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Exploring the Apoptotic and Cytotoxic Effects of Thymol-Induced Apoptosis in C6 Glioma

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Abstract
Background: Glioblastoma (GBM) is the most common and aggressive brain tumor associated with a poor prognosis, highlighting the need for new therapeutic strategies. The present study aimed to evaluate the anticancer properties of thymol in rat GBM C6 malignant cells.
Method: This in-vitro study, conducted to evaluate the effects of thymol on cell viability and apoptosis using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay and Annexin V-FITC/PI staining kit, respectively. Moreover, reactive oxygen species (ROS) generation was evaluated using the Dichlorodihydrofluorescein diacetate assay, and the mRNA levels of *Bax*, *Bcl2*, and *p53* were assessed using real-time. Data from three independent experiments were analyzed using GraphPad Prism 8.2.1 and compared for statistically significant differences ($P < 0.05$) using a one-way ANOVA with a Tukey post-hoc test.
Results: The half-maximal inhibitory concentration (IC_{50}) of thymol for C6 cells was 350 µM at 24 h and 260 µM at 48 h post-treatment. At the same time, lower cytotoxic effects were observed

on normal human foreskin fibroblast cells (IC_{50} = 675 μM). 350 Mm of thymol also caused a significant G2/M cell cycle arrest and early and late apoptosis compared with the non-treated control group. Thymol also caused a significant increase in *Bax* and *p53* expression, as well as ROS generation, when cells were treated with thymol compared with the non-treated control group.
Conclusion: The results indicate the anticancer properties of thymol including cytotoxic, and pro-oxidant effects with selectivity towards cancer cells. These preclinical findings provide a strong rationale for further exploration of thymol as a potential phytochemical agent in combating GBM.

Keywords: Glioblastoma multiforme, Thymol, Cancer, C6 cell line, Apoptosis

Introduction

Glioblastoma multiforme (GBM), the most common and malignant brain tumor, is classified by World Health Organization (WHO) as grade IV.[1] GBM is one of the deadliest tumors, with an average survival of 14 months for patients.[2, 3] Almost half of brain tumors are glioblastoma (GBM), and GBM accounts for 60% of primary central nervous system (CNS) malignancies. Additionally, 85–90% of GBM patients are adults.[4] According to CBTRUS statistics, the incidence rate is 3.97 Cases per 100,000 for men and 2.53 for women. This means that the overall prevalence of the tumor is 3 to 4 people per hundred thousand people, which in turn, is a high and alarming statistic.[5, 6] Surgery is the primary treatment; nonetheless, the tumor's prognosis remains unfavorable, even with the most intensive interventions, including radiotherapy, chemotherapy (temozolamide), and surgery.[7] Consequently, identifying an efficacious therapeutic approach to enhance efficacy and reduce adverse effects becomes imperative.[4, 8, 9] A viable strategy for addressing this issue involves using natural compounds. Several studies demonstrate the advantages of natural compound-based therapies for cancer treatment as they are proposed to be less toxic and potentially effective.[10-13]

Thymol (2-isopropyl-5-methylphenol) is a natural monoterpenoid phenol from cymene and an isomer of carvacrol, primarily found in Thymus vulgaris (thyme) and other plants like *Ocimum gratissimum* and *Origanum*.[14] Thymol has various applications in medicine, food, and cosmetics, and possesses significant medicinal properties, including antimicrobial, antioxidant, and anti-inflammatory effects, which are beneficial for respiratory, nervous, and cardiovascular disorders.[15] The anticancer properties of this compound are also unique and have been intensively studied in recent years. The anticancer role of thymol has been investigated in various cancers, including colon, bladder, breast, ovary, and several other types of cancer.[16] Thymol has been shown to exhibit anticancer efficacy through various mechanisms, including the inhibition of cell proliferation, induction of apoptosis, generation of intracellular reactive oxygen species (ROS), and activation of pro-apoptotic mitochondrial proteins such as *Bax*.[17] Thymol exhibits significant anticancer properties by modulating critical cellular pathways, including PI3K/AKT, ERK, mTOR, and Wnt/β-catenin. Through this mechanism, it can induce apoptosis, arrest the cell cycle, and suppress metastasis. Furthermore, studies indicate that thymol can boost the potency of the standard chemotherapeutic agent 5-fluorouracil in colorectal cancer models.[14, 17]

Nevertheless, the mechanism by which thymol induces these effects on C6 rat GBM cells and the corresponding underlying processes remains unclear. Given the inadequacy of the most complete treatment

methods and the high side effects of current treatment methods, it seems necessary to find a natural-based treatment method with low side effects. Therefore, the present study aimed to evaluate the anticancer effects of thymol on the C6 rat GBM cell line.

