

<https://doi.org/10.48047/AFJBS.6.16.2024.416-432>



African Journal of Biological Sciences

Journal homepage: <http://www.afjbs.com>



Research Paper

Open Access

## Targeting 5-Fluorouracil: Revealing pathways - Related DNA Hypermethylation and Diabetes

Gayathri S V<sup>1\*</sup>, Chinmaya DS<sup>1</sup>, Sadischa Panda<sup>1</sup>, Esha Khater<sup>1</sup>, Rohit Kambala<sup>1</sup>,  
Kuruba Mohan Krishna<sup>1</sup>, Manjunatha P Mudagal<sup>1</sup>, Uday Raj Sharma<sup>1</sup>, Suresh  
Janadri<sup>1</sup>, Surendra Vada<sup>1</sup>, T Hari Babu<sup>1</sup>, Nageena Taj<sup>1</sup>, Jyotsna S Kharvi<sup>1</sup>, N K  
Thanuja<sup>1</sup>, Ranjith Muniswamy<sup>1</sup>

Acharya & BM Reddy College of Pharmacy, Acharya Post Office, Soldevanahalli, Bengaluru – 560  
107, Karnataka, India.

### Address For correspondence

Mrs. Gayathri S V  
Assistant Professor

Acharya & BM Reddy College of Pharmacy, Acharya Post Office, Soldevanahalli,  
Bengaluru – 560 107, Karnataka, India.

[gayathri\\_2296@acharya.ac.in](mailto:gayathri_2296@acharya.ac.in)

9087838181, ORCID: 0009-0006-8866-4101

Volume 6, Issue 16, Nov 2024

Received: 15 Oct 2024

Accepted: 22 Nov 2024

Published: 04 Dec 2024

[doi: 10.48047/AFJBS.6.16.2024.416-432](https://doi.org/10.48047/AFJBS.6.16.2024.416-432)

### Abstract

The study aims to explore the molecular mechanisms of 5-Fluorouracil in the development of diabetes, specifically focusing on DNA hypermethylation. Previous research has suggested that 5-FU, a chemotherapeutic drug, induces hyperglycemia in patients. A network pharmacology-based approach was used to identify target proteins for 5-FU using STITCH, ChEMBL and Cytoscape databases. Docking studies were conducted on 5-FU against target proteins. *In-vivo* studies were performed on Swiss albino mice, where the treatment group received 5-FU (20 mg/kg, intraperitoneally). Fasting blood sugar levels were measured at intervals of 4, 8, 12, 16, and 20 days. According to the network pharmacology result TXNIP, EGFR and DNMT1 were identified as validated targets for 5-FU in hypermethylation and hyperglycemia. *In-silico* docking studies revealed that 5-FU displayed a binding energy of -4.7 kcal/mol, -4.9 kcal/mol, and -5.6 kcal/mol against target proteins TXNIP, EGFR and DNMT1 respectively. In *in vivo* studies the treatment group showed a significant increase in fasting blood sugar levels compared to the normal control group. Where n=6, \*\*\*p<0.001. Histopathological analysis confirmed the pancreatic degenerative response induced by 5-FU. The study suggests that 5-FU induces DNA hypermethylation, impacting glucose regulation through proteins like TXNIP, EGFR and DNMT1.

**Key Words:** 5-Fluorouracil, DNA hypermethylation, hyperglycemia, fasting blood sugar level

## Introduction

Diabetes is a most common metabolic disease in worldwide. The incidence ratio of people affected with diabetes are increased and population itself accepting this disorder as a part of their life after certain age. (Ogle GD *et al.*, 2022) reported that type I diabetes, with an estimated global incidence of 149,500 cases with the age range 0-19 diagnosed in 2021.

(Li Y *et al.*, 2011) detailed about the occurrence of LADA (Latent Autoimmune Diabetes in Adult) which is a subgroup of type 1 diabetes, and associated with autoimmune destruction of  $\beta$ -cells. It was observed that there is a significant rise in genomic DNA methylation in CD4<sup>+</sup> T cells of LADA patients. LADA is also known as type 1.5 diabetes since this pathological condition shows symptoms of both type 1 and type 2 diabetes mellitus. Most of the patients are able to withstand while taking oral hypoglycemic drugs in first one year, later their blood sugar levels only can be controlled by insulin administration, may be due to  $\beta$  cell degeneration of pancreas results lack of secretion of insulin.

(Santos DS *et al.*, 2022) mentioned about DNA methylation, which is an epigenetic mechanism concerned with reversible changes in gene expression or silencing that do not involve changes in DNA base sequences, but this changes are inheritable. DNA methylation, which results from the incorporation of a methyl group to the cytosine carbon 5, mostly in cytosine followed by guanine (CpG) and leading to the formation of 5-methylcytosine (5mC) catalysed by DNA-methyltransferase enzymes (DNMTs).

(Ruiz, 2015; Gaine, 2018 and Yu G, 2019) explained the essential role of epigenetic regulation for maintenance of basic life process. (Sapienza, 2016 and Picascia, 2015) reported that abnormalities in epigenetic regulations leads to disorders including cancers and autoimmunity. (Cerna 2019) mentioned that, gene expression was silenced in diseased conditions as a result of progressive increases in promoter methylation of selected genes. Since DNA hypermethylation induces T cell proliferation associated with autoimmunity, this epigenetic dysregulation also can be one of the reason behind type 1 diabetes or LADA.

To reveal the association between DNA hypermethylation and hyperglycemic effect, we mainly focused upon a drug 5-Fluorouracil, a chemotherapeutic agents especially used for treating colorectal cancer. (Nyce JW *et al.*, 1997) explains how drug-induced DNA hypermethylation can contribute to both cancer development and acquired drug resistance during chemotherapy. It shows that certain chemotherapy drugs, such as cytosine arabinoside (araC), 5- fluorouracil, and methotrexate, induce hypermethylation of tumor suppressor genes,

particularly at toxic concentrations. This hypermethylation silences these genes, disrupting normal cell growth and apoptosis, allowing cancer cells to proliferate unchecked. As a result, cancer progression occurs, and these cells become resistant to chemotherapy, leading to treatment failure. (Bilian Jin *et al.*, 2011) also reviewed the role of DNA methylation in the development of cancer.

