

TARGETING BREAST CANCER USING HYDROCORTISONE AND ITS IMPACT ON INFLAMMATORY GENE EXPRESSIONS AND LIPOGENESIS- An *In Silico* and *In Vitro* Study

Submitted in partial fulfillment of the requirement for the award of
Bachelor of Technology Degree in Biotechnology.

By

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SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **Juana Darlene Virgit J (40230017)** who carried out the project entitled "**TARGETING BREAST CANCER USING HYDROCORTISONE AND ITS IMPACT ON INFLAMMATORY GENE EXPRESSIONS AND LIPOGENESIS- An *In Silico* and *In Vitro* Study**" under the supervision of **Dr. M. Bavanilatha** from **October 2023 to March 2024**.

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I, **JUANA DARLENE VIRGIT J (40230017)** hereby declare that the project report entitled “**TARGETING BREAST CANCER USING HYDROCORTISONE AND ITS IMPACT ON INFLAMMATORY GENE EXPRESSIONS AND LIPOGENESIS- AN *IN SILICO* AND *IN VITRO* STUDY**” done by me under the guidance of **Dr. M.BAVANILATHA** at the **Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai-119** and the **International Research Centre (IRC) Sathayabama Institute of Science and Technology, Chennai-119** is submitted in partial fulfillment of the requirements for the award of **Bachelor of Technology Degree in Biotechnology**.

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ABSTRACT

Breast cancer remains a significant global health burden, necessitating the exploration of novel therapeutic strategies. This study investigates the potential of hydrocortisone in targeting breast cancer by modulating inflammatory gene expressions and lipogenesis. In vitro experiments using breast cancer cell lines revealed a significant reduction in the expression of key inflammatory markers, including NF- κ B, TNF- α , and IL-6, following hydrocortisone treatment. Concurrently, hydrocortisone treatment led to a substantial downregulation of FASN expression, implicating a disruption in lipid metabolism within breast cancer cells. Although the reduction in SREBP-1 expression was not as pronounced, it is reasonable to anticipate further suppression with prolonged drug exposure, suggesting a time-dependent effect of hydrocortisone on lipogenic pathways. Additionally, in-silico molecular docking studies confirmed favourable binding affinities between hydrocortisone and the p52 subunit of NF- κ B, as well as between hydrocortisone and SREBP-1, providing insights into the direct interaction between hydrocortisone and key regulators of inflammatory and lipogenic pathways. These findings underscore the potential of hydrocortisone as a therapeutic agent in breast cancer management, offering a promising approach to mitigate inflammation and aberrant lipid metabolism, two critical drivers of breast cancer progression.

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LIST OF ABBREVIATIONS

AO	Acridine Orange
ANOVA	Analysis Of Variance
CSC	Cancer stem cells
CO ₂	Carbon di-oxide
cDNA	Complementary DNA
C _t	Cycle threshold
DNA	Deoxyribonucleic acid
DMSO	Dimethyl sulfoxide
DMEM	Dulbecco's Modified Eagle Medium
EMT	Epithelial-to-mesenchymal transition
EB	Ethidium Bromide
FASN	Fatty Acid Synthase
FBS	Foetal bovine serum
FDA	Food and Drug Administration
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
IC ₅₀	Half maximal inhibitory constant
MRI	Magnetic Resonance Imaging
MSC	Mesenchymal stem cells
µg	Micro gram
µL	Micro litre
mg	Milli gram
MMLV	Moloney Murine Leukaemia Virus

NCCS	National Centre for Cell Science
NF- κ B	Nuclear Factor Kappa-B
OD	Optical density
PBS	Phosphate buffered saline
qRT-PCR	Quantitative Real Time Polymerase Chain Reaction
RNA	Ribonucleic Acid
SREBP-1	Sterol regulatory element binding protein 1
TBE	Tris Borate EDTA
TNF- α	Tumour Necrosis Factor-alpha
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide

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CHAPTER 1

INTRODUCTION

Cancers originating from the breast tissues, particularly in the inner lining of the milk ducts in the mammary glands and the lobules that supply the ducts with milk are collectively referred to as 'Breast cancer' (Sharma, G.N., *et al.* 2010) Worldwide, an estimated 25% of all women with cancer are diagnosed with breast cancer every year. In India, 1.38 million cases of breast cancer are diagnosed every year. Of these, 0.45 million people succumb to the disease. Every four minutes, a woman is diagnosed with breast cancer, and every 15 minutes, a woman dies from breast cancer in India. Breast cancer is incurable mostly because it is a metastatic cancer that frequently spreads to distant organs such the liver, brain, lung, and bone. Early diagnosis and detection can lead to a favourable prognosis and a high likelihood of survival . One widely used screening approach for early breast cancer detection is mammography , and it has been proved to effectively reduce mortality rates. Other screening techniques have also been used and researched, including MRI, which is more sensitive than mammography .

The main theme behind this study is drug repurposing. Drug repurposing – as the name suggests, is a process that involves identifying new applications for approved or unsuccessful/abandoned compounds in the treatment of different diseases. The need for drug repurposing arose due to the significant increase in the need for varied strategies which aim to safely bring new drugs to the market rapidly and cost-effectively (Pulley *et al.*, 2020). By avoiding the difficulties of de novo drug creation, such as safety evaluation and regulatory processes, a successful drug repurposing application can offer novel therapy protocols for a wide range of pathological illnesses.

Hydrocortisone is the name given to the hormone naturally produced by the adrenal glands called 'cortisol' when given as a medication(Becker KL 2001). It is a naturally produced corticosteroid commonly prescribed for a spectrum of autoimmune conditions, ranging from dermatitis to rheumatoid arthritis since its ability to modulate the body's immune response is well-established. In this study, we try to explore the potential of hydrocortisone as an anti-breast cancer agent, leveraging its anti-inflammatory attributes. Inflammation stands as a significant hallmark in the

progression of numerous cancers, including breast cancer. Therefore, the primary objective of this study is to investigate whether hydrocortisone's anti-inflammatory properties can be harnessed to suppress inflammation within breast cancer cell lines by assessing the expression of few inflammatory mediators, namely Nuclear Factor Kappa-B (NF- κ B), Tumour Necrosis Factor-alpha (TNF- α), and Interleukin-6 (IL-6), known to orchestrate inflammation within the cancer microenvironment. NF- κ B, a transcription factor intricately linked with inflammatory pathways, plays a multifaceted role in cancer development (Balkwill, 2006), influencing processes such as cell proliferation, apoptosis, and immune response. Moreover, it governs the transcription of key cytokines like TNF- α and IL-6, whose dysregulation causes chronic inflammation, conducive to tumorigenesis. TNF- α is a member of the TNF/TNFR cytokine superfamily. Its overexpression fuels chronic inflammation leading to cancer progression and hence is being investigated as a possible target for cancer treatment (Heikkilä, Ebrahim and Lawlor, 2008) . IL-6 is known to cause chronic inflammation, creating a favourable microenvironment for tumour growth thus playing a major role in the development of cancer (Heikkilä, Ebrahim and Lawlor, 2008). Additionally, this study explores the interplay between hydrocortisone and lipogenesis, a process intricately linked with breast cancer progression. Sterol regulatory element-binding protein 1 (SREBP-1) is A transcription factor of lipogenic genes like FASN and ACC, is often found to be overexpressed in breast cancer cell lines. Therefore, this study aims to check the ability of hydrocortisone to reduce the expression of SREBP-1 (Bao et al., 2016). Fatty Acid Synthase (FASN) is an enzyme involved in the De-novo synthesis of fatty acids. Its upregulation is common in most cancers including breast carcinomas. Overexpression of FASN is a universal hallmark of most human cancers as FASN catalysed lipogenesis aid in the growth and survival of cancer cells (J. Fernandez Menendez and Lupu, 2017). Therefore, we aim to study the ability of hydrocortisone to reduce the expression of FASN in the breast cancer cell line.

Thus, by amalgamating various computational approaches and *In Vitro* experimentations, this study aims to delve into the investigation of the potential of hydrocortisone to be a promising anti-breast cancer agent by checking its potential to target inflammation and lipogenesis – the major hallmarks of breast cancer.

CHAPTER 2

LITERATURE SURVEY

2.1 BREAST CANCER

Cancers originating from the breast tissues, particularly in the inner lining of the milk ducts in the mammary glands and the lobules that supply the ducts with milk are collectively referred to as 'Breast cancer' (Sharma, G.N *et al.*, 2010) . Breast tumours typically originate from excessive cell growth in the ducts, progressing from benign growths to potentially metastatic cancers due to continuous exposure to various cancer-causing factors (Maffini *et al.*, 2004). Breast cancer is incurable mostly because it is a metastatic cancer that frequently spreads to distant organs such the liver, brain, lung, and bone. Early diagnosis and detection can lead to a favourable prognosis and a high likelihood of survival (DeSantis *et al.*, 2015). One widely used screening approach for early breast cancer detection is mammography , and it has been proved to effectively reduce mortality rates. Other screening techniques have also been used and researched, including MRI, which is more sensitive than mammography (Drukteinis *et al.*, 2013). Common risk factors of breast cancer include sex, age, oestrogen, family history, gene mutations and unhealthy lifestyle,(Sun *et al.*, 2017). Breast cancer is known to occur 100 times more commonly in women than in men (Sun *et al.*, 2017a) . Another significant risk factor for breast cancer, apart from gender, is age. Research indicates that breast cancer is more common among women aged 40 and older, with higher prevalence rates seen in those over 60. This underscores the importance of mammography screening for women aged 40 and above (Siegel *et al.*, 2017).

Approximately one fourth of breast cancer cases have a hereditary component. Women with a family history of breast cancer, particularly if their mother or sister has been affected, are at an increased risk of developing the disease. This heightened risk is partly due to mutations in genes associated with breast cancer, specifically BRCA1 and BRCA2 (Brewer *et al.*, 2017). Factors such as early onset of menstruation, late onset of menopause, delayed first pregnancy, and low number of pregnancies can elevate the risk of breast cancer (Washbrook, 2006) (Horn & Vatten, 2017). Contemporary lifestyles, characterized by excessive alcohol consumption and high intake of dietary fats, also contribute to an increased likelihood of developing breast cancer (Makarem *et al.*, 2013). To date, mounting evidence suggests that smoking, particularly starting at a young age, significantly increases the risk of developing breast cancer (Catsburg *et al.*, 2014) (Gaudet *et al.*, 2016). According to a meta-analysis of 53 epidemiological studies, consuming 35-44 grams of alcohol per day can elevate the risk of breast cancer by 32%. Additionally, for each extra 10 grams of alcohol consumed

daily, there's a 7.1% increase in the relative risk of developing breast cancer ("Alcohol, Tobacco and Breast Cancer – Collaborative Reanalysis of Individual Data from 53 Epidemiological Studies, Including 58 515 Women with Breast Cancer and 95 067 Women without the Disease," 2002).

2.2 INFLAMMATION IN CANCER

Even with notable advancements in therapy, breast cancer continues to prevail as the predominant cancer among women in industrialized nations. The notion of the immune system's potential to regulate cancer has sparked debate for over a century. It is now widely acknowledged that the immune system can both impede and facilitate tumour growth, a concept encapsulated by the process of immunoediting, which unfolds in three phases: elimination, equilibrium, and escape.(Schreiber *et al.*, 2011; Vesely *et al.*, 2011). Elimination is achieved through identifying and destructing nascent transformed cells by acute tumour-inhibiting inflammation, characterized by infiltration of effector cells of the innate and adaptive immune system and the production of tumour-inhibiting cytokines. The escape phase is sustained by chronic tumour-promoting inflammation, which primarily involves immunosuppressive cells and soluble factors (Vesely *et al.*, 2011).

Evading immune destruction is now recognized as a hallmark of cancer (Hanahan and Weinberg, 2011) Persistent inflammation has been associated with multiple stages of tumour development, encompassing cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis (Mantovani, 2005). Inflammatory conditions within tumour microenvironments are marked by the presence of host leukocytes, found both in the supportive stroma surrounding tumours and within tumour regions themselves . In breast cancer, inflammation serves as a critical driver of tumour progression and metastasis, modulating various aspects of the disease pathology (Balkwill *et al.*, 2001). The tumour microenvironment, enriched with inflammatory cells and cytokines, orchestrates a pro-inflammatory milieu conducive to cancer cell survival and proliferation (Coussens *et al.*, 2013).Initially, the presence of pre-existing inflammation or infection was not recognized as a potential risk factor for breast cancer development. However, current understanding acknowledges that the infiltration of leukocytes can either inhibit or facilitate the progression of breast cancer (Coussens and Pollard, 2011).

2.3 GENES CAUSING INFLAMMATION IN CANCER

2.3.1 *NF-κB*

NF-κB represents a family of transcription factors that aid in the transcription of a wide variety of genes involved in the immune and inflammatory responses (Oeckinghaus and Ghosh, 2009). The family includes 5 structurally related subunits namely, NF-κB1 (also named p50), NF-κB2 (also named p52), RelA (also named p65), RelB and c-Rel. These subunits mediate the transcription of inflammatory genes by binding to the κB enhancer (Sun, Chang and Jin, 2013). These subunits are normally present in the cytoplasm and remain inactive by a family of inhibitory proteins called the IκB (Inhibitors of κB) (Sun, 2010). Activating NF-κB requires two key signalling pathways called the canonical and the non-canonical pathways. These pathways are known to be crucial for regulating immune and inflammatory responses (Vallabhapurapu and Karin, 2009). The canonical pathway is known to respond to diverse stimuli, like the ligands of various cytokine receptors, pattern recognition receptors (PRRs), TNF receptor (TNFR) superfamily members, as well as T-cell receptor (TCR) and B-cell receptor (Zhang and Sun, 2015).

The activation of the canonical pathway of NF-κB involves the degradation of IκBα through phosphorylation by IκB kinase (IKK) complex. IKK is activated by numerous stimuli, including cytokines, growth factors, mitogens, microbial components and stress agents (Karin and Delhase, 2000). Post activation, IKK phosphorylates IκBα at two N-terminal serines and, thereby, triggers ubiquitin-dependent IκBα degradation in the proteasome, resulting in the rapid translocation of the canonical NF-κB members (the p50/RelA and p50/c-Rel dimers) to the nucleus (Israel, 2009). The canonical NF-κB pathway is involved in almost all aspects of immune responses, while the non-canonical pathway cooperates with the canonical pathway in regulating the functions of the adaptive immune system (Sun, 2010). The major function of NF-κB has to be the regulation of inflammatory responses, followed by the mediation of transcription of proinflammatory genes in the innate immune cells (Vallabhapurapu and Karin, 2009). Any dysregulation in the activation of NF-κB results in chronic inflammation which is a hallmark for chronic inflammatory diseases like cancer. Hence it becomes essential to better understand the underlying mechanism

of the activation of the NF- κ B pathways to develop targeted therapeutic strategies for inflammatory diseases (Liu *et al.*, 2017).

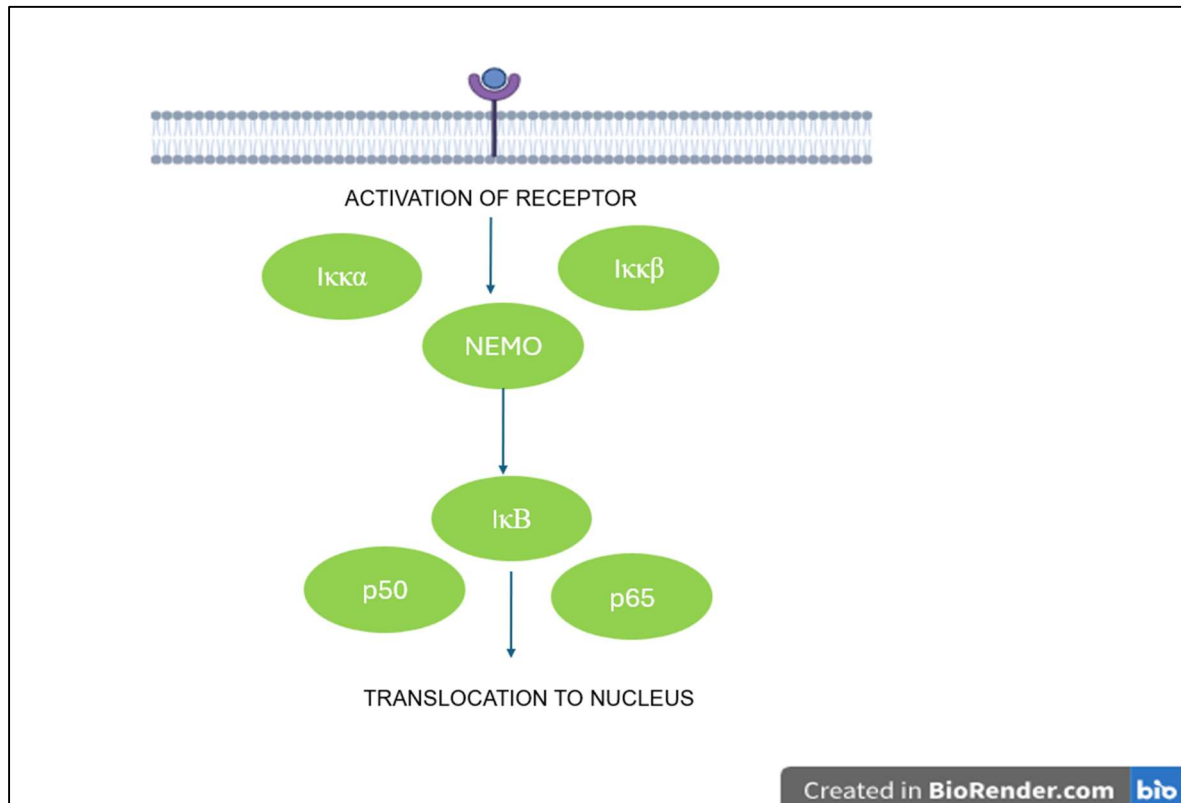


FIGURE 2.1 : CANONICAL PATHWAY OF NF- κ B

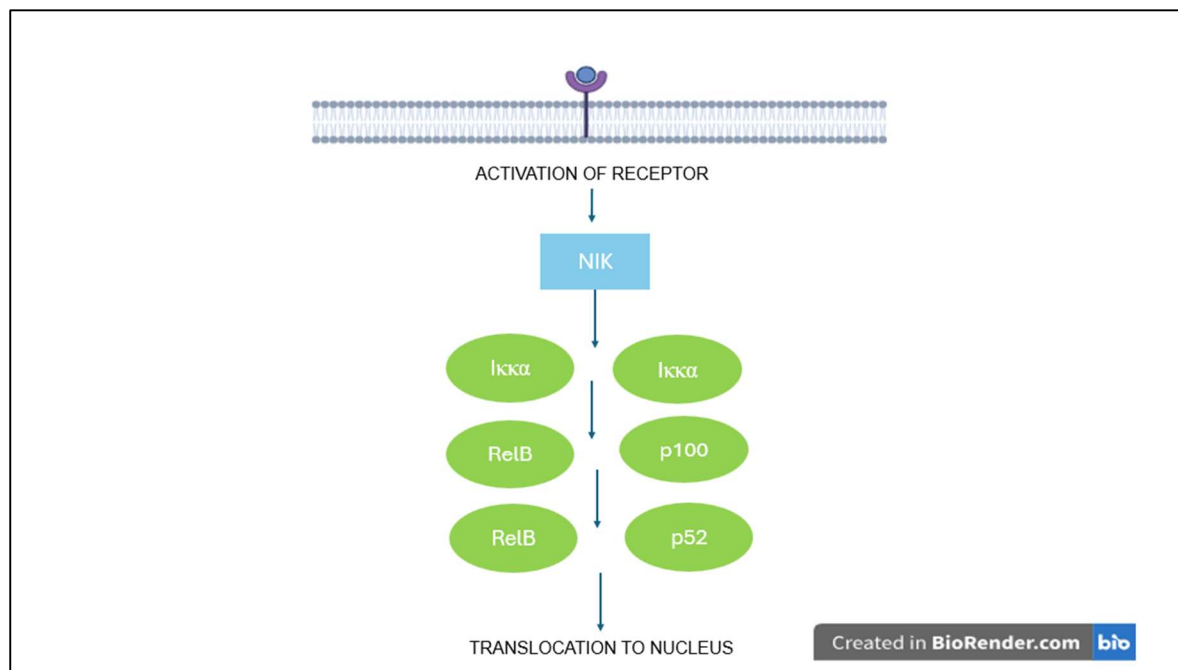


FIGURE 2.2 : NON- CANONICAL PATHWAY OF NF- κ B

2.3.1.1 ROLE OF NF- κ B IN CANCER

The activation of NF- κ B is found to be enhanced in most cancers including breast cancer. This enhancement is often due to the increased levels of cytokines like TNF α and interleukins in the tumour microenvironment (DiDonato *et al.*, 2012). An important function of NF- κ B is the regulation of cell survival. In cancer, the activation of NF- κ B leads to impaired apoptosis (Joyce *et al.*, 2001). In addition, NF- κ B is also known to regulate cell proliferation by activating genes that regulate cell proliferation (Kiraly *et al.*, 2015). In inflammatory immune cells such as macrophages and neutrophils, the activation of NF- κ B activates the expression of inflammatory cytokines such as TNF α , IL-1 β , and IL-6, thereby promoting the proliferation of malignant cells and tumour stromal cells (Kiraly *et al.*, 2015). It is widely acknowledged that NF- κ B is crucial in controlling both tumour cell growth and programmed cell death, with its roles and mechanisms being diverse and intricate.

NF- κ B is known to play a crucial role in tumorigenesis through various mechanisms like promoting tumour occurrence and progression by inducing DNA damage, chromosomal instability, and mutations (Pikarsky *et al.*, 2004a). Additionally, NF- κ B prevents apoptosis of DNA-damaged cells, leading to the accumulation of oncogenic mutations (Gudkov *et al.*, 2011). Furthermore, NF- κ B activation by viral infections such as HBV and EBV contributes to tumorigenesis through chronic inflammation and by encoding factors that stimulate NF- κ B activity (Gudkov *et al.*, 2011). Moreover, NF- κ B enhances tumour angiogenesis, epithelial-mesenchymal transition (EMT), and metastasis by regulating the expression of angiogenesis-related genes, EMT molecules, and metastasis-related genes (Wu, 2012b). NF- κ B also plays a central role in shaping the tumour microenvironment by regulating the expression of cytokines, chemokines, and inflammatory mediators from various cell types including tumour cells, stromal cells, and immune cells (DeBerardinis, 2020). It contributes to the production of tumour-promoting cytokines such as TNF α , IL-6, IL-1 α/β , IL-17A, and TGF- β , which foster inflammation, and tumour progression (Balkwill, 2009). NF- κ B also drives the expression of chemokines, recruiting immune cells and exacerbating the inflammatory response, while also promoting tumour growth and metastasis (Ammirante *et al.*, 2014). Overall, targeting NF- κ B in the tumour microenvironment holds promise as a potential therapeutic strategy (Grivennikov *et al.*, 2010).

2.3.1.2 THERAPEUTIC APPLICATION OF NF- κ B IN CANCER

The therapeutic application of NF- κ B in cancer treatment involves inhibiting its activation using various inhibitors, targeting either core pathway components or upstream/downstream molecules. Drugs like bortezomib, carfilzomib, and ixazomib are proteasome inhibitors used to treat multiple myeloma by preventing I κ B degradation and inhibiting NF- κ B activity (Obeng *et al.*, 2006). However, directly targeting NF- κ B has shown limited success in other cancers besides multiple myeloma and certain DLBCL subtypes, suggesting its complex and context-dependent role in tumorigenesis (Phelan *et al.*, 2018). Combining NF- κ B inhibitors with other treatments, such as chemotherapy or immunotherapy, or using multiple NF- κ B inhibitors in combination, shows promise in enhancing therapeutic efficacy (Wang *et al.*, 1997). Clinical trials targeting the NF- κ B pathway in cancer treatment are ongoing, reflecting the interest in exploiting NF- κ B as a potential therapeutic target (Dash *et al.*, 2020).

