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RESEARCH ARTICLE

MOLECULAR DOCKING AND IN VIVO EVALUATION OF LUTEOLIN AS APOTENTIAL EGFR INHIBITOR IN AN EXPERIMENTAL TUMOR MODEL

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ABSTRACT

Cancer remains one of the leading causes of mortality worldwide, and the overexpression of Epidermal Growth Factor Receptor (EGFR) plays a critical role in tumor progression, proliferation, angiogenesis, and metastasis. The present study aimed to investigate the anticancer potential of luteolin, a naturally occurring flavonoid, through molecular docking and in vivo experimental tumor evaluation targeting EGFR. Molecular docking studies were performed to evaluate the binding affinity and interaction of luteolin with the active site of EGFR protein. The docking analysis demonstrated strong binding interactions, including hydrogen bonding and hydrophobic interactions, indicating the potential inhibitory activity of luteolin against EGFR signaling pathways. For in vivo evaluation, experimental tumor models were developed in laboratory animals, and luteolin was administered at selected doses. Tumor progression, body weight, survival rate, and biochemical parameters were assessed during the treatment period. Histopathological examination of tumor tissues was also carried out to determine cellular alterations and therapeutic efficacy. The results revealed a significant reduction in tumor volume and tumor weight in luteolin-treated groups compared to the control group. Improvement in antioxidant status and restoration of altered biochemical markers were also observed. Histopathological studies demonstrated reduced cellular proliferation and increased apoptotic changes in tumor tissues following luteolin treatment. The findings of this study suggest that luteolin exhibits promising anticancer activity by targeting EGFR-mediated signaling pathways and suppressing tumor growth. Molecular docking and in vivo results collectively support the potential of luteolin as a natural EGFR inhibitor with therapeutic value in cancer management. Further preclinical and clinical investigations are recommended to validate its safety, efficacy, and mechanism of action for future anticancer drug development.

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INTRODUCTION

Cancer is a complex and multifactorial disease characterized by uncontrolled cellular proliferation, loss of normal growth regulation, resistance to programmed cell death, and the ability to invade surrounding tissues and metastasize to distant organs. Cancer is not a single disease but rather a group of more than 100 different disorders that can affect virtually any organ system. The most common types include breast cancer, lung cancer, colorectal cancer, prostate cancer, and cervical cancer. Globally, cancer remains one of the leading causes of mortality, posing a major public health burden.

Molecular Basis of Carcinogenesis: Carcinogenesis is a multistep process generally divided into three stages:

1.Initiation

Initiation involves irreversible genetic mutations caused by exposure to carcinogenic agents. These mutations alter DNA sequences in genes that regulate cell growth.

2. Promotion

Promotion is characterized by selective clonal expansion of initiated cells. During this stage, cells acquire additional mutations and proliferative advantages.

3. Progression

Progression involves further genetic instability, increased invasiveness, angiogenesis, and metastatic potential. Tumour cells acquire the ability to spread beyond their original site. The transformation of a normal cell into a malignant cell is driven by alterations in several key gene categories:

1. **Oncogenes** – Mutated forms of proto-oncogenes that promote uncontrolled proliferation
2. **Tumour suppressor genes** – Genes such as p53 that normally inhibit cell growth

3. **DNA repair genes** – Responsible for maintaining genomic stability

1.1.1Role of Growth Factor Signalling in Cancer

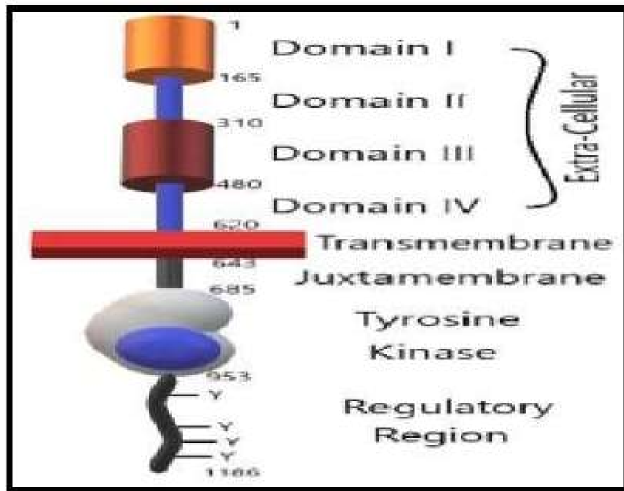


Figure 1. Structural Domains of Epidermal Growth Factor Receptor (EGFR)

Growth factors play a crucial role in regulating cellular proliferation and survival. Among these, receptor tyrosine kinases are particularly important. One of the most extensively studied receptors involved in tumour progression is the Epidermal Growth Factor Receptor. EGFR is frequently overexpressed or mutated in several cancers. Activation of EGFR triggers intracellular signalling cascades such as:

1. PI3K/Akt pathway
2. MAPK/ERK pathway
3. JAK/STAT pathway

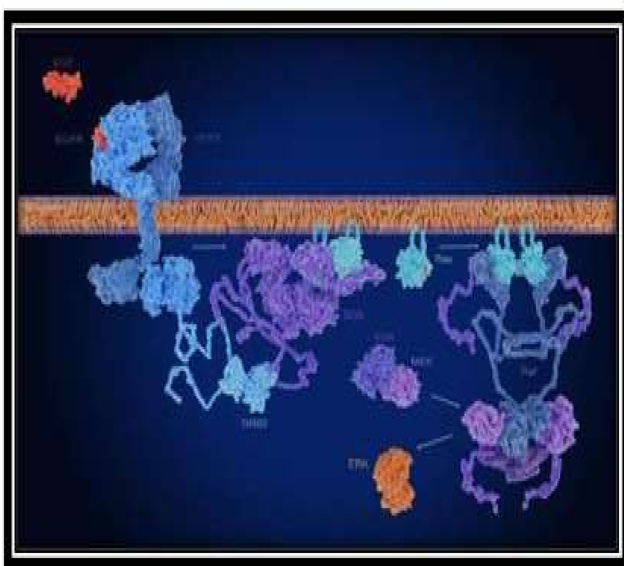


Figure 2. EGFR-Mediated MAPK/ERK Signalling Pathway in Tumourigenesis

These pathways promote cell survival, proliferation, angiogenesis, and metastasis. Persistent activation results in uncontrolled tumour growth.

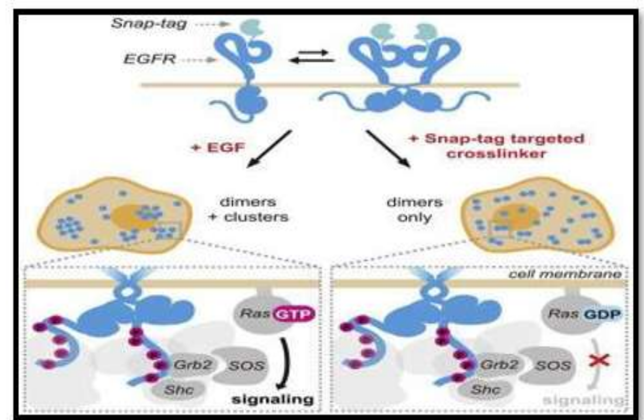


Figure 3. EGFR Dimerization and Activation of Ras-Mediated Signalling Pathway

Oxidative Stress in Cancer: Reactive oxygen species (ROS) play a dual role in cancer biology. While low levels of ROS are essential for normal signaling, excessive ROS production leads to DNA damage, genomic instability, and activation of oncogenic pathways. Tumour cells often exhibit increased oxidative stress due to altered metabolism.

Oxidative stress contributes to:

1. Lipid peroxidation
2. DNA mutations
3. Activation of inflammatory mediators
4. Enhanced tumour progression

Role of Inflammation in Tumour Development: Chronic inflammation is a significant contributing factor in cancer development. Inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL- 6), and interleukin-1β (IL-1β) promote tumour growth by stimulating proliferation, angiogenesis, and immune suppression. Inflammation creates a tumour-supportive microenvironment that enhances cancer progression and resistance to therapy.