(Feng JP *et al.*, 2013), highlights the important side effect of 5-fluorouracil chemotherapy. The research found that non-diabetic patients with colorectal cancer treated with 5-FU often developed new-onset diabetes. This chemotherapy not only affected cancer but also significantly increased blood sugar levels, leading to symptoms of hyperglycemia. The study underscores the importance of closely monitoring blood glucose levels in patients undergoing 5-FU treatment to manage this side effect and help prevent diabetes.

Epigenetic modifications influence the pathogenesis of diabetes. Knowledge about epigenetic changes in diabetes is essential to find potential biomarkers for prevention, diagnosis and individualized treatment of the disease. DNA methylation is an important epigenetic mechanism. Hypermethylated genes are found to be present in patient with autoimmune disease like T1DM. 5-FU is a chemotherapeutic agent mainly used for treating colorectal cancer. The major adverse drug reaction reported for 5-FU is the increased blood sugar level or hyperglycemia. Even though 5-FU used as an anti-cancer agent, this drug is also responsible for DNA hypermethylation. Therefore our study targeting 5-FU for revealing its molecular pathways relating DNA hypermethylation and diabetes.

## Materials and Methods

### Validation of targets of 5-FU (Network Pharmacology Approach)

The network pharmacology approach utilized for the study of 5-Fluorouracil (5-FU) followed a systematic and structured methodology aimed at identifying and validating the drug's potential targets and biological pathways. The initial step involved obtaining the canonical SMILES (Simplified Molecular Input Line Entry System — O=c1cc(F)[nH]c(=O)[nH]1) notation of 5-FU from databases such as PubChem and ChEMBL (ChEMBL ID: 4854670). This notation provided a simplified representation of the molecule's structure, forming the basis for subsequent computational analysis of the chemical properties of the drug. The second step involved the identification of genes interacting with 5-FU using the STITCH database. In the third step, Venn diagram software was employed to compare the gene lists from humans

and mice to identify common genes between the two species. Among these common genes identified the genes for 5-FU associated with DNA hypermethylation and hyperglycemia by literature survey (Miller, 2023; Orofiamma, 2022 and Gunes, 2018). Identified TXNIP and EGFR that are genes influencing the respective biological pathways. Since mechanism of 5-FU behind DNA hypermethylation was not well studied, we also considered molecular pathways associated with DNMT1 protein which are the important DNA methyl transferase enzyme which regulating DNA methylation (Jin and Robertson 2011). Biological pathways related to these genes were confirmed by using Cytoscape data base. After entering the genes into Cytoscape software and analyzed them using the NDEx integrated query, we successfully identified several pathways linked to hypermethylation and hyperglycemic effects. This analysis provided a deeper understanding of the molecular mechanisms involved, highlighting specific genes and interactions that may play a critical role in these processes. After identifying the common genes the PDB (Protein Data Bank) database was used to extract their corresponding PDB IDs. The PDB IDs provided three-dimensional structures of the proteins, which were essential for visualizing and analysing protein-drug interactions.

### ***In-silico investigation on 5- Fluorouracil***

#### **3D ligand preparation**

5-FU was selected as a ligand molecule based on data obtained from network pharmacological approaches and analyzed for compatibility with the proteins TXNIP and EGFR. The 3D structures of these ligands were prepared using Chem-Draw Pro 12.0 (Mendelsohn 2004). The computed structures were subsequently optimized to reach minimal geometric configurations using PyRx 1.0 software (Dallakyan and Olson 2015). Docking studies were conducted using these minimized structures. Protein Selection and Preparation Standard protein structures were sourced from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) in the .pdb format (Sussman 1998). The proteins TXNIP, EGFR and DNMT1, identified by PDBId 4GEI, 1M14 and 3PTA respectively, were chosen for docking studies. These proteins were selected based on their x-ray diffraction resolution, absence of mutations, and Ramachandran plot criteria (Khare 2020). The protein structures were refined and optimized, structures were saved in .pdb format (Madhavi, 2013 and O'Boyle 2011). Docking Procedure Molecular docking of 5-FU with the proteins was performed using PyRx 1.0 software, which combines AutoDock 4.2 and AutoDock Vina (Dallakyan and Olson 2015). The proteins TXNIP, EGFR and DNMT1 were loaded into PyRx, and their formats were

converted to .pdbqt using the "make macromolecule" option. Each 3D ligand was uploaded individually, converted to .pdbqt format. Both ligands and proteins were selected simultaneously to determine grid parameters covering the binding pocket. Docking was conducted using default software commands, and results were visualized using Discovery Studio 4.0 software (Dassault Systèmes BIOVIA, Discovery Studio Visualizer 2020).

### **In Vivo Animal Study**

The experiment was carried out according to the guidelines of CCSEA, New Delhi, India and approved by the Institutional Animal Ethical Committee (IAEC) of Acharya & BM Reddy College of Pharmacy with approval no. Ref. IAEC/ABMRCP/2024-2025/37.

