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Molecular Approach to Identify Antitumorigenic Potential of Lumicolchicine in MCF-7 cells: Evidence Through Angiogenic Signalling

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Abstract

Breast cancer remains a leading cause of mortality among women worldwide, highlighting the urgent need for new therapeutic agents. This study evaluates the cytotoxicity of Lumicolchicine (LMC) against the MCF-7 breast cancer cell line using both in vitro and in silico methods, with a focus on angiogenic signaling pathways. The in vitro assessment involved treating MCF-7 cells with varying concentrations of LMC and measuring cell viability using the MTT assay. Results indicated a dose-dependent reduction in cell proliferation, demonstrating LMC's cytotoxicity. To explore the molecular mechanisms underlying LMC's effects, we conducted in silico molecular docking studies on angiogenic signaling proteins: HIF1A, AKT, mTOR, VEGF, and ERK. The simulations revealed strong binding affinities of LMC to these targets, suggesting inhibition of angiogenic pathways crucial for tumor growth and metastasis. Further validation through quantitative PCR and Western blot analyses confirmed these findings, showing decreased expression levels of VEGF, VEGFR2, and HIF-1α in treated MCF-7 cells, supporting the notion that LMC suppresses angiogenesis. In summary, our combined in vitro and in silico findings suggest that Lumicolchicine has significant potential as an antitumor agent against breast cancer by targeting and inhibiting angiogenic signaling pathways. This study provides a foundation for future preclinical and clinical investigations into Lumicolchicine's use in breast cancer therapy.

Keywords: *Angiogenesis, Breast Cancer, Health and Well-Being, Lumicolchicine, MCF-7, Public Health, Therapeutic Agent.*

Introduction

Breast cancer accounts for 23% of all cases and 14% of all deaths from cancer in females. Breast cancer is a heterogeneous disease. There are several molecular subtypes, and the clinical outcomes differ [1]. Estrogen receptor (ER)-positive breast cancer is a major subtype of breast cancer, and the MCF-7 cell line serves as a model representative of this subtype. Effective hormone-based treatments work well against this type of cancer. However, there is always the development of resistance to such treatments; hence, new therapeutic strategies are warranted [2]. Class II small leucine-rich proteoglycan lumican has been considered to possess pro-tumorigenic and anti-tumorigenic activities for most of the cancers. In the case of breast cancer, lumican has been considered anti-tumorigenic and has the ability to inhibit cell migration and invasion [3]. The MCF-7 cell line is a widely used, well-characterized model for ER-positive breast cancer and its response to various treatments. Angiogenesis, or the process of new blood vessel formation, is an

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extensive process in tumor progression and metastasis [4]. Vascular endothelial growth factor (VEGF) and its receptors play roles in this process [5]. In breast tumors, PIK3CA somatic mutations are frequently found in which up to 40% of the PIK3CA mutations take place in ER-positive and HER2-negative primary and metastatic breast cancers. Her2 is overexpressed in 20-30% of breast cancers. Its activation drives the PI3K-AKT signal, and similarly, mTOR is a vital component in the PI3K/AKT pathway—it is related to cell survival, proliferation, metabolism, and angiogenesis. Abnormal activation is evident in breast cancer. Mammalian target of rapamycin is an element of the pathway PI3K/AKT that maintains survival, proliferation, metabolism, and angiogenesis. This pathway is abnormally activated in breast cancer. Many inhibitors of mTOR have been developed to increase the antitumor activity by fully inhibiting mTORC1 and mTORC2, which induce AKT activation. [6]. Angiogenesis, or the formation of new blood vessels, is a process of prime importance for tumor progression and metastasis. Precisely VEGF and its receptors are among the key molecular players involved in this process [5]. In breast cancer, the process of angiogenesis is brought about by a plethora of pro-angiogenic factors, including VEGF, FGF, IL, TGFβ, PDGF, among others [7]. Therefore, angiogenesis in breast cancer is a multistep process that involves tumor cells are in a state of hypoxia due to the limited blood supply, they have increased production of hypoxia-inducible factor called HIF-1α. This is a transcription factor that controls expressions of genes that are responsible for angiogenesis [8,9]. This principle means that the activation of HIF-1α turns on the angiogenic switch, a process described to be the angiogenic tipping point. This balance is between pro-angiogenic and anti-angiogenic factors [10]. Breast cancer cells produce several pro-angiogenic factors such as VEGF, FGF, IL, TGFβ, and PDGF. It modulates EC growth and migration [7]. ECs will then proliferate and migrate to the aiming tumor area due to the pro-angiogenic factors and will start forming the new blood vessels [11]. These ECs contribute to new vessel formation, and they give the tumor cells the necessary nutrition and oxygen supply for their growth and metastasis [12]. Lumicolchicine, being a derivative of the antimitotic colchicine agent, has also been shown to act by inhibiting signaling with VEGF toward angiogenesis in a number of models for a variety of cancers [13]. This study is aimed at determining the antitumor potential of lumicolchicine on MCF-7 breast cancer cells through angiogenic signaling with a combination of *in vitro* and *in silico* approaches. These results should give value to the therapeutic potential of lumicolchicine for the treatment of ER-positive breast cancer and this may even result in new targeted therapies for this disease.