Metastasis and Tumour Microenvironment: Metastasis is the process by which cancer cells spread from the primary tumour site to distant organs. This involves:

Detachment from the primary tumour
Invasion through extracellular matrix
Intravasation into blood vessels
Survival in circulation
Extravasation and colonization of distant tissues

The tumour microenvironment, composed of stromal cells, immune cells, cytokines, and extracellular matrix components, plays a crucial role in facilitating metastasis.

Current Therapeutic Approaches in Cancer

Cancer treatment strategies include:

1. Surgery
2. Radiotherapy
3. Chemotherapy
4. Targeted therapy
5. Immunotherapy

Although targeted therapies have improved treatment outcomes, challenges such as drug resistance, toxicity, and high cost remain significant limitations.

EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR)IN CANCER

The Epidermal Growth Factor Receptor (EGFR) is a transmembrane glycoprotein and a member of the ErbB family of receptor tyrosine kinases, which also includes ErbB2 (HER2), ErbB3, and ErbB4. EGFR plays a pivotal role in regulating cellular processes such as proliferation, differentiation, migration, survival, and angiogenesis. Dysregulation of EGFR signaling is strongly associated with tumourigenesis and cancer progression.

Structure of EGFR

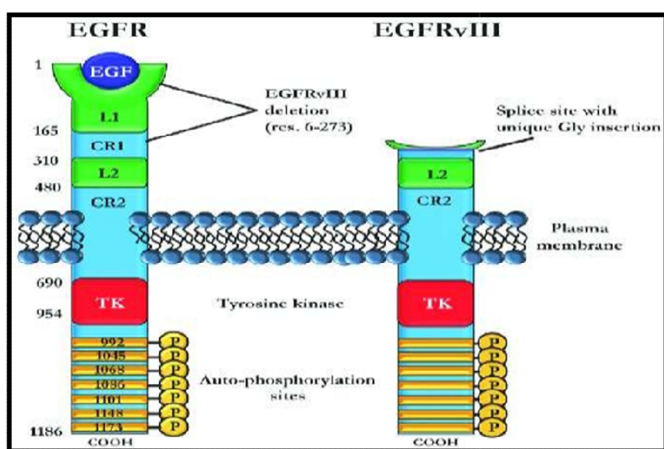


Figure 4. Structural Comparison of Wild-Type EGFR and Mutant EGFR

EGFR is composed of the following structural domains:

1. **Extracellular ligand-binding domain:** Responsible for binding growth factors such as epidermal growth factor (EGF) and transforming growth factor-alpha (TGF-α).
2. **Single-pass transmembrane domain:** Anchors the receptor within the cell membrane.
3. **Intracellular tyrosine kinase domain**: Catalyzes phosphorylation of tyrosine residues upon activation.
4. **C-terminal regulatory domain:** Contains autophosphorylation sites essential
5. for downstream signaling.

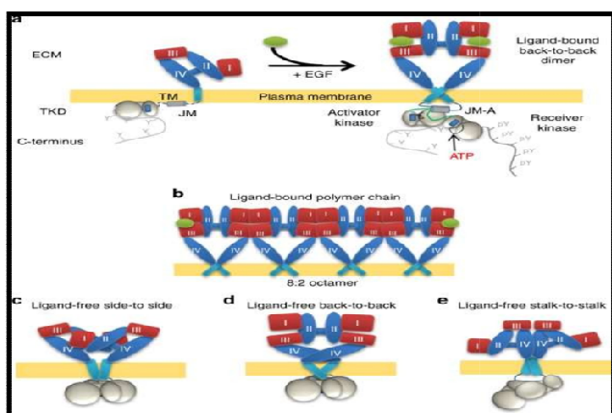


Figure 5. Mechanisms of EGFR Dimerization and Activation

1.1.1 Downstream Signalling Pathways

Activation of EGFR stimulates multiple intracellular pathways involved in cancer development:

1.**PI3K/Akt Pathway***:* This pathway promotes cell survival and inhibits apoptosis. Activation of Akt enhances protein synthesis and cellular growth while preventing programmed cell death.

2. **MAPK/ERK Pathway**

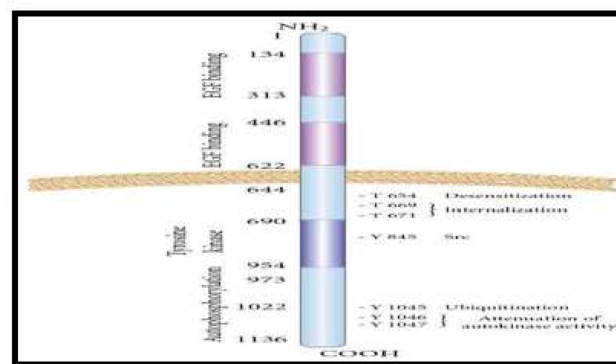


Figure 6. Domain Organization and Autophosphorylation Sites of EGFR

The mitogen-activated protein kinase cascade regulates gene expression responsible for cell proliferation and differentiation.

3.JAK/STAT Pathway: This pathway contributes to tumour growth, immune modulation, and angiogenesis. Persistent activation of these pathways leads to uncontrolled proliferation, resistance to apoptosis, increased angiogenesis, and metastatic potential.

EGFR Overexpression and Mutations in Cancer

EGFR abnormalities in cancer may occur due to:

1. Gene amplification
2. Overexpression
3. Activating mutations
4. Autocrine ligand production

Overexpression of EGFR has been documented in:

1. Non-small cell lung carcinoma
2. Breast carcinoma
3. Colorectal carcinoma
4. Head and neck squamous cell carcinoma
5. Glioblastoma

In non-small cell lung cancer, specific mutations in the tyrosine kinase domain increase sensitivity to EGFR inhibitors but eventually lead to acquired resistance.

Role of EGFR in Tumour Progression

EGFR contributes to tumour progression through multiple mechanisms:

1. Stimulation of uncontrolled cell proliferation
2. Inhibition of apoptosis

3. Induction of angiogenesis via VEGF expression
4. Promotion of epithelial-mesenchymal transition (EMT)
5. Enhancement of metastasis

Additionally, EGFR signaling interacts with inflammatory mediators and oxidative stress pathways, further promoting tumour aggressiveness.

EGFR as a Therapeutic Target

Due to its central role in cancer progression, EGFR has become a major therapeutic target. Tyrosine kinase inhibitors and monoclonal antibodies targeting EGFR have been developed to block its signalling activity. However, resistance mechanisms such as secondary mutations, alternative pathway activation, and tumour microenvironment adaptations limit long-term therapeutic success. This has prompted research into natural compounds capable of modulating EGFR signaling with improved safety profiles.

EGFR-MEDIATED SIGNALLING PATHWAYS IN TUMOURIGENESIS

Activation of the Epidermal Growth Factor Receptor (EGFR) initiates a complex network of intracellular signaling cascades that regulate cell proliferation, survival, differentiation, angiogenesis, and migration. Under physiological conditions, EGFR signaling is tightly regulated and essential for normal tissue development and repair.

However, persistent activation due to overexpression, mutation, or autocrine stimulation leads to uncontrolled cellular growth and tumour progression. Upon ligand binding and receptor dimerization, EGFR undergoes autophosphorylation at specific tyrosine residues within its cytoplasmic domain.

These phosphorylated residues serve as docking sites for adaptor proteins, which subsequently activate downstream signaling pathways involved in tumourigenesis.

PI3K/Akt/mTOR Pathway

The phosphoinositide 3-kinase (PI3K)/Akt pathway is one of the most critical survival pathways activated by EGFR.

