

Anticancer Potential of Ligustilide Derived from Extract of *Angelica sinensis* against Colon Cancer: *In vitro* and Computer Molecular Docking Studies

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ABSTRACT

Background: Colorectal cancer is a substantial global health issue, requiring the advancement of innovative therapeutic strategies beyond traditional methods. Despite progress in treatment options, colorectal cancer continues to be the second most fatal cancer worldwide, with anticipated increases in incidence and mortality rates over the coming decades. **Objectives:** The present study explored the anticancer effects of ligustilide against colon cancer HT-29 cells using *in vitro* and *in silico* studies. **Materials and Methods:** The cytotoxic effect of ligustilide at various concentrations (2.5-100 μ M) against HT-29 cells was investigated using the MTT assay. The apoptosis in the ligustilide-treated HT-29 cells was evaluated using a dual staining technique. The caspase enzyme activities in the ligustilide-treated cells were evaluated using kits. **Results:** The growth of HT-29 cells was considerably diminished after treatment with ligustilide at increasing dosages. The findings of the dual staining assay revealed that ligustilide treatment significantly elevated apoptotic incidences in the HT-29 cells. Moreover, the ligustilide treatment significantly elevated caspase enzyme activities in the HT-29 cells. The molecular docking studies revealed that the ligustilide could interact with the target's caspase-3, -8, and -9 with the binding affinity of -5.9 kcal/mol, -4.8 kcal/mol, and -4.0 kcal/mol with the RMSD of 2.109Å, 1.199Å, and 1.507Å through the hydrogen bonds. These *in vitro* and *in silico* results described that the ligustilide could potentially enhance the caspase-3, -8, and -9 expression. **Conclusion:** The findings of this study demonstrate that ligustilide treatment effectively suppresses growth and triggers apoptosis in colon cancer cells. Thus, it was clear that ligustilide has the capacity to induce caspase-dependent apoptosis in colon cancer cells.

Keywords: Colon cancer, Apoptotic caspases, Molecular docking, HT-29 cells, Ligustilide, Apoptosis.

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INTRODUCTION

Colorectal cancer, commonly referred to as colon cancer, is characterized by the uncontrolled proliferation of aberrant glandular epithelial cells within the colon or rectum. This malignancy is predominantly adenocarcinoma, accounting for over 90% of cases, though rarer forms like carcinoid tumors and lymphomas can also arise.¹ Globally, colorectal cancer stands as a challenging public health issue, ranking as the third most common cancer type and the second major cause of cancer-associated mortalities, with nearly 1.9 million new incidences and 904,000 mortalities reported annually. This escalating burden is further evidenced by a more than doubling of incident cases and deaths

from colorectal cancer worldwide between 1990 and 2019, with projections indicating the cancer-related burden could reach 28.4 million cases globally by 2040. This significant increase is partly attributable to an aging population and the rising prevalence of modifiable risk factors, including sedentary lifestyles, obesity, and detrimental dietary patterns.² Despite developments in screening and treatment, significant disparities in survival rates persist globally, with the overall 5-year survival rate estimated at 60%, varying considerably by stage at diagnosis and geographic region. The escalating case of colon cancer, particularly among younger demographics, underscores the urgent need for enhanced preventative strategies and more effective screening modalities to mitigate its global burden.³

The intricate pathophysiology of colon cancer involves a complex interplay of genetic predispositions, environmental causes, and lifestyle factors, ultimately ending in the conversion of normal cells into malignant phenotypes. The etiology of colorectal cancer is multifactorial, involving a combination of inherited genetic mutations, acquired somatic alterations, and modifiable lifestyle



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risk factors such as obesity, sedentary behavior, smoking, and unhealthy dietary habits.⁴ Approximately 65% of colorectal cancer cases progress sporadically without a clear family history or inherited genetic predisposition, arising from multiple acquired genomic and epigenetic alterations. Familial history, genetic vulnerabilities, and chronic inflammation are also recognized as significant contributors to the onset of colorectal cancer. A significant proportion of colorectal cancers arises through a multi-step process, often known as the adenoma-carcinoma sequence, where benign adenomatous polyps progressively transform into malignant adenocarcinomas.⁵ The progression from benign polyp to invasive adenocarcinoma involves unique genetic and molecular alterations within cells that characterize the specific colorectal cancer phenotype. The understanding of these genetic and epigenetic changes is crucial for developing targeted therapies and early detection strategies.⁶

