Chapter 5

Insilico assessment of cardioprotective potential of anthocyanin: Docking studies with β_1 adrenergic receptor

5.1 INTRODUCTION

G-protein-coupled receptors are involved in response to different neuro- and autocrine transmitters. Ahlquist (1948) was the first to differentiate the adrenergic receptors pharmacologically into α- and β-adrenergic receptors (AR). Several years later the adrenoceptors were distinguished using adrenergic agonists and antagonists into α1-, α1-, β1- and β2-AR subtypes. In the human heart β1AR is the predominant receptor with β1/β2 ratio 70–80% : 30–20% in the ventricle and in atrium the ratio is 60–70% : 40–30% (Brodde, 1991; McDevitt, 1989). β1 and β2AR are involved in positive inotropic and chronotropic effects on the myocardium induced by endogenous catecholamines such as adrenaline and noradrenaline. However, noradrenaline acts on β adrenoceptors more via the β1 than via the β2AR subtype (McDevitt, 1989). G-protein-coupled receptors contain three extracellular and intracellular loops. The first and second extracellular loop are coupled via a disulfide bridge and play an important role in the development of receptor conformation (Dixon et al., 1987; Wallukat et al., 1995). The receptor molecule also includes an extracellular N-terminal domain and a cytosolic C-terminal tail. The latter contains the phosphorylation sites of G-proteincoupled receptor kinases (GRKs). The phosphorylation site of PKA is localized on the third intracellular loop of the receptor (Benovic et al., 1985). Increased phosphorylation of the βAR is believed to play an important role in the agonist-promoted uncoupling which leads to rapid desensitization and later to the downregulation of the receptor (Wallukat, 2002). Therefore, phosphorylation of the receptor is an effective mechanism to modulate the responsiveness of the βAR mediated signal transduction cascade.

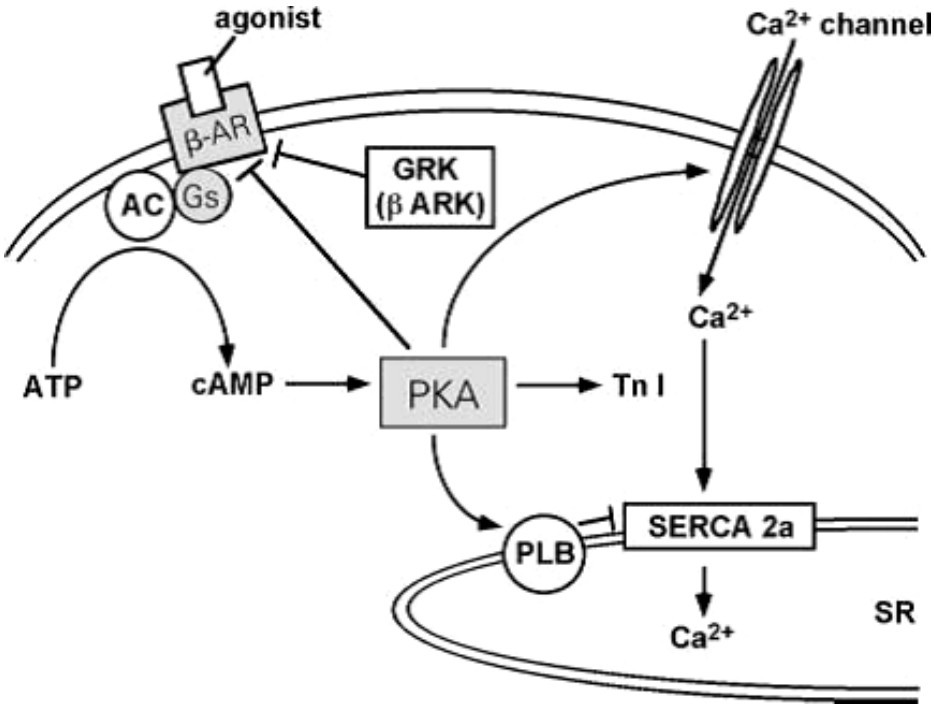


Figure 5.1: Schematic diagram of agonist mediated activation of β adrenergic receptor. (Perez & Karnik, 2005)

Transgenic mice overexpressing the β1AR develop heart failure similar to that observed in dilated cardiomyopathy in humans (Engelhardt et al., 1999). Also, of mice overexpressing the β1AR develop early impairment of calcium (Ca^{2+}) handling and altered expression of junctions in heart (Engelhardt et al., 2001). Such changes of Ca^{2+} transient have also been described for myocytes prepared from the human failing heart (Beuckelmann et al., 1992).

In the classical paradigm of GPCR signaling, ligand binding leads to conformational change of the receptor from an inactive state R into a single activated state R* that results in the coupling of the receptor to heterotrimeric G proteins. Receptor coupling facilitates the exchange of the bound GDP with GTP in α subunit of the G protein complex. This triggers dissociation of the complex into Gα and Gβγ subunits. They go on to activate their respective effectors such as AC, phospholipases and ion channels. These receptor mediated reactions often generate signaling

molecules called second messengers which activate or inhibit other components of the cellular machinery. Thus, receptor stimulation produces a multitude of cellular responses via the activation of the signal transduction pathways downstream of G proteins (Perez & Karnik, 2005).

