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In Silico Screening of Quercetin Analogues as Potential Inhibitors of Tumor Necrosis Factor-alpha for Diarrhea Mitigation in Irinotecan Cancer Treatment

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CERTIFICATE

I hereby certify that the Project Dissertation titled **"In Silico Screening of Quercetin Analogues as Potential Inhibitors of Tumor Necrosis Factor-alpha for Diarrhoea Mitigation in Irinotecan Cancer Treatment"** which is submitted by **Nivedita Das**, Roll No. **2K21/IBT/04** Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of requirement for the award of degree of Master of Technology, 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.

09/06/2023

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Place: Delhi

Date: 29 May 2023

CANDIDATE'S DECLARATION

I, **Nivedita Das**, Roll No. **2K21/IBT/04** student of M. Tech (Industrial Biotechnology), hereby declare that the Project Dissertation titled **"In Silico Screening of Quercetin Analogues as Potential Inhibitors of Tumor Necrosis Factor-alpha for Diarrhoea Mitigation in Irinotecan Cancer Treatment"** which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Master of Technology is original and not copied from any source without proper citation. The 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: 29 May 2023

(NIVEDITA DAS)

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Abstract

Diarrhea can be caused due to many reasons in an oncological patient. It could be due to radiation therapy, graft vs host infections, chemotherapeutic agents. Early diagnosis of causative agent can help to prevent diarrhea in cancer patients. Chemotherapeutic agents such as 5 fluorouracil with leucovorin, capecitabine and irinotecan are main causative agent of diarrhea. Loperamide and Octreotide are two recommended treatments for chemotherapy induced diarrhea. Loperamide can only treat grade 1 or 2 diarrhea, it becomes ineffective in severe cases of diarrhea. Octreotide is especially for treating grade 3 or 4 diarrhea but one drawback is that it requires hospitalization for fluid resuscitation due to dehydration in patients which thus increases the cost of treatment. The therapeutic potential of TNF- alpha inhibition for conditions including cancer, diabetes, and particularly autoimmune illnesses is significant. Even though there are several small molecule inhibitors of TNF- that have been discovered, no orally active treatment has yet been revealed that necessitates the urgent need for a small molecule drug against TNF- alpha.

This research paper presents a comprehensive analysis of autodocking results obtained from virtual screening experiments conducted on a library of plant natural compound as inhibitor - Quercetin. The objective was to identify potential lead compounds with high binding affinities and favourable binding modes against the Tumor Necrosis Factor – alpha. Our findings reveal promising candidates for further investigation as potential therapeutics in the treatment of chemotherapy induced diarrhoea.

CONTENTS

PAGE NO.

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND SYMBOLS

SYMBOLS/ABBREVIATIONS	MEANING
CPT - 11	Irinotecan Hydrochloride
CID	Chemotherapy induced diarrhea
TNF - α	Tumor Necrosis Factor - alpha
IL-1β, 6, 12	Interleukins – 1 beta, 6, 12
IFN-γ	Interferon - gamma
TNFR I and II	tumor necrosis factor receptor type I and II
SN 38	7-ethyl-10-hydroxycamptothecin
IBD	Inflammatory Bowel Disease

CHAPTER 1

INTRODUCTION

Chemotherapeutic drug induced diarrhea

Diarrhoea is a dose limiting toxicity induced by chemotherapeutic drugs used in cancer. Irinotecan Hydrochloride, Oxaliplatin, Capecitabine and 5- Fluorouracil are few chemotherapeutic drugs which are concerned with diarrhoea in patients. Diarrhoea is divided into early and late onset diarrhoea. Early onset diarrhoea is witnessed just after the administration of CPT -11 which is due to inhibition of acetylcholinesterase and it can be controlled by administration of atropine[2]. Late onset diarrhoea occurs after the exposure to SN 38 in intestine, which is the active form of prodrug CPT -11. Loperamide is a recommended medicine to treat irinotecan induced diarrhoea but not all patients respond to this medicine very well [1]. Loperamide at high doses relieves diarrhoea brought on by chemotherapy treatment. However, using it can have a number of negative side effects, such as severe constipation, stomach discomfort, disorientation, rashes, exacerbation of pre-existing bloating, nausea, and vomiting. When loperamide is ineffective after 48 hours despite loperamide dose escalation, patients are typically given octreotide as a second line treatment [4]. This medication is used to treat together complex diarrhoea and loperamide-refractory diarrhoea. Even while octreotide significantly reduces CID, over 10% of patients experience serious adverse effects include leisurely and/or irregular heartbeat, serious constipation, stomach discomfort, enlarged thyroid, vomiting, nausea, headache, as well as vertigo [3]. Thus, novel drugs or agents are required to alleviate CPT -11 induced diarrhoea. Even though chemotherapy has significantly increased the total survival in many cancer types, cytotoxiic side-effects remain a substantial barrier that severely restricts the clinical implementation of otherwise effective treatments. Furthermore, it has been noted that CID might persist for up to 10 years after treatment [6]. The histological alterations that take place through the GI tract in response to irinotecan administration have been investigated in various animal studies because diarrhoea is a well-known

side effect of irinotecan treatment. In the small and large intestines, there have been reports of pronounced crypt extirpation, villus diminishing, and epithelial atrophy, which has led to mucosal injury and deterioration being a prevalent topic throughout the literature regarding CID [5]. CID is still mainly thought to be a type, or by-product, of GI mucositis even though patients do not frequently undergo colonoscopy or endoscopy to diagnose the chemotherapy-induced mucosal swelling. Mucosal damage that manifests as inflammation and ulceration and leads to changes in the intestinal microbiota and GI secretion is known as mucositis [55].

