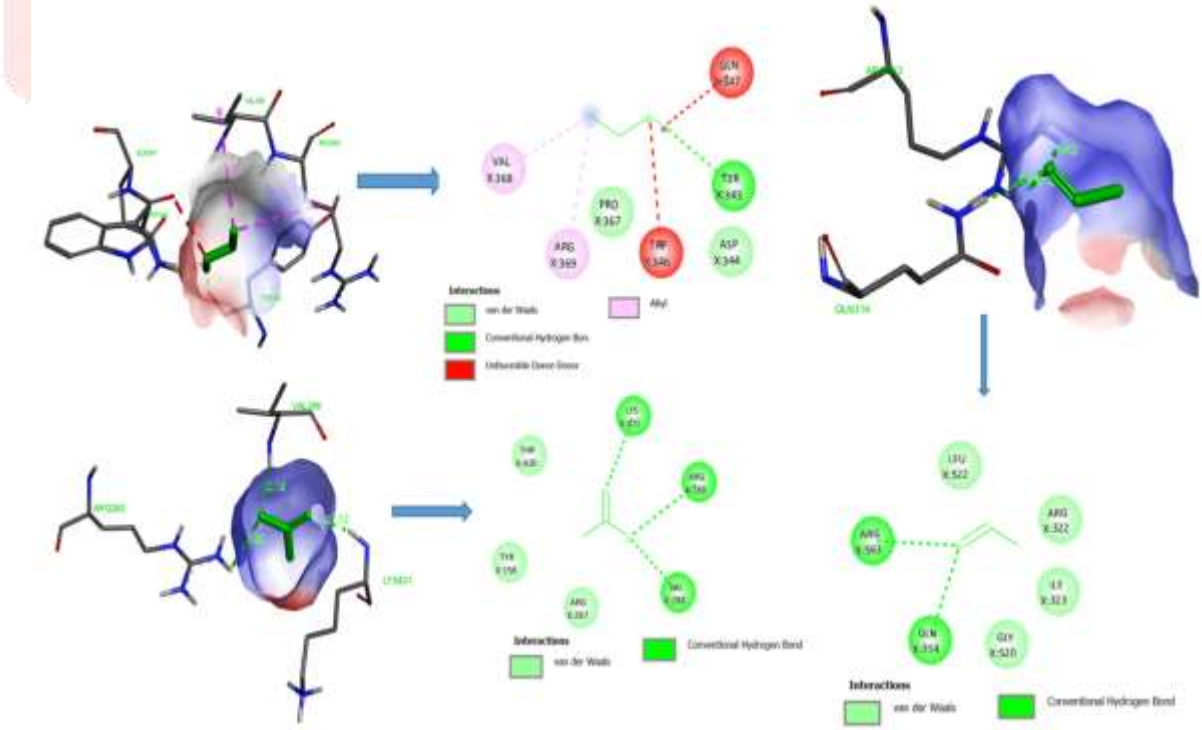


Figure 7. Molecular Binding interaction of Protein-4.9 with Ethanol, Acetate and Acetaldehyde