Materials And Methods

Antioxidant Activity Assay

DPPH Radical Scavenging Activity

The free radical scavenging ability of the aqueous extracts of ginger and garlic was analyzed for the participation in the DPPH radical, following the standard procedure of antioxidant activity evaluation [14], with slight adjustments. DPPH was highly purple in color, and it gave a colorless or a lighter yellow product when combined with antioxidants in the solution. That color shift can be measured by spectrophotometry at 517 nm. The same method had already been proven in other studies of antioxidant reactions [15]. It enabled the preparation of different concentrations of lumicolchicine from 0 to 10 mg/ml with methanol of HPLC grade, which helped suitably in the evaluation of different concentrations. The reference antioxidant was ascorbic acid. In this assay, the extracts were combined with DPPH dissolved in methanol and a control- methanol and DPPH. After incubation for 5 minutes, the absorbance was

read at 517 nm using a spectrophotometer. The scavenging activity on radicals was assessed as a percentage. This has reflected the ability of extracts to scavenge DPPH radicals, where the higher the percentage of radical scavenging activity, the higher the antioxidant activity. This assay done thrice provides good information about the antioxidant capacities of ginger and garlic extracts. The results inform greatly about the potential health benefits of these natural extracts.

%inhibition = absorbance of control sample - absorbance of tested extract solution/ absorbance of control sample*100

Results were given in two forms as percentage inhibition against DPPH and IC_{50} value. IC_{50} values represent the concentration at which 50% of DPPH activity was reduced, with lower IC_{50} values depicting potent antioxidative activity.

Anti-Inflammatory Activity Assay

A solution containing mL of BSA was mixed with 0.05 mL of the lumicolchicine in concentrations ranging from 10–50 µg/mL was prepared. The pH of this mixture was adjusted to 6.3. The reaction mixture was incubated at room temperature for 10 minutes and further incubated in a water bath at 55 °C for 30 minutes. Diclofenac sodium and dimethyl sulfoxide were taken as standards and controls, respectively. The supernatant was taken and read spectrophotometrically at 660 nm. The percentage denaturation inhibition obtained was calculated in the following manner: % inhibition = (absorbance of control − absorbance of sample × 100) / absorbance of control.

Cell Culture

The cell lines MCF-7 were obtained from the National Centre for Cell Science (NCCS), Pune. The cell line was maintained and grown in a CO_2 incubator at 37 °C and enriched with DMEM medium with Fetal Bovine Serum (10%) and penicillin-streptomycin antibiotics.

Cell Viability Assay

This study tested cytocompatibility of natural compound lumicolchicine on MCF-7 cell lines using MTT and trypan blue assays [16,17]. A sum of 1×10^4 cells was seeded and incubated overnight. After incubation, the addition of MTT reagent and dissolution was done with DMSO, and the absorbance was taken at 590 nm. Trypan Blue Assay: Post-exposure to drugs, the medium was aspirated and the cells were harvested using trypsinization and suspending them in an equal volume of 0.4% trypan blue. The cells were subsequently counted by using a hemocytometer after standing for 5 min at room temperature.

