TARGETING CHOLINERGIC SYNDROME IN PANCREATIC CANCER THROUGH NATURAL COMPOUNDS

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I, Muskaan Dhingra, Roll No. 2K20/MSCBIO/17 M.Sc. Biotechnology student declares that the project report titled "**TARGETING CHOLINERGIC SYNDROME IN PANCREATIC CANCER THROUGH NATURAL COMPOUNDS"** which has been submitted to the Department of Biotechnology, Delhi Technological University, New Delhi, is original and has not taken from any of the sources without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

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CERTIFICATE

I hereby certify that the dissertation project titled "**TARGETING CHOLINERGIC SYNDROME IN PANCREATIC CANCER THROUGH NATURAL COMPOUNDS**" which is submitted by Muskaan Dhingra, 2K20/MSCBIO/17, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of requirement for the award of the degree of Master of Science, is a record of work carried out by the student under my supervision. To the best of my knowledge, this work has not been submitted in part of any Degree or Diploma to this University or elsewhere.

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ABSTRACT

Pancreatic cancer is among the leading cancers worldwide responsible for the mortality rate increasing due to cancer deaths. The detection of pancreatic cancer is very difficult thus, making it the more fatal until the treatment for it begins.

The treatment for the cancer includes immunotherapy, surgical resection and chemotherapy. Chemotherapy has always been in research for introducing better drugs for cancer therapy. Various chemotherapeutic drugs have been given approval by Food and Drugs Association [FDA]. These include Olaparib, irinotecan hydrochloride liposome, mitomycin, erlotinib, paclitaxel, gemcitabine [the first drug] among others which have proven to be beneficial for the treatment of pancreatic cancer.

But these FDA approved drugs do have various side effects such as rash, diarrhea, vomiting, bradycardia, rhinitis, acute renal failure, hypotension and hypersalivation. In this article, the side effect which has been talked about is cholinergic syndrome which is a major side effect of onivyde [irinotecan hydrochloride liposome]. Thus, we need natural compounds to overcome these side effects which if not better are equivalent to these drugs. Through this research we are going to identify which drug suits the most with the FDA approved drugs by performing molecular docking. For performing docking, we underwent many steps from finding the structure to pharmacophore modelling which also will be mentioned in the paper. Isowogonin obtained from *Didymocarpus pedicellata* is the one which appeared to be the lead compound as it can be well tolerated with no or least side effects and considered as an alternative for various FDA approved drugs used in treatment of pancreatic cancer.

Keywords: pancreatic cancer, receptors, natural compounds, FDA approved drugs, docking, bioavailability testing, pharmacophore modelling.

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ABBREVIATIONS

5-FU: Fluorouracil

CEA: Carcinoembryonic antigen

CT: Computed tomography

dFdCTP: 20 ,20 -difluoro-20 -deoxycytidine triphosphate

DPD: Dihydropyrimidine dehydrogenase

EGFR: Epidermal Growth Factor Receptor

EMT: epithelial mesenchymal transition

ERCP: Endoscopic Retrograde Cholangiopancreatography

FDA: Food and Drug Administration

FdUMP: fluorodeoxyuridine monophosphate

FdUTP: fluorodeoxyuridine triphosphate

FUTP: fluorouridine triphosphate

GEM: Gemcitabine

HR: homologous recombination

IGRT: Image-Guided Radiotherapy

IMPPAT: Indian Medicinal Plants, Phytochemistry and Therapeutics

IMRT: Intensity-Modulated Radiotherapy

IPMN: Intraductal Papillary Mucinous Neoplasm

MAb: Monoclonal Antibodies

MCN: Mucinous Cystic Neoplasm

MRI: Magnetic Resonance Imaging

NIH: National Institute of Health

PanIN: Pancreatic Intraepithelial Neoplasia

PARP: Poly-Adenosine diphosphate Ribose Polymerase

PC: Pancreatic Cancer

PDB: Protein Data Bank

PET-CT: Positron Emission Tomography

RNR: Ribonucleotide Reductase
TGF-alpha: transforming growth factor-alpha
TK: tyrosine kinase
TKI- tyrosine kinase inhibitors
TOP1: Topoisomerase I
US: Ultrasound

CHAPTER-1: INTRODUCTION

1.1 PANCREATIC CANCER marks the most common type of cancer in humans. The cases of pancreatic cancer have been constantly increasing worldwide. It explains for about 4-5% in terms of deaths occurring because of cancer [20]. Due to its late diagnosis, the rate of survival still remains low as it was earlier. This leads to ineffective treatment of the cancer resulting in deaths and other severe issues [22]

RISK FACTORS:

Age: it is majorly diagnosed in people above the age of 55 years. People under 30 years are even rarely detected with this cancer. Thus, age is considered one of those risk factors which cannot be modified. [21]

Sex, genetic susceptibility and family history: According to the researches, males are more vulnerable to pancreatic cancer than females. If the cancer has been in one's family until second degree relatives, then there are chances that the case might occur in the family. People with family history are more prone to cancer than people who don't have family history. There might be cases of genetic susceptibility where people with BRCA2 and PALB mutations might increase the risk of developing pancreatic cancer. [21]

Smoking and alcohol: cigarette smoking is associated with the onset of pancreatic cancer. It has been shown that current and former smokers are more at risk of developing this cancer than non-smokers. Patients consuming alcohol more than 30 gm per day are on the higher urge of developing pancreatic cancer even though there has been no evidence of low or moderate alcohol consumers who have acquired cancer. [21]

Chronic pancreatitis: An inflammatory condition in pancreas which results in fibrosis followed by loss of islet and acinar cells. There are chances that group having chronic pancreatitis is surely going to acquire pancreatic cancer within their lifespan. Patients who have this infection and intake excessive alcohol are probably at the risk of developing pancreatic cancer. [21]

Diabetes and obesity: another major risk factor for pancreatic cancer is diabetes which increases the chances of cancer two-fold. There can be chances that pancreatic cancer might result in onset of diabetes thus resulting in HbA1c as the biomarker for detection of pancreatic cancer. Other than the type I diabetes, obesity is considered another risk factor. [21]

Infection: patients who already are infected with *Helicobacter pylori* or hepatitis-C infections have been observed to be at higher risk of increasing incidences related to pancreatic cancer. [21]

1.2 PATHOLOGY: pancreatic neoplasia occurs in three forms which are IPMN [neoplastic cells which produce mucin], MCN [contains mucin but isn't connected to pancreatic ductal system] and PanIN [non-invasive lesions]. [24]

DIAGNOSIS:

Histopathology: This method is considered to be the most standard among all the methods. Its applicable for all the patients except for those who have undergone surgical resection. There are various methods by which specimens can be obtained such as Computed tomography guided biopsy or exploratory biopsy under laparoscopy. [20]

Biomarkers: the only biomarker which is accepted by FDA is serum cancer antigen 19-9 [CA-19-9]. It monitors patient's response to the treatment and acts as a marker of recurrent disease. If its level is low then CA-19-9 plays zero role in screening of patients who are asymptomatic [5]. CA-19-9 when combined with CA-125 has greater sensitivity than CA-19-9. Other biomarker considered is CEA used in combination with CA-19-9. [20]

Imaging: these include techniques such as MRI, CT, PET-CT, US, ERCP among others. US is recommended where pancreatic lesions are small and have low sensitivity. CT is also considered vital technique but it has certain disadvantages like exposure to radiation. MRI is used to show pancreatic mass. For monitoring cancer post treatment, PET-CT is used in combination with modified version of CT. [24]

1.3 TREATMENT:

Surgery: the major treatment which provides cure for the pancreatic cancer. There are three criteria on which the procedure to be followed is based which are location of tumor, size of tumor and staging of tumor. The most common one out of all the procedures is Pancreatico-duodenectomy also known as Whipple's Procedure. This procedure works on eliminating pancreatic head along with duodenum curve, gall bladder and common bile duct. The procedure is performed on patients who are suffering with pancreatic head cancer and periampullary cancer. Other two procedures are distal and subtotal pancreatectomy which is advised for patients with cancer in pancreatic body and tail. [24]

Radiotherapy: for locally advanced tumors which can't be surgically resected, radiotherapy is applicable. Radiotherapy is used in killing of cancer cells and preventing growth and recurrence. Side effects of radiotherapy are gastrointestinal symptoms, fatigue and rashes on skin. IMRT and IGRT are two modified radiotherapies which can be used as an alternative for pancreatic cancer treatment as these are more successful and can be tolerated.[24]

Chemotherapy: this therapy is used for patients who cannot undergo surgical resection. Major combinations involved are GEM/erlotinib, GEM/capecitabine and FOLFIRINOX. GEM acts as a main drug for the treatment of pancreatic cancer. Erlotinib is an EGFR-tyrosine kinase inhibitor which is over-expressed at the time of cancer. Thus, receptor expression shows the efficiency of combined chemotherapy. FOLFIRINOX is applicable for patients who have advanced tumor and also who are younger since it has more toxicity than GEM. FOLFIRINOX is made by combining various drugs which include irinotecan, leucovorin, oxaliplatin and 5-fluorouracil. Capecitabine is a prodrug which is

administered orally followed by its conversion to 5-FU [fluorouracil]. GEM/NAB-paclitaxel is another combination which has importance in clinical trials as this combination helps in enhancing the sensitivity of GEM as NAB-P inhibits catabolic enzyme of GEM. [24]

CHAPTER 2: LITERATURE REVIEW

2.1 PANCREATIC CANCER TREATMENT

As mentioned in the introduction part, there are various treatment methods for pancreatic cancer including surgical resection, radiotherapy and chemotherapy. Chemotherapy is the most used method for treating PC where surgical resection doesn't work. Adjuvant or palliative chemotherapy are two approaches which work for curing PC. First line chemotherapy includes Gemcitabine and FOLFIRINOX as single agent or in combination. GEM is used in combination with 5-FU for treating patients with locally advanced or metastatic cancer. GEM results in increasing survival for over a year. GEM is more beneficial when combined with other chemotherapeutic drugs such as oxaliplatin and capecitabine. Oxaliplatin and Nab-paclitaxel are the two drugs used in combination therapy but these have grade ¾ toxicities causing polyneuropathy which makes this combination unsuitable for longer use. Second line chemotherapy includes use of irinotecan for cancer therapy. Combination of GEM with erlotinib followed by the second line treatment with capecitabine proved to have greater efficacy then the reverse combination. Targeted therapies have also been used which include monoclonal antibodies but these therapies failed in case of pancreatic cancer as the biomarkers were not specified in most of the studies. When talking about immunotherapy, pancreatic cancer was found to be poorly immunogenic. For locally advanced PC, chemoradiation has been applied. Chemoradiotherapy along with systemic chemotherapy might be a better option for locally advanced PC resulting in resectability in patients. Apart from these therapies, electroporation, ultrasound and microwave ablation have come into existence for cancer therapy.

