Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

# Impact of Pirfenidone Administration Routes on Colon Anastomosis and Healing in Rats

*Ali Duran, MD,*[a,*] *Ferhat Çay, MD,*[a] *Nelin Hacıoğlu, PhD,*[b] *Esra Tokay, PhD,*[b] *Feray Köçkar, PhD,*[b] *Eren Altun, MD,*[c] *Azad Gazi Şahin, MD,*[a] *Hüseyin Pülat, MD,*[d] *and Alev Çetin Duran, MD*[e]

[a] *Faculty of Medicine, Department of Surgery, Balıkesir University, Balıkesir, Turkey*
[b] *Faculty of Sciences and Arts, Department of Molecular Biology and Genetics, Balıkesir University, Balıkesir, Turkey*
[c] *Department of Pathology, Bagcılar Training and Research Hospital, University of Health Sciences, Istanbul, Turkey*
[d] *Mersin City Hospital, General Surgery Clinic, Mersin, Turkey*
[e] *Faculty of Medicine, Department of Microbiology, Balıkesir University, Balıkesir, Turkey*

ARTICLE INFO

*Article history:*
Received 11 August 2025
Received in revised form
18 November 2025
Accepted 19 November 2025
Available online 15 December 2025

*Keywords:*
Anastomotic strictures
Antifibrotic effects
Colorectal surgery
Pirfenidone
Wound healing

ABSTRACT

*Introduction:* Anastomotic strictures are a prevalent complication in colorectal surgery. This study aimed to investigate the antifibrotic effects of pirfenidone on colorectal anastomotic stenosis, abdominal wall wound healing, and adhesion formation in a rat model by comparing various routes of administration (intraperitoneal, oral, and rectal).

*Methods:* Forty female Wistar Albino rats were randomly allocated to four groups: control, intraperitoneal pirfenidone administration (IAP), oral pirfenidone administration, and rectal pirfenidone administration. Colonic anastomosis was performed followed by pirfenidone administration for 14 d. This study evaluated burst pressure, histopathological parameters, immunohistochemical analyses, and molecular analyses of transforming growth factor-β, vascular endothelial growth factor-A, and fibroblast growth factor-2 expression.

*Results:* Burst pressure increased significantly across all treatment groups, with IAP and rectal pirfenidone administration demonstrating a three-fold increase compared with the control. Histopathological analysis revealed enhanced inflammatory cell infiltration, fibroblastic activity, collagen deposition, and neoangiogenesis in the treatment groups. Transforming growth factor-βexpression increased in all treatment groups, whereas vascular endothelial growth factor-A and fibroblast growth factor-2 expressions decreased. Intra-abdominal adhesions decreased across all treatment groups, with IAP exhibiting the most substantial reduction. Anastomosis width increased in all treatment groups, with oral pirfenidone administration demonstrating the widest anastomosis.

*Conclusions:* Pirfenidone exhibited a significant antifibrotic effect by preventing colorectal anastomotic stenosis, reducing intra-abdominal adhesions, and promoting optimal wound healing. Oral administration resulted in the widest anastomotic lumen, suggesting its

* *Corresponding author*. Faculty of Medicine, Department of Surgery, Balıkesir University, Cagis Campus, Balıkesir 10145, Türkiye.
E-mail address: g.cerrahad@gmail.com (A. Duran).
0022-4804/$ – see front matter 
https://doi.org/10.1016/j.jss.2025.11.050

potential as the preferred route for clinical applications. Further research is necessary to elucidate the long-term impact of pirfenidone on anastomotic healing and optimize its therapeutic potential.



## Introduction

Anastomotic strictures are one among the most common complications of colorectal surgery, with an incidence ranging from 2.5% to 30%.[1] Although the pathophysiology of anastomotic strictures is not fully understood, potential causes include anastomotic leaks, radiotherapy, stapler use (surgical technique), bowel ischemia, male sex, and diversion stomas.[2] Anastomotic strictures can lead to significant clinical problems such as defecation disorders, fecal incontinence, and partial or complete bowel obstruction.[3] Although some anastomotic strictures may resolve spontaneously during fecal passage, others require endoscopic or fluoroscopic balloon dilation, stenting, repeat anastomosis, transanal stricturoplasty, electrocautery resection, or incision.[4] However, local procedures are associated with a high recurrence rate, leading to repeated procedures and surgeries, increased hospital admissions, anastomotic leaks, and increased postoperative morbidities. Consequently, due to persistent strictures, some patients may require a permanent stoma.[5]