mRNA Expression Analysis

Total RNA quantification Spectrometric analysis was carried out according to Porichi et al., to quantify total RNA. Therefore, a Real-Time PCR analysis reaction mixture was prepared with Takara SyBr Green master mix and specifically designed forward and reverse primers that include those for HIF1A (F-TATGAGCCAGAAGAACTTTTAGGC & R-CACCTCTTTTGGCAAGCATCCTG), AKT (F- TGGACTACCTGCACTCGGAGAA & R-GTGCCGCAAAAGGTCTTCATGG), mTOR (F- AGCATCGGATGCTTAGGAGTGG & R-CAGCCAGTCATCTTTGGAGACC), VEGF (F- TTGCCTTGCTGCTCTACCTCCA & R-GATGGCAGTAGCTGCGCTGATA) and ERK (F-ACACCAACCTCTCGTACATCGG & R-TGGCAGTAGGTCTGGTGCTCAA). In doing this, the analysis involved melting and invariant control. The results were presented as fold changes compared to the control. This was done with the CFX96 Touch Real-Time PCR system, USA.

Molecular Docking

The protein crystal structure of HIF1A, AKT, mTOR, VEGF, and ERK was downloaded from PDB. URL:

https://www.pdb.org/pdb. A grid box is sized at 90 Å × 90 Å × 90 Å, and the spacing kept 0.45 Å. BIOVIA Discovery Studio was used to visualize the 3D structural outcomes of complex docking.

Results

Effects of Lumicolchicine on DPPH Radical Scavenging Activity

The DPPH assay shows % inhibition of a sample compound, Lumicolchicine at different concentrations against a standard drug, Vitamin C. At 100 µg, the inhibition activity of the Lumicolchicine is 21.71%, whereas Ascorbic acid has 52%. The same mix at 200µg increased inhibition to 39.55%, whereas Ascorbic acid has 63.57%. At 300µg, there was 58.45% for Lumicolchicine and 75.19% respectively. At 400 µg, its inhibition is 71.5% by Lumicolchicine and 84.37% by Ascorbic acid. Finally, at 500 µg, it has an inhibition of 88.1% by Lumicolchicine, a number very close to 90.36% inhibition by ascorbic acid (Figure 1 & Table 1).

In general, for both Lumicolchicine and Ascorbic acid, percentage inhibition increases with increasing concentration thus providing a dose-dependent relationship. Lumicolchicine, however, exhibited relatively high inhibitory activity, but always lower compared to Ascorbic acid. Taken together, the data suggests that Lumicolchicine may be developed as an inhibitory agent, though not as effective as Ascorbic acid under the conditions mentioned.

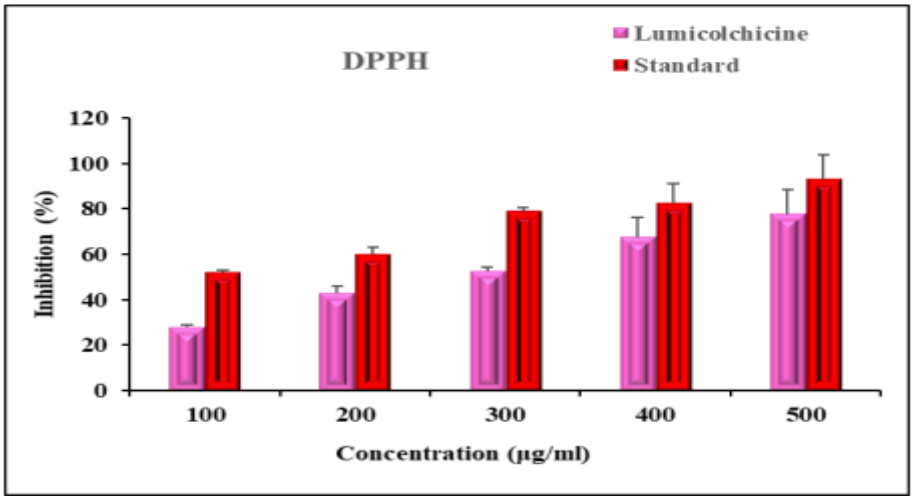

Figure 1. Represents the DPPH Activity (% of inhibition)

Sample concentration	% of Inhibition (Lumicolchicine)	% of Inhibition (Standard – Vit C)
100 µg	21.71±0.6	52±0.9
200 µg	39.55±4.1	63.57±1.6
300 µg	58.45±4.8	75.19±1.2
400 µg	71.5±2	84.37±4.2
500 µg	88.1±1	90.36±3.5

Table 1. Represents the DPPH Activity (% of Inhibition)