Despite significant developments in medical technology, colon cancer treatment continues to present considerable challenges, necessitating the development of novel therapeutic approaches. Current strategies, while offering notable clinical achievements, often face limitations such as drug resistance, severe systemic toxicities, and limited efficacy in advanced stages of the disease, underscoring the critical need for safer and effective alternatives.⁷ Specifically, the emergence of drug-resistant stem cells, which contribute to tumor initiation, progression, and metastasis, further complicates therapeutic outcomes and highlights the critical need for agents selectively targeting these resistant populations. The intrinsic heterogeneity of colorectal cancer, both inter-tumor and intra-tumor, also presents a substantial obstacle to universal therapeutic efficacy, compelling a shift towards more personalized treatment paradigms.⁸ Thus, there is a compelling impetus to assess and validate novel therapeutic candidates derived from natural sources, particularly plant-based compounds, given their proven multi-modal pharmacological activities and generally favorable safety profiles. These natural compounds often exhibit pleiotropic effects, simultaneously targeting multiple aberrant pathways involved in carcinogenesis and drug resistance, thereby presenting a promising avenue for enhanced therapeutic efficacy with reduced adverse effects.^{9,10} Ligustilide is a naturally occurring dihydropthalide compound found extensively in the medicinal plant *Angelica sinensis* with various biological activities.¹¹ It has been suggested that ligustilide showed anti-inflammatory,¹² anti-atherosclerosis,¹³ and neuroprotective¹⁴ properties. Several previous studies have already reported that ligustilide inhibited viability and triggered apoptosis in several tumor cells, including lung cancer,¹⁵ oral cancer,¹⁶ ovarian cancer,¹⁷ bladder cancer,¹⁸ and breast cancer.¹⁹ Furthermore, it inhibited the growth of colon cancer HCT-116 cells.²⁰ However, there are no such reports on how ligustilide inhibits other forms of colon cancer cells and initiates apoptosis in colon cancer cells. Therefore, this work explored the anticancer effects of ligustilide against colon cancer HT-29 cells.

MATERIALS AND METHODS

Chemicals

The ligustilide, which is sourced from the extract of *A. sinensis*,^{21,22} was procured from Sigma Aldrich, USA. The diagnostic kits for measuring biochemical markers were obtained from Elabscience, USA, respectively.

Collection and maintenance of cell line

The colon cancer HT-29 cells were purchased from ATCC, USA, and cultivated on DMEM medium with FBS (10%) and antibiotics (1%) in a 5% CO₂ environment. Cells were collected upon achieving 80% confluency and employed for subsequent fluorescence staining and biochemical assessments.

MTT cytotoxicity assay

The cytotoxicity of ligustilide on the proliferation of HT-29 cells was assessed using MTT assay. Cells were cultured on a 96-well plate for 24 hr. Then, cells were subjected to ligustilide treatment for an additional 24 hr at different concentrations of 2.5, 5, 10, 25, 50, 75, and 100 μ M. Following the ligustilide treatment, MTT (20 μ L) and DMEM (100 μ L) was mixed in the wells and incubated for 4 hr. After dissolving the formazan formations using DMSO (100 μ L), the absorbance was then measured at 570 nm.

Dual staining

The dual staining experiment was done on control and ligustilide-treated HT-29 cells to evaluate apoptosis. The HT-29 cells were grown for 24 hr and then exposed to 25 μ M of ligustilide for 24 hr. A solution of 100 μ g/mL AO/EB dyes was then introduced to the wells for a duration of 5 min in the absence of light. The presence of apoptotic cells was assessed using a fluorescent microscope.

Analysis of caspase enzyme activities

The HT-29 cells from both the control and treatment groups were collected, and their cell lysate was produced using cell lysis buffer to measure the activities of caspase enzymes. The activities of caspase-3, -8, and -9 were assessed in the cell lysates of HT-29 cells using commercial test kits as per the manufacturer's specifications (Elabscience, USA).

In silico molecular docking studies

Dataset retrieval for molecular docking analysis

The structures of caspase-3 (UniProt ID: P42574), caspase-8 (UniProt ID: Q14790), and caspase-9 (UniProt ID: P55211) were retrieved from the AlphaFold database²³ for molecular docking analysis, as full-length structures without mutations were not available in the Protein Data Bank. All the downloaded structures exhibited high confidence levels, with pLDDT scores of 85.5%, 81.05%, and 80.40%, respectively. The ligustilide 3D atomic coordinates was obtained from the PubChem compound

database in the SDF format,²⁴ were then converted into PDB format using Discovery Studio Visualizer (DSV) v19.1.0.18287 (www.accelerys.com).

Active site identification for the caspase targets

The active sites of the caspase-3, -8, -9 were identified by implementing the PrankWeb server (<https://prankweb.cz/> Accessed on: 16 September 2025).²⁵ The active sites ranked as one were selected for the further molecular docking studies.

Molecular docking and Visualization

The molecular docking studies were carried out for the selected targets that includes, caspase-3, caspase-8, and caspase-9, with the ligand ligustilide using Autodock Vina, an advanced tool with an integrated scoring algorithm compiled into the PyRx software²⁶ (Eberhardt *et al.*, 2021). The Autodock vina uses a new scoring mechanism,

$$C = \sum_{i < j} f_{titj}(r_{ij}),$$

C - Sum of intermolecular and intramolecular distance; Σ - Over all of the pairs of atoms; f_{titj} - Symmetric set of interaction functions; r_{ij} - Interatomic distance.

The specified caspase targets and ligand ligustilide have been converted into PDBQT format to initiate the docking process. After, that the predicted active sites of the targets were selected and assigned the grid boxes (caspase-3 = size_{x,y,z} = 31.01, 27.88, 25.94; caspase-8 = size_{x,y,z} = 27.07, 26.61, 24.88; caspase-9 = size_{x,y,z} = 20.95, 18.68, 16.48) respectively, and identified the most possible binding pose between the targets and the ligand. After running molecular docking, we analyzed the obtained raw files for the targets that interacted with the ligustilide in 3D and 2D using Discovery Studio Visualizer v19.1.0.1828, developed by Dassault Systèmes BIOVIA (URL: www.accelerys.com, Accessed on 16th September 2025).