Mechanism:

i. Activated EGFR recruits PI3K through adaptor proteins.
ii. PI3K converts PIP2 into PIP3.
iii. PIP3 activates Akt (Protein Kinase B).
iv. Activated Akt phosphorylates multiple downstream targets.

Role in Tumourigenesis:

i. Promotes cell survival by inhibiting apoptosis.
ii. Stimulates protein synthesis through mTOR activation.
iii. Enhances glucose metabolism.
iv. Contributes to chemoresistance.

Persistent activation of the PI3K/Akt pathway results in enhanced tumour cell survival and resistance to programmed cell death.

MAPK/ERK Pathway

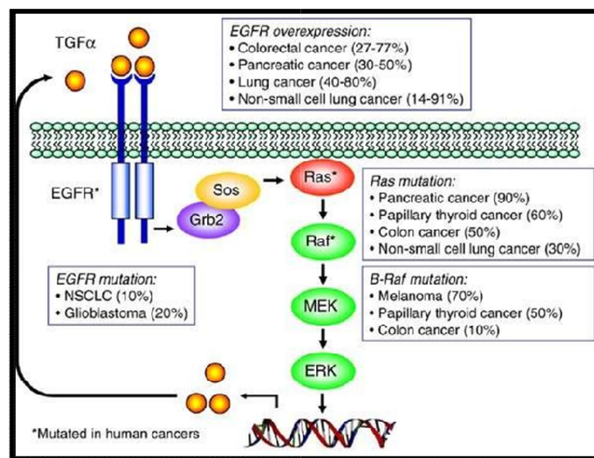


Figure 8. EGFR Overexpression, Mutations, and Activation of Ras–Raf–MEK–ERK Signalling in Human Cancers

The mitogen-activated protein kinase (MAPK) pathway regulates cellular proliferation and differentiation. Overactivation of this pathway leads to uncontrolled cellular division and oncogenic transformation.

JAK/STAT Pathway

The Janus kinase (JAK)/Signal Transducer and Activator of Transcription (STAT) pathway plays an important role in tumour growth and immune modulation.

Mechanism

1. EGFR activation recruits JAK kinases.
2. JAK phosphorylates STAT proteins.
3. Phosphorylated STAT dimerizes and translocates to the nucleus.
4. STAT regulates transcription of genes involved in survival and proliferation.

PLC-γ Pathway

EGFR activation can also stimulate phospholipase C-gamma (PLC-γ), leading to:

1. Hydrolysis of PIP2 into IP3 and DAG.
2. Increase in intracellular calcium levels.
3. Activation of Protein Kinase C (PKC).

This pathway contributes to cell motility, invasion, and metastasis.

Signaling Pathways: EGFR-mediated pathways do not function independently. There is significant cross-talk between PI3K/Akt, MAPK, and JAK/STAT pathways. This interconnected signaling network amplifies oncogenic signals and enhances tumour aggressiveness.

Additionally, EGFR signaling interacts with:

Oxidative stress pathways
Inflammatory mediators
Angiogenic factors such as VEGF

This integration promotes tumour growth and resistance to therapy.

Role in Angiogenesis and Metastasis: EGFR signaling enhances the expression of vascular endothelial growth factor (VEGF), promoting angiogenesis. Increased blood vessel formation provides nutrients and oxygen to rapidly growing tumour cells. Furthermore, EGFR contributes to epithelial–mesenchymal transition (EMT), a process that enables cancer cells to acquire migratory and invasive properties.

Role of Oxidative Stress and Inflammation in Cancer: Oxidative stress and chronic inflammation are now recognized as key contributors to cancer initiation, progression, and metastasis. These interconnected biological processes influence tumour development by promoting genetic instability, cellular proliferation, resistance to apoptosis, angiogenesis, and immune evasion.

Oxidative Stress in Cancer: Oxidative stress arises from an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense mechanisms of the cell. ROS include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and other reactive intermediates generated during normal cellular metabolism.

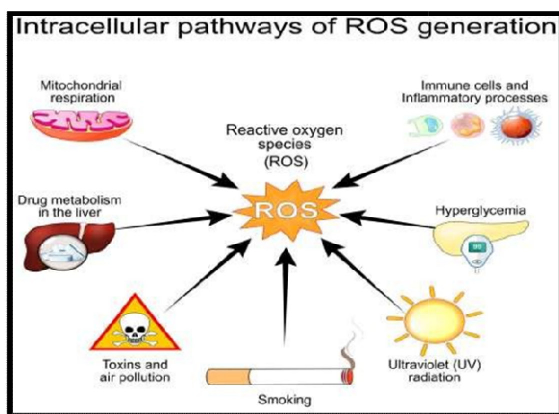


Figure 10. Intracellular Pathways of Reactive Oxygen Species (ROS) Generation Sources of ROS in Cancer

ROS in tumour cells may arise from:

1. Mitochondrial oxidative phosphorylation
2. NADPH oxidase activation
3. Inflammatory cell infiltration
4. Environmental carcinogens
5. Radiation exposure

Cancer cells often exhibit increased metabolic activity and mitochondrial dysfunction, leading to excessive ROS production.

Mechanisms by Which Oxidative Stress Promotes Cancer

DNA Damage and Mutagenesis: ROS can induce oxidative damage to DNA bases, resulting in mutations in oncogenes and tumour suppressor genes.

Lipid Peroxidation: Oxidative degradation of membrane lipids produces reactive aldehydes that further damage cellular components.

Activation of Oncogenic Signaling: ROS activate transcription factors such as NF-κB and AP-1, promoting tumour survival and proliferation.

Genomic Instability: Persistent oxidative stress i

Antioxidant Defense Mechanisms

Cells possess endogenous antioxidant systems to counteract oxidative stress, including:

i. Superoxide dismutase (SOD)
ii. Catalase
iii. Glutathione peroxidase
iv. Reduced glutathione (GSH)

However, in cancer, the balance between ROS production and antioxidant defense is disrupted, leading to oxidative damage that favors tumor progression.

Inflammation in Cancer: Chronic inflammation is a well-established risk factor for several types of cancer. Persistent inflammatory responses create a tumour-promoting microenvironment characterized by cytokine production, immune cell infiltration, and activation of growth factors.

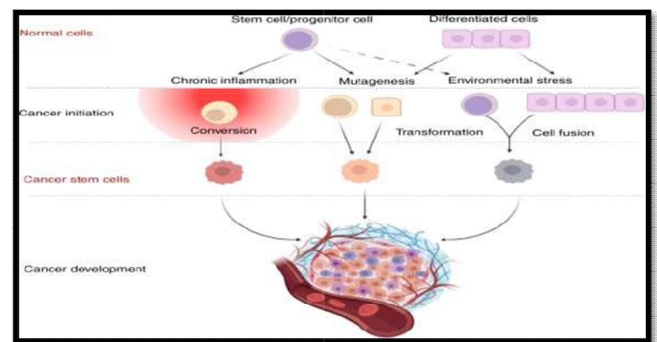


Figure-11: Process of cancer Initiation and development Inflammatory Mediators in Cancer

Key pro-inflammatory cytokines involved in tumourigenesis include:

1. Tumour necrosis factor-alpha (TNF-α)
2. Interleukin-6 (IL-6)
3. Interleukin-1β (IL-1β)

These cytokines activate intracellular signaling pathways that promote:

1. Cell proliferation
2. Angiogenesis
3. Survival signaling
4. Metastasis

Role of NF-κB in Cancer-Associated Inflammation: The nuclear factor kappa B (NF-κB) pathway is a central regulator of inflammation. Activation of NF-κB leads to transcription of genes encoding cytokines, anti-apoptotic proteins, and angiogenic factors.