2.2 FDA APPROVED DRUGS

There are various drugs which have been given a green signal for treatment of PC by FDA. These include Gemcitabine, Olaparib, Mitomycin, Irinotecan Hydrochloride liposome among others.

2.2.1 5-FU: it is considered to be the common out of all the drugs used for PC treatment. Other than pancreatic cancer, it is also most used drug for colorectal and breast cancers. This drug works by inhibiting thymidylate synthase and integrating the metabolites into the nucleic acids RNA & DNA. The metabolites which disturb the RNA are FdUMP, FdUTP and FUTP. Conversion via DPD is the rate limiting step in the catabolic activity of 5-FU. Capecitabine is the derivative of 5-FU which is administered orally in patients suffering from cancer. This drug when used with other anti-cancerous drugs may have chemo- brain whose symptoms include memory impairments and confusion along with effect on verbal memory function.

FIG 2.1- STRUCTURE OF 5-FU [PubChem]

2.2.2 GEMCITABINE: gemcitabine is a cytotoxic agent which plays the most important role in the treatment of PC. Apart from PC, this drug has been proved beneficial for breast cancer, non-small lung cancer and bladder cancer. The toxicity of GEM can result in anemia, neutropenic fever and thrombocytopenia. High number of patients with edema have also been reported. Other side effects include rash, fever, vomiting, diarrhea and anorexia among others. In case of PC, gemcitabine is

considered as first-line treatment and also used in combination with 5-FU and Irinotecan. GEM works on mechanism of inhibiting DNA synthesis. Incorporation of dFdCTP in DNA results in prevention of chain elongation. Thus, leading to masked chain termination which makes it difficult for DNA repair enzymes to remove GEM.

2.2.3 ERLOTINIB HYDROCHLORIDE: a low molecular-weight drug which is highly selective inhibits tyrosine kinase activity of EGFR. Erlotinib is administered orally and is bounded by protein. Side effects are rashes and diarrhea. Erlotinib when combined with GEM can be used for PC treatment only in cases where chemotherapy has not been performed. Erlotinib helps in potentiation the apoptosis induced by GEM in PC. Other toxicities include changes in eyelash, hair and nail color, pruritis and erythema.

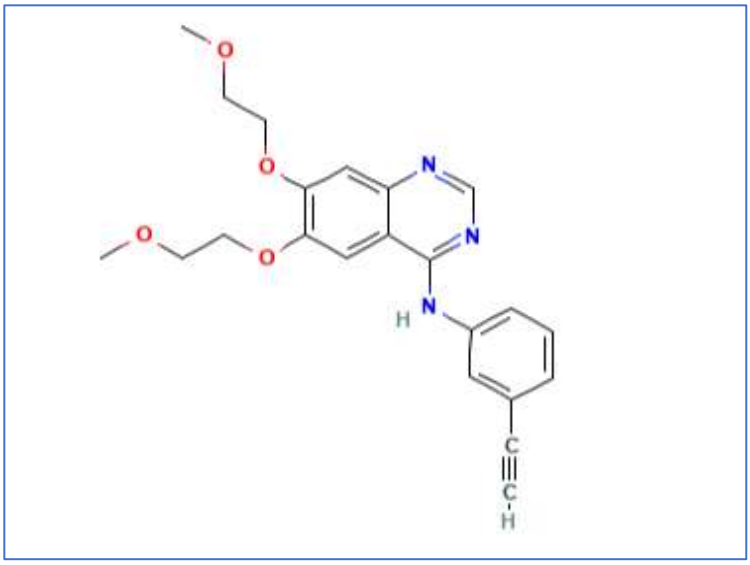

FIG 2.2- STRUCTURE OF ERLOTINIB HYDROCHLORIDE [PubChem]

2.2.4 IRINOTECAN HYDROCHLORIDELIPOSOME: it is another drug approved by FDA which is used in treatment of various cancers including colorectal cancer. Irinotecan is the derivative of camptothecin which is a plant alkaloid. Its liposomal formulation is the one which is modified and used specifically for pancreatic cancer treatment. The liposome formulation is given in the form of injection and is also used in combination with leucovorin and fluorouracil. The other name for this formulation is Onivyde. This drug is an inhibitor of topoisomerase 1 whose metabolite

SN-38 binds to DNA-top1 and prevents repairing of breaks leading to exposure and cell death. Onivyde is used as first line drug as combination known as FOLFIRINOX. Onivyde when used as single agent is considered for second line treatment and also used in gemcitabine-refractory pancreatic cancer. This liposomal formulation has greater half-life than the original irinotecan hydrochloride. Major side effects involved with onivyde are neutropenia, cholinergic syndrome, and neutropenic sepsis. Other toxicities include abdominal pain, mucositis, myelosuppression, and alopecia. Neutropenia and diarrhea come under grade ¾ toxicities. This can be controlled by monitoring blood cell count for patients with neutropenia whereas for patients with diarrhea, the onivyde dose shouldn't be given. The dosage can be reduced if complications are observed or even discontinued when hypersensitive reactions occur. In case of pregnancy, contraceptives which are effective should be provided as the onivyde administration may might result in fetal harm. Acute renal failure and septic shock are other serious toxicities which might take place. Irinotecan therapy should be stopped when the interstitial lung disease is detected. Proper monitoring and dose modifications are recommended for patients suffering from these side effects.

The cholinergic syndrome is the most severe one out of the above-mentioned toxicities. The article is based on using natural compound in order to reduce these effects on patients getting treated for PC. This syndrome is manifested during early diarrhea as well as diaphoresis. After irinotecan is administered, symptoms occur within 24 hours interval. Cholinergic reaction along with rhinitis and increased salivation is also associated with irinotecan liposome. Side effects of irinotecan related with cholinergic syndrome are bradycardia, diarrhea, hypersalivation and hypertension. The three mechanisms proposed to know the existence of this syndrome are as follows. The acetylcholinesterase taken from human erythrocytes proves that irinotecan hydrochloride has ability to directly inhibit enzymatic activity resulting in prevention of breakdown of acetylcholine which is a neurotransmitter into acetic acid and choline at cholinergic synapses' level. Another experiment suggests interaction of irinotecan with muscarinic receptors of cholinergic thus activating directly. Blandizzi also proposed that irinotecan activated nerve fibers followed by inducing vagal reflexes to trigger cholinergic response. Symptoms of irinotecan induced cholinergic syndrome

can thus be treated with anticholinergic formulations such as scopoline butyl bromide, atropine sulphate or loperamide.

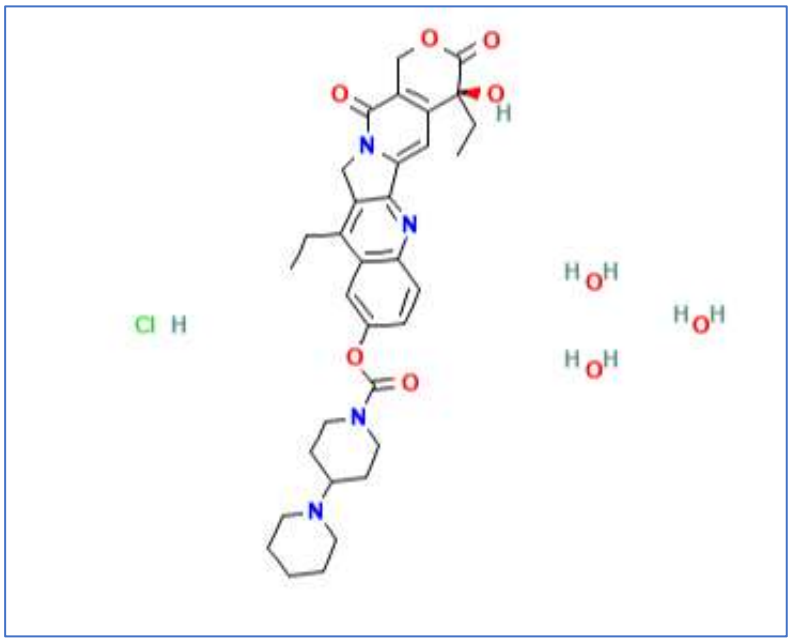

FIG2.3:- IRINOTECAN HYDROCHLORIDE [ONIVYDE] STRUCTURE [PubChem]