Statistical analysis

The statistical tests were done using Graphpad Prism software, and the data are given as Mean $\pm$ SD of triplicates. The results were assessed using one-way ANOVA and Tukey's *post hoc* tests, with a significance threshold of $p < 0.05$.

RESULTS

Effect of ligustilide on the colon cancer HT-29 cell viability

Figure 1 illustrates the results of the MTT cytotoxicity assay, highlighting the impact of ligustilide on the growth of HT-29 cells. The ligustilide treatment significantly ($p < 0.05$) reduced the HT-29 cell growth at increasing dosages (2.5-100 μ M).

The increased dosages of ligustilide revealed a reduction in the growth of HT-29 cells, as seen in Figure 1. The IC₅₀ concentration of ligustilide was established at 25 μ M against HT-29 cells, and this dosage was selected for subsequent studies.

Effect of ligustilide on the apoptosis in HT-29 cells

The dual staining was utilized to examine the apoptotic level in both control and ligustilide-treated HT-29 cells, with findings illustrated in Figure 2. After treatment with 25 μ M of ligustilide, the HT-29 cells had elevated levels of yellow/orange fluorescent cells, signifying the occurrence of both early and late-stage apoptosis. Therefore, it was clear that ligustilide can trigger apoptosis in HT-29 cells.

Effect of ligustilide on caspase activities in HT-29 cells

Figure 3 illustrates the activities of caspase enzymes in both control and ligustilide-treated HT-29 cells. The control cells exhibited moderate activities of caspase-3, -8, and -9. However, the treatment of HT-29 cells with 25 μ M of ligustilide resulted in a significant increase ($p < 0.05$) in caspase-3, -8, and -9 when compared with control cells. These data support the concept that ligustilide may facilitate caspase-mediated apoptosis in HT-29 cells.

Results of *in silico* molecular docking studies

The active sites for the obtained caspase-3, -8, and -9 were predicted to initiate the molecular docking study. In that, the ranked 1 cluster exhibited 18 residues as active sites for the caspase-3 which includes SER120, HIS121, GLY122, GLU123, PHE128, GLN161, CYS163, GLY165, THR166, TYR204, SER205, TRP206, ARG207, PHE256, MET61, THR62, SER63, and ARG64 with the probability of 0.785. Likely, the residues such as LEU254, ILE257, ARG258, ARG260, SER316, HIS317, GLY318, ASP319, TYR324, GLN358, CYS360, GLY362, ASP363, VAL410, SER411, TYR412, and ARG413 were identified as active sites of the caspase-8 with the probability of 0.744. Also, the residues such as GLY248, ALA249, ALA286, CYS287, GLY288, TYR345, THR347, and PRO349 were identified as the active sites of the caspase-9 with the probability of 0.021. The molecular docking results showed that caspase-3 and -8 interacted more than caspase-9. The resulting binding affinity, RMSD, and the types of bonds formed between the caspase-3, -8, and -9 with the ligustilide were detailed in Table 1.

Caspase-3

The caspase-3 residue ARG207 interacted with ligustilide through a hydrogen bond, with a binding affinity of -5.9 kcal/mol and an observed RMSD of 2.109 Å. Additionally, other residues such as LEU168, SER205, and SER251 formed hydrophobic interactions with ligustilide. Furthermore, residues like TYR204, TRP206, and PHE256 formed pi-alkyl, pi-pi T-shaped, and pi-sigma

interactions, which contributed to further stabilizing the complex (Figures 4A-B, 5A, and Table 1).

Caspase-8

Ligustilide interacted well with the residues of caspase-8 that includes GLY318 and CYS360 via hydrogen bonds and also interacted with other hydrophobic residues such as LEU254 and TYR324 with the binding affinity of -4.8 kcal/mol and the observed RMSD as 1.199Å. It also formed the pi-cation, alkyl, and pi-alkyl bonds with the residues HIS317, VAL140, ILE257, CYS360, and HIS317 which stabilizes the complex further (Figures 4 C, D, 5B, & Table 1).

Caspase-9

The caspase-9 residue GLN240 interacted with the ligustilide via the hydrogen bond and other residues such as SER242, HIS243, and PHE404 hydrophobically interacted with the binding affinity of -4.0 kcal/mol and the observed RMSD as 1.507Å. Likely other residues such as PRO247, PRO349, and PHE348 interacted via the pi-alkyl and alkyl bond (Figures 4 E, F, 5C, & Table 1).