Pirfenidone is an orally administered synthetic pyridine compound approved for the treatment of idiopathic pulmonary fibrosis (IPF).[6] It is an antifibrotic agent that has shown efficacy in reducing lung function decline, decreasing mortality, and improving progression-free survival in patients with mild-to-moderate IPF.[6,7] Interestingly, although pirfenidone was initially developed for lung fibrosis, its therapeutic potential has expanded to other areas. Studies have shown its effectiveness in kidney fibrosis[8] and its promise in eye care, including applications for corneal fibrosis, diabetic retinopathy, macular degeneration, and postoperative glaucoma interventions.[9] In addition, research has explored its potential for the treatment of cartilage injury and osteoarthritis.[8] Although it has a well-established role in IPF treatment, ongoing research continues to uncover its potential applications in various fibrotic conditions across different organ systems.

This study investigated the antifibrotic effects of pirfenidone on colorectal anastomotic stenosis, abdominal wall wound healing, and adhesion formation in rats. This study included immunohistochemical analyses, bursting pressure analysis, evaluation of histological parameters, and molecular analyses of genes such as Transforming Growth Factor-β, Vascular Endothelial Growth Factor-A, and Fibroblast Growth Factor-2 (TGF-β, VEGF-A, and FGF-2). This study also aimed to compare the efficacy of different administration routes of pirfenidone (intraperitoneal, oral, and rectal) and analyze the effects of its antifibrotic and anti-inflammatory properties on the anastomosis healing process. This study evaluated pirfenidone's potential to accelerate wound healing and enhance tissue regeneration while examining its ability to reduce complications associated with colon anastomosis.

## Methods

### *Animal model*

Forty female Wistar Albino rats, with an average weight of 320 g (range, 300-340 g), were obtained from the Balikesir University Experimental Animal Center. In studies involving experimental animals, the guidelines for the care and use of animals are made in accordance with the European Commission Directive 86/609/EEC on animal experiments.

The rats were housed under standard laboratory conditions with 55%-60% humidity and a room temperature of 22°C-24°C, following a 12-h light/dark cycle. The rats were provided a regular laboratory rat diet and tap water ad libitum. The rats were randomly divided into four groups of 10 rats each and housed in pairs per cage, allowing free movement. Study groups were organized as following: control group $n = 10$, intraperitoneal pirfenidone administration (IAP) group $n = 10$, oral pirfenidone administration (OAP) group $n = 10$, and rectal pirfenidone administration (RAP) $n = 10$.

Only female rats were used in this study to maintain consistency with established colonic anastomosis experimental models and to minimize variability associated with sex-related behavioral and hormonal influences on postoperative stress and wound healing. The study design aligns with current guidelines acknowledging sex as a biological variable.

### *Surgical procedure*

Before the operation, all rats were fasted for 12 h. Anesthesia was induced by intraperitoneal administration of 90 mg/kg xylazine hydrochloride (Rompun , 2% injectable solution, 20 mg/mL; Bayer, Canada) and 10 mg/kg ketamine hydrochloride (Ketalar, injectable solution 100 mg/mL; Pfizer, UK). The anterior abdominal area of the rats was shaved and the incision site was sterilized using povidone–iodine. A 3 cm vertical incision was made along the midline of the abdomen to enter the abdominal cavity. The sigmoid colon was excised. Intestinal samples were collected from all rats for molecular and histopathological analyses. Samples for molecular testing were stored in RNA-safe solution at −80°C, whereas those for histopathological examination were preserved in 10% formaldehyde. The intestine was reconnected using an end-to-end anastomosis technique using 5/0 vicryl sutures. The diameter of the anastomosis was measured using a surgical loupe and ruler. Following anastomosis, the groups were administered

different treatments: The IAP group received 350 mg/kg/d pirfenidone intraperitoneally (1 mL), the OAP group was administered 25 mg/kg/d pirfenidone orally using an oral gavage needle (1 mL), and the RAP group received 25 mg/kg/d pirfenidone rectally via a 4-F rectal catheter (1 mL). The control group received intraperitoneal saline (1 mL/d).

Absorbable sutures (4/0) were used to close the abdominal walls. After surgery, the subjects fasted for 12 h before resuming a standard diet and water intake. Ketoprofen (2-5 mg/kg) was injected intramuscularly twice daily for pain relief, and a single subcutaneous dose of cefazolin (15-25 mg/kg) was administered for antibiotic prophylaxis. For the next 14 d, groups IAP, OAP, and RAP continued to receive their specified drug dosages, while control group was administered an equivalent volume of saline.