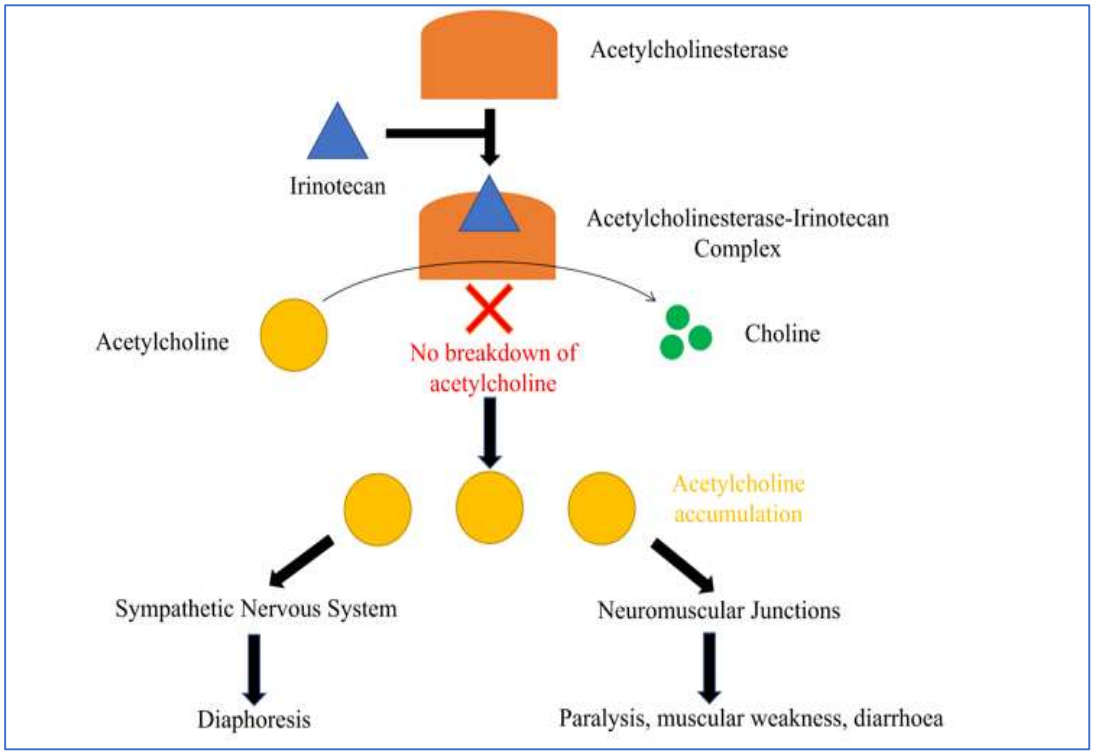

FIG 2.4- IRINOTECAN INDUCED CHOLINERGIC SYNDROME AND SIDE EFFECTS

2.3 PANCREATIC CANCER AND RECEPTORS:

2.3.1 EGFR: It is a mediator of cell growth and survival. This transmembrane glycoprotein has TK enzymatic activity. EGFR is expressed in tumors especially non-small cell lung cancer whereas overexpression is seen in colorectal, breast and pancreatic cancers. These receptors serve major role in increasing the pathogenesis of PC leading to its aggressiveness. The ligands which are important for EGFR are EGF and TGF-alpha. This ligand-receptor complex and dimerization is mutated which activates the receptor aberrantly during tumor progression. This mutation and overexpression manipulate the downstream signaling. Thus, EGFR is considered prominent target during the treatment of pancreatic cancer.

The alteration in the chromosome no.7 is primarily linked with increased expression of EGFR in human pancreatic carcinomas. Since there is an increase in transcription of gene, ERBB-1 gene is expressed in larger amount during tumor. Erlotinib and gefitinib are two EGFR inhibitors that reduce its overexpression in pancreatic cancer. Another mutation seen during PC is of KRAS oncogene which involves RAS protein but studies have not found any evidence linking it to be the biomarker in patients with advanced PC.

EGFR signaling activates Notch genes resulting in malignant transformation by expansion in undifferentiated cells. There are two approaches by which EGFR can be targeted i.e., MAbs against the extracellular domain as well as TKIs competing at the ATP binding site of TK domain. The first approach uses panitumumab which blocks the ligand binding and activation of receptor whereas the second approach uses erlotinib which prevents EGFR autophosphorylation along with downstream signalling. More of the research is needed to know the correlation of EGFR with pancreatic cancer better.

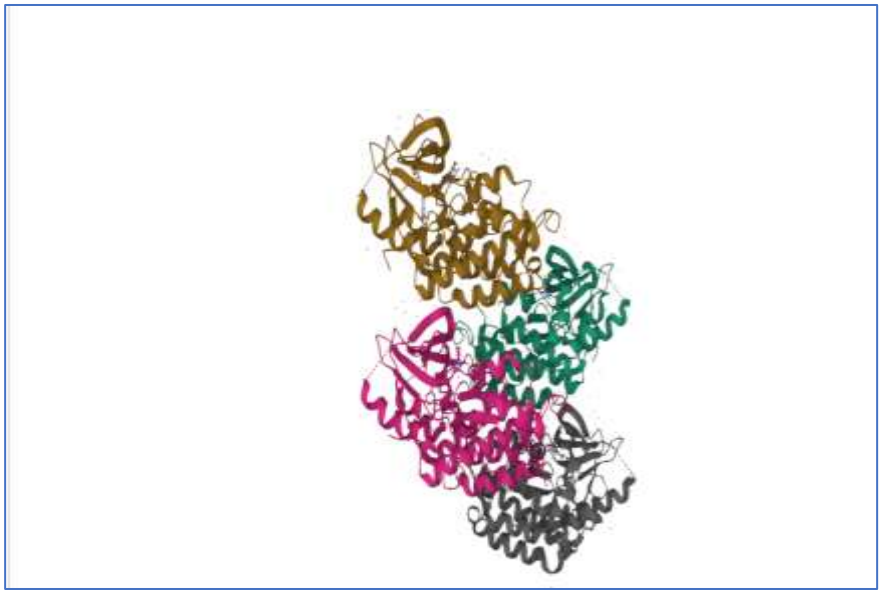

FIG 2.5- EGFR STRUCTURE [PDB ID:5GTY]

2.3.2 TOP1: topoisomerases play a role in relieving stress in DNA helix due to replication and other cellular processes. TOP1 is used to remove the negative supercoiling in DNA while topoisomerase 2 decatenates DNA cleaving both the DNA strands. Removal of supercoils by TOP1 is beneficial as it may result in the formation of DNA structures such as breaks and loops along with maintaining the genetic stability.

Irinotecan is the main inhibitor of TOP1 whose target is TOP1CC and it binds at its interface. The binding doesn't take place without one of the components whether it is DNA or TOP1. The degradation of TOP1 is beneficial for DNA repair enzyme, tyrosyl DNA phosphodiesterase for accessing and hydrolysing the crosslink between DNA and TOP1.

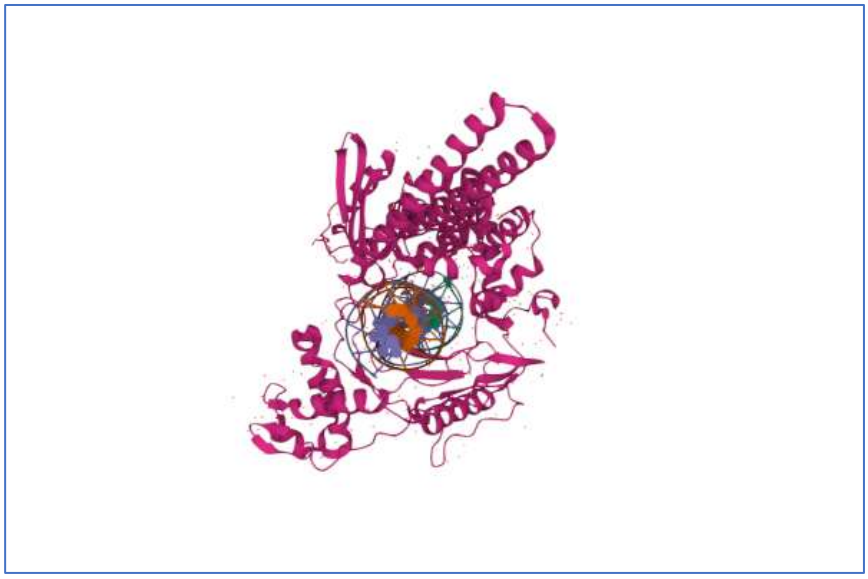

FIG 2.6- TOP1 STRUCTURE [PDB ID: 1K4T]

2.3.3 PARP: Both PARP1 and PARP2 act as DNA damage sensors by binding at the DNA damage site followed by resealing the ssDNA breaks during repairing process. PARP also plays a major role in ATM activation, important for HR. PARP-1 generates the PARP chains by catalysing ADP-ribose from NADC to other proteins resulting in recruitment of DNA repair proteins. Other processes which involve PARP are angiogenesis, chromatin remodelling, EMT, cancer metastasis and transcriptional regulation. The loss of BRCA allele results in increased risk of breast and pancreatic cancer. Three major inhibitors of PARP are Olaparib, rucaparib and niraparib. The accepted one is Olaparib which can be applied as the monotherapy for maintenance treatment in patients suffering from pancreatic cancer. This treatment is known as POLO trial which evaluates efficacy of Lynparza as first line treatment in maintenance therapy. The side effects of these PARPi are fatigue, nausea and neuropathy. PARPi help develop resistance against the homologous recombination deficiency which further progresses towards the therapy. There are experiments being performed to know more about PARP and pancreatic cancer relationship.

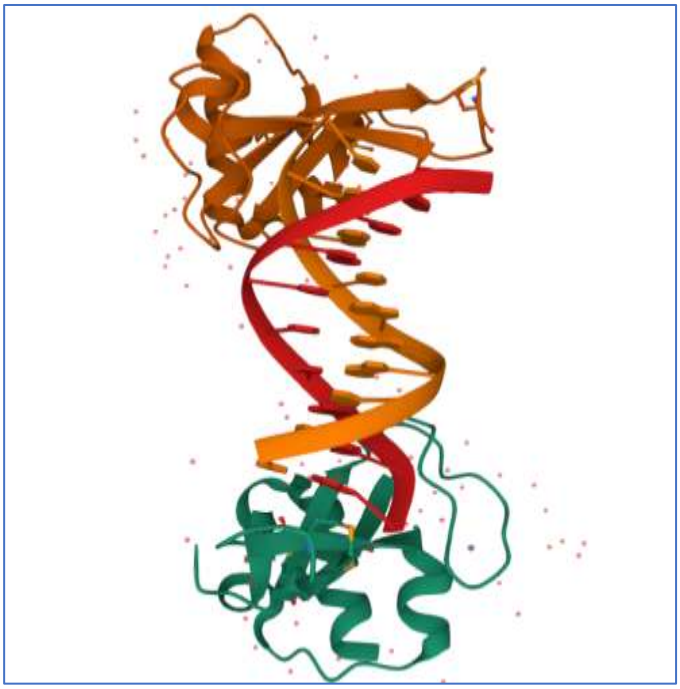

FIG 2.7- PARP-1 STRUCTURE [PDB ID: 3OD8]

2.3.4 RNR: RNR is a multi-subunit enzyme required for cell division and DNA repair. It plays a role in catalysing the rate-limiting step of synthesising dNTP by the removal of 2' hydroxyl ribose in order to generate deoxyribose. Since RNR is important agent in replication, it proves to be a beneficial target in cancer therapy. RNR inhibitors include fludarabine, cladrabine and gemcitabine. GEM is considered to be the first line drug for the treatment of pancreatic carcinomas. RNR increases the dNTP level in cells resulting in the decreased integration of dNTP into DNA thus, reducing the anti-tumour effect of GEM.