DISCUSSION

Colorectal cancer continues to pose a major global health problem, necessitating the advancement of novel therapeutic techniques beyond conventional approaches. Despite advancements in treatment modalities, colorectal cancer remains the second most lethal cancer globally, with its incidence and mortality rates projected to increase in the coming decades, particularly in developing countries.²⁷ This growing global burden is primarily driven by shifting demographics, including an aging population, and the widespread adoption of Westernized lifestyles characterized by sedentary habits and diets rich in processed and genetically modified foods. This pervasive incidence underscores the complex interplay of environmental, genetic, and epigenetic factors participating in its pathogenesis. Understanding these multifactorial contributions is essential for clarifying the underlying molecular mechanisms driving tumor initiation and development, thereby informing the development of targeted therapeutic interventions.²⁸ Despite developments in screening, diagnosis, and treatments, the prognosis for

colorectal cancer often remains poor because of late-stage detection, frequent metastasis, and recurrence.²⁹ While current therapeutic interventions, encompassing surgery, chemotherapy, and radiation, have shown efficacy in curbing cancer progression, they are frequently associated with severe side effects that compromise patient quality of life and present a low risk-benefit ratio. Consequently, there is an unmet need for safer, more effective, and well-tolerated therapeutic alternatives for colorectal cancer management.³⁰

The cytotoxic analysis is crucial for identifying potential therapeutic agents capable of selectively inducing apoptosis or necrosis in malignant cells while minimizing harm to healthy tissues. Such investigations are pivotal in early-stage drug discovery and development, guiding the selection of lead compounds for further preclinical and clinical evaluation.³¹ Reliable and accurate *in vitro* cytotoxicity assays are essential to predict *in vivo* responses, thereby streamlining the drug development pipeline. Understanding the exact mechanisms by which candidate drugs show their cytotoxicity is paramount, as this knowledge can inform subsequent modifications to enhance efficacy and mitigate off-target toxicity.³² The MTT assay is broadly employed to evaluate cell viability, making it an indispensable tool in cancer research for assessing the cytotoxic potential of novel drug candidates. Furthermore, the MTT assay is particularly valuable due to its cost-effectiveness, rapidity, and convenience, positioning it as a preferred technique for quantifying viable cells in culture for a wide array of cell types.³³ In this work, the findings of MTT assay demonstrated that the ligustilide treatment markedly reduced the HT-29 cell growth at increasing concentrations. These findings revealed the cytotoxic nature of ligustilide against colon cancer cells.

Apoptosis is an essential biological mechanism critical for maintaining tissue homeostasis and discarding injured or abnormal cells within an organism. This highly regulated process ensures the orderly dismantling and removal of cells without eliciting an inflammatory response, unlike necrosis.³⁴ In cancer, dysregulation of apoptosis is a hallmark of tumorigenesis, allowing tumor cells to escape apoptotic pathways, proliferate uncontrollably, and resist conventional therapies. Consequently, the triggering of apoptosis in tumor cells is a highly desirable

Table 1: The list of targets, UniProt ID, binding affinity (kcal/mol), RMSD (Å), and their interacting residues with ligustilide and the type of bonds.

Targets Name	Targets (UniProt ID)	Binding affinity (Kcal/mol)	RMSD (Å)	Hydrogen bond	Hydrophobic residues	Other bonds
Caspase-3	P42574	-5.9	2.109	ARG207	LEU168, SER205, SER251	TYR204 ^{PA} , TRP206 ^{PPS} , PHE256 ^{PS}
Caspase-8	Q14790	-4.8	1.199	GLY318, CYS360	LEU254, TYR324	HIS317 ^{PC} , VAL410 ^A , ILE257 ^{PA} , CYS360 ^{PA} , HIS317 ^{PA}
Caspase-9	P55211	-4.0	1.507	GLN240	SER242, HIS243, PHE404	PRO247 ^{PA} (2), PRO349 ^A (2), PHE348 ^{PA}

Note: A - Alkyl; PA - Pi-Alkyl; PPS - Pi-Pi T-shaped; PS - Pi-sigma; PC - Pi-cation.

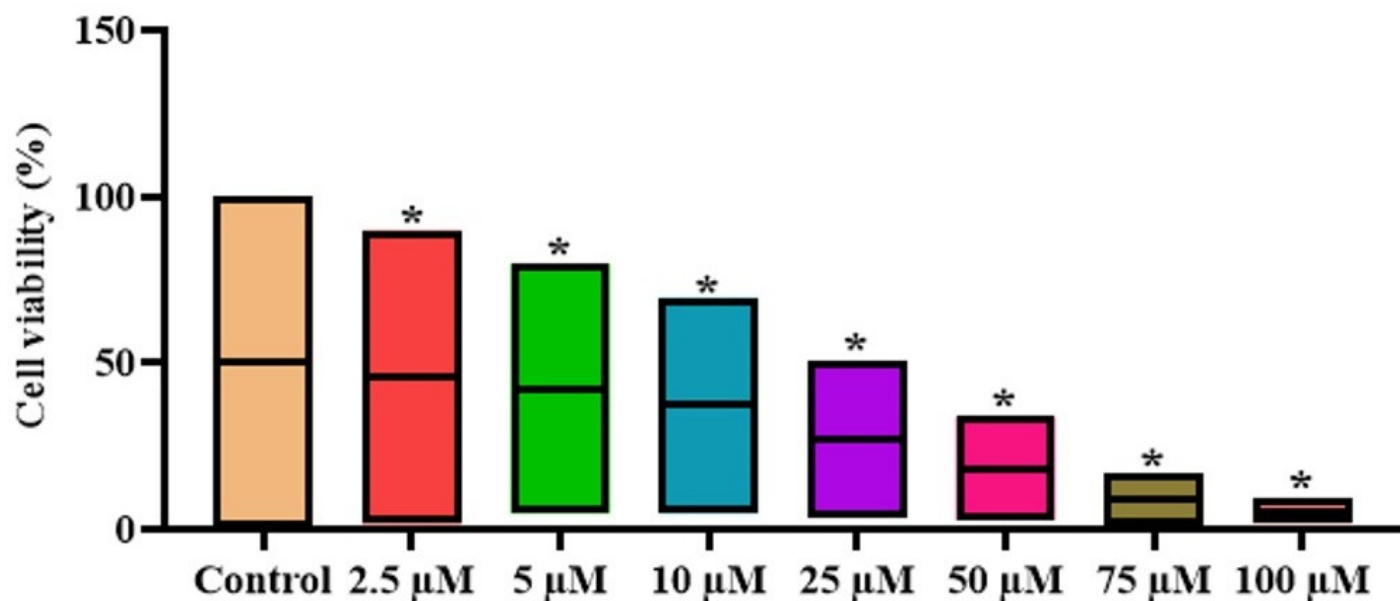
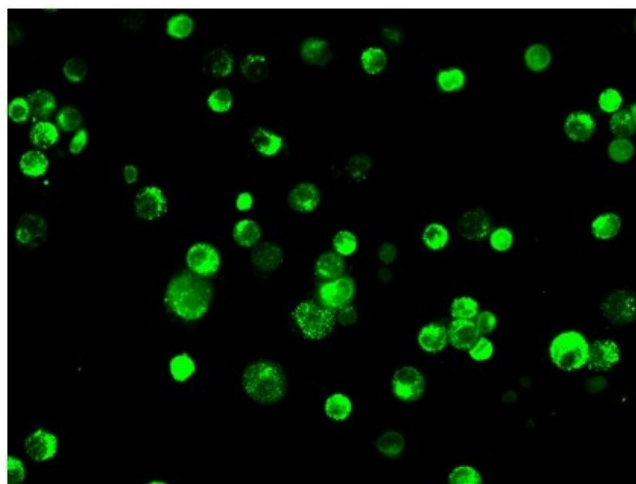