2.3.2 TNF- α

TNF- α , belonging to the TNF/TNFR cytokine superfamily, plays a role in the immune system regulation, inflammation, and defence mechanisms within the body. Although TNF- α is essential for both innate and adaptive immunity, excessive levels of this cytokine can lead to chronic inflammation, overall deterioration of health, and in cases of acute elevation, septic shock (Iocca & Isom, 2003). The release of pre-formed TNF- α functions as a positive autocrine feedback signal, stimulating the activation of NF- κ B. This activation, in turn, induces the production of additional TNF- α along with other cytokines like GM-CSF and IL-8 (Tsenova *et al.*, 1999). TNF- α plays a complex role in tumorigenesis, with evidence suggesting its involvement in tumour initiation through various mechanisms. Studies in mouse models have shown that TNF- α production by different cell types, including epithelial cells and stromal cells, contributes to tumour development in skin, liver, and other tissues (Keane *et al.*, 2001). TNF- α appears to promote tumorigenesis by facilitating the survival and proliferation of initiated cells, as well as by inducing inflammation and cell death resistance in the tumour microenvironment, potentially through NF- κ B activation. Elevated TNF- α levels in the serum of cancer patients, particularly those with advanced disease, have been observed in various cancers such as

prostate, renal cell, chronic lymphocytic leukaemia (CLL), metastatic breast cancer, and pancreatic cancer, correlating with disease severity and poor survival outcomes (Orosz *et al.*, 1995). Cancer cell lines also secrete TNF- α along with other inflammatory cytokines, contributing to constitutive NF- κ B activity and promoting tumour progression (Orosz *et al.*, 1995). Additionally, malignant cells exhibit differences in TNF- α mRNA stability compared to normal cells, with TNF- α production being at least partially responsible for IL-6 production in ovarian cancer cells (Pasparakis *et al.*, 1997). TNF- α production by cancer cells, even at low levels, plays a significant role in cancer progression by various mechanisms. It can induce DNA damage, promote anti-apoptotic or mitogenic activity, mediate tumour-stromal cell interactions, and induce the production of MMPs, cytokines, and chemokines that contribute to tumour development (Raza *et al.*, 2004). Additionally, TNF- α can synergize with growth factors like TGF- β and EGF to further enhance tumour progression (Szlosarek & Balkwill, 2003).

2.3.3 IL-6

IL-6 is a versatile inflammatory cytokine with multiple functions. Initially identified as a factor promoting the growth of B-cells, it is produced by various cell types, such as T-cells, macrophages, and stromal cells, upon stimulation by TNF- α and IL-1 (Hortobagyi, 2000). The activation of IL-6 in turn activates Janus kinases (JAK), signal transducers and activators of transcription (STATs). These are known to regulate cell proliferation and apoptosis (Dranoff, 2004). High concentrations of IL-6 are known to be associated with cancer due to its regulation of chronic inflammation setting up a suitable microenvironment for the progression of cancer (Brocke-Heidrich *et al.*, 2004). IL-6 is also known to play a role in the resistance towards apoptosis, by the induction of anti-apoptotic proteins like (BCL-2, BCL-X1, and MCL-1) that inhibit apoptosis (Leu *et al.*, 2003). Studies of IL-6 in breast cancer cell line MCF-7 revealed high levels of IL-6 (Conze *et al.*, 2001). Besides apoptosis, IL-6 is also found to promote breast cancer cell motility thereby playing a critical role in metastasis (Arihiro *et al.*, 2000).

2.3.3.1 ROLE OF IL-6 IN CANCER

The ability of IL-6 to be pro-tumorigenic are elicited by its effects on both intrinsic (for example, cell proliferation, survival, migration, invasion

and metastasis) and extrinsic (modulation of inflammation, immunosuppression, and angiogenesis) cancer cell activities thus shaping the tumour microenvironment (Ben-Baruch, 2002). IL-6 is also an important regulator of energy metabolism which is a hallmark for the initiation and progression of cancer (Flint *et al.*, 2016).

2.3.3.2 ROLE OF IL6 IN BREAST CANCER METASTASIS

IL-6 plays a crucial role in breast cancer metastasis by contributing to various processes associated with cancer cell dissemination and invasion. Studies have linked IL-6 to metastasis through multiple mechanisms, including epithelial-to-mesenchymal transition (EMT), increased cell invasion and migration, and the recruitment of mesenchymal stem cells (MSC) (Heinrich *et al.*, 2003). EMT, a process associated with embryonic development, is implicated in metastatic growth, and IL-6 has been shown to participate in EMT in human breast cancer cells (Chehboun *et al.*, 2017). This involvement in EMT is driven by embryonic signalling pathways and their downstream transcription factors, resulting in the transformation of epithelial-like cancer stem cells (CSC) into cells with a more aggressive mesenchymal-like phenotype. Additionally, IL-6 exposure has been demonstrated to induce EMT phenotype markers in breast cancer cells. Loss of the membrane adhesive protein E-cadherin, a critical step in EMT, leads to increased cell motility, and IL-6 has been shown to decrease cell adhesion in ER-positive breast cancer cell lines. Furthermore, IL-6 mediates the interaction between MSCs and CSCs, promoting tumour growth and facilitating metastatic dissemination (Taniguchi *et al.*, 2015). Breast cancer cells secrete IL-6 under hypoxic conditions, activating and attracting MSCs, which can be attenuated by blocking IL-6 signalling (Taniguchi *et al.*, 2015). Similarly, breast cancer cells incubated with media obtained from MSCs plus antibodies against IL-6 could strongly inhibit the migration of breast cancer cells induced by MSC conditioned media (Taniguchi *et al.*, 2015).

2.3.3.3 THERAPEUTIC STRATEGIES TARGETING IL-6

Therapeutic strategies targeting IL-6 cytokine family members encompass various approaches, including blocking monoclonal antibodies acting on the cytokine or its receptor, recombinant cytokine regimes, and small-molecule therapies interfering with cytokine receptor signalling via gp130 and the JAK–STAT

pathway (Dinarello, 2007). IL-6, in particular, has been a focus due to its involvement in various inflammatory conditions. Monoclonal antibodies such as tocilizumab and sarilumab, targeting IL-6 or its receptor, have shown efficacy in treating inflammatory arthritis, juvenile idiopathic arthritis, and other diseases (Choy *et al.*, 2013). Understanding IL-6's role in immune homeostasis is crucial for optimizing therapeutic outcomes (Hunter & Jones, 2015).

Moreover, IL-6-directed therapies have been explored in cancer treatment, albeit with mixed clinical responses in trials. Despite limited success in cancer treatment, IL-6-targeted therapies like tocilizumab have demonstrated utility in managing adverse symptoms associated with cancer immunotherapy, such as cytokine storm. Small-molecule designer drugs and JAK inhibitors, which interfere with the IL-6 signalling pathway, represent promising avenues for the treatment of autoimmune diseases, cancer, and other inflammatory conditions.

2.4 LIPOGENESIS AND ITS ROLE IN BREAST CANCER

Tumours are known to require high levels of energy for their growth and synthesis of cell membranes. This energy is provided by the *De Novo* synthesis of lipids (Medes *et al.*, 1952). Lipogenesis is known to occur in the liver as well as adipose tissues leading to *De Novo* fatty acid synthesis from acetyl CoA produced by glycolysis. Acetyl CoA is then carboxylated by ACC forming malonyl CoA. Malonyl CoA and acetyl CoA are further processed by FASN in palmitic acid, which is then transformed by Elv6 into stearic acid (Postic & Girard, 2008).

Expressions of lipogenic genes are under the control of the transcription factors like LXR and SREBP-1c (Postic & Girard, 2008). SREBP1 is a transcription factor known to aid in the transcription of lipogenic genes like ACC and FASN and is also known to be implicated in tumour growth. A high rate of lipogenesis is often associated with tumour development and progression. Increased expression of SREBP1 expression may be the reason for increased expression of lipogenic genes associated with malignancy. SREBP1c, an isoform of SREBP-1 is known to be involved in the process of normal cell transformation (Huang *et al.*, 2012).

An elevated FASN expression promotes the progression of cancer cells into the S phase (Wang *et al.*, 2001). On the flip side, reducing FAS expression not only slows down tumour growth but also triggers programmed cell death in cancer cells.

(Horiguchi *et al.*, 2008). Increased ACC expression is detected during the initial phase of breast cancer a Suppression of its expression leads to the inhibition of growth and apoptosis in cancer cells (Ma *et al.*, 2008).Hence, lipid synthesis likely furnishes energy to cancer cells, promoting cell proliferation and viability, thereby driving tumour expansion.

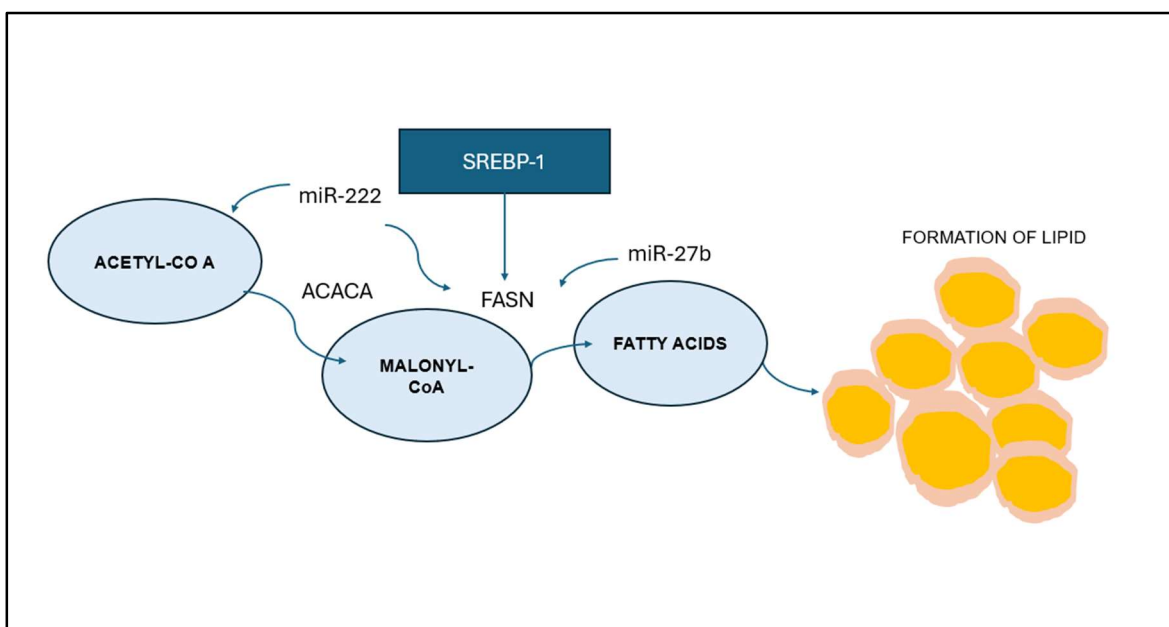


FIGURE 2.3 : LIPOGENESIS

2.5 LIPOGENIC GENES

2.5.1 STEROL REGULATORY ELEMENT BINDING PROTEIN 1(SREBP-1)

The lipogenic phenotype is now a major characteristic of cancer (Menendez & Lupu, 2007). Recent studies reveal a link between aberrant lipogenesis with the development and progression of breast cancer (Puig *et al.*, 2007). Sterol regulatory element-binding proteins (SREBPs) like including SREBP-1a, SREBP-1c and SREBP-2 are transcription factors important in regulating the expression of key enzymes involved in fatty acid synthesis like FASN and ACC (Nakakuki *et al.*, 2007). Excessive lipogenesis is found to be a key hallmark of cancer (Miyachi *et al.*, 2013). Intracellular lipid homeostasis is upheld through a delicate equilibrium between lipid biosynthesis and degradation(Ameer *et al.*, 2014). The heightened lipogenesis observed in tumour cells is characterized by a significant elevation in lipogenic enzyme levels, partly due to the transcriptional activation facilitated by SREBP-1 in cancer cells (Ameer *et al.*, 2014). Therefore, increased expression of

SREBP-1 significantly influences cancer advancement and metastasis (Yamashita *et al.*, 2009). Nevertheless, the influence of SREBP-1 on breast cancer progression is still not fully understood, and further research is needed to unravel its underlying mechanisms.

2.5.2 FATTY ACID SYNTHASE- (FASN)

Of all known lipogenic enzymes involved in tumour progression and metastasis, FASN (Fatty acid synthase) has been the most investigated. The activation of FASN is an early and nearly universal hall mark of most human cancers (Esslimani-Sahla *et al.*, 2006). Its expression increases in a stage dependent manner worsening patient survival (Gansler *et al.*, 1997). The prognosis and survival outcomes of cancer patients are primarily determined by the presence of metastasis, with increased FASN expression being linked to unfavourable prognoses in various hormone-dependent cancers including breast cancer (Milgraum *et al.*, 1997). A direct link between the over expression of FASN and tumour metastasis has been observed in breast carcinomas (Milgraum *et al.*, 1997). FASN has been linked to the alteration of breast cancer cells by influencing the expression of EGFR (HER2/neu isoform) (Menendez *et al.*, 2004). HER2/neu is a proto-oncogene linked to the progression of metastasis in breast cancer (Hartkopf *et al.*, 2012).

2.6 DRUG REPURPOSING FOR CANCER TREATMENT

For a novel drug to come into the market, it can take anywhere between 10 to 20 years making it an extremely lengthy and costly task to accomplish (Kaitin, 2012). Increase in the number of regulatory hurdles faces suggests that the process of bringing new drugs to the market will become more costlier and most time consuming (Pushpakom *et al.*, 2018). Most drugs or therapies which make it to human testing in clinical trials tend to fail (DiMasi *et al.*, 2010). Thus, there exists a growing need for novel strategies that aims to bring drugs rapidly and cost-effectively to the market all while keeping safety in mind. The term Drug repurposing is a strategy in which new applications are identified for existing drugs or therapies that maybe FDA approved or have been shown to be safe in phase 1–2 trials but never made it to the market due to reasons unrelated to safety (Ashburn & Thor, 2004).

The repurposing method of drug development has a higher likelihood of success since the repurposed drug or therapy has already had an established safety profile. As a bonus, drug repurposing strategies also consume less time as well as relatively lesser monetary investment (Pulley *et al.*, 2020). Drug repurposing involves two main strategies namely the computational approach and the experimental approach (Pushpakom *et al.*, 2018). Computational methods heavily rely on data analysis across various types of data, such as gene expression, chemical structures, genotypes, proteomic data, or electronic health records (EHRs). This analysis process can then generate hypotheses for repurposing (Hurle *et al.*, 2013). Computational methods involved various techniques like signature matching, molecular docking, pathway mapping, genetic association and retrospective clinical analysis. Signature matching entails comparing the unique characteristics, or "signature," of a drug, which can include transcriptomic, structural, or adverse effect profiles, with those of another drug or a disease phenotype (Keiser *et al.*, 2009). Molecular docking is a computational approach based on the structure of molecules. It predicts the compatibility between a ligand, like a drug, and a therapeutic target, usually a protein, by assessing the complementarity of their binding sites (Kitchen *et al.*, 2004). Genetic associations suggest that genes linked to a disease could serve as potential targets for drug development (Sanseau *et al.*, 2012). Pathway mapping involves utilizing network analysis with genetic, protein, or disease data to help identify potential repurposing targets and retrospective clinical analysis involves systematically examining electronic health records (EHRs), data from clinical trials, and post-marketing surveillance data. This comprehensive analysis can provide insights for drug repurposing efforts. Emerging data sources such as large-scale in vitro drug screens coupled with genomic data, biobanks linked to electronic health records (EHRs), and patient-reported data offer novel opportunities for leveraging drug repurposing efforts (Greene & Voight, 2016).

The experimental approaches include phenotypic screening which involves conducting high-throughput screening of compounds using *In Vitro* or *In Vivo* disease models (Moffat *et al.*, 2017). This approach can highlight compounds with potential for further clinical evaluation. Another approach are binding assays which can be employed to identify relevant interactions between targets and compounds.

Methods like affinity chromatography and mass spectrometry can uncover novel targets for known drugs.

2.7 HYDROCORTISONE AND ITS USES

Hydrocortisone is a glucocorticoid (naturally produced steroid hormone by the adrenal cortex) usually administered to treat conditions like endocrine disorders, immune conditions and allergic disorders (Jung et al., 2014). Hydrocortisone is being employed in the treatment of immune-related, inflammatory, and neoplastic disorders (*Food and Drug Administration (FDA) Approved Drugs.*, n.d.). Hydrocortisone was discovered in the 1930s, when Edward Kendall discovered a substance which he termed Compound F or 17-hydroxycorticosterone. (Simoni *et al.*, 2002)

The FDA approved hydrocortisone on August 5, 1952 . Hydrocortisone acts by binding to the glucocorticoid receptor and causes several downstream effects such as inhibition of phospholipase A2, NF- κ B, other inflammatory transcription factors, and promotes anti-inflammatory genes (Corticosteroid-related adverse effects, 1991)

2.7.1 MECHANISM OF ACTION OF HYDROCORTISONE

Corticosteroids exert short-term effects by reducing the vasodilation and permeability of capillaries, and by decreasing the migration of leukocytes to sites of inflammation. These effects are mediated through the binding of corticosteroids to the glucocorticoid receptor, which initiates changes in gene expression, leading to various downstream effects occurring over hours to days. Glucocorticoids inhibit the apoptosis and de-margination of neutrophils, suppress the activity of phospholipase A2, thereby reducing the production of arachidonic acid derivatives, and inhibit inflammatory transcription factors like NF- κ B. Additionally, they promote the expression of anti-inflammatory genes such as IL-10.

Lower doses of corticosteroids primarily exert an anti-inflammatory effect, while higher doses have immunosuppressive properties. Prolonged administration of high doses of glucocorticoids can result in binding to the mineralocorticoid receptor, leading to elevated sodium levels and decreased potassium levels in the body (Corticosteroid-related adverse effects, 1991).

2.7.2 METABOLISM OF HYDROCORTISONE

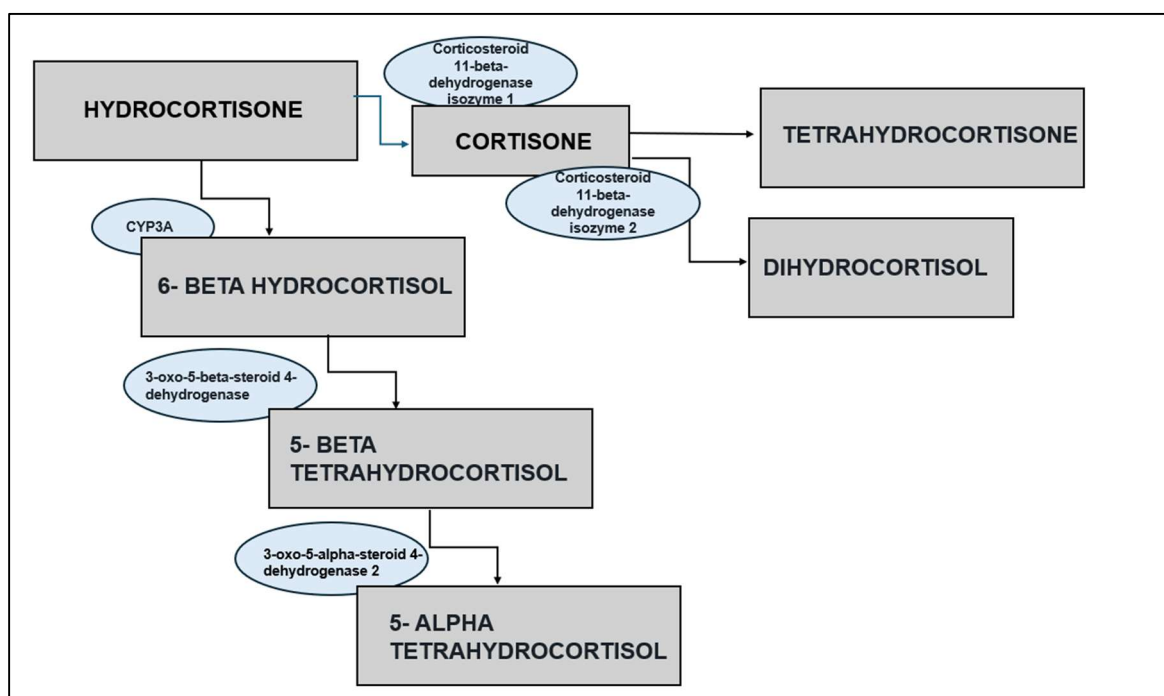


FIGURE 2.4 : Metabolism of hydrocortisone

Hydrocortisone is metabolised by undergoing several metabolic pathways resulting in the formation of various metabolites. Various metabolites produced during the metabolism of hydrocortisone include,

- 6-beta Hydro cortisol: This metabolite is formed via the action of the enzyme CYP3A.
- 5-beta Tetrahydro cortisol: Produced via the enzyme 3-oxo-5-beta-steroid 4-dehydrogenase.
- 5-alpha Tetrahydro cortisol: Formed by the action of the enzyme 3-oxo-5-alpha-steroid 4-dehydrogenase 2.
- Cortisone: This metabolite is generated through the actions of two enzymes, Corticosteroid 11-beta-dehydrogenase isozyme 1 and Corticosteroid 11-beta-dehydrogenase isozyme 2.
- Cortisone, one of the metabolites of hydrocortisone undergoes further metabolism. Cortisone is further metabolized into two other compounds:

- Tetra hydrocortisone: This is one of the products formed from cortisone metabolism.
- Dihydrocortisol: Another product resulting from the metabolism of cortisone.
- The metabolites of hydrocortisone then undergo a process called glucuronidation. Glucuronidation is a process in which glucuronic acid is conjugated with a drug or its metabolite, often to increase its solubility for excretion from the body (Sarkar *et al.*, 2015).

2.8 REPURPOSING HYDROCORTISONE FOR BREAST CANCER TREATMENT

Hydrocortisone, a glucocorticoid with well-established anti-inflammatory properties, presents a promising candidate for repurposing in breast cancer treatment. In this study, we try to leverage the anti-inflammatory properties of hydrocortisone and we try to investigate the potential of hydrocortisone to mitigate inflammation, a major hallmark of cancer by reducing the expression of inflammatory genes like NF- κ B, TNF- α and IL-6. Another major hallmark of cancer, most particularly of that of breast cancer is aberrant lipid synthesis. Therefore, exploring whether hydrocortisone can exert inhibitory effects on lipogenesis in breast cancer cell lines by reducing the expression of lipogenic genes like FASN and SREBP-1 holds considerable therapeutic potential in breast cancer management. Further research into the dual anti-inflammatory and anti-lipogenic properties of hydrocortisone could pave the way for novel treatment strategies in breast cancer.

CHAPTER 3

AIM AND SCOPE OF THE STUDY

3.1 AIM

To explore the effects of hydrocortisone on breast cancer cell lines, assessing its ability to reduce inflammation, inhibit lipogenesis, and determine its potential as a treatment for cancer.

3.2 SCOPE

- To investigate the anti-inflammatory properties of hydrocortisone on breast cancer cell lines by assessing changes in the expressions of pro-inflammatory gene levels.
- To evaluate the impact of hydrocortisone on lipogenesis in breast cancer cells, by examining alterations in the expression levels of genes involved in lipid metabolism.
- To assess the overall cytotoxic effects of hydrocortisone on breast cancer cell viability and to understand its potential therapeutic efficacy.

CHAPTER 4

MATERIALS AND METHODS

4.1 *IN SILICO* STUDY

4.1.1. *PROTEIN PREPARATION*

For the docking study the p52 subunit of NF- κ B and the transcription factor SREBP-1 was selected. Their structures were retrieved from the RCSB protein data bank with PDB ID 1A3Q and 1AM9 respectively. To begin the docking study, the proteins were first processed using the Molegro Molecular viewer. The water molecules and all non-standard residues were removed. Further, the binding sites of the p52 subunit and SREBP-1 were identified using the software Biovia Discovery studio and PyMol. The top binding site favourable for the docking study was identified.

4.1.2. *LIGAND PREPARATION*

The structure of the ligand Hydrocortisone was obtained from the Pubchem database with Pubchem ID 5754 in SDF format. The canonical SMILES, Molecular formula and molecular weight and other physical properties were also obtained from the Pubchem database. The Molecular properties and drug likeliness were obtained from the Molsoft webserver, and the Pharmacokinetic properties and medicinal chemistry of hydrocortisone were obtained from the SwissADME website. Toxicity prediction was done using the PROTOX-2 website. The compound was loaded into PyRx and made into a pdbqt file. This was done by clicking Load molecule, create as ligand pdbqt file.

4.1.3. *MOLECULAR DOCKING USING PyRx*

PyRx is a free and open-source software designed for molecular docking studies. PyRx provides a graphical user interface (GUI) for AutoDock Vina, which is a popular molecular docking software program. PyRx was used for the molecular docking between the p52 subunit of NF- κ B and hydrocortisone and SREBP-1 and hydrocortisone to investigate potential interactions and binding affinities (Katra Kolšek et al., 2014).

4.1.4. VALIDATION OF MOLECULAR DOCKING USING iGEM DOCK

Validating molecular docking studies is crucial for ensuring the accuracy and reliability of computational predictions. The docking results obtained from PyRx was validated using iGEM DOCK, an integrated graphical environment for molecular docking (Hsu et al., 2011).