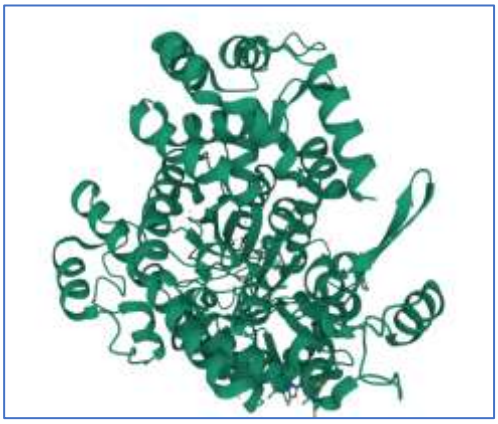

FIG 2.8- RNR STRUCTURE [PDB ID: 6LKM]

2.4 NATURAL COMPOUNDS:

The FDA approved drugs for cancer treatment are effective in the treatment but do have side effects which prove to be fatal enough for the patients. So, to overcome this problem use of natural compounds is beneficial for the purpose. These compounds serve an important part in cancer therapy and are obtained from natural sources such as plants, animals and microbes.

There have been evidences showing positive effects of using natural compounds such as antioxidants from green tea prove to be helpful in slowing down growth of breast cancer. Various natural compounds do possess antitumor activity which hinder with tumor cell proliferation, invasion and process of angiogenesis. Synthetic drugs are small molecules, which can bind to receptors other than target receptors, which can have unwarranted consequences. This supports the need for natural compounds, which have lesser side effects while being as effective, if not better, than the synthetic drugs. The examples of natural compounds are taxanes, curcumin, cannabinoids and resveratrol which are available for pancreatic cancer treatment. These drugs act as anti-proliferative and have proven beneficial for pre-clinical and clinical trials. Natural compounds slow down the resistance against cancer therapy. They also make cancer cells sensitive towards chemotherapeutic drugs. They may also result in accumulating drugs in cancer cells. Other main advantage of natural compounds is promotion of normal cell repairing due to damage by chemotherapeutic drugs.

This article emphasises on use of natural compounds and finding out the natural compound which has similar mode of action as the FDA approved drugs with no or least side effects.

CHAPTER 3: METHODOLOGIES

3.1.DATA COLLECTION:

We started by identifying those drugs which are approved by FDA. These drugs were collected from the NIH Cancer Portal which are listed below as Table I. (cancer.gov/about-cancer/treatment/drugs/pancreatic)

TABLE 3.1: DRUGS APPROVED BY FDA

S.No.	APPROVED DRUGS	PubChem CID
1.	5-FU (Fluorouracil Injection)	3385
2.	Gemcitabine Hydrochloride	60749
3.	Mitomycin	5746
4.	Olaparib	23725625
5.	Erlotinib Hydrochloride	176871
6.	Irinotecan Hydrochloride Liposome	60838
7.	Sunitinib Malate	6456015
8.	Paclitaxel Albumin-stabilized Nanoparticle Formulation	17716129
9.	Everolimus	6442177
10.	Lynparza (Olaparib)	23725625

Next, we performed a literature survey in order to determine which receptors these drugs bind to. This was done to obtain the natural compounds which bind to the receptors involved in pancreatic cancer.

Among all the receptors we selected TOP1, EGFR and PARP-1 for our analysis. Below is the Table II showing drugs approved by FDA and their targeted receptors which obtained from literature analysis.

TABLE 3.2: FDA APPROVED DRUGS AND THEIR RECEPTORS

Drugs	Target receptors	References
Irinotecan Hydrochloride	Topoisomerase 1	[1]
Erlotinib Hydrochloride	Epidermal Growth Factor Receptor	[2]
Olaparib	Poly (ADP-ribose) Polymerase	[3]
Gemcitabine Hydrochloride	Ribonucleotide Reductase [RNR]	[4]
Mitomycin	DNA, RNA	[5]
Sunitinib Maleate	Vascular Endothelial Growth Factor Receptor, c-kit	[22][23]

3.2. PHARMACOPHORE MODELLING:

For all the approved drugs, pharmacophore was created. Pharmacophore modelling is done with the help of a software called PharmaGist.[18] Pharmacophore acts as an influential model for various applications for drug design for instance, lead optimization, de-novo design among others. PharmaGist is a web-server tool which helps in the creation of pharmacophore.

First, we found the structures of the drugs from PubChem which were downloaded in the form of 3d sdf. Then those were converted to. Mol2 format using a software known as Open Babel GUI* which is compatible with PharmaGist.

After creating the pharmacophores, results were obtained, followed by selection of models with all the drugs and maximum energies.

*Open Babel GUI: an open-source toolbox which converts structures between various formats approximately over 110. Other positive points which make it a useful tool include depiction of 2D structures, conformer searching, searching of similarity among others. The weblink for this software is http://openbabel.org. [17]

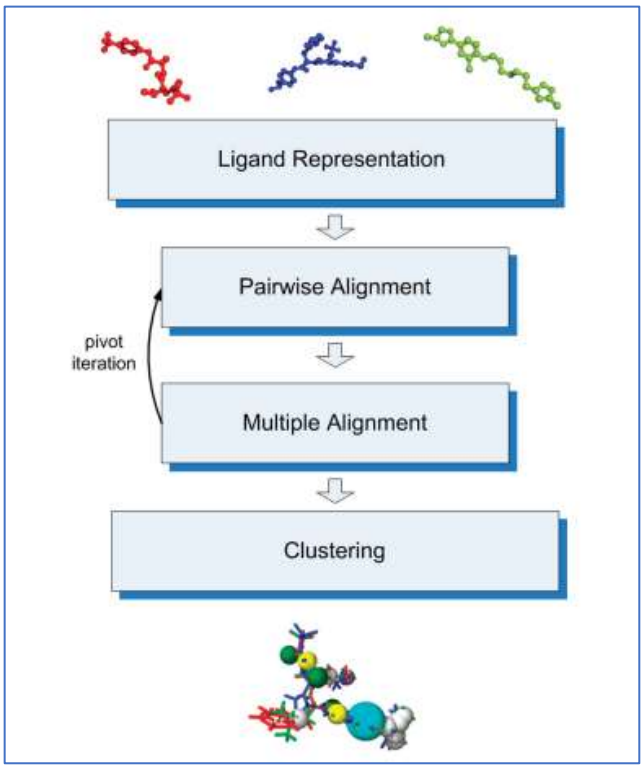

FIG 3.1- PHARMAGIST FLOW CHART

[Adapted from Schneidman-Duhovny, et.al., 2008]

3.3. COMPOUND SELECTION:

Using ZincPharmer*, which is a web-based tool, the pharmacophores obtained were uploaded in it. ZincPharmer then examines those uploaded pharmacophores against the Zinc Database. [14]

After the completion of analysis, matches of compounds which were structurally similar to the pharmacophore were provided. The top hit compound i.e., "**ZINC63409373"** was chosen which was run against the IMPPAT database**. Then on the basis of SMILES structure similarity, the natural compounds were found. [Table III]

*ZincPharmer: It offers mechanism for originating primary hypothesis related to pharmacophore directly from structures taken from PDB. [16]

FIG 3.2- ZINC PHARMER HOME PAGE [http://zincpharmer.csb.pitt.edu/]