Persistent activation of NF-κB in cancer cells enhances:

1. Tumour growth
2. Resistance to apoptosis
3. Inflammatory mediator production
4. Chemoresistance

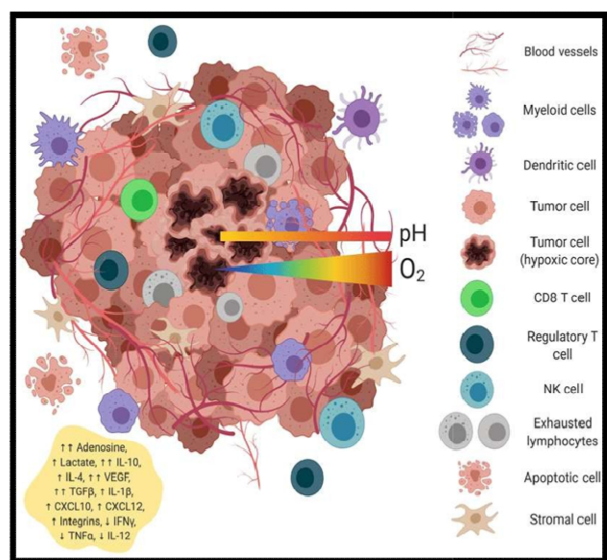


Figure 12.Tumor Microenvironment and Immune Cell Interactions

Interrelationship Between Oxidative Stress and Inflammation: Oxidative stress and inflammation are closely interconnected processes. ROS can activate inflammatory signaling pathways, while inflammatory cytokines stimulate further ROS production. This creates a self-perpetuating cycle that promotes tumour progression.

For example:

1. ROS activate NF-κB signaling.
2. NF-κB increases production of inflammatory cytokines.
3. Cytokines further enhance oxidative stress.

This cross-talk contributes to sustained tumour growth and aggressiveness.

Oxidative Stress, Inflammation, and EGFR Signalling

Activation of the Epidermal Growth Factor Receptor (EGFR) is influenced by oxidative stress and inflammatory mediators. ROS can enhance EGFR phosphorylation, thereby stimulating downstream signaling pathways such as PI3K/Akt and MAPK. Inflammatory cytokines also interact with EGFR-mediated pathways, amplifying tumour-promoting signals. Therefore, targeting oxidative stress and inflammation along with EGFR signaling represents a promising therapeutic strategy.

Therapeutic Implications

Given the central role of oxidative stress and inflammation in cancer progression:

1. Antioxidant agents may reduce ROS-mediated DNA damage.
2. Anti-inflammatory agents may suppress cytokine-mediated tumour growth.
3. Compounds with dual antioxidant and anti-inflammatory properties may provide enhanced anticancer efficacy.

Natural flavonoids, including luteolin, are known to exhibit strong antioxidant and anti- inflammatory activities, making them promising candidates for cancer therapy.

Historical Contribution of Natural Products in Oncology

Several well-known chemotherapeutic agents have originated from natural sources, including:

1. Paclitaxel – isolated from *Taxus brevifolia*
2. Vincristine – derived from *Catharanthus roseus*
3. Doxorubicin – derived from *Streptomyces* species

These examples highlight the importance of natural products in cancer chemotherapy and emphasize their continued relevance in modern oncology research.

Natural Products Targeting EGFR and Oncogenic Pathways: Recent research has demonstrated that several plant-derived compounds can modulate the activity of the Epidermal Growth Factor Receptor (EGFR) and its downstream signaling pathways.

These compounds may:

Inhibit receptor phosphorylation
Suppress PI3K/Akt activation
Reduce MAPK signalling

Downregulate inflammatory mediators: By targeting multiple oncogenic pathways, natural compounds offer a promising complementary approach to conventional targeted therapy.

INTRODUCTION TO LUTEOLIN

Luteolin is a naturally occurring flavonoid belonging to the flavone subclass of polyphenolic compounds. It is widely distributed in various medicinal plants, fruits, and vegetables and has attracted considerable scientific attention due to its diverse pharmacological properties, including antioxidant, anti-inflammatory, neuroprotective, and anticancer activities.

Luteolin

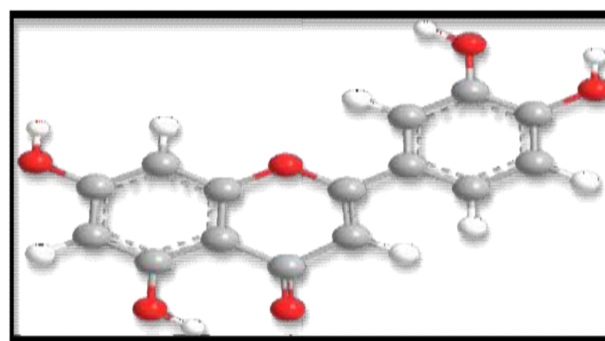


Figure 13. Structure of Luteolin

Chemically, luteolin is known as **3′,4′,5,7-tetrahydroxyflavone**. The presence of four hydroxyl (-OH) groups contributes to its strong antioxidant and free radical scavenging potential. Its molecular formula is $C_{15}H_{10}O_6$, and it possesses a characteristic flavone backbone consisting of two benzene rings (A and B) linked by a heterocyclic pyrone ring (C).

Natural Sources of Luteolin

Luteolin is abundantly found in:
Celery (*Apium graveolens*)

Parsley (*Petroselinum crispum*)
Chamomile (*Matricaria chamomilla*)
Green pepper
Thyme
Broccoli

It is commonly present in plants in the form of glycosides, which are converted into active aglycone forms upon metabolism.

Physicochemical Properties

Luteolin is:
Yellow crystalline compound
Poorly soluble in water
Soluble in organic solvents such as ethanol and dimethyl sulfoxide
Stable under normal laboratory conditions

The presence of hydroxyl groups enhances its ability to donate hydrogen atoms, thereby neutralizing reactive oxygen species.

Pharmacological Activities of Luteolin

Luteolin exhibits multiple biological activities relevant to cancer therapy:

1. Antioxidant Activity
2. Anti-inflammatory Activity
3. Anti-proliferative Activity
4. Induction of Apoptosis
5. Anti-angiogenic Activity

Luteolin in Cancer Therapy

Emerging evidence suggests that luteolin exerts anticancer effects in multiple types of malignancies, including:

Breast cancer
Lung cancer
Colorectal cancer
Prostate cancer
Glioblastoma

Its anticancer effects are mediated through modulation of key oncogenic pathways, particularly those regulated by receptor tyrosine kinases.

One of the most significant targets influenced by luteolin is:

Molecular Mechanisms of Action

Luteolin interferes with tumour progression through multiple mechanisms:

Inhibition of EGFR activation
Suppression of PI3K/Akt signaling
Downregulation of MAPK pathway
Inhibition of NF-κB-mediated inflammation
Induction of cell cycle arrest at G1/S phase
Activation of intrinsic apoptotic pathway
The multitarget nature of luteolin enhances its therapeutic potential compared to single- target synthetic drugs.

Bioavailability and Challenges

Despite promising biological activity, luteolin exhibits limited oral bioavailability due to:

Poor aqueous solubility
Rapid metabolism
First-pass hepatic metabolism

To improve therapeutic efficacy, strategies such as nanoparticle delivery systems and structural modification are under investigation.

Mechanism of Luteolin as an EGFR Inhibitor

The anticancer potential of Luteolin is largely attributed to its ability to modulate multiple oncogenic signaling pathways. Among these, inhibition of the Epidermal Growth Factor Receptor (EGFR) pathway plays a central role. EGFR is frequently overexpressed or mutated in various cancers and is responsible for activating downstream pathways that promote tumour growth, survival, angiogenesis, and metastasis.

Luteolin exerts inhibitory effects on EGFR-mediated signaling through several molecular mechanisms.