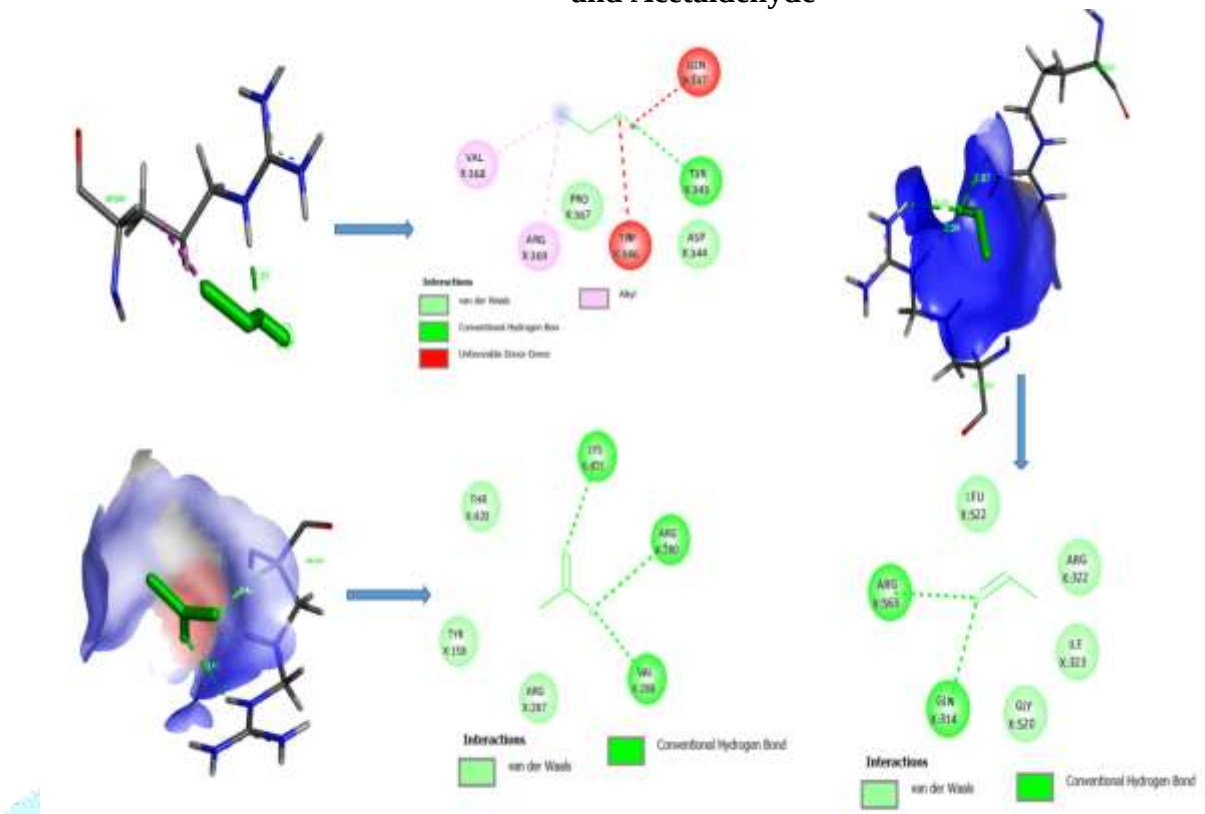


Figure 8. Molecular Binding interaction of NaK Atpase with Ethanol, Acetate and Acetaldehyde

