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Original Article

In-Silico Screening of Flavonoids Targeted for Death Receptors in Cancer by Using Hex Molecular Docking

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ABSTRACT

Objective: Docking is one of the major tools in the drug development process, here we have selected certain flavonoid molecules such as Formononetin, Tangeritin, Myricetin and Kaempferol and we docked against Death Receptors (DRs) for the prevention of cancer progression. **Materials and Methods**: In this study, Protein Ligand Docking, we have used HEX as a Docking Software. Receptor structure was obtained from Protein Data Bank (PDB) while the ligand is drawn by using the Chem Draw Software and docking was done according to the specified parameters. **Results**: All the investigatory molecules except tangeritin (DR5 -212.2; DR 2 -231.1) showed higher energy values on the Death Receptor 5 (Formononetin DR5 -197.8) which states, these flavonoid molecules are having higher affinity and steric compatibility to Death Receptor 5, where DR5 mediates the TRAIL mediated apoptosis. Tangeritin molecule shows higher energy values at DR2 -231.1, it may mediate its effect through the Fas apoptotic path. The Docked molecules are viewed with the help of chimera software to discover the interaction of molecules with the receptors. **Conclusion**: From the present study, it is confirmed that these investigatory molecules showed a predictable effect over Death Receptors, and further studies will be carried out with ADME/T (Absorption, Distribution, Metabolism, Elimination, Toxicity) Tool.

Key words: Flavonoids, Docking, Hex, Death Receptors.

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INTRODUCTION

Cancer is an unregulated uncontrolled growth of cells which occurs in any part of the body, which is one of the leading causes of death worldwide. According to International Agency for Research on cancer, it was projected about 6% of death in India is due to cancer.[1] Also World Health Organization (WHO) has proposed around 7,00,000 people were affected by cancer in India, which may be due to the increase in population, urbanization, industrialization and lifestyle changes.

Various treatment process for cancer like surgery, radiation and chemotherapy still exist, but has some drawbacks in eradicating cancer from human society, which may be due to lack of cell cycle specificity, toxicity of chemotherapy drugs and development of drug resistance. This made the researchers to work on the development of new molecules in cancer treatment.[2]

One of the targeted sites for the cancer treatment is activation of apoptosis either extrinsic or intrinsic pathway. Apoptosis is a highly programmed cell death for tissue remodeling, homeostasis and development of multicellular organisms.[3] Extrinsic pathway of apoptosis is associated with death receptor stimulation on the cell surface, these death receptors belong to the tumor necrosis factor receptor (TNFR) superfamily with TNF-R1, CD95 (APO-1, Fas), TRAMP (APO-3 DR-3), TNF-related apoptosis-inducing ligand (TRAIL)- Receptor 1 (TRAIL-R1 DR4) and TRAIL - R2 (APO-2, DR5). It is better to target the molecule on the death receptors (site specific) for producing better therapeutic action of the lead molecules and also helps in reducing the side effects of molecules at the other regions. Among the death receptors DR3 doesn't have enough detail mechanism in activation of apoptosis,[4] also evidences that DR3 shows activation of apoptosis in low, dense population of osteoblasts, while in high density population it doesn't show apoptosis.[5]

The receptor structures are obtained from Protein Data Bank (PDB) and the receptors are selected based on the previous studies. The Death Receptor 1 (1F3V),[6] Death Receptor 2 (3MX7),[7] Death Receptor 4 (1DG6),[8] Death Receptor 5 (1D4V).[9]

Many compounds which are in use today are the derivatives of natural products. Among the most widely used drugs which derived from plant alkaloids are the taxanes, Vinca alkaloids and topoisomerase inhibitors. Antitumor agents such as antibiotic, anthracyclines, Bleomycin and Mitomycin-C. Several antimetabolites also origins of natural products.[10] Flavonoids act as promising agents in combination with several chemotherapeutic agents against the growth of tumor cells.[11] Some mechanism involved in those activities are through cell cycle modulation, anti-angiogenesis, apoptosis induction, anti-oxidant, prevention of carcinogenic metabolic activation. Flavonoids in combination with chemotherapy to help overcome the problem of resistance of cancer cells and increases the effectiveness of chemotherapeutic agents.

