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IN SILICO STUDIES OF FLAVONOID'S PATTERN OF INHIBITION AGAINST WNT/ β-CATENIN CASCADE

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CANDIDATE'S DECLARATION

I, **Akhila K [2K21/MSCBIO/03],** student of **M. Sc. Biotechnology,** hereby declare that the project Dissertation titled **"In silico studies of flavonoid's pattern of inhibition against wnt/ β-catenin cascade"** which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirements for the award of the degree of Master of Science, is original and not copied from any source without proper citation, is done under the supervision of **Dr. Navneeta Bharadvaja.** This work has not previously formed the basis for the award of any Degree, Diploma Associateships, Fellowship or other similar title or recognition.

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CERTIFICATE

I, hereby certify that the Project Dissertation titled, **"In silico studies of flavonoid's pattern of inhibition against wnt/ β-catenin cascade"** which is submitted by, **Akhila K [2K21/MSCBIO/03],** Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, is a record of the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

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ABSTRACT

The Wnt signaling system is an intricate network of cellular communication pathway that is crucial for tissue homeostasis, development, and disease. It is named after a family of secreted proteins called Wnts, which binds to the receptor on the cellular surface to activate intracellular signaling cascades. This system has vital role in growth related conditions and tumours, particularly as a main factor in proliferation and spread of colorectal cancer (CRC), which is correlated with the build-up of β-catenin in cells due to glycogen synthase kinase-3β (GSK-3β) inactivation. Recently, several polyphenolic substances from the naturally occurring flavonoid family were examined for their potential to inhibit Wnt signaling and found that they exhibit potent anti-oxidant and anti-carcinogenic activities. This thesis identifies the mechanism by which the flavonoids like silibinin, eriodictyol and quercetin attaches to β-catenin and Wnt protein, determining more effective natural inhibitor of the disease-causing protein. For clarify the binding efficiency of flavonoids, a comparative molecular docking analysis was carried out and their interaction with the respective protein molecule is done in this study.

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Keywords: Wnt pathway, β-catenin, flavonoids, docking studies, colorectal cancer.

Abbreviations:

CRC- Colorectal cancer

GSK-3β- Glycogen synthase kinase-3β

CK-1α- Casein kinase-1α

TGF-β- Transforming growth factor-β

FZD receptors- Frizzled receptors

LRP5/6- Low density lipoprotein receptor-related protein 5 and 6

Dvl- Dishevelled

APC- Adenomatous polyposis coli

TCF- T cell factor

LEF- Lymphoid enhancer binding factor

CHAPTER 1
INTRODUCTION

One among the important cellular signaling cascade, the Wnt cascade being a predominant pathway in the progression and occurrence of colorectal cancer [1]. When Wnt ligand attach to Frizzled receptors on surface of cell, the pathway is triggered. It can cause the beta-catenin to become more stable and accumulate in the cytoplasm. This causes it to go inside the nucleus and can activate TCF/LEF transcription factors [2]. Expression of certain genes important in cell division, survival, and differentiation then occurs after the transcription 'on' signal. The Wnt cascade controls tissue homeostasis and a number of embryogenic developmental processes through β-catenin transcriptional activator. The dysregulation of Wnt/β-catenin cascade and the accumulation of β-catenin brought on by the inactivation of glycogen synthase kinase-3β have both been related to the onset of cancer [3].

1.1 Wnt signaling cascade

In most CRC patients, the Wnt signal transduction pathway, mainly promotes tumour invasion, recurrence, and metastasis, and is inappropriately active and plays a significant function in the onset and development of CRC [4].

Wnt pathway is made up of nineteen secreted-glycoproteins that can transmit signals intracellularly from exterior through cell receptors on the surface. As a result, the proteins take part in an increased range of biological procedures, including development of embryo and organ formation, proliferation of cells, differentiation of cells, and self-renewal of stem cells [5] [6]. β-catenin independent non-canonical cascade and beta-catenin dependent canonical cascade are two general divisions of Wnt signalling cascade [7]. Wnt cascade is a desirable therapeutic target for the CRC, one among the cancers with highest rates mortality and morbidity; yet bringing out safety and efficacy is very difficult [8].

In the western world, CRCs are a primary contributor in non-smoking-related cancer deaths. It is found that about 90% of the CRC have a canonical Wnt cascade mutation, which will eventually cause β-catenin to stabilize and accumulate in a cell's nucleus [9]. An active canonical cascade is characterised by nuclear β-catenin, which is present in even the minute observable lesions brought on by Wnt mutations [10].

Canonical Inactivation of glycogen synthetase kinase-3β that results in the building of beta-catenin in cells, which is correlated with Wnt signaling's contribution to the proliferation of tumour cells. However, unchecked production of β-catenin results in the fibromatosis development, sarcoma, and mesenchymal tumours. Numerous carcinomas are caused by β-catenin, in conjunction with trans-acting LEF-1 or TCF [11]. Recently, several polyphenolic substances from the naturally occurring flavonoid family were examined for their ability to block Wnt signaling.

The cascade begins by binding of Wnt ligands to receptors on surface of cell, that causes triggering of a series of downstream signaling events that could ultimately leads to variations in expression of genes and cellular behaviour. The main components of the Wnt pathway includes:

1. **Wnt ligands:** There are 19 kinds of Wnt ligands that have been found in humans. These ligands are secreted glycoproteins that could bind to the cell surface receptors to initiate the Wnt pathway.

2. **Frizzled receptors:** FZD receptors are the seven-trans-membrane proteins that can act as a main receptor for Wnt ligands. FZD receptors are able to activate several different downstream signaling cascades, depending on the type of Wnt ligand and the cellular context.

3. **LRP5/6 co-receptors:** The LDL receptor-related protein 5 and 6 co-receptor are required for the Wnt signaling pathway activation by many Wnt ligands. When the Wnt ligand bind to FZD receptor, the LRP5/6 co-receptor is recruited to the complex, that causes the activation of downstream signaling events.

4. **Dishevelled (Dvl):** Dvl is a cytoplasmic protein that plays a prominent mechanism in activating the Wnt cascade. On binding to the receptors by the Wnt ligands, Dvl is recruited to the complex and undergoes conformational changes that activate downstream signaling events.

5. **β-catenin:** A significant Wnt cascade downstream effector is beta-catenin. When Wnt ligands are not present, beta-catenin is phosphorylated and is then targeted by proteasome for destruction. Whereas, beta-catenin is stabilised and its concentration rises in the cytoplasm when the Wnt cascade occurs, allowing it to go to the nucleus and control gene expression.

6. **TCF/LEF transcription factors:** TCF/LEF transcription factors are nuclear proteins that interact with beta-catenin and regulate the expressions of targeted genes. When the Wnt cascade is activated, beta-catenin reaches the nucleus and interact with the TF to carry out expression of the genes.

Wnt cascade is a complex signaling cascade that includes a variety of different components, containing cytoplasmic proteins, ligands, receptors, co-receptors, and nuclear transcription factors. The precise details of the pathway can change based on the particular context and the type of Wnt ligand involved.

