Short Communication

IN SILICO INTERACTION STUDIES OF RESVERATROL AND SILIBININ WITH DIFFERENT PROTEIN TARGETS USING MOLECULAR DOCKING

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ABSTRACT

Resveratrol and silibinin are natural phytochemicals with considerable anticancer properties. They induce apoptosis of cancer cells by binding to various protein targets. Hence in this study, in silico molecular interaction studies were made between ligand resveratrol and ligand silibinin with NF-kB p50 subunit and p65 subunit, MMP9, caspase 3 and Bax proteins. The in silico molecular interaction revealed that silibinin and resveratrol interacted with five receptors with a high binding affinity and docking score making them novel inhibitors against various types of diseases.

Key words: Resveratrol, Silibinin, *in silico.*

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INTRODUCTION

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a stilbenoid, a form of natural phenol, and a phytoalexin synthesized by a variety of plants in reaction to the damage caused by the pathogens like bacteria or fungi. Grape skin, blueberries, raspberries, mulberries, and peanuts are all good sources of resveratrol in diet. Resveratrol has antioxidant, anti-inflammatory, immunomodulatory, glucose and lipid regulatory, neuroprotective, and cardiovascular protective effects, and thus can protect against a variety of chronic diseases, including cancer, liver diseases, obesity, diabetes, Alzheimer's disease, and Parkinson's disease (Berman *et al.,* 2017).

The plant milk thistle's (*Silybum marianum*) active extract, silymarin, is made up of four flavonolignans: silibinin, silychristin, silydianin, and isosilybin. Silibinin (also known as silybin) is the most common bioactive of these chemicals, principally recognised for its hepatoprotective characteristics and

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having been utilised to treat a variety of acute and chronic liver disorders since the 1970s (Adetuyi *et al.,* 2021).

Hence in this study, silibinin and resveratrol were chosen and their effect *in silico* against different protein targets were studied.

MATERIALS AND METHODS

Protein preparation

The 3D structures of NF-kBp50 subunit, NF-kBp65 subunit, Bax, MMP9 and Caspase 3 were retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank. The protein name and their PDB ID were shown below.

Protein	PDB ID
Nf-kB p50	1NFK
Nf-kB p65	6QHL
MMP9	1L6J
Bax	4S0O
Caspase3	4JJ8

Ligand preparation

The 3D structures of resveratrol and silibinin was retrieved from the chemical data base "PubChem" which is a repository of chemicals. According to Lipinski rule of five, the compound was filtered as follows:

Sl. No.	Compound name	Compound ID	Molecular formula	Molecular weight (g/mol)	H acceptors and donors	Log P-Value
1	Resveratrol	CID 445154	C14H12O3	228.24	3, 3	3.2
2	Silibinin	CID 31553	C25H22O10	482.4	10, 5	2.63

Molecular docking

The molecular docking between the downloaded ligand and proteins was done using the commercial software Biovia Discovery Studio version 4.0 using CHARM force field. The protocol for receptor ligand interaction namely Libdock was used for molecular docking. The dock score, relative energy and RMSD value were observed for analysis.

ADMET analysis

The identified lead compound was subjected to ADMET analysis for toxicity studies using bioinformatics online tool PREADMET.

RESULTS AND DISCUSSION

The *in silico* molecular interaction studies made between ligand resveratrol and ligand silibinin with NF-kB p50 subunit and p65 subunit, MMP9, Caspase 3 and Baxprotein using BIOVIA discovery studio version 4 is presented in Table 1 and Fig.1.

The *in silico* molecular interaction revealed that silibinin and resveratrol interacted with five receptors with a high binding affinity. Silibinin and resveratrol found to interact with P50 subunit of NF-kB with a dock score of 150.67, 84.66 and binding energy of -11.5, -7.8 kcal/mol and amino acids LEU 167; ARG 161, GLY 182, ASP 183 respectively. With p65 subunit of NF-kB, silibinin and resveratrol had a dock score of 130.54, 79.89 and binding energy of -9.2, -6.4 kcal/mol and amino acids VAL 46; ASN 50 respectively. These findings are in concurrence with the results of Mulakayala *et al.* (2013) who reported the interaction of resveratrol to ARG 161 and silibinin to LEU 167 which was found to be a key residue involved in binding of Nf-kB p50 subunit with DNA at the consensus sequences through hydrogen bonding. Activation of Nf-kB is associated with the generation and progression of cancers. It targets many genes that facilitate tumour progression, inflammation, cellular mortality, cell survival, angiogenesis, proliferation, tumour promotion and metastasis. Hence NF-kB is considered as an attractive target for therapy using silibinin and resveratrol by binding to p50 and p65 subunits with high binding efficiency (Nishikori, 2005).

The results of the present study showed that resveratrol and silibinin could bind to Bax, Caspase 3 and MMP-9 with a docking score of 85.06, 137.53; 72.15, 123.04; 78.28, 131.47 and binding energy of -5.8, -7.6; -7.1, -10.4; -7.4, -8.1 respectively. These findings are in accordance with the previous reports suggesting that resveratrol and silibinin had better binding properties towards various cancer targets (Safdari *et al.,* 2015).

The pharmacokinetic features of resveratrol and silibinin were satisfactory with human intestinal absorption value of 88.48, 78.55, MDCK cell permeability value of 76.74, 0.06 skin permeability value of -3.15, -4.23, pure water solubility of 338.99 mg/L, 1.09 mg/L, plasma protein binding value of 100, 87.75 and blood barrier penetration value of 1.74, 0.061 respectively for resveratrol and silibinin. The toxicity studies revealed that resveratrol and silibinin were found to be non-mutagen as well as non-carcinogen as predicted by Ames test and carcinogenicity mice model test respectively. Drug likeness predictions showed that resveratrol and silibinin had better drug like properties and fulfilling all the five rules (Daina *et al.,* 2017).