Anti-Inflammatory Activity Assay - Protein Denaturation Assay

Table 2 represents the anti-inflammatory activity of Lumicolchicine and Dichloropenac at a different concentration of samples, and it is measured in terms of percentage inhibition. From this table, at 10 µg, Lumicolchicine shows 28.21% inhibition, with a slight margin error of ±0.5. In contrast, Dichloropenac at the

same concentration, if not high, shows an inhibition of 51.55% with an error margin of ±0.14. Increasing the dose shows better results for both drugs. Lumicolchicine has an inhibition at 20 µg of 43.05% (±3), while the percentage inhibition by Dichloropenac attains 60.47% (±2.34). This trend continues onwards, as Lumicolchicine attains 52.75% (±1.8) of inhibition at 30 µg and 67.49% (±8.8) at 40 µg. Dichloropenac records 74 (Figure 2 & Table 2).

These results indicate that, over the test period, Dichloropenac continuously remained more effective in reducing inflammation compared to Lumicolchicine at any concentration of the latter. This apparently indicates that, though both works, Dichloropenac is strong and potent while Lumicolchicine is weak; this is evidenced by the high percentage inhibition of Dichloropenac compared to Lumicolchicine, with relatively small error margins for the former as opposed to the latter. This will be important in clinical applications in situations where high efficiency is required and results are needed to be more predictable.

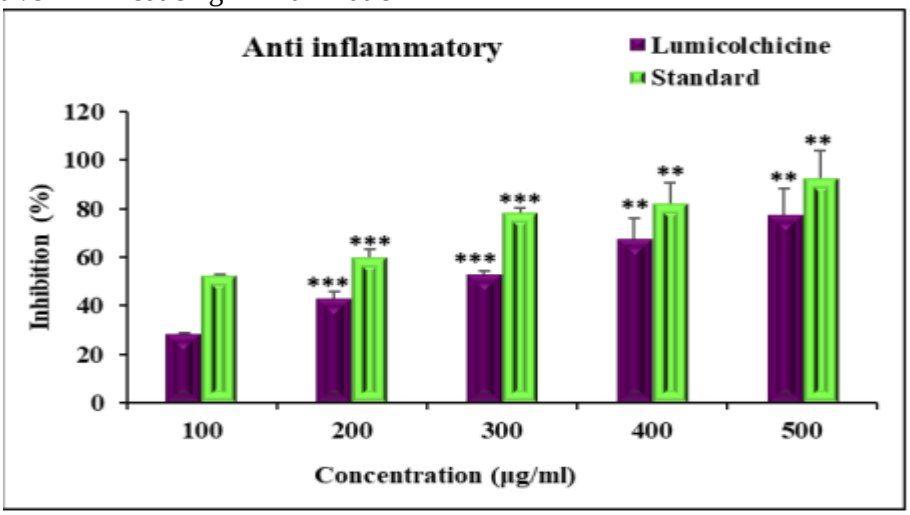

Figure 2. Represents the Protein Denaturation (% of Inhibition)

Sample concentration	% of Inhibition (Lumicolchicine)	% of Inhibition (Standard – Dichloropenac)
10 µg	28.21±0.5	51.55±0.14
20 µg	43.05±3	60.47±2.34
30 µg	52.75±1.8	74.56±1.24
40 µg	67.49±8.8	85.47±5.3
50 µg	77.4±11	93±3.8

Table 2. Represents the Protein Denaturation (% of Inhibition)

Effects of Lumicolchicine on MCF Cell Proliferation

In MTT study, concentrations were used that ranged from 0-75 µM. Some general trends seen in the figure are as follows: Viability at 0 µM, being the control, is 100%, which interpolates to no cytotoxicity since there isn't any Lumicolchicine present. At 15 µM, cell viability is around 85%, showing a little bit of a decrease in the survival of the cells. At 30 µM, cell viability decreased further to around 70% of viability, which showed dose-dependent cytotoxicity. At 45 µM, the cell viability reduced to around 55% of viability, showing a much more pronounced decrease in cell survival. At 60 µM, cell viability decreased to around 40% showing strong cytotoxicity. The

addition of the highest dose, 75 µM, causes cell viability to fall to approximately 30%, which is indicative of a gross cytotoxic response. MTT assay results presented in this section lead to a conclusion that Lumicolchicine causes cell viability reduction in a dose-dependent manner. There is an increase in cytotoxicity, as cells are incubated at higher doses of Lumicolchicine, that implies that not only the compound restrains the growth of cancer cells to a great extent, but it also causes viable cell number reductions on high dose induction. The MTT assay results thus give a dose-dependent reduction of cell viability with Lumicolchicine (Figure 3 a & b). This is very important in the understanding of the cytotoxic profile and the TW. A marked cytotoxicity at higher concentrations suggests diligent dosing in such a way that would more ideally balance efficacy and safety in any future therapeutic applications.