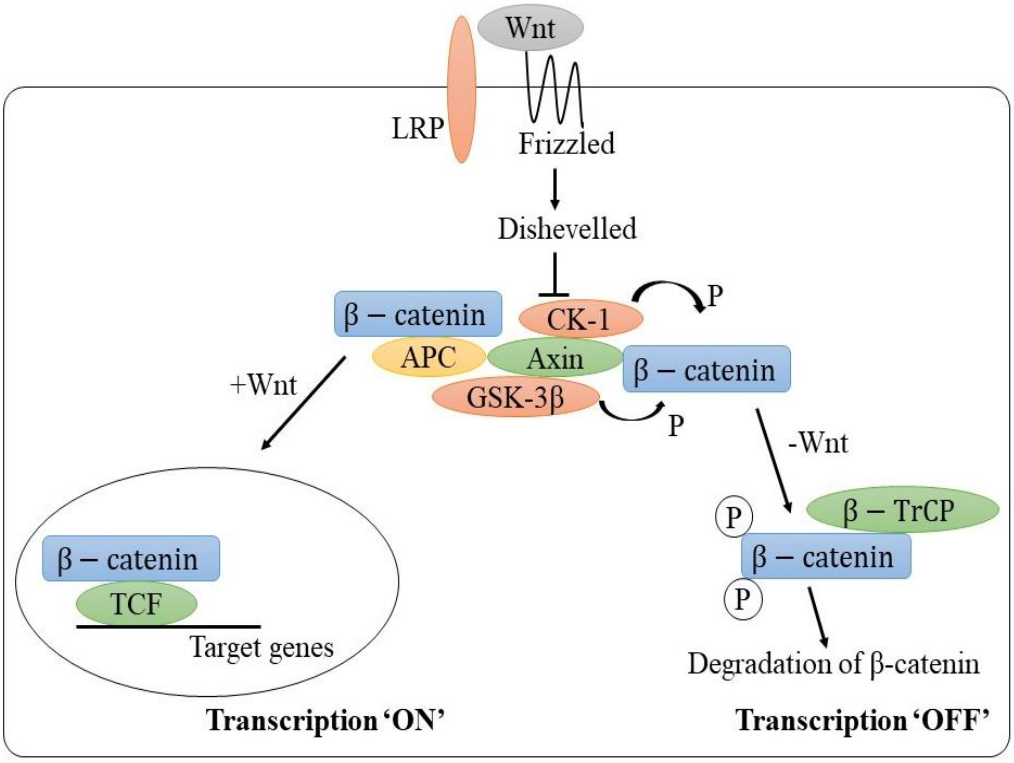

Fig. 1.1 +/-Wnt pathway

The canonical Wnt signal transduction cascade, which was first identified during the development of vertebrate and non-vertebrate embryos, has now been linked to the emergence of numerous distinct tumour forms, mostly with gastrointestinal origins. Target genes are been activated by the classical mechanism, known as canonical Wnt cascade through nuclear stabilization of β-catenin.

DNA binding protein of the TCF/LEF-1 family are directed in their transcriptional abilities by the canonical Wnt signalling cascade, which regulates cell behaviour. The Wnt cascade is triggered by stabilization of cytosolic β-catenin, that binds to TCFs to activate target genes. Wnts are glycoproteins that attach to the frizzled. Main co-receptors of the Wnt ligands are LRP 5 and 6. Wnt signalling inside the cell causes the cytosolic β-catenin to stabilize. Casein kinase 1 α phosphorylates β-catenin at serine residue 45 when Wnts are not present, which then allows GSK-3β to phosphorylate threonine or serine residues 37, 41 and 33. The final two amino acids, when

phosphorylated, causes beta-catenin to be ubiquitinated by β-TrCP and then degraded in the proteasomes [1].

Dishevelled, a cytoplasmic component, prevents β-catenin degradation in the presence of Wnts by an unidentified mechanism. The transcription of genes is triggered by the β-catenin as it enters to nucleus and binds with transcription factors. Beta-catenin is stabilized and target genes are activated in tumours as a result of either excess expression of Wnts (that is not common in human tumours) or mutation is said to be one of the substances involved in β-catenin's breakdown. Because β-catenin enters the nucleus and interacts with the transcription factor there, the target genes are activated when the Wnt pathway is active. A constant flow of non-phosphorylated β-catenin will be delivered to the nucleus because the destructive complex remains inactive leading to the overexpression of the genes [12]. Thus, it is possible to block uncontrolled transcription in cancer that is brought on by β-catenin activity; either by suppressing β-catenin that are non-phosphorylated or by introduction of a competitive inhibitor for transcription factor to hinder its association with β-catenin.

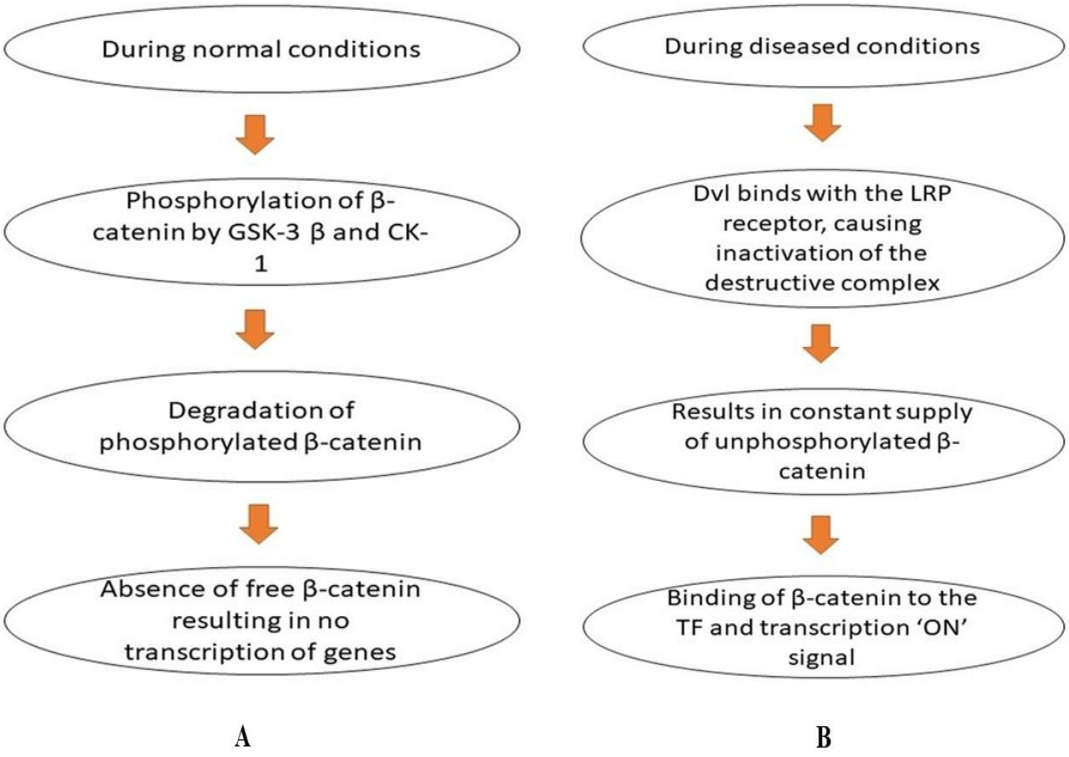

Fig. 1.2 Flowchart showing (A) Wnt signaling cascade 'off' during normal conditions and (B) Wnt signaling cascade 'on' during diseased condition.

