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# Lapatinib prevents and ameliorates dermal fibrosis in bleomycin induced experimental scleroderma model

## Lapatinib bleomisin ile oluşturulmuş deneysel skleroderma modelinde dermal fibrozu önler ve iyileştirir

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## ABSTRACT

**Background:** Scleroderma is a connective tissue disease characterized by endothelial damage and diffuse interstitial fibrosis. Lapatinib, a tyrosine kinase inhibitor, is a 4-anilinoquinol derivative. It inhibits many important signalling pathways including MAPK and PI3K. As a result, it affects cell cycle progression, apoptosis, angiogenesis and cell adhesion.
**Materials and Methods:** Mice with an average age of 6 weeks and a weight of 20-25 g were divided into 6 equal groups (n=10 in each group). Mice in the control group (group A and group D), which were not treated with bleomycin (BLM), received sc phosphate buffered saline (PBS) daily. BLM was dissolved in FTS and administered to mice in groups B and C for 3 weeks, and to mice in groups E and F at a dose of sc 100 µL (100 µg) daily for 6 weeks. Mice in groups A, B and C were sacrificed at the end of week 3; mice in groups D, E and F were sacrificed at the end of week 6 and tissue samples were collected for further analysis. The mRNA expressions of TGF-β1 and fibronectin-1 were determined by RT-PCR**.**
**Results:** Repeated subcutaneous administration of BLM caused dermal inflammatory cell infiltration, increased skin thickness and dermal fibrosis at early and late stages. TGF-β1 and fibronectin-1 mRNA expressions were also evidently increased. In both prophylactic and therapeutic applications of lapatinib, TGF-β1 and fibronectin-1mRNA expressions decreased markedly. In addition, histopathological dermal necro- inflammation and fibrosis were reduced.
**Conclusions:** Lapatinib may exert anti-fibrotic effects in BLM-induced dermal fibrosis model. Studies show that lapatinib is a potential therapeutic agent, but it needs to be confirmed with in vivo studies.
**Keywords:** Experimental scleroderma, lapatinib, tyrosine kinase

## ÖZET

**Amaç:** Skleroderma, endotelyal hasar ve diffüz interstisyel fibroz ile karakterize bir bağ dokusu hastalığıdır.Lapatinib, 4-anilinoquinoline türevi olan bir tirozin kinaz inhibitörüdür. Böylece, MAPK ve PI3K gibi önemli pek çok yolağın akışını durdurur. Sonuç olarak, hücre siklus ilerlemesi, apoptoz, anjiogenez ve hücre adhezyonunu etkilemektedir.Çalışmamızın amacı, bleomisin (BLM) ile oluşturulmuş deneysel skleroderma modelinde lapatinib uygulamalarının profilaktik ve teröpatik etkinliklerinin belirlenmesidir
**Materyal ve Metot:** Çalışmaya ortalama 6 hafta yaşında ve 20-25 gram ağırlıklarında, 6 eşit gruba ayrıldı (her grupta n=10).Bleomisin uygulanmayacak olan kontrol grubu farelere (grup A ve grup D), her gün sc fosfat ile tamponlanmış salin (FTS) uygulandı. BLM, FTS içerisinde çözündürülerek, B ve C grubundaki farelere 3 hafta boyunca, E ve F grubundaki farelere 6 hafta boyunca her gün sc 100 µL (100 µg) dozunda uygulandı. A, B ve C grup fareler 3. hafta; D, E ve F grubu fareler 6. hafta sonunda sakrifiye edildi ve yapılacak analizler için doku örnekleri alındı. Doku TGF-β1, ve fibronektin-1 mRNA ekspresyonları RT-PCR yöntemi ile belirlendi.
**Bulgular:** Tekrarlanan subkutan BLM uygulamaları sonucunda; erken ve geç evrede, dermal inflamatuar hücre infiltrasyonu, dermal fibroz ve dermal kalınlıkta artış meydana geldi. Benzer şekilde TGF-β1 ve fibronektin-1 mRNA ekspresyonları belirgin artı. Lapatinibin hem profilaktik hem de teröpatik uygulamalarında TGF-β1, ve fibronektin-1 mRNA ekspresyonları belirgin azaldı. Ek olarak, histopatolojik olarak dermal nekro inflamasyon ve dermal fibrozda gerileme tespit edildi.
**Sonuç:** Lapatinib BLM ile uyarılmış dermal fibroz modelinde anti-fibrotik etkiler sergileyebilmektedir.
**Anahtar Kelimeler:** Deneysel skleroderma, lapatinib, tirozin kinaz