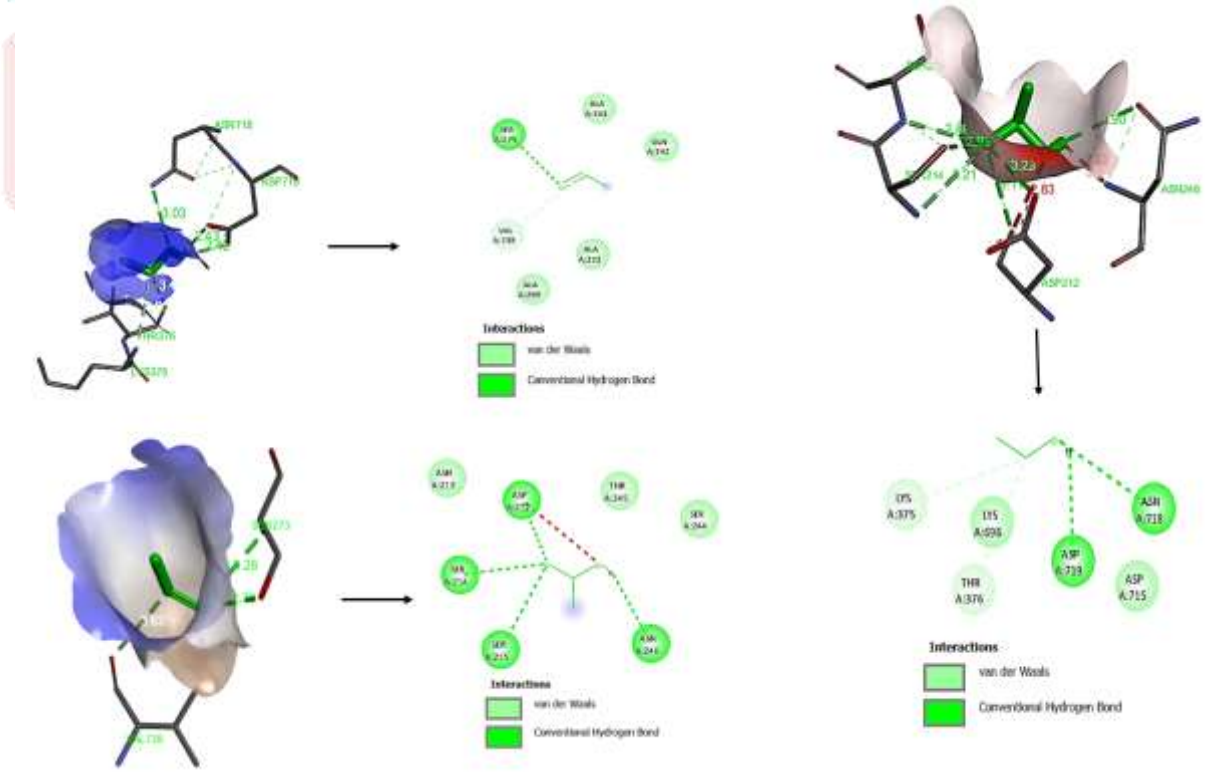


Figure 9. Molecular Binding interaction of Mg Atpase with Ethanol, Acetate and Acetaldehyde