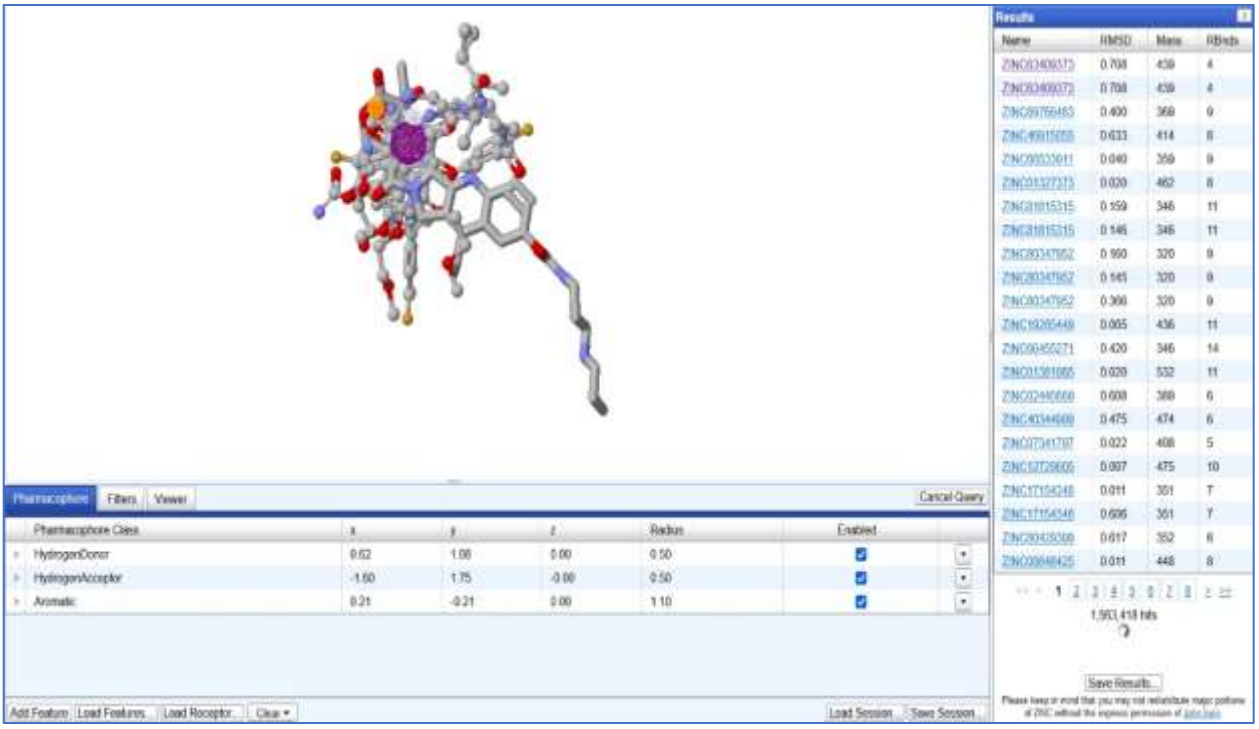

FIG 3.3- ZINC PHARMER SHOWING PHARMACOPHORE AND TOP HIT ZINC63409373

**IMPPAT database: [15]

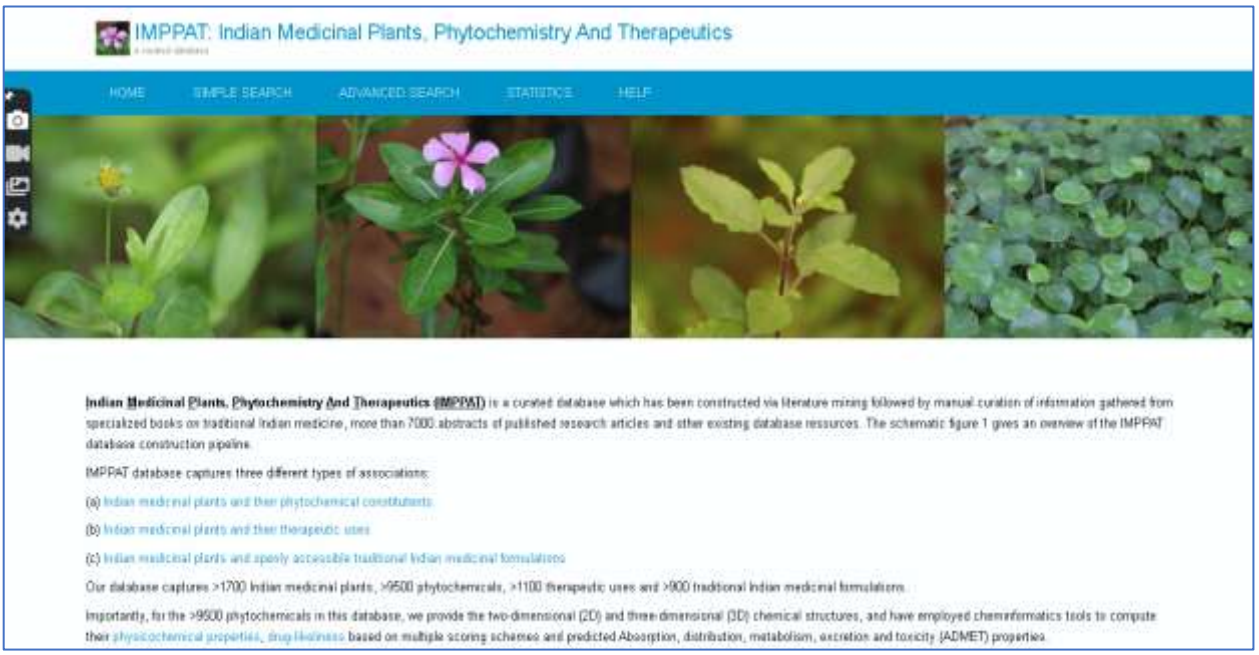

FIG 3.4- HOME PAGE OF IMPPAT DATABASE [https://cb.imsc.res.in/imppat/home]

TABLE 3.3: NATURAL COMPOUNDS TAKEN FROM IMPPAT DATABASE

PubChem ID	Source
CID:20489	*Didymocarpus pedicellata*
CID:115025	*Lolium temulentum*
CID:445040	*Aloe vera*
CID:71447337	*Murraya exotica*
CID:22382	*Passiflora edulis*
CID:11980943	*Camellia sinensis*
CID:442884	*Adhatoda zeylanica*
CID:44257	*Portulaca grandiflora*
CID:156437	*Pongamia pinnata*
CID:610735	*Daemonorops draco*
CID:12305449	*Pongamia pinnata*
CID:520130	*Triticum aestivum*
CID:156338	*Tephrosia purpurea*

Now we go ahead with checking the bio-availability of compounds with the help of SwissADME [13] software. Several parameters related to compounds are checked through this program which majorly include lead-likeness and Lipinski's rule of 5 violations. Out of the total 13 compounds obtained we discovered 4 compounds only which met with the parameters. Table IV shows below those four compounds with PubChem CID.

TABLE 3.4: LIST OF FAVOURABLE COMPOUNDS OBTAINED

PubChem ID	Name
CID:115025	Perloline
CID:442884	Anisotine

CID:610735	Nordracorhodin
CID:20489	Isowogonin

FIG 3.5- HOME PAGE OF SWISS ADME [http://www.swissadme.ch/]

3.4. DOCKING ANALYSIS:

Now we move on to the final step i.e., docking whose first step involves preparation of protein and ligand samples. Out of all the receptors mentioned, we selected topoisomerase I as our target receptor because bring DNA replication to a halt is a very efficient method in order to arrest the proliferation of cancer cell.

The receptor – inhibitor complex i.e., Topoisomerase 1/Camptothecin (RCSB PDB ID: 1T8I) [12] and Acetylcholinesterase/Donepezil (RCSB PDB ID: 4EY7) [11] was selected from PDB. [10]. This was followed by identifying active site coordinates using the Biovia DS Visualizer. The heteroatoms were removed leaving only the receptor structure, followed by energy minimization using another database i.e., Swiss-PDB Viewer [9] and the result was saved in .pdb format.

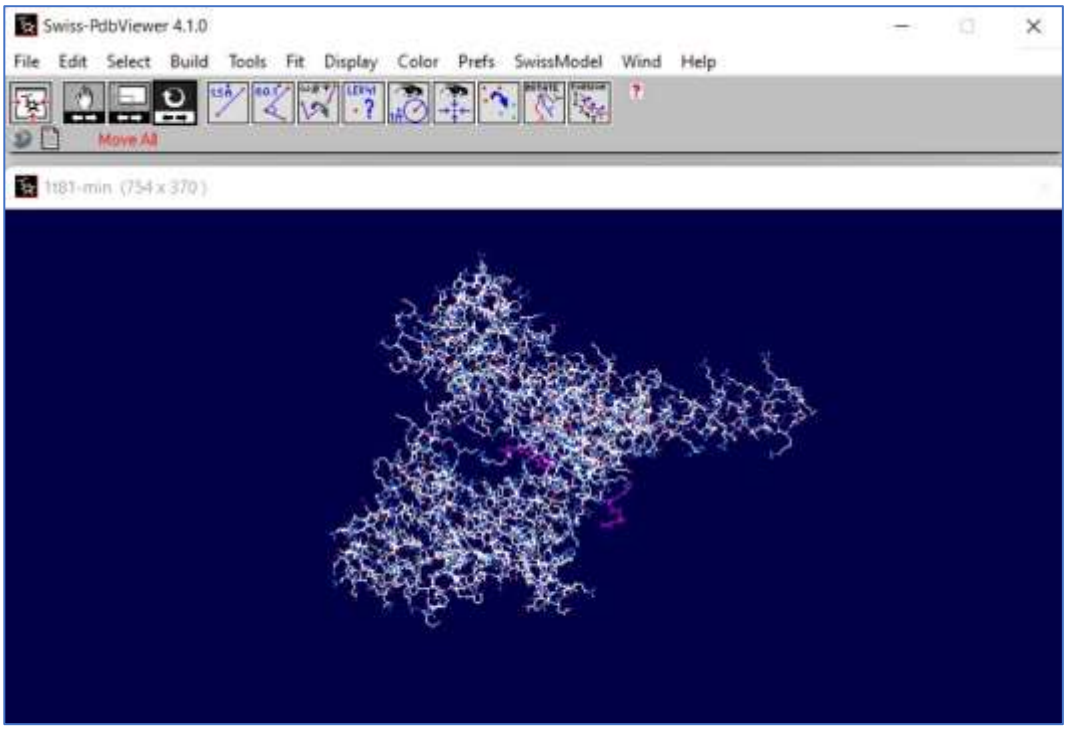

FIG 3.6- ENERGY MINIMIZATION USING SWISS PDB VIEWER

Using the Open Babel GUI software, the downloaded ligands were converted to .pdb format. Auto Dock v4.2.6, with UI support by AutoDockTools [8] was used for performing docking analysis. The receptors were loaded and were optimized by adding polar hydrogens and Kollman charges and removing non-polar hydrogens. When working on ligands, apart from Kollman charges, Gasteiger charges were also added.

The grid parameters were set by using Auto Grid function for affinity and docking was started using the Lamarckian GA as search parameter. As the results were displayed for all the conformations, the one with lowest binding energy for all the ligands was chosen. After performing docking for Topoisomerase, I against ligands, ligands with greater binding affinity than irinotecan were made to undergo another docking with acetylcholinesterase using the same protocol as above.

CHAPTER 4: RESULTS

4.1 TOPOISOMERASE 1

Post docking analysis, it was inferred that Isowogonin possesses greater binding affinity than the FDA approved drug Irinotecan Hydrochloride. Whereas the rest of the natural compounds showed lesser binding affinity for the target receptor Topoisomerase 1.

TOP1 interactions with both Irinotecan Hydrochloride and Isowogonin were visualized using Biovia DS Visualizer.

Fig.12A shows Topoisomerase 1 interactions with Irinotecan Hydrochloride where it can be seen that Irinotecan forms two conventional hydrogen bonds with ARG364, a pi-cation bond asASP533. Alkyl bonds are also formed TYR426 and MET428.

Fig.12B shows Topoisomerase II interactions with Isowogonin which confirms that Isowogonin forms a total of four bonds, along with conventional hydrogen bond at TYR426, and three pi-alkyl bonds which includes two with MET428 and one with ALA351.