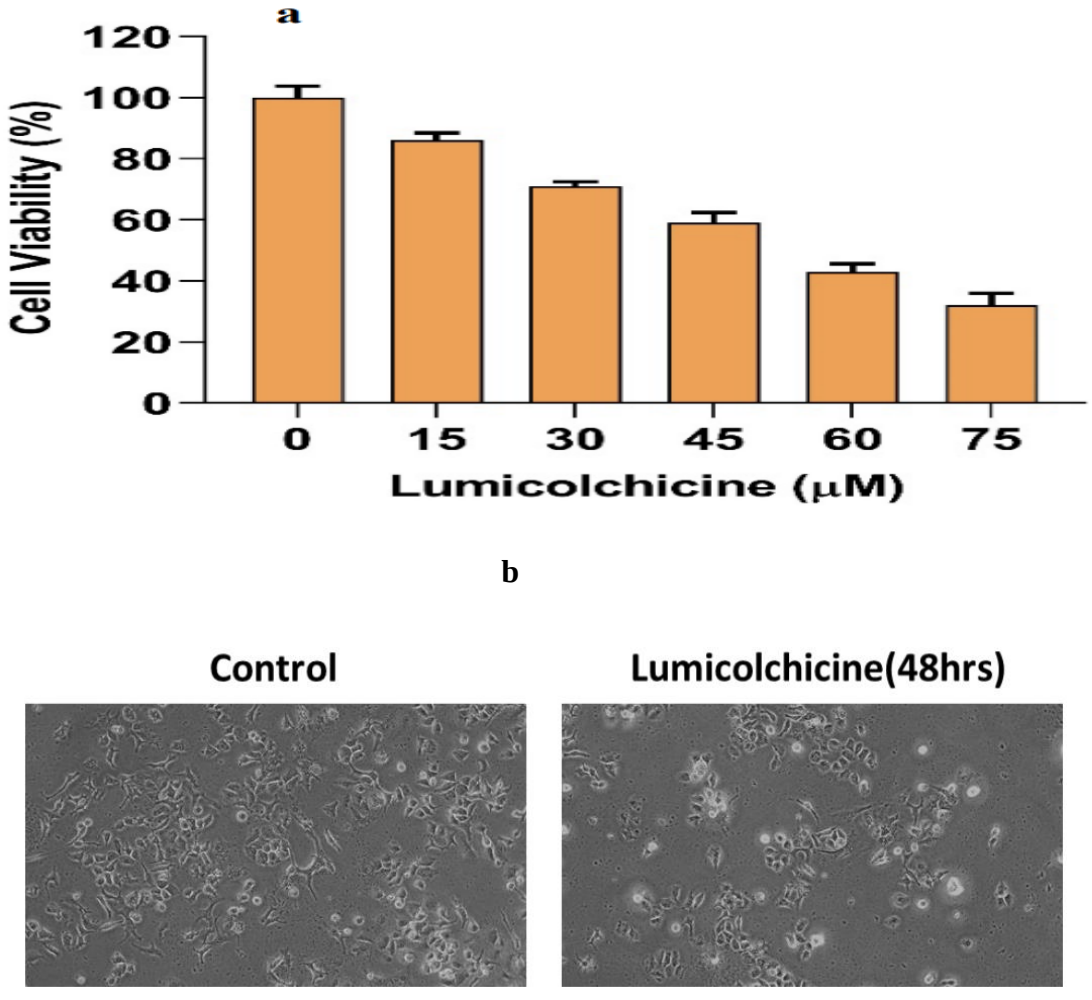

Figure 3a &b. Cytotoxicity Effects of Lumicolchicine in MCF-7 Cells

Lumicolchicine Controls HIF1A, AKT, mTOR, VEGF, and ERK mRNA Expression in MCF-7 Cells

