LOKMAN HEKIM HEALTH SCIENCES

DOI: 10.14744/lhhs.2025.66605
Lokman Hekim Health Sci 2025;5(1):6–15

lokmanhekimhs.com

ORIGINAL ARTICLE

Investigation of the Cytotoxic Effects of *Thymbra Spicata* Polysaccharides on MCF-7 Breast Cancer Cell Proliferation and Migration *in vitro* Conditions

Thymbra spicata Polisakkaritlerinin MCF-7 Meme Kanseri Hücrelerinin Proliferasyonu ve Göçü Üzerine Sitotoksik Etkilerinin in vitro Koşullarda Araştırılması

Caner Özbey[1], Işıl Aydemir[2], Emre Çetindağ[2], Oktay Özkan[3]

[1]Department of Pathology, Niğde Ömer Halisdemir University, Training and Research Hospital, Niğde, Türkiye
[2]Department of Histology and Embryology, Niğde Ömer Halisdemir University Faculty of Medicine, Niğde, Türkiye
[3]Department of Pharmacology, Niğde Ömer Halisdemir University Faculty of Medicine, Niğde, Türkiye

Abstract

Introduction: *Thymbra spicata* species is a widely used plant, especially in the Eastern Mediterranean region, and is known to have many health benefits. However, the effects of its polysaccharides on tumor cells have not been investigated. We aimed to evaluate the biological effects of *Thymbra spicata* polysaccharides in MCF-7 breast cancer cells.

Methods: MTT test was performed to determine the cytotoxicity levels of polysaccharides and doxorubicin in MCF-7 and L929 fibroblast cells. The expression levels of VEGF and GSK-3β were examined by immunocytochemistry. For the in vitro wound healing assay, the scratch wound model was created in the shape of plus (+), and the percentage of closure was calculated.

Results: *Thymbra spicata* polysaccharides and doxorubicin had a cytotoxic effect on MCF-7 cells depending on the dose increase. The percentage of wound closure also decreased in correlation with the MTT results. In L929 cells, there was no significant difference in VEGF and GSK-3β immunoreactivity after polysaccharides and doxorubicin treatments, but a significant decrease in VEGF and GSK-3β expression was observed in MCF-7 cells.

Discussion and Conclusion: We demonstrated that polysaccharides exert toxic effects by suppressing VEGF and GSK-3β molecules. In addition, the polysaccharides inhibited cell proliferation and migration and delayed in vitro wound healing at high concentrations.

Keywords: Breast cancer; Cell migration; Cytotoxicity; Proliferation; *Thymbra spicata* Polysaccharides

Cite this article as: *Özbey C, Aydemir I, Çetindağ E, Özkan O. Investigation of the Cytotoxic Effects of Thymbra Spicata Polysaccharides on MCF-7 Breast Cancer Cell Proliferation and Migration in vitro Conditions. Lokman Hekim Health Sci 2025;5(1):6–15.*

Correspondence: Caner Özbey, M.D. Niğde Ömer Halisdemir Üniversitesi, Eğitim ve Araştırma Hastanesi, Patoloji Kliniği, Niğde, Türkiye
E-mail: canerozbey@hotmail.com **Submitted:** 16.12.2024 **Revised:** 20.01.2025 **Accepted:** 29.01.2025

Breast cancer in women is an important health problem due to its high mortality and morbidity, and therefore, intensive research is needed for the treatment of this cancer type.[1] Breast cancer cell lines are widely used in both *in vitro* and *in vivo* breast cancer studies, such as Michigan Cancer Foundation-7 Cells (MCF-7) human breast cancer cell line. MCF-7 was first isolated in 1970 from the pleural effusion of a female patient whose breast cancer had metastasized to the lung. MCF-7 cells are positive for ERs and PRs and, therefore, useful for *in vitro* breast cancer studies.[2,3] At the same time, other molecular pathways such as vascular endothelial growth factor (VEGF), phosphotidylinositol 3-kinase (PI3K), protein kinase B (AKT), mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK), mechanistic target of rapamycin (mTOR), nuclear factor-kappaB (NF-kappaB), glycogen synthase kinase-3beta (GSK-3β) are the factors that determine the aggressive and metastatic behavior of tumor cells.[4–8] Cancer treatment involves the use of agents (synthetic or herbal products) that target these molecules.[9–11]

VEGF is a factor involved in embryonic and adult vasculogenesis and angiogenesis. It has a dual role, while it triggers the formation of blood vessels in healthy tissues, it also facilitates the spread of the tumor cells. Due to this feature, it is one of the targeted molecules in cancer therapies.[4,12,13] In addition to regulating the glycogen metabolism, GSK-3β, together with other molecules such as β-catenin, NF-κB, AKT, phosphatase and tensin homolog (PTEN), has been determined to have a role in tumor cell proliferation and drug resistance.[14,15]

Many chemotherapeutic agents and herbal products with anti-tumorogenic properties are used in *in vitro* and *in vivo* studies. The demonstration of the anticarcinogenic effects of herbal products or extracts will pave the way for their use as supportive agents in cancer treatment. *Thymbra spicata* belongs to the Lamiaceae family. It grows in countries in the Eastern Mediterranean region and is used as a spice and herbal tea by the local people for different purposes, such as antitussive, carminative, etc. In studies, the antioxidant, antiseptic, antihypercholesterolaemic, anti-inflammatory, antifungal, antiviral, antimicrobial, and cytotoxic properties of *Thymbra spicata* of Thymbra genus, their extracts, essential oils or components, have been demonstrated.[16–26] However, there were no data on the cytotoxicity of *Thymbra spicata* polysaccharides on cancer cells. Studies have mostly been carried out with *Thymbra spicata* or essential oils containing all active agents or compounds. Our aim here then is to examine the effect on cancer cells only its polysaccharides *in vitro* condition.

Therefore, the aim of this study was to investigate the possible cytotoxic effects of *Thymbra spicata* polysaccharides on MCF-7 breast cancer cells and their effects on cell migration, distribution of VEGF, and GSK-3beta.