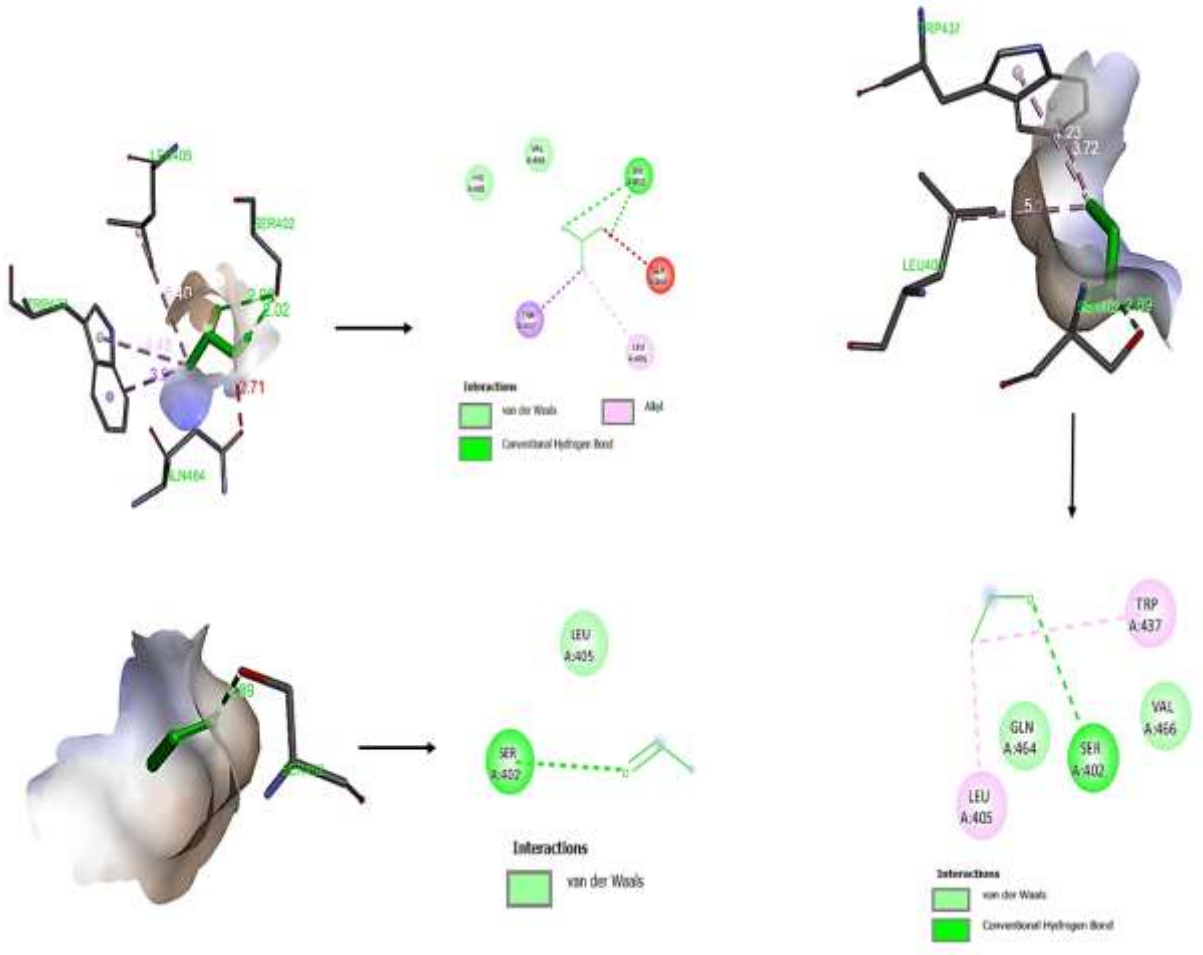
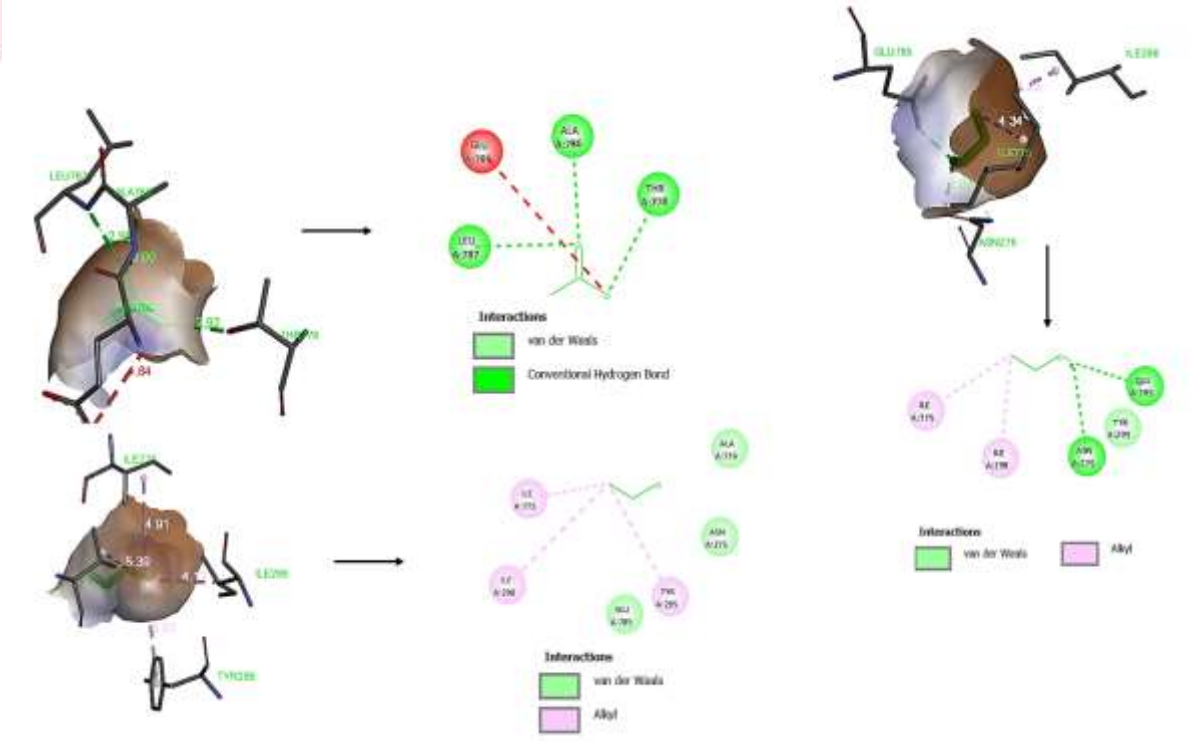


Figure.10. Molecular Binding interaction of Ca Atpase with Ethanol, Acetate and Acetaldehyde