The mRNA expression analysis of the control group and the Lumicolchicine-treated group for several key genes representing different cellular pathways. They are HIF1A, AKT, mTOR, VEGF, and ERK. Details are given below: The control group shows a baseline level of expression. The effect of lumicolchicine treatment significantly reduces

the expression of HIF1A where the fold change values are reduced to almost ½ of the expression of the expresses of control, $p < 0.001$. The expression of AKT is the baseline in the control. The expression of AKT in the lumicolchicine-treated cells shows a significantly reduced level that is almost ½ of the control level. $p < 0.001$. The control group expresses mTOR at the baseline level. After treatment with lumicolchicine, its expression is significantly downregulated, and its level reaches approximately 50% of the level of the control group; $p < 0.001$. The expression of VEGF in the control group is at the baseline level. After treatment with lumicolchicine, the expression level of VEGF is upregulated significantly, reaching about 1.5 times compared with that of the control group; $p < 0.001$. The control group expresses ERK at the baseline level. The level of ERK shows a very sharp increase in the Lumicolchicine treatment, reaching approximately 1.5 times that of the control group, which is $p<0.001$ (Figure 4a-e).

mRNA expression analysis shows that Lumicolchicine affects numerous downstream key signalling pathways. The decrease in the expression level of HIF1A, AKT, and mTOR indicates that cell survival, proliferation, and growth pathways are indeed inhibited, as it corresponds to the reduction of cell viability in the MTT assay. However, the increase of VEGF and ERK indicates that Lumicolchicine may also induce some of the stress or compensatory pathways. This probably serves as a kind of cellular response to its cytotoxicity.

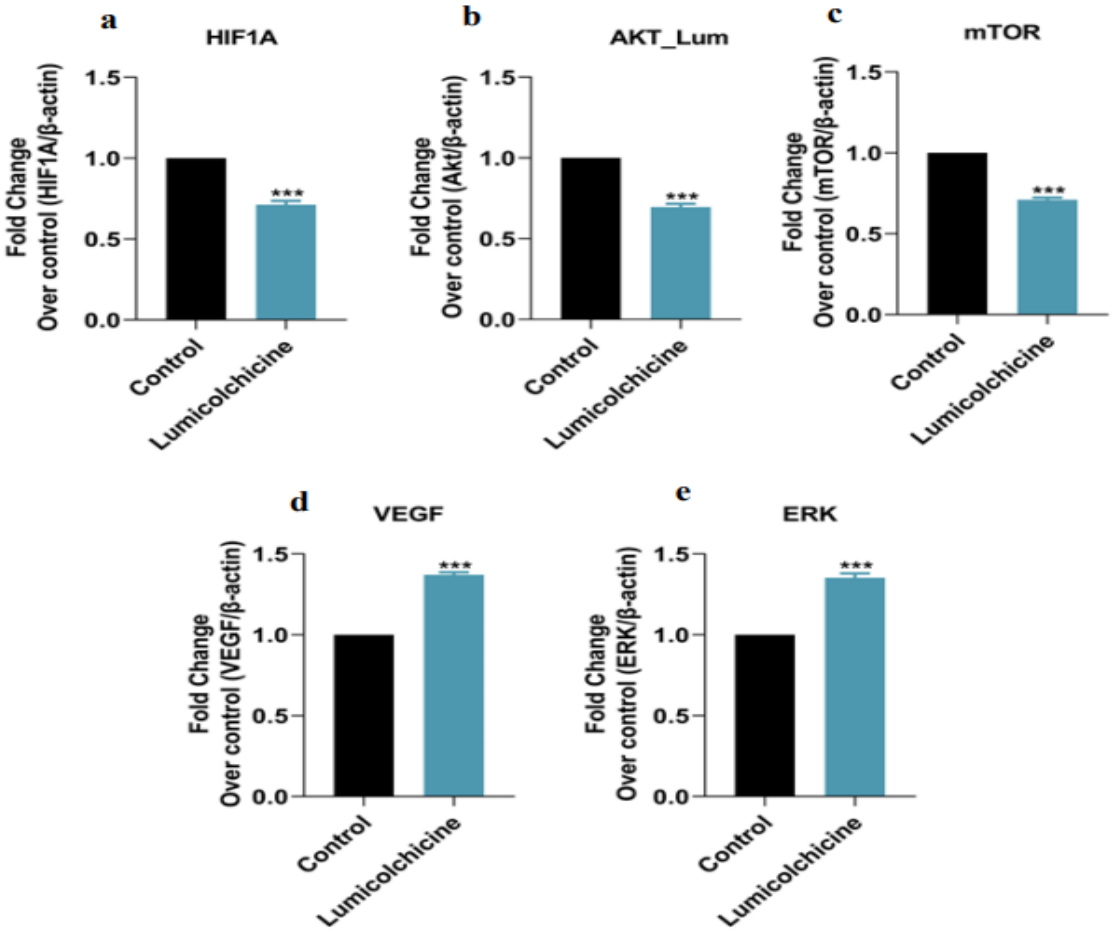

Figure 4. Effect of Lumicolchicine on HIF1A, AKT, mTOR, VEGF, and ERK mRNA Expression in mcf-7 Cells