Materials and Methods

Preparation of Agents

After the *Thymbra spicata* plant was commercially obtained, polysaccharide isolation was performed by hot water extraction and ethanol precipitation. The dried *Thymbra spicata* samples (200 gr) were defatted with absolute alcohol for 2 hours. After defatting that extraction with distilled water (1:10, w/v) at 80 °C by using a soxhlet apparatus for 3 hours in a rotary evaporator, the water extracts were concentrated under reduced pressure to 30% of the original volume and then centrifuged at 2000 rpm for 15 min. The supernatant was collected and 3 times the volume of 95% alcohol was added slowly with stirring and then left overnight at +4 °C to form polysaccharide pellets, which were then centrifuged at 4000 rpm for 15 minutes. Polysaccharide pellets were washed with 15 ml of ethanol, acetone and ether, respectively. Then completely dissolved in distilled water and left for two days, concentrated again and deproteinized by mixing with Sevag reagent (CHCl3: BuOH = 4:1, v/v) for 30 min. Finally, it was centrifuged, and the supernatant was lyophilized in a freeze dryer.[27] Also, doxorubicin was commercially available (Saba, Türkiye). Both polysaccharides and doxorubicin were dissolved in cell culture media to apply to cells.

Cell Culture

In the study, the MCF-7 breast cancer cell line (Cat. No: 00092502) was used as tumor cells, and L929 fibroblast cells (Cat. No: 92123004) were used as control. Both cells were obtained from Şap Institute, Ankara, Türkiye. MCF-7 cells were cultured in Roswell Park Institute-1640 (RPMI-1640) media (Wisent, Canada) containing 10% fetal bovine serum (FBS, Biowest, France), 2 mM L-glutamine (Biosera, France), 100 UI/ml penicillin/streptomycin (Capricorn Scientific GmbH, Germany), whereas L929 fibroblast cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, USA) containing 10% fetal calf serum, 50 µg/ml gentamicin (Gibco, USA), 100 UI/ml penicillin, 100 UI/ml streptomycin at 37 °C and 5% CO_2 condition.[28]

MTT Assay

To detect the cytotoxic effects of *Thymbra spicata* polysaccharides and doxorubicin, 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyltetrazolium bromide (MTT, Serva Electrophoresis GmbH, Germany) assay was performed.

Cells were grown to monolayer (70-80% confluence) in 96-well plates ($45x10^3$ cells/well) and treated with *Thymbra spicata* polysaccharides (0 µg/ml, 0.1 µg/ml, 1 µg/ml, 10 µg/ml, 100 µg/ml and 1000 µg/ml) and doxorubicin (0 µM, 0.1 µM, 1 µM, 10 µM, 100 µM and 1000 µM) for 24, 48 and 72 h. The media containing polysaccharides and doxorubicin were discarded, and 100 µl of fresh culture medium with 10 µl of 12 mM MTT stock solution was added to each well. After 4 hours of incubation at 37 °C, the medium containing MTT was removed by pipetting, and dimethyl sulfoxide (DMSO, A3672, AppliChem, Darmstadt, Germany) was put into each well. Absorbance values were determined at a wavelength of 570 nm using a UV visible microplate reader (EPOCH2, BioTek, USA). The cytotoxicity levels and the half-maximal inhibitory concentration (IC50) doses of two agents were identified for each cell. The protocol was performed thrice.[29]

In vitro Wound Healing Assay

The *in vitro* wound healing experiment, known as the scratch wound model, was performed to determine the effects of *Thymbra spicata* polysaccharides on cell migration. MCF-7 and L929 cells were seeded in the 6-well plate (25x104 cells/well), and 90-95% confluency was reached. The *in vitro* wound was created using a 200 µl pipette tip in the shape of plus (+). Then *Thymbra spicata* polysaccharides (0 µg/ml, 0.1 µg/ml, 10 µg/ml, 1000 µg/ml) and doxorubicin (0 µM, 0.1 µM, 10 µM, 1000 µM) were then applied to both cells for 48 h. At hours 0 and 48, images were taken under the camera-attached inverted microscope (Zeiss, Germany), and the closure percentage was measured via ImageJ.1.47 software for each concentration.[29]

Immunocytochemistry

The distribution of VEGF and GSK-3β was determined by immunocytochemical staining. Both cells were cultured in a 24-well plate ($2.5x10^5$ cells/well) until confluency. Cells were exposed to three concentrations of *Thymbra spicata* polysaccharides (0 µg/ml, 0.1 µg/ml, 10 µg/ml, 1000 µg/ml) and doxorubicin (0 µM, 0.1 µM, 10 µM, 1000 µM) for 48 h. After fixation in 4% paraformaldehyde, 0.1% Triton X-100 (AppliChem, Darmstadt, Germany) was used for permeabilization. After washing with PBS, 3% hydrogen peroxide (Merck, Darmstadt, Germany) was used to inhibit the endogenous peroxidase activity. The primary antibodies, anti-VEGF (NB100-664, Novus, USA) and anti-GSK-3beta (NBP1-47470, Novus, USA) were treated in cells for 18 h. For negative control, the primary antibody was not applied to the cells. After the stage of secondary antibodies, biotinylated secondary antibodies, and peroxidase-conjugated streptavidin (Histostain kit, 85-9043, Zymed, Carlsbad, USA), the immunoreactivity was made visible using diaminobenzidine/hydrogen peroxide (DAB, İnvitrogen, CA, USA). And the counterstaining was carried out using Mayer's hematoxylin (ScyTek, UT, USA). Samples were mounted with an aqueous medium (DBS, Pleasanton, USA). They were evaluated under a light microscope with a camera attachment microscope (IX71 inverted-florescence phase microscope, Olympus, Japan). The results of staining were given as H-score; firstly, five different areas were chosen in the images, and immunoreactivities were determined as weak (+), moderate (++), and strong (+++) respectively. The H-score was calculated using the formula: Σ Pi (intensity of staining + 1). Pi means the percentage of immunopositive cells for each intensity (from 0% to 100%).[29]

Ethical Statement

This study was approved by the Niğde Ömer Halisdemir University Clinical Research Ethics Committee (approval number: 06.09.2019-2019/22). In our experimental study, we only cultured the cell lines, MCF-7 breast cancer cell line and L929 fibroblast cell line; no tissue samples isolated from patients or animals were used. Artificial intelligence-enabled technologies were not used in our study. The study was conducted in compliance with the Declaration of Helsinki.