Fig. -1

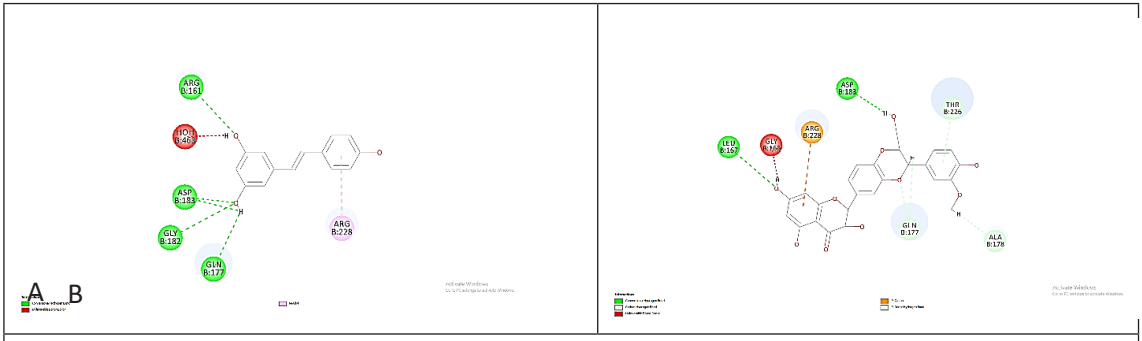
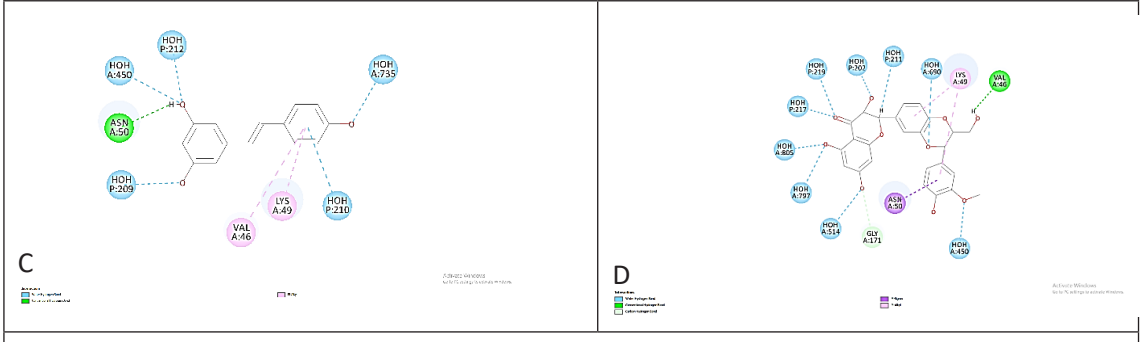


Fig. A. 2D interaction structure of Resveratrol with 1NFK (NF-kB P50 subunit); **B.** 2D interaction structure of Silibinin with 1NFK (NF-kB P50 subunit)



C. 2D interaction structure of Resveratrol with 6QHL (NF-kB P65 subunit)
D. 2D interaction structure of Silibinin with 6QHL (NF-kB P65 subunit)

CONCLUSION

The *in silico* molecular interaction studies between ligand resveratrol and ligand silibinin with NF-kB p50 subunit and p65 subunit, MMP9, caspase 3 and Bax proteins revealed that silibinin and resveratrol interacted with five receptors with a high binding affinity. The pharmacokinetic features of resveratrol and silibinin were satisfactory with human intestinal absorption value, MDCK cell permeability value, skin permeability value, pure water solubility, plasma protein binding and blood barrier penetration value. Hence, from this study it can be ascertained that compounds of resveratrol and silibinin can be used against various diseases like cancers. The promising compounds of resveratrol and silibinin can be translated clinically as candidates of drug molecule against various cancers which further necessitates studies.

Table 1. *In silico* interaction studies of resveratrol and silibinin with different protein targets

Protein	PDB ID	Active sites	Ligand	Dock Score	Absolute Binding Energy (kcal/ mol)	No. of H atoms	H bond length (A°)	Amino acids	RMSD value
Nf-kB p50 subunit	1NFK	31	Resveratrol	84.66	-7.8	1501	0.67- 3.71	ASP 183 GLY 182 ARG 161	0.8
			Silibinin	150.67	-11.5	5010	0.10- 4.19	LEU 167	0.8
Nf-kB p65 subunit	6QHL	2	Resveratrol	79.89	-6.4	764	1.55- 3.19	ASN 50	1
			Silibinin	130.54	-9.2	1528	1.8- 3.19	VAL 46	1
Bax	4S0O	17	Resveratrol	85.06	-5.8	1822	1.37- 3.1	----	1
			Silibinin	137.53	-7.6	1215	1.35- 3.07	ARG 109 LEU 47	1
Caspase 3	4JJ8	20	Resveratrol	72.15	-7.1	1462	0.64- 3.18	ILE 159	1
			Silibinin	123.04	-10.4	748	1.42- 3.18	GLN 184 CYS 186 GLY 145 ARG 87 ARG 233	1
MMP 9	1L6J	6	Resveratrol	78.28	-7.4	714	1.63- 4.07	---	1
			Silibinin	131.47	-8.1	714	1.6- 3.07	ARG 332	1

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