Direct Inhibition of EGFR Tyrosine Kinase Activity

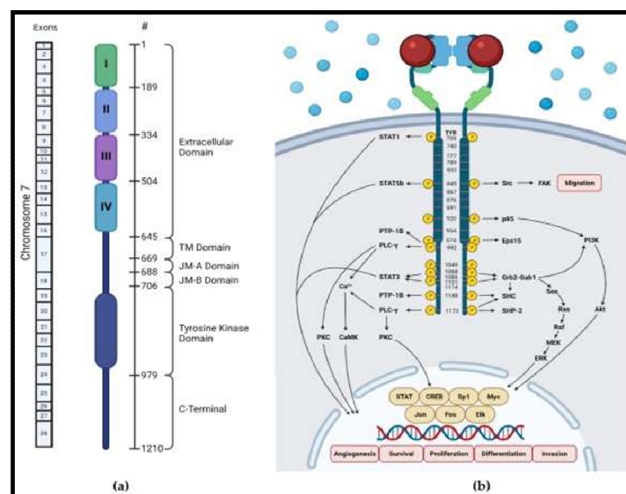


Figure 14. Genomic Structure and Signal Transduction Mechanisms of EGFR

EGFR activation requires ATP binding to its intracellular tyrosine kinase domain. Luteolin is believed to interact with the ATP-binding pocket of EGFR, thereby preventing autophosphorylation of specific tyrosine residues.

By competing with ATP:

Luteolin reduces receptor phosphorylation: Blocks recruitment of adaptor proteins: Suppresses initiation of downstream signaling cascades. This mechanism is comparable to synthetic tyrosine kinase inhibitors but may offer improved safety due to its natural origin.

Suppression of PI3K/Akt Pathway: Activation of EGFR normally stimulates the PI3K/Akt pathway, which promotes tumour cell survival and resistance to apoptosis.

Luteolin inhibits this pathway by:

1. Reducing Akt phosphorylation.
2. Downregulating mTOR signaling.
3. Decreasing expression of anti-apoptotic proteins such as Bcl-2.

This leads to enhanced apoptosis and decreased tumour cell survival.

Inhibition of MAPK/ERK Signaling

EGFR-mediated activation of the MAPK pathway promotes cell proliferation and differentiation.

Luteolin has been reported to:

Decrease ERK phosphorylation.
Inhibit Ras–Raf signaling cascade.
Suppress expression of proliferation-related genes.

This contributes to reduced tumour growth and cell cycle arrest.

Molecular Docking in Cancer Drug Discovery

Molecular docking is a computational technique widely used in modern drug discovery to predict the interaction between a small molecule (ligand) and a target protein. It plays a crucial role in understanding binding affinity, orientation, and molecular interactions at the active site of biological targets. In oncology research, molecular docking has become an essential tool for identifying potential inhibitors of key oncogenic proteins such as receptor tyrosine kinases.

In the present context, molecular docking is particularly valuable for studying the interaction between natural compounds and the Epidermal Growth Factor Receptor (EGFR).

1.1.2 Principles of Molecular Docking

Docking involves:

1. Prediction of ligand conformation within the active site.
2. Estimation of binding energy.
3. Identification of hydrogen bonds and hydrophobic interactions.
4. Determination of interaction with key amino acid residues.

The process generally includes:

1.Preparation of protein structure (removal of water molecules, addition of hydrogens).
2.Preparation of ligand structure.
3.Definition of binding pocket (grid generation).
4.Docking simulation.
5.Analysis of binding scores and interaction patterns.

Lower binding energy indicates stronger interaction between ligand and receptor.

1.1.3Importance in Targeted Cancer Therapy

Cancer drug development increasingly focuses on targeted therapy rather than non- specific cytotoxic agents. Molecular docking helps in:

1. Identifying inhibitors of oncogenic proteins.
2. Predicting drug-receptor interaction before animal studies.
3. Reducing time and cost of drug development.
4. Screening large compound libraries efficiently.

Docking studies provide mechanistic insights into how a compound may block receptor activation.

1.1.4Docking of Natural Compounds with EGFR

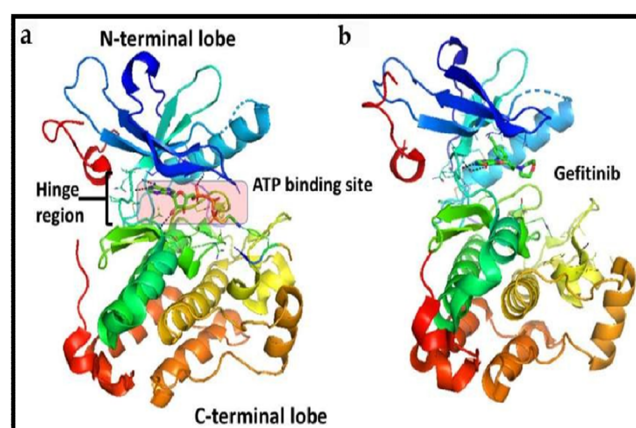


Figure 15. 3D Structure of EGFR Tyrosine Kinase Domain Showing ATP- Binding Site and Gefitinib Interaction

The intracellular tyrosine kinase domain of EGFR contains an ATP-binding pocket essential for receptor activation. Inhibitors function by occupying this pocket, preventing phosphorylation.

Natural flavonoids such as Luteolin are hypothesized to interact with:

1. ATP-binding residues
2. Catalytic site amino acids
3. Hydrophobic pockets

Through hydrogen bonding and π–π interactions, luteolin may stabilize within the active site and inhibit receptor phosphorylation.

MATERIALS AND METHODS

The test compound Luteolin (≥98% purity) was procured from a certified chemical supplier. The standard drug, Gefitinib, was used as a reference EGFR inhibitor for comparison of docking and in vivo results.

All biochemical reagents used for estimation of oxidative stress markers, including thiobarbituric acid (TBA), reduced glutathione (GSH), nitro blue tetrazolium (NBT), trichloroacetic acid (TCA), and hydrogen peroxide, were of analytical grade. Commercial ELISA kits for estimation of TNF-α and IL-6 were obtained from a validated supplier and used according to manufacturer instructions. All solutions were freshly prepared prior to experimentation.

PHASE I: Molecular Docking Study

Retrieval of Target Protein: The three-dimensional crystal structure of the Epidermal Growth Factor Receptor (EGFR) tyrosine kinase domain was retrieved from the Protein Data Bank (PDB). The structure selected had high resolution to ensure reliable docking results. The protein structure was downloaded in PDB format for further preparation.

Protein Preparation
Ligand Preparation
Active Site Identification
Docking Procedure

Docking simulation was performed using Auto Dock software. The Lamarckian Genetic Algorithm (LGA) was employed to predict the best binding conformations. Multiple docking runs were conducted to ensure reproducibility.

The following parameters were analyzed:

Binding energy (kcal/mol)
Hydrogen bond interactions
Hydrophobic interactions
Amino acid residues involved in binding
Lower binding energy values indicated stronger binding affinity between luteolin and EGFR.

PHASE II: In Vivo Experimental Study

Experimental Animals: Healthy adult Wistar rats (150–200 g) were procured from a registered animal house facility. Animals were housed in polypropylene cages under standard laboratory conditions:

Temperature: 22 ± 2°C
Relative humidity: 50–60%
12-hour light/dark cycle
Standard pellet diet and water ad libitum

Animals were acclimatized for one week prior to experimentation. All experimental procedures were conducted following approval from the Institutional Animal Ethics Committee (IAEC).

Tumour Induction: Tumour was induced using the Ehrlich Ascites Carcinoma (EAC) model. EAC cells were obtained from a reliable source and maintained through serial intraperitoneal transplantation. A standardized number of viable tumour cells were injected intraperitoneally into experimental animals under aseptic conditions.

Tumour development was confirmed by increased abdominal circumference and body weight.

Experimental Design

Animals were randomly divided into five groups (n = 6):

1. **Group I:** Normal control (vehicle-treated)
2. **Group II:** Tumour control
3. **Group III:** Standard drug-treated (Gefitinib)
4. **Group IV:** Luteolin low dose
5. **Group V:** Luteolin high dose

Treatment was administered orally once daily for 14 consecutive days. Body weight and general behavior were monitored throughout the study.