TNF- alpha as potential target for mitigating chemotherapy drug induced diarrhea

Cytokines are a class of tiny signaling proteins secreted by cells to facilitate communication between cells and to initiate an immune response. Major groups of cytokines include interleukins, lymphokines, tumor necrosis factor, and interferon. Proinflammatory cytokines include TNF-, IL-1, 6, 12, and IFN-. Proinflammatory cytokines are shown to be elevated in response to CPT -11 and SN 38. Tumor necrosis factor alpha (TNF-alpha) is a pleiotropic inflammatory cytokine. Lack of regulation of this protein has been related to a variety of diseases, despite the fact that it acts as the key biological regulator of important immunological functions. TNF- is a macrophage-derived pro-inflammatory cytokine that can activate macrophages, mobilize neutrophils, and upregulate other pro-inflammatory mediators to initiate tissue inflammation [7]. Edema and barrier dysfunction deterioration may also develop if it increases cell permeability. Two forms of TNF have been discovered: tumor necrosis factor beta and tumor necrosis factor alpha. Both are physically and temporally connected, and they compete against each other for binding to their respective receptor2. TNF- triggers a wide range of physiological signaling cascades after connecting with its two receptors, tumor necrosis factor receptor type I (TNFR1 or p55) and tumor necrosis factor receptor type II (TNFR2 or p75) [9]. In a normal physiological setting, TNFR1 is found on virtually all nucleated cells and plays a vital role in activating the TNF- pathway of signaling [8]. In contrast, TNFR2 is exclusively found on immune cells, endothelial cells, and nerve cells. Upon binding to receptors, TNF-alpha initiates a cascade of key signaling pathways that includes c-Jun N-terminal

kinase, MAP kinase, extracellular signal-regulated kinase, and transcription factor NF-B4. Infliximab, Certolizumab, Etanercept, Golimumab, and Adalimumab are only a few examples of the many TNF- antagonists that have changed the way rheumatologic inflammatory illnesses are treated [10]. Drugs like these target the TNF- dimer interface, where they build a complex that blocks the receptor-TNF interaction necessary for inflammation and other signal transduction. Inflammatory colitis is characterized by an increase in TNF-, which plays a key role in mucosal inflammation [11]. Anti-TNF medicines, for example, have been demonstrated to be helpful in the treatment of inflammatory bowel disease (IBD) because of the role this cytokine plays in the development of this condition. Furthermore, in acute shigellosis, TNF- alpha values in stool are higher than in other viral or bacterial causes of diarrhoea. To date, the only drugs with widespread adverse effects have been high-molecular-weight proteins or antibodies, which have been linked to conditions like tuberculosis, congestive heart failure, lupus, demyelinating illness, injection-site reactions, autoantibody generation, and other systemic issues. Symptoms of Crohn's disease, such as diarrhea, have been shown to improve when the inflammatory mediator TNF is blocked, according to clinical research. In fact, Crohn's disease, graft-versus-host disease, celiac sprue are all associated with elevated levels of TNF in affected tissues [18]. High levels of TNF have been detected in the feces of patients suffering from diarrhea due to an intestinal illness. Specifically, a Phase I research using recombinant TNF infusion in cancer patients indicated that it caused watery diarrhea, fever, chills, and flu-like symptoms. All of these findings taken together implicate TNF as a significant contributor to diarrhea. Cancer, diabetes, and especially autoimmune diseases have a lot to gain from TNF- alpha suppression as a therapeutic strategy. The dire need for a small molecule medication against TNF-alpha exists despite the discovery of multiple TNF- small molecule inhibitors [34]. So far, no orally active therapy has been developed [25].

Autodocking technology's incorporation into the drug discovery process is crucial and has many benefits. Drug development and optimization can be sped up significantly with the use of autodocking, a computer technique used to anticipate the binding interactions between tiny drug compounds and target proteins. Autodocking can save a lot of time and money in the drug discovery pipeline by eliminating the need to manually identify potential drug candidates. This used to entail producing and testing

an enormous number of chemicals, which was not only expensive but also time-consuming. On the other side, autodocking allows for the virtual screening of a huge database of compounds against a target protein, helping scientists to determine which compounds are most likely to have a positive effect on the protein and hence which should be pursued for experimental validation. The drug discovery process as a whole is sped up thanks to this fast screening method for locating promising lead compounds. Autodocking improves drug design productivity and precision as well. The binding affinity and method of interaction between a therapeutic molecule and its target protein can be predicted, providing researchers with valuable information for optimizing and improving the drug's chemical composition. This information improves the odds of future medication development by facilitating the design of more effective and selective therapeutic candidates [48]. The molecular mechanisms of drug-protein interactions can be better understood with the help of autodocking as well. By analyzing docking results, scientists can learn about the structural requirements for an effective drug-target interaction and investigate which amino acid residues play a role in the binding process. These findings not only improve our knowledge of disease pathways and molecular targets, but also pave the way for more precise and efficient treatment approaches. The optimization of drug candidates is another important area where autodocking plays a significant role [51]. Researchers can optimize the chemical structure of potential medications to improve their binding affinities with the target protein by repeatedly running docking simulations and assessing the binding affinity of changed molecules. Successful clinical outcomes are more likely as a result of this iterative optimization procedure, which boosts the drug's efficacy, selectivity, and pharmacokinetic features [65].