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## INTRODUCTION

Scleroderma (systemic sclerosis [SSc]) is a connective tissue disease of unknown etiology characterized by the progressive development of fibrosis of the skin and internal organs. Although it affects many systems, the severity of organ involvement is variable. Depending on the development of microvascular pathology and fibrosis, the heart, lung, kidney and gastrointestinal tract can be affected (Hughes et al., 2019). Three main mechanisms are thought to be responsible for the clinical and pathological findings: vascular injury (vasculopathy), activation of the immune system/inflammation, and fibrosis (Cutolo et al.,2019).

Vascular dysfunction is believed to be the earliest stage in the pathogenesis of SSc (Hughes et al., 2019; Cutolo et al., 2019; Allanore et al., 2015). Endothelial damage, particularly in the microcirculation, occurs with cytokines produced by activated lymphocytes, and antibodies developed in response to endothelial cells (Mostmans et al., 2017). Activated lymphocytes secrete transforming growth factor (TGF)-β, collagen tissue growth factor (CTGF), and platelet-derived growth factor (PDGF). TGF-β leads to endothelial damage, inducing the expression of MHC and intercellular adhesion molecule (ICAM)-1. PDGF stimulates endothelial cell proliferation and reduces vascular endothelial growth factor (VEGF) levels, consequently inducing neovascularisation (Hughes et al., 2019; Cutolo et al., 2019; Allanore et al.,2015).

Inflammatory cell infiltration, especially T lymphocytes, mast cells and macrophages, has been detected in skin biopsies of scleroderma patients. In addition, serum levels of cytokines such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-2, IL-6, and IL-8 have been shown to be elevated and correlated with the severity of skin involvement in scleroderma patients (Gu et al., 2008). Activated endothelial cells and inflammatory cells can activate fibroblasts directly through cell-cell interactions and indirectly through the production of various cytokines, adhesion molecules and growth factors. TGF-β and other growth factors play important roles in these stages (Varga et al.,2009).

Lapatinib is a tyrosine kinase inhibitor, a 4-anilinoquinoline derivative (Schroeder et al.,2014). It reversibly binds to the intracellular tyrosine kinase domains of epidermal growth factor receptor (EGFR)-1 and -2 and inhibits substrate phosphorylation (Rusnak et al.,2001). Thus, lapatinib affects the cell cycle, apoptosis, angiogenesis, and cell adhesion, which play a role in the pathogenesis of SSc (Rusnak et al., 2001; Hirata et al.,2002).

These effects of lapatinib on expressed receptors, intracellular pathways and cell behavior suggest that it may be effective in the treatment of SSc. The aim of our study was to determine the prophylactic and therapeutic efficacy of lapatinib in a bleomycin (BLM)-induced experimental model of scleroderma.

## MATERIALS AND METHODS

This study was initiated with the approval of the Animal Experimentation Ethics Committee of Firat University (FUHADEK) with the date of 06.02.2013 and the number of 2013/01-10. The study was conducted an the Experimental Research Center of Fırat University in accordance with the ethical principles of standard animal experimental studies.

Sixty Balb/c female mice with an avarage age of 6 weeks and a weight of 20-25 g were included in the study. As skin thickness scleroderma is more common in females, female mice were included in the study. Since skin thickness also varies according to gender and weight of the mouse, Balb/c mice with an average age of 6 weeks and a weight of 20-25 g were selected and housed in specially prepared cages in a room with 12 hours of sunlight. A designated area on the dorsal side of all mice was shaved for subcutaneous (sc) administration.

This study tested the efficacy of lapatinib treatment in the early and late (established fibrosis) stages of a BLM-induced experimental scleroderma model. Six groups were created as follows: early stage groups (groups A [control group], B [BLM group], C [lapatinib group]) and late stage groups (groups D [control group], E [BLM group], F [lapatinib group]) (Figure 1). In the early stage groups, fibrosis stimulation by BLM and lapatinib treatment was initiated on the same day of BLM injections. In the late stage groups, BLM alone was administered for the first 3 weeks, and after day 21, BLM and lapatinib were administered together (Figure 1).