Natural compounds or compounds that are obtained from plants can be used as a preventive aid for several types of cancer, one such type is the flavonoids [13]. Phenolic chemicals like the flavonoids are the secondary metabolites that protects plants against harmful environmental factors, ultraviolet rays, and microbial infections [14].

It is generally recognized that flavonoids may both prevent and treat diseases. The basic chemical structure of flavonoids has three carbon rings. These polyphenols are found in organically growing plants and vegetables and these polyphenol-rich fruits and vegetables are known to produce functional health advantages. They exhibit a molecular structure of C_6-C_3-C_6 [15]. Based on variances in chemical structure, they are divided into many subclasses, such as isoflavones, flavones, flavonols, and flavanones.

CHAPTER 2
LITERATURE REVIEW

Surgery, chemotherapy, and radiotherapy were the primary treatments for CRC in the past, but recurrence and distant metastases following treatment continue to be difficult to treat [16]. The Wnt/β-catenin system is activated in certain cases and in most of the CRC patients, inhibiting this route may prevent the growth, spread and metastasis of CRC. The research community is currently interested in the therapeutic development of medicines that targets the Wnt/β-catenin cascade. Wnt/β-catenin signaling cascade is therefore anticipated to be the primary focus of CRC treatment. It is therefore anticipated to be the primary focus of CRC treatment [12].

2.1 WNT PROTEIN

Different signal pathways are activated by wnt proteins, which are secreted glycoproteins. Cancer is one of many diseases that can be brought on by improper Wnt signalling control. Wnt proteins have an N-terminal signal sequence.
The stabilisation and then nuclear translocation of β-catenin protein, which is being regulated by Wnt protein, is the main mechanism behind Wnt's induction of the stimulation of tumor-cell proliferation and neoplasia [17].

GSK-3β, CK-1α, APC, and Axin may work together to increase the ubiquitination of beta-catenin in the absence of activation of Wnt pathway [18]. But when Wnt is activated, GSK-3β is released from the destruction complex by binding to the Frizzled receptor, LRP5 and 6 coreceptors. DVL and Axin build up in the cellular membrane at the same time, preventing the development of the destructive complex [19] [20].

The TCF/LEF transcription factors can bind to the beta-catenin molecule. As a result, β-catenin tend to be stabilised, gather within the cytoplasm, and undergoes nuclear translocation. The transcription of genes necessary for the ultimate activity of Wnt is induced by this binding [21].

The residues of β-catenin that bind with TCF4 to create a complex includes Tyr306, Gly307, Lys312, Arg386, Asn387, Asn426, Cys429, Lys335, Lys345, Arg376 Lys435, Arg474, Lys508 etc [22].

2.2 ANTI-CRC THERAPY TARGETING WNT CASCADE

The majority of CRC cases have mutations in Wnt/β-catenin cascade, which is a major cause for the progression and for the development of CRC [4] [23]. The build-up of β-catenin protein in nucleus may result from abnormal Wnt/β-catenin pathway activation, which would greatly increase cell proliferation [24]. Adenomas are first developed, however with enough mutations, adenomas can grow into CRC [21] [25]. Adenomas first arise as a result of this, but with enough mutations, adenomas can progress into CRC. Studies aiming to block the signal transduction and target the Wnt signaling cascade have revealed that the route aids in stabilizing the development of the destroying complex [26] [27].

Treatments that helps patients to get benefit with CRC by enhancing the standard of life and extending the time of survival may be developed as a result of research aimed at blocking the Wnt/β-catenin cascade [17]. By enhancing the instability of β-catenin, the β-catenin inhibitor KYA1797K may bind Axin and decrease tumours [28]. A new β-catenin inhibitor called BC2059 may lower cytoplasmic as well as nuclear level of β-catenin as well as the TCF4/LEF transcriptional activity [29]. The target gene of the small chemical β-catenin/TCF inhibitor ICG-001 encourages the growth and spread of colorectal tumour cells.

2.3 FLAVONOIDS AGAINST WNT CASCADE

Acting on several targets in Wnt/β-catenin cascade, specific natural substances found in traditional therapeutics have been shown to alter the pathophysiology of CRC and can effectively treat them. Furthermore, research have shown that these natural substances have lower toxicity and less adverse patient events [30] [31].

It is important to note that when some of the natural substances are coupled with chemo-therapeutic medications, they may work together to reduce the side effects of

chemotherapy while still exerting antitumor effects. Further investigation into the anti-CRC mechanisms of these natural chemicals is necessary in order to understand how they help CRC patients [7] [32]. On the ability of polyphenol to chemo protect against cancer, numerous investigations have been conducted [33]. There have been carried out many animal researches and cell culture techniques that have revealed many evidences that pointed to CRC prevention effects [34] [30].

2.3.1 Silibinin

Silibinin is a flavonoid, specifically said to be a flavonolignan that is obtained from the milk thistle plant (*Silybum marianum*) which has long been valued for its therapeutic benefits and uses. It is found to be a potential antioxidant, silibinin is also known for its ability to fight cancer, viruses, and inflammation. Silibinin is commonly used as a natural remedy for a wide range of liver disorders, including cirrhosis and hepatitis, because of its ability to protect liver cells from injury. Silibinin have other potential uses such as in the treatment of diabetes, cardiovascular disease, and skin problems, and their other therapeutic effects have been investigated [35].

Its possible anticancer characteristics have been investigated in relation to a variety of cancer forms, including colorectal cancer. Silibinin induces apoptosis and cell cycle arrest, which stop the growth of human colorectal cancer cells. It can also prevent the migration and invasion of colorectal cancer cells by inhibiting the activity of certain proteins that are involved in cell motility and invasion [7] [36].

It was investigated that dietary supplementation with silibinin reduced the formation of colorectal tumours in rats that had been exposed to a carcinogen. Silibinin prevent the formation of precancerous lesions in colon of mice that had been treated with a carcinogen.

2.3.2 Eriodictyol

Eriodictyol is another plant pigment that have been utilized to treat several significant medical disorders. Eriodictyol is obtained from Yerba Santa (*Eriodictyon californicum*), *Millettia duchesnei* twigs and many therapeutic plants. Eriodictyol is a crucial component of dietary supplements, and its presence in food has exceptional antioxidant

properties that lower the likelihood of developing any health problems. Eriodictyol, is a functional food with a high antioxidant content and is a crucial component of dietary supplements [7] [30].