In conclusion, autodocking technology has become an indispensable part of the drug discovery pipeline, dramatically improving the earlier stages of the process (candidate identification and optimization). It has greatly aided the efficacy and success of drug development efforts due to its capacity to speed up compound screening, facilitate rational drug design, and provide crucial insights into molecular interactions. Autodocking is projected to play an increasingly important role in the discovery of novel therapies, leading to the development of more effective and targeted treatments for a wide range of diseases as computational tools continue to evolve [38].

CHAPTER 2

METHODOLOGY

Docking using Autodock vina mgl was done to search for potential inhibitors of Tumor Necrosis Factor – alpha.

Bioinformatic Tools Used

1. Autodock Vina mgl
2. Pymol
3. Open Babel GUI
4. Perl, IDE

Ligand Preparation

A list of ligands i.e., herbal compounds which are being used as traditional medicine were gathered from literature search using PubMed and Google Scholar. The structure of each ligand i.e., herbal compound was downloaded from ZINC database in .sdf file format which was then changed to. pdbqt file format using OPEN BABEL GUI. Each file of ligand was then renamed as quer, quer_1, quer_2, quer_3, quer_4, quer_5, quer_6, quer_7, quer_8, quer_9, quer_10, quer_11, quer_12, quer_13, quer_14, quer_15, quer_16, quer_17, quer_18, quer_19 **…….**quer_29.

Protein Preparation

The crystal structure of cytokine TNF - alpha was downloaded from Research Collaboratory for Structural Bioinformatics, Protein Data Bank in .pdb file format. The protein structure which was downloaded from PDB was of TNF-alpha (PDB_ID – 1TNF). Since the binding site was already known so site specific docking was performed instead of blind docking. TNF – alpha is a trimeric protein in which only two subunits participate in formation of active site for the ligands. The amino acid residues which are present in the binding site of TNF- alpha are Leu57, Tyr59, Ser60, Gln61, Tyr119, Leu120, Gly121, and Tyr151 from chain A and B and Gly122 from

chain A. Chain C was manually removed from the pdb file of TNF -alpha as it is not participating in binding site formation.

Grid Box Setting

Grid box with centre 19.968, 49.675, 39.930 and spacing 0.375 was set.

Docking and Visualization

Multiple docking was done and best hits were recorded. Lastly, the outputs were visualized in Pymol.

CHAPTER 3

RESULT AND DISCUSSION

Virtual Screening

Our virtual screening campaign involved the autodocking of a library of 30 quercetin analog inhibitors. The compounds were ranked based on their docking scores, representing their predicted binding affinities. The top-ranked compounds were further analyzed for their binding modes and interactions with the TNF – alpha.

Compound	ZINC ID	Mode	Affinity (kcal/mol)	RMSD l.b.	RMSD u.b.
Quer_4	ZINC4096846	1	-8.6	0.000	0.000
		2	-8.6	2.211	8.197
Quer_7	ZINC4349687	1	-8.6	0.000	0.000
		2	-8.2	1.705	9.582
Quer_14	ZINC13479091	1	-8.2	0.000	0.000
		2	-7.9	21.548	24.730
Quer_15	ZINC13515662	1	-7.9	0.000	0.000
		2	-7.9	22.487	26.482
Quer_2	ZINC3973253	1	-7.9	0.000	0.000
		2	-7.7	21.493	25.465

Quer_1	ZINC517261	1	-7.9	0.000	0.000
		2	-7.6	2.241	3.074
Quer_8	ZINC4654812	1	-7.8	0.000	0.000
		2	-7.6	12.698	17.348
Quer_5	ZINC4175638	1	-7.7	0.000	0.000
		2	-7.7	25.649	29.117
Quer_13	ZINC13479087	1	-7.7	0.000	0.000
		2	-7.7	10.683	15.515
Quer_3	ZINC4096845	1	-7.6	0.000	0.000
		2	-7.5	11.954	16.495
Quer_6	ZINC4349592	1	-7.5	0.000	0.000
		2	-7.2	1.667	4.965

Table 1: Docking Scores

Table 1 presents the top 11 compounds identified through virtual screening, along with their respective docking scores of best 2 hits. Compound quer_4 and quer_7 exhibited the highest docking score of -8.6 kcal/mol, indicating a strong binding affinity towards TNF – alpha target. Other compounds also demonstrated favorable docking scores, suggesting their potential as promising inhibitors.

Target Protein TNF - alpha and Ligand Quercetin Analogs

TNF – alpha is a trimeric protein but only 2 of its chains which is Chain A and chain B are involved in formation of its active site. The amino acid residues which are in the binding site of TNF- alpha are Leu57, Tyr59, Ser60, Gln61, Tyr119, Leu120, Gly121, and Tyr151 from chain A and B and Gly122 from chain A. Fig 1 shows chain A and chain B ribbon structure of TNF -alpha which is visualised on Pymol.