Statistical Analysis

The results of the experiments were analyzed by repeated measures of the Analysis of Variance (ANOVA) test using GraphPad software (San Diego, CA, USA). The differences between the groups were revealed by the Tukey-Kramer multiple comparisons test. The data was given as mean±standard deviation, and the $p<0.05$ was accepted as statistically significant.[29]

Results

MTT Assay

It was determined that *Thymbra spicata* polysaccharides and doxorubicin had cytotoxic effects on MCF-7 cells depending on the dose increase (Fig. 1). The IC50 doses of *Thymbra spicata* polysaccharides were found to be 14.75 µg/ml for MCF-7 breast cancer cells and 65.04 µg/ml for L929 fibroblast cells, while the IC50 doses of doxorubicin were 12.50 µM for MCF-7 breast cancer cells and 25.45 µM for L929 fibroblast cells (Fig. 1). After MTT analysis, the changes at 48 hours were examined for *in vitro* wound

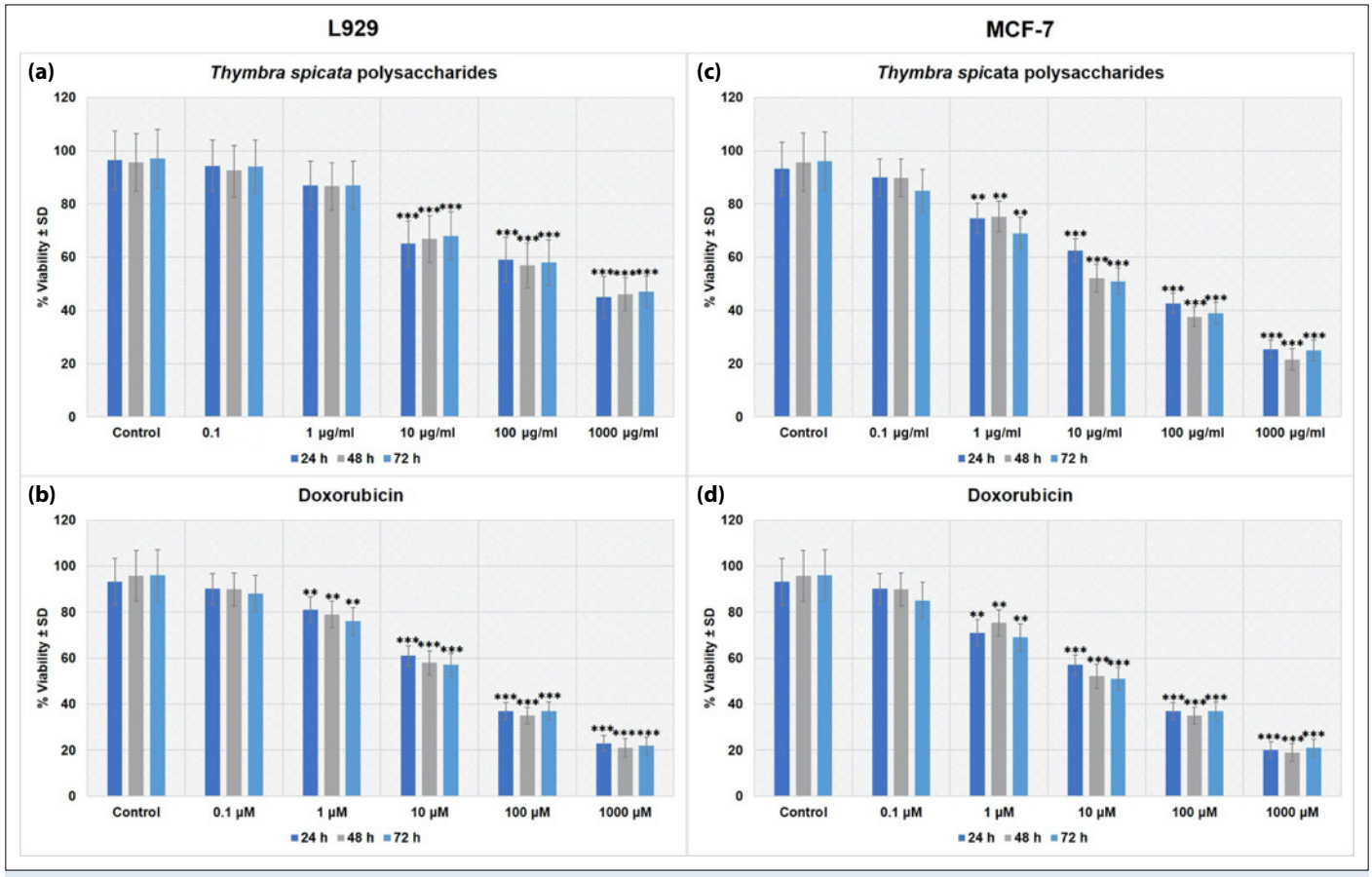

Figure 1. Cytotoxicity levels of *Thymbra spicata* polysaccharides and doxorubicin on L929 fibroblast cells **(a, b)** and MCF-7 **(c, d)** cells. **p<0.01, and ***p<0.001.

healing assay and and immunocytochemistry. Because, in MTT results, the cell proliferation levels were very close to each other at 24 and 48 hours. And also, cell viability rate was below 50% at 72 hours (Fig. 1). Therefore, evaluation of the changes at 48 hours was more effective in revealing the results of the study.

In Vitro Wound Healing

In our study, we found the statistically significant inhibitory effect of *Thymbra spicata* polysaccharides and doxorubicin on cell migration at 48 h (Fig. 2). The cells were treated with two agents for 48 h (*Thymbra spicata* polysaccharides; 0 µg/ml, 0.1 µg/ml, 10 µg/ml, 1000 µg/ml and doxorubicin; 0 µM, 0.1 µM, 10 µM, 1000 µM). In control groups, closure rates were 90.5% for L929 and 85.0% for MCF-7 cells at 48 h. After 10 and 1000 µg/ml *Thymbra spicata* polysaccharides applications, closure rates of MCF-7 were diminished compared to the control group, 55.4% and 42.3%, respectively (***p<0.001) (Fig. 2c). In L929 cells, these rates were 80.4% and 73.0%. So, there was no significant difference between groups in L929 cells. Wound closure rates as a result of exposure to 10 and 1000 µM doxorubicin were 50.1% and 25.1% in MCF-7 breast cancer cells, whereas they were 78.1% and 55.3% in L929 fibroblast cells. As a result of the experiments, it was determined that cell proliferation and thus wound closure were suppressed depending on the dose increase.

Immunocytochemistry Results

After applications of *Thymbra spicata* polysaccharides and doxorubicin, the distributions of VEGF and GSK-3β were determined by immunocytochemical staining in L929 and MCF-7 cells at 48 h.