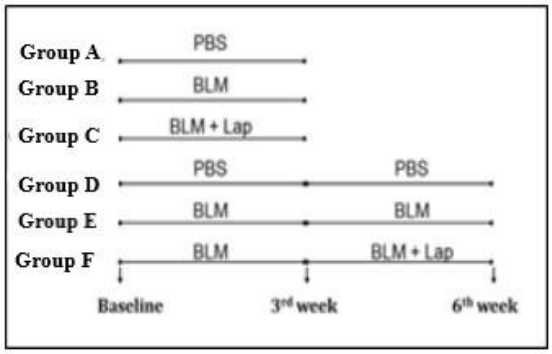


**Figure 1.** Schematics of the experimental applications in the study groups. PBS; phosphate buffered saline, BLM; bleomycin.

**Control Groups**

Phosphate buffered saline (PBS) was administered subcutaneously and daily to mice in the early (group A) and late (group D) stage control groups through a shaved area of the dorsal skin. Mice in the group A received PBS for the first 3 weeks, while the mice in the group D received PBS for 6 weeks.

**Bleomycin and Lapatinib Administrations**

100 µg BLM dissolved in 100 µL of PBS was administered subcutaneously to mice in the groups B and C for 3 weeks and to mice in the groups E and F for 6 weeks, as previously described (Yamamoto et al., 2002; Sritmatkandada et al., 2008). In addition to BLM, lapatinib (dissolved in 30% DMSO and 70% physiological saline) was administered subcutaneously at a dose of 30 mg/kg to mice in the group C for the first 3 weeks and to mice in the group F from day 21 until the end of the study (Taskar et al., 2012).

At doses of 1 µg and above, repeated BLM applications have been shown to induce scleroderma-like histopathological and biochemical findings after 3-4 weeks, and scleroderma-like findings persist for at least 6 weeks after BLM applications are terminated. Inflammatory cell infiltration is observed in the tissues in the early stages of the disease and inflammation is replaced by marked fibrosis in the later stages. Thus, the BLM-induced dermal fibrosis model and the data obtained from studies with other models mimic the early stage of scleroderma and do not provide sufficient information about the later stages of the disease. When 50 µg of BLM was administered every other day for 6 weeks, fibrosis was observed to develop at week 3. The researchers interpreted the first 3 weeks of the 6-week experiment as the early stage of the disease and the next 3 weeks as the late stage (established fibrosis stage), and the treatment applications were made in the next 3 weeks. This modification is a suitable method for assessing treatment response in the late stage of the disease (Yamamoto et al.,2002; Sritmatkandada et al.,2008; Taskar et al.,2012).

**Collection of Tissue Samples**

The mice in the early stage groups (A, B and C groups) were sacrificed by decapitation at the end of week 3, while the mice in the late stage groups (D, E and F groups) were sacrificed by decapitation at the end of week 6, 24 hours after the last treatments. The injected dorsal skin samples of the mice were excised for further examination. The tissue samples were divided into two parts for histopathological examination and real-time polymerase chain reaction (RT-PCR) analysis. One part of the tissue was placed in 10% formalin solution, and the other part, reserved for RT-PCR analysis, was placed in aluminium foil and stored at -80 $^0$C until the day of the study.

**Histopathological and İmmunohistochemical Analyses**

On the same day, paraffin blocks were prepared from the tissue samples placed in formalin solution. Slices taken from the blocks were stained with Hematoxylin-Eosin and Masson-Trichrome, and the degree of inflammatory cell infiltration and fibrosis was determined by light microscopy (Olympus BX-50) at X40, X100, X200 and X400 magnification (by an expert pathologist). For dermal thickness (the distance from the epidermal-dermal junction to the dermis-subcutaneous adipose tissue junction), the average of at least 5 different measurements was taken in at least two different specimens at X100 magnification in each subject.

**Assessment of Tissue mRNA Expression Levels**

Using an appropriate RNA isolation kit, tissue homogenate was obtained for RT-PCR analysis, and the mRNA expression of TGF-β1 and fibronectin-1 were assessed. Trizol (Invitrogen, Carlsbad, CA, USA) was used to isolate RNA from mouse skin samples. The Qubit® RNA Assay Kit for use with the Qubit® 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA) was used to measure RNA. The amount of RNA was measured in micrograms per millilitre. For cDNA synthesis, the lowest RNA value read was used as a reference to equalize the RNA amounts. Samples from each group were combined to create an RNA pool for complementary DNA synthesis(cDNA). Complementary DNA Synthesis was performed using a High-Capacityc DNA Kit for Reverse Transcription (Applied Biosystems, Foster City, CA, USA). The cDNAs obtained by reverse transcription were amplified using Tag Man Master Mix (Applied Biosystems, Foster City, CA, USA) in the ABI Prism 7500 Fast Real Time PCR instrument (Applied Biosystems, Foster City, CA, USA) when primers specific for a given sequence are present. The temperature was adjusted to 50$^o$C, for two minutes, 95$^o$C, for ten minutes X 40 cycles, 90$^o$C,5 for fifteen seconds, and 60 $^o$C for one minute. Real-time PCR was performed three times. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a housekeeping (control) gene. The comparative Ct (ΔCt) approach was used to determine the level of gene expression.

