ABSTRACT

Multi-Drug-Resistance (MDR) exerted by tumor cells is one of the barriers to successful chemotherapy in colon cancer patients. Overexpression of ATP Binding Cassette (ABC) transporters such as ABCB1/MDR1/P-glycoprotein (P-gp) is a major contributor to the development of MDR in tumor cells. Although several P-gp inhibitors have been identified in the past decades, their clinical utility has been limited due to their unpredictable pharmacokinetic interactions and undesirable toxicity. Hence a combination of potential non-toxic P-gp inhibitors along with anti-cancer drugs has been a great choice for reversing MDR in tumor cells. Compounds identified from natural sources are sought to effectively reverse MDR in tumors with a higher specificity and a lesser cross-reactivity. One such group of compounds, Annonaceous Acetogenins (AGEs) are structurally related phytocompounds present in the plants of Annonaceae family were explicitly reported for their anticancer activity. Few preliminary studies have reported the usage of AGEs in hepatic, epithelial cell, and breast carcinomas. However, the MDR reversal capability of AGEs and its mechanism of action in colon cancer is yet to be investigated.

Our present study aims at identifying a potent MDR reversing candidate among the AGEs of *Annona muricata* through computational approaches and to explore its inhibitory effect on P-gp in reversing oxaliplatin-resistance in human colon cancer cells. *In silico* study: The molecular interactions of AGEs and human P-gp were analyzed using the Schrodinger modeling suite. A total of twenty-four AGEs were selected from the literature and screened for their drug-likeness properties. The inward-facing conformation of human P-gp was modeled and its structure was

validated using Modeller and other relevant tools. Molecular docking of AGEs was carried out at the two active domains- Drug Binding Domain (DBD) and Nucleotide Binding Domain (NBD) of human P-gp (inward and outward-facing conformations (PDB ID:6C0V)). In addition, the binding free energies (MMGB-SA) of AGEs were also predicted and the protein: ligand complexes were subjected to molecular dynamics simulation for stability analysis. *In vitro* studies: The lead molecule annonacin A predicted through computational screening, was isolated from the leaves of *A. muricata* and further characterized by chromatographic methods. The fraction enriched with annonacin A was tested for its cytotoxicity, clonogenicity, anti-apoptosis, and multi-drug reversal activity on parental (SW480) and oxaliplatin drug-resistant colon cancer ($SW480^{R}$) cell lines. Additionally, P-gp expression studies were also performed in the annonacin A-treated cells.

AGEs except solamin were predicted to be drug-like candidates with acceptable pharmaceutical properties. Docking of AGEs at the DBD of the protein (outward-facing conformation) was not favorable due to the structural constraint created by the protein's helical regions. However, AGEs (annohexocin: -10.49 kcal/mol, annomuricin B: -9.13 kcal/mol, and annonacin A: -8.10 kcal/mol) interacted at the NBD1 of the protein with a greater affinity. The modeled inward-facing conformation of P-gp showed a greater affinity with AGEs (annonacin A: -13.489 kcal/mol, annohexocin -13.404 kcal/mol, and annomuricin E: -10.354 kcal/mol) at the DBD than at the NBD. Moreover, the binding free energies (MMGB-SA) of AGEs (annohexocin: -122.69 kcal/mol, annonacin A: -113.76 kcal/mol, and annomuricin E: -105.27 kcal/mol) were also higher at the protein's DBD compared to the chemotherapeutic drug (oxaliplatin: -32.42 kcal/mol) and well reported P-gp inhibitor (verapamil: -88.32 kcal/mol). Of the AGEs, P-gp: annonacin A complex was found to be stable throughout the entire

simulation trajectory. Annonacin A significantly reduced the expression of P-gp and induced apoptosis (2.6 folds) in $SW480^{R}$. The colony-forming ability of $SW480^{R}$ cells was also effectively inhibited by annonacin A. The combinatorial treatment of oxaliplatin and annonacin A increased the intracellular accumulation of P-gp substrate (calcein-AM).

The findings of this study suggest that AGEs could inhibit the efflux of chemotherapeutic drugs by P-gp and might help reverse MDR in colon cancer cells. As annonacin A competes with P-gp more effectively than P-gp substrates, it could act as a potential lead molecule for MDR reversal. Further *in vivo* studies are required to decipher the underlying mechanism of AGEs in treating multi-drug resistant cancers.