On postoperative day 14, general anesthesia was induced again using intraperitoneal injections of xylazine hydrochloride (90 mg/kg) and ketamine hydrochloride (10 mg/kg). The previous incision scar was excised to reopen the abdominal cavity. The scar tissue was divided, with one half preserved in RNA-safe solution at −80°C for molecular analysis and the other half fixed in 10% formaldehyde for histopathological examination. The abdominal cavity was inspected for adhesions, which were scored. The anastomotic site was identified and its diameter was measured using a surgical loupe and ruler. The anastomotic line, including the 2 cm margins on both sides, was resected.

### Burst pressure measurement

A specialized setup was created to measure the burst pressure.[10] The resected intestinal segment, which included 2 cm proximal and distal to the anastomosis, was carefully prepared while preserving the surrounding adhesions. One end of the intestinal segment was tied with a 3/0 silk suture, and the other end was attached to the measurement apparatus. A steady flow of an isotonic solution mixed with methylene blue was administered at a rate of 3 mL/min. The anastomotic site was also closely observed. The pressure at the point where methylene blue first leaked from the anastomosis was recorded as the burst pressure.

### Evaluation of peritoneal adhesions

After the subjects were sacrificed, the abdominal cavity was opened through a midline incision, and peritoneal adhesions were examined macroscopically. Adhesions were assessed based on two parameters: extent and severity. This classification was performed according to a scoring system previously described and widely used in the literature.[11]

For extent, 0: no adhesion; I: adhesion covering 1%-25% of the traumatized area; II: adhesion covering 26%-50% of the traumatized area; III: adhesion covering 51%-75% of the traumatized area; and IV: adhesion covering 76%-100% of the traumatized area.

For severity, 0: no adhesion; I: single, thin, and easily detachable adhesion; II: less widespread but weak and traction-insensitive adhesion; III: multiple, widespread visceral adhesions without visceroparietal extension; and IV: multiple, widespread, and dense adhesions extending to the abdominal wall, involving the mesentery, intestine, and omentum.

The extent and severity of adhesions were scored separately for each animal. The scoring process was performed by a researcher who was blinded to the experimental groups.

### Collection and analysis of tissue samples

#### *Tissue sample collection*

After measuring burst pressure, the anastomotic line was split into two halves. One half was placed in an RNA-safe solution for molecular analysis and stored at −80°C. The remaining half was placed in 10% formaldehyde for pathological examination. These samples were examined along with those obtained from the incision scars.

#### *Molecular analysis*

RNA was isolated from the samples using a Trizol Reagent kit. The quantity of RNA was determined and visualized using an RNA gel. Complementary DNA (cDNA) synthesis was performed using the recommended amounts of reaction buffer, oligo(dT), a ribolock inhibitor, deoxynucleotide triphosphates, and reverse transcriptase from Thermo Fisher Scientific. The functionality of the synthesized cDNA was verified by examining the expression of an internal gene using human β-microglobulin gene primers. An agarose gel with a concentration of 2% was used to visualize the DNA. Real-time polymerase chain reaction was performed in a final volume of 10 μL, containing 5 μL SYBR Green PCR Master Mix, 1 μL cDNA, 0.5 μL each of forward and reverse primers (100 ng/μL stock concentration), and 3 μL $dH_2O$.[12,13] Results were evaluated using the Livak method.[14,15] Each cDNA sample was analyzed in triplicate for the target genes and the internal control glyceraldehyde 3-phosphate dehydrogenase primers (Table 1). The gene expression of FGF-2, VEGF-A, and TGF-β was analyzed at the messenger RNA (mRNA) level, correlating with immunohistochemical studies.

#### *Histological and immunohistochemistry analysis*

All tissue samples for histological examination were processed routinely. The samples were embedded in paraffin blocks, sectioned at 5 mm, and stained with hematoxylin and

**Table 1 – Primers that are used in real-time PCR reaction.**

| Primer name | Sequence (5′-3′) |
|---|---|
| FGF-2 | F: 5′-ACTTCGCTTCCCGCACTGC-3′<br>R: 5′-CCAGTTGGTATGTGGCACTG-3′ |
| VEGF-A | F: 5′-CCCATGAAGTGGTGAAGTTC-3′<br>R: 5′-GAACAAGGCTCACAGTGAAC-3′ |
| TGF-β | F: 5′-GCTCAGTCTGTCTACCTGCA-3′<br>R: 5′-GGCGGGATGGCATCAAGGTA-3′ |
| GAPDH | F: 5′CTGGAGAAACCTGCCAAGTATG-3′<br>R: 5′-GGTGGAAGAATGGGAGTTGCT-3′ |

FGF-2 =fibroblast growth factor-2; VEGF-A = vascular endothelial growth factor-A, TGF-β = transforming growth factor-β; GAPDH = glyceraldehyde 3-phosphate dehydrogenase.

eosin. The samples were qualitatively examined under a light microscope for necrosis, granulation tissue, fibrous tissue, and vascularization.