Since eriodictyol can scavenge free radicals and preserve the antioxidant system, pre-treatment with it has led to a rise in the amount of anti-oxidant enzymes. In conjunction with hesperidin, the lemon fruit compound eriodictyol has known to decrease the oxidative stresses. For the purpose of preventing the development of certain diseases like cancer especially CRC, eriodictyol modifies the cellular biochemistry and molecular pathways. By inducing apoptosis and cell cycle arrest, eriodictyol can stop the growth of human colorectal cancer cells. It can also inhibit CRC cells from migrating and invading by inhibiting the activity of a protein called matrix metalloproteinase-9, which is involved in cancer cell metastasis.

However, researches on the chemo-preventive potential of eriodictyol in colorectal cancer is still limited and further many studies are required to fully understand its action mechanisms and estimate potential clinical applications. Also, the bioavailability of eriodictyol from dietary sources and supplements may vary, which can impact its potential effectiveness as a chemo preventive agent.

2.3.3 Quercetin

The flavonoid component quercetin, which is found in many fruits, vegetables, and grains, is what gives fruits, flowers, and vegetables their vivid colours. They are also well known for having anti-inflammatory and anti-oxidant characteristics, which may have positive effects on health.

Several foods, including onions, apples, citrus fruits, berries, green tea, and red wine contain quercetin. It has undergone testing for its significant health advantages, such as lowering inflammation, enhancing heart health, and preventing cancer. Quercetin has been shown to offer other potential health advantages, including enhancing athletic performance and easing allergy and asthma symptoms. Numerous research have looked into its potential to be used as a chemo preventive therapy for colorectal cancer. Quercetin may have anticancer effects in colorectal cancer cells, according to several studies [37].

Quercetin suppresses the growth of human colorectal cancer cells in vitro by inducing apoptosis (programmed cell death), according to a 1995 study. By preventing the Wnt cascade, which is typically dysregulated in colorectal cancer, from working, quercetin can also stop the proliferation of colo-rectal cancer cells in vitro [38].

Comparing its potential effect on colorectal cancer cells, quercetin has also been found in clinical studies as a potent chemo preventive agent acting against colorectal cancer [39] [40]. A controlled trial published in 2007 found that daily supplementation with quercetin and vitamin C reduced the risk of advanced colorectal adenomas (precancerous growths) in patients who are suffering from colorectal cancer [41].

The risk of CRC has been shown to be inversely correlated with flavonoid consumption in recent years [42] [43]. The interest in flavonoids has lately increased because to their strong antioxidant activity against oxidative stresses, and have been shown that they have anti-carcinogenic capabilities against various types of cancer. They are helpful in the reduction of CRC because they act as antiproliferative agents, sensitize cancer cells, or lessen the oxidative stress brought on by the pharmaceuticals employed in these treatments [44].

The flavonoids could prevent the β-catenin/Transcription factor complex by occupying the binding sites. To prevent the β-catenin and TCF interaction from occurring and to inhibit the transcription of targeted genes, it can be estimated that chemicals from the flavonoid family would bind with the TCF binding residues of the β-catenin [45]. Here the inhibitory effect of some of the flavonoids like silibinin and eriodictyol that comes under the superclass flavanone; and quercetin that comes under the superclass flavonol on β-catenin are studied using docking mechanism and their ability to be used as a curative agent in colorectal cancer is been examined [46].

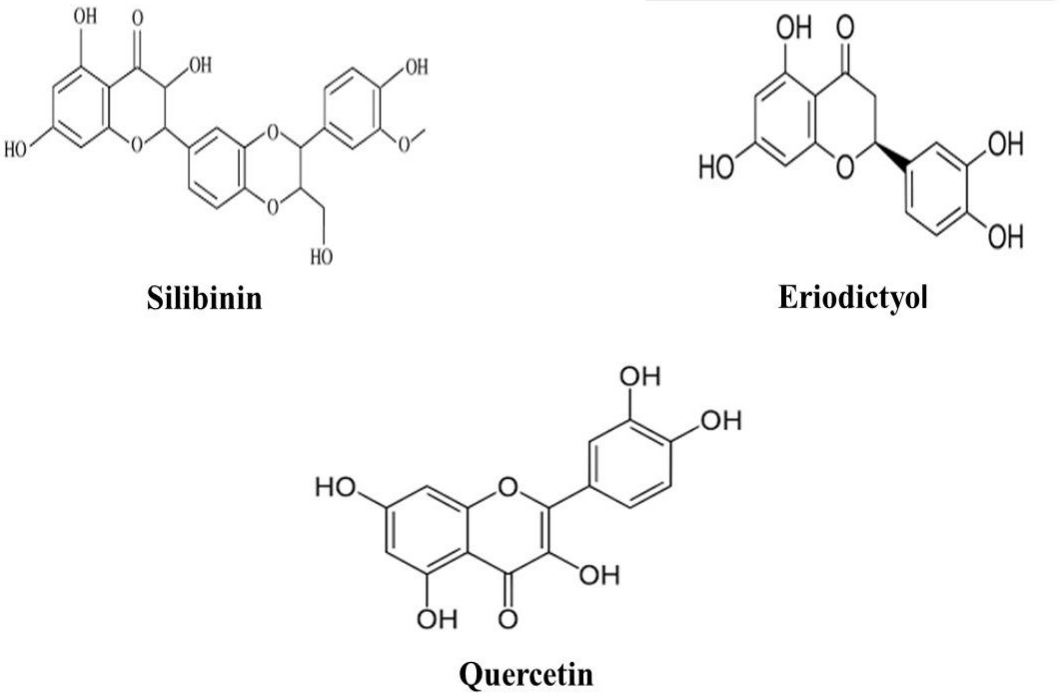

Fig. 2.3 Chemical structure of flavonoids like silibinin, eriodictyol and quercetin.

There are several clinical experiments conducted over the use of flavonoids for the prevention of CRC. Study published in the journal Cancer Prevention Research in 2015 found the use of flavonoid-rich supplement on colon cancer biomarkers in patients with colorectal adenomas (precancerous growths in the colon). The study found that the supplement reduced levels of a biomarker associated with colon cancer risk.

A flavonoid-rich cocoa beverage's effects on risk indicators for colorectal cancer in healthy persons were also investigated. According to the study, drinking cocoa lowered inflammation markers and enhanced antioxidant status indicators, which may have a preventive effect against colorectal cancer. Flavonoids may have a preventive impact against colorectal cancer, according to a review of numerous studies that was published in the journal Nutrients in 2016.

Overall, it can be said that flavonoids may have a protective impact against CRC; however, further investigation is required to confirm this and to identify the best flavonoid kinds and dosages for cancer prevention. Additionally, without seeking the advice of a medical practitioner, flavonoids should not be used as a substitute for medical care or as a means of cancer prevention. Preclinical studies investigating the connection between flavonoids and the Wnt cascade have also been conducted.