The obtained results showing, based on docking studies, clearly exhibit which compounds are having more affinity towards various erythrocyte membrane proteins. The Protein α-Spectrin has a docking score

of -3.1 (kcal/mol) which forms three hydrogen bonds with Arg1789, Ser1715 bond (Figure 1). The Protein β-Spectrin has a docking score of -3.2 (kcal/mol) which forms two hydrogen bonds with Tyr278, Gln191 and Arg233 bond (Figure 2). The Protein Ankyrin has a docking score of -2.7 (kcal/mol) which forms three hydrogen bonds with Arg1265, Thr1188 and Gln1164 bond (Figure 3). The Protein Protein-4.1 has a docking score of -3. (kcal/mol) which forms four hydrogen bonds with Lys1455, Tyr1543, Arg1349 and His1345 bond (Figure 4). The Protein Protein-4.2 has a docking score of -3.5 (kcal/mol) which forms three hydrogen bonds with Lys421, Arg280 and Val288 bond (Figure 5). The Protein Protein-4.9 has a docking score of -2.4 (kcal/mol) which forms three hydrogen bonds with Lys421, Arg280 and Val288 bond (Figure 6). The Protein Band-3 has a docking score of -3.1 (kcal/mol) which forms three hydrogen bonds with Asp277, Ser106 and His275 bond (Figure 7). NaK Atpase has a docking score of -3.1 which forms three hydrogen bonds with four hydrogen bonds Asn246, Ser215, Ser215 and Asp212 (Figure 8). Mg Atpase has a docking score of -2.3 which forms three hydrogen bonds with three hydrogen bonds Ser402, Trp437 and Leu405 (Figure 9). Ca Atpase has a docking score of -3.1 which forms three hydrogen bonds with three hydrogen bonds Lue787, Ala786 and Thr778 (Figure 10).

Table :4. Analysis of Docking studies of Erythrocyte membrane proteins (α-Spectrin, β- Spectrin, Ankyrin, Band-3, Protein-4.1, Protein-4.2, Protein-4.9, NaK Atpase, Mg Atpase, Ca Atpase) against alcohol breakdown compounds)

Protein name	Compound	Docking score (kcal/mol)	Interacting residue	Type of bond	Bond distance (Aº)
α-Spectrin	Ethanol	-2.6	Ala273, Ile276 Leu278.	H-bond Alkyl	2.49, 2.77 4.42
	Acetate	-3.1	Tyr278, Gln191, Arg233.	H-bond	2.31, 2.35, 3.04
	Acetaldehyde	-2.4	Tyr278, Arg233.	H-bond	2.04, 2.64
β- Spectrin	Ethanol	-2.5	Arg1733, Thr1726	H-bond	2.66, 3.48
	Acetate	-3.2	Arg1789, Ser1715	H-bond	1.99, 2.65
	Acetaldehyde	-2.4	Arg1789, Ser1715.	H-bond	2.25, 2.48
Ankyrin	Ethanol	-2.6	Asp1319 Met1316	H-bond Alkyl	2.50, 4.55
	Acetate	-2.7	Arg1265, Thr1188, Gln1164.	H-bond	1.96, 1.91, 2.91.
	Acetaldehyde	-2.6	Arg1265, Thr1188, Gln1166.	H-bond	2.05, 2.14, 2.31
Protein-4.1	Ethanol	-2.8	Asp1710, Val1708	H-bond	
	Acetate	-3.3	Lys1455, Tyr1543, Arg1349, His1345	H-bond	2.00, 2.01, 2.40, 2.56
	Acetaldehyde	-2.6	Arg1349,	H-bond	1.97, 2.37