The evaluation of immunoreactivities was made using the H-score method (Fig. 3). In L929 cells, the VEGF and GSK-3β immunoreactivities did not change after the applications of *Thymbra spicata* polysaccharides and doxorubicin. So, there was no statistically significant difference between the groups (p>0.05) (Fig. 3a).

In MCF-7 cells, the decreased expression levels of those two molecules were detected in 10 and 1000 µg/ml *Thymbra spicata* polysaccharides, 10 and 1000 µM

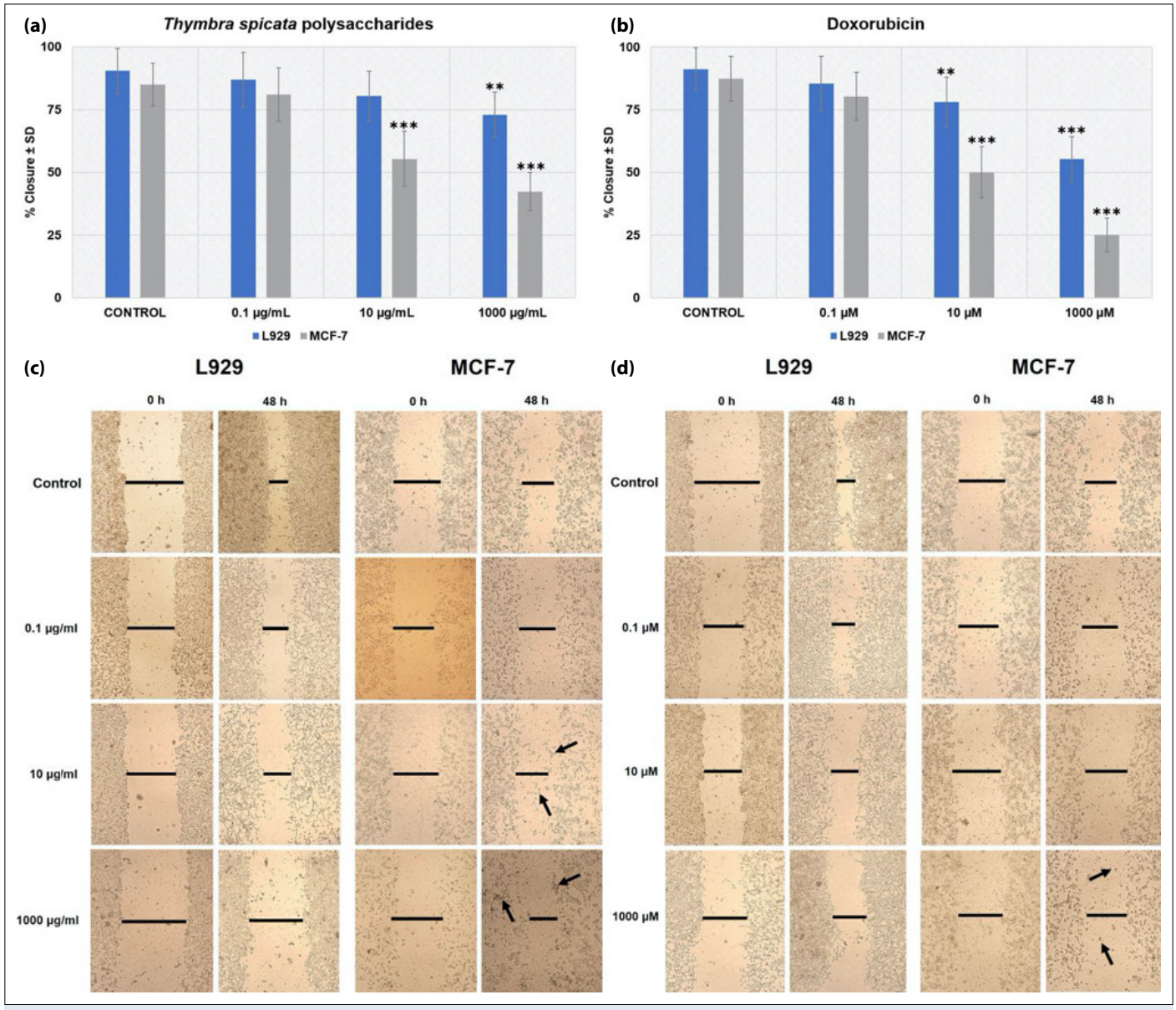

Figure 2. The closure rates of *in vitro* wound healing after the applications of *Thymbra spicata* polysaccharides **(a, c)** and doxorubicin **(b, d)** on MCF-7 and L929 fibroblast cells for 48 h. **p<0.01, and ***p<0.001. Arrows: Dead cells. Magnification: x100.

doxorubicin applications (Fig. 3–5). The expressions of VEGF and GSK-3β were close to each other in control, 0.1 µg/ml *Thymbra spicata* polysaccharides and 0.1 µM doxorubicin groups. There was not a significant difference between these groups (p>0.05). In a dose of 10 µg/ml polysaccharides, immunoreactivities of VEGF and GSK-3β were decreased significantly, 245.22±17.35 and 235.45±15.70, respectively (***p<0.001). Their decrease at 1000 µg/ml was more pronounced compared to control and 0.1 µg/ml groups, the VEGF rate was 121.65±16.00, and GSK-3β was 126.15±15.00 (***p<0.001). In the 10 µM doxorubicin group, rates of VEGF and GSK-3β were 255.25±18.45 and 243.00±17.00.

Discussion

With this study, we revealed that the *Thymbra spicata* polysaccharides have a significant cytotoxic effect on the MCF-7 breast cancer cell line depending on the dose increase. And we also used an antineoplastic drug, doxorubicin, to compare the cytotoxic effect of *Thymbra spicata* polysaccharides. The antiproliferative effect of this agent was detected in the *in vitro* wound healing assay. The decrease in VEGF and GSK-3β molecules, which play a role in tumor progression, also supported its inhibitory effect in MCF-7 cells.