Materials and Methods

Cell culturing and chemical reagents

The Apsala C6 malignant rat GBM cell line and the human foreskin fibroblast (HFF) non-neoplastic fibroblast cell line were obtained from the National Iranian Cell Bank at the Pasteur Institute in Tehran, Iran. These cells were grown in DMEM (Gibco, Karlsruhe, Germany) supplemented with 10% FBS and antibiotics (100 µg/ml streptomycin and 100 U/ml penicillin). Thymol was provided by Golexir Pars Co (Mashhad, Iran), and the Annexin V-FITC propidium iodide (PI) kit was supplied by Cayman Chemical Company (USA). The Dichlorodihydrofluorescein diacetate (DCFDA) assay kit was sourced from Abcam (Cambridge, UK). Additionally, resazurin powder, PI, and DMSO were purchased from Sigma-Aldrich.

Cell viability assessment

Cell viability was assessed using the resazurin assay, which measures the conversion of resazurin to the fluorescent compound resorufin by viable cells. C6 and HFF cells were seeded at a density of 1×10^4 cells per well in 96-well plates and allowed to incubate overnight. The next day, the cells were treated with thymol at concentrations of 15.7, 31.25, 62.5, 125, 250, 500, 1000, and 2000 µM for 24 and 48 hours. After treatment, resazurin (0.01 mg/mL in PBS) was added, and resorufin was excited at 530 nm; the emission was recorded at 590 nm using a VICTOR X5 Multimode Plate Reader (PerkinElmer, Waltham, MA, USA). The data were analyzed using GraphPad Prism software to calculate the half-maximal inhibitory concentration (IC_{50}) for each compound.

Quantitative real-time Polymerase Chain Reaction (qRT-PCR)

Total RNA was isolated from cells treated with thymol using Favorgen (Taiwan) protocols. The quality and integrity of RNA were verified through agarose gel electrophoresis and a NanoDrop spectrophotometer. RNA was then reverse-transcribed into cDNA using a kit from Yekta Tehiz Azma, followed by qRT-PCR with SYBR Green master mix (Ampliqun, Denmark) and forward and reverse primers for *B2M*, *Bax*, *Bcl-2*, and *p53* (Table 1). All experiments were performed in triplicate, and the results were normalized to the *B2M* control.

Cell cycle analysis

The cell cycle was analyzed in C6 cells, which were plated at a density of 10^5 cells per well in 6-well plates and exposed to thymol at concentrations of 175 and 350 µM for 24 hours. Flow cytometry was used to evaluate the DNA content using PI. The cells were collected, washed twice with cold PBS, and fixed in ice-cold 70% ethanol for 2 hours at 4°C. To eliminate double-stranded RNA, the cells were treated with RNase A (free of DNase) for 30 minutes at 37 °C. Then, they were resuspended in PI-Triton X-100 solution in the dark for 30 minutes, and DNA distribution was analyzed using FACS on a BD FACSCALIBUR™ FLOW CYTOMETER, with data being processed using FlowJo software. All experiments were conducted in triplicate.

Annexin V-FITC assay

Apoptosis in C6 GBM cells caused by thymol exposure (175 and 350 µM) was assessed using the Annexin V/PI staining kit (Cayman, USA). This one-step technique quantifies apoptotic rates by detecting phosphatidylserine on the cell membrane. Specifically, 10^5 cells were treated with thymol for 24 hours, then collected and washed twice with PBS. The cells were

resuspended in 200 μL of Annexin V binding buffer, followed by the addition of 100 μL of Annexin V/PI. After a 5-minute incubation at room temperature in the dark, the cells were analyzed using a BD FACSCALIBUR™ FLOW CYTOMETER, and the data were processed with FlowJo software. All experiments were conducted in triplicate.

Measurement of ROS activity

ROS activity was evaluated using a 2′,7′-dichlorodihydrofluorescein diacetate assay kit to measure thymol-induced ROS production. Cellular esterases convert DCFDA into the non-fluorescent compound 2′,7′-dichlorodihydrofluorescein (H2DCF), which turns into fluorescent DCF in the presence of ROS. C6 cells (25×10^3) were plated in 96-well plates and incubated overnight. After 24 hours, the cells were washed with PBS and stained with H2DCFDA for 45 minutes in the dark. Following treatment with thymol (175 and 350 μM) and the positive control Tert-butyl hydroperoxide (TBHP), fluorescence was measured at 485/535 nm using a Victor X5 Multiplate Reader, with all treatments conducted in triplicate.

Statistical analysis

Experimental results were analyzed using GraphPad Prism® 8.2.1, presenting measurements from three independent experiments as mean ± standard deviation. Groups were compared using one-way ANOVA with Tukey post-hoc test, considering $P < 0.05$ to be statistically significant.