Molecular docking provides structural insights into interaction between receptor and ligand using structural database and molecular docking software. Molecular interaction study with flavanoid-3-glucoside and flavanoid-3-glycosyl transferase showed the stable interaction between both ligand and receptor with 3-OH group elucidates that the environment in the close vicinity of the acceptor is conserved in all the members of this group and that these amino acids favor the binding of a flavonoid with glycosylation at 3-OH group over other (Sharma et al., 2014). Alterations in these regions may affect the acceptor specificity. Thus, the biochemical and pharmaceutical properties of flavonoids can be altered to prepare more effective drug molecules in a desired chemical form. Quercitin showed more binding energy (−8.5 kcal/mol) with angiotensin converting enzyme compared to the standard drug enalapril (−7.0 kcal/mol) (Muhammad & Fatima, 2015). Also, docking study with anthocyanin showed the possible role in mitigating retinosis pigmentosa by inhibiting retinotis pigmentosa (RP2) and alzheimers by docking at PPIase and activating FKBP52, as FKBP52 inhibit the aggregation of Tau protein (Sharma et al., 2014). Kanakis et al. 2006 and Tang et al. 2014 had shown molecular interaction of BSA/HSA with the hydroxyl groups in the B ring of cyanidin-3-glucoside and delphinidin-3-glucoside. Hence the present study was designed to decipher the possible interactions between β1AR and anthocyanins which may help to determine the therapeutic effectiveness of active ingredients.

Table 5.1: List of known β1AR antagonist with pharmacodynamics and side effects.

Name	Pharmacodynamics	Side effects
Carvedilol	Nonselective β receptor antagonist, no intrinsic sympathomimetic activity, restore inotropic and prevent OH^- radical induced decrease in sarcoplasm reticulum Ca^{2+}-ATPase activity.	Dairrhea, nausea and tiredness
Pindolol	Nonselective β receptor antagonist and no intrinsic sympathomimetic activity	
Propranolol	Nonselective β receptor antagonist and no intrinsic sympathomimetic activity	
Metaprolol	Selective β receptor antagonist and no intrinsic sympathomimetic activity	
Atenolol	Selective β receptor antagonist and no intrinsic sympathomimetic activity	
Bisprolol	Selective β receptor antagonist and no intrinsic sympathomimetic activity	
Acebutolol	Selective β 1 receptor antagonist, mild intrinsic sympathomimetic activity	

Reference: www.drugbank.ca

Aim: *Insilico approach to study the possible interaction between anthocynain and β1AR*

5.2 MATERIALS AND METHODS

Structure retrieval of target protein and Ligand

The sequence rat β1AR was retrieved from National Center for Biotechnology Information (NCBI) sequence database (NP_036833.1). The ligand files were acquired from the NCBI pubchem database that include cyanidin-3,5-diglucoside (CID 441688), cyanidin-3-glucoside (CID 92131208), delphinidin-3-glucoside (CID 443650) and Isoproterenol (CID 5807)

Preparation of target protein and Homology modeling

CPHmodels-3.2 Server was used for formation of three-dimensional (3D) model of β1AR and alignment of rat β1AR (residue 49 to 380) with the template sequence of Turkey β1AR (PDB ID: 3ZPR). CPHmodels-3.2 Server uses profile-profile alignment method guided by secondary structure and exposure prediction to find out best template structure for model building. Further, stereochemical quality of the modeled structure was evaluated through Ramachandran plot analysis. To get better relaxation and more correct arrangement of the side chain and main chain atoms, optimization was performed with protein preparation wizard in Maestro 9.3 using OPLS 2005 force field with a .3 Å RMSD tolerance of backbone atoms. Finally, models were energy minimized using steepest descent followed by conjugate gradient method in Discovery Studio 2.5 software.

Prediction of active sites

A 20 Å grid box was generated at the active site of the β1AR using three active site residues N352, S228 and D138 retrieved from the co-crystal structure of quinoline with turkey β1AR (PDB ID : 3ZPR).

Molecular Docking studies

Molecular docking of cyanidin-3-glucoside, cyanidin-3,5-diglucoside delphinidin-3-glucoside and isoproterenol with rat β1AR model was performed using Glide program in Schrodinger with Extra Precision (XP) method for calculations. The protein and the ligand molecules were prepared for docking using protein preparation wizard and LigPrep respectively. PyMol was used for visualization of molecular interactions.

5.3 RESULTS

Homology modeling and identification of active site

Alignment of rat β1AR (residue 49 to 380) with the template sequence of turkey β1AR (PDB ID : 3ZPR) showed that the loop structure in rat β1AR (residues 258-298) is quite different from turkey β1AR but rest of the residues are more or less conserved and having 69.9 % sequence identity. The Ramachandran plot analysis of the model showed that all the residues are in the allowed regions and out of which 91.5% residues are in most favored regions (Figure 5.2). The query-template alignment used in this purpose is given in Figure 5.4 and the 3D structure of the modeled protein is shown in Figure 5.3 which consists of seven helical structures in a bundle formation. This 3D structure consists of a flexible loop between Helix-5 (H5) and Helix-6 (H6), residue number 258 to 298.