Swiss albino mice weighing 22-25 g were used for the experiments. The Animals were kept at a constant room temperature of 27 degree Celsius and fed with free access to water. Fasting blood sugar level was determined for all 12 animals, minimum of 12 hrs of fasting. Fasting blood sugar level was determined by using glucometer, consist of glucose chip, commonly known as test strip. Collected blood by cutting off 1 to 2 millimeters of the tail tip. Placed a drop of this blood on the glucose chip and recorded the baseline blood sugar level. Next day (0 day) each animals in the treatment group were administered with 20 mg/Kg of 5-FU intraperitoneal route. This was repeated for every day in the first week of study and alternative day drug administration for second and third weeks of the study. Control group was treated with normal saline in respective days. The fasting blood sugar level was determined in an intervals of 4<sup>th</sup> , 8<sup>th</sup> , 12<sup>th</sup> , 16<sup>th</sup> and 20<sup>th</sup> day until we observed elevated FBS level in treatment group. At the end of the study animals were sacrificed and pancreases were collected for histopathological examination.

## **Results and Discussion**

### **Identification of Molecular Targets for 5-FU**

The SMILES notation of 5-FU, sourced from PubChem and ChEMBL, was utilized to perform initial computational analyses. This provided a simplified molecular structure for further investigation of the drug's chemical properties and interactions. Using the STITCH database, we identified key genes interacting with 5-FU in both humans and mice.(Fig.1 and 2).This revealed the drug's mechanisms, potential therapeutic effects, and side effects, helping us understand how 5-FU affects biological pathways in both species.

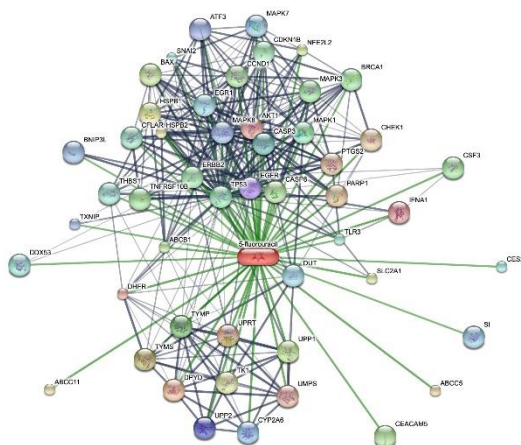


**Fig 1.** Interactions of 5-FU in human

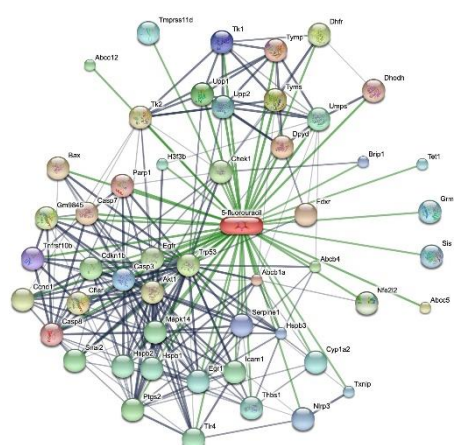
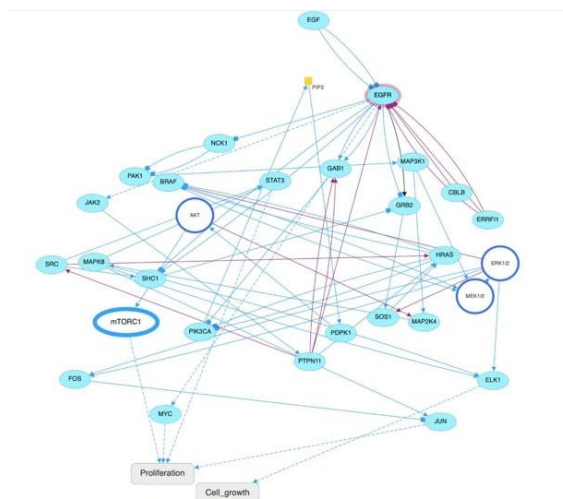


**Fig 2.** Interactions of 5-FU in mice

By comparing the gene interaction lists between humans and mice with Venn diagram software, we identified common genes. This was essential for ensuring the relevance of mouse model findings to human biology, aiding in the translation of preclinical results to clinical applications. TXNIP and EGFR were the gene identified as interlinked with 5-FU in DNA hypermethylation and hyperglycemia. Apart from that molecular pathways of 5-FU associated with gene DNMT1 also was explored while using Cytoscape data base.



**(a)**

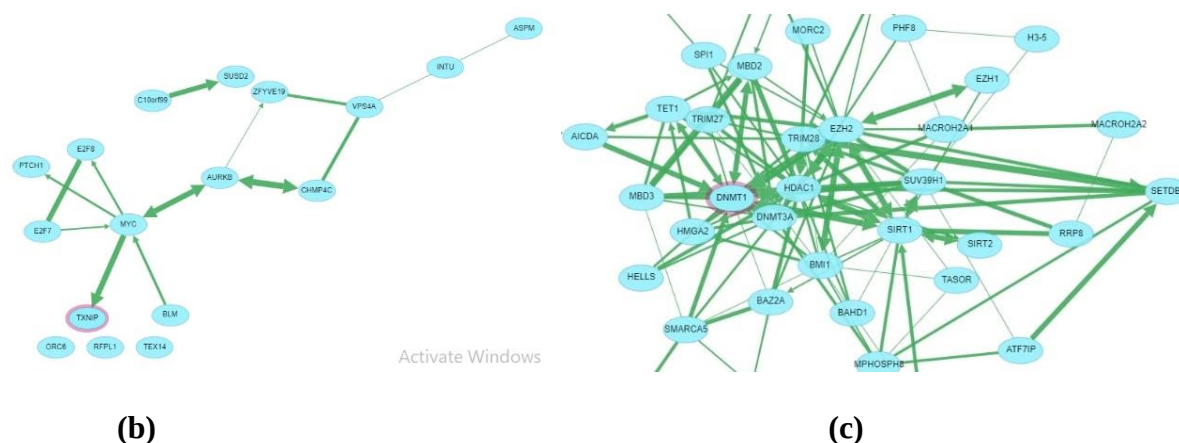


**Fig 3:** (a) Biological pathways associated with EGFR; (b) Biological pathways associated with TXNIP; (c) Biological pathways associated with DNMT – Cytoscape data base generated pathways.