TABLE 4.1: BINDING ENERGY OF COMPOUNDS WITH TOP1

Ligand	Auto-Dock Binding Energy(kcal/mol)
Irinotecan Hydrochloride^	-5.22
Perloline	-5.00
Anisotine	-4.66

Nordracorhodin	-4.61
Isowogonin	-5.64

^: FDA Approved Drug

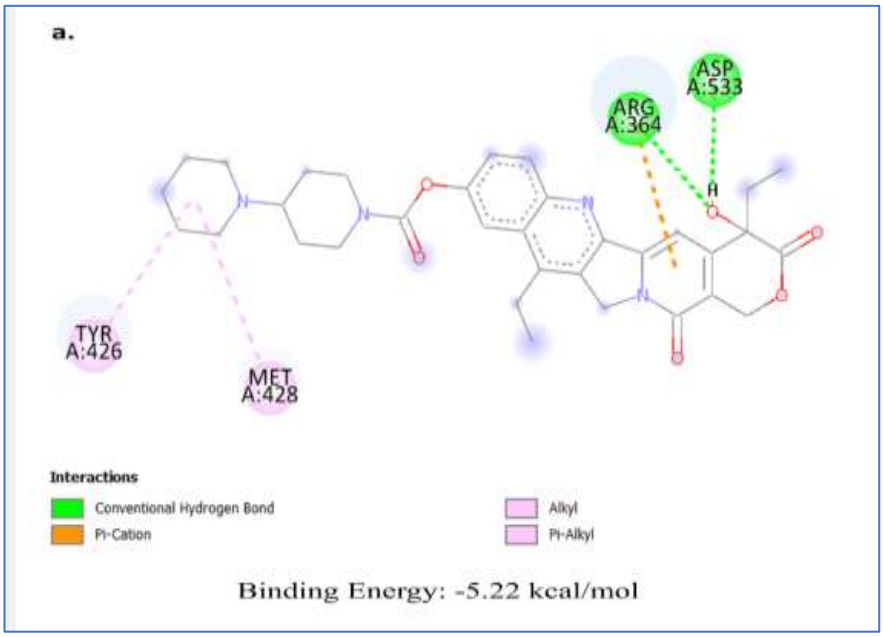

FIG 4.1(a)- TOPOISOMERASE 1 INTERACTION WITH IRINOTECAN HYDROCHLORIDE

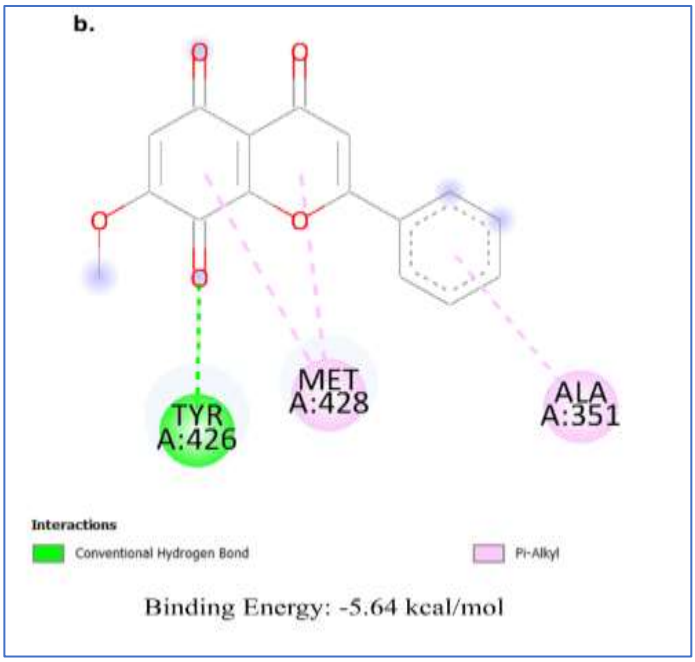

FIG 4.1(b)- TOPOISOMERASE 1 INTERACTION WITH ISOWOGONIN

4.2 ACETYLCHOLINESTERASE

The docking results showed that Isowogonin had greater binding energy (-9.29 kcal/mol) when compared with Irinotecan Hydrochloride (-11.59 Kcal/mol) for acetylcholinesterase receptor.

This also proved that Isowogonin had lesser affinity for binding to acetylcholinesterase. The interactions were then visualized using Biovia DS Visualizer.

In case of irinotecan, two carbon-hydrogen bonds are formed at ASP74 and TYR341along with five alkyl bonds which are TYR72, LEU76, VAL294, PHE338 and HIS447. Apart from this, three pi-pi interactions were also visualized which consists of three with TYR341, two with TRP286 and one with TYR124 [fig.13A].

In case of Isowogonin, two conventional hydrogen bonds are formed with ARG296. Pi-sigma and pi-alkyl bonds are also formed with VAL294. Pi-Pi interactions with TRP286 and TYR341, and a pi-donor hydrogen bond with PHE295 were other two bonds observed [fig.13B].

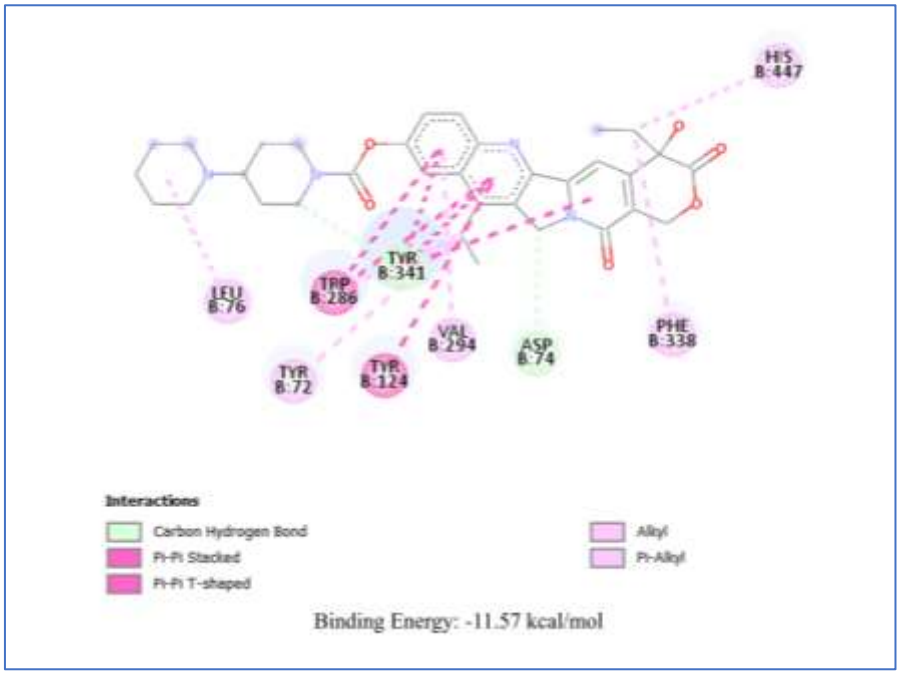

FIG 4.2(a)- ACETYLCHOLINESTERASE INTERACTION WITH IRINOTECAN HYDROCHLORIDE

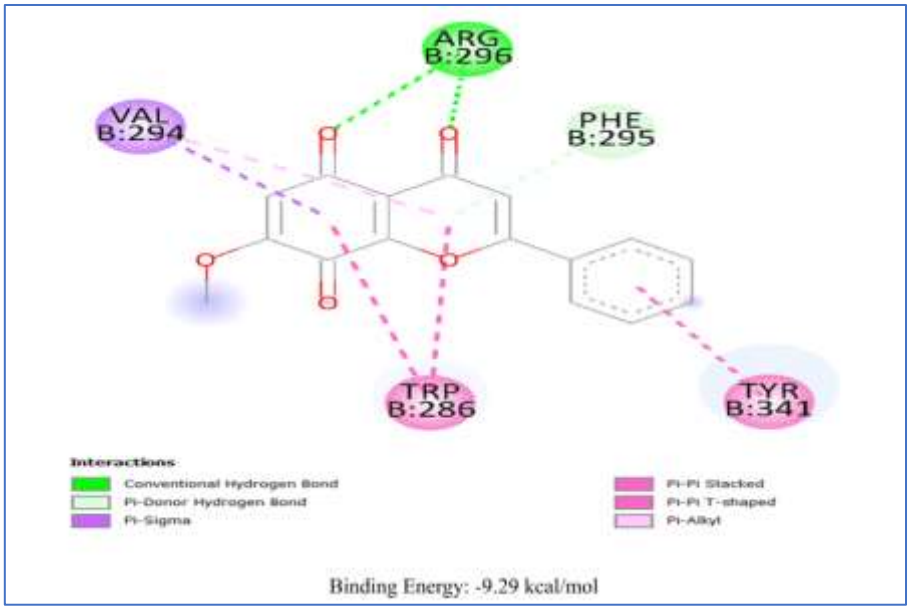

FIG 4.2(b)- ACETYLCHOLINESTERASE INTERACTIONS WITH ISOWOGONIN

TABLE 4.2: BINDING ENERGY OF COMPOUNDS WITH ACETYLCHOLINESTERASE

Ligand	Auto-Dock Binding Energy(kcal/mol)
Irinotecan Hydrochloride^	-11.59
Perloline	-10.87
Anisotine	-10.34
Nordracorhodin	-9.14
Isowogonin	-9.29

^FDA approved drug

CHAPTER-5: DISCUSSION

Topoisomerase 1acts as a regulator of DNA supercoiling and cell cycle progression. So it is considered to be the main target when treatment of pancreatic cancer is talked about.

Topoisomerase 1 relaxes the negative supercoiling of DNA strands and also prevents the breakage of strands due to any torsional strain.

For fulfilling this purpose, synthetic drugs such as irinotecan hydrochloride or Onivyde which is liposomal formulation of irinotecan approved by FDA are used.

The drugs act as inhibitors of TOP1 and lead to inhibition by binding to topoisomerase 1 which results in non-removal of negative supercoils causing torsional stress in DNA strand followed by strand breakage and bringing cellular replication to the halt.

Fig:14 shows what happens when irinotecan is administered into the body and how it affects the replication of cancer cell. The first part shows that no supercoiling is resolved and breakage of strand is not observed leading to cell replication but as soon as irinotecan acts upon the topoisomerase 1-DNA complex, the negative supercoiling is unresolved resulting in strand breaks leading to cell death.