Inflammation and wound healing parameters, including fibrin, bleeding, edema, congestion, polymorphonuclear leukocytes, fibrous tissue increase, fibroblasts, lymphocytes, macrophages, granulation tissue, vessel proliferation, and necrosis development, were evaluated. Immunohistochemical analyses of FGF, VEGF-A, and TGF-β were performed. Inflammatory cell infiltration, fibroblastic activity, collagen deposition, and neoangiogenesis in the samples were evaluated using a modified Ehrlich-Hunt scale as follows: 0, no evidence; 1, minimal and scattered; 2, minimal and in all areas; 3, large amounts but scattered; and 4, large amounts and in all areas. All samples were evaluated by a pathologist who was blinded to the groups.[10,16]

All macroscopic evaluations, adhesion scoring procedures, and histological analyses were performed under fully blinded conditions. All samples and tissue sections were coded by an independent researcher, and the investigators responsible for scoring and evaluation were unaware of the treatment group assignments throughout the entire assessment process.

### *Statistical analysis*

Statistical analyses were performed using GraphPad Prism 8 software. Data distribution was assessed using the Shapiro–Wilk normality test. Since the variables did not meet the assumption of normal distribution, results are presented as median (Q1-Q3). Comparisons among groups were conducted using the Kruskal–Wallis test followed by Dunn post hoc test with multiplicity adjustment. A $P$ value $< 0.05$ was considered statistically significant.

## Results

### *Burst pressure*

To assess the effect of pirfenidone on burst pressure, pirfenidone was administered intraperitoneally (IAP), orally (OAP), and rectally (RAP) at a specified dose. The specified dose was determined using a previous literature.[17] Burst pressures were measured for 9 d after administration, and the averages were calculated.[10,16] The results are shown in Figure 1. The study found that burst pressure increased three-fold compared to that in the control group following intraperitoneal and rectal administration, whereas oral administration resulted in a two-fold increase. All treatments resulted in a statistically significant increase in burst pressure, suggesting that intraperitoneal and rectal administration are recommended as appropriate options (Fig. 1). Significant differences in bursting pressure were observed among the groups (Kruskal–Wallis test, $P < 0.0001$). According to Dunn multiple comparison analysis, the IAP and RAP groups demonstrated significantly higher bursting pressures compared with the control group (both $P < 0.0001$). The OAP group showed bursting pressure values comparable to the control group ($P = 0.3649$). In addition, the IAP group exhibited significantly higher bursting pressure than the OAP group ($P = 0.0419$), and the RAP group

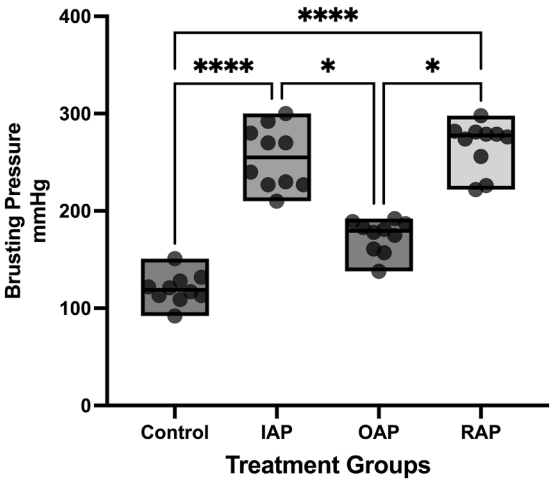


**Fig. 1 – The average burst pressure values were as follows: C_1st (control group at the first day), IAP, OAP, and RAP. Data are presented as median (Q1-Q3). Statistical analysis was performed using Kruskal–Wallis test followed by Dunn post hoc multiple comparisons test. $P < 0.05$ was considered statistically significant. IAP = intraperitoneal pirfenidone administration; OAP = oral pirfenidone administration; RAP = rectal pirfenidone administration.**

was also significantly higher than the OAP group ($P = 0.0124$). However, there was no significant difference between the IAP and RAP groups ($P > 0.9999$).