Biological pathways reveals that EGFR (epidermal growth factor receptor) responsible for cell growth and survival in biological system, over expressed or mutated during cancer progression. 5-FU induced DNA hypermethylation may influence the overexpression of EGFR, which results high risk of metastasis recurrence and also develops 5-FU resistance in human. Cancer cells demands higher aerobic glycolysis for rapid proliferation by PI3k – Akt (phosphatidylinositol 3-kinase) mediated phosphorylation which results degradation of TXNIP (thioredoxin interacting protein). Gene TXNIP regulating glucose uptake in human skeletal muscle. Akt phosphorylation of TXNIP leads to dissociation of TXNIP from GLUT1 which results enhancement of glucose influx. Tumor suppressor properties of TXNIP was studied in thyroid cancer. Longer metastasis-free intervals in breast cancer patients are associated with TXNIP expression compared those with low TXNIP expression. These data implicate TXNIP as a tumor suppressor in a variety of cancers. Pharmacology network studies reveals that hypomethylation TXNIP leads to increased blood sugar level. Therefore preserving hypermethylation level of TXNIP gene may hold promise as a potential preventive tool against T2DM. The exact molecular interaction behind 5-FU and TXNIP was not clear. But biological pathways reveals that DNA hypermethylation property of 5-FU can be leads to over expression of EGFR, which causes silencing of TXNIP. Degradation TXNIP protein results 5-FU resistance in cancer patients and hyperglycemia.

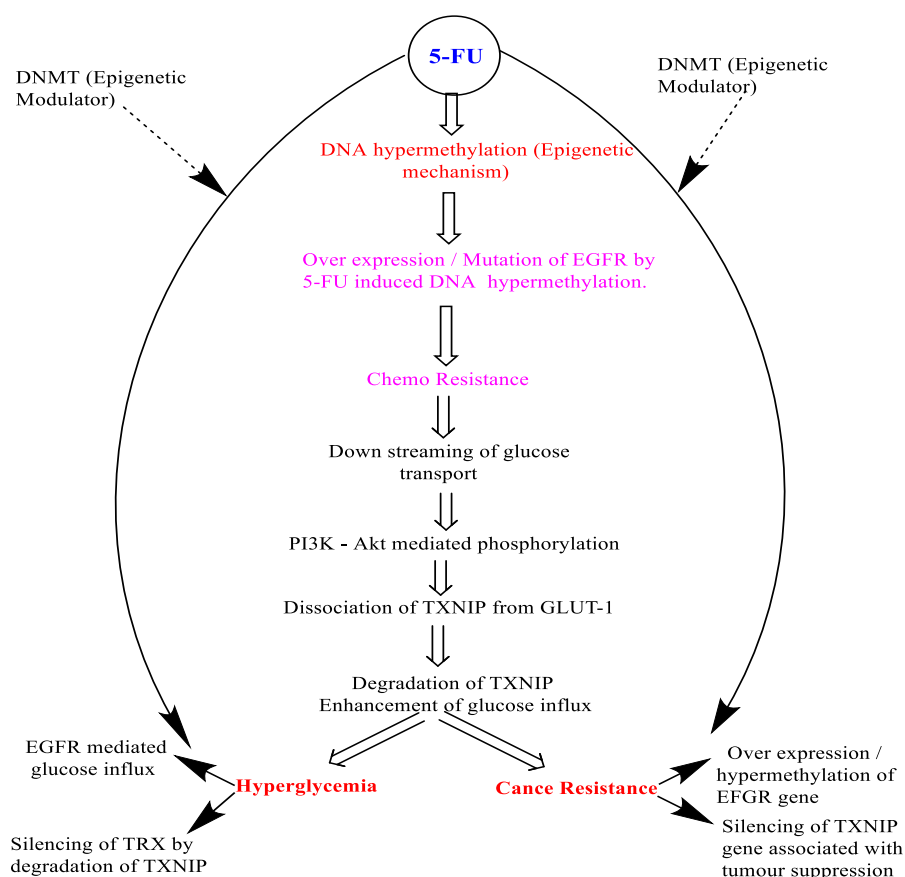


**Fig 4.** Overview of molecular pathways associated with 5-FU

### Docking Study

The outcomes of the docking studies for 5-FU is detailed in Table 1. The 2D and 3D configurations of the ligand-protein interactions were illustrated using Discovery Studio Visualizer software. Hydrogen bond analysis considered distances up to 4 Å. 5-Fluorouracil displayed a binding energy of -4.7 kcal/mol with protein TXNIP, stabilizing the docking complex by forming two hydrogen bonds with Asp831 and Glu738. The binding energy was -4.9 kcal/mol for 5-fluorouracil with the target protein EGFR, forming four hydrogen bonds with Leu78, Thr83, Pro82, and Tyr99. 5-FU displayed a highest binding energy of -5.6 kcal/mol with protein DNMT1 forming hydrogen bonds with Ser1146, Gly1150, and Leu1151.