**Statistical Analysis**

At the end of the research, the statistical analysis of the obtained information was performed in the SPSS statistics software. The significance of possible differences between groups was assessed using the Post Hoc Mann-Whitney U and Kruskal-Wallis tests. P-values less than 0.05 were considered statistically significant.

## RESULTS

Induced dermal fibrosis by BLM: The histopathological evaluation of mice in the groups A and D (early and late stage control groups) that received PBD injection for 3 and 6 weeks showed no dermal fibrosis (Figure 2). However, both 3 and 6 weeks of BLM administration caused dermal and subcutaneous inflammatory cell infiltration and dermal fibrosis (Figure 2).

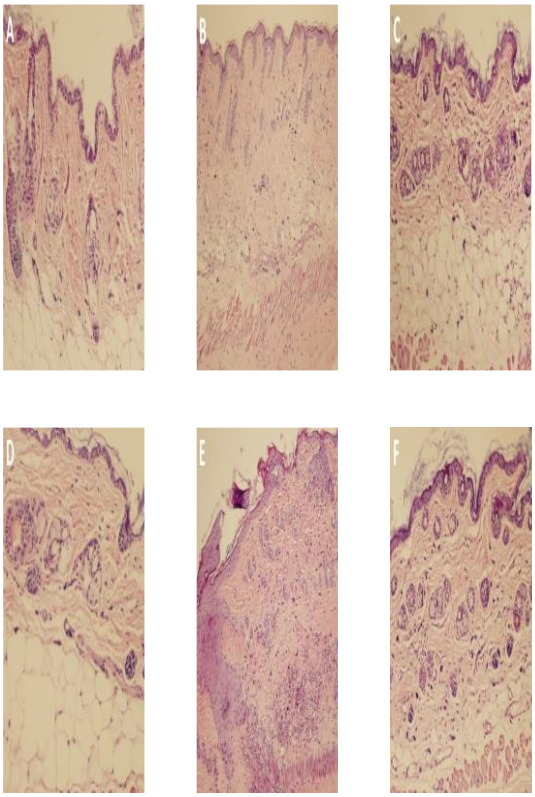


**Figure 2.** Histopathological appearances of skin sections (H&E, x200). Histopathological appearances were normal in the control groups (A and D). Bleomycin caused to thickened collagen bundles in dermis and definite skin fibrosis in the sham groups (B and E). Prophylactic and therapeutic applications of octreotide ameliorated dermal fibrosis and decreased infiltrations of inflammatory cells (C and F, respectively).

Group B had a significantly higher mean inflammatory cell count than group A, whereas group C had a significantly lower mean inflammatory cell count than group B (Figure 3). The mean dermal inflammatory cell count was 5.7 ± 1.9 in the early stage control group, while it was 31.3 ± 12.7 in the group receiving BLM alone, with a significant increase (p=0.003).

The mean dermal inflammatory cell count was 8.1 ± 5.2 in the group receiving prophylactic lapatinib simultaneously with BLM, with a significant decrease compared to the early phase placebo group (p=0.001). The mean dermal inflammatory cell count was 4.7 ± 2.3 in the

latter phase control group, while it was 17.4 ± 6.8 in the late-stage BLM (placebo) group, with a notable increase (p=0.001). The mean dermal inflammatory cell count was 9.4 ± 4.1 in the group that received therapeutic lapatinib simultaneoulsy with BLM, with a significant decrease compared to the late stage placebo group (p=0.001).

