Chapter 5

CONCLUSIONS

5.1 *In-Silico* studies

On the basis of research conducted for the molecular target identification for quercetin and taxifolin by *in–silico* studies following conclusion can be made:

5.1.1 Molecular target 1: Bcl-2

Comparative analysis of Bcl-2-ligand complex after 10 ns molecular dynamics simulation with crystal structure of Bcl-2 revealed that binding of the quercetin and taxifolin bring significant conformational changes in the Bcl-2 structure and the collapse of hydrophobic groove in the ligand bound Bcl-2. However taxifolin showed higher effect on the groove and found to be completely disappear in taxifolin bound Bcl-2. All these results indicated that polyphenols have potential to inhibit the Bcl-2 by binding with apoptotic protein and distorting structure of hydrophobic groove. Apart of this, it was found that taxifolin and quercetin disrupted the Bcl-2-Bax heterodimer during 12 ns MD simulation. Taxifolin and quercetin interacted with residues were Arg 65, Asp 68, Glu 69, and Ser 72 of Bax and Arg 110, and Asp 111 of Bcl-2 which are known to be responsible for interaction between Bcl-2 and Bax. Taxifolin and quercetin binding to complex induced the mobility in protein by disrupting stable interaction of interface residues as shown by RMSF profiles. Further, validated by the MD simulation of

ligand unbound Bcl-2-Bax and obatoclax bound Bcl-2-Bax showed stability during simulation.

5.1.2 Molecular target 3: Heat Shock Protein 90

Taxifolin binds to ATP binding site of Hsp90. It is evident from MD simulation studies that taxifolin stabilized the open lid conformation of Hsp90 NTD. The loop of ‘‘ATP-lid’’ segment was pulled toward the taxifolin. A comparative investigation of ADP, geldanamycin and taxifolin bound Hsp90 NTD revealed the significant similarity in the overall structure. This study significantly contributes to ligand-based modulation of the Hsp90 NTD conformational dynamics and supports the mechanism of the active site lid as a nucleotide sensitive conformational switch. On the other hand, taxifolin interrupts the interaction of interface residues of Hsp90 and Cdc37 complex and provides a novel mechanism for chaperoning process inhibition. During simulation studies it was found that taxifolin binding to Hsp90–cdc37 complex disrupt the key interaction required for functional complex formation. A strong interaction between Glu 47 of Hsp90 and Arg167 of Cdc37 bridged by water molecule was vanished in presence of taxifolin. Gln 133 of Hsp90 showed polar interaction with Arg166 and Arg167 of Cdc37 during MD simulation of taxifolin unbound form. Binding of taxifolin to Hsp90–cdc37 complex disrupt this interaction as evidenced by MD simulation. A network of polar contact was found Lys 116 and Glu 120 of Hsp90 with peptide bond (CONH) of Ala 204 and Leu 205 of Cdc37 which was vanished in presence of taxifolin. In taxifolin unbound complex Gln 133 of Hsp90 also found to form H-bond with Asp 170 of Cdc37 which was found absent in taxifolin bound form.

The dual action of taxifolin on chaperoning process may lead to significant development of drug candidates to target the folding of oncogenic proteins.

5.1.3 Molecular target 4: MDM2

The p53 interacts with MDM2 by inserting its hydrophobic residues (Phe19, Trp23 and Leu26) into a deep groove in MDM2. Quercetin and taxifolin bound to the hydrophobic groove of MDM2 and alter the conformation of groove as evidenced by 65 ns molecular dynamics simulation. Both compounds were found to inhibit the MDM2 and p53 interaction as evidenced by molecular dynamic simulation. The interaction of MDM2 and p53 is dominantly governed by the hydrophobic residues (Phe19, Trp23 and Leu26). Taxifolin and quercetin efficiently mask these interactions led to separation of p53. The hydropobic aromatic group system of ligands mainly contributed to this action. The 2D plot of taxifolin and MDM2-p53 interaction at different time interval of MD simulation showed and confirmed that the hydrophobic interactions were dominated during simulation. Initially, taxifolin showed π-π interaction with both MDM2 and p53 which finally, completely, switched to π-π and, cation-π interaction between taxifolin aromatic ring A and Tyr 51 and, Lys 47 respectively. In this way interaction of Trp 23 of p53 and Tyr 51 of MDM2 transformed into Trp 23-B-C ring and, Tyr 51-A ring interaction and finally in to A ring of taxifolin and Tyr 51and, Lys 47 of MDM2. The 2D plot of quercetin and MDM2-p53 interaction also showed that initially, quercetin involved in π-π interaction with MDM2 by C ring. At 12000 ps B and, C-ring of quercetin found in π-π interaction wirh Tyr 51. Being the natural compounds and their bioavailability in natural food products,

these phytocemicals can be used to target MDM2 and p53 interaction in apo as well as complex form.