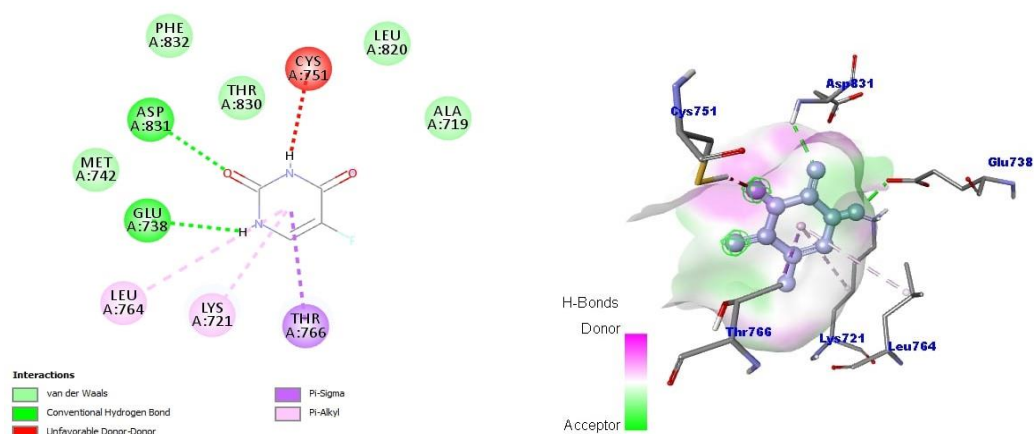


Fig. 5 2D &amp; 3D images of 5-FU with TXNIP

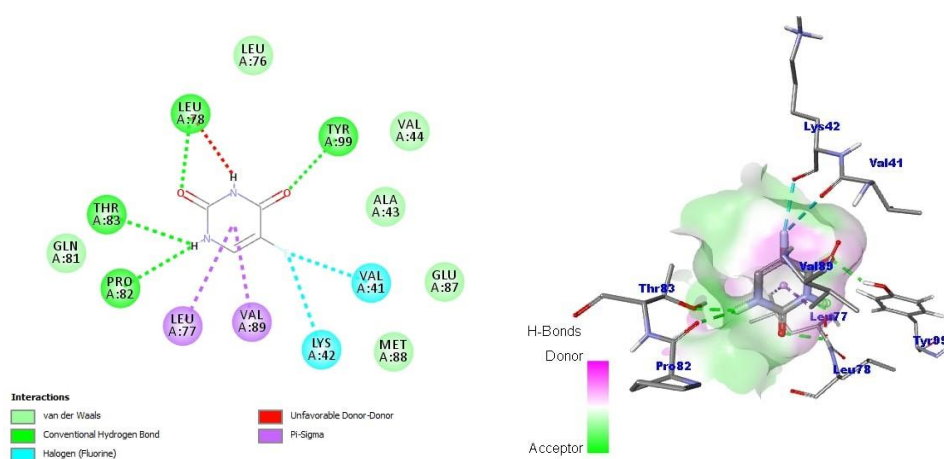


Fig. 6 2D &amp; 3D images of 5-FU with EGFR

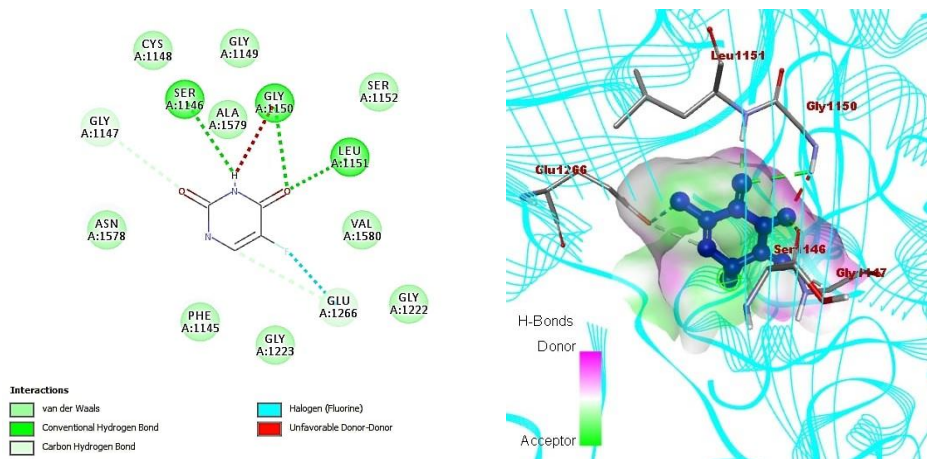


**Fig. 7** 2D & 3D images of 5-FU with DNMT1**Table 1** : Docking results of 5-FU with key target molecules

| Compound | Gene  | PDB ID | Docking Score (Kcal/mol) | Hydrogen Bond | Amino Acid Involved              |
|----------|-------|--------|--------------------------|---------------|----------------------------------|
| 5-FU     | TXNIP | 4GEI   | -4.7                     | 2             | Asp831<br>Glu738                 |
|          | EGFR  | 1M14   | -4.9                     | 4             | Leu78<br>Thr83<br>Pro82<br>Tyr99 |
|          | DNMT1 | 3PTA   | -5.6                     | 3             | Ser1146<br>Gly1150<br>Leu1151    |

### Animal Study

**Table 2:** Fasting blood sugar level (FBS Level)

| Sl. No | Group   | Drug and Route of administration | BLOOD SUGAR LEVEL (in mg/dl) |                     |                      |                      |                      |
|--------|---------|----------------------------------|------------------------------|---------------------|----------------------|----------------------|----------------------|
|        |         |                                  | 4 <sup>th</sup> day          | 8 <sup>th</sup> day | 12 <sup>th</sup> day | 16 <sup>th</sup> day | 20 <sup>th</sup> day |
| 1.     | Control | Normal saline (10 ml/Kg – PO)    | 81.50 ±0.99                  | 83.17 ±1.62         | 81.33± 2.08          | 83.17± 1.08          | 82.17± 1.48          |
| 2.     | Test    | 5FU (20 mg/Kg – IP)              | 82.83 ±0.95                  | 90.17 ±0.95         | 99.50± 1.52          | 113.70 ±2.12         | 122.70 ±2.32         |

The result was expressed as Mean $\pm$ SEM values. Where n=6, \*\*\*p<0.001; compared with control group. Data was analysed using unpaired student t test. Values ranging from p<0.05 were considered to be statistically significant compared to normal control.