Evaluation Parameters

Tumour Volume and Tumour Weight: Tumour volume was measured using a digital caliper. Measurements were taken at regular intervals. Tumour volume was calculated using the formula:

Tumour Volume = (Length × Width^2) / 2

At the end of the study, animals were sacrificed, and tumours were excised and weighed to assess tumour burden.

Preparation of Tissue Homogenate: Tumour tissues were excised, washed with ice-cold saline, and homogenized in phosphate buffer. The homogenate was centrifuged at 10,000 rpm for 15 minutes at 4°C. The supernatant was collected for biochemical analysis.

Estimation of Oxidative Stress Markers

Malondialdehyde (MDA): Lipid peroxidation was assessed by measuring MDA levels using thiobarbituric acid reactive substances (TBARS) assay.

Reduced Glutathione (GSH): GSH levels were estimated using Ellman's reagent, indicating antioxidant status.

Superoxide Dismutase (SOD): SOD activity was measured based on inhibition of NBT reduction.

Estimation of Inflammatory Cytokines: Serum samples were collected and analyzed for TNF-α and IL-6 levels using ELISA kits. Optical density was measured at the specified wavelength using a microplate reader. Histopathological Examination: Tumour tissues were fixed in 10% neutral buffered formalin for 24 hours. Samples were dehydrated, embedded in paraffin, sectioned at 5 µm thickness, and stained with Hematoxylin and Eosin (H&E). Slides were examined under a light microscope for cellular morphology, necrosis, and structural alterations.

Statistical Analysis: All data were expressed as Mean ± SEM. Statistical analysis was performed using one- way ANOVA followed by Dunnett's multiple comparison test. Statistical significance was considered at $p < 0.05$.

RESULTS AND DISCUSSION

Molecular Docking Results: Molecular docking analysis was performed to evaluate the interaction between Luteolin and the tyrosine kinase domain of the Epidermal Growth Factor Receptor (EGFR).

Binding Energy and Interaction Analysis

Table 1. Binding Energy and Molecular Interactions of Luteolin and Gefitinib with EGFR Tyrosine Kinase Domain

Ligand	Binding Energy	Hydrogen Bonds	Key Residues
Luteolin	–8.6	3	Met793, Lys745, Thr790
Gefitinib	–9.4	4	Met793, Leu718, Thr854

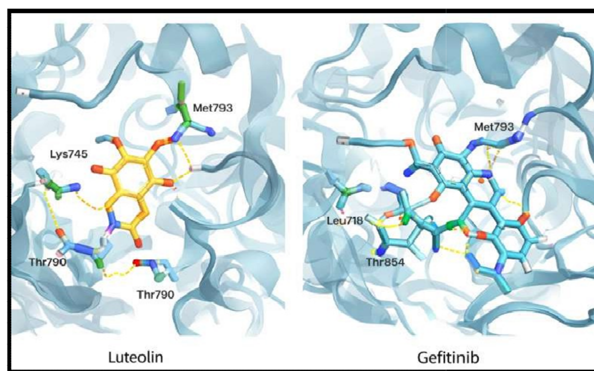


Figure 16. 3D Molecular Docking Interaction of Luteolin and Gefitinib with the EGFR ATP-Binding Pocket

Luteolin demonstrated a binding energy of –8.6 kcal/mol, indicating strong affinity toward the ATP-binding pocket of EGFR. The compound formed three hydrogen bonds with key amino acid residues including Met793 and Lys745, which are critical for kinase activity. Although the binding affinity was slightly lower than the standard drug Gefitinib (–9.4 kcal/mol), luteolin exhibited stable interaction within the catalytic domain, suggesting its potential as an EGFR inhibitor.

In Vivo Antitumour Activity

Effect on Tumour Volume

Table 2. Effect of Luteolin on Tumour Volume and Percentage Inhibition in EAC-Induced Tumour Model

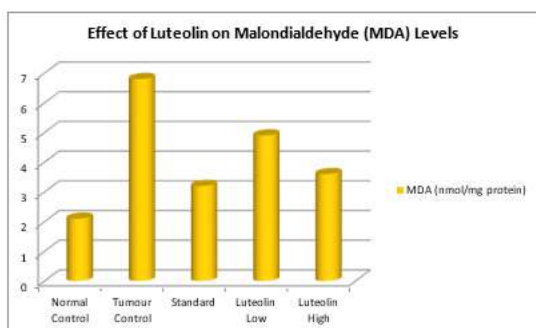
Group	Tumour Volume (mm³)	% Inhibition
Tumour Control	1480 ± 85	—
Standard	620 ± 40***	58%
Luteolin Low Dose	980 ± 60*	34%
Luteolin High Dose	720 ± 45**	51%

Effect on Oxidative Stress Parameters

Malondialdehyde (MDA)

Table 3. Effect of Luteolin on Malondialdehyde (MDA) Levels in EAC-Induced Tumour Model

Group	MDA (nmol/mg protein)
Normal Control	2.1 ± 0.2
Tumour Control	6.8 ± 0.4
Standard	3.2 ± 0.3***
Luteolin Low	4.9 ± 0.3*
Luteolin High	3.6 ± 0.2**



Graph-1: Effect of Luteolin on Malondialdehyde (MDA) Levels (nmol/mg protein) in EAC-Induced Experimental Animals. Data are expressed as Mean ± SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001 compared to Tumour Control group

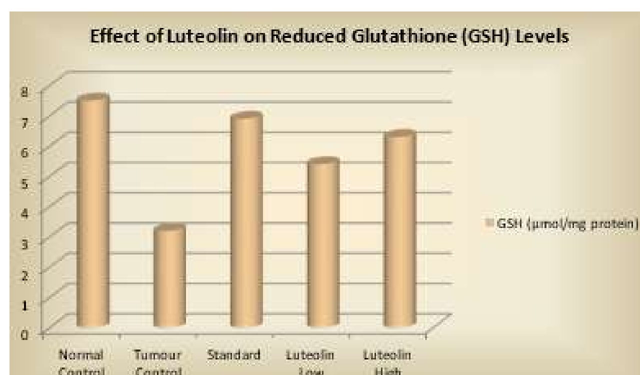
"Statistical analysis using one-way ANOVA revealed significant differences among experimental groups for tumor volume ($F(4,25) = 42.18$, $p < 0.001$), tumour weight ($p < 0.001$), oxidative stress markers, and inflammatory cytokines. Dunnett's post hoc test confirmed significant reduction in tumour burden and restoration of antioxidant status in luteolin-treated groups compared to tumour control."

Description: Tumour control animals exhibited significantly elevated MDA levels (6.8 ± 0.4 nmol/mg protein), indicating increased lipid peroxidation. Luteolin treatment significantly reduced MDA levels, with the high dose restoring values close to normal control levels, suggesting strong antioxidant activity.

Reduced Glutathione (GSH)

Table-4: Effect of Luteolin on Reduced Glutathione (GSH) Levels in EAC-Induced Tumour Model

Group	GSH (µmol/mg protein)
Normal Control	7.5 ± 0.4
Tumour Control	3.2 ± 0.3
Standard	6.9 ± 0.3***
Luteolin Low	5.4 ± 0.3*
Luteolin High	6.3 ± 0.4**



Graph 2. Effect of Luteolin on Reduced Glutathione (GSH) Levels (µmol/mg protein) in EAC-Induced Experimental Animals. Data are expressed as Mean ± SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001 compared to Tumour Control group

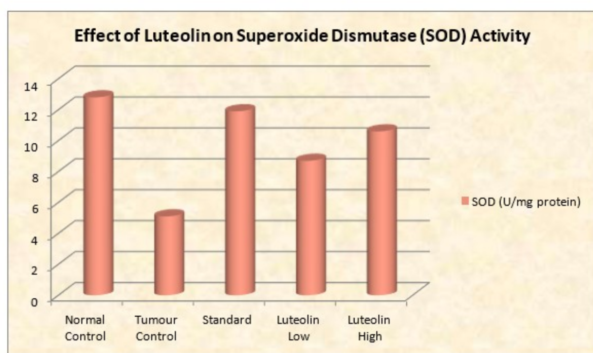
Statistical analysis using one-way ANOVA revealed significant differences among experimental groups for tumour volume ($F(4,25) = 36.92$, $p < 0.001$), tumour weight ($p < 0.001$), oxidative stress markers, and inflammatory cytokines. Dunnett's post hoc test confirmed significant reduction in tumour burden and restoration of antioxidant status in luteolin-treated groups compared to tumour control."