### *Histopathological analyses of pirfenidone treatment on colonic anastomoses*

To histologically determine the effect of different pirfenidone administration strategies (IAP, OAP, and RAP) on colonic anastomosis site wound healing, histological tissue samples were examined for inflammatory cell infiltration, fibroblastic activity, collagen deposition, and neoangiogenesis. The study found a significant and statistically meaningful increase in these parameters in wound tissues on day 14 compared to the control group on day 1. Specifically, rectal administration of pirfenidone notably increased the inflammatory cell infiltration rate compared with that observed on day 14 (Fig. 2). There is no statistically significant effect of fibroblastic activity, collagen deposition, and neoangiogenesis in three different administration routes, IAP, OAP, and RAP. In addition, there is no impact in epithelization recovery in wound closure.

Immunohistochemical analyses were conducted to evaluate protein expression of TGF-β, FGF-2, and VEGF-A on 14th d. As seen in Figure 3, all related proteins, immunohistochemistry of TGF-β, FGF-2, and VEGF-A proteins, showed diffuse intracytoplasmic expression in the stroma (arrow) in the anastomosis line. The representative photo of immunochemistry is shown in Figure 3. TGF-β expression increased in all treatment groups compared to the control group on day 14. For the other genes, no changes were observed in the protein expressions of FGF-2 and VEGF-A.