Figure 1: Effect of ligustilide on the colon cancer HT-29 cell viability. The viability of colon cancer HT-29 cells was significantly reduced with the treatment with increasing doses (2.5-100 μM) of ligustilide. The IC_{50} value of ligustilide for HT-29 cells was determined at 25 μM. The results are shown as the Mean±SD of triplicates. The results are evaluated by one-way ANOVA and Tukey's post hoc analysis utilizing GraphPad Prism software. A symbol '*' indicates that the values are statistically significant at $p < 0.05$ compared to the control group.

Control



25 μM

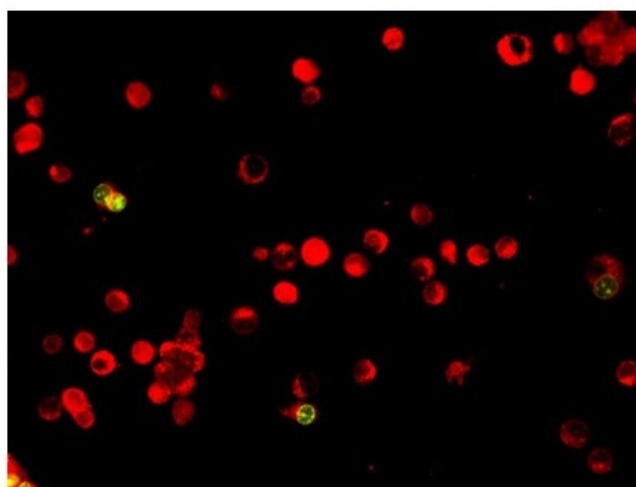


Figure 2: Effect of ligustilide on the apoptosis in colon cancer HT-29 cells. The colon cancer HT-29 cells exhibited heightened yellow/orange fluorescence after treatment with 25 μM of ligustilide. This observation confirmed the occurrence of early and late apoptosis in the ligustilide-treated HT-29 cells.