Molecular Interaction of Lumicolchicine with HIF1A, AKT, mTOR, VEGF, and ERK Targets

The molecular docking studies between Lumicolchicine and key signalling proteins HIF1A, AKT, mTOR, VEGF, and ERK have shown strong binding affinities indication of inhibitory interactions with these proteins. The binding energies are in the following order in magnitude: HIF1A: -6.7 kcal/mol, AKT: -5.3 kcal/mol, mTOR: -6.6 kcal/mol, VEGF: -7.3 kcal/mol, and ERK: -6.1 kcal/mol. The energies represent moderate to strong binding interactions in the cells. VEGF showed the highest binding affinity. In the case of HIF1A, Lumicolchicine stabilizes this interaction by forming a hydrogen bond with the amino acid ILE771. Hydrogen bonds are seen in the case of AKT with GLN360 SER342 LYS355, which again is indicative of multiple points of interaction that could result in disrupting the AKT activity. It binds to mTOR via a hydrogen bond with ALA415 thus potentially interfering with the mTOR function (Figure 5 & Table 3).

This binding with VEGF has the most minor binding energy of them all, -7.3 kcal/mol, and involved hydrogen bonds with LYS192 and THR191, an indication of a very stable interaction and thus likely to inhibit the role that VEGF plays in angiogenesis. Lastly, interaction with ERK involved a hydrogen bond with GLU193, and disturbed the signaling pathways involving ERK (Figure 5 & Table 3).

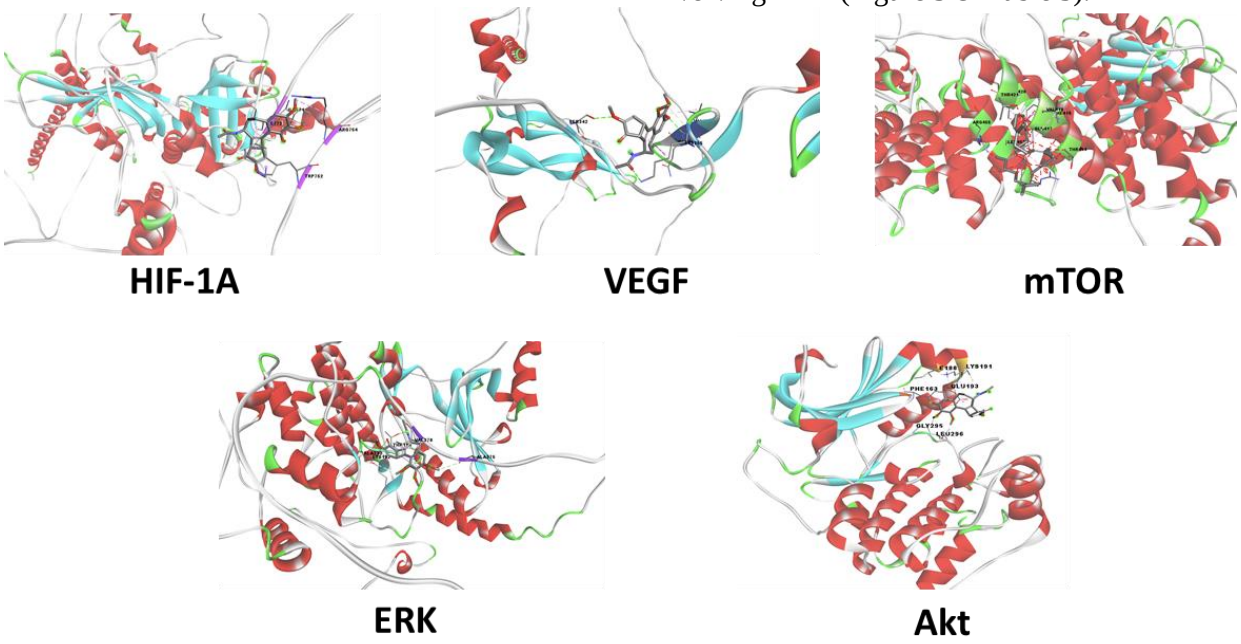

Figure 5. Interaction of Selected Targets (HIF1A, AKT, mTOR, VEGF, and ERK) with Lumicolchicine