5.1.4 Molecular target 5: VEGFR-2 kinase

Taxifolin inhibit the VEGFR-2 kinase by binding at ATP-binding pocket revealed by molecular docking study. Further, stability of VEGFR-2 kinase-taxifolin complex is validated by molecular dynamic simulation. RMSD analysis for 3800 ps confirmed the stability of complex. Furthermore, thermodynamic stability was evidenced by stable total energy, potential energy, and, temperature and pressure profile. After MD simulation taxifolin was found to stably interact with pocket residues Cys 917 and Lys 1053 along with water molecules. Taxifolin was found to bind at the ATP-binding site on VEGFR-2 kinase with large value of binding energy and act as type I competitive inhibitor as revealed by molecular docking study. These studies confirmed the potential role of taxifolin in cancer chemoprevention which should be further studied for modulation of other signaling pathways which play crucial role in cancer and development of second generation type II inhibitors.

5.2 *In-vitro* studies

In vitro studies on HeLa cells revealed following conclusions:

1. Cell viability test showed that quercetin and taxifolin have IC_{50} value 90 and 400 μM.

2. Treatment of cells with quercetin (90 µM) and taxifolin (400 µM) for 24 h resulted in cell shrinkage, chromosomal condensation, nuclear and plasma membrane convulsion and nuclear fragmentation indicating that the cytotoxic action of quercetin and taxifolin was due to its ability to induce apoptosis.
3. Cells treated with quercetin (80 µM) and taxifolin (400 µM) undergo DNA fragmentation as shown by DNA ledder was in agarose gel electrophoresis.
4. RT-PCR analyses revealed a significant increase in the expression of Bax, cytochrome c and caspase -9 with decrease in the expression of Bcl-2 and in cells treated with quercetin and taxifolin compared to untreated control.

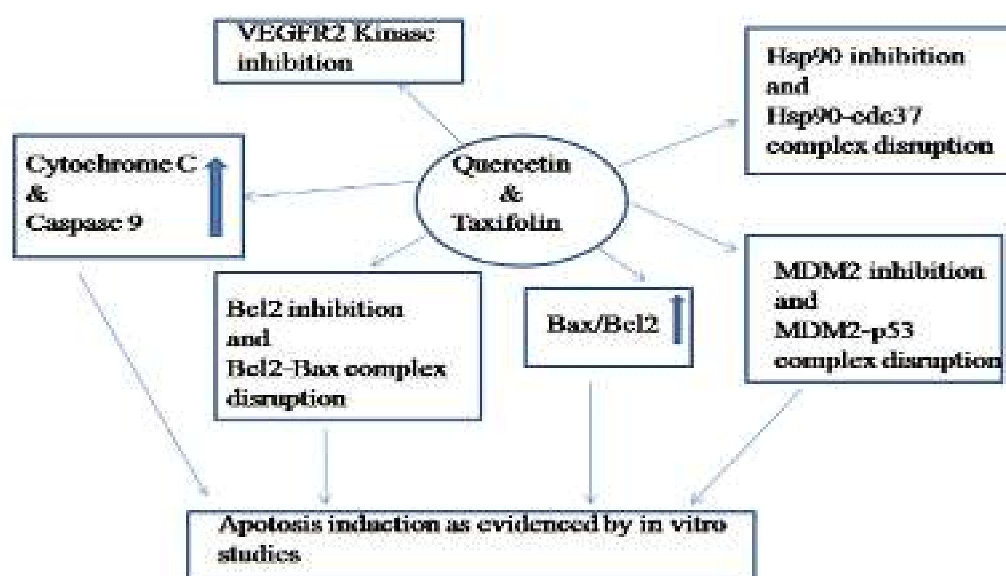

Fig. 5.1. Schematic representation of in silico and in vitro studies of quercetin and taxifolin on different molecular target and HeLa cells.