CHAPTER 3
METHODOLOGY

3.1 INTERACTION BETWEEN β-CATENIN AND FLAVONOIDS

3.1.1 Data collection

3D structure of the protein of interest, 3SLA (First four repeats of human beta-catenin) (PDB DOI: 10.2210/pdb3SLA/pdb) was obtained from RCSB Protein Data Bank in PDB format (https://www.rcsb.org), and 3D conformer compounds in the flavonoid family, silibinin and eriodictyol from flavonone subclass, quercetin from flavonol subclass were obtained in SDF format from PubChem database (https://pubchem.ncbi.nlm.nih.gov).

3.1.2 Protein and ligand preparation for docking

The target protein β-catenin was prepared using BIOVIA discovery studio. Protein opened in BIOVIA, viewed the hierarchy, and removed water molecules and hetatms. Later polar hydrogen atoms then added to protein and saved the file in PDB format.

PyRx virtual screening tool was used for docking. It is a multiple ligand docking software with AutoDock, Vina Wizard, AutoDock Wizard and Open Babel embedded in it that provides easy conversion of ligand from SDF format to PDB format. Protein was loaded in PyRx screening tool and converted from PDB to PDBQT format after clicking on AutoDock and then selecting 'Make macromolecule.' After loading the ligand to PyRx, the energy of all were minimized by clicking 'Minimize All.' Later all the ligands were converted to PDBQT format.

3.1.3 Molecular docking

Move to Vina Wizard and click on start and then select the protein and ligands, the whole molecule will be covered with the grid box. Auto-grid was used for making the grid and points on the grid were placed to cover the entire inner cavity of the receptor which constitute the ligand and then clicked on forward. Docking was then completed and the scores were recorded in excel format by clicking on 'Save as Comma separated values.' Best score was then identified and the conformation of ligand with maximum binding affinity from all the conformations generated were identified by viewing the display in AutoDock. The display of the ligand was then changed into molecular surface and then saved in PDB format.

3.2 INTERACTION BETWEEN WNT PROTEIN AND FLAVONOIDS

3.2.1 Data collection

3D structure of the protein of interest, 6AHY (Wnt signaling protein) (PDB DOI: 10.2210/pdb6AHY/pdb) was obtained from RCSB Protein Data Bank in PDB format (https://www.rcsb.org), and 3D conformer compounds in the flavonoid family, silibinin and eriodictyol from flavonone subclass, quercetin from flavonol subclass were obtained in SDF format from PubChem database (https://pubchem.ncbi.nlm.nih.gov).

3.2.2 Wnt protein and ligand preparation for docking

The target protein Wnt was prepared using BIOVIA discovery studio. Protein opened in BIOVIA, viewed the hierarchy, and removed water molecules and hetatms. Later polar hydrogen atoms added to the Wnt protein and saved the file in PDB format.

PyRx virtual screening tool was used for docking. It is a multiple ligand docking software with AutoDock, Vina Wizard, AutoDock Wizard and Open Babel embedded in it that provides easy conversion of ligand from SDF format to PDB format. Protein was loaded in PyRx screening tool and converted from PDB to PDBQT format after clicking

on AutoDock and then selecting ‘Make macromolecule.’ After loading the ligand to PyRx, the energy of all were minimized by clicking ‘Minimize All.’ Later all the ligands were converted to PDBQT format.

3.2.3 Molecular docking of Wnt protein with flavonoids

Move to Vina Wizard and click on start and then select the Wnt protein and ligands, the whole molecule will be covered with the grid box. Auto-grid was used for making the grid and points on the grid were placed to cover the entire inner cavity of the receptor which constitute the ligand and then clicked on forward. Docking was then completed and the scores were recorded in excel format by clicking on ‘Save as Comma separated values.’

Best score was then identified and the conformation of ligand with maximum binding affinity from all the conformations generated were identified by viewing the display in AutoDock. The display of the ligand was then changed into molecular surface and then saved in PDB format.

CHAPTER 4
RESULT

β-CATENIN AND FLAVONOIDS

4.1 Binding affinity between β-catenin and flavonoids

After docking was performed using AutoDock Vina, the result came out to be positive, showing highest affinity between the protein β-catenin and silibinin. The highest docking score of -8.4 Kcal/mol shows that silibinin binds to significant receptor sites of the β-catenin molecule. Second highest docking score was obtained by eriodictyol (-7.4 Kcal/mol) and lowest docking score was obtained by quercetin (-7.2 Kcal/mol). More the negative energy, greater the interaction between the molecule. In general, binding energy scoring with AutoDock Vina is utilized to estimate many receptor-ligand complexes while lowering processing costs. By using free energy modelling tools, the prediction of binding energy in these receptor-ligand interactions was assessed. The binding scores of flavonoids are given in "Table 4.1"

TABLE 4.1 BINDING AFFINITY OF FLAVONOIDS WITH β-CATENIN

Sl. No.	PubChem CID	Name of Flavonoid	Binding Affinity (Kcal/mol)
1.	31553	Silibinin	-8.4
2.	440735	Eriodictyol	-7.4
3.	5280343	Quercetin	-7.2

4.2 Docking interactions of amino acid residues

4.2.1 Interacting residues of receptor and the silibinin ligand

It was found that the interaction of silibinin with β-catenin is through the formation of H bonds at transcription factor interacting regions involving residue Lys281. Lys288 was involved in pi-cation interaction and Gly245 in pi-sigma interaction.

The structure (A) shows the protein β-catenin in red ribbon whereas the ball and line structure between them represents the ligand silibinin where it interacts with the receptor site of β-catenin.

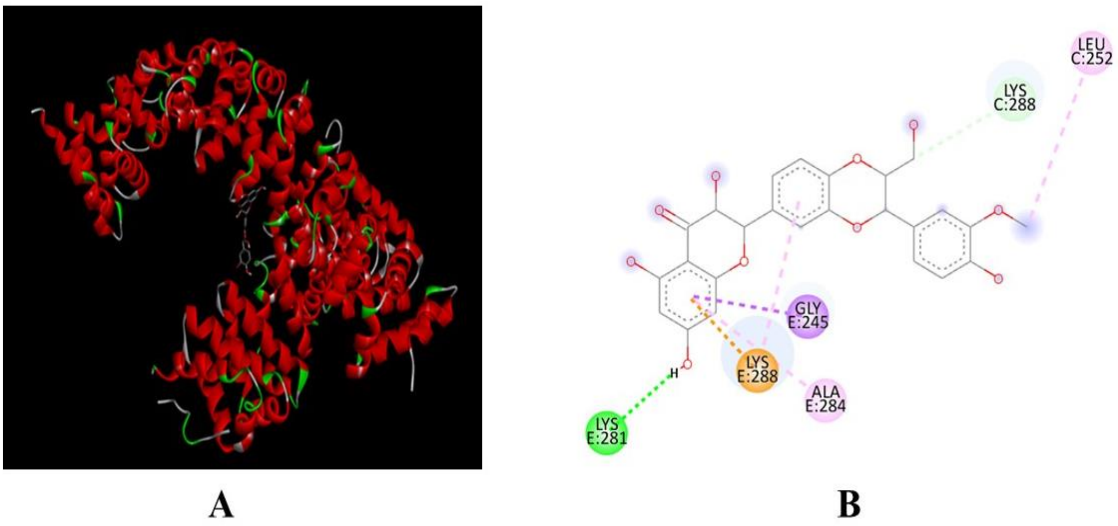

Fig. 4.2.1 (A) 3D structure-based docking of silibinin with the protein β-catenin and (B) Interacting residues of receptor and the silibinin ligand.