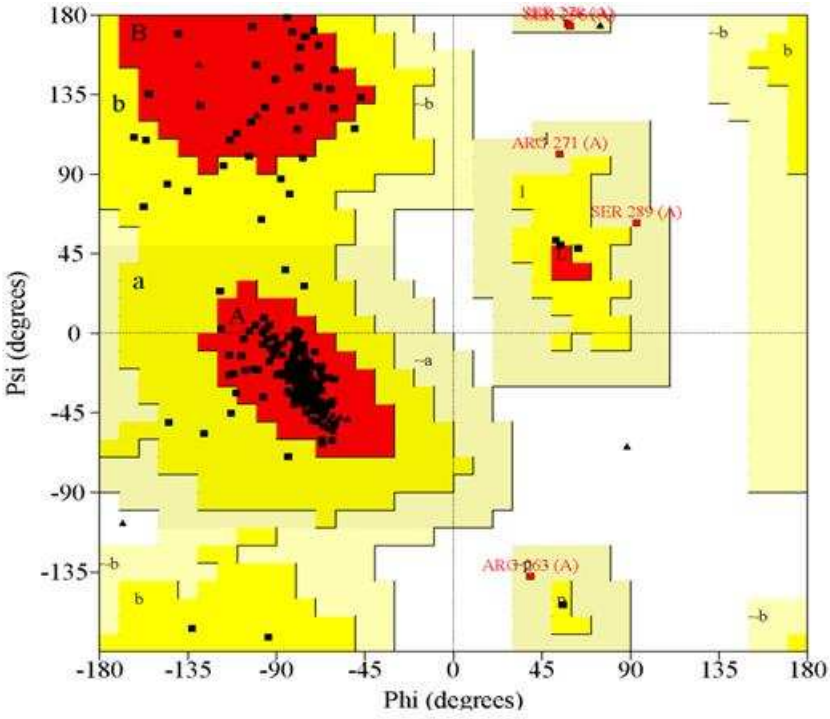
The active site or ligand binding site of rat β1AR is positioned at the opposite side of the loop structure, the residues contributing to active site are mainly from three helices H3, H5 and H7. The critical residues D138, S228a and N352 which plays important role in quinoline binding in turkey β1AR are found to be conserved in rat β1AR as evident from the query-template alignment (Figure 5.3 & 5.4).

Molecular docking

Molecular docking of cyanidin-3-glucoside, cyanidin-3,5-diglucoside, delphinidin-3-glucoside and isoproterenol with rat β1AR showed that they were well accommodated within the active site and interacted through the hydrophobic and electrostatic bonds at a distance of 2.5 to 3.2 Å. Hydrogen bonding and hydrophobic interactions between anthocyanin and amino acid residue of β1AR is mentioned in Table 5.3. These

compounds accounted -36.40 kJ/mol (Figure 5.5A and B), -26.22 kJ/mol (Figure 5.6A and B), -35.56 kJ/mol (Figure 5.7A and B) and -30.54 kJ/mol (Figure 5.8A and B; Table 5.2) Glide XP (docking) scores respectively.

5.4 FIGURES



Plot statistics

Residues in most favoured regions [A,B,L]	269	91.5%
Residues in additional allowed regions [a,b,l,p]	20	6.8%
Residues in generously allowed regions [~a,~b,~l,~p]	5	1.7%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	294	100.0%
Number of end-residues (excl. Gly and Pro)	1	
Number of glycine residues (shown as triangles)	14	
Number of proline residues	23	
Total number of residues	332	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