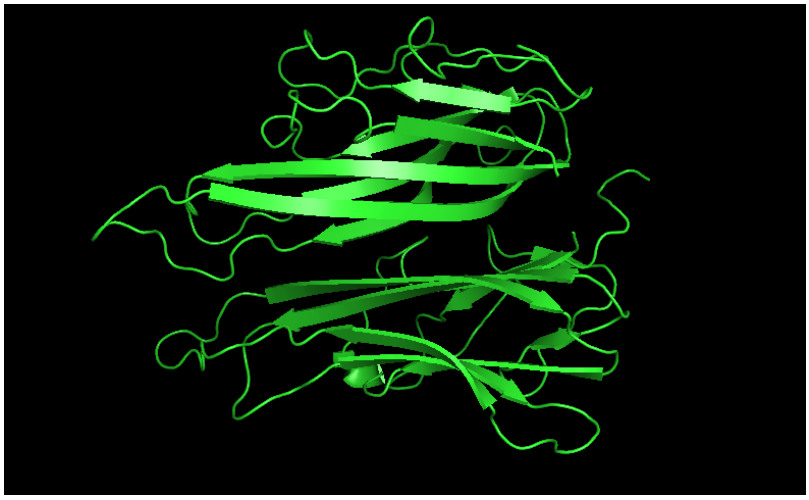

Fig 3.1: Dimer of TNF – alpha (1TNF)

Following are the structures of top 11 quercetin analogs obtained from Zinc database