4.2.2 Interacting residues of receptor and the eriodictyol ligand

It was found that the interaction of eriodictyol with β-catenin is through the formation of H bonds at transcription factor interacting regions involving residue Asp249 and Lys281. Phe293, Asn290, Val248, Ser246 and Leu244 are involved in van der Waals interaction with the ligand molecule. Pi-Sigma interaction is formed by Thr289, Gly245 and Lys288. Leu252, Leu285 and Ala284 forms Pi-Alkyl interactions.

The structure (A) shows the protein β-catenin in red ribbon whereas the ball and line structure between them represents the ligand eriodictyol where it interacts with the receptor site of β-catenin.

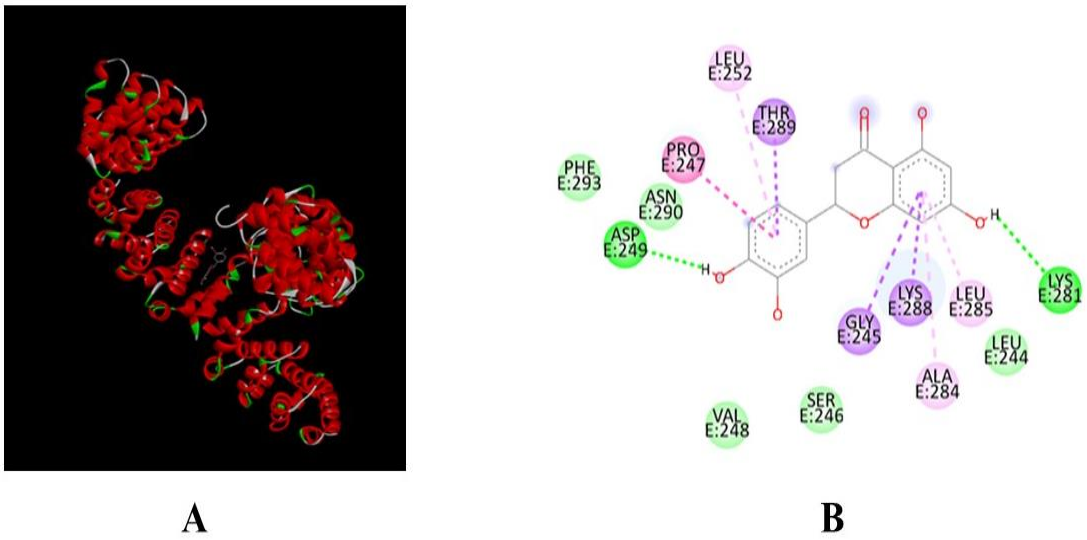

Fig. 4.2.2 (A) 3D structure-based docking of eriodictyol with the protein β-catenin and (B) Interacting residues of receptor and the eriodictyol ligand.

4.2.3 Interacting residues of receptor and the quercetin ligand

It was found that the interaction of quercetin with β-catenin is through the formation of H bonds at transcription factor interacting regions involving residue Ala171, Pro154, Ile153, Glu155 and Ser184. Leu178 was involved in pi-sigma interaction. Val175, Met174 and Ala152 was involved in pi-alkyl interactions.

The structure (A) shows the protein β-catenin in red ribbon whereas the ball and line structure between them represents the ligand quercetin where it interacts with the receptor site of β-catenin.

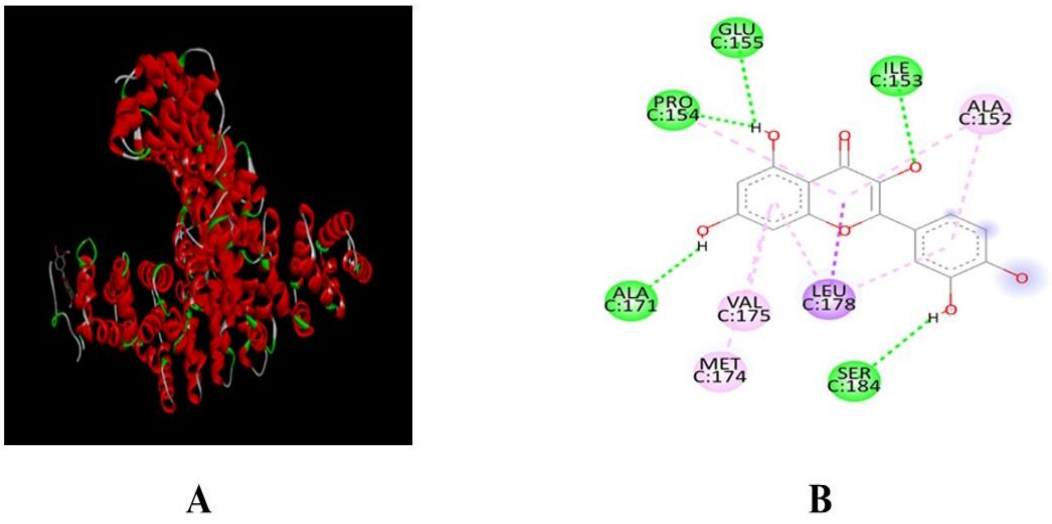

Fig. 4.2.3 (A) 3D structure-based docking of quercetin with the protein β-catenin and (B) Interacting residues of receptor and the quercetin ligand.

WNT PROTEIN AND FLAVONOIDS

4.3 Binding affinity between Wnt protein and flavonoids

After docking was performed using AutoDock Vina, the result came out to be positive, showing highest affinity between the Wnt protein and silibinin. The highest docking score of -9.3 Kcal/mol shows that silibinin binds to significant receptor sites of the Wnt protein molecule. Second highest docking score was obtained by quercetin (-8.3 Kcal/mol) and lowest docking score was obtained by eriodictyol (-8.1 Kcal/mol). More the negative energy, greater the interaction between the molecule. In general, binding energy scoring with AutoDock Vina is utilized to estimate many receptor-ligand complexes while lowering processing costs. By using free energy modelling tools, the prediction of binding energy in these receptor-ligand interactions was assessed. The binding scores of flavonoids are given in “Table 4.3”

TABLE 4.3 BINDING AFFINITY OF FLAVONOIDS WITH WNT PROTEIN

Sl. No.	PubChem CID	Name of Flavonoid	Binding Affinity (Kcal/Mol)
1.	31553	Silibinin	-9.3
2.	440735	Eriodictyol	-8.1
3.	5280343	Quercetin	-8.3

4.4 Docking interactions of amino acid residues

4.4.1 Interacting residues of receptor and the silibinin ligand

It was found that the interaction of silibinin with Wnt protein is through the formation of H bonds at transcription factor interacting regions involving residues Pro144, Cys330, Cys148, Asp150 and Asn152. Cys148 was involved in pi-sulphur interaction and Leu147 in pi-alkyl interaction.