Studies show that different species belonging to the genus Thymbra or *Thymbra spicata* species have antioxidant,

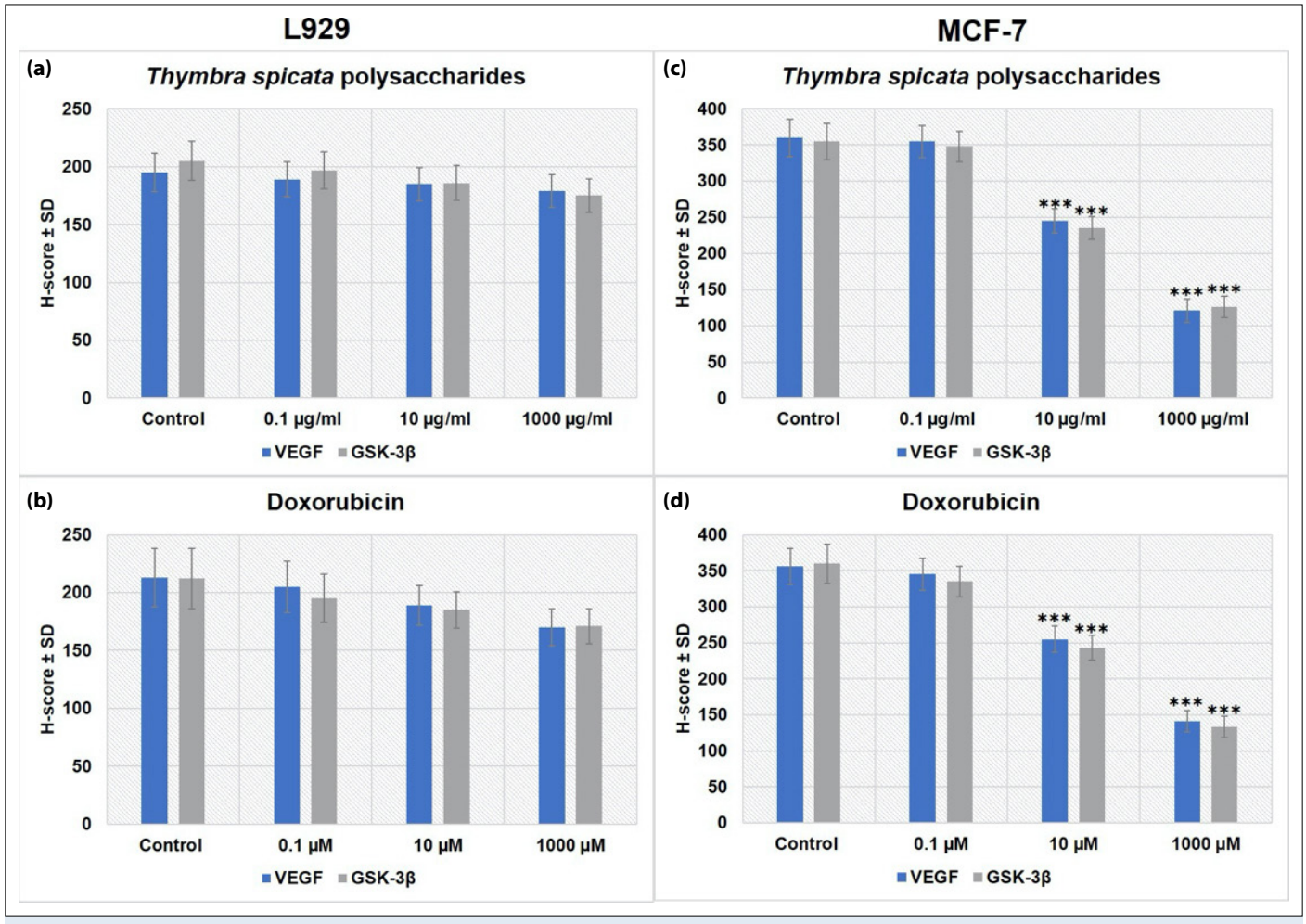

Figure 3. The H-Score rates of immunocytochemical staining of VEGF and GSK-3β in L929 fibroblast cells **(a, b)** and MCF-7 **(c, d)** cells after applications of *Thymbra spicata* polysaccharides and doxorubicin for 48 h. ***p<0.001.

antibacterial, or cytotoxic properties *in vivo* and *in vitro* conditions.[16–26,30] The ethanolic extract of *Thymbra spicata* has been shown to have a cytotoxic effect in MCF-7 cells via the production of reactive oxygen species (ROS) and apoptosis, and its half-maximal inhibitory concentration (IC50) has been reported as 80±5.6 µg/ml.[30] According to the wound healing assay, 25 µg/ml ethanolic extract inhibited the migration of MCF-7 cells. The ethanolic extract of *Thymbra spicata* has also been demonstrated to reduce the phosphorylation of signal transducer and activator of transcription 3 (STAT3) and NFκB molecules, which play a crucial role in tumor formation and metastasis. In the same study, the ethanolic extract of *Thymbra spicata* also showed cytotoxic effects on other tumor cell lines, such as human breast epithelial cell spontaneously immortalized (MCF-10A), non-small-cell lung cancer cells (A549), human cervix carcinoma cells (HeLa), acute T cell leukemia cells (Jurkat), and human prostate adenocarcinoma cells (PC3).[30] In another study, thymol was isolated from *Thymbra spicata*, and its antiproliferative effects were searched in olfactory ensheathing cells (OECs) derived from rat pups at *in vitro* conditions. When its effects on these cells in normal and high glucose environment was examined, it was reported that it protected the cells from high glucose. At the same time, it has been determined that the thymol in the extract of *Thymbra spicata* has a similar effect to thymol. After applications of two agents, decreased reactive oxygen species (ROS) and nitric oxide (NO) levels were observed, and Western blot analysis revealed a reduction in VEGF and an increase in brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) levels in OECs under high glucose condition. Despite the protective effects of both agents against high glucose, it has been reported to have a cytotoxic effect at high doses in normal glucose levels, lethal concentration 50% (LC50) doses were 121±5.3 µM thymol and 125±5.8 µM thymol in extract.[21] Unlike these studies, we used the *Thymbra spicata* polysaccharides and ascertained their effects on the MCF-7 breast cancer cell