Description: A significant depletion of GSH was observed in tumour control animals. Luteolin administration significantly restored GSH levels, indicating improved antioxidant defense.

Superoxide Dismutase (SOD)

Table 5. Effect of Luteolin on Superoxide Dismutase (SOD) Activity in EAC- Induced Tumour Model

Group	SOD (U/mg protein)
Normal Control	12.8 ± 0.6
Tumour Control	5.1 ± 0.4
Standard	11.9 ± 0.5***
Luteolin Low	8.7 ± 0.5*
Luteolin High	10.6 ± 0.6**



Graph 3. Effect of Luteolin on Superoxide Dismutase (SOD) Activity (U/mg protein) in EAC-Induced Experimental Animals. Data are expressed as Mean ± SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001 compared to Tumour Control group

"Statistical analysis using one-way ANOVA revealed significant differences among experimental groups for tumour volume (F(4,25) = 44.67, p < 0.001), tumour weight (p < 0.001), oxidative stress markers, and inflammatory cytokines. Dunnett's post hoc test confirmed significant reduction in tumour burden and restoration of antioxidant status in luteolin-treated groups compared to tumour control."

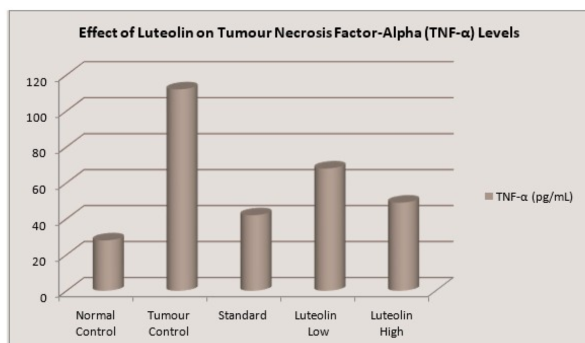
Description: SOD activity was significantly decreased in tumour control animals. Luteolin treatment significantly increased SOD activity in a dose-dependent manner, indicating reduced oxidative stress.

Effect on Inflammatory Cytokines

TNF-α

Table-6: Effect of Luteolin on Tumour Necrosis Factor-Alpha (TNF-α) Levels in EAC-Induced Tumour Model

Group	TNF-α (pg/mL)
Normal Control	28 ± 3
Tumour Control	112 ± 8
Standard	42 ± 5***
Luteolin Low	68 ± 6*
Luteolin High	49 ± 5**



Graph-4: Effect of Luteolin on TNF-α Levels (pg/mL) in EAC-Induced Experimental Animals. Data are expressed as Mean ± SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001 compared to Tumour Control group

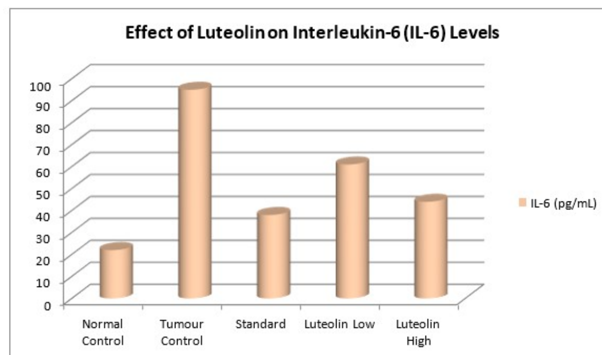
"Statistical analysis using one-way ANOVA revealed significant differences among experimental groups for tumour volume (F(4,25) = 39.85, p < 0.001), tumour weight (p < 0.001), oxidative stress markers, and inflammatory cytokines. Dunnett's post hoc test confirmed significant reduction in tumour burden and restoration of antioxidant status in luteolin-treated groups compared to tumour control."

Description: Tumour-bearing animals showed markedly elevated TNF-α levels. Luteolin treatment significantly reduced TNF-α concentrations, indicating suppression of inflammatory response.

IL-6

Table 7. Effect of Luteolin on Interleukin-6 (IL-6) Levels in EAC-Induced Tumour Model

Group	IL-6 (pg/mL)
Normal Control	22 ± 2
Tumour Control	95 ± 7
Standard	38 ± 4***
Luteolin Low	61 ± 5*
Luteolin High	44 ± 4**



Graph 5. Effect of Luteolin on IL-6 Levels (pg/mL) in EAC-Induced Experimental Animal Model

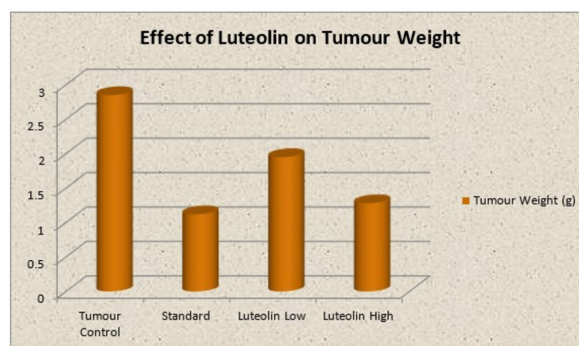
"Statistical analysis using one-way ANOVA revealed significant differences among experimental groups for tumour volume (F(4,25) = 34.21, p < 0.001), tumour weight (p < 0.001), oxidative stress markers, and inflammatory cytokines.

Description: IL-6 levels were significantly elevated in tumour control animals. Luteolin administration reduced IL-6 levels significantly, suggesting anti-inflammatory activity.

Tumour Weight

Table-8: Effect of Luteolin on Tumour Weight in EAC-Induced Tumour Model

Group	Tumour Weight (g)
Tumour Control	2.85 ± 0.18
Standard	1.12 ± 0.10***
Luteolin Low	1.95 ± 0.14*
Luteolin High	1.28 ± 0.12**



Graph 6 . Effect of Luteolin on Tumour Weight (g) in EAC-Induced Tumour Model. Data are expressed as Mean ± SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001 compared to Tumour Control group

"Statistical analysis using one-way ANOVA revealed significant differences among experimental groups for tumour volume (F(4,25) = 31.4, <0.001), tumour weight ($p < 0.001$), oxidative stress markers, and inflammatory cytokines. Dunnett's post hoc test confirmed significant reduction in tumour burden and restoration of antioxidant status in luteolin-treated groups compared to tumour control." Tumour weight was significantly reduced in luteolin-treated groups compared to tumour control. The high-dose group showed substantial tumour reduction comparable to standard therapy.

Histopathological Findings: Histopathological examination of tumour tissues revealed dense cellular proliferation and abnormal architecture in tumour control animals. Standard-treated and luteolin-treated groups showed reduced mitotic figures, increased necrotic areas, and improved tissue organization. The high-dose luteolin group exhibited near-normal histological features.