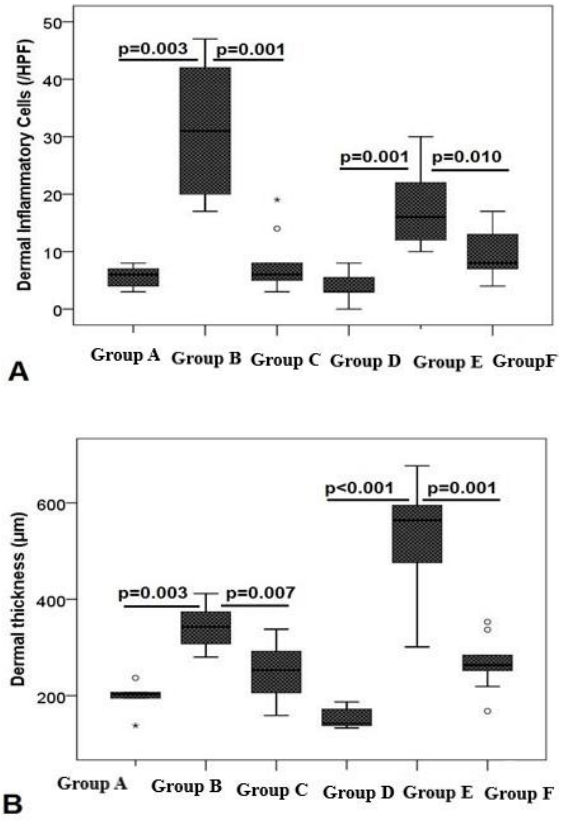


**Figure 3.** Dermal inflammatory cell counts (A) and dermal thicknesses (B) in the study groups.

Group B had a significantly higher dermal thickness than group A, group C had a much lower dermal thickness than group B, and group E had a significantly higher dermal thickness than group D (Figure 3). While the mean dermal thickness was 197.2 ± 32.5 µm in the early stage control group, it was 342.6 ± 47.3 µm in the group that received only BLM (p=0.003). The dermal thickness was 246.7 ± 57.4 µm in the group that received prophylactic lapatinib simultaneously with BLM, with a significant decrease compared to the late stage placebo group (p=0.007).

The dermal thickness was measured as 152.5 ± 21.8 µm in the late stage control group, whereas it was 524.7 ± 116.9 µm in the late stage group that received BLM only, and it was again measured to be increased ($p<0.001$). On the other hand, unlike the increase in inflammatory cell count, the increase in dermal thickness in the late stage BLM group was greater than both the late control group and the early stage BLM group. The dermal thickness was 267.7 ± 53.1 µm in the group that received therapeutic lapatinib simultaneously with BLM, with a significant decrease compared to the late stage placebo group ($p=0.001$).

Treatment of BLM-induced dermal fibrosis with lapatinib**:** There was a histopathological decline in dermal necroinflammation, dermal inflammatory cell count, and dermal fibrosis in both group C (prophylactic lapatinib group), which started receiving lapatinib with BLM administrations and in group F (therapeutic lapatinib group), which started receiving lapatinib from week 3 and continued until week 6 (Figures 2a). Mean dermal thicknesses and the prophylactic and therapeutic lapatinib groups exhibited a significant reduction in mean dermal inflammatory cell count and thickness when compared to the bleomycin groups. (Figures 3a and 3b). Similarly, both prophylactic and therapeutic administrations of lapatinib significantly reduced TGF-β1 and fibronectin-1 mRNA expression (Figures 4a and 4b).

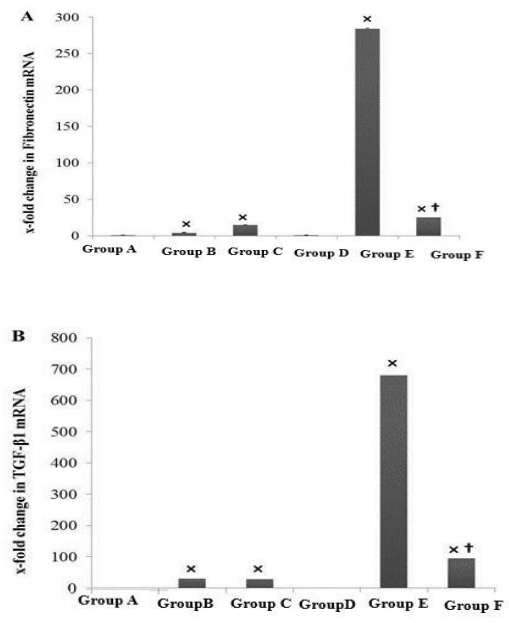


**Figure 4**. Dermal tissue mRNA expressions of fibronectin(A), and TGF-β1 (B). Results are expressed as mean ± SD. The gene values were normalized to the GAPDH level. TGF; transforming growth factor. ˣP value was < 0.05, when compared to the own Cntrl group.†P value was < 0.05, when compared to the own Sham group.