4.1.5 VISUALIZATION OF DOCKING USING BIOVIA DISCOVERY STUDIO

Visualization of docking results is crucial for understanding protein-ligand interactions and guiding the design of novel therapeutic agents. BIOVIA Discovery Studio was used to visualize the docking of the p52 subunit with hydrocortisone and SREBP-1 with hydrocortisone. High quality images were generated using BIOVIA Discovery Studio.

4.2 *In Vitro* STUDY

4.2.1 CELL CULTURE

MDA-MB-231 triple negative breast cancer cell line was purchased from NCCS (National Centre for Cell Science) Pune. The cells were maintained in DMEM medium containing 100 mg/L penicillin and 100 mg/L streptomycin supplemented with 10% foetal bovine serum at 37°C in a 5% CO₂ atmosphere. Subculturing was done after the cells reached 80% - 90% confluency. Prior to passaging, the media was discarded, and the cells were washed using 2ml of PBS. The cells were then trypsinized with 2 ml of trypsin and incubated in a CO₂ incubator for 2 minutes to encourage cell detachment. 2 ml of serum containing media was added to the flask to inactivate the trypsin. The cells were centrifuged at 900 rpm for 3 minutes. The supernatant was discarded, and the pellet was collected by gentle aspiration. The pellet was suspended in growth media supplemented with 10% FBS. The cells were then seeded into 6 well and 96 well plates and incubated for 24 hours .



FIGURE 4.1 : MDA-MB-231 Cells seeded on a 96 well plate

4.2.2. STOCK PREPARATION OF HYDROCORTISONE

Hydrocortisone (white crystalline powder form) was purchased from Himedia. The drug stock was prepared by dissolving 10mg of Hydrocortisone in 100 μ l of ethanol. 10 μ l from this stock is taken and dissolved in 1 ml of DMEM medium and rapidly vortexed. This stock was then stored at 4°C for future use.



FIGURE 4.2 : Stock preparation of Hydrocortisone

4.2.3 ASSESMENT OF CYTOTOXICITY OF HYDROCORTISONE – MTT ASSAY ON C₂C₁₂ CELL LINE

C₂C₁₂ fibroblast cells were plated in 96-well microplates, with DMEM culture medium supplemented with 10% FBS and incubated at 37°C and 5% CO₂, overnight. The next day, the cells were subject to treatment with hydrocortisone, for periods of 24 and 48 hours in varying concentrations from 5 to 50µg/ml. The MTT dye was prepared by mixing 5 mg/ml of MTT in PBS . 50 µL of MTT solution was added to each well of the 96 well plate, The plate was wrapped in aluminium foil and incubated at 37°C for 3 hours. Over the formazan precipitates formed, DMSO, was added to each well to solubilize the crystals. The absorbance was taken at 570 nm using a microplate reader. The data obtained were then plotted in a graph with concentration on the x axis and % inhibition on the y axis. This serves as a control to check the comparative effect of Hydrocortisone on breast cancer as well as normal cells (Tolosa, Donato and Gómez-Lechón, 2015).

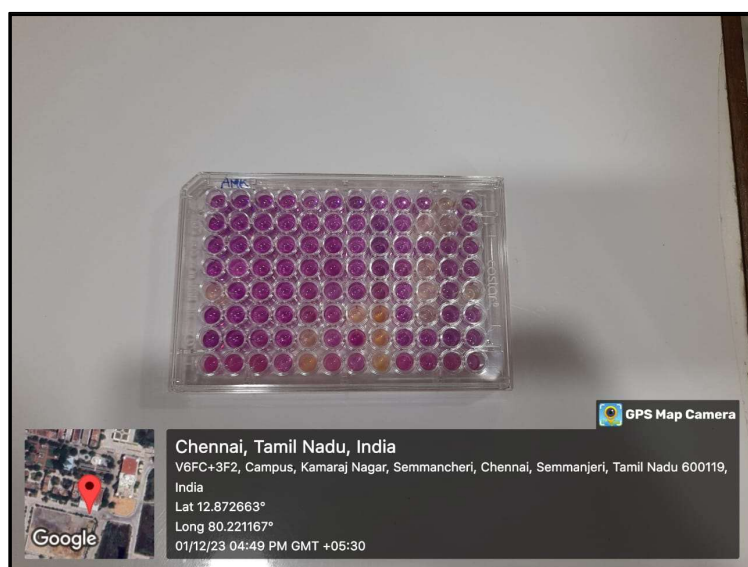


FIGURE 4.3 : Formation of purple formazan crystals after addition of MTT

4.2.4 ASSESMENT OF ANTI-CANCER ACTIVITY OF HYDROCORTISONE – MTT ASSAY ON MDA-MB-231 CELL LINE

MDA-MD-231 cells were plated in 96-well microplates, with DMEM culture medium supplemented with 10% FBS and incubated at 37°C and 5% CO₂, overnight. The next day, the cells were subject to treatment with hydrocortisone, for periods of 24 and 48 hours in varying concentrations from 5 to 50µg/ml. The MTT dye was prepared by mixing 5 mg/ml of MTT in PBS . 50 µL of MTT solution was added to each well of the 96 well plate, The plate was wrapped in aluminium foil and incubated at 37°C for 3 hours. Over the formazan precipitates formed, DMSO, was added to each well to solubilize the crystals. The absorbance was taken at 570 nm using a microplate reader. The data obtained were then plotted in a graph with concentration on the x axis and % inhibition on the y axis and the IC₅₀ value was found (Tolosa, Donato and Gómez-Lechón, 2015).

4.2.5 APOPTOSIS DETECTION – AO/EB STAINING

Acridine orange (AO) and ethidium bromide (EB) staining is a widely used technique for evaluating cell viability and assessing apoptotic and necrotic cell populations within a sample. In breast cancer cell lines, AO/EB staining can provide valuable information about cell health, proliferation, and response to treatment. AO permeates both live and dead cells, emitting green fluorescence upon binding to double-stranded DNA, while EB only enters cells with compromised membrane integrity, emitting red fluorescence upon binding to nucleic acids. Live cells typically exhibit green fluorescence, whereas apoptotic cells may display a mix of green and orange fluorescence, and necrotic cells appear predominantly red or yellow. By analysing the relative proportions of these fluorescent populations under a fluorescence microscope we can gain insights into the efficacy of anti-cancer treatments, mechanisms of cell death, and overall cell viability in breast cancer cell lines (Smith et al., 2012).

4.2.6 TOTAL RNA ISOLATION

Total RNA isolation was done by the TRIzol method. A T25 flask seeded with MDA-MB-231 cells was washed with PBS. 1ml of TRIzol was added and the cells were scraped using a disposable cell scraper. TRIzol was removed and the cell lysate was added to an Eppendorf tube and incubated at room temperature for 5

minutes. 250 μ L of chloroform was added and shaken vigorously for 15 seconds followed by incubation at room temperature for 5 minutes. The cell lysate was then centrifuged at 10,000 rpm for 5 minutes. Now 3 distinct layers will be visible: a top clear aqueous layer with RNA, an interphase with white precipitated DNA and a bottom layer which is the pink organic phase which contains the pelleted proteins. The aqueous phase was gently removed and added to another Eppendorf tube. 550 μ L of isopropanol was added to the tube and centrifuged at 14,000 rpm for 20 – 30 minutes. Isopropanol was discarded and 1ml of 75% ethanol was added to the tube and centrifuged at 9500 RPM for 5 minutes. Ethanol was discarded and the pellets were allowed to air dry. 15-25 μ L of DEPC treated water was added to the tubes and the isolated RNA was subject to quantification (Mukhopadhyay and Roth, 1998).

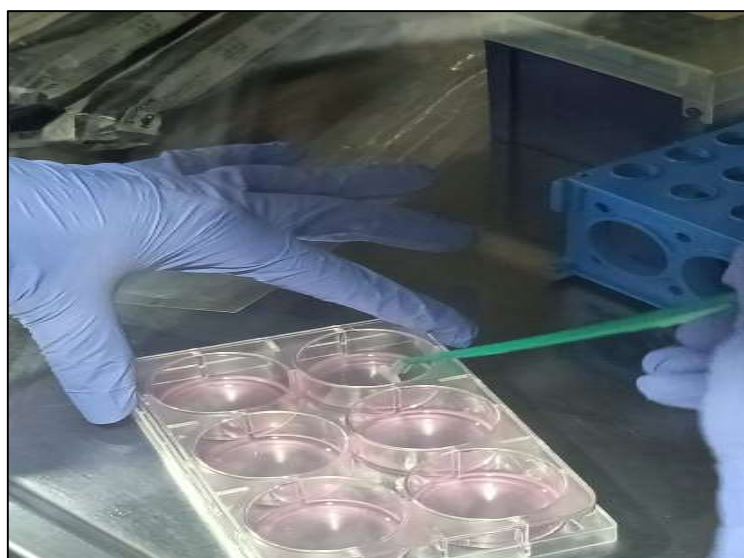


FIGURE 4.4: Scrapping of cells and addition of TriZol for RNA isolation

4.2.7 QUANTIFICATION OF RNA

The isolated RNA was quantified using BioTek micro-volume plate reader . The purity of RNA was determined by measuring the absorbance at 260 nm . The ratio of OD₂₆₀/ OD₂₈₀ should be greater than 1.8. If the ratio is less than 1.5, the RNA is partially degraded. Lower ratios are suggestive of DNA or Thiocyanate contamination. The BioTek micro-volume plate reader was used to analyse 2 μ L of the RNA sample, displaying the RNA quantity in nanograms per microliter of the loaded sample. This measurement represents the amount of RNA present in 1 μ L of the sample in nanograms. Using this measurement, the amount of RNA template to be added for cDNA conversion was calculated.

4.2.8 AGAROSE GE ELECTROPHORESIS

Agarose gel electrophoresis was done to check the integrity of the isolated RNA. To perform this procedure 1% agarose gel in 100 ml TBE (Tris-borate-EDTA) buffer is used. Firstly, agarose powder is added to the buffer solution and heated until it dissolves completely. The mixture is then cooled to around 60°C and poured into a gel mould, with combs inserted to create wells for loading the RNA samples. Once the gel solidifies, the combs are removed, and the gel is submerged in a buffer-filled electrophoresis chamber. RNA samples mixed with loading dye are carefully pipetted into the wells, and an electric current is applied across the gel. RNA molecules migrate through the gel matrix at rates inversely proportional to their sizes, with smaller fragments traveling farther. After electrophoresis, the gel is stained with the RNA-binding dye ethidium bromide and visualized under UV light to observe the separated RNA bands (Rio et al., 2010).

4.2.9 cDNA CONVERSION

The quantified RNA was converted into cDNA using a cDNA synthesis kit from BioRad. The BioRad cDNA conversion kit facilitates the efficient conversion of RNA into complementary DNA (cDNA). This kit utilizes reverse transcription technology, employing a blend of engineered MMLV (Moloney Murine Leukaemia Virus) reverse transcriptase and RNase H to ensure high fidelity and yield. The process begins with RNA denaturation and priming using random hexamers or oligo(dT) primers, followed by reverse transcription to generate cDNA. This cDNA can then be utilized in downstream applications such as PCR, qPCR, or gene expression analysis (Ross *et al.*, 2000).

4.2.10 DESIGNING OF PRIMERS FOR QUANTITATIVE REAL TIME POLYMERASE CHAIN REACTION

Primers are short, single-stranded DNA sequences that bind to complementary sequences in the target DNA region, initiating DNA amplification or sequencing. The FASTA sequence for inflammation promoting genes (TNF-ALPHA, IL-6, and NF-κB) and genes responsible for lipogenesis (FASN and SREBP-1) were obtained from the NCBI database for primer designing. The primers were then designed using the Eurofins Genomics online primer designing tool. They were

designed with a GC content of 45% and an annealing temperature of 54 -57 °C and then given for synthesising to Barcode Biosciences Pvt Ltd. The primers were then diluted according to the given dilutions for further use (Dieffenbach *et al.*, 1993).

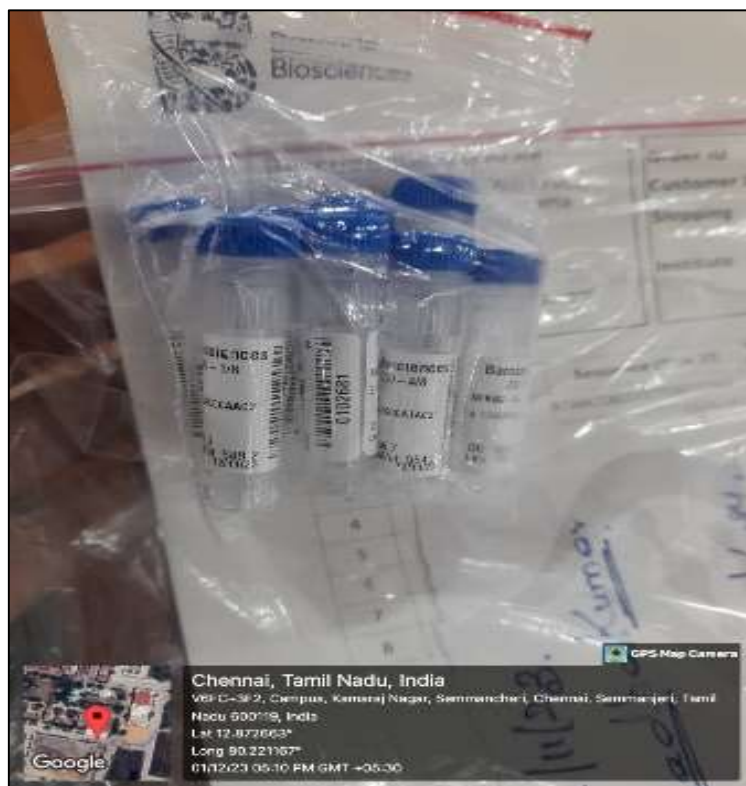


FIGURE 4.5 : Primers for qRT- PC

TABLE 4.1 Primers designed for inflammatory and lipogenic genes

SNO	GENE	FORWARD PRIMER	REVERSE PRIMER
1.	Interleukin 6	5'-ATAACCACCCCTGACCCAAC-3'	5'-ATACCTCAAACCTCCAAAAGACC-3'
2.	Nuclear factor kappa B	5'-CGAATGGACAAGACAGCAG-3'	5'-CCGGAACACAATGGCATAC-3'
3.	TNF-alpha	5'-AACCATTCTCCTTCTCCCC-3'	5'-TTTCACCTCCATCCATCCTC-3'
4.	FASN	5'-CGCGTGGCCGGCTACTCCTAC-3'	5'-TCAGGAAGGCTTCAAGAGAG-3'
5.	SREBP-1	5'- TCAACAACCAAGACAGTGAC- 3'	5'-TCAGGAAGGCTTCAAGAGAG-3'

4.2.11 q RT-PCR

The amount of cDNA in the loaded sample was measured using quantitative real-time polymerase chain reaction with the Bio-Rad SYBR Green master mix. The cDNA template containing the target sequence and particular primers was mixed with the master mix. When double-stranded cDNA attaches to SYBR Green dye, it releases fluorescence, which intensifies with further DNA amplification. The real-time monitoring of SYBR Green's fluorescence during cycling enables accurate determination of gene expression levels (Derveaux *et al.*, 2010).



FIGURE 4.6 : Thermocycler



FIGURE 4.7 : Biorad Protocol for qRT-PCR

4.3 STATISTICAL ANALYSIS

Various statistical analyses were done using GraphPad Prism Version 10. The Student's T-Test (MacFarland & Yates, 2020) was done to check for the significance of the % Inhibition on the MDA-MB -231 cell line after 24 and 48 hours of drug treatment. Student's T-Test was also done to check for the significance between the % Inhibition on the MDA-MB -231 cell line and the non- cancerous C₂C₁₂ cell line after 24 as well as 48 hours of drug treatment. Chi square test (Franke et al., 2011) was performed on the images obtained after the AO/EB dual staining, and one way- analysis of variance (ANOVA) was performed on the gene expression results obtained after qRT-PCR to check if there is significant reduction in the expression of inflammatory genes and lipogenic genes after treatment with hydrocortisone (A. Ross & Willson, 2017).

CHAPTER 5

RESULTS AND DISCUSSION

5.1 *IN SILICO* STUDY

TABLE 5.1 General properties of Hydrocortisone obtained from PubChem

SMILES ID	<chem>CC12CCC(=O)C=C1CCC3C2C(CC4(C3CCC4(C(=O)CO)O)C)O</chem>
PubChem ID	5754
Molecular formula	C ₂₁ H ₃₀ O ₅
Molecular weight (g/mol)	362.5
Number of hydrogen bond acceptors	35
Number of hydrogen bond donors	3
Number of atoms	56
Number of bonds	59
Number of rotatable bonds	2
Molecular refractivity	97.53
Topological Polar Surface Area	94.83
Octanol/water partition coefficient (logP)	1.78
MolLogP	2.68
MolLogS	-2.84 (in Log(moles/L)) 375.19 (in mg/L)
MolPSA	43.86 Å ²
MolVol	262.08 Å ³
pKa of most basic/Acidic group	-3.04 / 3.75
Number of stereo isomers	0

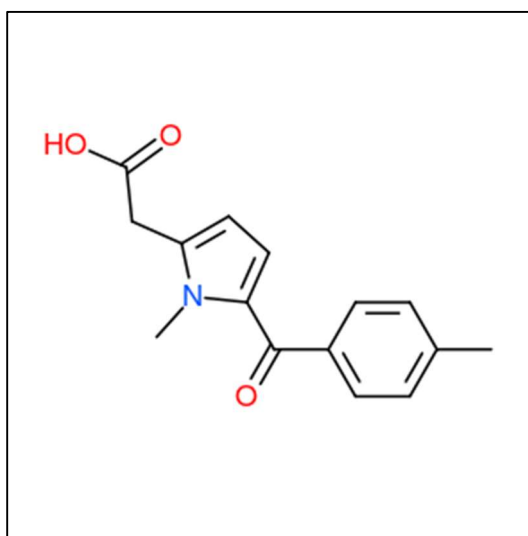


FIGURE 5.1: 2D Chemical structure of Hydrocortisone

TABLE 5.2: Water solubility of Hydrocortisone

Log S (ESOL)	-2.97
Solubility	3.89e-01mg/ml; 1.07e-03 mol/l
Class	Soluble
Log S (Ali)	-3.21
Solubility	2.22e-01 mg/ml; 6.12e-04 mol/l
Class	Soluble
Log S (SILICOS-IT)	-2.63
Solubility	8.42e-01 mg/ml; 2.32e-03 mol/l
Class	Soluble

5.1.1 INTERPRETATION OF TABLE 2

Information about the water solubility of hydrocortisone predicted using various methods was obtained from the SwissADME website.

Log S (ESOL): This value is -2.97. It represents the logarithm of the aqueous solubility of hydrocortisone, predicted by the ESOL (Estimated SOLubility) model. A negative log S value typically indicates low solubility. In this case, the negative value

suggests that hydrocortisone is predicted to have low aqueous solubility according to the ESOL model.

Solubility: The predicted solubility is 3.89e-01 mg/ml or 1.07e-03 mol/l.

Log S (Ali): This value is -3.21. It represents the logarithm of the aqueous solubility of hydrocortisone, predicted by another model. Similar to the ESOL prediction, this negative value suggests low solubility.

Solubility: The predicted solubility is 2.22e-01 mg/ml or 6.12e-04 mol/l.

Log S (SILICOS-IT): This value is -2.63. It represents the logarithm of the aqueous solubility of hydrocortisone, predicted by the SILICOS-IT model. Again, the negative value suggests low solubility.

Solubility: The predicted solubility is 8.42e-01 mg/ml or 2.32e-03 mol/l.

TABLE 5.3 : Lipophilicity of Hydrocortisone

Log P ₀ /w (WLOGP)	1.78
Log P ₀ /w (MLOGP)	1.39
Log P ₀ /w (SILICOS-IT)	2.47
Consensus Log P ₀ /w	1.84

5.1.2 LIPOPHILICITY

Information about the lipophilicity of hydrocortisone predicted using various methods was obtained from the SwissADME website.

Lipophilicity refers to the ability of a compound to dissolve in lipids or fats. Determining the lipophilicity of a drug is important for several reasons:

1. It can give us information on how well a drug is absorbed through biological membranes, such as those in the gastrointestinal tract. Drugs that are too hydrophilic may be poorly absorbed while excessively lipophilic drugs may have difficulty in reaching their target tissues.

2. Lipophilicity also affects drug distribution throughout the body. For example, lipophilic drugs may be distributed more readily through fatty tissues when compared with hydrophilic drugs.
3. Lipophilicity can influence the metabolism of drugs. Highly lipophilic drugs may be more prone to metabolism by enzymes in the liver, potentially leading to faster clearance from the body.
4. The degree of lipophilicity can impact the bioavailability of a drug, which refers to the fraction of the administered dose that reaches systemic circulation. Optimal lipophilicity can enhance bioavailability by facilitating absorption and distribution to target tissues.
5. Lipophilicity can influence interactions between drugs. Drugs with similar lipophilic properties may compete for binding sites on proteins or enzymes, affecting their pharmacokinetics and efficacy.
6. Excessive lipophilicity can lead to increased accumulation of a drug in fatty tissues, potentially causing toxicity. On the other hand, drugs that are too hydrophilic may be rapidly eliminated from the body, leading to reduced efficacy.

5.1.3 INTERPRETATION OF TABLE 5.3 :

Log P_{0/w} (iLOGP): This value is 1.93. It represents the calculated logarithm of the partition coefficient between octanol and water, which is a common measure of lipophilicity. A higher value indicates higher lipophilicity, suggesting hydrocortisone has a moderate to high affinity for lipids.

Log P_{0/w} (XLOGP3): This value is 1.61. This is another variation in the calculation of the logarithm of the partition coefficient between octanol and water. Again, it suggests moderate lipophilicity for hydrocortisone.

Log P_{0/w} (WLOGP): This value is 1.78. This value also represents the logarithm of the partition coefficient between octanol and water. This suggests moderate lipophilicity as well.

Log P_{0/w} (MLOGP): This value is 1.39. It's another measure of the logarithm of the partition coefficient between octanol and water. This indicates slightly lower lipophilicity compared to the other measures.

Log P_{0/w} (SILICOS-IT): This value is 2.47. It is also the calculation of the logarithm of the partition coefficient between octanol and water, possibly utilizing a different computational method. This suggests relatively higher lipophilicity compared to the other measures.

Consensus Log P_{0/w}: This value is 1.84. It represents the consensus prediction obtained by considering all the individual predictions. It provides an overall estimate of the lipophilicity of hydrocortisone, which seems to be moderate to moderately high based on the range of individual predictions.

From these interpretations, it is found that Hydrocortisone has moderate to high affinity for lipids indicating that it has good absorption in the gastrointestinal tract.

TABLE 5.4: Pharmacokinetic properties of Hydrocortisone

GI absorption	High
BBB permeant	No
P-gp substrate	Yes
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log k _p (Skin permeation)	-7.37 cm/s

The following pharmacokinetic properties were obtained from the SwissADME website.

5.1.4 INTERPRETATION OF TABLE 5.4

GI absorption: This property indicates that hydrocortisone is readily absorbed in the gastrointestinal tract, suggesting it can effectively enter systemic circulation when administered orally.

BBB permeant: Hydrocortisone is indicated as not permeating the blood-brain barrier (BBB). This suggests that hydrocortisone may not readily enter the brain tissue from the bloodstream.

P-gp substrate: Hydrocortisone is identified as a substrate for P-glycoprotein (P-gp), an efflux transporter found in various tissues including the intestine and blood-brain barrier. This suggests that P-gp may affect the absorption and distribution of hydrocortisone in the body.

CYP enzyme inhibitors: Hydrocortisone is not indicated as an inhibitor of cytochrome P450 (CYP) enzymes such as CYP1A2, CYP2C19, CYP2C9, CYP2D6, or CYP3A4. This suggests that hydrocortisone is unlikely to significantly interfere with the metabolism of other drugs metabolized by these enzymes.

Log kp (Skin permeation): This value of -7.37 cm/s represents the logarithm of the permeability coefficient for skin permeation. A negative value suggests that hydrocortisone has low permeation through the skin, indicating it may not be efficiently absorbed through this route.

5.1.5 TOXICITY PREDICTION -PRO TOX II

PREDICTED TOXICITY CLASS 5 – (Relatively low toxicity or a low potential for causing harm)

TABLE 5.5: Toxicity prediction - PRO TOX II

CLASSIFICATION	TARGET	SHORTHAND	PREDICTION	PROBABILITY
Organ toxicity	Hepatotoxicity	dili	Inactive	0.99
Toxicity end points	Carcinogenicity	carcino	Inactive	0.94
Toxicity end points	Immunotoxicity	immuno	Active	0.96
Toxicity end points	Mutagenicity	mutagen	Inactive	0.53
Toxicity end points	Cytotoxicity	ctyo	Inactive	0.76

5.1.6 INTERPRETATION OF TABLE 5.5:

Hepatotoxicity (dili): Predicted as inactive with a high probability (0.99). This suggests that hydrocortisone is unlikely to cause liver toxicity.