Figure 5.2: Ramachandran plot analysis of β1AR

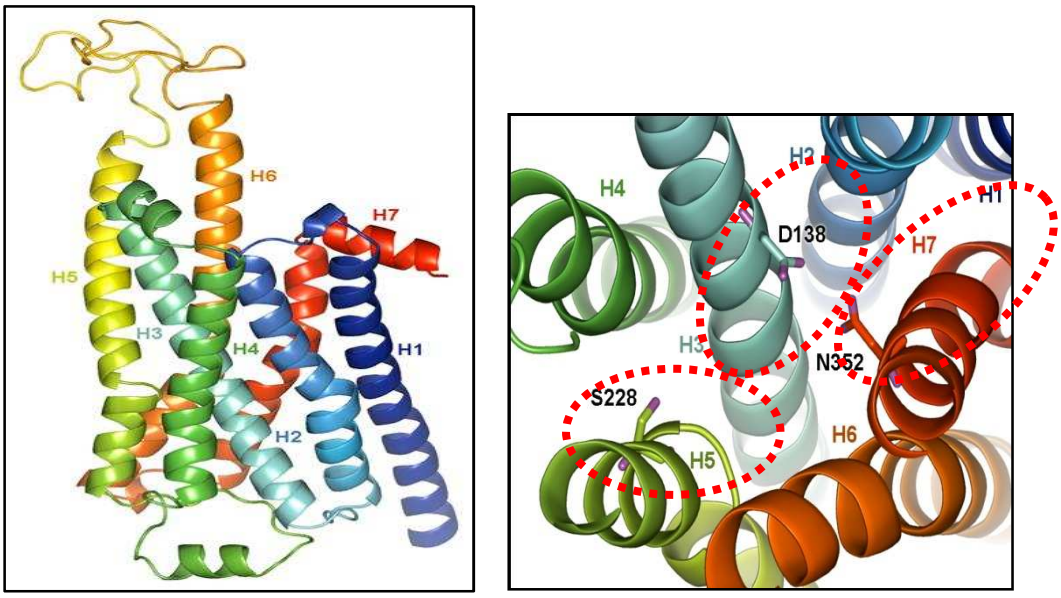


Figure 5.3: 3D model of rat β1AR. Red circle represents active site present in β1AR.

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Query: 49   GSAPLSQQWTAGMGLLLALIVLLIVVGNVLVIVAIAKTPRLQTLTNLFIMSLASADLVMG 108
            G+  LSQQW AGM LL+AL+VLLIV GNVLVI AI  T RLQTLTNLFI SLA ADLV+G
Templ: 1    GAELLSQQWEAGMSLLMALVVLLIVAGNVLVIAAIGSTQRLQTLTNLFITSLACADLVVG 60

                                      138
Query: 109  LLVVPFGATIVVWGRWEYGSFFCELWTSVDVLCVTASIETLCVIALDRYLAITLPFRYQS 168
            LLVVPFGAT+VV G W +GSF CELWTS-DVLCVTASIETLCVIA+DRYLAIT PFRYQS
Templ: 61   LLVVPFGATLVVRGTWLWGSFLCELWTSIDVLCVTASIETLCVIAIDRYLAITSPFRYQS 120
                                      121                              228
Query: 169  LLTRARARALVCTVWAISALVSFLPILMHWWRAESDEARRCYNDPKCCDFVTNRAYAIAS 228
            L+TRARA+ ++CTVWAISALVSFLPI+MHWWR E  +A +CY DP CCDFVTNRAYAIAS
Templ: 121  LMTRARAKVIICTVWAISALVSFLPIMMHWWRDEDPQALKCYQDPGCCDFVTNRAYAIAS 180
                                                                      211
Query: 229  SVVSFYVPLCIMAFVYLRVFREAQKQVKKIDSCERRFLTGPPRPPSPAPSPSPGPPRPAD 288
            S++SFY+PL IM FV LRV+REA++Q++KID                A
Templ: 181  SIISFYIPLLIMIFVALRVYREAKEQIRKIDR---------------A------------ 213

Query: 289  SLANGRSSKRRPSRLVALREQKALKTLGIIMGVFTLCWLPFFLANVVKAFHRDLVPDRLF 348
                   SKR+ SR++ +RE KALKTLGIIMGVFTLCWLPFFL N+V  F+RDLVPD LF
Templ: 213  -------SKRKTSRVMLMREHKALKTLGIIMGVFTLCWLPFFLVNIVNVFNRDLVPDWLF 266
                 352
Query: 349  VFFNWLGYANSAFNPIIYCRSPDFRKAFQRLL 380
            V FNWLGYANSA NPIIYCRSPDFRKAF+RLL
Templ: 267  VAFNWLGYANSAMNPIIYCRSPDFRKAFKRLL 298
                 329
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Figure 5.4: Sequence alignment of rat β1AR and turkey β1AR. Highlighted sequence represents similarity between rat and turkey β1AR active site.