There are various methods available for the drug development like combinatorial chemistry, High Throughput Screening, Denovo synthesis, Computational methods, etc. Bioinformatics is an interdisciplinary field which is comprised of Science, Computer Science, Statistics, Mathematics and engineering, helps in development of methods and software tools for understanding biological data. Bioinformatics has been used for *In Silico* analyses of biological queries using mathematical and statistical techniques.[12]

Computational Biology and Bioinformatics contributes their role, not only speeding up the drug discovery process,also helps in reducing the costs invested in designing of drugs. Rational Drug Design (RDD) helps

to facilitate and speed up the drug designing process, which involves a variety of methods to identify novel compounds.[8] One such method is the docking of the drug molecule with the receptor (target). The site of drug action, which is ultimately responsible for the pharmaceutical effect, is a receptor. Docking is the process by which two molecules fit together in 3D space. Here we have used Hex as Docking Software version 8.0.0 and the interaction of the ligand-protein binding was studied with software chimera version 1.10.2.

The purpose of the study was to identify the potential phytochemicals, which binds to the death receptors for the activation of apoptosis in cancer cells. The data collected here shows the possibility of flavonoids, which successfully binds to the death receptors for initiation of cell death in cancer cells. This data may break through the lead molecules for further investigation in cancer studies.

MATERIAL AND METHODS

Bioinformatics tools such as PubMed Central (PMC), Drug Bank, PDB (Protein Data Bank) and the software's like Hex, Chem draw was used in the present study. Chem draw is a professional tool for researchers to design and communicate the chemical structures also it helps to draw the chemical molecules and calculates chemical properties.

Hex is an interactive molecular graphics tool for calculating and displaying feasible docking modes of pairs of protein and DNA molecules. Hex also calculates protein-ligand Docking, assuming ligand is rigid and it can superimpose molecules in 3D shapes. It uses spherical polar Fourier correlations to accelerate the calculations and it's still one of the few docking programs which has built-in graphics to view the results.[13]

Drug bank is an online database containing information regarding drug and drug targets. Drug Bank provides complete information of chemical, pharmacological, pharmaceutical, sequencing of a drug target, structure and pathway of molecular interactions. It is widely used by the drug industry, Pharmacist and physicians.[14]

Three dimensional structural database of the biological molecules such as nucleic acids, proteins was provided by the Protein Data Bank (PDB). PDB is online free accessible key resource in structural biology and structural genomics, which provides information, regarding the protein obtained by X-ray crystallography, NMR spectroscopy and Cyro - electron microscopy.[15]

Among the digital media, which publishes scholarly articles in the field of biomedical and life science journal, PubMed Central (PMC) plays a significant role in providing information of journals, scientific research data's which is a major research database in suite of resources developed by the National center for Biotechnology Information (NCBI).

The structure of Death Receptors (DR) was obtained from the PDB, It is shown in the Figure 2, while the structure of flavonoids was sketched using Chem draw and the docking analysis of flavonoids with Death Receptors was carried by using the HEX docking Software.

The parameters used in the docking process were

- Correlation type - Shape Only
- FTT mode - 3D Fast lite
- Grid Dimension - 0.6
- Receptor Range - 180
- Ligand Range - 180
- Twist Range - 360
- Distance Range - 40

The flavonoids are docked using the above conditions with the death receptors.

RESULTS AND DISCUSSION

Docking results are tabulated in Table 1, between the flavonoids and Death Receptors Hex software allows the receptor molecule to rotate on the Z axis,[16] from the obtained results Tangeritin molecule has a higher energy value of all the Death receptors than compared to any other molecule. Its shown in Table 1.

The circled parts depicted in Figure 1 distinguish the difference between the flavonoid molecules. The receptor family was selected from the Protein Data Bank (PDB). Death Receptor 3 was not selected for the present study because of the DR3