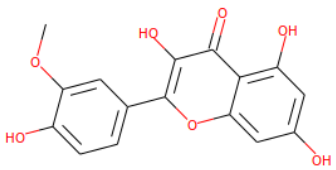

Fig 3.2: Quer_1

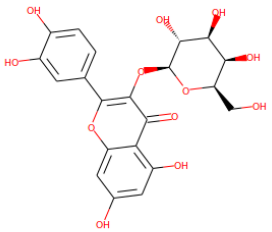

Fig 3.3: Quer_2

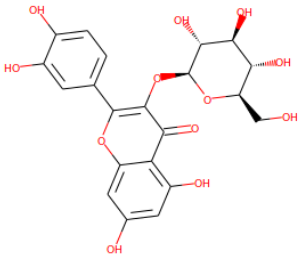

Fig 3.4: Quer_3

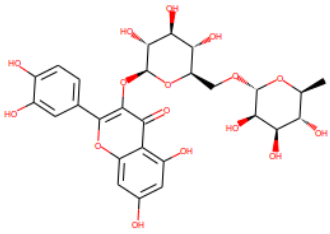

Fig 3.5: Quer_4

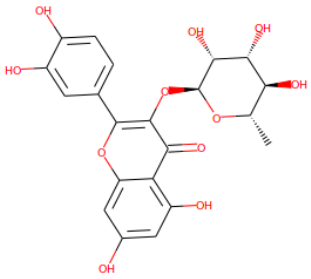

Fig 3.6: Quer_5

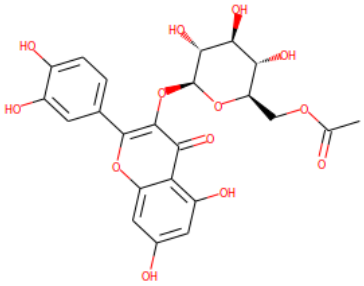

Fig 3.7: Quer_6

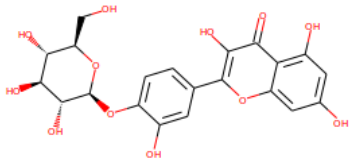

Fig 3.8: Quer_7

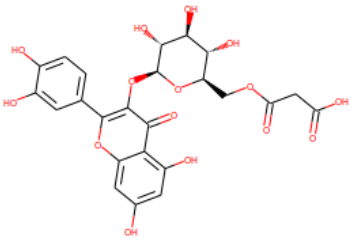

Fig 3.9: Quer_8

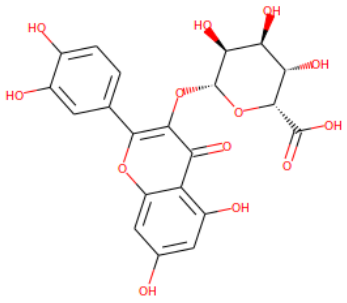

Fig 3.10: Quer_13

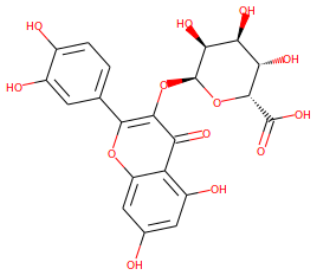

Fig 3.11: Quer_14

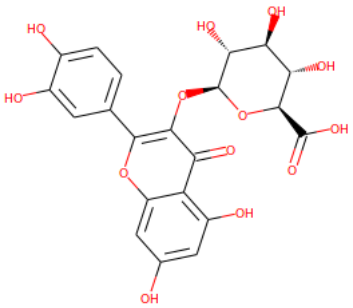

Fig 3.12: Quer_15

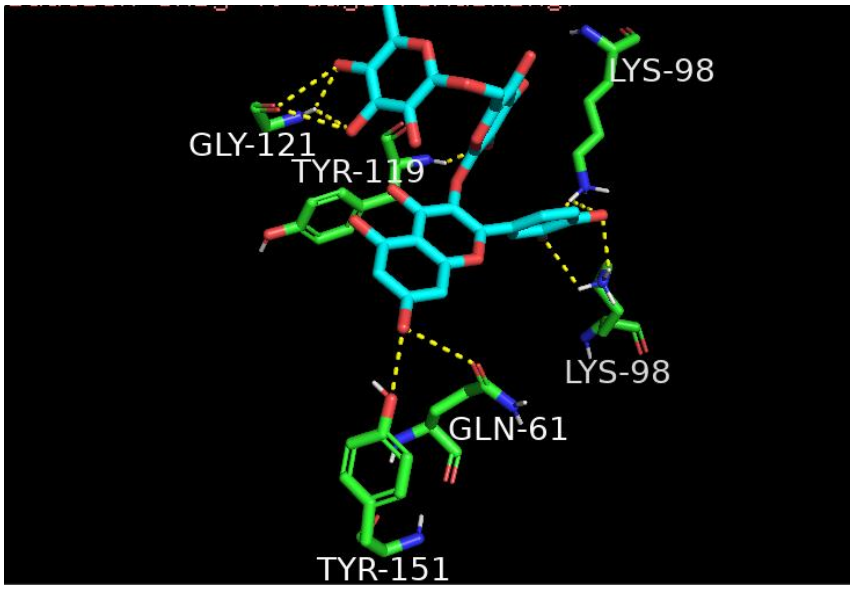

Fig 3.13: Amino acid residues of TNF – alpha making polar contact with quer_4 quercetin analog

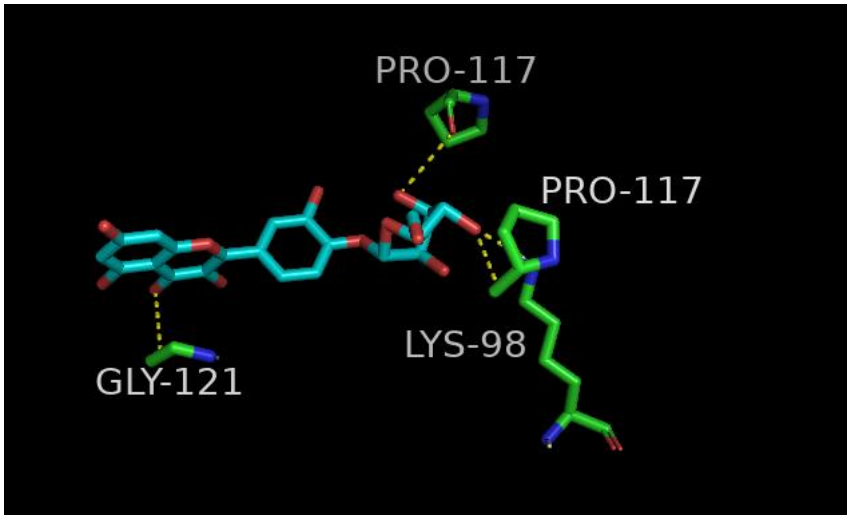

Fig 3.14: Amino acid residues of TNF – alpha making polar contact with quer_7 quercetin analog

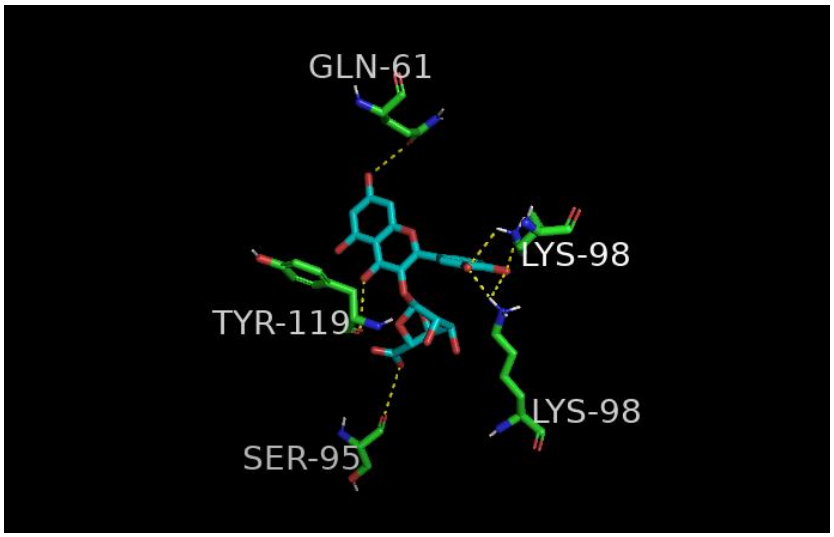

Fig 3.15: Amino acid residues of TNF – alpha making polar contact with quer_14 quercetin analog