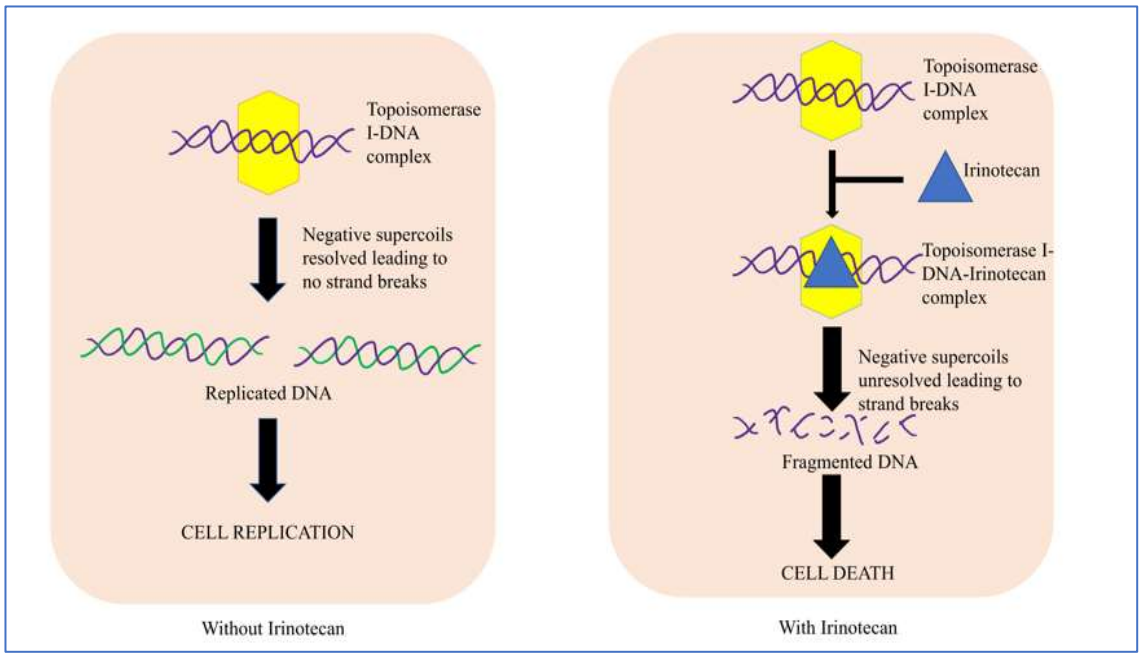

FIG 5.1- IRINOTECAN EFFECT ON CANCER CELL REPLICATION

Irinotecan plays a beneficiary role in the treatment of refractory pancreatic cancer. This is because the liposomal formulation proves to be more efficient in terms of drug release and stays in the body for a longer time period.

But there is a disadvantage that irinotecan may bind to some of the non-target receptors for example acetylcholinesterase receptor.

When acetylcholinesterase binds with irinotecan, inhibition occurs and is unable to breakdown acetylcholine to choline and acetic acid.

This results in acetylcholine accumulation which works on the nervous system leading to the side effects including muscle weakness, fatigue, diarrhea, paralysis among others.

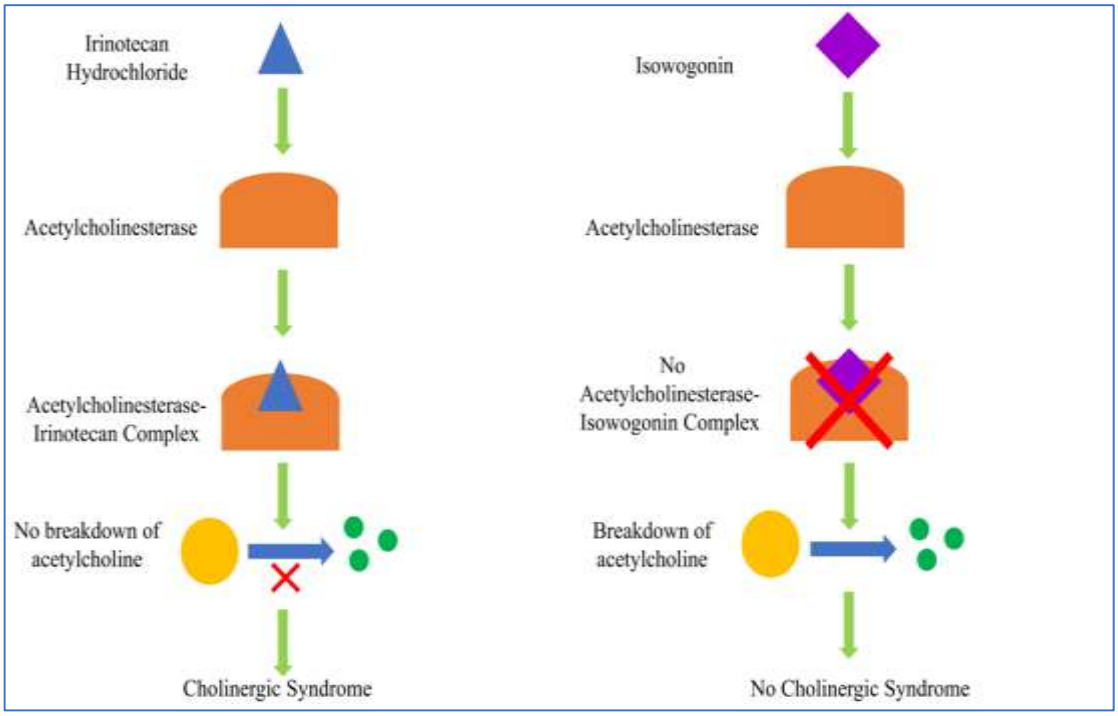

FIG 5.2- ACETYLCHOLINESTERASE BINDING WITH IRINOTECAN VS ISOWOGONIN

For rectifying this problem, Isowogonin came on the board which is a natural compound obtained from plant source *Didymocarpus pedicellata*. Isowogonin bonded more efficiently with topoisomerase 1 but failed to bind with acetylcholinesterase, which led to prevention of symptoms pointing towards cholinergic syndrome. Thus, this resulted in considering Isowogonin as an alternative of irinotecan for the second line treatment of pancreatic cancer. Fig:15 shows the outcome when irinotecan binds with acetylcholinesterase in comparison with outcome when Isowogonin binds with acetylcholinesterase. On binding with irinotecan, acetylcholinesterase forms a complex leading in no breakdown of acetylcholine thus resulting in cholinergic syndrome. On the other hand, Isowogonin on binding with acetylcholinesterase forms no complex and results in breakdown of acetylcholine which doesn't allow its accumulation so no syndrome is detected.

CHAPTER 6: CONCLUSION

Pancreatic cancer is being one of the most fatal among the all the other cancers. Most of the times, pancreatic cancer remains undetected which increases the rate of mortality and severity. The treatment for PC is either surgical resection or use of chemotherapeutic drugs. Some of the drugs such as erlotinib, mitomycin, irinotecan hydrochloride liposome, Olaparib among others have been approved by FDA. These drugs play an important role as chemotherapeutic agents by controlling the DNA synthesis or blocking the signaling pathways which halts the cancer progression.

But these drugs do have certain side effects from acute diarrhea to fatal cholinergic syndrome and from nausea to bradycardia which prove to be severe. So, to overcome these side effects, natural compounds have been found which play similar role in cancer therapy as these approved drugs. Among all the drugs analyzed, Isowogonin obtained from *Didymocarpus pedicellata* is the one which has greater binding energy for TOP1 then irinotecan and has lesser or no side effects when compared with irinotecan. Many more analysis will be performed so that more natural compounds can be used for treatment of cancer which may be equivalent to FDA approved drugs if not better than them.

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APPENDICES

Appendix A: SUPPLEMENTARY INFORMATION

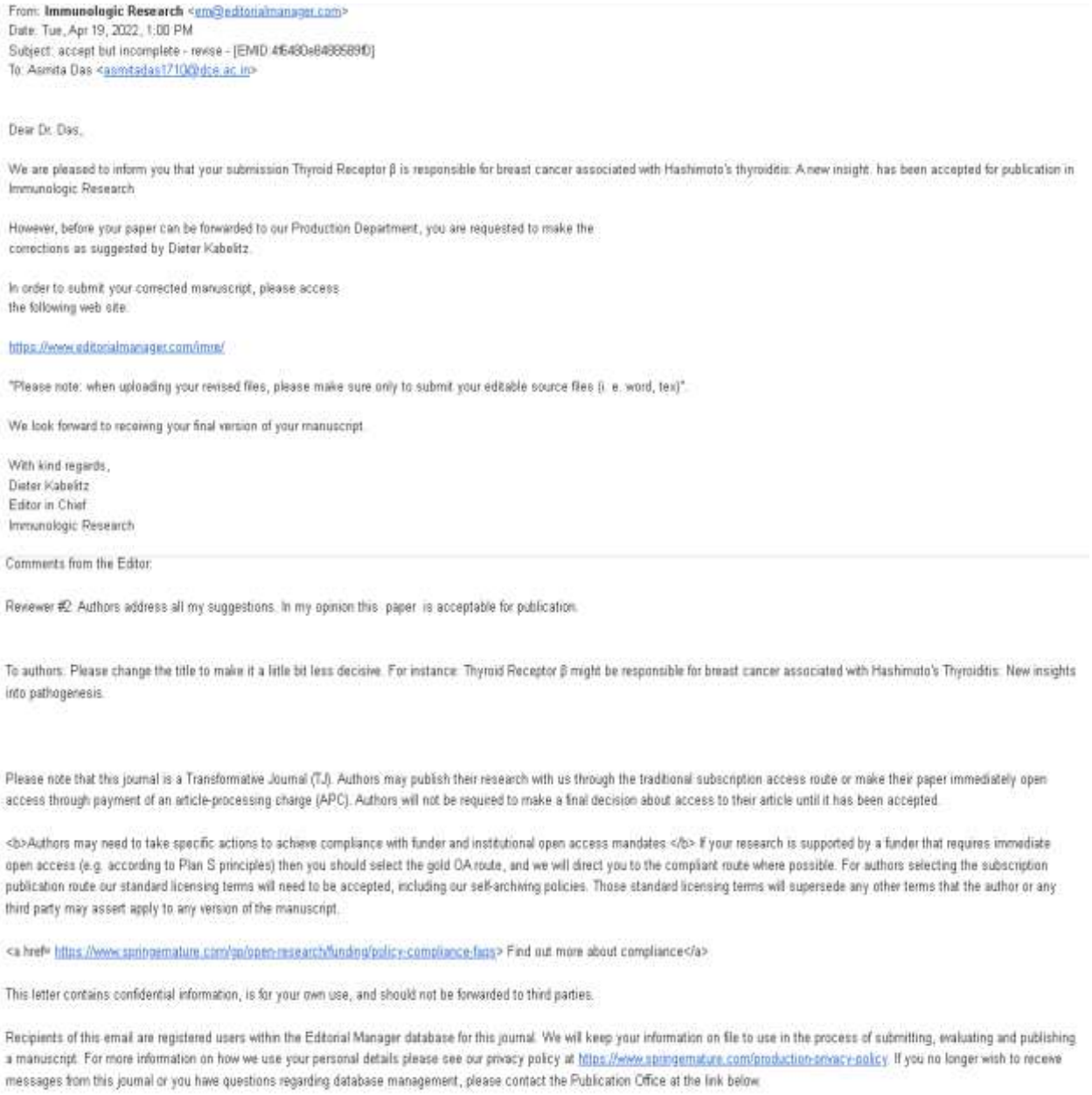
Molecule	MW	Lipinski #violations	Bioavailability Score	Leadlikeness #violations	Synthetic Accessibility
4H-Furo(2,3-h)-1-benzopyran-4-one, 2-(2-methoxyphenyl)-	292.29	0	0.55	1	3.16
Nordracorhodin	252.26	0	0.55	0	3.25
Kanjone	292.29	0	0.55	1	3.19
8-methoxycoumarin	176.17	0	0.55	1	2.73
1-(5-acetyl-4-methyl-1,3-thiazol-2-yl)-4-hydroxy-2-(4-hydroxyphenyl)-3-(7-methoxy-1-benzofuran-2-carbonyl)-2H-pyrrol-5-one	504.51	1	0.11	2	4.5
Apollinine	362.38	0	0.55	2	3.82
Isowogonin	284.26	0	0.55	0	3.15
6-Methoxyflavone	252.26	0	0.55	1	2.89
Karanjin	292.29	0	0.55	1	3.21
Rhodamine 123	380.82	0	0.55	1	3.63
4-pyridone analogue,34	472.44	0	0.55	2	3.18