Carcinogenicity (carcino): Predicted as inactive with a high probability (0.94). This indicates that hydrocortisone is not expected to be carcinogenic.

Immunotoxicity (immuno): Predicted as active with a high probability (0.96). This suggests that hydrocortisone may have immune-toxic effects, indicating potential impacts on the immune system.

Mutagenicity (mutagen): Predicted as inactive with a moderate probability (0.53).

Cytotoxicity (ctyo): Predicted as inactive with a moderate probability (0.76).

Therefore, hydrocortisone is predicted to have low to no toxicity in terms of hepatotoxicity, carcinogenicity, mutagenicity, and cytotoxicity. However, it shows potential for immune-toxic effects according to the prediction. This is useful as hydrocortisone is being investigated in this study as an anti-inflammatory substance, to reduce the expression of inflammation causing genes thereby reducing inflammation in cancer.

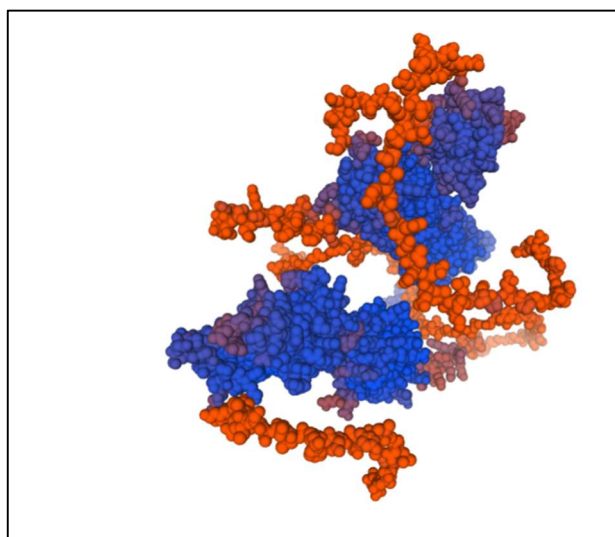


FIGURE 5.2 : 3D model of the p52 subunit of NF-KB built using SWISS- MODEL

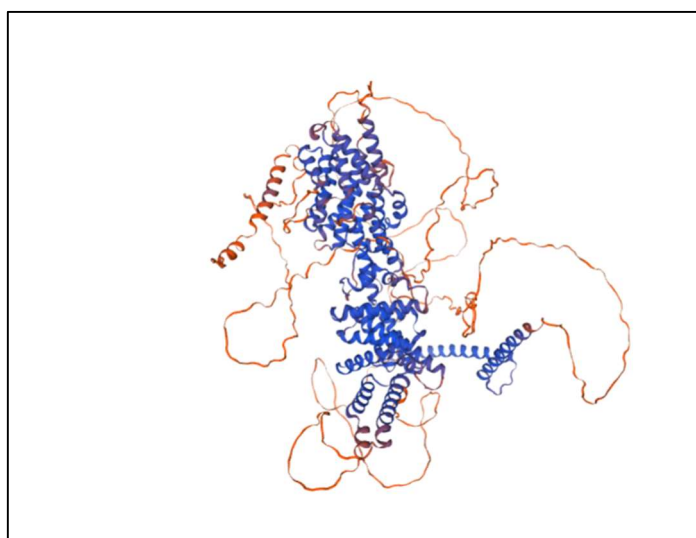


FIGURE 5.3 : 3D model of SREBP-1 built using SWISS- MODEL

5.1.7 DOCKING RESULTS FROM PyRx

TABLE 5.6: Docking results from PyRx – Docking of *Hydrocortisone* to the *p52* subunit of *NF-κB*

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
Processed_p52_5754_uff_E=571.16	-6.2	0	0
Processed_p52_5754_uff_E=571.16	-6.2	21.448	18.69
Processed_p52_5754_uff_E=571.16	-6.1	22.652	20.475
Processed_p52_5754_uff_E=571.16	-6.1	35.137	32.17
Processed_p52_5754_uff_E=571.16	-6	35.194	32.131
Processed_p52_5754_uff_E=571.16	-5.8	22.667	18.499
Processed_p52_5754_uff_E=571.16	-5.8	36.484	33.426
Processed_p52_5754_uff_E=571.16	-5.7	22.924	18.31
Processed_p52_5754_uff_E=571.16	-5.6	24.457	22.06

Ligand: This column represents different conformations or poses of the ligand (hydrocortisone) docked with the p52 subunit OF NF- κ B.

rmsd/ub: This column likely represents the root mean square deviation (RMSD) of the ligand pose compared to an upper bound (ub). A lower RMSD value indicates a closer match between the docked pose and the reference structure.

rmsd/lb: This column likely represents the RMSD of the ligand pose compared to a lower bound (lb). Similarly, a lower RMSD value indicates a closer match.

TABLE 5.7: Docking results from PyRx – Docking of *Hydrocortisone* to *SREBP-1*

Ligand	Binding Affinity	rmsd/ub	msd/lb
Processed_SREBP_5754_uff_E=571.16	-6.2	0	0
Processed_SREBP_5754_uff_E=571.16	-6	6.849	2.345
Processed_SREBP_5754_uff_E=571.16	-5.3	5.425	3.433
Processed_SREBP_5754_uff_E=571.16	-5.2	7.24	1.734
Processed_SREBP_5754_uff_E=571.16	-5.1	5.92	3.87
Processed_SREBP_5754_uff_E=571.16	-4.7	4.561	2.458
Processed_SREBP_5754_uff_E=571.16	-4.7	15.884	14.187
Processed_SREBP_5754_uff_E=571.16	-4.7	12.729	8.867
Processed_SREBP_5754_uff_E=571.16	-4.7	18.937	16.798

Ligand: This column represents different conformations or poses of the ligand (hydrocortisone) docked with SREBP-1

Binding Affinity: This column represents the binding affinity of the ligand to the target receptor. The values are negative, indicating that lower values correspond to stronger binding affinity. For example, a value of -6.2 indicates a stronger binding affinity than a value of -5.1.

rmsd/ub: This column likely represents the root mean square deviation (RMSD) of the ligand pose compared to an upper bound (ub). A lower RMSD value indicates a closer match between the docked pose and the reference structure.

rmsd/lb: This column likely represents the RMSD of the ligand pose compared to a lower bound (lb). Similarly, a lower RMSD value indicates a closer match.

5.1.8 INTERPRETATIONS OF DOCKING RESULTS- NF- κ B

For each ligand pose, the RMSD values are provided for both upper and lower bounds.

Lower RMSD values indicate that the ligand pose is closer to the reference structure.

The first row with an RMSD of 0 likely represents the best pose with a perfect match to the reference structure.

As the RMSD values increase, the deviation from the reference structure also increases, indicating potential changes in the ligand's conformation or orientation within the binding site.

Overall, these results provide insights into the quality of the docking poses of hydrocortisone with the p52 subunit of NF- κ B. The Low RMSD values suggest good alignment and stronger binding interactions between hydrocortisone and the p52 subunit of NF-KB.

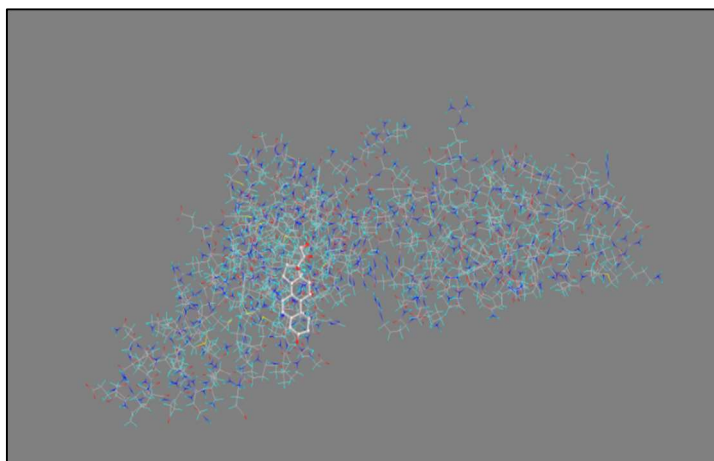


FIGURE 5.4 : Binding of Hydrocortisone to the p52 subunit of NF- κ B – image from PyRx

5.1.9 INTERPRETATIONS OF DOCKING RESULTS- SREBP-1

Row 1: Ligand with a binding affinity of -6.2, with no significant deviation from the unbound state (rmsd/ub = 0) or the bound state (rmsd/lb = 0).

Row 2: Ligand with a binding affinity of -6, with moderate deviations from both the unbound (6.849) and bound (2.345) states.

Rows 3-9: Ligands with varying binding affinities and rmsd values from both unbound and bound states.

Given that the binding affinities are negative values, with more negative values indicating stronger binding, it seems that hydrocortisone binds favourably to SREBP-1. The first row specifically shows a binding affinity of -6.2, suggesting a relatively strong binding interaction.

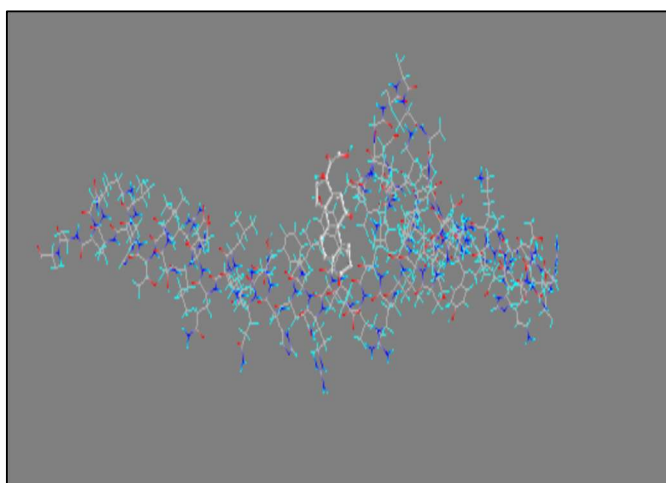


FIGURE 5.5 : Binding of Hydrocortisone to SREBP-1 – image from PyRx

5.1.10 VALIDATION OF DOCKING USING iGEM DOCK:

TABLE 5.8: Docking validation of hydrocortisone to the p52 subunit of *NF-κB* using iGEM

#Ligand	TotalEnergy	VDW	HBond	Elec	AverConPair
Processed p52-hydro drug bank-0.pdb	-96.6839	-75.4343	-21.2495	0	23.4615

TABLE 5.9: Docking validation of hydrocortisone to SREBP-1 using iGEM

#Ligand	TotalEnergy	VDW	HBond	Elec	AverConPair
Processed SREBP-hydro drug bank-0.pdb	-78.1662	70.4193	7.74688	0	15.7308

5.1.11 INTERPRETATIONS OF iGEM DOCKING:

The docking of hydrocortisone with the p52 subunit using iGEM dock appear favourable.

- **Total Energy:** This is the overall binding energy between the ligand and the receptor. A lower total energy score indicates a more favourable interaction. In this case, the total energy is -96.6839, which is a relatively low score.
- **VDW:** This stands for van der Waals energy, which is a type of non-covalent interaction between atoms. A lower VDW energy indicates a more favourable interaction. Here, the VDW energy is -75.4343.
- **HBond:** This represents the hydrogen bond energy, another type of non-covalent interaction. A lower HBond energy indicates a more favourable interaction. In this case, the HBond energy is -21.2495.
- **Elec:** This refers to the electrostatic energy, which is the energy of attraction or repulsion between charged atoms. A lower Elec energy indicates a more favourable interaction. Here, the Elec energy is 0.
- **AverCor:** This stands for the average correction energy.

The docking of hydrocortisone with SREBP-1 using iGEM dock appear favourable.

- **Total Energy:** The total energy is negative, indicating that the interaction between hydrocortisone and SREBP is energetically favourable.
- **VDW (Van der Waals) Energy:** The predominantly favourable Van der Waals energy suggests that hydrocortisone forms strong non-covalent interactions with SREBP, contributing significantly to the overall binding energy.

- **HBond (Hydrogen Bond) Energy:** The presence of a positive hydrogen bond energy indicates that hydrogen bonding also contributes to the binding between hydrocortisone and SREBP. Hydrogen bonds are typically important for stabilizing ligand-receptor interactions.
- **Elec (Electrostatic) Energy:** The absence of electrostatic energy suggests that electrostatic interactions play a minor role in the binding of hydrocortisone to SREBP in this simulation.
- **AverConPair (Average Contact Pair Energy):** This value represents the average strength of interactions between the atoms of hydrocortisone and SREBP. The positive value suggests that overall, the contacts between the ligand and the receptor are favourable.

Overall, the binding between hydrocortisone and SREBP appears to be favourable, with significant contributions from Van der Waals and hydrogen bonding interactions.

5.1.12 VISUALISATION OF DOCKING AND BINDING SITES USING BIOVIA DISCOVERY STUDIO :

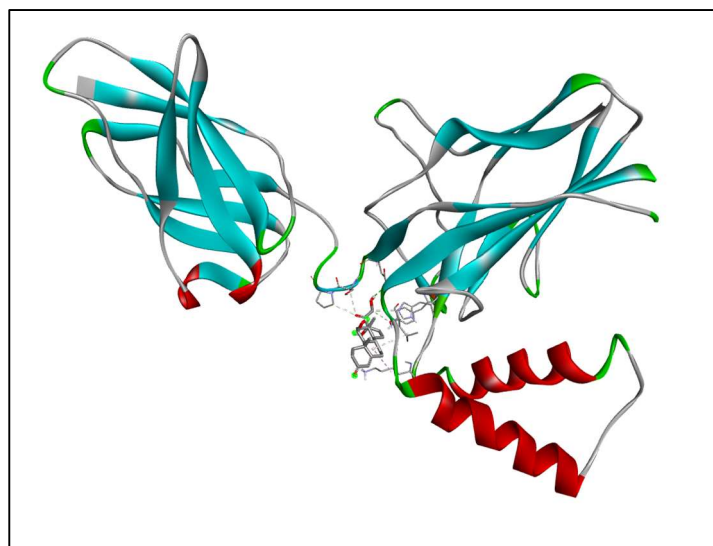


FIGURE 5.6 3D image of the binding of Hydrocortisone to the p52 subunit of NF- κ B

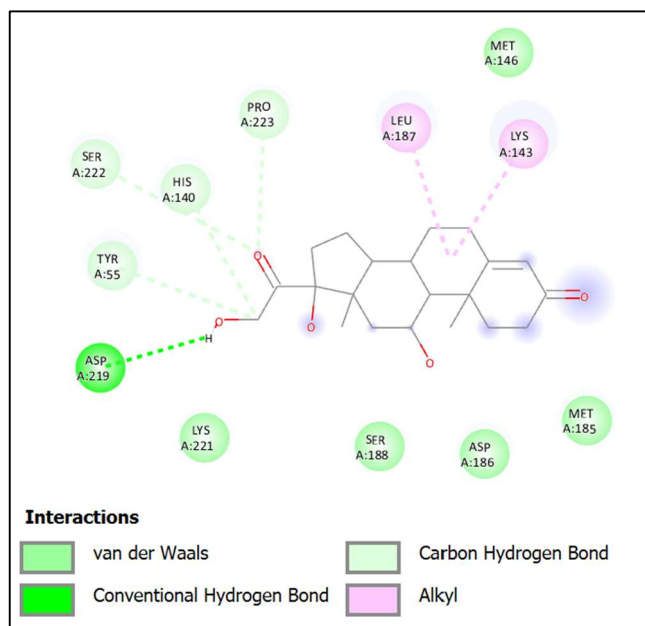


FIGURE 5.7 2D image showing the various interactions between the binding of Hydrocortisone to the p52 subunit of NF- κ B

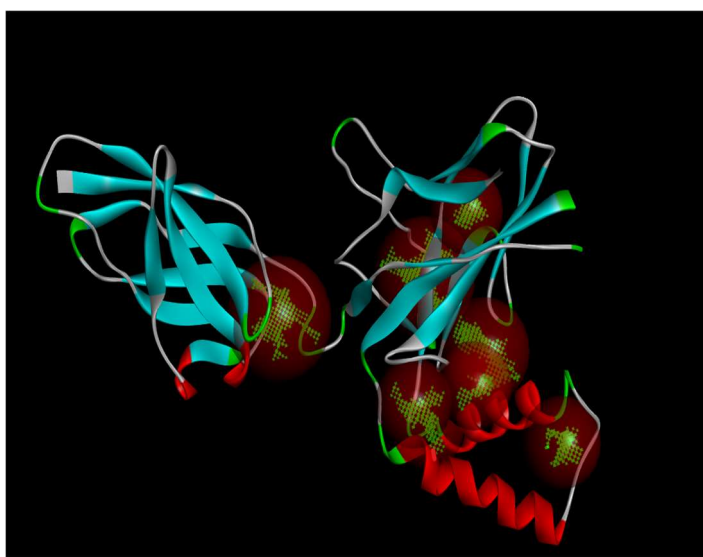


FIGURE 5.8 : Possible binding sites of Hydrocortisone on the p52 subunit of NF- κ B – Image from Biovia Discovery studio

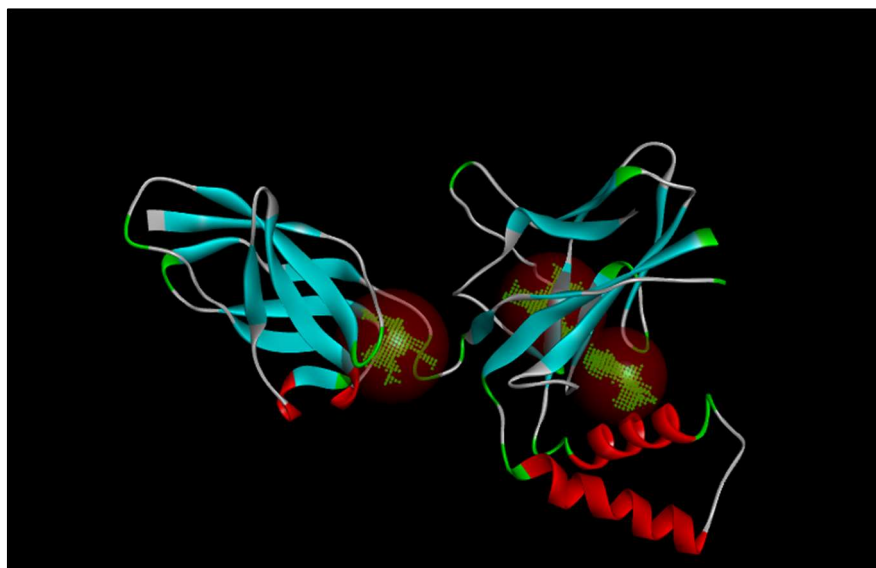


FIGURE 5.9: Top 3 binding sites of Hydrocortisone on p52 subunit of NF-κB

TABLE 5.10 XYZ Coordinates of the top 3 binding sites of Hydrocortisone on the p52 subunit of NF-κB

BINDING SITE	XYZ COORDINATES
SITE 1	1.059000 70.169000 8.556993
SITE 2	15.568275 54.634852 12.931503
SITE 3	15.568275 54.634852 12.931503

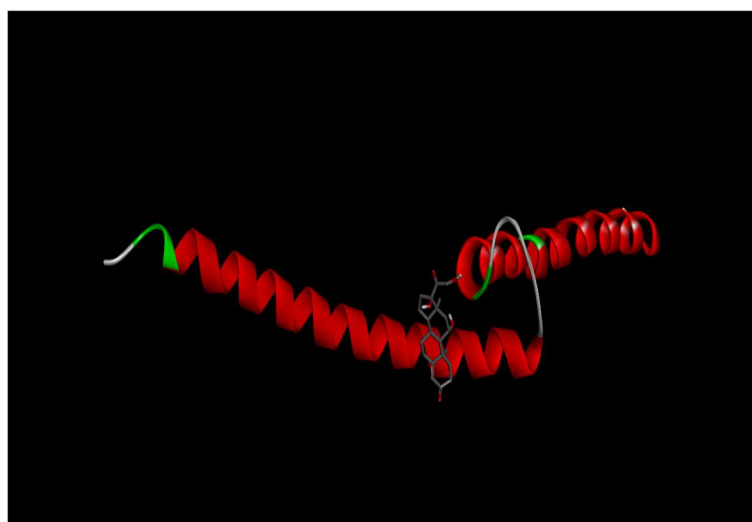


FIGURE 5.10 Image of the binding of Hydrocortisone to SREBP-1

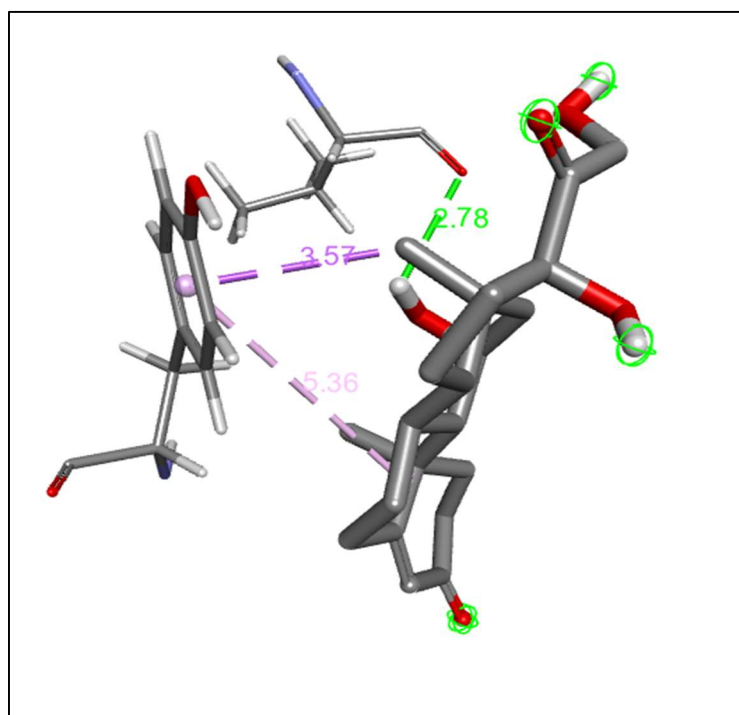


FIGURE 5.11 3D image of the binding of Hydrocortisone to SREBP-1

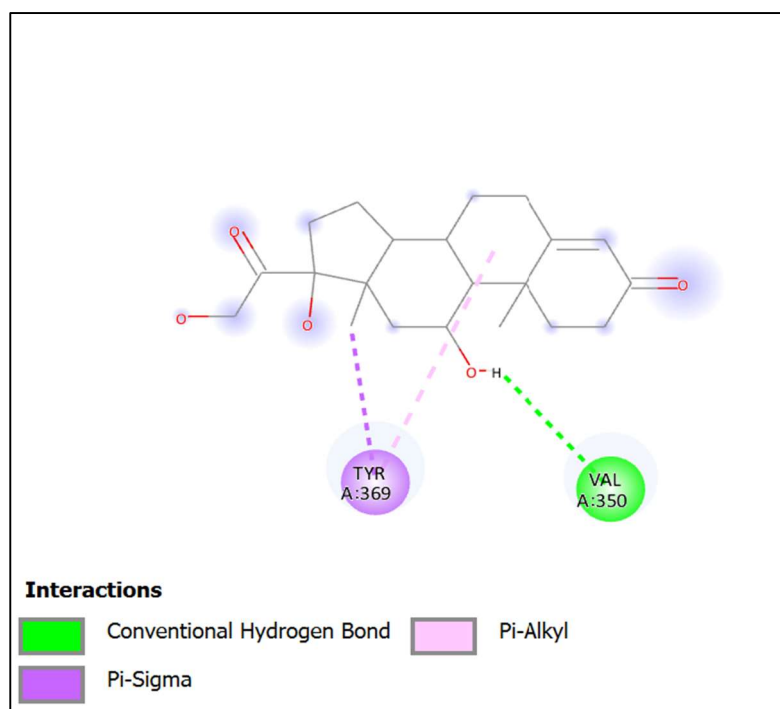


FIGURE 5.12 2D image showing the various interactions between the binding of Hydrocortisone to SREBP-1

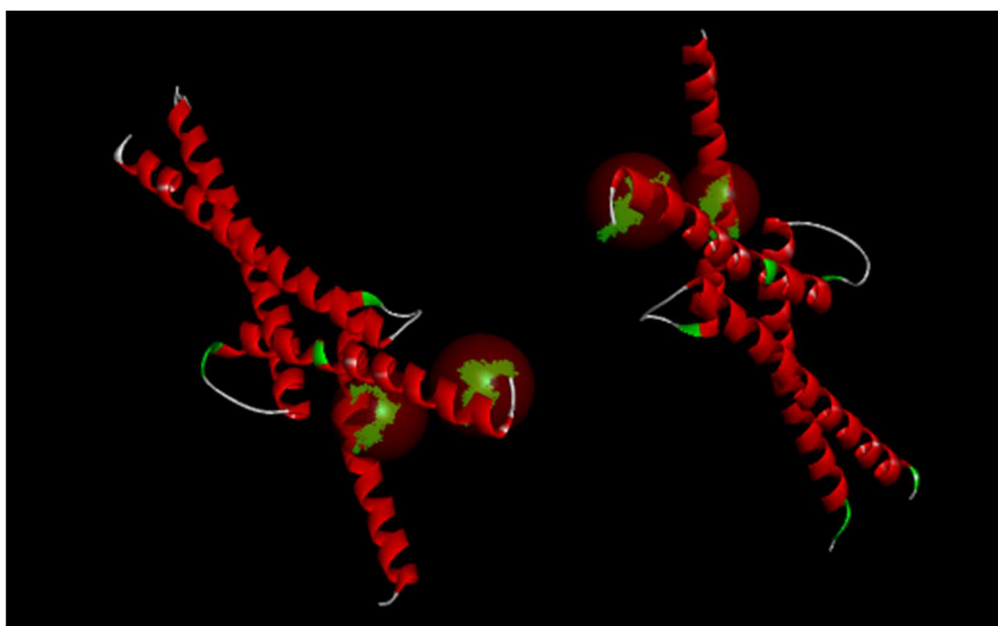


FIGURE 5.13 :Top 4 binding sites of Hydrocortisone on SREBP-1

TABLE 5.11 XYZ Coordinates of the top 4 binding sites of Hydrocortisone on SREBP-1

BINDING SITE	XYZ COORDINATES
SITE 1	57.603160 43.118000 169.235000
SITE 2	60.103160 39.368000 137.985000
SITE 3	43.603160 46.868000 178.735000
SITE 4	59.353160 24.368000 129.735000

5.2 In Vitro STUDIES

5.2.1 ASSESMENT OF CYTOTOXICITY OF HYDROCORTISONE – MTT ASSAY ON C₂C₁₂ CELL LINE

50 μ L of MTT solution was added to each well of the 96 well plate, The plate was wrapped in aluminium foil and incubated at 37°C for 3 hours. Over the formazan precipitates formed, Dimethyl sulfoxide DMSO, was added to each well to solubilize the crystals. The absorbance was taken at 570 nm using a microplate reader. The data obtained were then plotted in a graph with concentration on the x axis and % inhibition on the y axis and the IC₅₀ value was found .