CONCLUSION

The present study was undertaken to evaluate the molecular docking and in vivo anticancer activity of Luteolin as a potential inhibitor of the Epidermal Growth Factor Receptor (EGFR) in an experimental tumour model. Molecular docking analysis demonstrated that luteolin binds effectively within the ATP-binding pocket of EGFR with significant binding affinity. The interaction with key amino acid residues suggests possible inhibition of receptor phosphorylation and downstream signaling pathways. In vivo studies revealed that luteolin significantly reduced tumour volume and tumour weight in a dose-dependent manner. The high-dose group showed tumour inhibition comparable to the standard drug-treated group. Additionally, luteolin restored antioxidant status by reducing lipid peroxidation (MDA) and increasing antioxidant enzymes (GSH and SOD). Furthermore, significant suppression of pro-inflammatory cytokines (TNF-α and IL-6) was observed in treated groups. The correlation between molecular docking findings and experimental tumour inhibition supports the hypothesis that luteolin exerts its anticancer effect through EGFR inhibition along with modulation of oxidative stress and inflammatory pathways. Overall, the present study concludes that luteolin possesses significant multitarget anticancer activity and may serve as a promising natural therapeutic candidate for EGFR-mediated cancers.

REFERENCES

1. Aggarwal, B. B., Shishodia, S., Sandur, S. K., Pandey, M. K., & Sethi, G. (2006). Inflammation and cancer: How hot is the link? *Biochemical Pharmacology, 72*(11), 1605–1621.
2. Arteaga, C. L., & Engelman, J. A. (2014). ERBB receptors: From oncogene discovery to basic science to mechanism-based cancer therapeutics. *Cancer Cell, 25*(3), 282–303.
3. Balkwill, F., & Mantovani, A. (2001). Inflammation and cancer: Back to Virchow? *The Lancet, 357*(9255), 539–545.
4. Baselga, J., & Swain, S. M. (2009). Novel anticancer targets: Revisiting ERBB2 and discovering ERBB3. *Nature Reviews Cancer, 9*(7), 463–475.
5. Berman, H. M., Westbrook, J., Feng, Z., *et al*. (2000). The Protein Data Bank. *Nucleic Acids Research, 28*(1), 235–242.
6. Bhattacharya, S., & Dash, R. (2016). Role of oxidative stress in cancer progression. *Free Radical Research, 50*(7), 785–801.
7. Bollag, G., Tsai, J., Zhang, J., *et al*. (2012). Vemurafenib: The first drug approved for BRAF-mutant cancer. *Nature Reviews Drug Discovery, 11*(11), 873–886.
8. Bos, J. L. (1989). Ras oncogenes in human cancer: A review. *Cancer Research, 49*(17), 4682–4689.
9. Cheng, H., & Force, T. (2010). Why do kinase inhibitors cause cardiotoxicity? *Current Opinion in Cardiology, 25*(4), 372–378.
10. Coussens, L. M., & Werb, Z. (2002). Inflammation and cancer. *Nature, 420*(6917), 860– 867.
11. Cragg, G. M., & Newman, D. J. (2013). Natural products: A continuing source of novel drug leads. *Biochimica et Biophysica Acta, 1830*(6), 3670–3695.
12. Dhillon, S. (2015). Afatinib: A review in advanced non-small cell lung cancer. *Drugs, 75*(11), 1263–1274.
13. Fidler, I. J. (2003). The pathogenesis of cancer metastasis. *Nature Reviews Cancer, 3*(6), 453–458.
14. Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: The next generation. *Cell, 144*(5), 646–674.
15. K. Pavan Kumar and M. Kannadasan. Therapeutic Horizons of Autophagy and Apoptosis in Colorectal Cancer: A Translational Perspective from Molecules to Medicine. International Journal of Pharmaceutical Biological and Chemical Sciences. 2025 14(1): 36-46.
16. K. Pavan Kumar and M. Kannadasan. Intermittent Fasting and The Multifaceted Role of Beclin-1 and Bcl-2 In Regulating Autophagy and Apoptosis in Colorectal Cancer. Journal of Chemical and Pharmaceutical Sciences. 2025 18 (2): 103-114.
17. K. Pavan Kumar and Amit Kumar. Modulation Of Beclin-1 and Bcl-2 Expression by Intermittent Fasting In CRC: Links to Autophagy and Apoptosis. International Journal of Pharmacy and Biological Sciences. 2025 15 (4): 91-115.
18. K. Pavan Kumar and Amit Kumar. Intermittent Fasting-Induced Autophagy and Apoptosis in Colorectal Carcinoma: The Role of Beclin-1 and Bcl-2. International Journal of Pharmaceutical Biological and Chemical Sciences. 2025 14 (4): 26-38.
19. Kokkula Pavan Kumar, Rajeev Imadi, Prasad Garrepally, S Nikitha Anthelmintic Activity of Some Medicinal Plants: A Short Review, International Journal of Pharmacy and Biological Sciences,9(2):605-611(2019). (UGC CARE).
20. Kokkula Pavan Kumar, Ampati Srinivas and Prasad Garrepally, Hyperthermia has consistently improved the efficacy of radiotherapy and chemotherapy for many types of cancers, Journal of Stem cell Research and Therapeutics International, 1(003):1-3(2019).
21. Kokkula Pavan Kumar, Ampati Srinivas and Prasad Garrepally, Subcutaneous DL technique has proven to be an adequate host for human embryonic stem cells, Journal of Stem cell Research and Therapeutics International, 1(004):1-3(2019).
22. Kokkula Pavan Kumar, Boda Rambabu, Investigational studies on carcinoma in male SD rats, International Journal of Research and Analytical Reviews,6(2):654-662(2019).

23. K. Pavan Kumar, G. Venkateshwarlu, Navaneeth Kumar, Mukesh Sharma, Rekha Pal, Taniya Rawat, E. Rajeswari, Wound healing activity of Indian Medicinal Plants, High Technology Letters, 28(2):1-7(2022).
24. K. Pavan Kumar, G. Venkateshwarlu, Mukesh Chandra Sharma, Govind pal, Rekha pal, Taniya Rawat, Invitro anthelmintic activity of alcoholic and methanolic extracts of seeds of Panicum miliaceum in Indian adult earthworm, High Technology Letters, 28(1): 800-809(2022).
25. Lynch, T. J., Bell, D. W., Sordella, R., *et al*. (2004). Activating mutations in EGFR underlying responsiveness of non-small-cell lung cancer to gefitinib. *New England Journal of Medicine, 350*(21), 2129–2139.
26. Mantovani, A., Allavena, P., Sica, A., & Balkwill, F. (2008). Cancer-related inflammation.
27. *Nature, 454*(7203), 436–444.
28. Newman, D. J., & Cragg, G. M. (2016). Natural products as sources of new drugs over the last 25 years. *Journal of Natural Products, 79*(3), 629–661.
29. Paez, J. G., Jänne, P. A., Lee, J. C., *et al*. (2004). EGFR mutations in lung cancer: Correlation with clinical response to gefitinib therapy. *Science, 304*(5676), 1497– 1500.
30. Pratheeshkumar, P., Son, Y. O., Wang, X., *et al*. (2012). Luteolin inhibits NF-κB activation and inflammatory responses. *Toxicology and Applied Pharmacology, 262*(2), 105–112.
31. Reuter, S., Gupta, S. C., Chaturvedi, M. M., & Aggarwal, B. B. (2010). Oxidative stress, inflammation, and cancer. *Free Radical Biology and Medicine, 49*(11), 1603–1616.
32. Seelinger, G., Merfort, I., & Schempp, C. M. (2008). Anti-oxidant and anti-inflammatory activities of luteolin. *Molecular Nutrition & Food Research, 52*(1), 26–42.
33. Wang, L., Tu, Y. C., Lian, T. W., Hung, J. T., Yen, J. H., & Wu, M. J. (2017). Luteolin inhibits tumour growth and angiogenesis. *International Journal of Oncology, 50*(2), 550–560.
34. Yarden, Y., & Pines, G. (2012). The ERBB network: At last, cancer therapy meets systems biology. *Nature Reviews Cancer, 12*(8), 553–563.
35. Zhang, Q., Zhao, X. H., & Wang, Z. J. (2020). Luteolin attenuates oxidative stress and apoptosis in colorectal cancer. *Biomedicine & Pharmacotherapy, 123*, 109789.

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