## DISCUSSION

This study investigated the potential preventive and therapeutic benefits of lapatinib in an experimental model of scleroderma caused by BLM. Dermal thickness, myofibroblastic cell activity, and inflammatory cell infiltration increase with repeated doses of BLM. In this experimental paradigm, lapatinib treatment halts the development of dermal fibrosis, myofibroblastic cell activity, and inflammatory cell infiltration.

Scleroderma is a chronic inflammatory disease with diffuse fibrosis of the skin and internal organs. However, the pathogenesis of scleroderma is not fully understood and there is no approved treatment protocol yet. Vascular injury is thought to be the earliest stage in the pathogenesis of scleroderma (Allanore et al.,2015). Cytokines produced by activated lymphocytes and antibodies in response to endothelial cells cause endothelial damage in the microcirculation (Mostmans et al., 2017). When lymphocytes are activated, CTGF, TGF-β, and PDGF are secreted. Secreted TGF-β causes endothelial damage and the expression of ICAM-1. PDGF causes neovascularization by stimulating endothelial cell proliferation and reducing the amount of VEGF (Allanore et al., 2015). Following endothelial damage and activation, vasomotor tone is lost due to an increase in the amount of ET-1, a vasoconstrictor, and due to a decrease in the amount of NO, a vasodilator mediator (Mostmans et al., 2017). Inflammatory cells migrate to the site of injury by chemoattraction and adhere to the vessel wall (Kuryliszyn-Moskal et al.,2004). Platelets adhere to the subendothelium with which they come into contact, leading to fibrin accumulation and intravascular thrombus formation. The muscle cells present in the vessel wall are also activated and progress towards the intima and transform into myofibroblasts.

In scleroderma, vasculopathy and immune activation begin years before dermal fibrosis. It is known that in the pathogenesis of scleroderma, fibrosis occurs with the excessive synthesis and storage of collagen and other ESM molecules (Kuryliszyn-Moskal et al., 2004). Active fibroblasts (myofibroblasts) are responsible for the production of ESM (Krieg et al., 2007; Postlethwaite et al., 2004). Fibroblasts can be directly activated by activated endothelial cells and inflammatory cells through cell-to-cell contact, and they can also be indirectly activated by these cells by producing various cytokines, adhesion molecules, and growth factors. TGF-β plays an important role in these stages (Varga et al., 2009).

The serum levels of cytokines such as IL-2, IL-4, and IL-6 have been reported to increase in scleroderma and may contribute to the disease process. A number of cytokines have demonstrated efficacy in the experimental scleroderma model produced by BLM.

BLM increases the synthesis and release of IL-4 and IL-6. IL-4 and TGF-β are major fibrogenic cytokines in scleroderma. Fibroblasts produce more collagen and TGF-β when exposed to IL-4. TGF-β inhibits metalloproteinase degradation while promoting the synthesis of collagen and matrix proteins, thus preventing collagen destruction. Anti-TGF-β antibody treatment has been shown to inhibit skin fibrosis in the experimental scleroderma model induced by BLM. (Gu et al.,2008; Varga et al.,2009; Schroeder et al.,2014; Rusnak et al.,2001). The results of our investigation showed that the BLM-induced cutaneous fibrosis model had elevated TGF-β1 mRNA expression. In addition, determination of an increase in inflammatory cell levels with BLM administration suggests that immune activation contributes to the disease process.

Lapatinib is a dual selective inhibitor that prevents signal conduction by inhibiting the HER2/ErbB2 tyrosine kinase (TK) (Spector et al.,2005). Lapatinib exerts its effect by binding intracellularly to the ATP-binding site of TK. It inhibits the phosphorylation and activation of TK. In this way, the activation of many pathways such as extracellular signal-dependent kinase-1 (ERK1) and phosphatidylinositol-3 kinase (PI3K) is inhibited by blocking the signaling from the onset. EGFR-1 carries the TK receptor family characteristics similar to the VEGF receptor. HER1 is one of the 19 subgroups of EGFR (Wood et al., 2004; Slamon et al., 1987). Activation of this family is critical for cell proliferation, activation, and continuity. Many substances can activate HER1. These include EGF and TGF-α. The combination of EGFR with the ligand leads to its dimerization. Homodimers and heterodimers are formed by dimerization in activation of the EGFR receptor family. The phosphoinositol-3-OH kinase and protein kinase pathways are activated by HER1 stimulation through TK activation. (Arteaga et al.,2001). Research has demonstrated that HER1 activation induces neovascularization and effectively inhibits cell migration, proliferation, adhesion, and apoptosis.It is well known that increasing the release of angiogenic chemicals such as VEGF with EGFR activation accelerates angiogenesis. It has been shown that angiogenesis can be inhibited by EGFR inhibitors (Van et al., 2005; Camp et al., 2005).