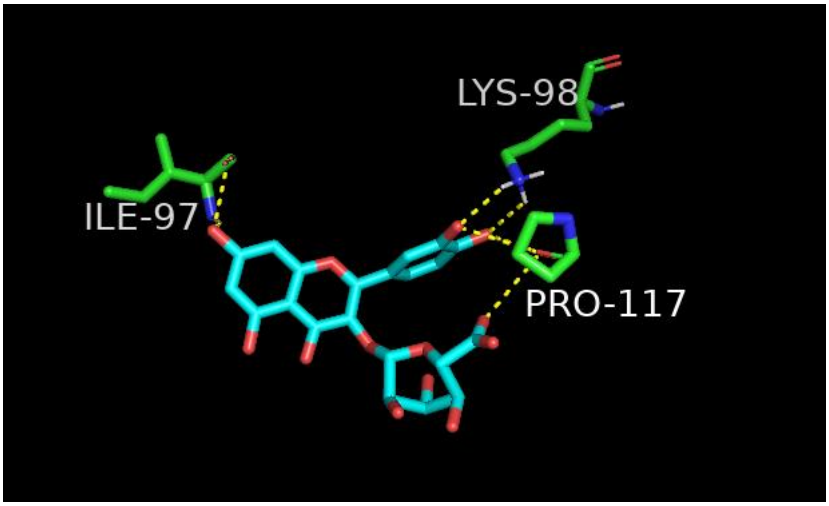

Fig 3.16: Amino acid residues of TNF – alpha making polar contact with quer_15 quercetin analog

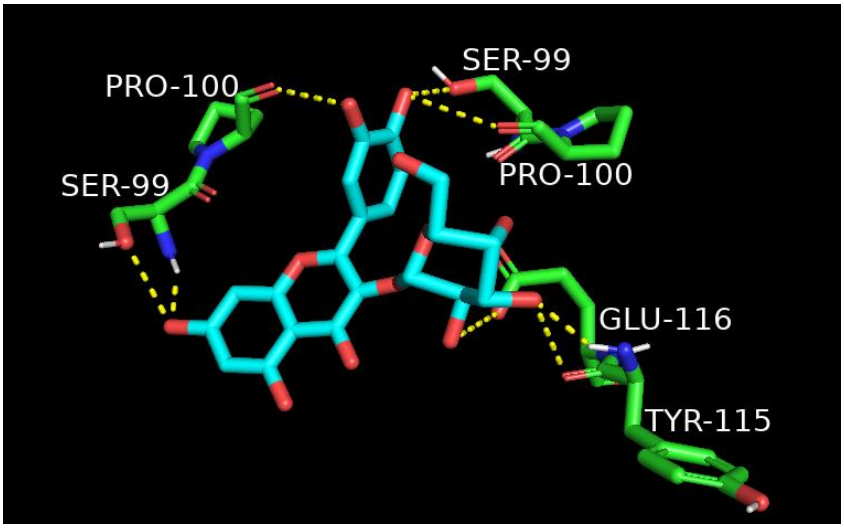

Fig 3.17: Amino acid residues of TNF – alpha making polar contact with quer_1 quercetin analog

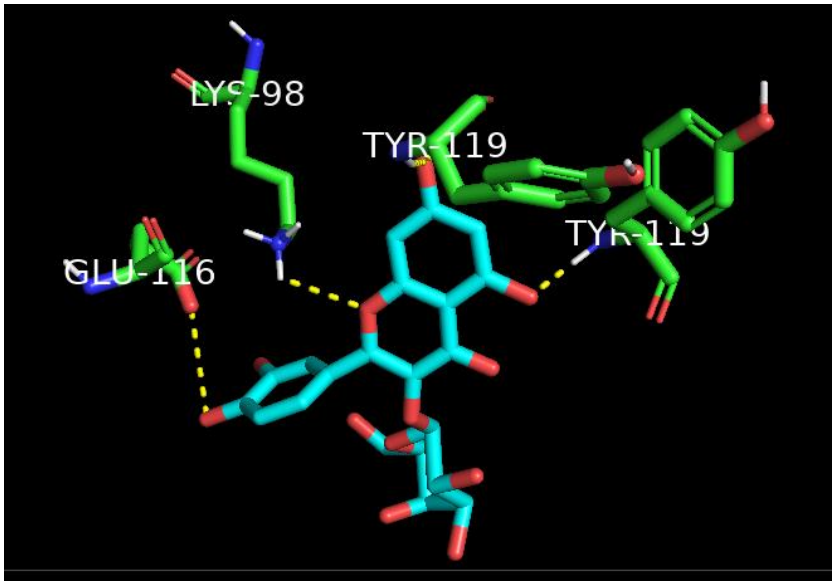

Fig 3.18: Amino acid residues of TNF – alpha making polar contact with quer_2 quercetin analog

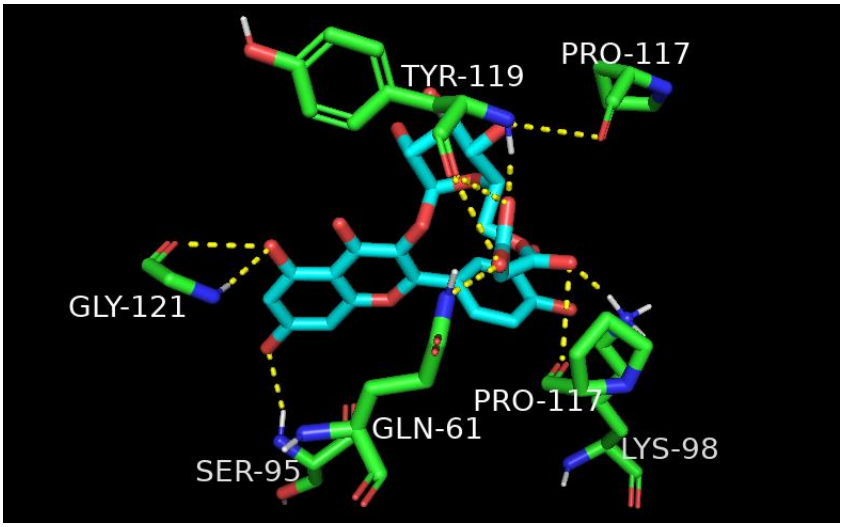

Fig 3.19: Amino acid residues of TNF – alpha making polar contact with quer_8 quercetin analog