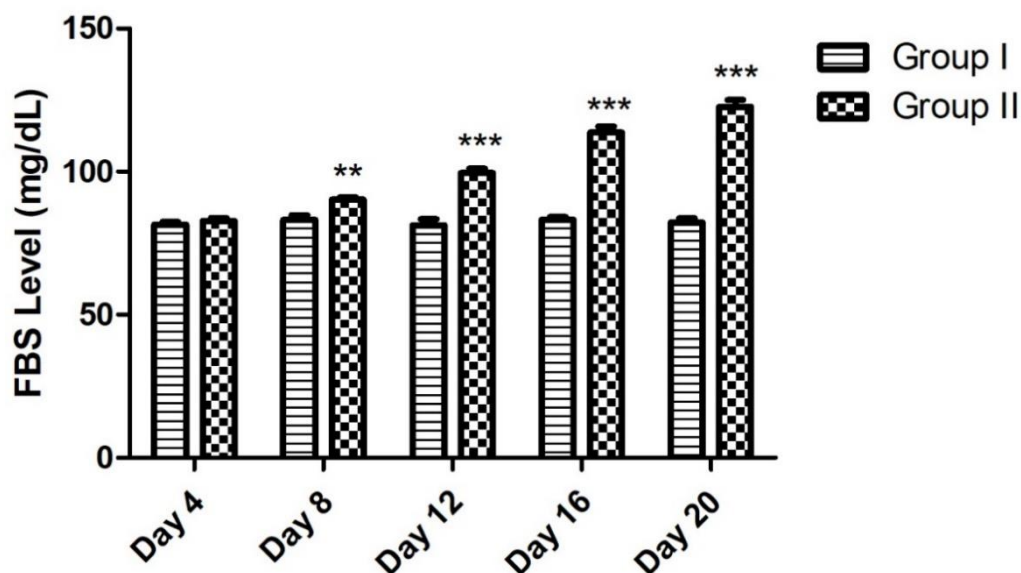


**Fig 8.** Graphical representation of fasting blood sugar level of animals

Group I: normal control- Normal saline (10 ml/Kg – PO), Group II: treatment- 5FU (20 mg/Kg –IP)

Treatment group showed significant rise in FBS level on 12<sup>th</sup>, 16<sup>th</sup> and 20<sup>th</sup> day compared to normal control group.

### Histopathology Result

Histopathological examination showed that, in mice treated with 5-FU the acinar cells were notably swollen, with small vacuoles present in nearly all of them. The interlobular ducts displayed a lining of flattened epithelial cells. There was a near-total loss of islet  $\beta$ -cells. Alterations in fatty acid composition could play a crucial role in explaining the destruction of pancreatic  $\beta$ -cells.

The pancreatic antioxidative enzyme activities (GPx and SOD) are reduced in the islet cells of diabetic animals, as  $\beta$ -cells have low antioxidant defenses and are vulnerable to oxidative stress. The pancreatic islets in these animals exhibited degeneration, with shrunken cell sizes,  $\beta$ -cell hyperplasia, and a reduction in the number of islets, indicating the presence of latent autoimmune diabetes in the mice. Normal pancreas with  $\beta$  cells and acinar cells were observed in normal control group.

**Table 3:** Histopathological examination of pancreas

| Group                           | Histopathological evaluation of Pancreas                                                                                                                                                            | Gradation/ Scoring |
|---------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------|
| Normal saline<br>(10 ml/Kg -PO) | Mice Pancreas showing islets & β cells with acinar cells - <b>normal</b>                                                                                                                            | <b>NAD+ normal</b> |
| 5FU<br>(20 mg/Kg - IP)          | Arterial fibrosis with inflammation & Congestion - moderate 3+<br>In Islets - vacuolar changes*<br>Shrunk islets with beta cell hyperplasia<br>- It is a degenerative response*- <b>moderate 3+</b> | <b>Moderate 3+</b> |