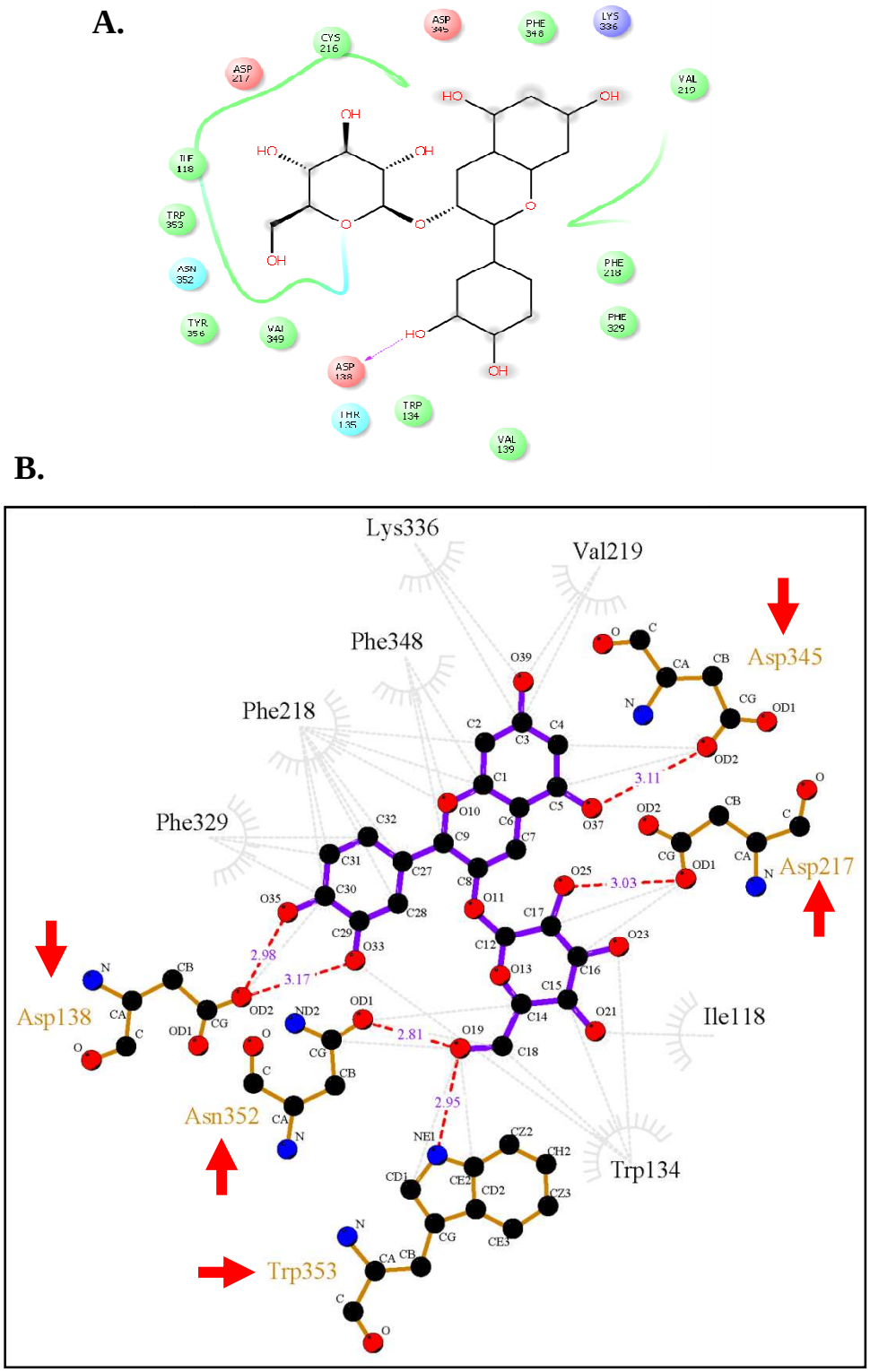

Figure 5.5: (A) Molecular interaction of cyanidin-3-glucoside with β1AR and (B) Ligplot analysis. Red arrow represents hydrogen bonding between amino acid residue and anthocyanin