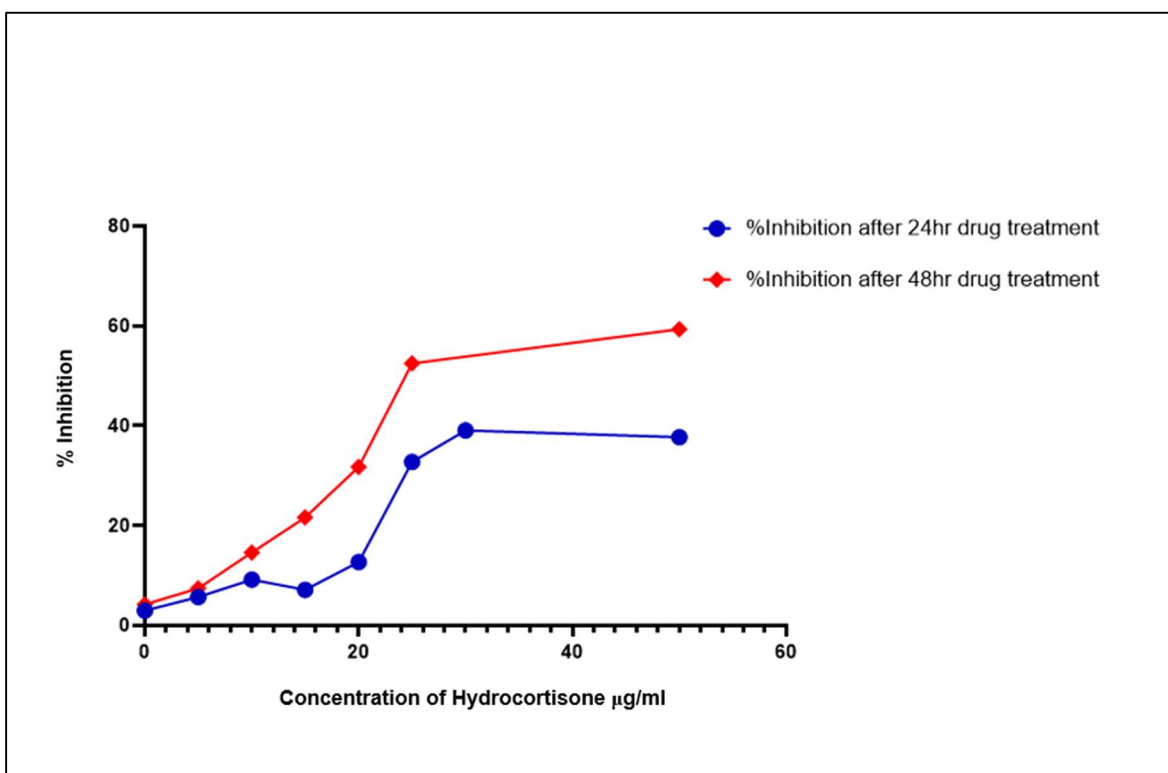


FIGURE 5.14 : Assessment of cytotoxicity of hydrocortisone on C₂C₁₂ cell line using MTT assay

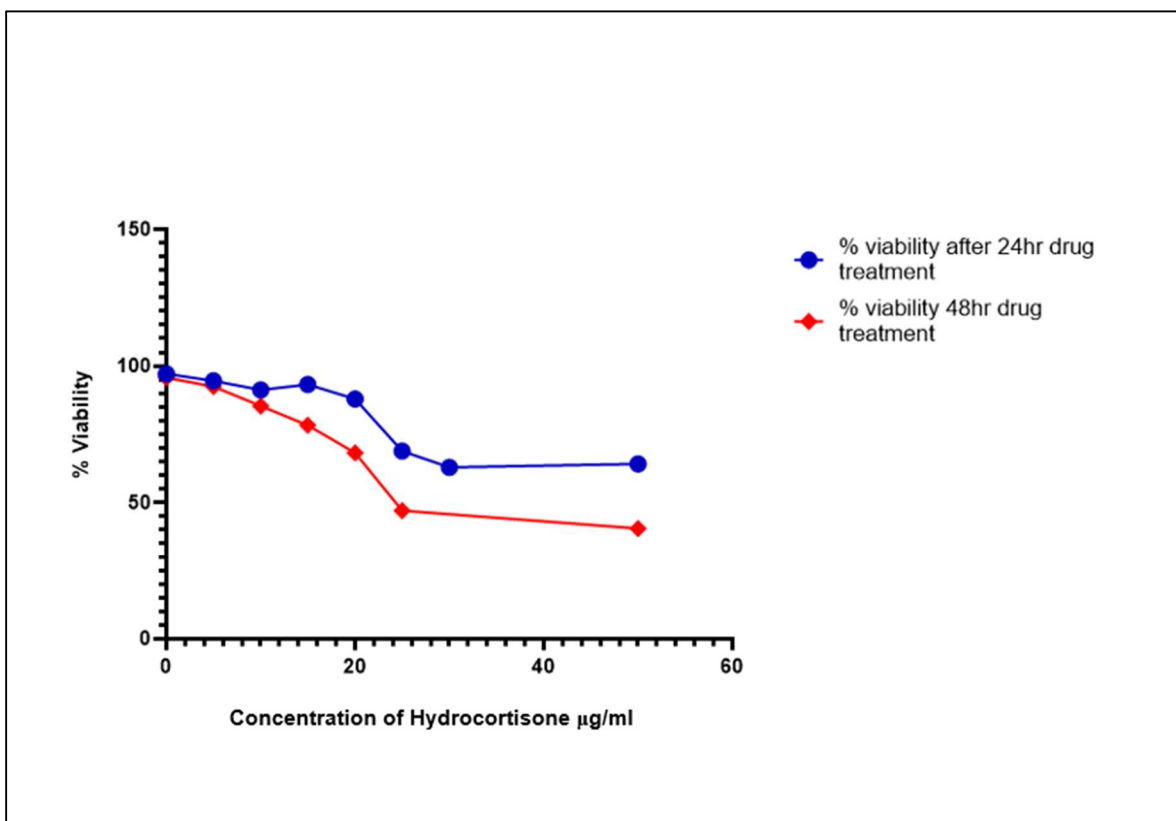


FIGURE 5.15 : Assessment of viability of C_2C_{12} cell line post hydrocortisone treatment using MTT assay

5.2.2 ASSESMENT OF ANTI-CANCER ACTIVITY OF HYDROCORTISONE -MTT ASSAY ON MDA-MB-231 CELL LINE

50 µL of MTT solution was added to each well of the 96 well plate, The plate was wrapped in aluminium foil and incubated at 37°C for 3 hours. Over the formazan precipitates formed, DMSO, was added to each well to solubilize the crystals. The absorbance was taken at 570 nm using a microplate reader. The data obtained were then plotted in a graph with concentration on the x axis and % inhibition on the y axis and the IC_{50} value was found . The IC_{50} value was found to be 14 µg/ml after 24 hours of drug treatment and 25 µg/ml after 48 hours of drug treatment.

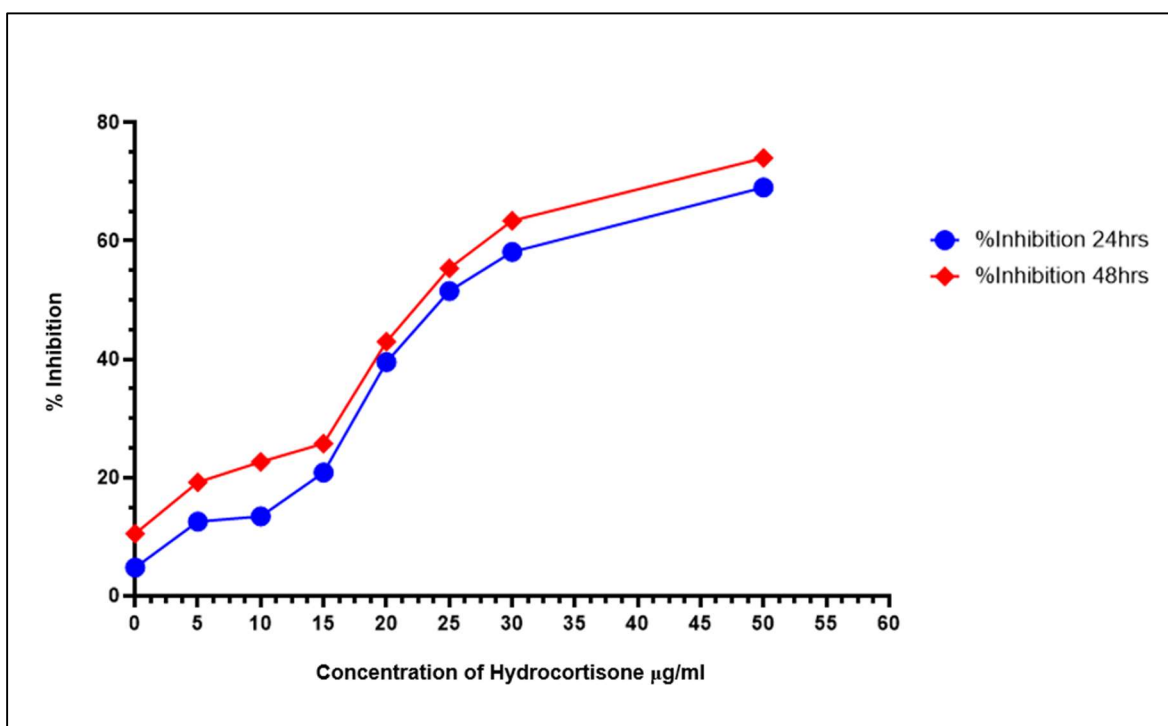


FIGURE 5.16: Assessment of anti-cancer activity of hydrocortisone on MDA-MD-231 cell line using MTT assay

5.2.3 APOPTOSIS DETECTION – AO/EB STAINING

AO/EB dual staining was done on the MDA-MD-231 after 24hrs and 48 hrs of drug treatment and images were taken using a fluorescence microscope. AO permeates both live and dead cells, emitting green fluorescence while EB only enters cells with compromised membrane integrity, emitting red fluorescence upon binding to nucleic acids. The bright greens dot represents cells in the early-apoptotic stage with fragmented nuclei while the necrotic cells appear yellow.

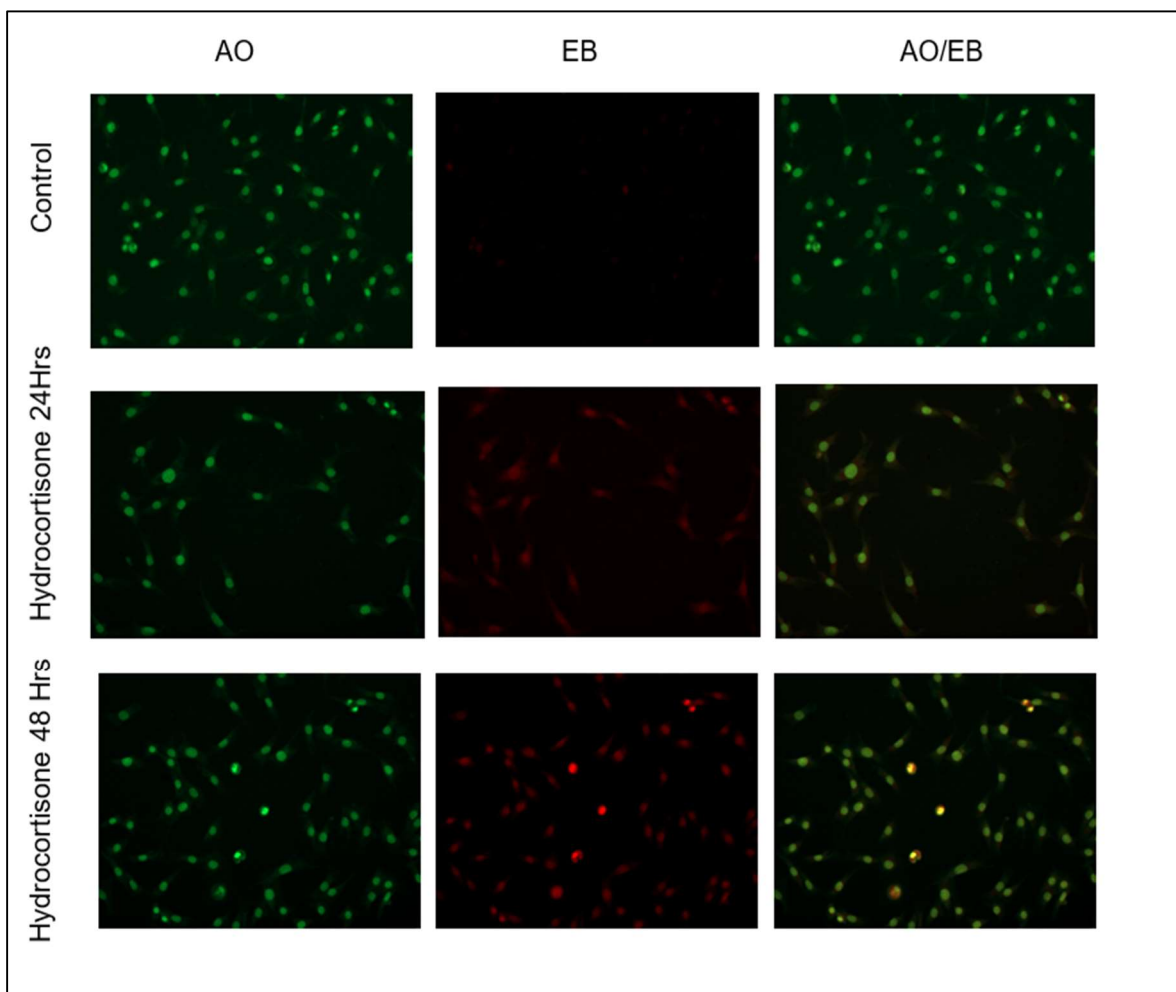


FIGURE 5.17 : AO/EB staining on MDA-MB-231 cell line

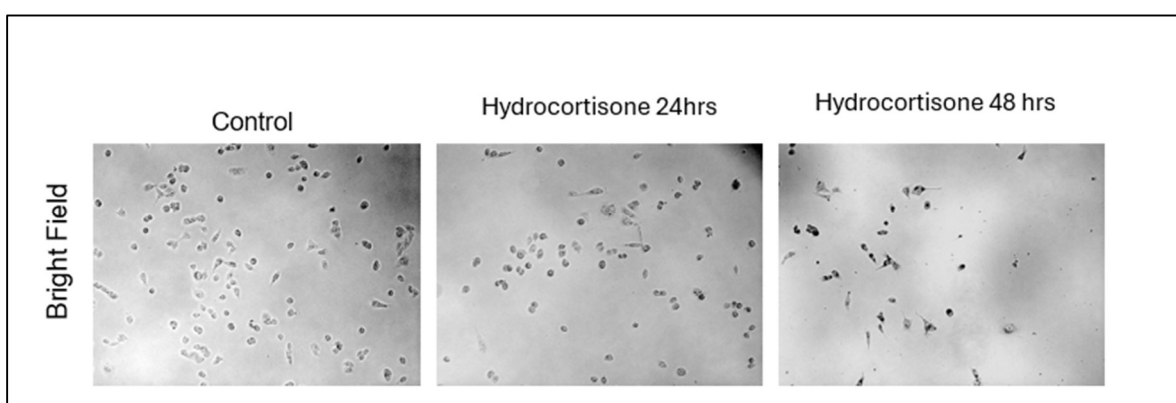


FIGURE 5.18 : Bright Field Microscopy images of the MDA-MB-231 cell line

5.2.4 TOTAL RNA ISOLATION AND QUANTIFICATION

The total RNA present in the cells were isolated using the TRizol method. The isolated RNA was then quantified using BioTek micro plate reader and stored at - 20°C for further conversion into cDNA.



FIGURE 5.19 : RNA isolation by TriZol method

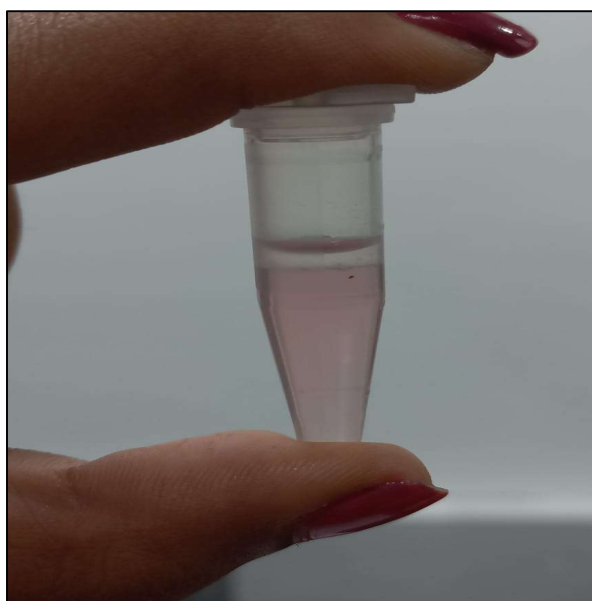


FIGURE 5.20 : Separation of the cell lysate into three phases after addition of chloroform

The ratio of OD at 260 to the OD at 280 was found to be 1.5. i.e., $260_{OD} / 280_{OD} = 1.5$. The amount of RNA in nanograms in 1 μ L was displayed using which the amount of RNA template to be added which will contain 1000 nanograms of RNA was calculated and used for further conversion into cDNA.

5.2.5 cDNA CONVERSION

The quantified RNA was converted into cDNA using a thermocycler using a reaction mix with the following components.

TABLE 5.12 : Components for cDNA conversion

COMPONENT	VOLUME PER REACTION μ L
5X iScript Reaction Mix	4
iScript Reverse Transcriptase	1
RNA template	15
Total volume	20

The above complete reaction mix was incubated in a thermal cycler using the following protocol:

TABLE 5.13 : cDNA conversion protocol

Priming	5 min at 25°C
Reverse Transcription	20 min at 46 °C
RT inactivation	1 min at 95 °C
Optional step	Hold at 4 °C



FIGURE 5.21: Converted cDNA

5.2.6 *q RT-PCR*

q RT-PCR was done to check the expression of genes responsible for inflammation (NF- κ B, TNF- α , and IL-6). These genes are found to be commonly overexpressed in most cancers including breast cancer. The expression of lipogenic genes ie, FASN and SREBP-1 were also checked. The cycle threshold values were obtained, and the fold change (Relative quantification) was calculated using the following formulae to check if the expression of the genes have been upregulated or downregulated.

FORMULAE USED

1. $\Delta C_t = C_t (\text{House-keeping gene}) - C_t (\text{gene of interest})$
2. $\Delta\Delta C_t = \Delta C_t - \text{Average } \Delta C_t$
3. $RQ = 2^{(\Delta\Delta C_t)}$

The calculated fold change value (RQ) of the controls, 24-hour drug treatment and 48-hour drug treatments were plotted in on bar graphs using Graphpad prism. The interpretations were as follows.

1. From the graph, it is evident that the expressions of the inflammatory genes NF- κ B, TNF- α , and IL-6 have been significantly reduced after the 24hr and 48 hr drug treatment.
2. The expression of Fatty acid synthase gene (FASN) was also found to be significantly reduced.
3. However, the expression of SREBP-1 was found to be upregulated after the 24hr drug treatment and slightly downregulated after 48 hours. This is because SREBP-1 is a transcription factor found bound to the endoplasmic reticulum and gets translocated to the nucleus where it aids the transcription of lipogenic genes like FASN . This translocation would take 72 hours and so we would be sure to see a significant reduction in its expression after 72 hours of drug treatment.