Results

Decreased Viability of C6 Cells by thymol

The resazurin assay evaluated the effect of thymol on the viability of C6 cells over 24 and 48 hours, revealing a dose-dependent response. Thymol significantly decreased the viability of C6 cells, with IC_{50} values of 350 μM at 24 hours and 260 μM at 48 hours (Figure 1A). In addition, as shown in Figure 1B, thymol caused shrinkage and detachment of C6 cells at doses of 350 and 500 μM, representing the toxic effects of thymol against C6 glioma cells.

Lower toxicity of thymol against HFF cells compared with cancer C6 cells

Treatment of normal HFF fibroblast cells with thymol for 24 hours exhibited reduced toxicity, as evidenced by the microscopic image demonstrating that thymol is less toxic at the IC_{50} level for C6 cells (Figure 1B). In addition, a higher IC_{50} of 675 μM for HFF cells after 24 h, compared with 350 μM for C6 cells, suggested selective toxicity of thymol against cancer cells (Figure 2A).

G2/M phase cell cycle arrest by thymol in C6 GBM cells

To investigate the detrimental effects of thymol on C6 cell survival, we assessed the percentage of cells that remained arrested in the cell cycle following thymol treatment. Figure 3A demonstrates that thymol markedly triggers cell cycle arrest in the G2/M phase at two distinct doses (175 and 350 μM), resulting in 14.63% and 16.1% arrest, respectively. These results indicate that at the IC50 concentration, thymol caused a significant G2/M cell cycle arrest of C6 GBM cells (Figure 3B).

Apoptosis induction by thymol in C6 GBM cells

The initiation of apoptosis in C6 cells exposed to thymol was evaluated using annexin V-FITC and PI staining, with analysis conducted via flow cytometry. As presented in Figure 4, 2.96% and 55.3% of cells treated with 175 and 350 μM thymol entered early and late apoptosis, respectively. In the control group, only 4.32 % of C6 GBM cells displayed signs of apoptosis (Figure 4A). These results indicate that thymol, at the IC_{50} concentration, caused significant

apoptosis of C6 cells compared with the control and 175 µM thymol groups (Figure 4B).

Increased ROS production by thymol

The ROS assay revealed that C6 cells treated with 350 µM thymol exhibited significantly elevated levels of ROS compared with those treated with 175 µM thymol and the control groups (Figure 5).

Effects of thymol on apoptosis-related gene expression in C6 cells

To better understand the mechanism of thymol, we examined the expression of critical apoptosis-related genes in C6 cells. The expression levels of *Bax* (fold change = 4.84 ± 1.49, *P*-value = 0.046) significantly increased in cells treated with 350 µM of thymol compared with the control group (Figure 6A). Furthermore, no notable alteration was observed in the *Bcl2* gene expression at both concentrations of thymol treatment (*P*-value < 0.05) (Figure 6B). However, the level of *Bax/Bcl2* ratio significantly increase after treatment with high concentration of thymol (fold change = 5.029 ± 0.14, *P*-value = 0.0101) (Figure 6C). Moreover, the expression levels of *p53* (2.05 ± 0.29, *P*-value = 0.047) significantly increased in cells treated with 350 µM of thymol compared with the control group (Figure 6D).

Discussion

Our study established that thymol has an antiproliferative effect and promotes apoptosis in C6 GBM cells in a dose-dependent manner. The IC_{50} of thymol against C6 cells was determined to be 350 µM after 24 h of exposure. After a 48-hour exposure period, the IC_{50} value decreased to 260 µM, indicating a greater inhibitory effect with prolonged exposure.

Similarly, several other types of studies showed that thymol could decrease the viability of cancer cells. For example, in the case of breast cancer, the MB-231 and MDA-MB-436 cell lines, thymol showed an IC_{50} between 0.92 and 1.70 µg/ml,[18] while B16-F10 melanoma cells showed an IC_{50} of 88 µg/ml.[19] In prostate cancer, using the PC-3 cell line, IC_{50} values were found to be between 350 and 500 µM.[12] Additionally, a recent study reported an IC_{50} value of 208.36 µM for MDA-MB-231 at 72 hours.[20] However, it has been shown that thymol could have selective toxicity toward cancer cells with lower toxicity for normal cells.[21] Consistent with available literature, our study also demonstrates that thymol have lower cytotoxic effect on normal HFF cells with higher IC_{50} after 24 h of treatment (675 µM), suggesting a degree of selectivity towards cancer cells. Furthermore, it is essential to mention that thymol did not display significant cytotoxic effects on human peripheral blood mononuclear cells, indicating a degree of selectivity in its action against cancerous cells.[22] Notably, consistent with our results, the anticancer properties of thymol was investigated in human GBM cells and the results showed that thymol is able to increase the effectiveness of Temozolomide, standard chemotherapy for GBM, in GBM cells [14].