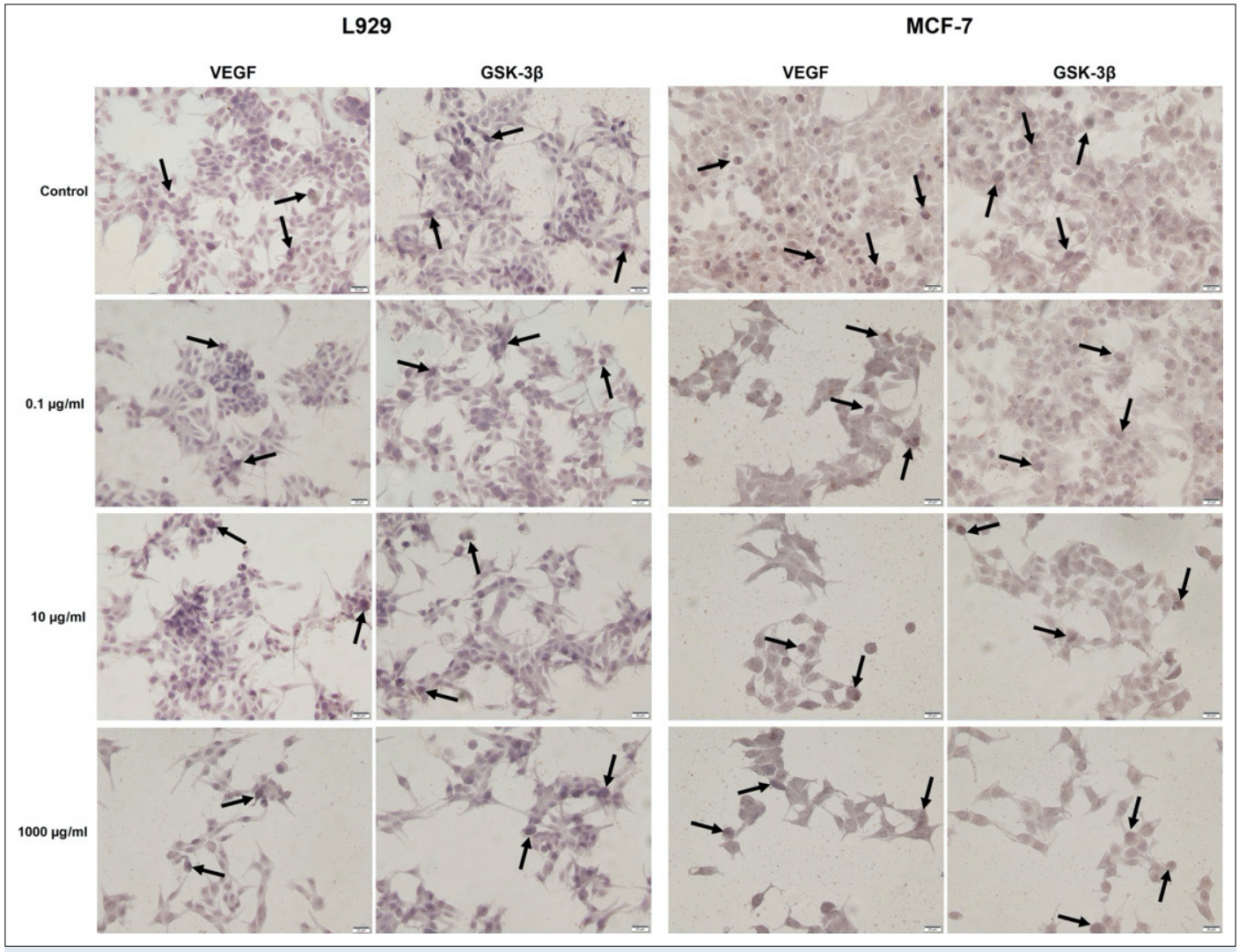

Figure 4. The immunocytochemical images of VEGF and GSK-3β after the applications of *Thymbra spicata* polysaccharides in L929 fibroblast cells and MCF-7 cells at 48. h. Arrows: Immunpositive cells. Scale bars: 20 µm.

line in our study. We detected its IC50 dose as 14.75 µg/ml for MCF-7 cells at normal glucose levels. We found that it has a cytotoxic effect on MCF-7 cells with a decrease in VEGF and GSK-3β molecule levels and inhibited cell proliferation and migration.

In another study with proven cytotoxic effect of *Thymbra spicata* extract, extracts prepared with water and ethanol were compared, and it was observed that the aqueous solution did not show any toxicity in three cancer cell lines, MDA-MB-231 breast adenocarcinoma cell line, A375 melanoma cell line and HCT116 colorectal carcinoma cell line. However, the ethanolic extract was found to be significantly toxic in these cell lines by MTT analysis. Its IC50 doses were 58.447 µg/ml for MDA-MB-231 cells, 110.238 µg/ml for HCT116 cells, and 31.443 µg/ml for A375 cells at 24 h. Also, the ethanolic extract was subjected to enzymatic processes (α-salivary amylase, pepsin, and pancreatin with bile salts) *in vitro* conditions, then its toxic effects were compared with the ethanolic extract that did not undergo any treatment. In that comparative experiment, it was revealed by the increase in IC50 as a result of MTT analysis that enzymatically treated ethanolic extract lost some of its toxic effects. Besides, carvacrol, a known toxic agent, was used as a positive control.[23] In our experiment, we used the polysaccharides, which obtained a powder form as a result of isolation, by dissolving it into cell culture media. Therewithal, we did not apply any enzymatic treatment to the polysaccharides. Therefore, it eliminated the toxic effect of ethanol or similar solvents, as well as any treatment. And, an antineoplastic drug, doxorubicin, was used as a positive control in our experiments.