Human EGFR-2 is the second receptor on which lapatinib acts and plays a crucial role in angiogenesis. The impact of HER2 on angiogenesis has been extensively studied compared to HER1. EGF binds to the receptor in response to HER2 activation, and several cellular signaling pathways are activated, including the PI3K and mitogen activated protein kinase cascades. The HER2 receptor is essential for normal cell proliferation and differentiation.

Activation of these receptors is associated with increased cell proliferation, invasion, increased tumor cell motility, angiogenesis, and inhibition of apoptosis (Schlessinger et al., 2000). Lapatinib blocks the action of the HER1 and HER2 receptors, according to in vitro research, and research suggests that lapatinib is more effective than HER1 receptor blockers when used alone as a HER1 and HER2 receptor blocker (Diaz et al., 2010).

The study by Roque et al. demonstrating the antitumor and antiangiogenic effects of the epidermal growth factor receptor and HER-2 TK inhibitor lapatinib in a lung cancer simulation showed that lapatinib induced cell cycle arrest, apoptotic cell death and decreased levels of cyclin A and B1, which regulate the S and G2/M phases of the cell cycle, respectively, during the G1 phase. In vivo studies have shown that rodents bearing tumor cells treated with lapatinib have a significant reduction in tumor cell growth compared to the control group. In addition, reduced angiogenesis has been shown in lapatinib-treated tumor bearing mice (Diaz et al., 2010).

Another study by Huang et al. found that in human chronic myeloid leukaemia (CML) using K562 cells as an experimental model, lapatinib concurrently caused morphological alterations resembling apoptosis, autophagy, and megakaryocytic differentiation. Lapatinib induces apoptosis by two distinct mechanisms: first, it decreases the transmembrane potential of mitochondria, and second, it activates the caspase-dependent pathway. Lapatinib-induced apoptosis and autophagic cell death are more common in human CML-K562 cells (Huang et al., 2011).

The present study demonstrated the efficacy of lapatinib, a TK inhibitor, in a BLM-induced experimental scleroderma model. Recent studies have shown that blockade of the EGFR pathway ameliorates experimental renal fibrosis and peritoneal fibrosis (Son et al.,2021; Wang et al., 2016). In addition to lapatinib, erlotinib and gefitinib are also TKs that inhibit EGFR. It has been reported that erlotinib regresses renal fibrosis. while gefitinib ameliorates BLM-induced pulmonary fibrosis (Gonçalves et al.,2021; Li et al.,2018; Rayego et al.,2018). These results reveal the pathogenic effects of the EGFR pathway in fibrotic diseases, particularly scleroderma, and support it as a potential therapeutic target. In the experimental model of renal fibrosis, Rayego-Mateos et al.reported that, the effects of CTGF, which has long been known to affect the fibrotic process, were associated with the EGFR pathway (Rayego et al.,2018).

## CONCLUSION

In conclusion, our study demonstrated that lapatinib histopathologically reduced dermal necroinflammation and dermal fibrosis with in both

prophylactic and therapeutic administrations. It was also shown that TGF-β1 and fibronectin-1 mRNA expressions were decreased. In a model of cutaneous fibrosis produced by BLM, lapatinib has the potential to exert anti-fibrotic effects. These anti-fibrotic effects may be attributed to the suppression of TGF-β production. Limitations of the study include that although the groups in animal studies were generally small, larger studies are needed to generalise. Multicenter and prospective studies should be conducted on the antifibrotic use of lapanitib in clinical practice in diseases with fibrosis such as scleroderma.

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**Ethics Committee Approval:** Permission was obtained from Animal Experiments Ethics Committee of Firat University (FUHADEK) with the date of 06.02.2013 and the number of 2013/01-10.

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**Conflict of Interest:** The authors declare that there is no conflict of interest regarding the publication of this article.

## Author Contributions

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