Further investigation into the mechanism of action of thymol in C6 cells revealed several key effects on cellular processes related to apoptosis and cell cycle regulation. Specifically, thymol treatment resulted in an increase in early and late apoptosis in C6 GBM cells. Similarly, increase in apoptosis was observed after treatment of esophageal cancer, colorectal cancer, bladder cancer, and so on.[21, 23, 24]

Apoptosis induction is one of the most basic foundations of anticancer effects. Apoptosis occurs through two main pathways: the mitochondrial (intrinsic) pathway and the death receptor (extrinsic) pathway. The basis of this process is the caspase enzyme, which

is classified as a cysteine protease. Typically, the mitochondrial pathway is dominant, and its function involves proteins such as cytochrome c.[25] Alterations in the permeability of the mitochondrial membrane result in the release of cytochrome c, which subsequently initiates caspase activation and apoptosis. BCL family proteins regulate permeability; specifically, *BCL-2* and *BCL-XL* help maintain membrane stability, whereas proapoptotic proteins such as *BAK* and *BAX* create pores that allow cytochrome c to escape, thereby activating caspase 9 and promoting apoptosis. In the death receptor pathway, the FAS ligand from activated T cells binds to FAS on target cells, activating caspase 8 and leading to cell death.[17] The present study demonstrated that thymol increased the levels of *Bax* and *p53*, and also *Bax/Bcl2* ratio. These changes in the expression of apoptosis-related proteins collectively induced programmed cell death, or apoptosis, in C6 glioma cells. Similarly, it has been reported that thymol also exhibited concentration-dependent cytotoxicity in HL-60 leukemia cells, potentially causing apoptosis.[16] In MDA-MB-231 and HCT-8 cell lines, the level of *Bcl-xL* decreased, and caspase-9 and -3 were activated.[26] Previous studies indicate that thymol may cause cytochrome c release and MMP depolarization in HCT-8 and MDA-MB-231 cells, although we have not assessed this.[27] MCF-7 breast cancer cell lines also demonstrated higher levels of *Bax* and lower levels of *Bcl-2* in laboratory settings and live models, with thymol enhancing apoptosis in basal alveolar epithelial cells and acute promyelocytic leukemia. Western blot analysis revealed a dose-dependent increase in *Bax*, a decrease in *Bcl-2*, and the activation of caspases 9, 8, and 3, as well as PARP cleavage, indicating that apoptosis occurs through a caspase-dependent mechanism.[28] In another similar study , for GBM, but this time using the U87 cell line, thymol increased *Bax* and *p53* expression, leading to cell death due to apoptosis.[14] Moreover, it has been shown that cytochrome c release from mitochondria and induction of apoptosis could largely be mediated by direct or indirect ROS action.[29] The results of the present study also demonstrate that thymol is able increase ROS production in C6 cells.

ROS are small, reactive molecules capable of oxidizing proteins, lipids, and DNA. They are formed from the partial reduction of oxygen and encompass species such as superoxide anions, hydroxyl radicals, and peroxides, with hydrogen peroxide (H2O2) being a notable example.[30, 31] The introduction of ROS into cancer cells, which destroys their cellular structures, represents a fundamental and critical strategy in anticancer therapies, particularly those that involve targeted drug delivery mechanisms.[32] Our experimental results using DCFDA staining indicated that treatment with thymol resulted in a dose-dependent increase in ROS production, specifically in GBM cells. Similarly, it was found that thymol is able to increase the level of lipid peroxide and protein carbonyl in A549 lung cancer cells. Notably, this effect could be reversed with pretreatment using N-acetylcysteine, which is known for its antioxidant properties.[33] Consistent with our findings, previous research has confirmed that thymol significantly increases ROS levels in various cancer cells, including those of the liver,[34] stomach,[35] ovary,[36] and breast origin,[26] highlighting its potential as a valuable therapeutic agent. In contrast, the effects of thymol, thymoquinone, and dithymoquinone were systematically evaluated in terms of their impact on ROS. This evaluation used both chemiluminescence and spectrophotometric methods for measurement and validation. Thus, it was demonstrated that all three compounds had the ability to scavenge different types of ROS, and the rate constant for the quenching activity of thymol toward