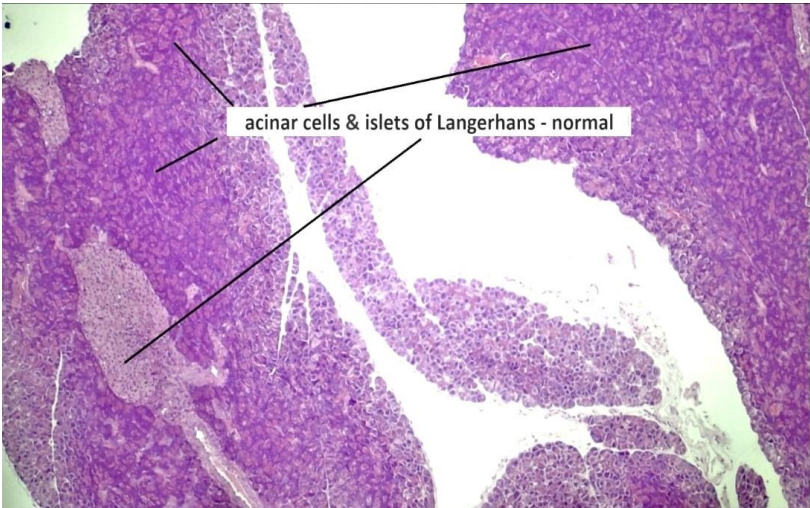


**Fig 9. Normal Control group:** Pancreas showing-normal Architecture of acinar cells & islets of Langerhans NAD + (X50)

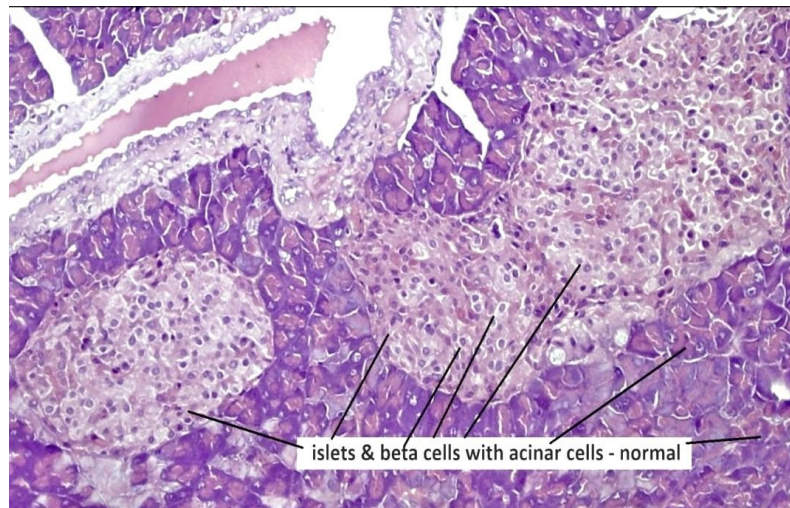


**Fig 10. Normal Control group:** Pancreas showing islets & beta cells with acinar cells normal NAD + (X100)

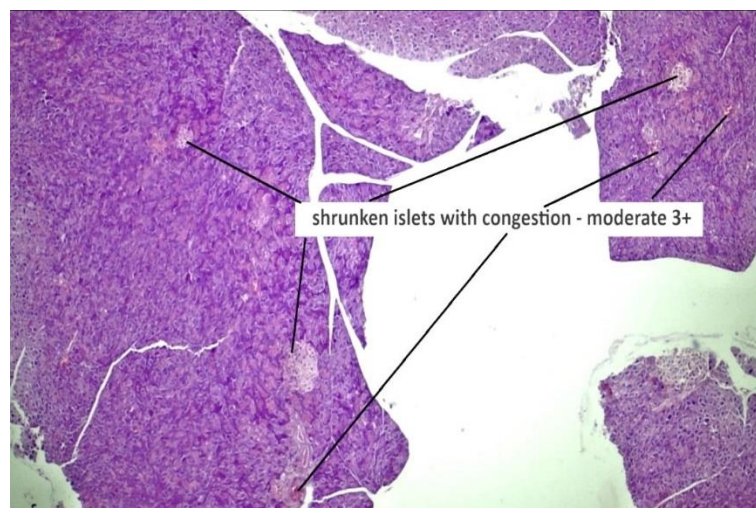


**Fig 11. 5-FU Treated – Mice Pancreas:** showing: shrunken islets with congestion - it is a degenerative response\*- moderate 3+ (X50)

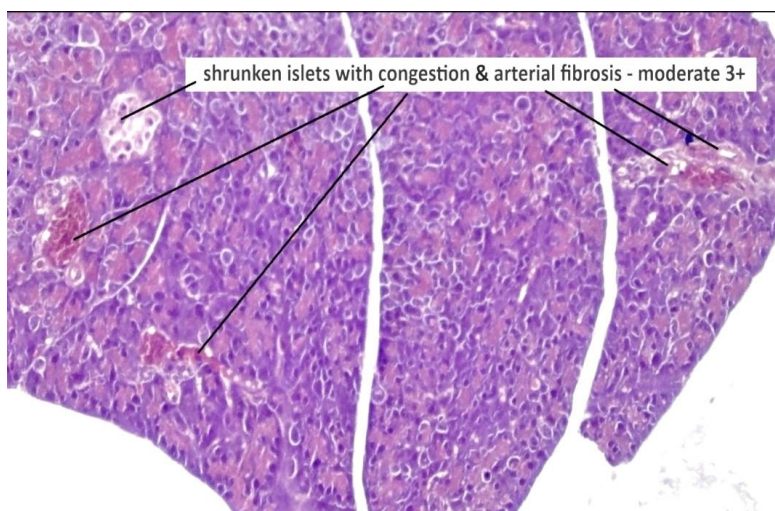


**Fig 12. 5-FU Treated – Mice Pancreas:** showing: shrunken islets with congestion & arterial fibrosis - it is a degenerative response\* - moderate 3+ (X100)

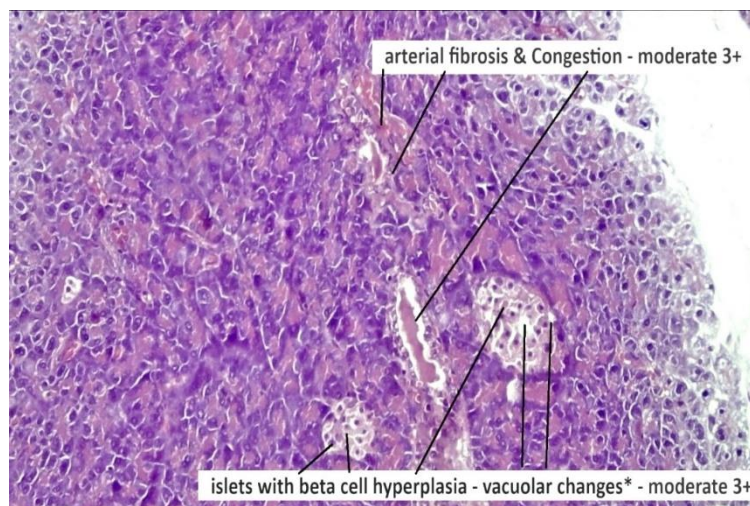


**Fig 13. 5-FU Treated – Mice Pancreas:** arterial fibrosis & Congestion with vacuolar changes\* in islets with beta cell hyperplasia - it is a degenerative response\*- moderate 3+ (X100)

This study reveals that 5-FU acting on non-cancerous animals may increase blood sugar level by DNA hypermethylation of gene EGFR followed by silencing of TXNIP proteins through DNMT enzymes. Therefore this type of hyperglycemic condition may develop LADA (Latent Autoimmune Diabetes in Adult) in patients. The extent of hyperglycemic effect needed to be compared with cancerous animals, since the expression of genes may vary in both cases. DNA sequencing/RT-PCR of samples are required for the future studies to confirm the epigenetic changes occurs in DNA of mice treated with 5-FU.