			His1345		
Protein-4.2	Ethanol	-2.9	Tyr343Val368, Arg369	H-bond Alkyl	2.01, 4.33, 4.88
	Acetate	-3.5	Lys421, Arg280, Val288	H-bond	2.12, 2.12, 2.80
	Acetaldehyde	- 2.7	Arg563, Gln314	H-bond	2.15, 2.20
Protein-4.9	Ethanol	-2.5	Arg40	H-bond	1.89
	Acetate	-3.0	Arg40	H-bond	2.42
	Acetaldehyde	-2.4	Arg66, Arg40	H-bond	2.04, 2.48
Band-3	Ethanol	-2.6	Ala273, Ile276, Ile189	H-bond Alkyl	2.50, 2.77 4.46
	Acetate	-3.1	Asp277, Ser106, His275	H-bond	1.93, 2.91, 3.27
	Acetaldehyde	-2.4	Tyr278, Arg233	H-bond	2.04, 2.64
NaK Atpase	Ethanol	-2.5	Asn718, Thr376, Asp719, Lys696, Lys375	H-bond	2.43,2.42, 3.03, 3.44
	Acetate	-3.1	Asn246, Ser215, Ser215, Asp212	H-bond	2.89, 2.95, 3.11, 2.90
	Acetaldehyde	-2.5	Ser273, Val739	H-bond	2.70, 3.62
Mg Atpase	Ethanol	-2.3	Ser402, Trp437, Leu405	H-bond Alkyl	2.89, 3.72, 4.23
	Acetate	-2.8	Ser402,Leu 405	H-bondAlkyl	2.984.48
	Acetaldehyde	-2.2	Ser402	H-bond	2.89
Ca Atapase	Ethanol	-2.6	Asn275, Glu785, Ile775, Ile285	H-bond Alkyl	2.66, 2.15 4.34, 4.22
	Acetate	-3.1	Lue787, Ala786, Thr778	H-bond	2.92, 2.96, 3.00
	Acetaldehyde	-2.5	Ile775, Tyr295, Ile298	Alkyl	4.12, 4.91, 5.39

The obtained docking results showed that the compounds exhibit more affinity towards RBC membrane proteins. The Protein α-Spectrin has a docking score of -3.1 (kcal/mol) which forms three hydrogen bonds with Arg1789, Ser1715 bond. The Protein β-Spectrin has a docking score of -3.2 (kcal/mol) which forms two hydrogen bonds with Tyr278, Gln191 and Arg233 bond. The Protein Ankyrin has a docking score of -2.7 (kcal/mol) which forms three hydrogen bonds with Arg1265, Thr1188 and

Gln1164 bond. The Protein Protein-4.1 has a docking score of -3. (kcal/mol) which forms four hydrogen bonds with Lys1455, Tyr1543, Arg1349 and His1345 bond. The Protein Protein-4.2 has a docking score of -3.5 (kcal/mol) which forms three hydrogen bonds with Lys421, Arg280 and Val288 bond. The Protein Protein-4.9 has a docking score of -2.4 (kcal/mol) which forms three hydrogen bonds with Lys421, Arg280 and Val288 bond. The Protein Band-3 has a docking score of -3.1 (kcal/mol) which forms three hydrogen bonds with Asp277, Ser106 and His275 bond. NaK Atpase has a docking score of -3.1 which forms three hydrogen bonds with four hydrogen bonds Asn246, Ser215, Ser215 and Asp212. Mg Atpase has a docking score of -2.3 which forms three hydrogen bonds with three hydrogen bonds Ser402, Trp437 and Leu405. Ca Atpase has a docking score of -3.1 which forms three hydrogen bonds with three hydrogen bonds Lue787, Ala786 and Thr778. In conclusion, our results revealed that acetaldehyde causes biochemical alterations even at low concentrations. A closely related challenge is the development of effective methods to predict the binding propensity for series of compounds or flexible peptides to a given receptor (Lybrand *et al.*, 1986; Gilson *et al.*, 1997).

Approaches based on scoring functions or compound libraries require large amounts of data to be available a priori. These methods include computational virtual screening, docking and similarity searching. As the binding propensity defining a given molecular association reflects the relative stabilities of the possible conformations of the receptor, effective drug-design protocols should be based on a distribution of receptor conformations. Crucial role of docking in drug design has been proven by expertise in pharmaceutical companies as well as researchers in scientific field of bioinformatics.

It is essential to study interactions of newly designed lead molecules with specified receptor or enzymes. Recent work carried out by Anuradha and co-workers have been designed antimicrobial agents for *Mycobacterium tuberculosis* of MurC and serine hydroxyl methyl transferase enzymes. They had studied the new anti tuberculosis agents docked (AutoDock 3.0) with MurC (Anuradha *et at.*,2010) and (AutoDock 4.0) with serine hydroxyl methyl transferase. These docking studies are crucial for drug design in less time and cost. In conclusion, our studies revealed that acetaldehyde causes more alterations in erythrocyte fluidity and rigidity even at low concentrations.

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