	A	B	C	D	E	F	G	H	I	J	K
1	TNF ALPHA										
2	NUMBER	SAMPLE	Ct GAPDH	Ct TNF	delta ct	Avg dCt	ddct	RQ	RQ avg	std dev	SE
3		1 CONTROL	21.43	34.64	-13.21	-13.035	0	1	1		
4		2 CONTROL	22.35	35.21	-12.86						
5		3 HYDROCOR	19.34	37.35	-18.01		-4.975	0.0317962	0.0311486	0.0009159	0.0006476
6		4 HYDROCOR	20.28	38.35	-18.07		-5.035	0.030501			
7		5 HYDROCOR	19.34	35.65	-16.31		-3.275	0.1033063	0.0615077	0.0591122	0.0417986
8		6 HYDROCOR	20.28	38.98	-18.7		-5.665	0.019709			
9											
10	IL6										
11	NUMBER	SAMPLE	Ct GAPDH	Ct IL6	delta ct	Avg dCt	ddct	RQ	RQ avg	std dev	SE
12		1 CONTROL	21.43	34.64	-13.21	-13.035	0	1	1		
13		2 CONTROL	22.35	35.21	-12.86						
14		3 HYDROCOR	19.34	37.82	-18.48		-5.445	0.0229558	0.0472505	0.034358	0.0242948
15		4 HYDROCOR	20.28	37.12	-16.84		-3.805	0.0715453			
16		5 CONTROL	21.43	23.55	-2.12	-1.66	0	1	1		
17		6 CONTROL	22.35	23.55	-1.2			1			
18		7 HYDROCOR	19.34	23.78	-4.44		-2.78	0.1455917	0.2124567	0.0004959	0.0003507
19		8 HYDROCOR	20.28	23.78	3.5		1.84	0.2793218			
20											
21	NF-KB										
22	NUMBER	SAMPLE	Ct GAPDH	Ct NF-KB	delta ct	Avg dCt	ddct	RQ	RQ avg	std dev	SE
23		1 CONTROL	21.43	34.64	-13.21	-13.035	0	1	1		
24		2 CONTROL	22.35	35.21	-12.86						
25		3 HYDROCOR	19.34	36.34	-17		-3.965	0.0640348	0.1170995	0.0750449	0.0530647
26		4 HYDROCOR	20.28	35.87	-15.59		-2.555	0.1701643			
27		5 HYDROCOR	19.34	34.92	-15.58		-2.545	0.1713479	0.2500418	0.11129	0.0786939
28		6 HYDROCOR	20.28	34.92	-14.64		-1.605	0.3287357			
29											

FIGURE 5.22: Fold change calculations for inflammatory genes

	A	B	C	D	E	F	G	H	I	J	K
30	FASN										
31	NUMBER	SAMPLE	Ct GAPDH	Ct FASN	delta ct	Avg dCt	ddct	RQ	RQ avg	std dev	SE
32		1 CONTROL	21.43	23.55	-2.12	-2.12	0	1			
33		2 CONTROL									
34		3 HYDROCOR	19.34	26.55	-7.21		-5.55	0.0213444	0.0305872	0.0130713	0.0092428
35		4 HYDROCOR	20.28	26.59	-6.31		-4.65	0.03983			
36		5 HYDROCOR	19.34	25.89	-6.55		-4.43	0.0463914	0.0676972	0.0301311	0.0213059
37		6 HYDROCOR	20.28	25.89	-5.61		-3.49	0.0890031			
38											
39	SREBP-1										
40	NUMBER	SAMPLE	Ct GAPDH	Ct NF-KB	delta ct	Avg dCt	ddct	RQ	RQ avg	std dev	SE
41		1 CONTROL	21.43	23.55	-2.12	-1.66	0	1			
42		2 CONTROL	22.35	23.55	-1.2						
43		3 HYDROCOR	19.34	19.93	-0.59		1.07	2.0994334	3.1059443	1.4234215	1.006511
44		4 HYDROCOR	20.28	19.9	0.38		2.04	4.1124553			
45		5 HYDROCOR	19.34	19.78	-0.44		1.22	2.3294672	3.3993079	1.5129832	1.0698407
46		6 HYDROCOR	20.28	19.78	0.5		2.16	4.4691486			
47											

FIGURE 5.23 : Fold change calculations for lipogenic genes

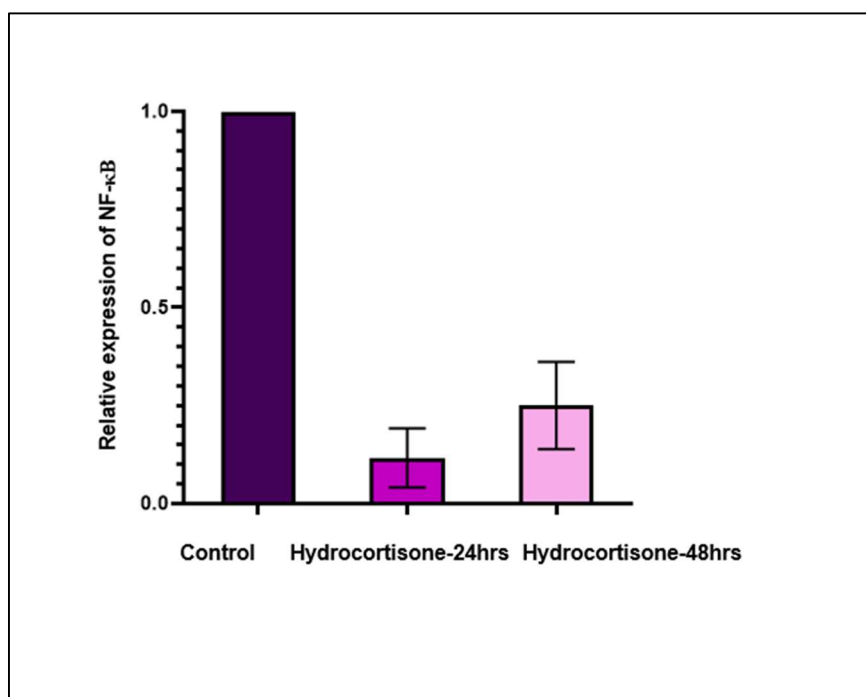


FIGURE 5.24 *Relative expression of NF-κB after 24 and 48 hrs of Hydrocortisone treatment*

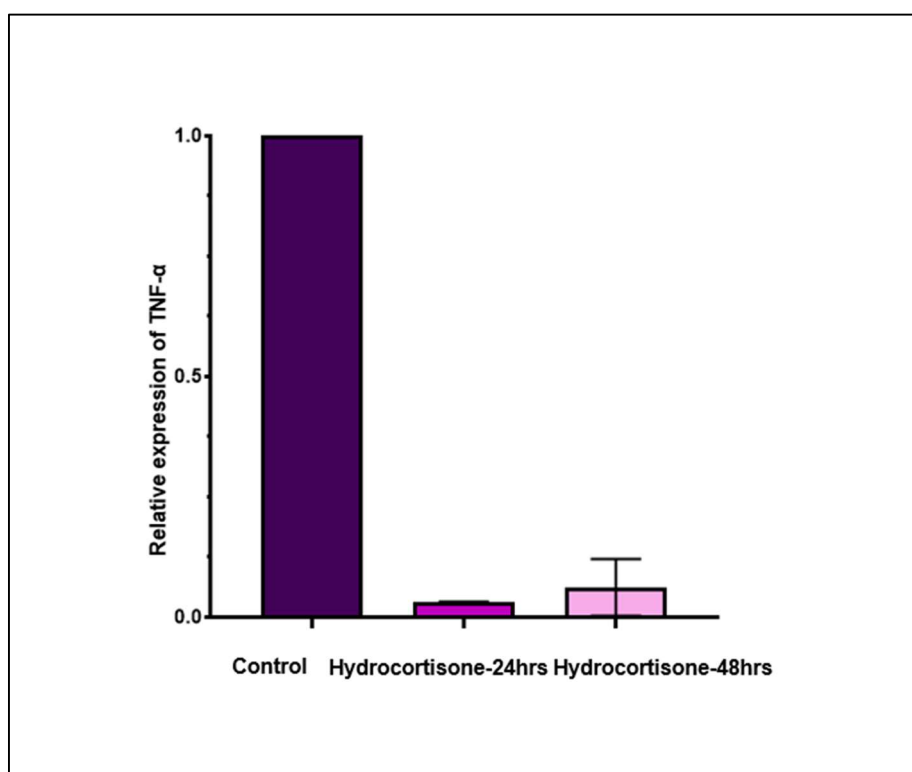


FIGURE 5.25 *Relative expression of TNF-α after 24 and 48 hrs of Hydrocortisone treatment*

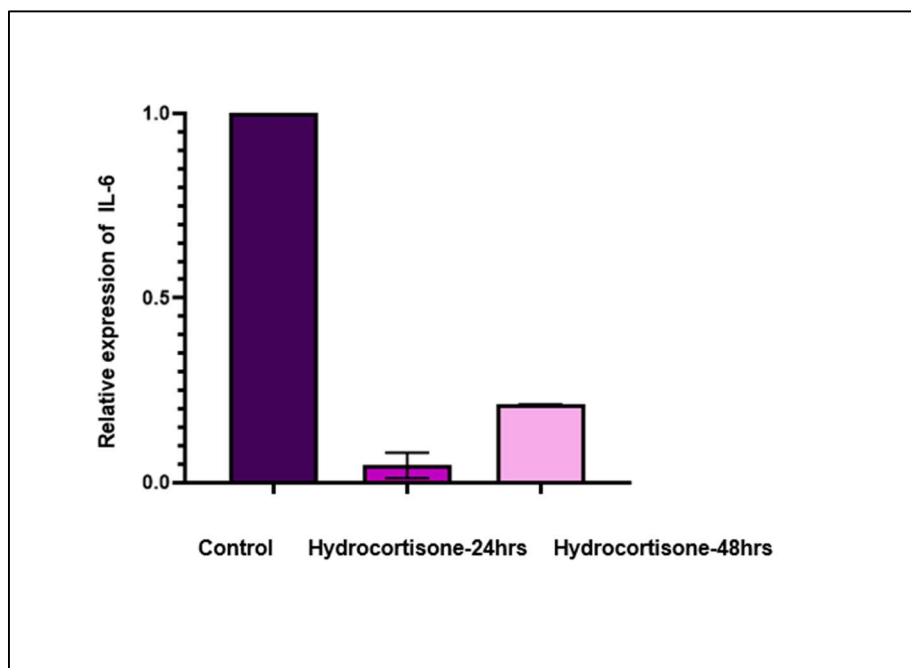


FIGURE 5.26 *Relative expression of IL-6 on MDA-MB 231*

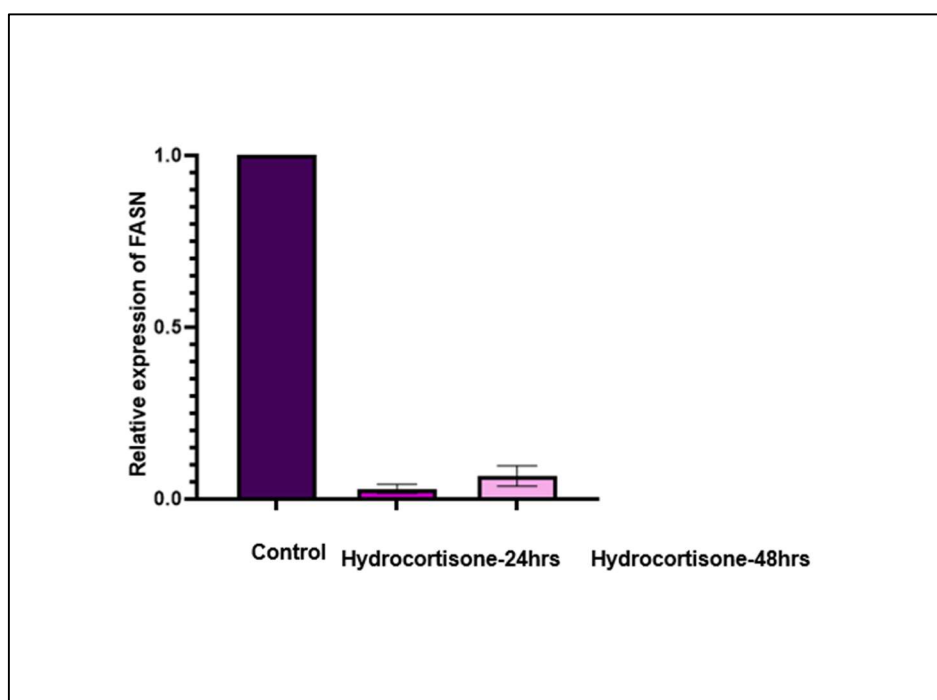


FIGURE 5.27 *Relative expression of FASN on MDA-MB 231*

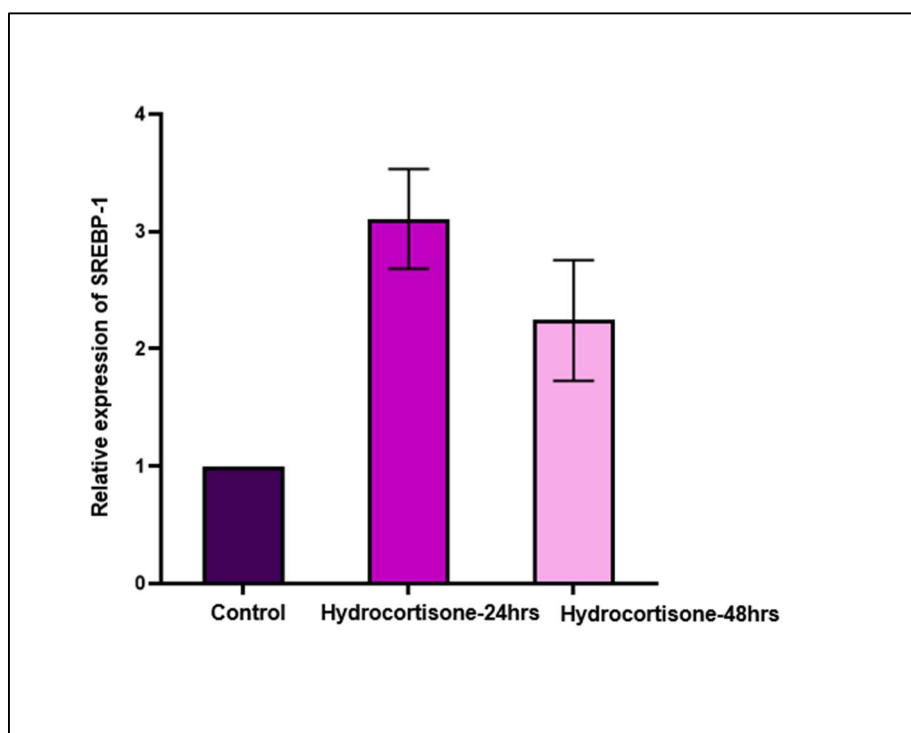


FIGURE 5.28 Relative expression of SREBP-1 on MDA-MB 231

5.3 STATISTICAL ANALYSIS

- Student's T-Test on the % inhibition of Hydrocortisone after 24 and 48hrs of treatment on the MDA-MB-231 cell line:

Null hypothesis H_0 = There was no significant changes in the % Inhibition of the MDA-MB-231 cell line after 24 and 48 hr of Hydrocortisone treatment.

Alternate Hypothesis H_a = There are significant changes in the % Inhibition of the MDA-MB-231 cell line after 24 and 48 hr of Hydrocortisone treatment.

Statistical analysis of the % inhibition of Hydrocortisone after 24 and 48hrs of treatment on the MDA-MB-231 cell line was done using the one tailed student's t-test with a significance level of 95%. (ie, P value of 0.05). The p value indicated by # between the control and treatment groups, was found to be the most significant ($p=0.001757 < 0.05$). This signifies that the null hypothesis can be rejected and that the drug (Hydrocortisone) has significant effect on the % inhibition of the MDA-MB-231 cell line after 24 and 48 hrs of treatment.

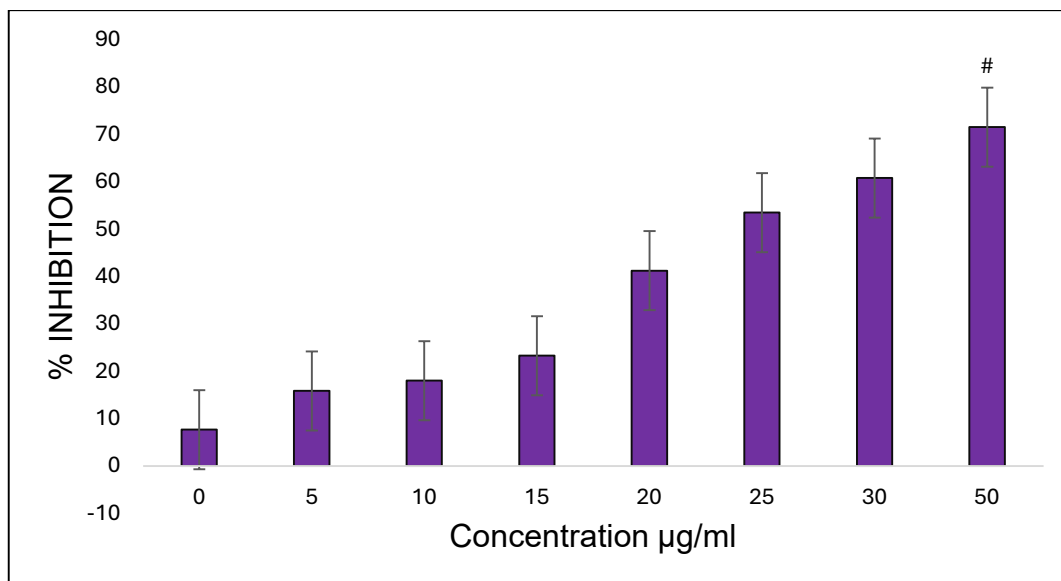


FIGURE 5.29: Student's T-Test on the % inhibition of Hydrocortisone after 24 and 48hrs of treatment on the MDA-MB-231 cell line

5.3.1 Chi square test on AO/EB staining images

Chi square test was done on the images obtained after AO/EB dual staining using Graph pad prism version 10 with a **p** value of **0.05** and was found to be **significant**. Cell counts to perform the Chi square test was done using the software ImageJ.

- The number of cells stained by **AO** gives us the **live cell** count.
- The number of cells stained by **EB** gives us the number of cells in the **early apoptosis** stage.
- The cell counts after overlaying **AO and EB**-stained images and counting the number of yellow cells gives us the number of necrotic cells.

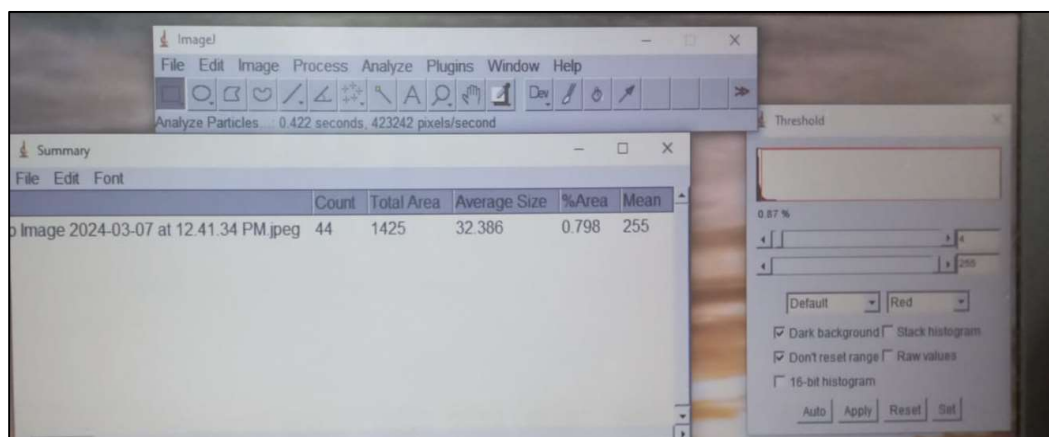


FIGURE 5.30: Cell count obtained using ImageJ

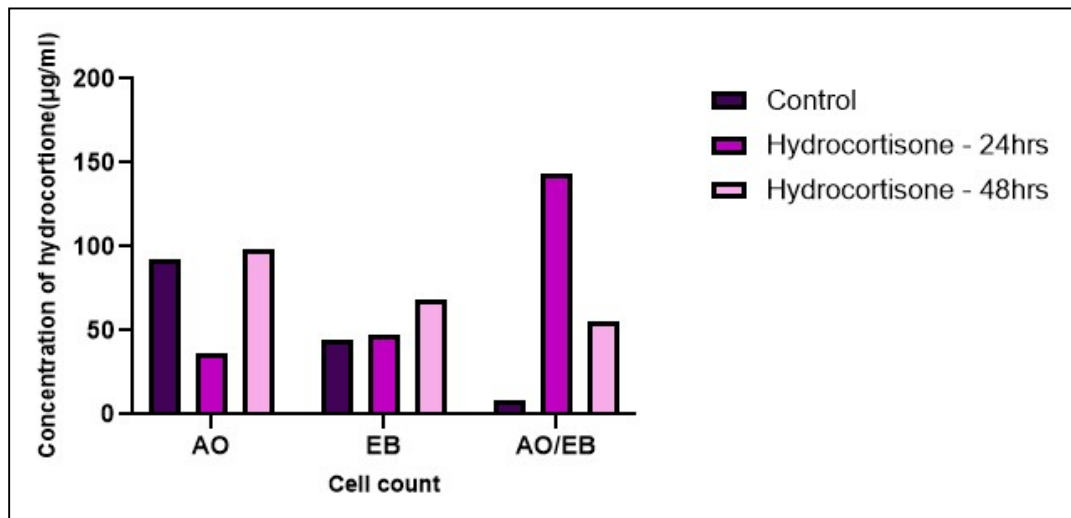


FIGURE 5.31 Chi square test on AO/EB dual staining images

Contingency		A
1	Table Analyzed	AOEB Chi square
2		
3	P value and statistical significance	
4	Test	Chi-square
5	Chi-square, df	175.2, 4
6	P value	<0.0001
7	P value summary	****
8	One- or two-sided	NA
9	Statistically significant (P < 0.05)?	Yes
10		
11	Data analyzed	
12	Number of rows	3
13	Number of columns	3

FIGURE 5.32 Chi square test results using GraphPad Prism version 10

5.3.2 Analysis of variance (ANOVA) on gene expression data

Statistical analysis of the gene expression data was done using one way Analysis of variance (ANOVA) with a significance level of 95% ie, P value of 0.05 using GraphPad Prism version 10. The data was found to be significant for NF- κ B, TNF- α , IL-6 and FASN.

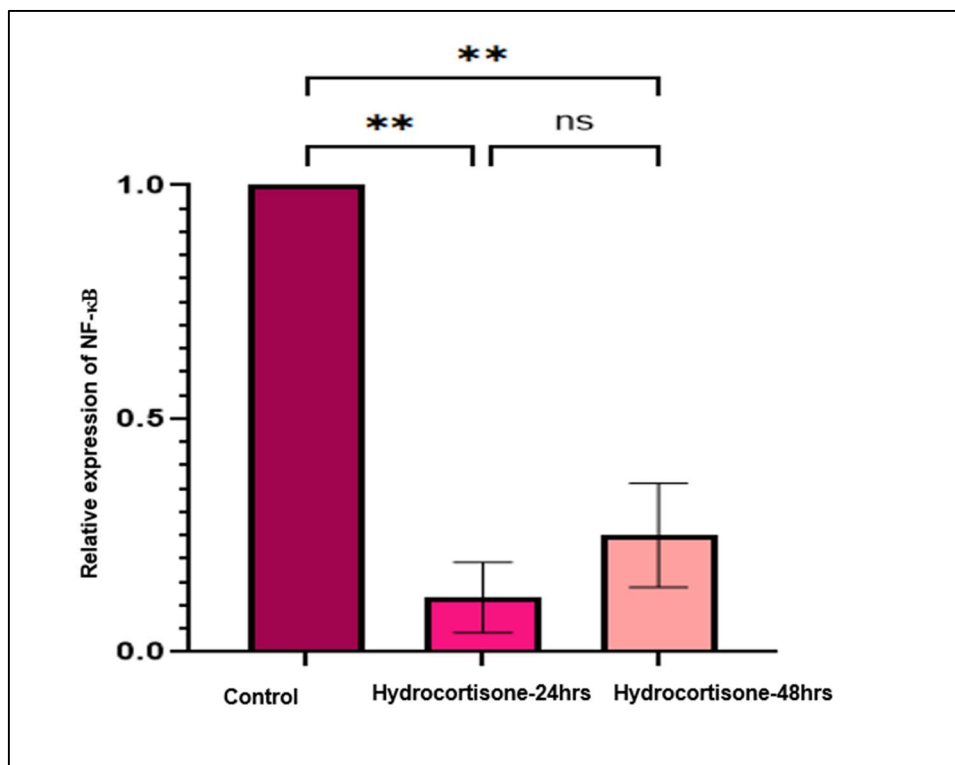


FIGURE 5.33 One-way ANOVA on the relative expression of NF- κ B using GraphPad Prism version 10 was found to be significant

Ordinary one-way ANOVA		
1	Table Analyzed	Anova-nfkb
2	Data sets analyzed	A-C
3		
4	ANOVA summary	
5	F	75.46
6	P value	0.0027
7	P value summary	**
8	Significant diff. among means (P < 0.05)?	Yes
9	R squared	0.9805

FIGURE 5.34 One-way ANOVA test results (NF- κ B) using GraphPad Prism version 10

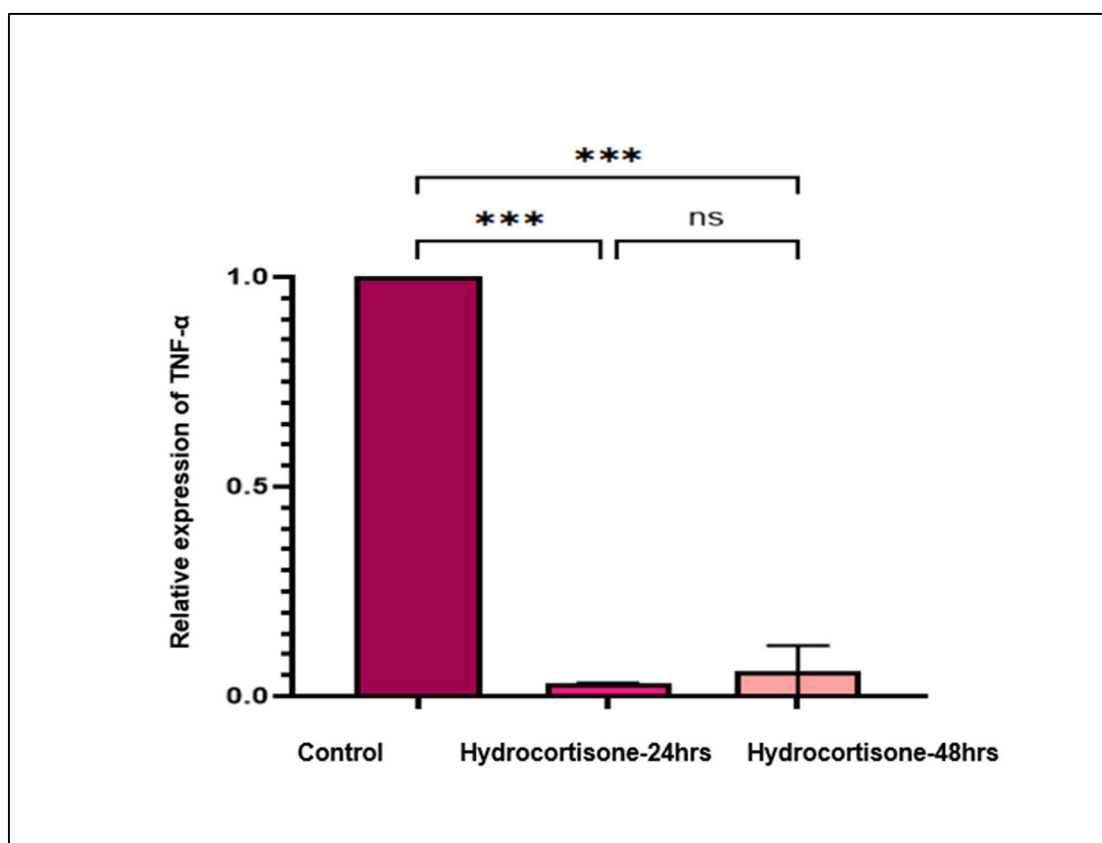


FIGURE 5.35 One-way ANOVA on the relative expression of TNF- α using GraphPad Prism version 10 and was found to be significant