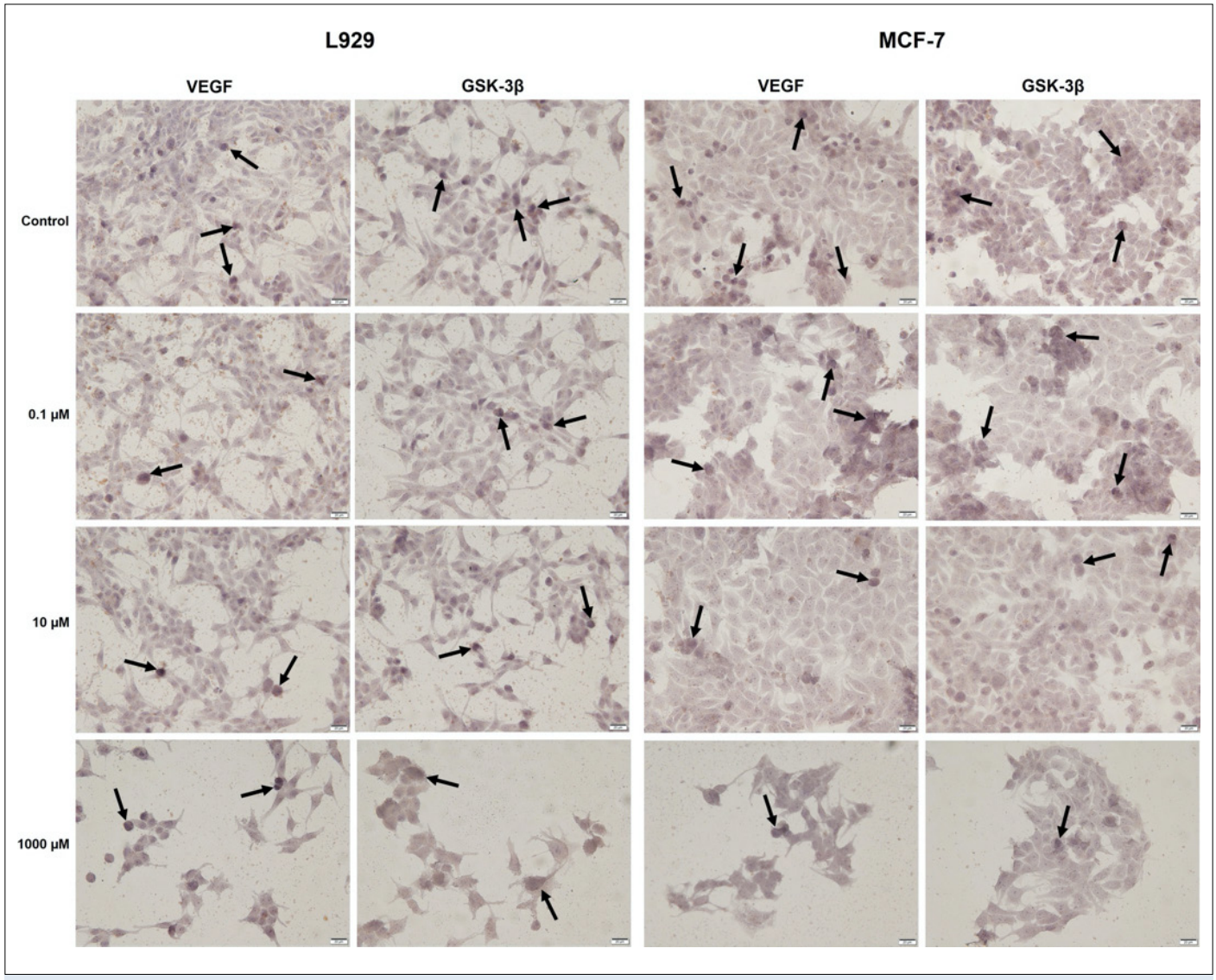

Figure 5. The immunocytochemical images of VEGF and GSK-3β after the applications of doxorubicin in L929 fibroblast cells and MCF-7 cells at 48. h. Arrows: Immunopositive cells. Scale bars: 20 µm.

In recent years, studies in which plant products are loaded into nanoparticles (NPs) have become widespread. Erci et al.[19] investigated the cytotoxic effect of *Thymbra spicata* extract loaded into silver NPs at different dilutions (1 ml and 5 ml plant extract loaded). They found their toxic effect at 50 µg/ml for 1 ml extract-loaded NPs and 150 µg/ml for 1 ml extract-loaded NPs. Again the same researchers carried out a similar study by loading *Thymbra spicata* extract (40 and 80 ml aqueous form) into the copper oxide NPs and reported that its extract had a cytotoxic effect at high concentrations.[20] In both studies, they demonstrated the cytotoxic effect of *Thymbra spicata* extract at high concentrations only in L929 fibroblast cells. In the current study, we aimed to determine the antitumorogenic effect of *Thymbra spicata* polysaccharides, and for this purpose, we used the MCF-7 breast cancer cell line, and L929 cells as were control group for our experiment. According to our results, the polysaccharides were more toxic in MCF-7 cells than in L929 cells.

Conclusion

Thymbra spicata polysaccharides have a cytotoxicity effect on MCF-7 breast cancer cells by reducing the VEGF and GSK-3β molecules. Also, they inhibited the migration of MCF-7 breast cancer cells. For the products of *Thymbra spicata* to be used in cancer treatment, it is necessary to determine the molecular signaling pathways that determine the cytotoxic effect via *in vitro* or *in vivo* studies by advanced molecular techniques.

Ethics Committee Approval: The Niğde Ömer Halisdemir University Clinical Research Ethics Committee granted approval for this study (date: 06.09.2019, number: 2019/22).

Authorship Contributions: Concept: CÖ, IA; Design: CÖ, IA; Supervision: CÖ, IA; Data Collection or Processing: IA, EÇ, OÖ; Analysis or Interpretation: CÖ, IA; Literature Search: CÖ, IA; Writing: CÖ, IA, EÇ; Critical Review: IA.

Conflict of Interest: None declared.

Use of AI for Writing Assistance: Not declared.

Financial Disclosure: This study was supported by the Scientific Research Committee of Niğde Ömer Halisdemir University (SAT 2020/3-BAGEP).

Peer-review: Double blind peer-reviewed.