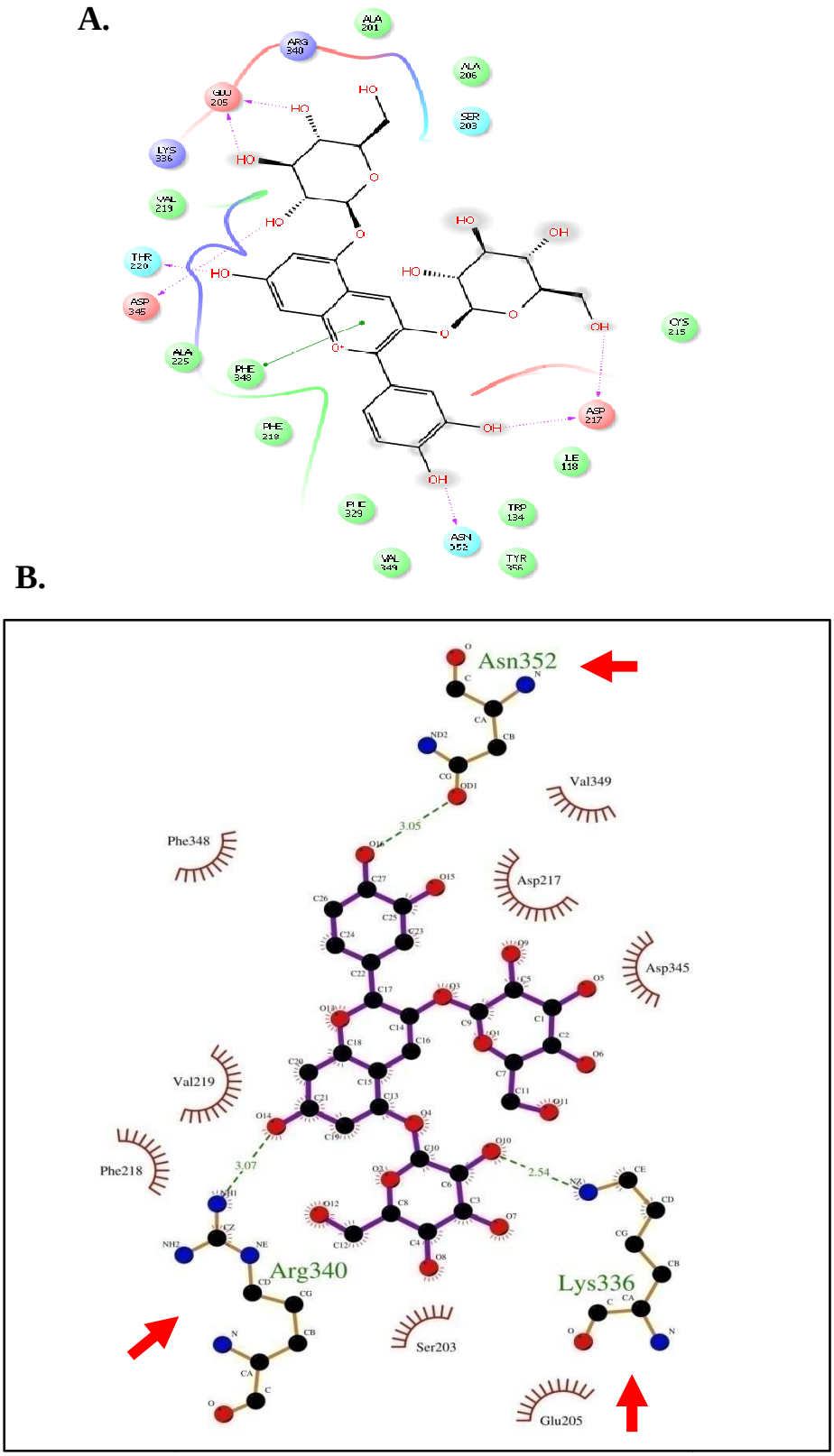

Figure 5.6: (A) Molecular interaction of cyanidin-3,5-diglucoside with β1AR and (B) Ligplot analysis. Red arrow represents hydrogen bonding between amino acid residue and anthocyanin.

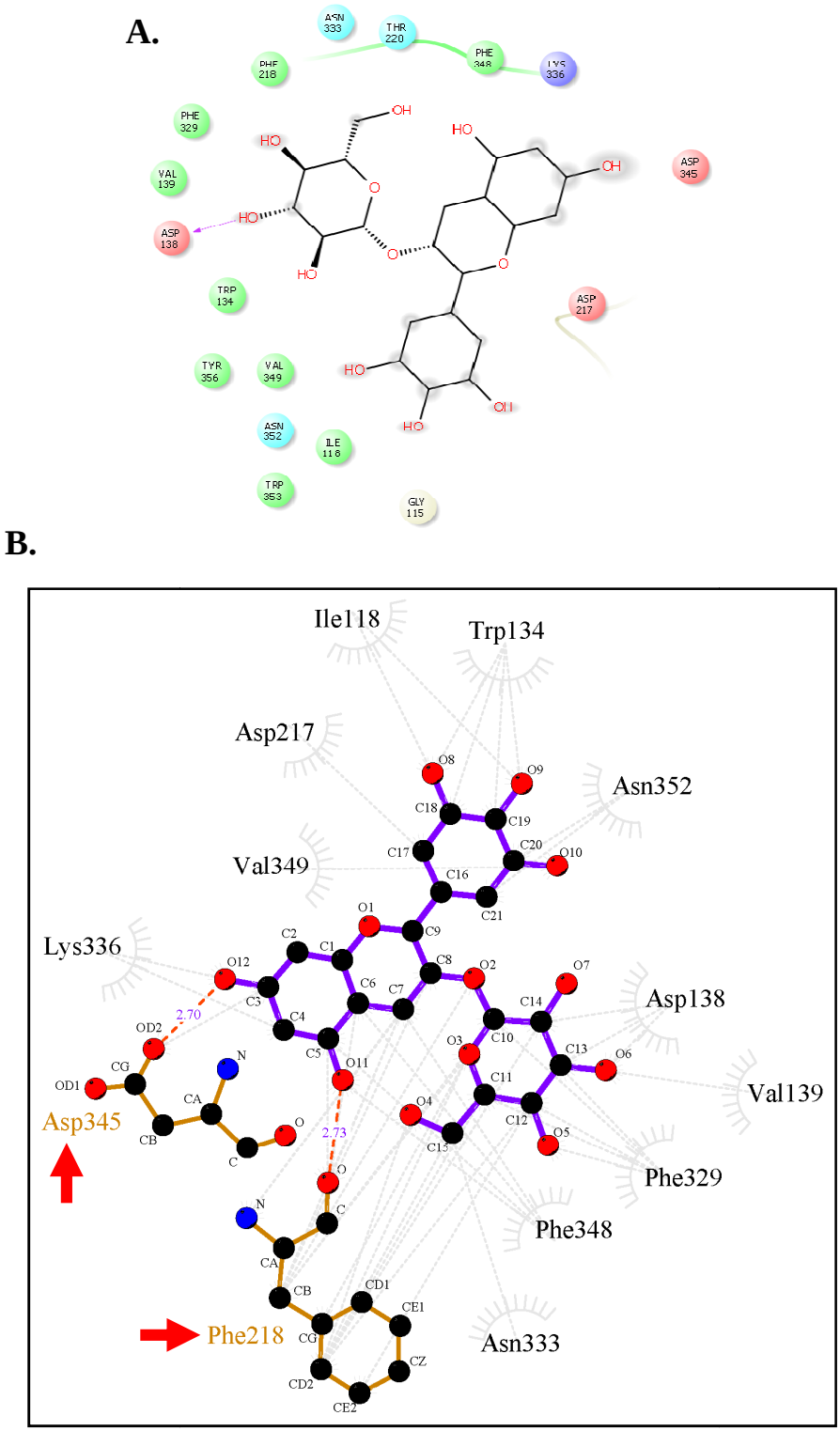

Figure 5.7: (A) Molecular interaction of delphinidin-3-glucoside with β1AR and (B) Ligplot analysis. Red arrow represents hydrogen bonding between amino acid residue and anthocyanin.