## Conclusion

The study results concluded that 5-fluorouracil induced DNA hypermethylation can be one of the etiology behind hyperglycemia. The molecular pathways of drug associated with TXNIP, EGFR and DNMT proteins are responsible for elevated blood sugar level. The molecular targets are validated by network pharmacological studies. *In silico* investigation results the interaction of 5-FU with DNMT1, which is DNA methyltransferase enzyme. Hypermethylated EGFR gene may causes silencing of TXNIP leads to increased glucose influx. Since TXNIP is a tumor suppressor gene, it can be one of the reason behind 5-FU induced cancer resistance. 5-Flurouracil induced hyperglycemia was confirmed by animal studies using Swiss albino mice. Degeneration  $\beta$  cells of pancreas in 5-FU treated animals reveals the chances of occurrence of LADA.

**Acknowledgements:** Authors are would like to thank to the management and Principal of Acharya & BM Reddy College of Pharmacy, Bengaluru, for support and providing infrastructure facility and chemicals to do the work.

**Conflicts of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## References

- Ogle GD, James S, Dabelea D (2022). Global estimates of incidence of type 1 diabetes in children and adolescents: Results from the International Diabetes Federation Atlas. *Diabetes Res Clin Pract.*183:109083.
- Li Y., Zhao M., Hou C., Liang G., Yang L., Tan Y., Wang Z., Yin H., Zhou Z., Lu Q (2011). Abnormal DNA methylation in CD4<sup>+</sup> T cells from people with latent autoimmune diabetes in adults. *Diabetes Res. Clin. Pract.* 2011.94, 242–248.
- Santos DS, Rocha MA, Mello MLS (2022). Epigenetic studies in insects and the valproic acid perspective. *Braz J Biol.* 84:e256045.
- Ruiz-Hernandez A., Kuo C.-C., Rentero-Garrido P., Tang W.-Y., Redon J., Ordovas J.M., Navas-Acien A, Tellez-Plaza M (2015). Environmental chemicals and DNA

methylation in adults: A systematic review of the epidemiologic evidence. *Clin. Epigenet.* 7, 55.

- Gaina M.E., Chatterjee S., Abel T (2018). Sleep deprivation and the epigenome. *Front. Neural Circuits.* 12, 14.
- Yu G., Wu Q., Gao Y., Chen M., Yang M (2019). The epigenetics of aging in invertebrates. *Int. J. Mol. Sci.* 20:4535.
- Sapienza C., Issa J.P (2016). Diet, nutrition, and cancer epigenetics. *Annu. Rev. Nutr.* 36,665–681.
- Picascia A., Grimaldi V., Pignalosa O., De Pascale M.R., Schiano C., Napoli C (2015). Epigenetic control of autoimmune diseases: From bench to bedside. *Clin. Immunol.* 157, 1–15.
- Cerna M. Epigenetic Regulation in Etiology of Type 1 Diabetes Mellitus (2019). *Int J Mol Sci.* 21(1), 36.
- Nyce JW (1997) Drug-induced DNA hypermethylation: a potential mediator of acquired drug resistance during cancer chemotherapy. *Mutat Res.* 386(2),153-161.
- Jin B, Li Y, Robertson KD (2011). DNA methylation: superior or subordinate in the epigenetic hierarchy? *Genes Cancer.* 2(6), 607-17.
- Feng JP, Yuan XL, Li M (2013). Secondary diabetes associated with 5-fluorouracil based chemotherapy regimens in non-diabetic patients with colorectal cancer: results from a single-centre cohort study. *Colorectal Dis.* 15(1), 27-33.
- Miller RG, Mychaleckyj JC, Onengut-Gumuscu S, Orchard TJ, Costacou T (2023). TXNIP DNA methylation is associated with glycemic control over 28 years in type 1 diabetes: findings from the Pittsburgh Epidemiology of Diabetes Complications (EDC) study. *BMJ Open Diabetes Res Care.* 11(1).
- Orofiamma LA, Vural D, Antonescu CN (2022). Control of cell metabolism by the epidermal growth factor receptor. *Biochim Biophys Acta Mol Cell Res.* 1869(12), 119359.
- Gunes A, Bagirsakci E, Iscan E, Cakan-Akdogan G, Aykutlu U, Senturk S, Ozhan G, Erdal E, Nart D, Yilmaz Barbet F, Atabey N (2018). Thioredoxin interacting protein promotes invasion in hepatocellular carcinoma. *Oncotarget.* 9(96), 36849-36866.
- Jin B, Li Y, Robertson KD (2011). DNA methylation: superior or subordinate in the epigenetic hierarchy? *Genes Cancer.* 2(6), 607-17.

- Mendelsohn LD (2004). ChemDraw 8 ultra, windows and macintosh versions. *J Chem Inf Comput Sci.*1;44(6), 2225–6.
- Dallakyan S, Olson AJ (2015). Small-molecule library screening by docking with PyRx. In: Chemical biology: methods and protocols, methods in molecular biology, p. 243–50.
- Sussman JL, Lin D, Jiang J, Manning NO, Prilusky J (1998). Protein Data Bank (PDB): database of three-dimensional structural information of biological macromolecules. *Int Union Crystallogr.*1078–84.
- Khare N, Jha AK (2020). Structure based docking of secondary metabolites against DrpE1 to treat tuberculosis. *Int J Res Appl Sci Eng Technol.* 8.
- Madhavi Sastry G, Adzhigirey M, Day T, Annabhimoju R, Sherman W (2013). Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. *J Comput Aided Mol Des.* 27(3), 221–34.
- O’Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR (2011). Open Babel: an open chemical toolbox. *J Cheminf.* 3(33), 1–14.
- Dassault Systèmes BIOVIA, Discovery Studio Visualizer, v20.1.0.19295, San Diego: Dassault Systèmes, 2020.