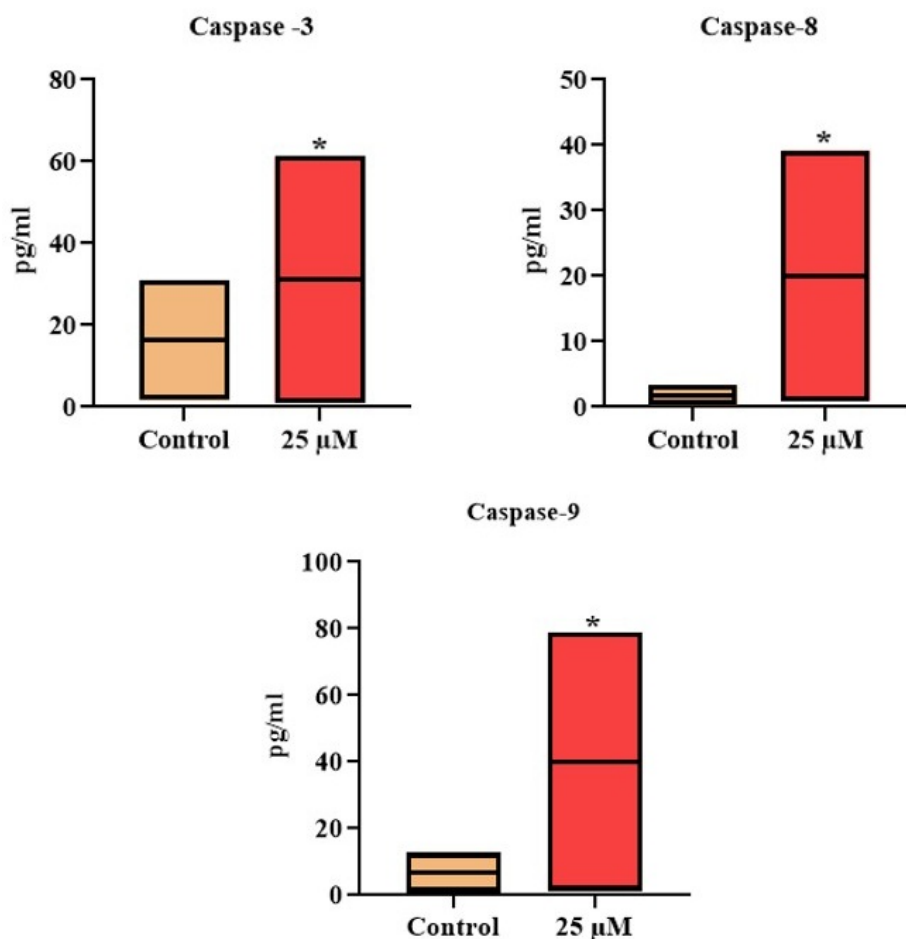


Figure 3: Effect of ligustilide on the caspase activities in the HT-29 cells. The results are shown as the ZMean $\pm$ SD of triplicates. The results are evaluated by one-way ANOVA and Tukey's post hoc analysis utilizing GraphPad Prism software. A symbol '*' indicates that the values are statistically significant at $p < 0.05$ compared to the control group.

therapeutic strategy, as it aims to restore this critical regulatory mechanism, leading to selective cancer cell elimination and improved treatment outcomes.³⁵ Targeting the delayed or inhibited apoptotic pathways in cancerous cells represents a significant and active area of oncological research. This approach seeks to leverage the intrinsic cellular machinery to trigger self-destruction in neoplastic cells, thereby circumventing the challenges associated with non-specific cytotoxicity often observed with traditional chemotherapy. Indeed, many oncogenic mutations confer resistance to proapoptotic insults, enabling tumor cells to bypass the natural cellular defense mechanisms.³⁶

Understanding the intricacies of apoptosis mechanisms and how cancer cells evolve to counteract apoptosis has therefore aimed research on novel techniques designed to trigger apoptosis specifically in tumor cells. This involves identifying therapeutic agents that can reactivate dormant apoptotic pathways or bypass existing resistance mechanisms within malignant cells.³⁷ Defects in apoptosis can result in the uncontrolled growth of neoplastic cells, underscoring the importance of targeting this pathway for effective cancer therapy. A major contributing factor in the onset of cancer is indeed the defective apoptosis, as tumor

cells often develop the capacity to escape apoptosis, playing an essential role in their resistance to therapies. Thus, the restoration of apoptotic signaling pathways or the introduction of novel pro-apoptotic triggers represents a critical avenue for developing more effective and selective cancer therapies.³⁸ This strategy capitalizes on the notion that while normal cells maintain robust apoptotic machinery, cancer cells often exhibit specific defects in these pathways, making them inherently vulnerable to targeted apoptotic interventions. Therefore, considerable research has focused on elucidating the molecular mechanisms governing apoptosis and identifying therapeutic agents that can selectively trigger apoptosis in tumor cells.³⁹ In this work, the findings of the dual staining assay demonstrated that ligustilide treatment induced apoptosis in HT-29 cells. Therefore, it was evident that ligustilide can decrease colon cancer progression via inducing apoptosis in colon cancer cells.

Understanding the intricate mechanisms that govern apoptosis, especially the central role of caspase enzymes, is crucial for developing effective anticancer strategies. Caspases, a family of cysteine-dependent proteases, are indispensable orchestrators of apoptosis, existing as inactive proenzymes (procaspases)