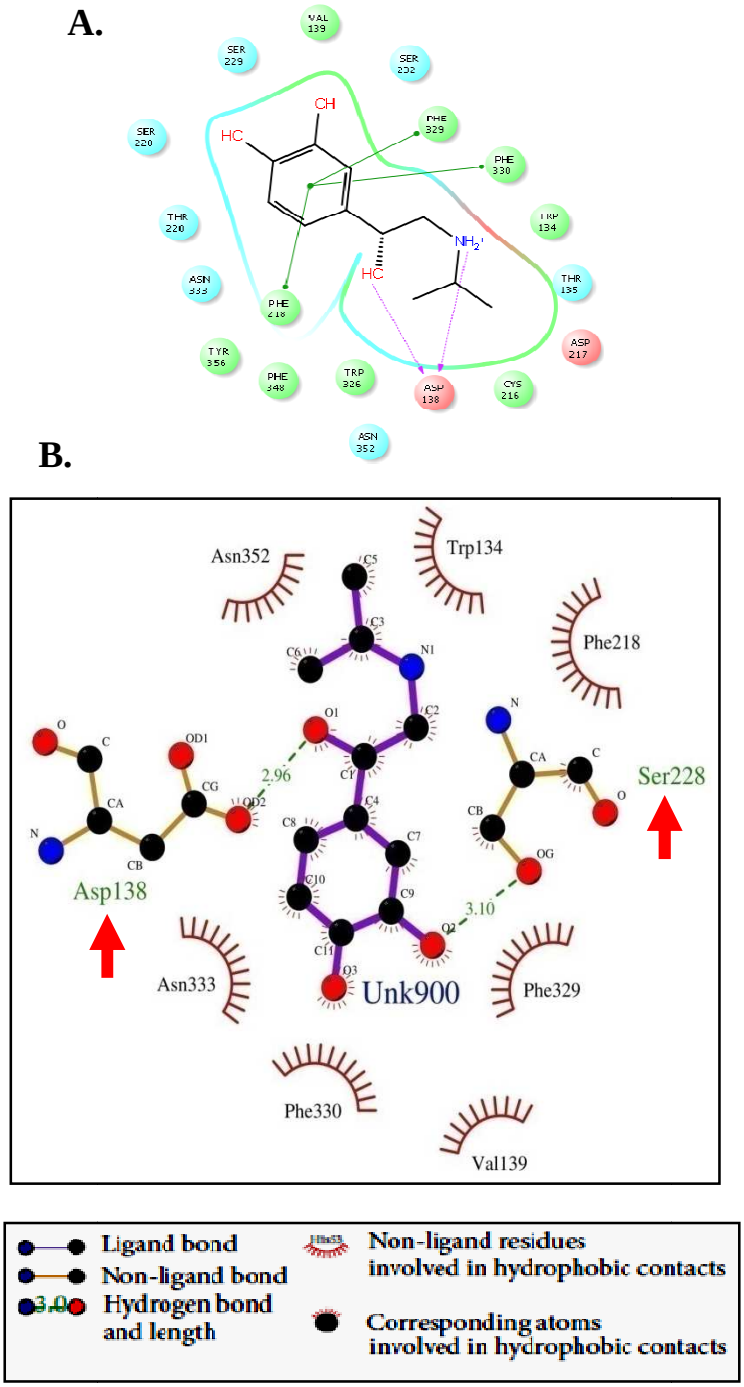

Figure 5.8: (A) Molecular interaction of isoproterenol with β1AR and (B) Ligplot analysis. Red arrow represents hydrogen bonding between amino acid residue and anthocyanin.

Table 5.2: Docking score of anthocyanin and isoproterenol with β1AR.

Name of the compound	Binding Score
Cyanidin-3-glucoside	-36.40 kJ/mol
Cyanidin-3,5-diglucoside	-26.22 kJ/mol
Delphinidin-3-glucoside	-35.56 kJ/mol
Isoproterenol	-30.54 kJ/mol

Table 5.3: List of amino acid residues involved in β1AR and test compound interactions.