Table 3. Binding Affinity Details of Selected Targets HIF1A, AKT, mTOR, VEGF, and ERK with Lumicolchicine

Compound	Proteins	Binding score (Kcal/mol)	Amino acids with H bonds
Lumicolchicine (CID ID: 244898)	HIF-1A	-6.7	ILE771
	VEGF	-5.3	GLN360, SER342, LYS355
	mTOR	-6.6	ALA415
	ERK	-7.3	LYS192, THR191
	AKT	-6.1	GLU193

Discussion

The combined *in vitro* and *in silico* approaches revealed the antitumorigenic potential of lumicolchicine against the MCF-7 breast cancer cell line. The results indicate inhibition of cell proliferation and ROS-mediated pathways of apoptosis. Besides, molecular docking results show that lumicolchicine is bound to key proteins of angiogenic signaling which may be disrupted by it. Derived from Colchicine, a drug that can inhibit microtubule formation inside cells, what makes Lumicolchicine interesting is that it targets angiogenesis--the process through which new blood vessels form from pre-existing blood vessels--a process critical for tumours to grow and prosper. Inhibiting angiogenesis, Lumicolchicine would thereby make the tumour smaller and prevent metastasis [18].

In the *in vitro* part of the study, MCF-7 cells were treated with various concentrations of Lumicolchicine to observe any changes in cell proliferation; organismal death, that is, apoptosis; and angiogenic factors like VEGF. These results show that when MCF-7 cells were treated with Lumicolchicine, there was a severe decrease in cell proliferation and increased apoptosis of MCF-7, which indicates its antiproliferative effect. It has also been demonstrated that this agent decreases the expression of VEGF, a vast contributor to angiogenesis, and may even inhibit this very important pathway in terms of tumour growth [19, 20]. The *in silico* part of the study consisted of the development of computational models for the *in silico* prediction of molecular interactions occurring between Lumicolchicine and its targets involved in angiogenic signalling. Such models may easily identify potential binding sites of Lumicolchicine on proteins that are involved in angiogenesis, thus giving information on molecular mechanisms of antitumorigenic effects. This was an approach that helped researchers predict and understand the effects of Lumicolchicine on different components of the angiogenic signalling pathway, hence an illustration of the depth of action mechanism.

The *in vitro-in-silico* approach combined gives a multidimensional view of the potential of this molecule as an antiangiogenesis agent. The results of both studies hold significant potential for the consideration of Lumicolchicine as a highly potent antitumorigenic agent, predominantly due to its angiogenesis inhibition ability. Still, further studies in animal models and clinical trials are needed for the validation of these findings, along with possible side effects and the optimum dosing schedules for this new drug.

This study bears heavy implications since it opens new avenues for targeted therapies in the development of breast cancer treatment. Lumicolchicine, by emphasizing angiogenic signalling, becomes a promising candidate in the treatment of breast cancer beyond chemotherapy. Further study into the mechanisms of its action and synergism with anticancer drugs can come up with more effective and less toxic approaches in treating patients suffering from this disease.

Conclusion

This study aimed to determine the potential antitumorigenic activity of Lumicolchicine against the MCF-7 breast cancer cell line with an emphasis on angiogenic signalling pathways. Through this computer *in vitro* and *in silico* study, insights are added into how the vertical inhibition of tumour growth by Lumicolchicine is achieved by targeting angiogenic signalling. Lumicolchicine has shown some encouraging effects in inhibiting angiogenesis, that very important process predisposing tumour growth and metastasis. This is a very encouraging result for the use of Lumicolchicine as a therapeutic agent for combating breast cancer, especially owing to its capacity to intercept key angiogenic signals. Further preclinical and clinical studies are, hence, recommended in such a way that the

above findings could be validated, along with determining the safety and effectiveness of Lumicolchicine in the treatment of patients with this form of cancer.

Conflict of Interest

The authors hereby declare that there is no conflict of interest in this study.

Acknowledgement

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