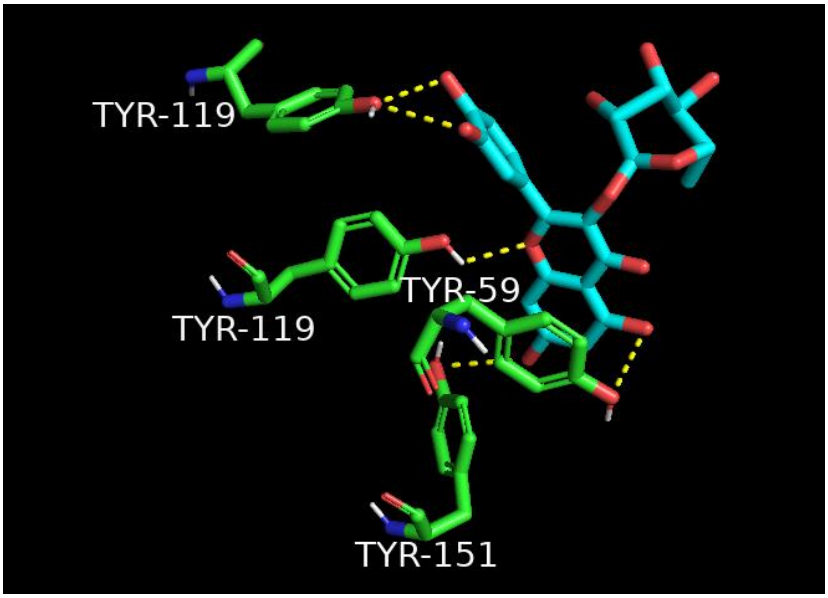

Fig 3.20: Amino acid residues of TNF – alpha making polar contact with quer_5 quercetin analog

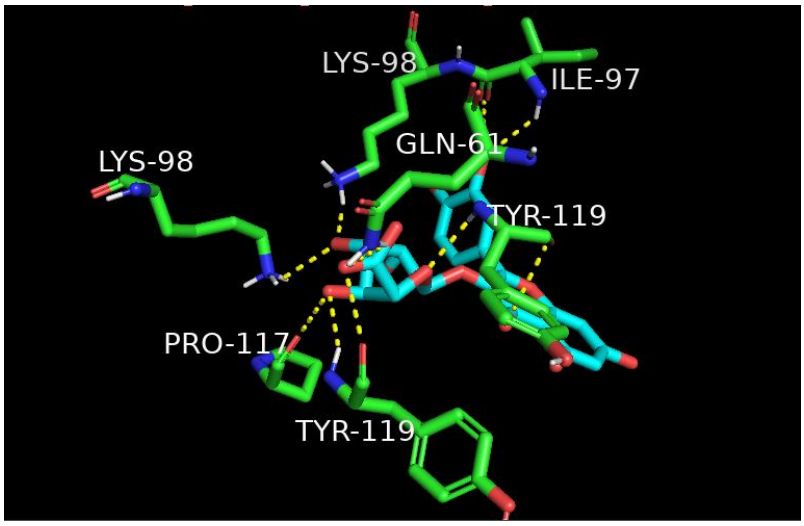

Fig 3.21: Amino acid residues of TNF – alpha making polar contact with quer_13 quercetin analog

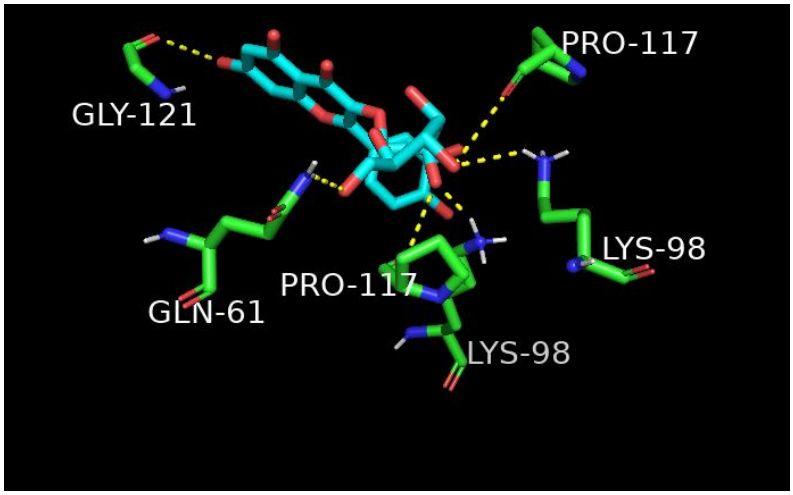

Fig 3.22: Amino acid residues of TNF – alpha making polar contact with quer_3 quercetin analog