Name of the compound	Amino acid residue involved in hydrogen bonding	Amino acid residue involved in hydrophobic interaction
Cyanidin-3-glucoside	Asp138, Asn352, Trp353, Asp345, Asp217	Lys336, Val219, Phe329, Ile 1118, Trp134
Cyanidin-3,5-diglucoside	Asn352, Arg340, Lys336	Val349, Asp217, Asp345, Phe348, Ser203, Glu205
Delphinidin-3-glucoside	Phe218, Asp345	Lys336, Val349, Asp217, Ile118, Trp134, Asn352, Asp138, Val139, Phe329, Phe348, Asn333
Isoproterenol	Asp138, Ser228	Asn352, Trp134, Phe218, Phe329, Val132, Phe330, Asn333

5.5 DISCUSSION

Insilico docking provides an insight into the level of possible interactions between drug and target molecules. Cyanidin and delphinidin-3-glucoside had been widely investigated for its bioavailability and therapeutic benefits. Cyanidin-3-glucoside had been investigated for its antioxidant, antifibrotic, anti-inflammatory and antidiabetic properties (Li et al., 2016). While Delphinidin-3-glucoside had been investigated for its activity against platelet aggregation, atherothrombosis and oxidized LDL induced modification in endothelial cells (Chen et al., 2010; Xie et al., 2012). Tang et al. (2014) and Zuo et al. (2015) had shown that cyanidin-3-glucoside and delphinidin-3-glucoside can bind to site-I of BSA via hydroxyl group of B-ring which is responsible for the antioxidant activity of anthocyanin. In our study cyanidin-3-glucoside, delphinidin-3-glucoside, cyanidin-3,5-diglucoside, cyanidin-3-diglucoside-5-glucoside, cyanidin-3-(p-coumaroyl)-diglucoside-5-glucoside, cyanidin-3-(feruloyl)-diglucoside-5-glucoside and cyanidin-3-(sinapoyl)-diglucoside-5-glucoside were docked with β1AR. Among these only cyanidin-3-glucoside, delphinidin-3-glucoside and cyanidin-3,5-diglucoside showed an interaction with β1AR. Cyanidin-3-glucoside and delphinidin-3-glucoside showed interaction at very less binding free energy viz, -36.40 and -35.56 kJ/mol respectively, while cyanidin-3,5-diglucoside showed interaction at -26.22 kJ/mol. Also, interaction between β1AR and isoproterenol accounted with -30.54 kJ/mol binding energy. Ligplot analysis showed that isoproterenol binds at the active site of β1AR with hydrophobic interactions and hydrogen bonding at Asp138, Ser228 residues. Also, cyanidin-3-glucoside binds at active site with hydrophobic interactions and hydrogen bonding at Asp138, Asp217, Asp345 and Trp353 residues, which shows that binding of cyanidin-3-glucoside may prevent the binding of isoproterenol at the active site of β1AR. Also, Ligplot analysis

of cyanidin-3-glucoside shows more and strong hydrogen and hydrophobic interactions then other representatives. Delphinidin-3-glucoside showed hydrophobic interactions and hydrogen bonding at Phe218 and Asp345 residues of β1AR. Asp345 was found to be common amino acid involved in hydrogen bonding with A-ring of cyanidin and delphinidin-3-glucoside. Hence, strong hydrophobic, electrostatic and hydrogen bonding of cyanidin and delphinidin-3-glucoside may prevent the binding of isoproterenol or oxidized product and prevent the overstimulation of β1AR and further cellular damage. Similarly, Hung et al. (2014) had observed strong hydrophobic interactions and hydrogen bonding of FKBP52 with anthocyanins then FK506 in search of an alternative against FK506 due to its side effects in prevention of alzhiemer's disease. Also, Vanni et al. (2011) had reported detailed description about isoproterenol and β1AR interactions using microsecond MD stimulations, which describes about role of hydrogen and hydrophobic interactions and its behavior on extracellular loop of receptor. β antagonist listed in Table 1 are used for acute myocardial infarction (MI), heart failure, angina pectoris and mild to moderate hypertension. Among these some are selective and nonselective to β1AR. Also, some antagonist are having intrinsic sympathomimetic activity, which by preventing the binding of agonist mimic the activity of agonist in very low level to avoid the extreme decrease in heart beat. Carvedilol has been widely studied antagonist not only as β1AR blocker but also can prevent OH^- radical induced decrease in sarcoplasm reticulum Ca^{2+}-ATPase activity. Combination of antagonist activity and antioxidant activity can be the powerful agent to fight against pathologies of heart failure. Hence, by known antioxidant activity and by knowing more about molecular interactions between anthocyanin and β1AR may provide alternative therapeutic strategies against pathophysiology of heart disease.

5.6 SUMMARY

In this study cyanidin-3-glucoside, cyanidin-3,5-diglucoside, delphinidin-3-glucoside and isoproterenol were docked with β1AR to determine the possibilities of its interaction with receptor and preventing the binding of isoproterenol. Cyanidin-3-glucoside and delphinidin-3-glucoside recorded less free energy than cyanidin-3,5-diglucoside on interaction with β1AR. Subsequently, ligplot analysis showed strong hydrophobic interaction and hydrogen binding of cyanidin-3-glucoside and delphinidin-3-glucoside then cyanidin-3,5-diglucoside and isoproterenol. Also, ligplot analysis recorded similar amino acid residues involved in cyanidin-3-glucoside and isoproterenol interaction with β1AR, hence binding of cyanidin-3-glucoside can inhibit the binding of isoproterenol to the active site of β1AR and can prevent the isoproterenol induced overstimulation of β AR.