these ROS was carefully determined.[37] Similarly, immunofluorescence assays demonstrated that thymol reduced LPS-induced ROS levels in a dose-dependent manner.[38] The discrepancy in outcomes may be attributed to differences in the dosages delivered; notably, lower doses of thymol appear to reduce ROS levels, while higher doses trigger apoptosis in cancer cells, resulting in elevated intracellular ROS levels. In addition, thymol induced cell cycle arrest at the G2/M phase, thereby preventing the cells from continuing the cell division process. Cell cycle regulation and apoptosis are crucial in tumor progression, as disrupting the cell cycle can hasten apoptosis, eliminating damaged cells. The *p53* protein is an essential regulator of the cell cycle and a significant tumor suppressor that helps maintain cell integrity by responding to cellular damage.[39] Our PI labeling and flow cytometry investigation demonstrated that thymol triggers G2/M phase cell cycle arrest and markedly diminishes *p53* protein expression in C6 GBM cells. In alignment with our findings, a related study on bladder cancer demonstrated that thymol effectively halted cell cycle progression at the G2/M checkpoints, mirroring our observations.[40] The impact of thymol was also recorded in other cancer cell lines, including MCF-7 (breast cancer),[26] HCT116 (colon cancer),[41] and AGS (gastric cancer),[42] as well as KYSE-30 (esophageal cancer).[43] In addition, thymol exhibited specific effects on leukemia cells, particularly in cell lines such as K-562, HL-60, and CEM.[16] Thymol also affected P815 mastocytoma cells,[44] A549 lung cancer cells,[45] and U87 GBM cells,[14] demonstrating that cell cycle arrest can occur in the G0/G1 phase.[14] The observed differences in phase arrest may arise from intrinsic differences in the specific cell types involved in these studies.

While our study provides valuable insights into the apoptotic and cytotoxic effects of thymol on C6 malignant glioblastoma cells, certain limitations warrant consideration. First, the study was conducted in-vitro, which may not fully replicate the complex tumor microenvironment present in-vivo; thus, future studies should aim to validate these findings in animal models better to understand the therapeutic potential of thymol in a physiological context. Furthermore, while the mechanisms of action were investigated, a detailed exploration of the signaling pathways involved in thymol-induced apoptosis could provide deeper insights into its molecular effects. Lastly, future research should consider assessing the pharmacokinetics and bioavailability of thymol, as well as potential combination therapies with other chemotherapeutic agents, to develop effective treatment protocols that maximize its therapeutic efficacy.

Conclusion

The present study show that thymol has the ability to induce apoptosis, disrupt the cell cycle, and produce intracellular ROS, all of which contribute to its anticancer properties. Given that thymol is a natural substance, it has the potential to be considered an excellent cancer treatment, both highly effective and with few adverse chemical effects. As research continues to explore the mechanisms of action and potential synergy of thymol with existing therapies, supplementation may ultimately contribute to more effective and safer cancer treatments with fewer side effects, paving the way for its integration into clinical practice. In this study, the effects of thymol on the C6 mouse GBM cell line were investigated. Given the fact that no in-vivo studies have been conducted on the effects of thymol on GBM, our results may provide a valuable basis for future in-vivo research. However, further clinical and preclinical research is needed to examine the anticancer benefits of thymol.

Data Availability

All data generated or analyzed during this study are included in this published article. More details and raw data will be available upon request.

Acknowledgments

Not applicabale.

Authors' Contribution

N.Gh., P.H., and S.A.: data acquisition, data analysis and interpretation, drafting and critical revision of the manuscript; B.B., M.J., Gh.B., S.S.A. and R.S.: conception and design of the work, drafting and critical revision of the manuscript; A.R.A.: conception and design of the work, supervision, critical revision of the manuscript. All authors have read and approved the final manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Conflict of Interest

None declared.

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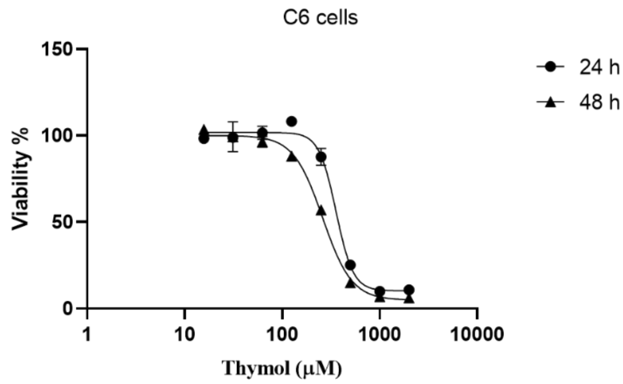
Table 1. The list of primer sequence

Gene symbol	Primers (5′ → 3′)
Bax	Forward: AAACTGGTGCTCAAGGCCC Reverse: GTCCAATGTCCAGCCCATGA
Bcl2	Forward: GTCATGTGTGTGGAGAGCGTC Reverse: CCGTACAGTTCCACAAAGGCATC
P53	forward: TGGGACGGAACAGCTTTGAG Reverse: TGTTGTTGGGCAGTGCTCG
B2M	Forward: AGGCTATCCAGCGTACTCCA Reverse: TGTCGGATGGATGAAACCCA

P53: Tumor protein p53; *Bax*: BCL2-associated X protein; *Bcl-2:* B-cell lymphoma 2; *B2M:* Beta-2-microglobulin (reference/housekeeping gene)