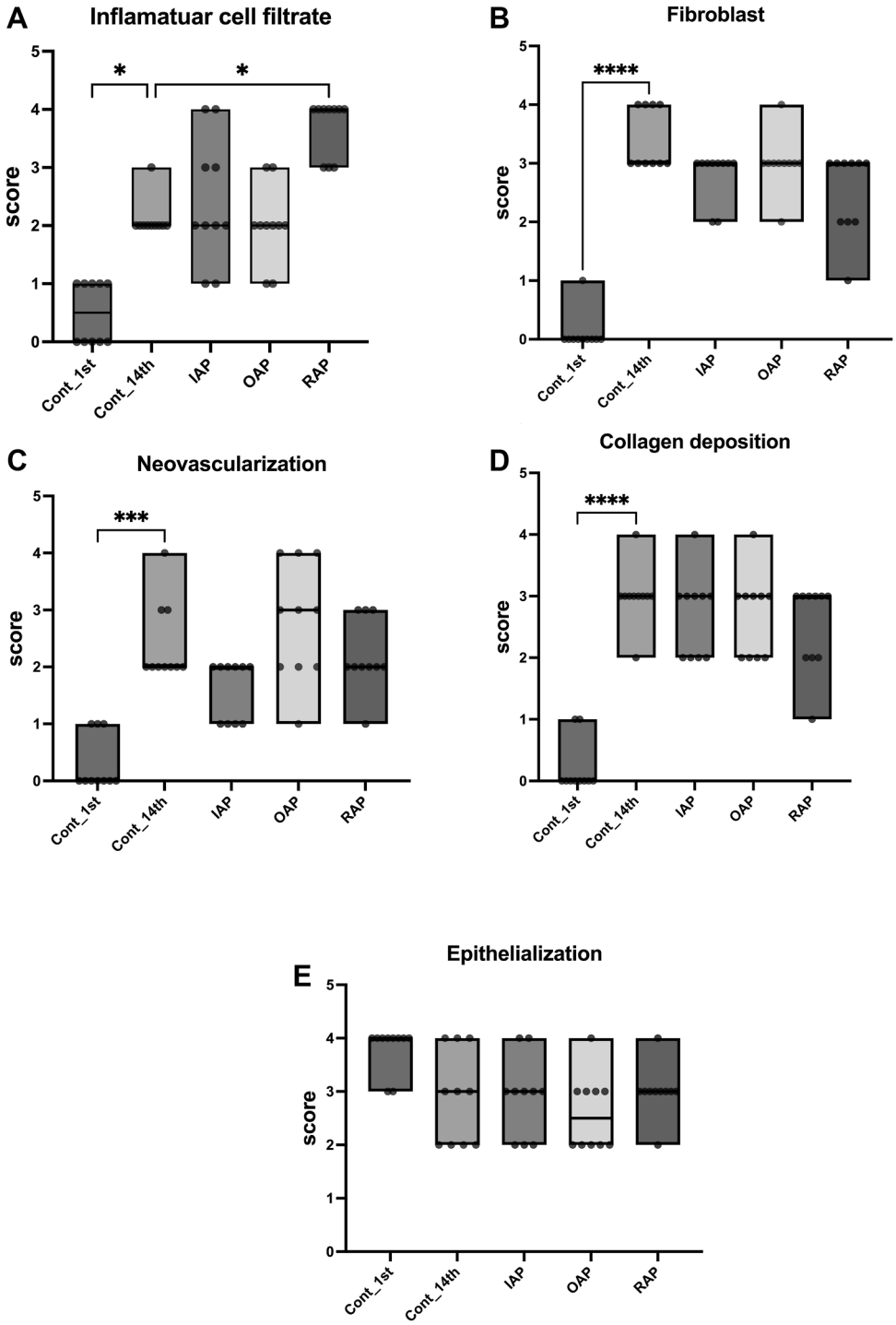


**Fig. 2 – Histological data of pirfenidon application on the colonic anastomoses Box-and-whisker plots (min to max) showing histological scores of (A) inflammatory cell infiltration, (B) fibroblast proliferation, (C) neovascularization, (D) collagen deposition, and (E) epithelialization in anastomotic tissue. Data are presented as median (Q1-Q3). Group comparisons were performed using the Kruskal–Wallis test followed by Dunn post hoc correction. $P < 0.05$ was considered significant. Cont_1st, control day 1; cont_14th, control day 14; IAP, intraperitoneal pirfenidone administration; OAP, oral pirfenidone administration; RAP, rectal pirfenidone administration. Exact adjusted P values for all pairwise comparisons are provided in Supplementary Table S1.**

Adhesion and anastomotic diameter measurements in the colonic anastomosis region are summarized in Table 2. On postoperative day 14, a significant reduction in adhesion scores was observed in all pirfenidone treatment groups compared with the control group. The IAP group showed the greatest decrease, with median adhesion scores reduced from