Ordinary one-way ANOVA		
ANOVA results		
1	Table Analyzed	Anova -tnfa
2	Data sets analyzed	A-C
3		
4	ANOVA summary	
5	F	520.8
6	P value	0.0002
7	P value summary	***
8	Significant diff. among means (P < 0.05)?	Yes
9	R squared	0.9971

FIGURE 5.36 One-way ANOVA test results (TNF- α) using GraphPad Prism version 10

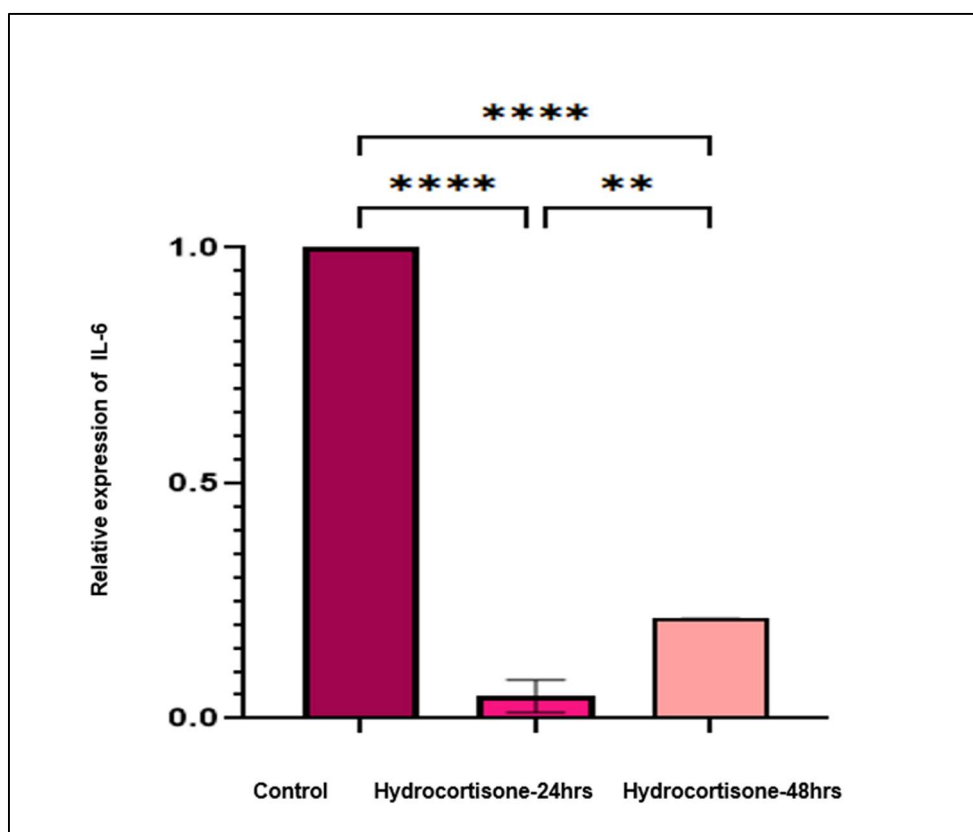


FIGURE 5.37 One-way ANOVA on the relative expression of IL-6 using GraphPad Prism version 10 and was found to be significant

ANOVA results		
Multiple comparisons		
Ordinary one-way ANOVA		
ANOVA results		
1	Table Analyzed	Anova- il6
2	Data sets analyzed	A-C
3		
4	ANOVA summary	
5	F	514.2
6	P value	<0.0001
7	P value summary	****
8	Significant diff. among means (P < 0.05)?	Yes
9	R squared	0.9942

FIGURE 5.38 One-way ANOVA results (IL – 6) using GraphPad Prism version 10

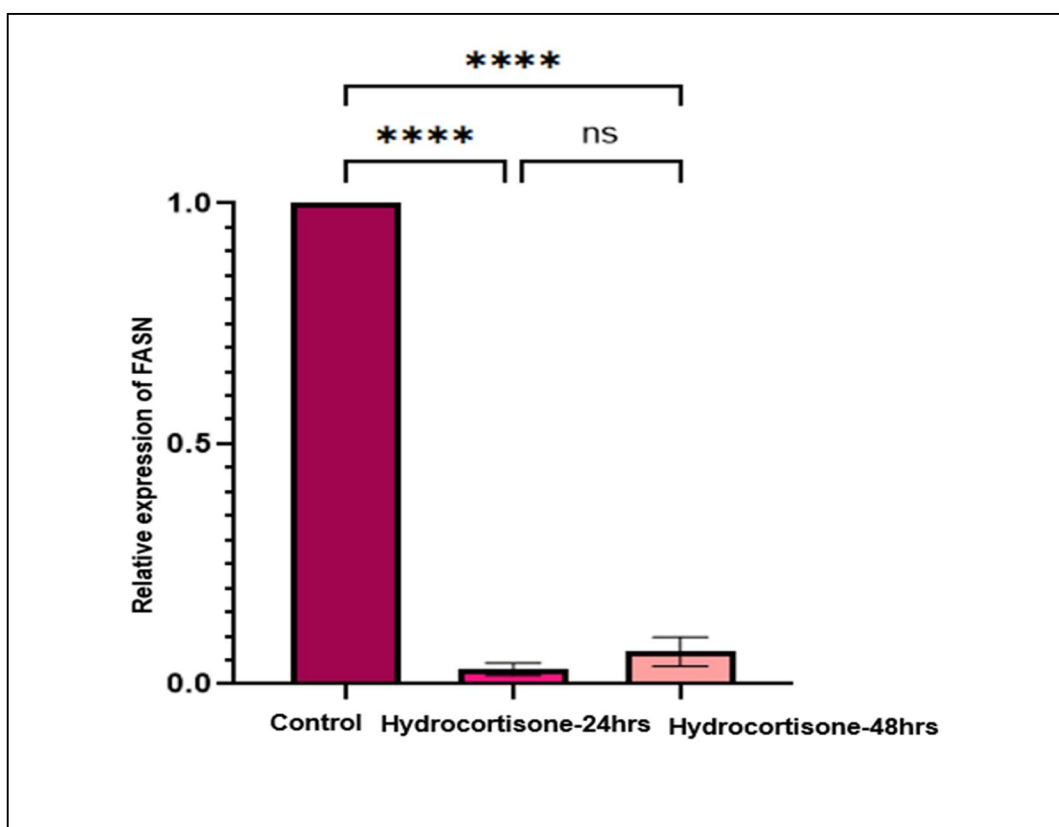


FIGURE 5.39 One-way ANOVA on the relative expression of FASN using GraphPad Prism version 10 and was found to be significant

1	Table Analyzed	Anova -FASN
2	Data sets analyzed	A-C
3		
4	ANOVA summary	
5	F	1678
6	P value	<0.0001
7	P value summary	****
8	Significant diff. among means (P < 0.05)?	Yes
9	R squared	0.9991

FIGURE 5.40 One-way ANOVA results (FASN) using GraphPad Prism version 10

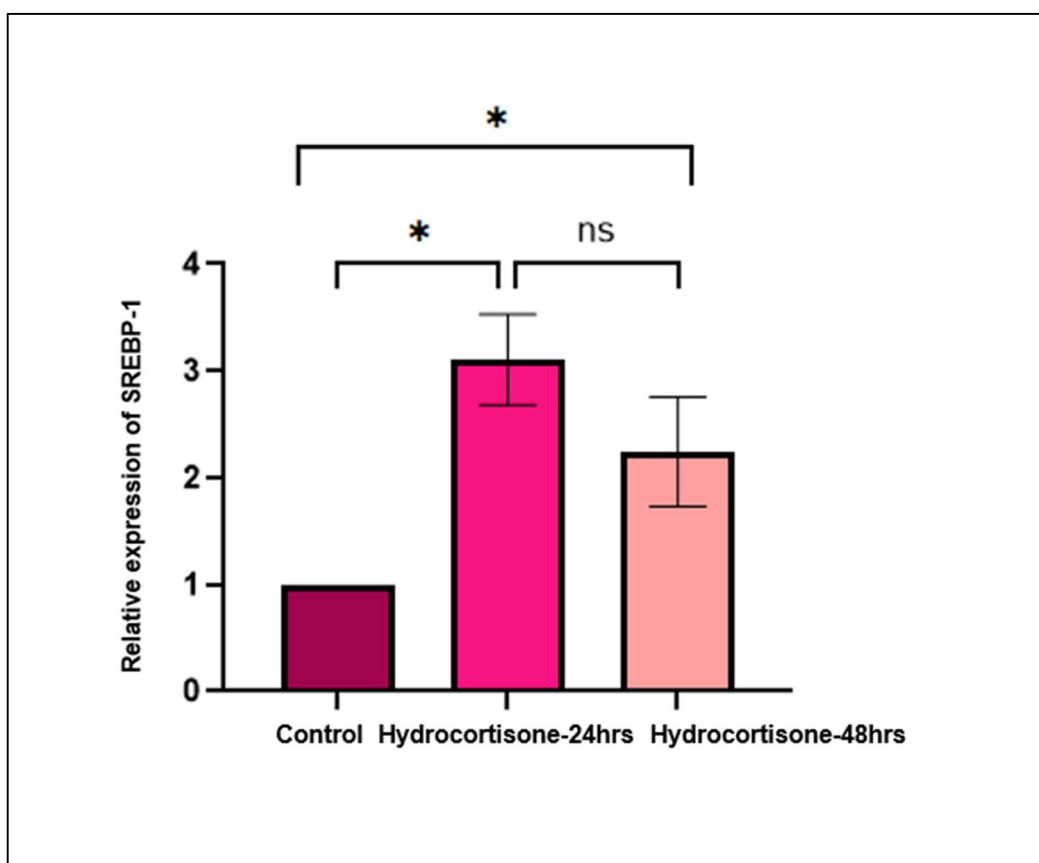


FIGURE 5.41 One-way ANOVA on the relative expression of SREBP-1 using GraphPad Prism version 10

1	Table Analyzed	anova sreb
2	Data sets analyzed	A-C
3		
4	ANOVA summary	
5	F	15.20
6	P value	0.0269
7	P value summary	*
8	Significant diff. among means ($P < 0.05$)?	Yes
9	R squared	0.9102

FIGURE 5.42 One-way ANOVA results (SREBP-1) using GraphPad Prism version 10

5.4 DISCUSSION

This study represents a significant step towards the understanding and treatment of breast cancer, aligning with the **United Nations Sustainable Development Goal 3 of promoting good health and well-being**. The findings shed light on the potential therapeutic role of hydrocortisone in targeting breast cancer by modulating inflammatory gene expressions and lipogenesis. The investigation began with the analysis of the expression of major inflammatory markers namely NF- κ B, TNF- α , and IL-6, which are known to play crucial roles in causing breast tumour-associated inflammation, a major hallmark of breast cancer, ultimately leading to the progression and metastasis of breast cancer (Balkwill et al., 2001). **By employing high-throughput Biotechnology techniques and *In Vitro* cell culture techniques**, this study validated the efficacy of hydrocortisone in targeting breast cancer cells while sparing normal cells. The significant reduction observed in the expression of these genes following hydrocortisone treatment for 24 and 48 hours corroborates previous studies suggesting the tumour-related anti-inflammatory properties of glucocorticoids (Corticosteroid-related adverse effects, 1991). This suggests a potential mechanism through which hydrocortisone may exert its anti-cancer effects by dampening the expression of inflammatory genes within breast cancer cells. Furthermore, this study delved into the impact of hydrocortisone on genes associated with lipogenesis, yet another major hallmark associated with breast cancer. Lipogenesis, regulated by key enzymes like FASN and transcription factors like SREBP-1, contributes to tumour growth and aggressiveness (Menendez & Lupu, 2007). The findings demonstrate a significant downregulation of FASN expression following hydrocortisone treatment, indicating a potential disruption of lipid metabolism within breast cancer cells. Although the reduction in SREBP-1 expression was not as pronounced, it is reasonable to anticipate further suppression with prolonged drug exposure, suggesting a time-dependent effect of hydrocortisone on lipogenic pathways. This validation not only meets global research standards but also underscores **the importance of engaging in lifelong learning and adopting an investigative approach to address complex health challenges**.

By elucidating the molecular mechanisms underlying the anti-breast cancer-related inflammation and anti-lipogenic effects of hydrocortisone in breast cancer

cells, this study offers valuable insights for the development of novel therapeutic strategies for repurposed cancer drugs and this investigation was further backed up by the good binding scores obtained from the *in silico* molecular docking studies. Targeting inflammation and aberrant lipid metabolism represents a promising approach to combatting breast cancer progression and improving patient outcomes. As a committed technologist, **the multidisciplinary thinking** applied in this study demonstrates the potential to enhance the quality of life for individuals affected by breast cancer.

Future directions of this research include exploring the expression profiles of additional inflammatory and lipogenic genes, thereby providing a more comprehensive understanding of the molecular effects of hydrocortisone. Furthermore, *in vivo* studies utilizing animal models, such as zebrafish, could offer valuable insights into the systemic effects of hydrocortisone treatment and its impact on tumour growth and metastasis. We also aim to explore the development of nanoparticles as a novel drug delivery system for hydrocortisone in breast cancer treatment. By encapsulating hydrocortisone within nanoparticles, we can potentially enhance its bioavailability, improve its stability, and achieve targeted delivery to breast cancer cells while minimizing off-target effects. Thus, we aim to advance towards more effective and personalized treatment strategies for breast cancer patients.

CHAPTER 6

SUMMARY AND CONCLUSION

This study investigated the therapeutic potential of hydrocortisone in breast cancer treatment through *in vitro* experimentation and *in silico* molecular docking studies. Results revealed significant reductions in inflammatory markers NF- κ B, TNF- α , and IL-6 following hydrocortisone treatment, indicating its anti-inflammatory effects. Moreover, hydrocortisone downregulated FASN expression, disrupting lipid metabolism in breast cancer cells, with potential for further suppression of SREBP-1 expression. Molecular docking studies supported these findings, highlighting hydrocortisone's binding affinity with key regulatory proteins. These results suggest hydrocortisone's potential as a complementary therapy in breast cancer management by targeting inflammation and lipid metabolism. Despite limitations, including the need for clinical validation, this study offers promising insights for future research to explore additional molecular targets and conduct *in vivo* studies for a comprehensive understanding of hydrocortisone's effects.

REFERENCES

- Alcohol, tobacco and breast cancer – collaborative reanalysis of individual data from 53 epidemiological studies, including 58 515 women with breast cancer and 95 067 women without the disease. (2002). *British Journal of Cancer*, 87(11), 1234–1245.
- Ammirante, M., Shalapour, S., Kang, Y., Jamieson, C. A. M., & Karin, M. (2014). Tissue injury and hypoxia promote malignant progression of prostate cancer by inducing CXCL13 expression in tumor myofibroblasts. *Proceedings of the National Academy of Sciences*, 111(41), 14776–14781.
- Ashburn, T. T., & Thor, K. B. (2004). Drug repositioning: Identifying and developing new uses for existing drugs. *Nature Reviews Drug Discovery*, 3(8), 673–683.
- Balkwill, F. (2006). TNF- α in promotion and progression of cancer. *Cancer and Metastasis Reviews*, 25(3), pp.409–416.
- Balkwill, F. (2009). Tumour necrosis factor and cancer. *Nature Reviews Cancer*, 9(5), 361–371.
- Balkwill, F., & Mantovani, A. (2001). Inflammation and cancer: Back to Virchow? *The Lancet*, 357(9255), 539–545.
- Bao, J., Zhu, L., Zhu, Q., Su, J., Liu, M. and Huang, W. (2016). SREBP-1 is an independent prognostic marker and promotes invasion and migration in breast cancer. *Oncology Letters*, 12(4), pp.2409–2416.
- Barkett, M., & Gilmore, T. D. (1999). Control of apoptosis by Rel/NF- κ B transcription factors. *Oncogene*, 18(49), 6910–6924.
- Becker KL (2001). *Principles and Practice of Endocrinology and Metabolism*. Lippincott Williams & Wilkins. p. 762. ISBN 978-0-7817-1750-2. Archived from the original on 14 September 2016.
- Becker, C., Fantini, M. C., Schramm, C., Lehr, H. A., Wirtz, S., Nikolaev, A., Burg, J., Strand, S., Kiesslich, R., Huber, S., Ito, H., Nishimoto, N., Yoshizaki, K., Kishimoto, T., Galle, P. R., Blessing, M., Rose-John, S., & Neurath, M. F. (2004). TGF- β Suppresses Tumor Progression in Colon Cancer by Inhibition of IL-6 trans-Signaling. *Immunity*, 21(4), 491–501.
- Ben-Baruch, A. (2002). Host microenvironment in breast cancer development: Inflammatory cells, cytokines and chemokines in breast cancer progression:

Reciprocal tumor–microenvironment interactions. *Breast Cancer Research*, 5(1).

Ben-Neriah, Y., & Karin, M. (2011). Inflammation meets cancer, with NF- κ B as the matchmaker. *Nature Immunology*, 12(8), 715–723.

Bottex-Gauthier, C., Pollet, S., Favier, A. and Vidal, D.R. (2002). Les facteurs de transcription Rel/NF-B : rôle complexe dans les régulations cellulaires. *Pathologie Biologie*, 50(3), pp.204–211.

Brewer, H. R., Jones, M. E., Schoemaker, M. J., Ashworth, A., & Swerdlow, A. J. (2017). Family history and risk of breast cancer: An analysis accounting for family structure. *Breast Cancer Research and Treatment*, 165(1), 193–200.

Brocke-Heidrich, K., Kretzschmar, A. K., Pfeifer, G., Henze, C., Löffler, D., Koczan, D., Thiesen, H.-J., Burger, R., Gramatzki, M., & Horn, F. (2004). Interleukin-6–dependent gene expression profiles in multiple myeloma INA-6 cells reveal a Bcl-2 family–independent survival pathway closely associated with Stat3 activation. *Blood*, 103(1), 242–251.

Catsburg, C., Miller, A. B., & Rohan, T. E. (2014). Active cigarette smoking and risk of breast cancer. *International Journal of Cancer*, 136(9), 2204–2209.

Cerhan, J. R., Anderson, K. E., Janney, C. A., Vachon, C. M., Witzig, T. E., & Habermann, T. M. (2003). Association of aspirin and other non-steroidal anti-inflammatory drug use with incidence of non-hodgkin lymphoma. *International Journal of Cancer*, 106(5), 784–788.

Chehboun, S., Labrecque-Carbonneau, J., Pasquin, S., Meliani, Y., Meddah, B., Ferlin, W., M., Tormo, A., Masson, J.-F., & Gauchat, J.-F. (2017). Epstein-Barr virus-induced gene 3 (EBI3) can mediate IL-6 trans-signaling. *Journal of Biological Chemistry*, 292(16), 6644–6656.

Choy, E. H., Kavanaugh, A. F., & Jones, S. A. (2013). The problem of choice: Current biologic agents and future-prospects in RA. *Nature Reviews Rheumatology*, 9(3), 154–163.

Conze, D., Weiss, L., Regen, P. S., Bhushan, A., Weaver, D., Johnson, P., & Rincón, M. (2001). Autocrine production of interleukin 6 causes multidrug resistance in breast cancer cells¹. *Cancer Research*, 61(24), 8851–8858.

Correction: An LXR agonist promotes glioblastoma cell death through inhibition of an egfr/akt/srebp-1/ldlr–dependent pathway. (2012). *Cancer Discovery*, 2(2), 190–191.

Corticosteroid-related adverse effects. (1991). *Reactions Weekly*, &NA;(334), p.12.

Coussens, L. M., Zitvogel, L., & Palucka, A. K. (2013). Neutralizing tumor-promoting chronic inflammation: A magic bullet? *Science*, 339(6117), 286–291.

DeBerardinis, R. J. (2020). Tumor Microenvironment, Metabolism, and Immunotherapy. *New England Journal of Medicine*, 382(9), 869–871.

Derveaux, S., Vandesompele, J., & Hellemans, J. (2010). How to do successful gene expression analysis using real-time PCR. *Methods*, 50(4), 227–230.

DeSantis, C.E., Fedewa, S.A., Goding Sauer, A., Kramer, J.L., Smith, R.A. and Jemal, A. (2015). Breast cancer statistics, 2015: Convergence of incidence rates between black and white women. *CA: A Cancer Journal for Clinicians*, 66(1), pp.31–42.

DiDonato, J. A., Mercurio, F., & Karin, M. (2012). NF-κB and the link between inflammation and cancer. *Immunological Reviews*, 246(1), 379–400.

Dieffenbach, C. W., Lowe, T. M., & Dveksler, G. S. (1993). General concepts for PCR primer design. *Genome Research*, 3(3), S30–S37.

DiMasi, J. A., Feldman, L., Seckler, A., & Wilson, A. (2010). Trends in risks associated with new drug development: Success rates for investigational drugs. *Clinical Pharmacology & Therapeutics*, 87(3), 272–277.

Dinareello, C. A. (2007). Historical insights into cytokines. *European Journal of Immunology*, 37(S1), S34–S45.

Dranoff, G. (2004). Cytokines in cancer pathogenesis and cancer therapy. *Nature Reviews Cancer*, 4(1), 11–22.

Drukteinis, J.S., Mooney, B.P., Flowers, C.I. and Gatenby, R.A. (2013). Beyond Mammography: New Frontiers in Breast Cancer Screening. *The American Journal of Medicine*, 126(6), pp.472–479.

Esslimani-Sahla, M., Thezenas, S., Simony-Lafontaine, J., Kramar, A., Lavaill, R., Chalbos, D., & Rochefort, H. (2006). Increased expression of fatty acid synthase and progesterone receptor in early steps of human mammary carcinogenesis. *International Journal of Cancer*, 120(2), 224–229.

Figure 6: Food and Drug Administration (FDA) approved drugs. (n.d.).

Flint, T. R., Janowitz, T., Connell, C. M., Roberts, E. W., Denton, A. E., Coll, A. P., Jodrell, D. I., & Fearon, D. T. (2016). Tumor-Induced IL-6 reprograms host metabolism to suppress anti-tumor immunity. *Cell Metabolism*, 24(5), 672–684.

Franke, T. M., Ho, T., & Christie, C. A. (2011). The chi-square test. *American Journal of Evaluation*, 33(3), 448–458.

Gansler, T. S., Hardman, W., III, Hunt, D. A., Schaffel, S., & Hennigar, R. A. (1997). Increased expression of fatty acid synthase (OA-519) in ovarian neoplasms predicts shorter survival. *Human Pathology*, 28(6), 686–692.

Google Scholar. (n.d.). [http://refhub.elsevier.com/S0303-7207\(13\)00241-4/h0090](http://refhub.elsevier.com/S0303-7207(13)00241-4/h0090)

Greene, C. S., & Voight, B. F. (2016). Pathway and network-based strategies to translate genetic discoveries into effective therapies. *Human Molecular Genetics*, 25(R2), R94–R98

Grivennikov, S. I., Greten, F. R., & Karin, M. (2010). Immunity, inflammation, and cancer. *Cell*, 140(6), 883–899.

Gudkov, A. V., Gurova, K. V., & Komarova, E. A. (2011). Inflammation and p53: A Tale of Two Stresses. *Genes & Cancer*, 2(4), 503–516.

Guttridge, D. C., Albanese, C., Reuther, J. Y., Pestell, R. G., & Baldwin, A. S., Jr. (1999). NF- κ B Controls Cell Growth and Differentiation through Transcriptional Regulation of Cyclin D1. *Molecular and Cellular Biology*, 19(8), 5785–5799.