The structure (A) shows the Wnt protein in red and blue ribbon whereas the ball and line structure between them represents the ligand silibinin where it interacts with the receptor site of Wnt protein.

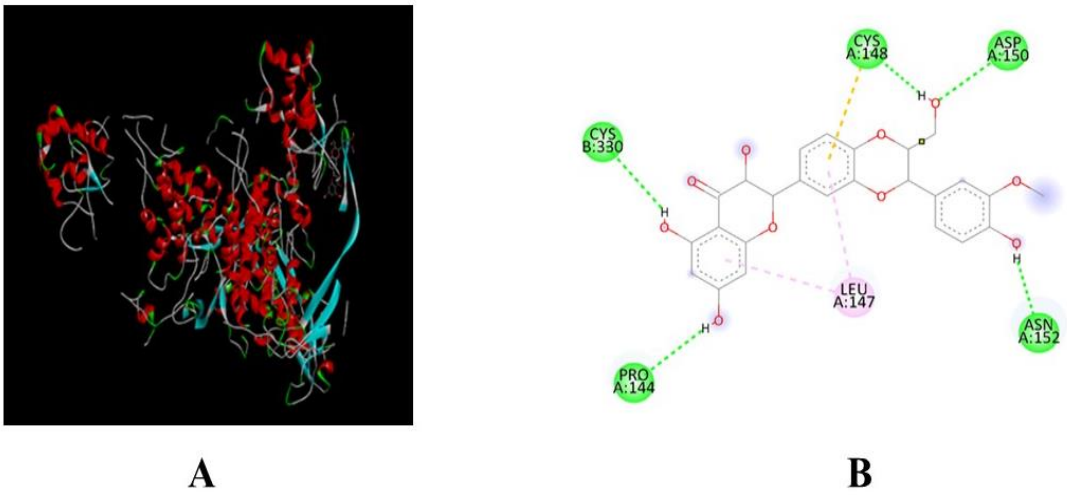

Fig. 4.4.1 (A) 3D structure-based docking of silibinin with Wnt protein and (B) Interacting residues of receptor and the silibinin ligand.

4.4.2 Interacting residues of receptor and the eriodictyol ligand

It was found that the interaction of eriodictyol with Wnt protein is through the formation of H bonds at transcription factor interacting regions involving residues Glu111, Thr290, Gly291, Thr93 and Asp95. Trp89 was involved in pi-pi stacked interaction and Ile94 in pi-sigma interaction.

The structure (A) shows the Wnt protein in red and blue ribbon whereas the ball and line structure between them represents the ligand eriodictyol where it interacts with the receptor site of Wnt protein.

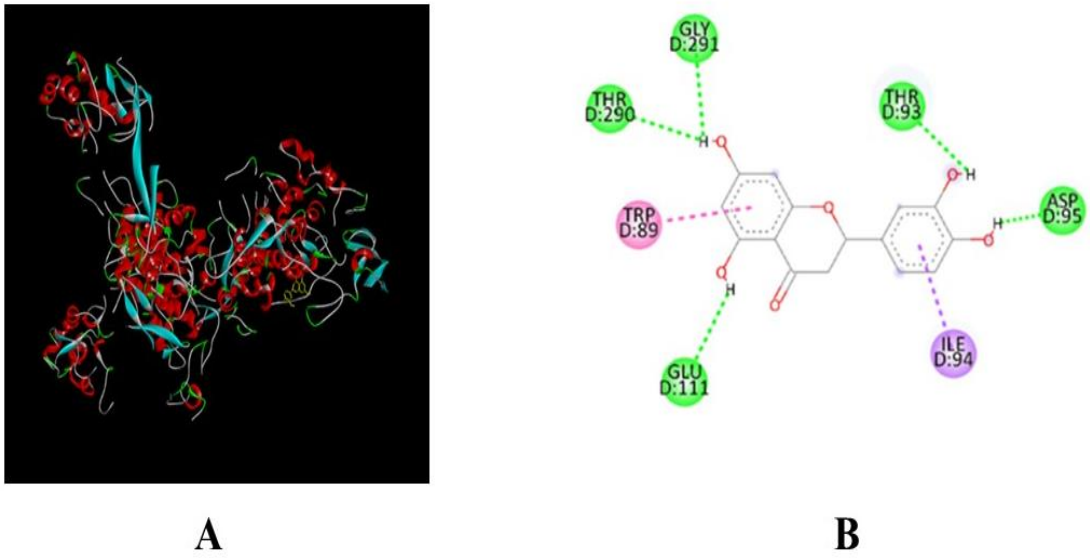

Fig. 4.4.2 (A) 3D structure-based docking of eriodictyol with Wnt protein and (B) Interacting residues of receptor and the eriodictyol ligand.

4.4.3 Interacting residues of receptor and the quercetin ligand

It was found that the interaction of quercetin with Wnt protein is through the formation of H bonds at transcription factor interacting regions involving residues Ile138, Pro180 and Gly134. Trp255, Thr137 and Cys139 was involved in pi-pi stacked interaction and Arg57 in amide-pi stacked interaction. Gly140 forms van der Waals interaction with the ligand. Pi-cation interaction was formed by Arg179.

The structure (A) shows the Wnt protein in red and blue ribbon whereas the ball and line structure between them represents the ligand quercetin where it interacts with the receptor site of Wnt protein.

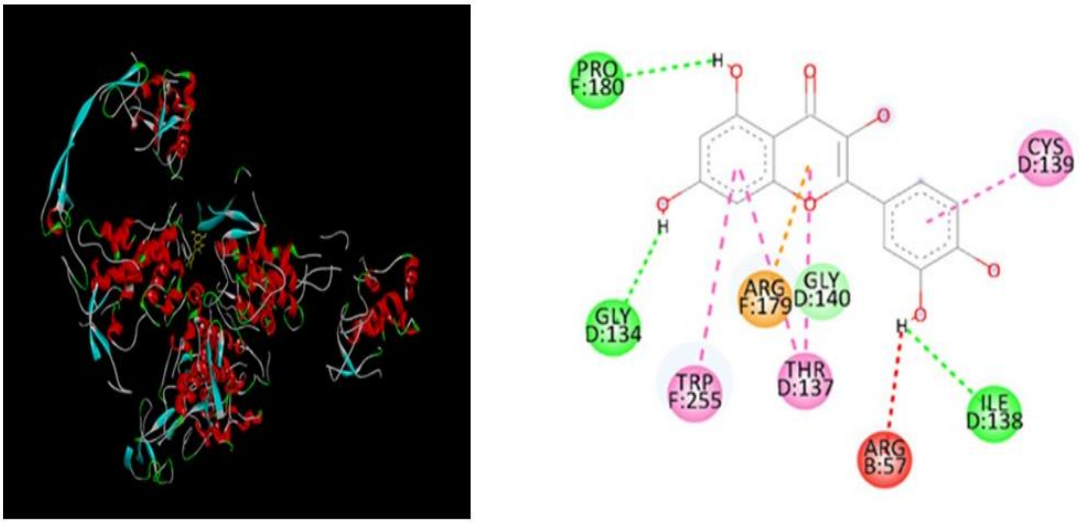

Fig. 4.4.3 (A) 3D structure-based docking of quercetin with Wnt protein and (B) Interacting residues of receptor and the quercetin ligand.