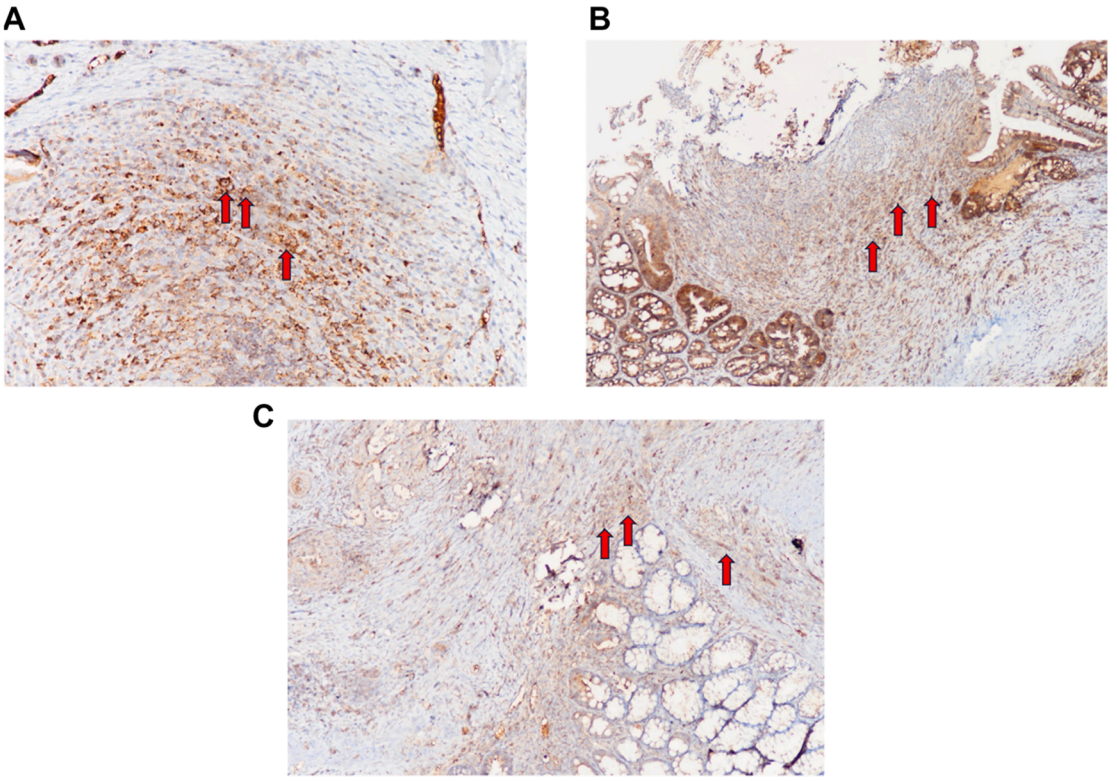


**Fig. 3 – Immunohistochemical expression of TGF-β, VEGF-A, and FGF-2 in the anastomotic tissue on postoperative day 14 following pirfenidone treatment. (A) TGF-β: increased intracytoplasmic stromal expression along the anastomosis line (arrows) (anti-TGF-β, × 200). (B) VEGF-A: diffuse stromal cytoplasmic staining in the anastomotic region (arrows) (anti-VEGF-A, × 100). (C) FGF-2: prominent cytoplasmic expression in stromal fibroblasts (arrows) (anti-FGF-2, ×100). FGF-2 = fibroblast growth factor-2; VEGF-A = vascular endothelial growth factor-A, TGF-β = transforming growth factor-β.**

3 (Q1-Q3: 3-3) in the control group to 0.5 (Q1-Q3: 0-1) ($P < 0.001$). The OAP group demonstrated a median adhesion score of 1 (Q1-Q3: 1-1), corresponding to a significant reduction compared with the control ($P < 0.001$). Similarly, the RAP group exhibited a median adhesion score of 1 (Q1-Q3: 1-1) ($P < 0.001$). These results indicate that pirfenidone reduced postoperative adhesions, with the magnitude of effect being intraperitoneal, oral, and rectal, respectively.

In the evaluation of anastomotic width, the control group on day 14 had a median diameter of 6 mm (Q1-Q3: 6-6). This measurement significantly increased in all pirfenidone-treated groups. The OAP group showed the largest increase, with a median width of 12 mm (Q1-Q3: 9-13) ($P < 0.001$). The IAP group reached a median width of 11 mm (Q1-Q3: 9-12) ($P < 0.001$), while the RAP group displayed a more moderate increase, reaching 8 mm (Q1-Q3: 7-9) ($P < 0.01$). These findings suggest that pirfenidone enhances anastomotic diameter, most prominently via the oral and intraperitoneal routes.

### *Molecular analyses*

On the 14th d, following the sacrifice of rats, RNA isolation and cDNA synthesis were performed on tissue samples taken from the colon to determine the effect of OAP, IAP, and RAP administration of pirfenidone on the mRNA levels of VEGF-A, TGF-β, and FGF-2 at the site of colon anastomosis (Fig. 4). Real-time analysis was conducted using specific primers to compare the expression of VEGF-A, TGF-β, and FGF-2 relative to the control tissue, with glyceraldehyde 3-phosphate dehydrogenase used as a normalizer. The results of this study demonstrated that TGF-β expression increased with IAP, OAP, and RAP administration, showing a 3-fold increase with RAP administration compared to the control. However, the expression of VEGF and FGF-2 was significantly decreased in all treatment groups.

## Discussion

Anastomotic stenosis is observed in up to 30% of colorectal surgeries and can cause morbidity and mortality. An important cause of anastomotic stenosis is anastomotic leakage.[2] Several methods and molecules have been used to prevent leakage. In experimental studies, the bursting pressure values were measured to determine the resistance and strength of the anastomosis line. In an experimental study conducted by Mathew *et al.*[18] on rats, it was shown that butyrate increased the mechanical strength of colonic anastomosis, and a significant increase in bursting pressure was detected with the system they made. In another study conducted on rabbits, a two-fold increase in bursting pressure was detected using leukocyte- and platelet-rich rich fibrin.[19]