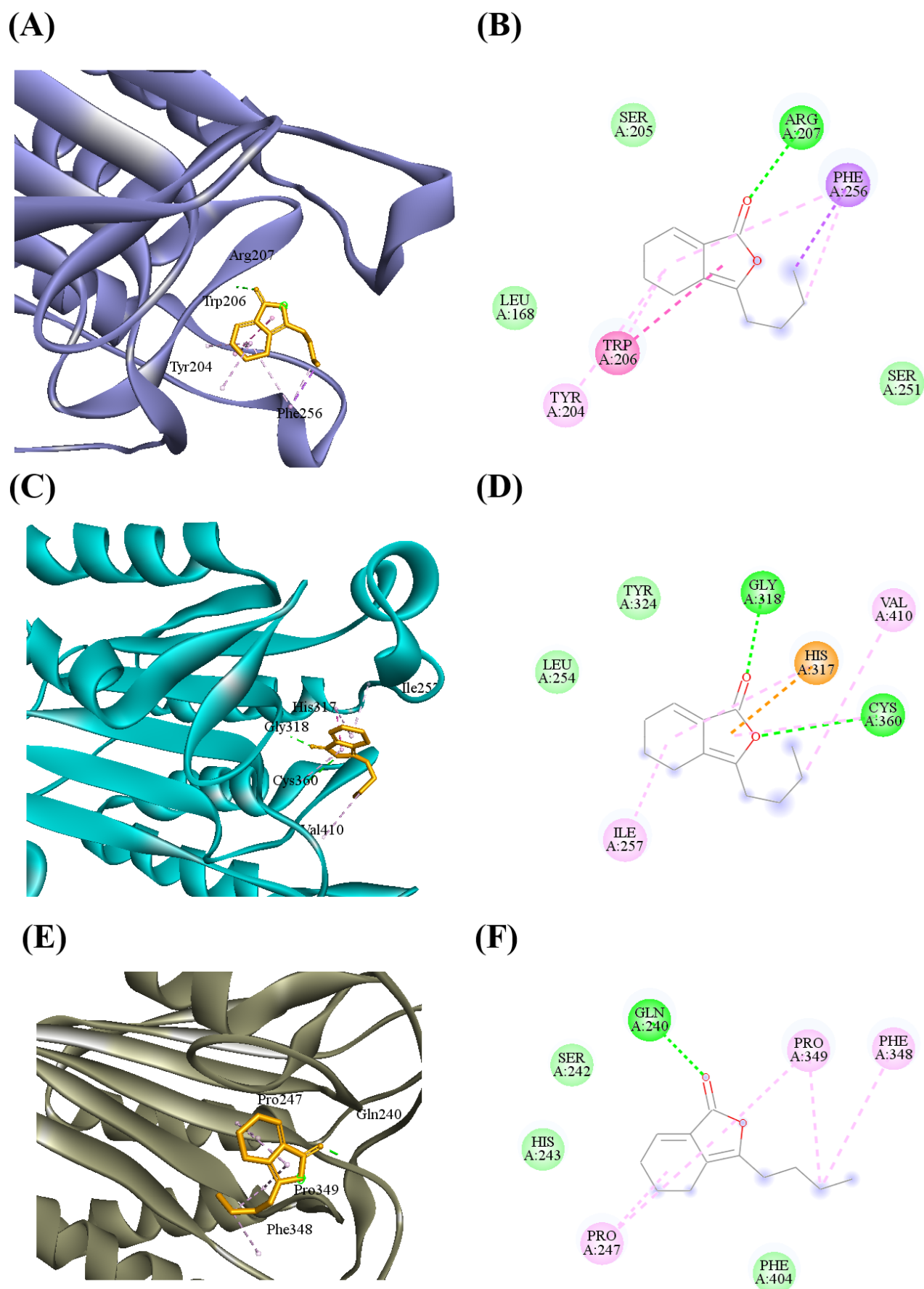


Figure 4: The docking pose of the ligustilide with the selected targets in 3D and 2D (A, B) Caspase-3 (A, B), Caspase-8 (C, D), and Caspase-9 (E, F).