Perloline	333.36	0	0.55	0	2.49
6-Biopterin	237.22	0	0.55	1	3.13
Asteropusazole A	367.2	0	0.55	2	2.08
HARMINE, HYDROCHLORIDE, DIHYDRATE	248.71	0	0.55	2	2.55
Tea extract	1122.94	3	0.17	2	8.67
Rhodamine 123	380.82	0	0.55	1	3.63
Guanine	151.13	0	0.55	1	1.8
Anisotine	349.38	0	0.55	0	3.38
Sapropterin	241.25	1	0.55	1	3.75

Appendix B: LIST OF PUBLICATIONS

- Dhingra M*., Mahalanobis S.* and Das A. Thyroid Receptor β might be responsible for breast cancer associated with Hashimoto's Thyroiditis: A new insight into pathogenesis. Accepted in Immunologic Research (Springer). Acceptance Date: April 19, 2022

  *Shared first authorship



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Date: Tue, Apr 19, 2022, 1:00 PM
Subject: accept but incomplete - revise - [EMID:4f6480e84666589f0]
To: Asmita Das <asmitadas1710@dce.ac.in>

Dear Dr. Das,

We are pleased to inform you that your submission Thyroid Receptor β is responsible for breast cancer associated with Hashimoto's thyroiditis: A new insight. has been accepted for publication in Immunologic Research.

However, before your paper can be forwarded to our Production Department, you are requested to make the corrections as suggested by Dieter Kabelitz.

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We look forward to receiving your final version of your manuscript.

With kind regards,
Dieter Kabelitz
Editor in Chief
Immunologic Research

Comments from the Editor:

Reviewer #2: Authors address all my suggestions. In my opinion this paper is acceptable for publication.

To authors: Please change the title to make it a little bit less decisive. For instance: Thyroid Receptor β might be responsible for breast cancer associated with Hashimoto's Thyroiditis: New insights into pathogenesis.

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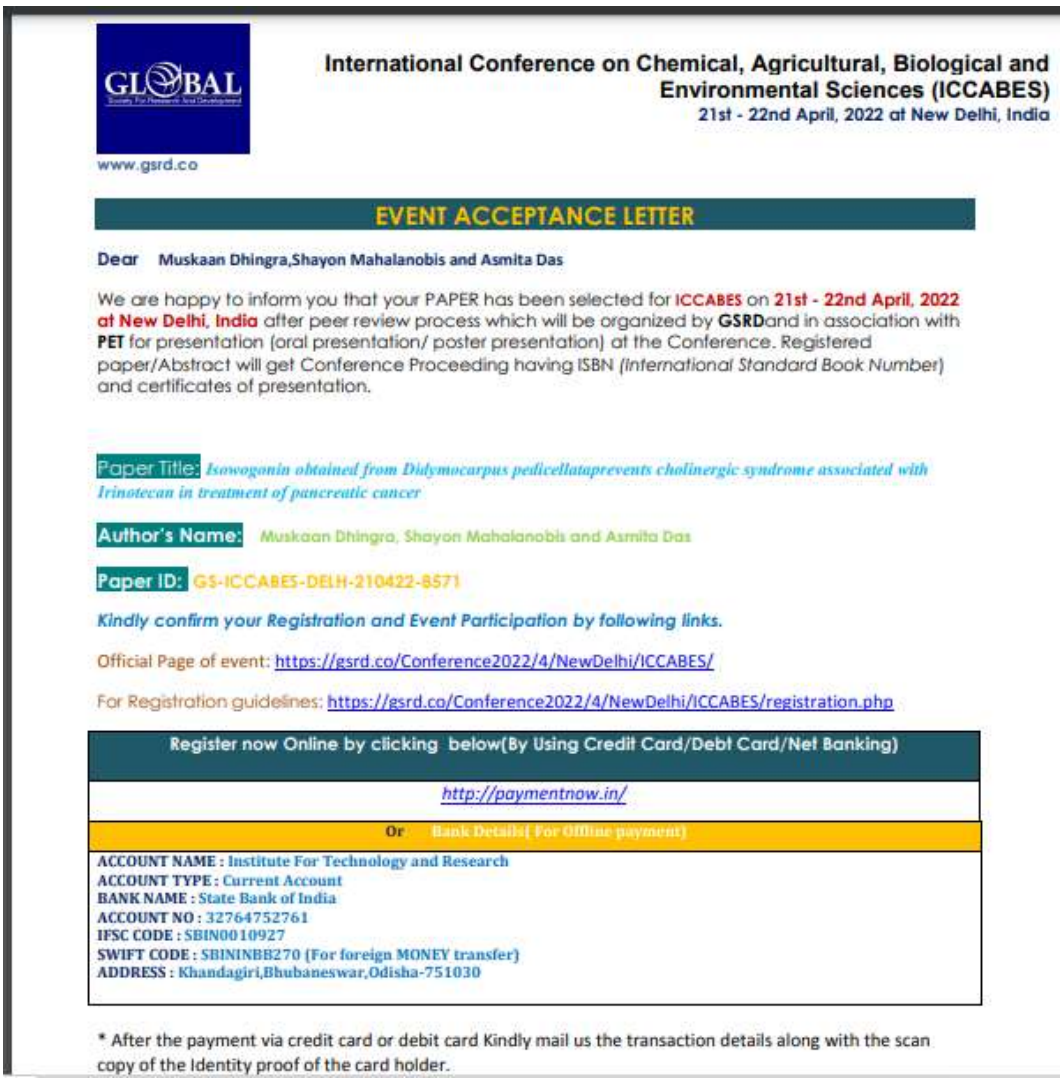
- Dhingra M., Mahalanobis S. and Das A. Isowogonin obtained from Didymocarpus pedicellata prevents cholinergic syndrome associated with Irinotecan in treatment of pancreatic cancer. Submitted in International Conference on Chemical, Agricultural, Biological and Environmental Sciences (24 April 2022), New Delhi.



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21st - 22nd April, 2022 at New Delhi, India

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Dear Muskaan Dhingra,Shayon Mahalanobis and Asmita Das

We are happy to inform you that your PAPER has been selected for ICCABES on 21st - 22nd April, 2022 at New Delhi, India after peer review process which will be organized by **GSRD**and in association with **PET** for presentation (oral presentation/ poster presentation) at the Conference. Registered paper/Abstract will get Conference Proceeding having ISBN *(International Standard Book Number)* and certificates of presentation.

Paper Title: *Isowogonin obtained from Didymocarpus pedicellataprevents cholinergic syndrome associated with Irinotecan in treatment of pancreatic cancer*

Author's Name: Muskaan Dhingra, Shayon Mahalanobis and Asmita Das

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- Dhingra M., Rawat P., Kosta K., and Das A., "Microflora impacts immune system and its anti-tumor function," in Microbial Crosstalk with Immune system. To be published by Elsevier

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I, Muskaan Dhingra, Roll No. 2K20/MSCBIO/17 M.Sc. Biotechnology student declares that the project report titled **"TARGETING CHOLINERGIC SYNDROME IN PANCREATIC CANCER THROUGH NATURAL COMPOUNDS"** which has been submitted to the Department of Biotechnology, Delhi Technological University, New Delhi, is original and has not taken from any of the sources without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

PLACE: Delhi

Date: 05 MAY 2022

Muskaan Dhingra

MUSKAAN DHINGRA
2K20/MSCBIO/17

DEPARTMENT OF BIOTECHNOLOGY
DELHI TECHNOLOGICAL UNIVERSITY
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CERTIFICATE

I hereby certify that the dissertation project titled "**TARGETING CHOLINERGIC SYNDROME IN PANCREATIC CANCER THROUGH NATURAL COMPOUNDS**" which is submitted by Muskaan Dhingra, 2K20/MSCBIO/17, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of requirement for the award of the degree of Master of Science, is a record of work carried out by the student under my supervision. To the best of my knowledge, this work has not been submitted in part of any Degree or Diploma to this University or elsewhere.

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DATE:

5/5/22

Dr. ASMITA DAS
(SUPERVISOR)
Assistant professor
Department of Biotechnology
Delhi Technological University

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Muskaan Dhingra

Muskaan Dhingra

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