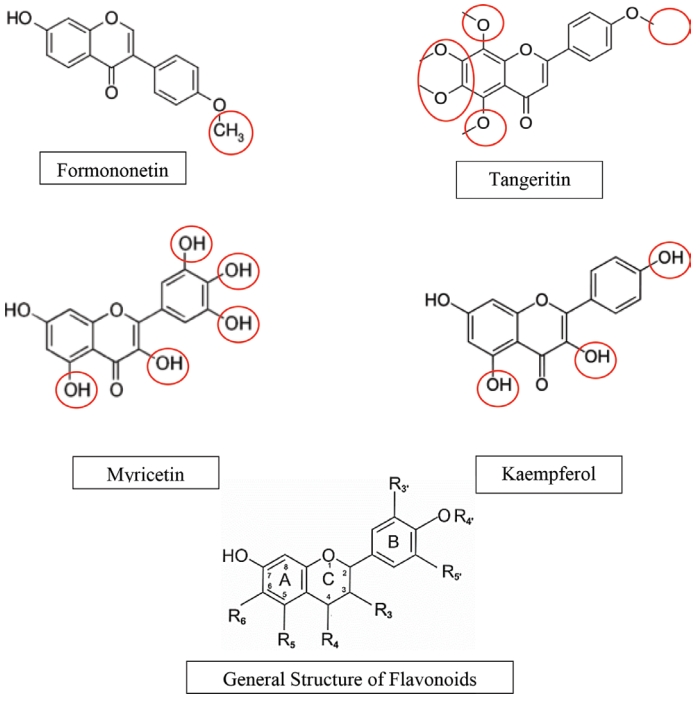

Figure 1: Structure of flavonoids.

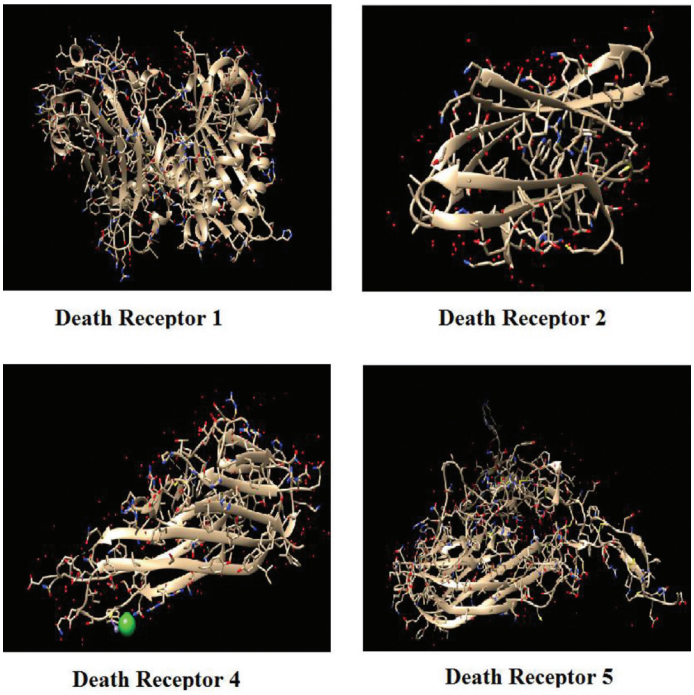

Figure 2: Structure of Death Receptors.