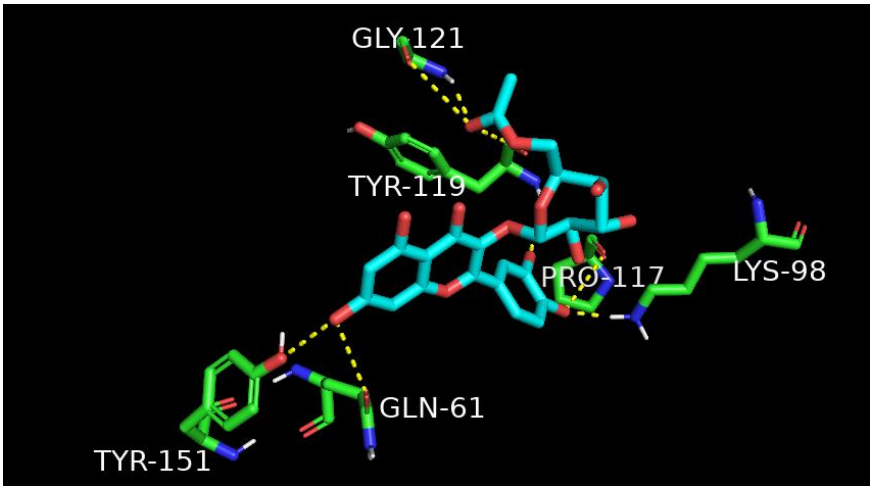

Fig 3.23: Amino acid residues of TNF – alpha making polar contact with quer_6 quercetin analog

Fig 13 – 23 represents the amino acid residues which are getting involved in binding to quer_4, quer_7, quer_14, quer_15, quer_1, quer_2, quer_8, quer_5, quer_13, quer_3, quer_6 quercetin analogues. The predicted binding site involved residues Leu57, Tyr59, Ser60, Gln61, Tyr119, Leu120, Gly121, and Tyr151 from chain A and B and Gly122. New binding sites has also been observed upon binding of quercetin analogues to the target protein which are further mentioned in discussion section.

DISCUSSION

The objective of our study was to find potential inhibitor of TNF – alpha which is a key factor in diarrheal conditions. Quercetin is one of the most important bioflavonoids found in over 20 plant species. It is renowned for its anti-inflammatory, antihypertensive, vasodilator, anti-obesity, anti-hypercholesterolemic, as well as anti-atherosclerotic properties. Quercetin is primarily found in onions, grapes, berries, cherries, broccoli, and citrus fruits. It is a powerful antioxidant flavonoid and more specifically a flavanol. It is a versatile antioxidant known to possess tissue-protective properties against drug-induced tissue damage.

Many studies suggest that chain A and chain B of trimeric TNF -alpha participate in active site formation. Our docking studies with quercetin analogs made additional polar bond with amino acids other than of primary binding site on TNF – alpha. Thus, this indicates that all the quercetin analogs has additional binding interactions or affinity for these amino acids. In figure 13 many of the amino acids of primary binding site are involved in polar bond formation between quer_4 and TNF -alpha such as Gly 121, Tyr 119, Tyr 151, Gln 61 along with additional binding site amino acid Lys 98. In figure 14 Gly 121 is one of amino acids which matches the predicted binding site amino acid residue of TNF -alpha. Similarly, in figure 15, Gln 61 and Tyr 119 were of predicted binding site of TNF -alpha whereas Lys 98 and Ser 35 were newly bounded sites After docking 30 different quercetin analogs, we narrowed our selection down to 11 of the most promising quercetin analogs based on the relative strength of their binding affinities to the other analogs The optimal docking score was somewhere in the range of -8.6 to -7.7 kcal/mol. It is possible to conduct additional research on quercetin and its analogs in order to provide more evidence of effectiveness of this plant compound as a TNF – alpha inhibitor.

CHAPTER 4

CONCLUSION

TNF -alpha is a pro – inflammatory cytokine which is found in high concentration in tissues of people with diseases like Crohn's disease, graft-versus-host disease, small-bowel transplant rejection, and celiac sprue that cause mucosal inflammation and diarrhoea. Patients with diarrhoea caused by an infection in the intestine have been found to have relatively high amounts of TNF in their stools. Thus, TNF -alpha can be targeted for mitigating chemotherapeutic drug induced diarrhoea. In future, other pro – inflammatory cytokines such as IL -6 and IL-1β can also be targeted to alleviate drug induced diarrhoea.

30 quercetin analogs were docked and we chose best 11 quercetin analogs on the basis of their comparatively good binding affinities of other analogs. The best docking score ranged between -8.6 to -7.5. Our study revealed the the potential of quercetin analog as inhibitors of TNF – alpha. Inhibition of TNF -alpha is not only mitigating diarrhoea but it is also affecting the tumor growth, invasion and metastasis negatively because of its role in cancer progression. Quercetin and its analogs can be further studied in wet laboratory in order to further cement its efficacy as TNF -alpha inhibitor.

There are many benefits to using autodocking technology in the drug discovery process. By facilitating virtual screening of compounds, it shortens the process of identifying new therapeutic candidates, saving both time and money. Autodocking improves drug design by predicting binding interactions, leading to more effective and selective medications. It also enhances our comprehension of disease mechanisms and molecular mechanisms. Autodocking improves drug candidates' characteristics through iterative tuning,

leading to better clinical outcomes. Drug discovery is sped up, rational design is made easier, and better therapies are developed all because of autodocking.

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