References

1. Barzaman K, Karami J, Zarei Z, Hosseinzadeh A, Kazemi MH, Moradi-Kalbolandi S, et al. Breast cancer: Biology, biomarkers, and treatments. Int Immunopharmacol 2020;84:106535. [CrossRef]
2. Comşa Ş, Cîmpean AM, Raica M. The story of MCF-7 Breast cancer cell line: 40 years of experience in research. Anticancer Res 2015;35(6):3147-54.
3. Rezano A, Ridhayanti F, Rangkuti AR, Gunawan T, Winarno GNA, Wijaya I. Cytotoxicity of simvastatin in human breast cancer MCF-7 and MDA-MB-231 cell lines. Asian Pac J Cancer Prev 2021;22(S1):33-42. [CrossRef]
4. Melincovici CS, Boşca AB, Şuşman S, Mărginean M, Mihu C, Istrate M, et al. Vascular endothelial growth factor (VEGF) - Key factor in normal and pathological angiogenesis. Rom J Morphol Embryol 2018;59(2):455-67.
5. Khongthong P, Roseweir AK, Edwards J. The NF-KB pathway and endocrine therapy resistance in breast cancer. Endocr Relat Cancer 2019;26(6):R369-80. [CrossRef]
6. Vijay GV, Zhao N, Den Hollander P, Toneff MJ, Joseph R, Pietila M, et al. GSK3β regulates epithelial-mesenchymal transition and cancer stem cell properties in triple-negative breast cancer. Breast Cancer Res 2019;21(1):37. [CrossRef]
7. Peng Y, Wang Y, Zhou C, Mei W, Zeng C. PI3K/Akt/mTOR pathway and its role in cancer therapeutics: Are we making headway? Front Oncol 2022;12:819128. [CrossRef]
8. Zhang Z, Richmond A, Yan C. Immunomodulatory properties of PI3K/AKT/mTOR and MAPK/MEK/ERK inhibition augment response to immune checkpoint blockade in melanoma and triple-negative breast cancer. Int J Mol Sci 2022;23(13):7353. [CrossRef]
9. Song M, Bode AM, Dong Z, Lee MH. AKT as a therapeutic target for cancer. Cancer Res 2019;79(6):1019-31. [CrossRef]
10. Hashem S, Nisar S, Sageena G, Macha MA, Yadav SK, Krishnankutty R, et al. Therapeutic effects of curcumol in several diseases; An overview. Nutr Cancer 2021;73(2):181-95. [CrossRef]
11. Tewari D, Patni P, Bishayee A, Sah AN, Bishayee A. Natural products targeting the PI3K-Akt-mTOR signaling pathway in cancer: A novel therapeutic strategy. Semin Cancer Biol 2022;80:1-17. [CrossRef]
12. Apte RS, Chen DS, Ferrara N. VEGF in Signaling and disease: Beyond discovery and development. Cell 2019;176(6):1248-64. [CrossRef]
13. Ahmad A, Nawaz MI. Molecular mechanism of VEGF and its role in pathological angiogenesis. J Cell Biochem 2022;123(12):1938-65. [CrossRef]
14. Patel P, Woodgett JR. Glycogen synthase kinase 3: A kinase for all pathways? Curr Top Dev Biol 2017;123:277-302. [CrossRef]
15. Lin J, Song T, Li C, Mao W. GSK-3β in DNA repair, apoptosis, and resistance of chemotherapy, radiotherapy of cancer. Biochim Biophys Acta Mol Cell Res 2020;1867(5):118659. [CrossRef]
16. Akdemir Evrendilek G. Empirical prediction and validation of antibacterial inhibitory effects of various plant essential oils on common pathogenic bacteria. Int J Food Microbiol 2015;202:35-41. [CrossRef]
17. Haroun MF, Al-Kayali RS. Synergistic effect of Thymbra spicata L. extracts with antibiotics against multidrug- resistant Staphylococcus aureus and Klebsiella pneumoniae strains. Iran J Basic Med Sci 2016;19(11):1193-200.
18. Al Hafi M, El Beyrouthy M, Ouaini N, Stien D, Rutledge D, Chaillou S. Chemical composition and antimicrobial activity of satureja, thymus, and thymbra species grown in Lebanon. Chem Biodivers 2017;14(5):e1600236. [CrossRef]
19. Erci F, Cakir-Koc R, Isildak I. Green synthesis of silver nanoparticles using Thymbra spicata L. var. spicata (zahter) aqueous leaf extract and evaluation of their morphology-dependent antibacterial and cytotoxic activity. Artif Cells Nanomed Biotechnol 2018;46:150-8. Erratum in: Artif Cells Nanomed Biotechnol 2018;46:392. [CrossRef]
20. Erci F, Cakir-Koc R, Yontem M, Torlak E. Synthesis of biologically active copper oxide nanoparticles as promising novel antibacterial-antibiofilm agents. Prep Biochem Biotechnol 2020;50(6):538-48. [CrossRef]
21. Karimi E, Abbasi S, Abbasi N. Thymol polymeric nanoparticle synthesis and its effects on the toxicity of high glucose on OEC cells: Involvement of growth factors and integrin-linked kinase. Drug Des Devel Ther 2019;13:2513-32. [CrossRef]
22. Toker EB, Yeşilbağ K. In vitro antiviral activity of Thymbra spicata L. extract on bovine respiratory viruses (BCoV, BPIV-3, BRSV, BVDV and BoHV-1). J Appl Microbiol 2022;132(4):2625-32. [CrossRef]
23. Diab F, Khalil M, Lupidi G, Zbeeb H, Salis A, Damonte G, et al. Influence of simulated in vitro gastrointestinal digestion on the phenolic profile, antioxidant, and biological activity of Thymbra spicata L. extracts. Antioxidants 2022;11(9):1778. [CrossRef]
24. Gur T, Meydan I, Seckin H, Bekmezci M, Sen F. Green synthesis, characterization and bioactivity of biogenic zinc oxide nanoparticles. Environ Res 2022;204:111897. [CrossRef]
25. Gedikoğlu A. The effect of Thymus vulgaris and Thymbra spicata essential oils and/or extracts in pectin edible coating on the preservation of sliced bolognas. Meat Sci 2022;184:108697. [CrossRef]

26. Kerem S, Koşar N, Tekin F, Güreser AS, Özbek Ö. Investigation of antimicrobial activities and molecular characterization of the species belong to Origanum, Thymus and Thymbra genera by ISSR. Mol Biol Rep 2023;50(1):289-98. [CrossRef]
27. Lv Y, Yang X, Zhao Y, Ruan Y, Yang Y, Wang Z. Separation and quantification of component monosaccharides of the tea polysaccharides from Gynostemma pentaphyllum by HPLC with indirect UV detection. Food Chem 2009;112(3):742-6. [CrossRef]
28. Aydemir İ. The inhibition effect of curcumin on MCF-7 breast cancer cells via GSK-3beta and VEGF signals. CBU-SBED 2021;8(2):337-42.
29. Ünsal ÜÜ, Mete M, Aydemir I, Duransoy YK, Umur AŞ, Tuglu MI. Inhibiting effect of oleocanthal on neuroblastoma cancer cell proliferation in culture. Biotech Histochem 2020;95(3):233-41. [CrossRef]
30. Khalil M, Khalifeh H, Baldini F, Serale N, Parodi A, Voci A, et al. Antitumor activity of ethanolic extract from Thymbra Spicata L. aerial Parts: Effects on cell viability and proliferation, apoptosis induction, STAT3, and NF-kB signaling. Nutr Cancer 2021;73(7):1193-206. [CrossRef]