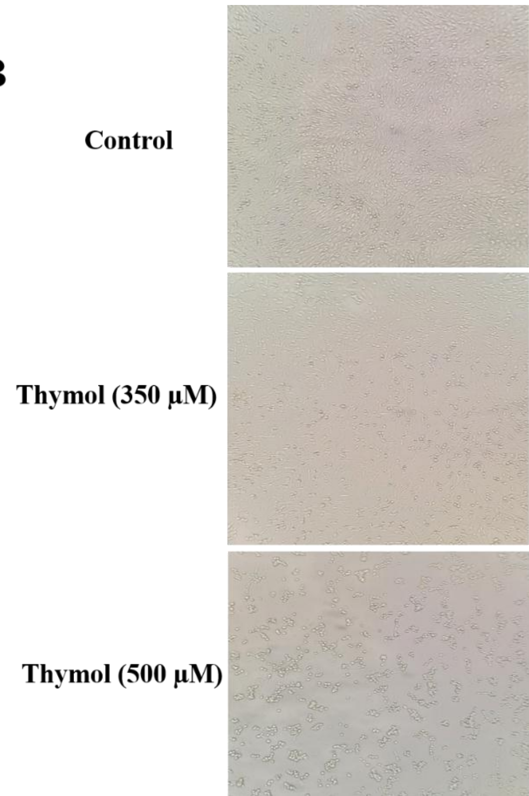
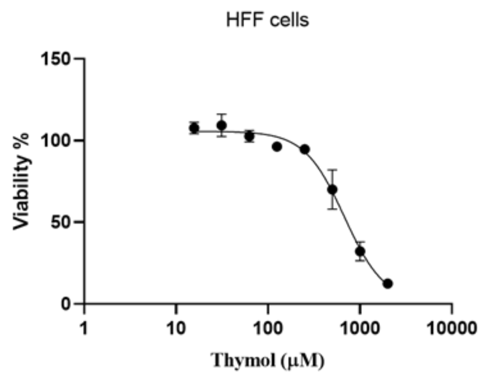


Figure 1. The cytotoxic effects of thymol on C6 GBM cells were examined using the resazurin indicator after 24 and 48 hours of thymol administration. (A) The IC_{50} for thymol in C6 cells was 350 µM at 24 hours and 260 µM at 48 hours. (B) Phase contrast images demonstrated thymol's inhibitory effects on C6 cells after 24 hours.

C6: rat glioma cell line; GBM: Glioblastoma multiforme; IC_{50}: Half-maximal inhibitory concentration; µM: Micromolar

A



B

Control

Thymol (500 µM)

Figure 2. The cytotoxic effects of thymol on HFF cells were examined using the resazurin indicator after 24 hours of thymol administration. (A) The IC_{50} for thymol in HFF cells was 675 µM at 24 hours. (B) Phase contrast images demonstrated low toxicity after 24 hours.

HFF: Human foreskin fibroblasts; IC_{50}: Half-maximal inhibitory concentration; µM: Micromolar