Hallmarks of cancer: The next generation. (n.d.). *Cell*, 144(5), 646–674.

Hartkopf, A. D., Banys, M., & Fehm, T. (2012). HER2-Positive dtcs/ctcs in breast cancer. In *Recent Results in Cancer Research* (pp. 203–215). Springer Berlin Heidelberg.

Heikkilä, K., Ebrahim, S. and Lawlor, D.A. (2008). Systematic review of the association between circulating interleukin-6 (IL-6) and cancer. *European Journal of Cancer*, 44(7), pp.937–945.

Heinrich, P. C., Behrmann, I., Haan, S., Hermanns, H. M., Müller-Newen, G., & Schaper, F. (2003). Principles of interleukin (IL)-6-type cytokine signalling and its regulation. *Biochemical Journal*, 374(1), 1–20.

Horiguchi, A., Asano, T., Asano, T., Ito, K., Sumitomo, M., & Hayakawa, M. (2008). Pharmacological inhibitor of fatty acid synthase suppresses growth and invasiveness of renal cancer cells. *Journal of Urology*, 180(2), 729–736.

Horn, J., & Vatten, L. (2017). Reproductive and hormonal risk factors of breast cancer: A historical perspective. *International Journal of Women's Health*, Volume 9, 265–272.

Hortobagyi, G. N. (2000). Developments in chemotherapy of breast cancer. *Cancer*, 88(S12), 3073–3079. [https://doi.org/10.1002/1097-0142\(20000615\)88:12+<3073::aid-cnrcr26>3.0.co;2-r](https://doi.org/10.1002/1097-0142(20000615)88:12+<3073::aid-cnrcr26>3.0.co;2-r)
Hunter, C. A., & Jones, S. A. (2015). IL-6 as a keystone cytokine in health and disease. *Nature Immunology*, 16(5), 448–457.

Huang, W.-C., Li, X., Liu, J., Lin, J., & Chung, L. W. K. (2012). Activation of androgen receptor, lipogenesis, and oxidative stress converged by SREBP-1 is responsible for regulating growth and progression of prostate cancer cells. *Molecular Cancer Research*, 10(1), 133–142.

Hurle, M. R., Yang, L., Xie, Q., Rajpal, D. K., Sanseau, P., & Agarwal, P. (2013). Computational drug repositioning: From data to therapeutics. *Clinical Pharmacology & Therapeutics*, 93(4), 335–341.

Israel, A. (2009). The IKK Complex, a Central Regulator of NF- κ B Activation. *Cold Spring Harbor Perspectives in Biology*, 2(3), a000158–a000158.

J. Fernandez Menendez and Lupu, R. (2017). Fatty acid synthase (FASN) as a therapeutic target in breast cancer. *Expert Opinion on Therapeutic Targets*, 21(11), pp.1001–1016.

Joyce, D., Albanese, C., Steer, J., Fu, M., Bouzahzah, B., & Pestell, R. (2001). NF- κ B and cell-cycle regulation: The cyclin connection. *Cytokine & Growth Factor Reviews*, 12(1), 73–90.

Jung, C., Greco, S., Nguyen, H. H., Ho, J. T., Lewis, J. G., Torpy, D. J., & Inder, W. J. (2014). Plasma, salivary and urinary cortisol levels following physiological and stress doses of hydrocortisone in normal volunteers. *BMC Endocrine Disorders*, 14(1).

Kaitin, K. I. (2012). Translational research and the evolving landscape for biomedical innovation. *Journal of Investigative Medicine*, 60(7), 995–998.

Karin, M. and Delhase, M. (2000). The I κ B kinase (IKK) and NF- κ B: key elements of proinflammatory signalling. *Seminars in Immunology*, 12(1), pp.85–98.

Keiser, M. J., Setola, V., Irwin, J. J., Laggner, C., Abbas, A. I., Hufeisen, S. J., Jensen, N. H., Kuijer, M. B., Matos, R. C., Tran, T. B., Whaley, R., Glennon, R. A., Hert, J., Thomas, K. L. H., Edwards, D. D., Shoichet, B. K., & Roth, B. L. (2009). Predicting new molecular targets for known drugs. *Nature*, 462(7270), 175–181.

Kiraly, O., Gong, G., Olipitz, W., Muthupalani, S., & Engelward, B. P. (2015). Inflammation-Induced cell proliferation potentiates DNA damage-induced mutations in vivo. *PLOS Genetics*, 11(2), e1004901.

Kitchen, D. B., Decornez, H., Furr, J. R., & Bajorath, J. (2004). Docking and scoring in virtual screening for drug discovery: Methods and applications. *Nature Reviews Drug Discovery*, 3(11), 935–949.

Lawrence, T. (2009). The Nuclear Factor NF- κ B Pathway in Inflammation. *Cold Spring Harbor Perspectives in Biology*, 1(6), a001651–a001651.

Leu, C.-M., Wong, F.-H., Chang, C., Huang, S.-F., & Hu, C. (2003). Interleukin-6 acts as an antiapoptotic factor in human esophageal carcinoma cells through the activation of both STAT3 and mitogen-activated protein kinase pathways. *Oncogene*, 22(49), 7809–7818.

Liu, T., Zhang, L., Joo, D. and Sun, S.-C. (2017). NF- κ B signaling in inflammation. *Signal transduction and targeted therapy*, 2(17023).

M. M., Carter, B. D., Brinton, L. A., Falk, R. T., Gram, I. T., Luo, J., Milne, R. L., Nyante, S. J., Weiderpass, E., Beane Freeman, L. E., Sandler, D. P., Robien, K., Anderson, K. E., Giles, G. G., Chen, W. Y., Feskanich, D., Braaten, T., Isaacs, C., Butler, L. M., ... Gapstur, S. M. (2016). Pooled analysis of active cigarette smoking and invasive breast cancer risk in 14 cohort studies. *International Journal of Epidemiology*, dyw288.

Ma, J., Yan, R., Zu, X., Cheng, J.-M., Rao, K., Liao, D.-F., & Cao, D. (2008). Aldo-keto reductase family 1 B10 affects fatty acid synthesis by regulating the stability of acetyl-coa carboxylase- α in breast cancer cells. *Journal of Biological Chemistry*, 283(6), 3418–3423

MacFarland, T. W., & Yates, J. M. (2020). Student's t-Test for Independent Samples. In *Using R for Biostatistics* (pp. 141–240). Springer International Publishing

Maffini, M. V., Soto, A. M., Calabro, J. M., Ucci, A. A., & Sonnenschein, C. (2004). The stroma as a crucial target in rat mammary gland carcinogenesis. *Journal of Cell Science*, 117(8), 1495–1502

Makarem, N., Chandran, U., Bandera, E. V., & Parekh, N. (2013). Dietary fat in breast cancer survival. *Annual Review of Nutrition*, 33(1), 319–348.

Mantovani, A. (2005). Inflammation by remote control. *Nature*, 435(7043), 752–753.

Medes, Grace., Thomas, Alice., & Weinhouse, Sidney. (1952). NUTRITIONAL FACTORS IN FATTY ACID SYNTHESIS BY TISSUE SLICES IN VITRO. *Journal of Biological Chemistry*, 197(1), 181–191.

Menendez, J. A., Vellon, L., Mehmi, I., Oza, B. P., Ropero, S., Colomer, R., & Lupu, R. (2004). Inhibition of fatty acid synthase (FAS) suppresses HER2/neu(erbB-2) oncogene overexpression in cancer cells. *Proceedings of the National Academy of Sciences*, 101(29), 10715–10720.

Milgraum, L.Z., Witters, L.A., Pasternack, G.R. and Kuhajda, F.P. (1997). Enzymes of the fatty acid synthesis pathway are highly expressed in in situ breast carcinoma. *PubMed*, 3(11), pp.2115–20.

Moffat, J. G., Vincent, F., Lee, J. A., Eder, J., & Prunotto, M. (2017). Opportunities and challenges in phenotypic drug discovery: An industry perspective. *Nature Reviews Drug Discovery*, 16(8), 531–543.

Oeckinghaus, A. and Ghosh, S. (2009). The NF- κ B Family of Transcription Factors and Its Regulation. *Cold Spring Harbor Perspectives in Biology*, [online] 1(4), pp.a000034–a000034

Pikarsky, E., Porat, R. M., Stein, I., Abramovitch, R., Amit, S., Kasem, S., Gutkovich-Pyest, E., Urieli-Shoval, S., Galun, E., & Ben-Neriah, Y. (2004a). NF- κ B functions as a tumour promoter in inflammation-associated cancer. *Nature*, 431(7007), 461–466.

Postic, C., & Girard, J. (2008). The role of the lipogenic pathway in the development of hepatic steatosis. *Diabetes & Metabolism*, 34(6), 643–648.

Pulley, J.M., Rhoads, J.P., Jerome, R.N., Challa, A.P., Erreger, K., Meghan Morrison Joly, Lavieri, R.R., Perry, K.E., Zaleski, N.M., Shirey-Rice, J.K. and Aronoff, D.M. (2020). Using What We Already Have: Uncovering New Drug Repurposing Strategies in Existing Omics Data. 60(1), pp.333–352.

Pushpakom, S., Iorio, F., Eyers, P. A., Escott, K. J., Hopper, S., Wells, A., Doig, A., Williams, T., Latimer, J., McNamee, C., Norris, A., Sanseau, P., Cavalla, D., & Pirmohamed, M. (2018). Drug repurposing: Progress, challenges and recommendations. *Nature Reviews Drug Discovery*, 18(1), 41–58.

Ross, A., & Willson, V. L. (2017). One-Way anova. In *Basic and Advanced Statistical Tests* (pp. 21–24). SensePublishers.

Ross, D. T., Scherf, U., Eisen, M. B., Perou, C. M., Rees, C., Spellman, P., Iyer, V., Jeffrey, S. S., Van de Rijn, M., Waltham, M., Pergamenschikov, A., Lee, J. C. F., Lashkari, D., Shalon, D., Myers, T. G., Weinstein, J. N., Botstein, D., & Brown, P. O. (2000). Systematic variation in gene expression patterns in human cancer cell lines. *Nature Genetics*, 24(3), 227–235.

Sanseau, P., Agarwal, P., Barnes, M. R., Pastinen, T., Richards, J. B., Cardon, L. R., & Mooser, V. (2012). Use of genome-wide association studies for drug repositioning. *Nature Biotechnology*, 30(4), 317–320.

Sarkar, U., Rivera-Burgos, D., Large, E. M., Hughes, D. J., Ravindra, K. C., Dyer, R. L., Ebrahimkhani, M. R., Wishnok, J. S., Griffith, L. G., & Tannenbaum, S. R. (2015). Metabolite profiling and pharmacokinetic evaluation of hydrocortisone in a perfused three-dimensional human liver bioreactor. *Drug Metabolism and Disposition*, 43(7), 1091–1099.

Schreiber, R. D. (2011). Cancer immunoediting: Integrating immunity's roles in cancer suppression and promotion. *Science*.

Sharma, G.N., Dave, R., Sanadya, J., Sharma, P. and Sharma, K.K. (2010). Various types and management of breast cancer: an overview. *Journal of advanced pharmaceutical technology & research*, [online] 1(2), pp.109–26. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3255438/>.

Siegel, R. L., Miller, K. D., & Jemal, A. (2017). Cancer statistics, 2017. *CA: A Cancer Journal for Clinicians*, 67(1), 7–30.

Simoni, R. D., Hill, R. L., & Vaughan, M. (2002). The Isolation of Thyroxine and Cortisone: The work of Edward C. Kendall. *Journal of Biological Chemistry*, 277(21), 21–22. [https://doi.org/10.1016/s0021-9258\(20\)85219-3](https://doi.org/10.1016/s0021-9258(20)85219-3)Arihiro, K., Oda, H., Kaneko, M., & Inai, K. (2000). Cytokines facilitate chemotactic motility of breast carcinoma cells. *Breast Cancer*, 7(3), 221–230.

Sun, S. (2012). The noncanonical NF- κ B pathway. *Immunological Reviews*, 246(1), 125–140.

Sun, S.-C. (2010). Non-canonical NF- κ B signaling pathway. *Cell Research*, 21(1), 71–85.

Sun, S.-C., & Liu, Z.-G. (2011). A special issue on NF- κ B signaling and function. *Cell Research*, 21(1), 1–2.

Sun, S.-C., Chang, J.-H. and Jin, J. (2013). Regulation of nuclear factor- κ B in autoimmunity. *Trends in Immunology*, [online] 34(6), pp.282–289.

Sun, Y.-S., Zhao, Z., Yang, Z.-N., Xu, F., Lu, H.-J., Zhu, Z.-Y., Shi, W., Jiang, J., Yao, P.-P. and Zhu, H.-P. (2017). Risk Factors and Preventions of Breast Cancer. *International Journal of Biological Sciences*, [online] 13(11), pp.1387–1397.

Sutterwala, F. S., Haasken, S., & Cassel, S. L. (2014). Mechanism of NLRP3 inflammasome activation. *Annals of the New York Academy of Sciences*, 1319(1), 82–95.

Taniguchi, K., Wu, L.-W., Grivennikov, S. I., de Jong, P. R., Lian, I., Yu, F.-X., Wang, K., Ho, S. B., Boland, B. S., Chang, J. T., Sandborn, W. J., Hardiman, G., Raz, E., Maehara, Y., Yoshimura, A., Zucman-Rossi, J., Guan, K.-L., & Karin, M. (2015). A gp130–Src–YAP module links inflammation to epithelial regeneration. *Nature*, 519(7541), 57–62

Tsenova, L., Bergtold, A., Freedman, V. H., Young, R. A., & Kaplan, G. (1999). Tumor necrosis factor α is a determinant of pathogenesis and disease progression in mycobacterial infection in the central nervous system. *Proceedings of the National Academy of Sciences*, 96(10), 5657–5662.

Vesely, M. D. (2011, March 22). Natural innate and adaptive immunity to cancer. *Annual Reviews*.

Wang, Y. (Yongqiang), Kuhajda, F. P., Li, J. N., Pizer, E. S., Han, W. F., Sokoll, L. J., & Chan, D. W. (2001). Fatty acid synthase (FAS) expression in human breast cancer cell culture supernatants and in breast cancer patients. *Cancer Letters*, 167(1), 99–104

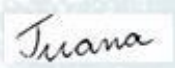
Washbrook, E. (2006). Risk factors and epidemiology of breast cancer. *Women's Health Medicine*, 3(1), 8–14.

Wu, P. (2012). Cancer Related Inflammation and Tumor Angiogenesis. In *Tumor Angiogenesis*. InTech.

Zhang, H. and Sun, S.-C. (2015). NF- κ B in inflammation and renal diseases. *Cell Bioscience*, 5(1).

ANNEXURE

■ GATE Scorecard

	GRADUATE APTITUDE TEST IN ENGINEERING 2024 अभियांत्रिकी स्नातक अभिक्षमता परीक्षा - २०२४ ORGANISING INSTITUTE: INDIAN INSTITUTE OF SCIENCE, BENGALURU	
SCORE CARD		
Name of the Candidate JUANA DARLENE VIRGIT J		
Name of the Parent/Guardian J JUDE RAJESH		
Registration No.	BT24557404239	
Test Paper	Biotechnology (BT)	
Date of Examination	February 10, 2024	
GATE Score	530	
Marks out of 100	51.0	
All India Rank (AIR) in the test paper	603	
Qualifying Marks		
General	38.9	
EWS/OBC-NCL	35.0	
SC/ST/PwD	25.9	
Number of candidates appeared for the test paper	17078	
		
Prof. Chandra Sekhar Seelamantula Organising Chairperson, GATE 2024 On behalf of NCB-GATE Ministry of Education (MoE)		
		
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A candidate is considered qualified if the marks secured are greater than or equal to the qualifying marks mentioned for the category, for which a valid category certificate, if applicable, must be produced along with this Score Card.		
This Score Card is valid up to 31 st March 2027.		

GATE SCORE COMPUTATION

The GATE 2024 score is calculated using the formula

$$\text{GATE Score} = S_0 + (S_1 - S_0) \frac{(M - M_0)}{(M_1 - M_0)}$$

where

M is the marks obtained by the candidate in the paper mentioned on the GATE 2024 Score Card

M_0 is the qualifying marks for general category candidates in the paper

M_1 is the mean of marks of top 0.1% or top 10 (whichever is larger) of all the candidates who appeared for the test paper

$S_0 = 350$, is the score assigned to M_0

$S_1 = 900$, is the score assigned to M_1

M_0 is 25 marks (out of 100) or $\mu + \sigma$, whichever is greater. Here, μ is the mean and σ is the standard deviation of marks of all the candidates who appeared for the test paper.

Qualifying in GATE 2024 does not guarantee admission to a postgraduate program or scholarship/financial assistance. Admitting institutes may conduct additional tests or interviews for final selection of candidates.

Graduate Aptitude Test in Engineering (GATE) 2024 was organised by Indian Institute of Science, Bengaluru, on behalf of National Coordination Board (NCB) - GATE for the Department of Higher Education, Ministry of Education (MoE), Government of India.

- **ABSTRACT PUBLISHED IN AN INTERNATIONAL CONFERENCE
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(P011)

A Network Pharmacology Approach to Investigate the Effects of Phytosterols on Uterine Fibroids

Juana Darlene Virgit J

Sathyabama Institute of Science and Technology

Uterine fibroids, a prevalent condition in gynaecology, present significant health challenges for women globally. These benign tumours are the most common in the reproductive tract and can manifest as symptoms like anaemia, dysmenorrhea, menorrhagia, and pelvic discomfort. Hormonal medication and surgical resection are the primary treatments. Phytosterols, plant-derived bioactive compounds such as β -sitosterol, Campesterol, Stigmasterol, Stigmastanol, Campestanol, Brassicasterol, and Cycloartenol, structurally resemble animal cholesterol. These phytosterols show promise in influencing hormonal and inflammatory pathways. This study employs a network pharmacology approach to understand how phytosterols impact uterine fibroids. Utilizing databases and bioinformatics, we built a comprehensive model to explore the interactions between phytosterols and key biological targets related to uterine fibroid growth. Information on various phytosterols and their target areas was gathered from diverse sources. We then delved into potential mechanisms by analysing KEGG pathway enrichment and GO biological processes. Molecular docking of key phytosterol targets was performed to validate network pharmacology findings. Experimental and clinical studies are necessary to validate these in-silico findings, potentially leading to novel phytosterol-based interventions for uterine fibroid management. This research contributes to the field of network pharmacology by providing a systematic approach to understanding the complex interactions between natural compounds and disease-related molecular networks.



CERTIFICATE OF PARTICIPATION

This certifies that

JUANA DARLENE VIRGIT J

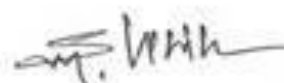
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■ CEETA SCORE CARD

CEETA 2024 COMMON ENGINEERING ENTRANCE TEST & ADMISSIONS ANNA UNIVERSITY, CHENNAI 600025 SCORE CARD		
Registration Number	11553286	
Name of the Candidate	JUANA DARLENE VIRGIT J	
Date of Birth	25/08/2002	
Gender	Female	
Community	BC	
Nativity	Tamil Nadu	
Nationality	Indian	Signature of the candidate
PERCENTILE SCORE		
PROGRAMME	IN FIGURES	IN WORDS
M.E./M.Tech/M.Arch/M.Plan	95.073	Nine Five Point Zero Seven Three
SECRETARY, TANCET		
IMPORTANT NOTE		
1. CEETA-PG (M.E./M.Tech./M.Arch./M.Plan) candidates have to exercise the choice of college & branch in the web portal after notifications from Anna University. 2. Hall Ticket should be produced with this Score Card during admission. 3. This Score Card is valid only for the Academic Year 2024-2025.		
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- Project presented at Technovation 2024





SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)

TARGETING BREAST CANCER USING HYDROCORTISONE AND ITS IMPACT ON INFLAMMATORY GENE EXPRESSIONS AND LIPOGENESIS AN IN-SILICO AND IN-VITRO STUDY

JUANA DARLENE VIRGIT J, B TECH BIOTECHNOLOGY (2020-2024)
UNDER THE GUIDANCE OF DR. M. DAVANILATHA



BREAST CANCER - ALARMING STATISTICS

1 In 28 women suffer from the disease
Every 4 Minutes an Indian woman is diagnosed with breast cancer
53-60% of women diagnosed with breast cancer in India do not survive more than a year

OVER VIEW

Aim of the study:

To Repurpose hydrocortisone, a corticosteroid hormone, as a potential therapeutic agent for breast cancer treatment.

Drug repurposing is the identification of new applications for existing compounds. It addresses the need for rapid and cost-effective treatment strategies.

KEY FEATURES

- To investigate the anti-inflammatory properties of hydrocortisone on breast cancer cell lines
- To evaluate the impact of hydrocortisone on lipogenesis in breast cancer cells
- To assess the overall cytotoxic effects of hydrocortisone on breast cancer cell viability and to understand its potential therapeutic efficacy

METHODS

IN-SILICO STUDY

- Molecular docking studies was done using PyRx to assess the binding affinity between hydrocortisone and the p52 subunit of NF- κ B and SREBP-1
- Visualization of the docking results was performed using BIOVIA Discovery Studio.

IN-VITRO STUDY

- Cytotoxicity of hydrocortisone was assessed using MTT assays on both MDA-MD-231 and the C₆ normal cell line to evaluate its selective toxicity
- RNA isolation and cDNA conversion enabled gene expression analysis of inflammatory markers (NF- κ B, TNF- α , IL-6) and lipogenic genes (FASN, SREBP-1) via qRT-PCR

STATISTICAL ANALYSIS

The following statistical tests were done and found to be significant:

Student's T-test on

- % Inhibition 24 vs 48hrs on MDA-MD-231
- % Inhibition on 24 hr /48 hr drug treatment- cancer vs normal cell line

Chi square test on AO/EB staining images

One way ANOVA on PCR results

CONCLUSION

These findings underscore the feasibility of repurposing hydrocortisone for breast cancer therapy, offering a cost-effective and readily available option for patients.

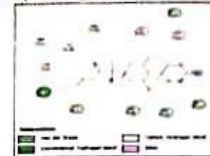
Further clinical studies are warranted to validate its efficacy and safety, ultimately paving the way for improved treatment strategies in breast cancer management

FUTURE DIRECTIONS

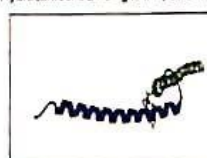
- Explore expression profiles of additional inflammatory and lipogenic genes to enhance understanding of hydrocortisone's molecular effects
- Develop nanoparticle-based drug delivery systems for targeted hydrocortisone delivery to breast cancer cells, reducing off-target effects
- Utilize in-vivo studies with animal models like zebrafish to investigate systemic effects of hydrocortisone treatment on tumour growth and metastasis

RESULT

Hydrocortisone demonstrated effective binding affinity to the targeted proteins

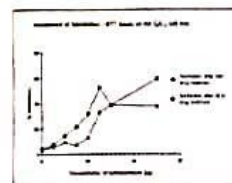
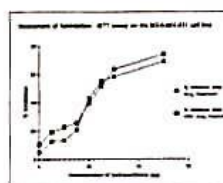


Hydrocortisone binding to the p52 subunit of NF- κ B (binding affinity -6.2 Kcal/mol)



Hydrocortisone binding to SREBP-1 (binding affinity -6.0 Kcal/mol)

ASSESSMENT OF CYTOTOXICITY - MTT ASSAY



ASSESSMENT OF APOPTOSIS*

BRIGHT FIELD

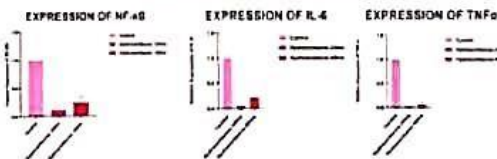


HYDROCORTISONE - 24HRS



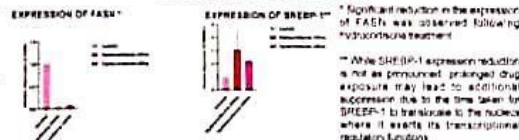
Normal healthy cells (Control normal cells)

ANALYSIS OF GENE EXPRESSION - qRT-PCR EXPRESSION OF INFLAMMATORY GENES

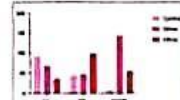


Significant reduction in the expression was observed following hydrocortisone treatment

EXPRESSION OF LIPOGENIC GENES



STATISTICAL ANALYSIS



T-test ON THE % INHIBITION

CHI SQUARE TEST ON AO/EB IMAGES

* Significant reduction in the expression of FASN was observed following hydrocortisone treatment

** While SREBP-1 expression reduction is not as pronounced, prolonged drug exposure may lead to additional suppression due to the time taken for SREBP-1 to translocate to the nucleus where it exerts its transcriptional regulatory functions