Table 1: Effect of Investigatory molecules over the Receptor

S.No	Investigatory Molecule	Pub Chem ID	Receptor	E – Value	Receptor Interactions
1.	Formononetin	5280378	Death Receptor 1	-176.3	Formononetin molecule Carbon at 16th position interacts with Serine amino acid at 7 Å and glycine at 136 Å. Also Carbon at 3rd position interacts with Glycine at 67 Å, while with Serine 7 Å at Carbon 9th position
			Death Receptor 2	-188.0	Formononetin has some clash point with the receptors at Tryptophan at 8 Å and Leucine 86 Å. It has a contact point with Asparagine 82 Å with the oxygen at the 3rd position and also with carbon in the 2nd position of the formononetin
			Death Receptor 4	-168.7	Formononetin has a contact point with the receptor at Methionine 268 Å with the oxygen held at the 3rd position also with the carbon at 1st, 5th and 6th position. Second contact point of Formononetin with the receptors are Isoleucine 151 Å with the carbon position held at the 13th, 14th, 15th, 16th. It has one clash point at the Arginine 158 Å
			Death Receptor 5	- 197.8	Formononetin Carbon at the 16th position it has a contact with Aspargine at two places 152 Å and the other at 134Å. Where carbon at the 5th and 6th position has the contact point with Isoleucine 151 Å, while oxygen at 3rd position has a contact point with Isoleucine present at the 266 Å. Apart from these contact points Carbon at the 12th position of formononetin has a contact point with Serine 153 Å. Finally, it has a clash point at Threonine 188 Å.
2.	Tangeretin	68077	Death Receptor 1	- 208.8	Tangeritin has two contact points, one is in the Carbon 11th position, has attached to the receptor with Arginine 393 Å and also Carbon 19th position has attachment with selenomethionine 463 Å. Tangeritin has a clash point with Lysine 25 Å
			Death Receptor 2	-231.1	Tangeritin has contact points with carbon 12th position with leucine 86 Å and Carbon 17th position with isoleucine 81 Å. Also Carbon 11th position has an attachment with Leucine 79 Å. It has no clash points with receptors.
			Death Receptor 4	-190.1	Tangeritin has a clash point with arginine 158 Å. Tangeritin has a contact point at the carbon 12th position with glutamine 263 Å and Valine 260 Å.
			Death Receptor 5	-212.2	Tangeritin has a clash point at Threonine 188 Å and has many more contact points than other receptor. Tangeritin at carbon 13th, 14th, 15th and 19th position has attached to Leucine 136 Å, asparagine 134 Å and also with asparagine 152 Å. Tangeritin at Carbon 14th and 15th position has same contact point of Asparagine 152 Å, while carbon 17th and 18th position has a contact point with serine 153 Å Tangeritin at carbon 12th has a contact point with Valine 260 Å and glutamine 263 Å, Carbon 9th and 10th position of tangeritin shows contact with Isoleucine 266 Å, Carbon 11th position of tangeritin shows contact with methionine 268 Å.
3.	Myricetin	5281672	Death Receptor 1	-188.0	It has clash pojnt at lysine 25 Å. Oxygen at 8th position of myricetin with glutamine 74 Å at the receptor, also at the carbon 11th position has a attachment at the serine 14 Å.
			Death Receptor 2	-184.1	Myricetin has a contact point at the carbon 6th position with leucine 4 Å, also oxygen at 2nd position and carbon at 3rdposition has an attachment point with alanine 6 Å
			Death Receptor 4	-170.5	Myricetin has a clash point at Arginine 158 Å. It has contact point carbon 8th position with leucine 169 Å and also carbon 6th position with glycine 172 Å.
			Death Receptor 5	-192.0	It has a no Contact point only clash point at tryptophan 189 Å
4.	Kaempferol	5280863	Death Receptor 1	-181.3	It has clash point with lysine 25 Å. Comparable has a contact point at the carbon 6th position with histidine 65 Å, also carbon 8th position has a contact point lysine 63 Å.
			Death Receptor 2	-195.7	It has a Clash point with Lysine 87 Å, it has a contact point at the carbon 12th position with valine 10 Å and also oxygen 4th position with leucine 79 Å
			Death Receptor 4	-168.1	It has a clash point at arginine 158 Å. It has contact points with oxygen at the 2nd position with glutamine 268 Å, also oxygen at the 6th position, carbon at 13th and 14th position has a same contact point isoleucine 266 Å. Kamepherol molecule at the carbon 11th and 12th position with isoleucine 151 Å while Oxygen at the 4th position with aspargine 152 Å
			Death Receptor 5	-195.1	Kampherol has a contact point with carbon at 7th position and oxygen at 4th position with cysteine 116 Å. It has only clash point threonine 188 Å.

do not relate in regulating the cell death. DR 3 plays a major role in the development of experimental autoimmune encephalomyelitis, allergic lung inflammation, inflammatory arthritis.[17]

From the results, all the investigatory molecules except tangeritin (DR5 -212.2; DR 2 -231.1) showed higher energy values on the Death Receptor 5 (Formononetin DR5 -197.8) which states, these flavonoid molecules are having higher affinity and steric compatibility to Death Receptor 5, where DR5 mediates the TRAIL mediated apoptosis. Tangeritin molecule shows higher energy values at DR2 -231.1, it may mediate its effect through the Fas apoptotic path. The Docked molecules are viewed with the help of chimera software to discover the interaction of molecules with the receptors.

CONCLUSION

For the Structural based drug designing, protein-ligand interaction plays a significant role, so in the present work we have selected the receptors and the investigatory molecule was used against the Death Receptors. When molecules docked with death receptors except Tangeritin all other molecules showed higher energy state at Death Receptor 5 while Tangeritin showed higher energy value at Death Receptor 2. Further, these works may be extended for ADME/T (Absorption, Distribution, Metabolism, Excretion / Toxicity) of these compounds using commercially available ADME/T tools which helps in the Drug Discovery and Drug development process.

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CONFLICT OF INTEREST

The Authors declare that there is no conflict of interest.

ABBREVIATION USED

DR: Death Receptor; **NCBI:** National Centre for Biotechnology Information; **ADME:** Absorption, Distribution, Metabolism, Excretion.

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