Studies revealed that certain known inhibitors (catechin, luteolin, coumestrol, and β-naphthoflavone) have binding affinities that vary from -6.50 to -5.22 Kcal/mol. Compared to the known inhibitors, this experiment showed that flavonoids like silibinin, eriodictyol and quercetin were well placed and had strong interactions inside the binding pocket of β-catenin. By having similar binding residues, it can be concluded that T cell factors 4 (TCF4) and members of the flavonoid family compete for β-catenin binding.

Other flavonoid family members are been examined by comparative modes of interacting studies and many inhibitory effects on the beta-catenin complex and Wnt signalling protein were noted down.

CRC can be prevented by hindering the interaction of Wnt proteins with the cell surface receptors with the binding of flavonoids with the Wnt proteins. It is found that the binding affinity of flavonoids with the Wnt protein is higher than the binding affinity between flavonoids and β-catenin.

CHAPTER 5

FUTURE PROSPECTS

To determine flavonoids therapeutic potential for the reduction of immunological disorders, more research on the substance's precise mechanism is needed. It is generally recognized that flavonoids may both prevent and treat disease. In addition to the ongoing studies, several flavonoid-related features should be investigated in the future before flavonoids are tested in human clinical experiments for the treatment of CRCs. One of the key challenges to be addressed in many animal models in the future is the acute as well as long-term toxicity of flavonoids and to further confirm the health benefit claims made for the flavonoids, further standardization and recording of the clinical trial data are required. Additional research will provide fresh perspectives and undoubtedly usher in a new era of pharmaceutical and nutraceutical products based on flavonoids for the treatment of several diseases caused by oxidative stress.

CHAPTER 6

CONCLUSION

Activation of the Wnt cascade in colorectal cancer has been linked to the development of adenomas and carcinomas, as well as resistance to chemo-therapy and poor prognosis. Targeting the Wnt signaling is a promising method for the treatment of CRC. Several drugs that target sites of the pathway, such as porcupine inhibitors and beta-catenin inhibitors, are currently in clinical development. But the efficacy and safety of these drugs in human need to be further evaluated in clinical trials. Additionally, these drugs may also have side effects and may not be suitable for all patients. To overcome these problems, Use of natural compounds like flavonoids can be used, which could prevent and treat diseases like CRC.

In this study, in-silico docking studies was primarily used to identify optimal binding conformations of flavonoid members to β-catenin/Transcription factor. The interaction of β-catenin with the three flavonoids like silibinin, eriodictyol and quercetin were evaluated in this study. It was observed that silibinin had a higher binding affinity and can be administered as an anti-cancer agent and had a more potent inhibitory effect against the Wnt pathway. Although many of the molecular pathways behind Wnt signaling have been discovered, it is still difficult to create therapeutic approaches that specifically targets the Wnt signaling cascade in CRC and further studies should be made to understand the interactions between the flavonoids and the colorectal cancer pathways.

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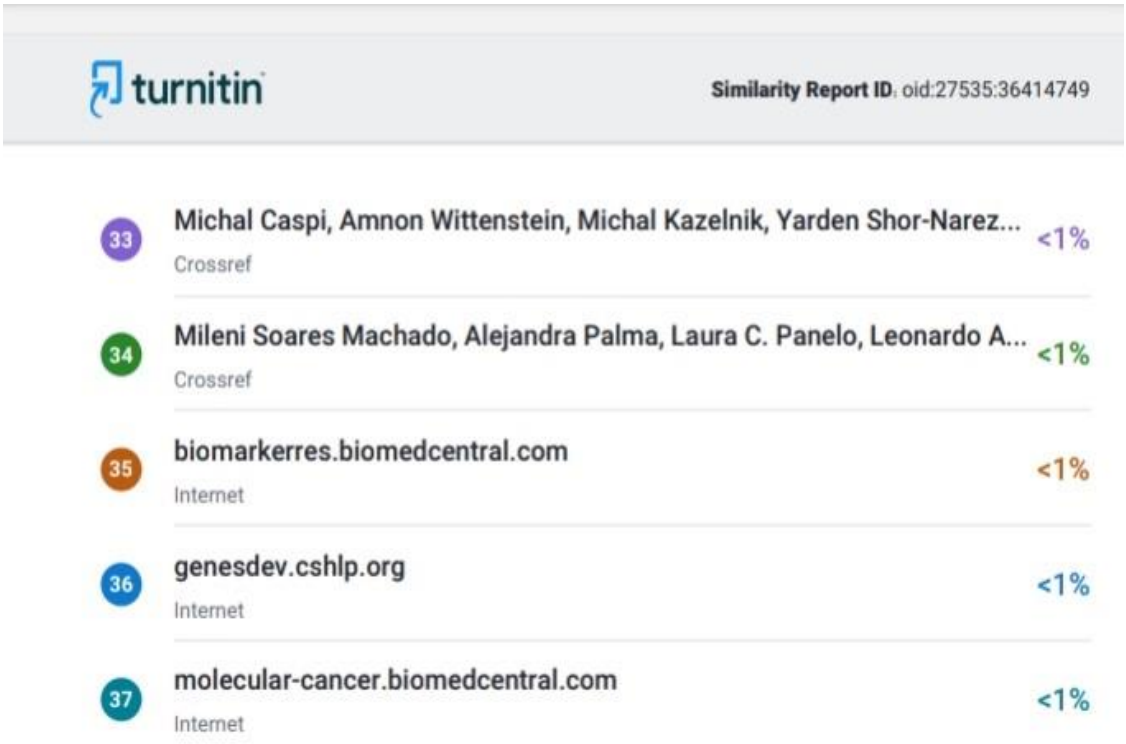
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I, **Akhila K [2K21/MSCBIO/03],** student of **M. Sc. Biotechnology,** hereby declare that the project Dissertation titled **"In silico studies of flavonoid's pattern of inhibition against wnt/ β-catenin cascade"** which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirements for the award of the degree of Master of Science, is original and not copied from any source without proper citation, is done under the supervision of **Dr. Navneeta Bharadvaja.** This work has not previously formed the basis for the award of any Degree, Diploma Associateships, Fellowship or other similar title or recognition.

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Author's name: Akhila K and Navneeta Bharadvaja

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AKHILA K

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CERTIFICATE

I, hereby certify that the Project Dissertation titled, **"In silico studies of flavonoid's pattern of inhibition against wnt/ β-catenin cascade"** which is submitted by, **Akhila K [2K21/MSCBIO/03],** Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, is a record of the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

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Date: 30/05/2023

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