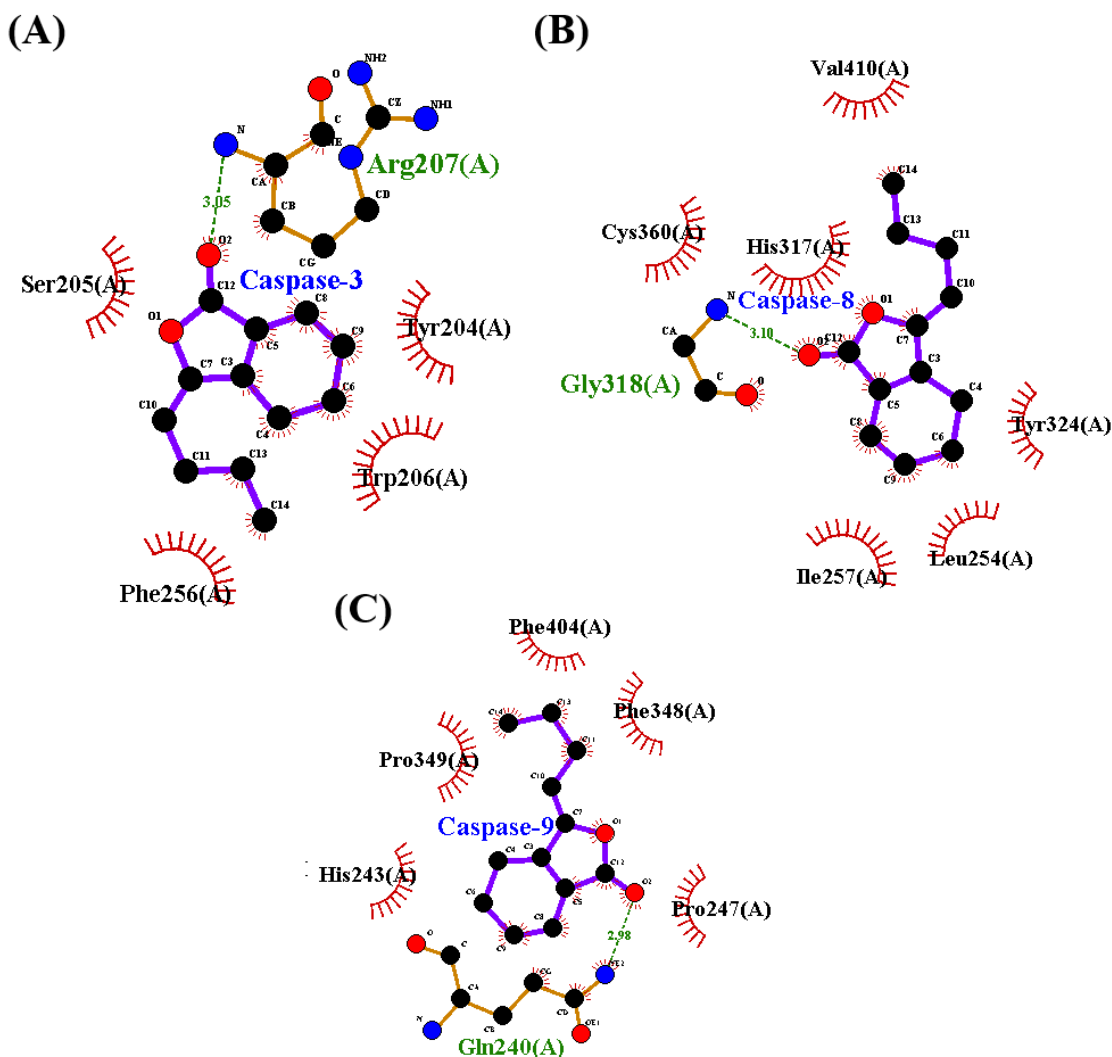


Figure 5: The LigPlot+v2.2.9 interactions for the selected caspase targets with the ligustilide. (A). Caspase-3 interacted with the ligustilide (B). Caspase-8 interacted with the ligustilide (C). Caspase-9 interacted with the ligustilide.

under normal physiological conditions and requiring proteolytic cleavage for activation.⁴⁰ There are fourteen identified mammalian caspases, which are broadly categorized into inflammatory caspases and apoptotic caspases, with the latter being central to the controlled execution of cellular demise. Apoptotic caspases are further classified as initiator and effector caspases, which function sequentially to amplify death signals and dismantle cellular components. The activation of these caspases marks a "point of no return" for a cell, ensuring the irreversible progression of apoptosis.⁴¹ Specifically, initiator caspases (caspase-8 and -9) become activated in response to pro-apoptotic stimuli, subsequently cleaving and activating downstream effector caspases like caspase-3. This hierarchical activation ensures a highly regulated and amplified proteolytic cascade, resulting in the systematic degradation of cellular constituents and the development of apoptotic bodies. This intricate interplay of caspase activation is fundamental to both the extrinsic and intrinsic pathways, representing critical targets for therapeutic intervention in cancer.⁴² These pathways converge on common machinery involving members of the caspase family, with initiator

caspases activating in response to stress or injury, subsequently cleaving and activating effector caspases. The caspase-9 activation consequently degrades the downstream effector caspases, most notably caspase-3, orchestrating the final stages of cellular dismantling.⁴³ The activation of caspase-3 is a pivotal step, as it subsequently cleaves numerous cellular substrates, resulting in the characteristic morphological alterations connected with apoptosis. Dysregulation of apoptosis in cancer cells, often leading to resistance to current treatment modalities, necessitates a deeper understanding of these regulatory mechanisms to bypass therapeutic resistance.⁴⁴ The findings of this study unveiled that the ligustilide treatment markedly enhanced the caspase-3, -8, and -9 activities in the HT-29 cells. These findings highlight that ligustilide treatment facilitates caspase-mediated apoptosis in colon cancer cells. Furthermore, the molecular docking results confirmed that ligustilide exhibited significantly stronger interactions with caspase-3 and caspase-8 compared to caspase-9, as evidenced by lower binding energies and a greater number of hydrogen bonds. These findings suggest that ligustilide may induce the expression of caspase-3, caspase-8, and caspase-9,

thereby contributing to the inhibition of colorectal cancer cell proliferation.

CONCLUSION

The findings of this work demonstrate that ligustilide treatment effectively suppresses growth and induces apoptosis in HT-29 cells. Furthermore, ligustilide significantly enhanced the activities of caspase-3, -8, and -9 in the HT-29 cells. Thus, it was evident that ligustilide has the potential to trigger caspase-mediated apoptosis in colon cancer cells. Furthermore, additional research is still crucial to clarify the specific molecular mechanisms underlying ligustilide-induced apoptosis in colon cancer cells.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

Å: Angstrom; **CASP:** Caspase; **CASP-3:** Caspase-3; **CASP-8:** Caspase-8; **CASP-9:** Caspase-9; **CRC:** Colorectal Cancer; **DAPI:** 4',6-Diamidino-2-Phenylindole; **HT-29:** Human Colon Adenocarcinoma Cell Line; **kcal/mol:** Kilocalories per Mole; **LIG:** Ligustilide; **MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; **μM:** Micromolar; **RMSD:** Root Mean Square Deviation; **ROS:** Reactive Oxygen Species.

SUMMARY

The study revealed that ligustilide effectively inhibited the proliferation of HT-29 colon cancer cells and induced apoptosis. Treatment with ligustilide notably increased the activities of caspase-3, -8, and -9, indicating activation of the caspase-dependent apoptotic pathway. These findings suggest that ligustilide possesses significant anticancer potential through the induction of caspase-mediated apoptosis in colon cancer cells. However, further investigations are necessary to elucidate the detailed molecular mechanisms involved in ligustilide-induced apoptosis.

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