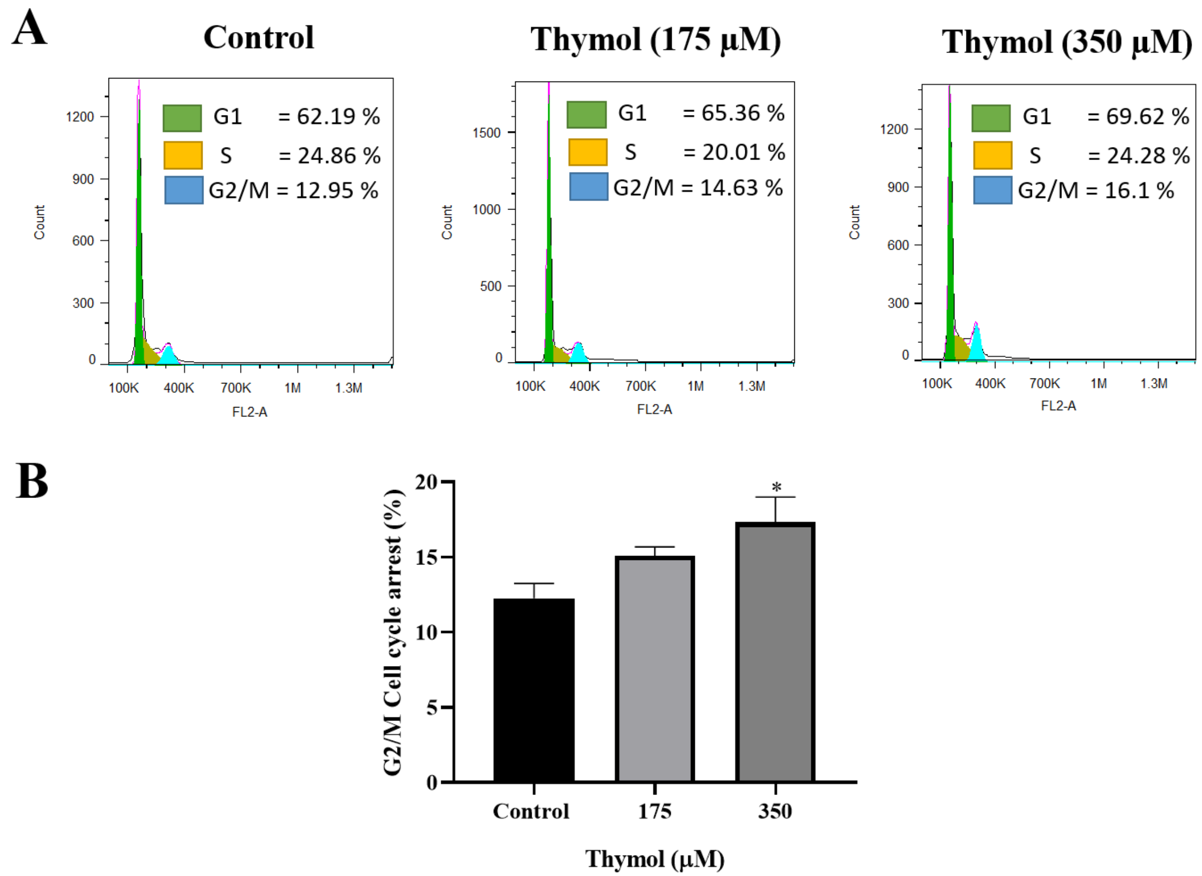


Figure 3. (A) The cell cycle in C6 GBM cells was evaluated after 24 h incubation with thymol (175 and 350 µM). (B) Thymol significantly induced G2/M phase arrest. Each column represents the mean ± standard deviation from three independent experiments ($^{*}P < 0.05$).

C6: Rat glioma cell line; GBM: Glioblastoma multiforme; h: Hour; µM: Micromolar

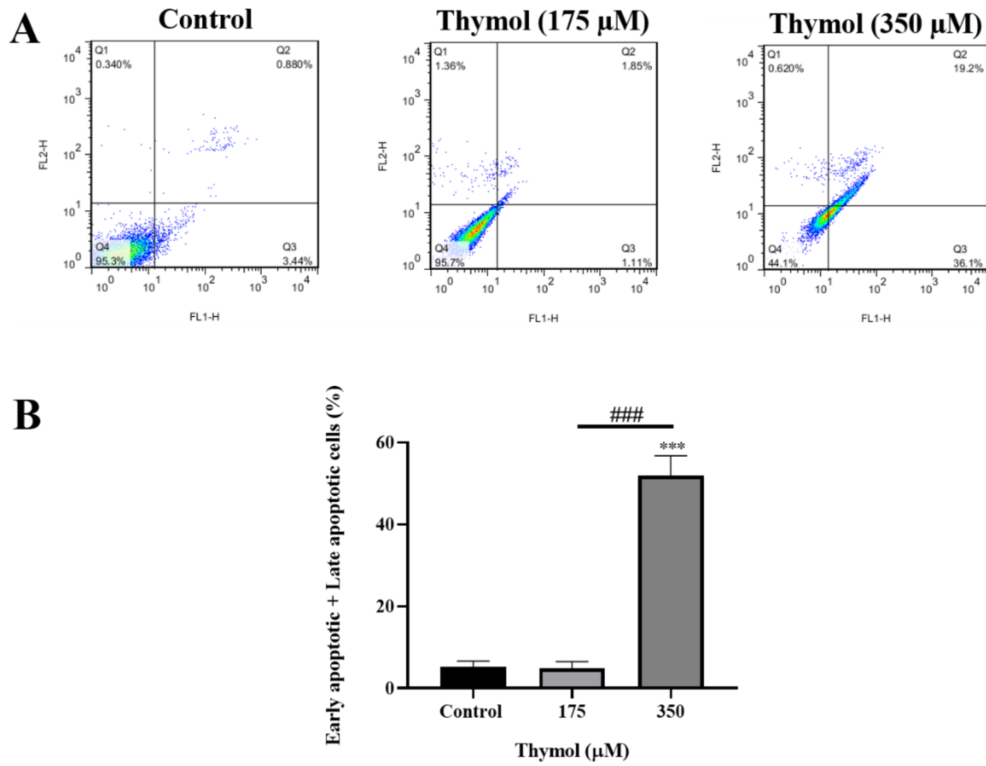


Figure 4. (A) Flow cytometry analysis of thymol-induced apoptosis in C6 cells. Diagrams Q4 to Q1 represent live, early apoptotic, late apoptotic, and necrotic cells. (B) Thymol-treated C6 cells exhibited significantly higher early and late apoptosis than control and 175 µM thymol. Each column shows mean ± SD from independent experiments. (***indicate $P < 0.001$ compared to control and $^{###}P < 0.001$ vs 175 µM thymol).

C6: Rat glioma cell line; Q: Quadrant (flow cytometry); µM: Micromolar; SD: Standard deviation

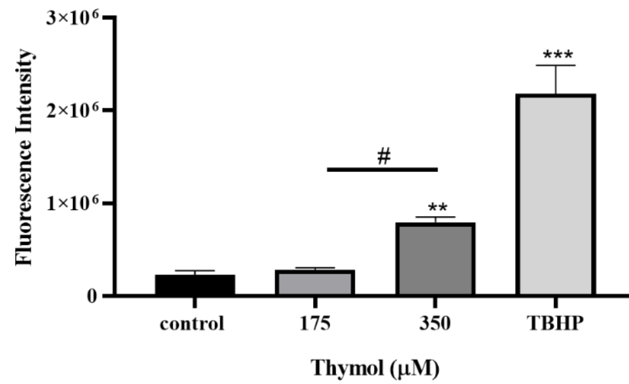


Figure 5. ROS levels in thymol-treated C6 cells were measured after 4 hours using DCFDA. Thymol significantly increased ROS compared to control and 175 μM thymol. TBHP, as a positive control, increased ROS levels. Results are shown as means ± standard deviation (** $P < 0.01$ and *** $P < 0.001$ vs. control and $^{\#}P < 0.05$ compared to 175 μM thymol).

ROS: Reactive oxygen species; C6: Rat glioma cell line; DCFDA: 2′,7′-dichlorodihydrofluorescein diacetate; TBHP: Tert-butyl hydroperoxide; μM: Micromolar

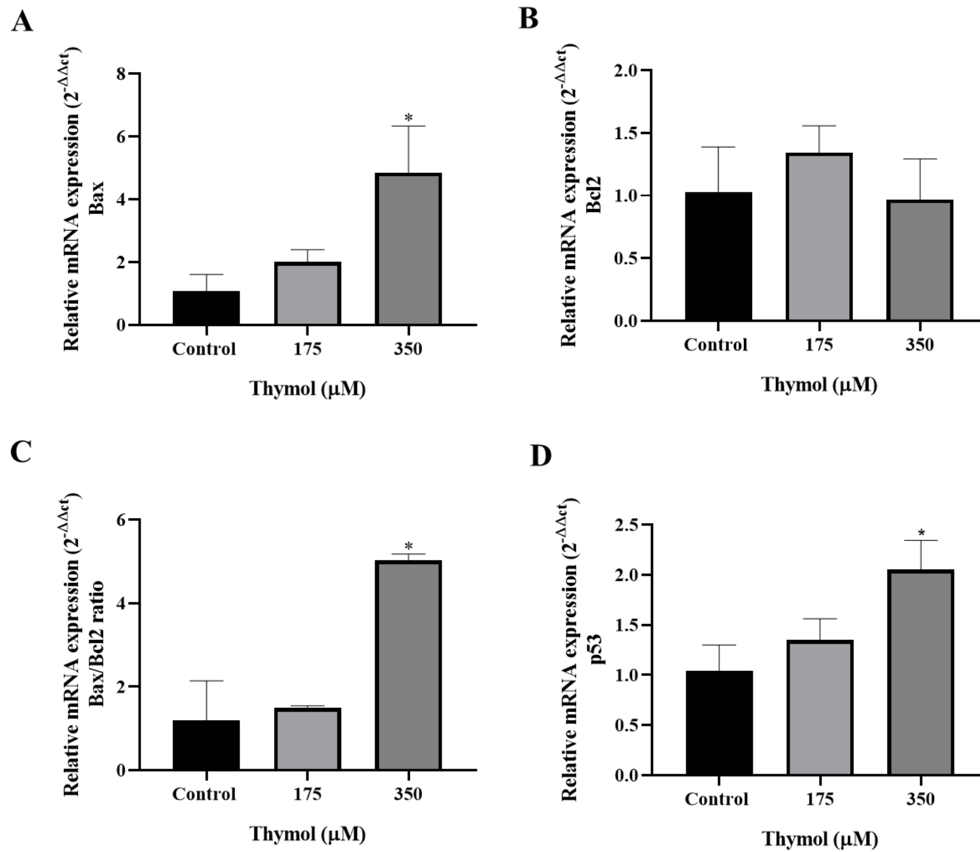


Figure 6. The effects of thymol on mRNA levels of apoptotic markers p53, Bax, and Bcl-2, and Bax/Bcl2 ratio were studied. C6 cells were treated with 175 and 350 μM of thymol for 24 hours before RT-PCR. Results are expressed as means ± standard deviation ($^{*}P < 0.05$ vs. control).
mRNA: Messenger RNA; *p53*: Tumor protein p53; *Bax*: BCL2-associated X protein; *Bcl-2:* B-cell lymphoma 2; C6: Rat glioma cell line; μM: Micromolar; RT-PCR: Reverse transcription polymerase chain reaction