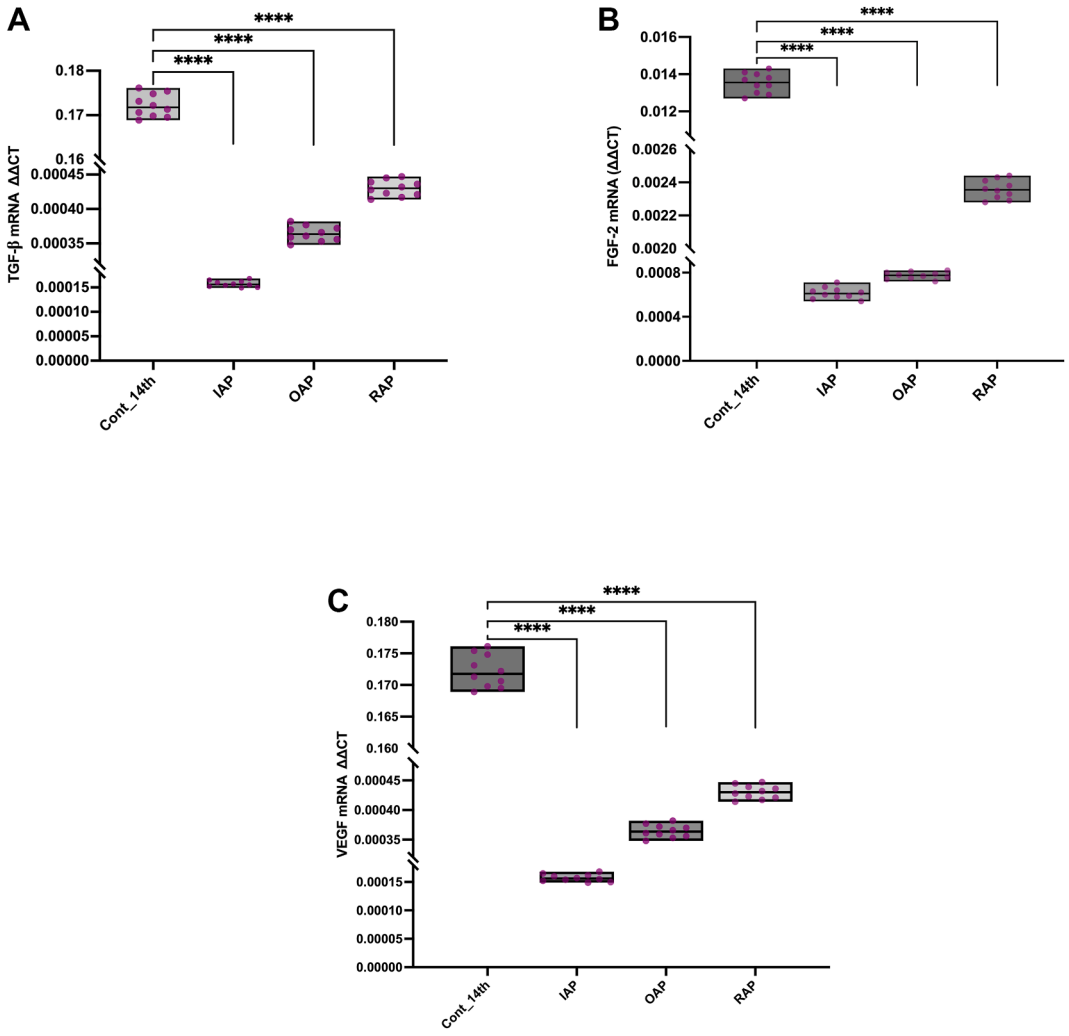


**Fig. 4 – Box-and-whisker plots (min to max) showing relative mRNA expression levels of TGF-β (A), FGF-2 (B), and VEGF-A (C) in anastomotic tissue on postoperative day 14. Gene expression was quantified using real-time PCR and normalized using the ΔΔCt method. Data are presented as mean ± SD. Group comparisons were performed using the Kruskal–Wallis test followed by Dunn post hoc correction. (*P < 0.05, **P < 0.01, ***P < 0.001). IAP, intraperitoneal pirfenidone administration; OAP, oral pirfenidone administration; RAP, rectal pirfenidone administration; PCR, polymerase chain reaction; SD, standard deviation; mRNA, messenger RNA.**

**Table 2 – Adhesion and anastomosis with data from the colonic anastomoses.**

| Parameters | Control 14th d | IAP 14th d | OAP 14th d | RAP 14th d |
|---|---|---|---|---|
| Adhesion | 3 (3-3) | 0,5 (0-1)* | 1 (1-1)* | 1 (1-1)* |
| Anastomosis width | 6 (6-6) | 11 (9-12)* | 12 (9-13)* | 8 (7-9)* |

Data are presented as median (Q1-Q3). Statistical comparisons were performed using the Kruskal–Wallis test followed by Dunn post hoc analysis.
IAP = intraperitoneal pirfenidone administration; OAP = oral pirfenidone administration; RAP = rectal pirfenidone administration.
**P < 0.01, **P < 0.001 *versus* control group on postoperative day 14.
*P < 0.05.

Pirfenidone was chosen after surgery for several reasons related to its antifibrotic and anti-inflammatory properties. Pirfenidone has shown efficacy in reducing fibrosis and inflammation in various tissues, including the lungs, liver, kidneys, and eyes.[8,9] Its ability to inhibit profibrotic cytokines, such as TGF-β, contributes to improved wound healing outcomes.[20] Studies have found that pirfenidone does not increase surgical complications or impair wound healing when continued until transplantation.[20,21] This suggests that it can be safely used in the postsurgical period without compromising healing. Therefore, we investigated the antifibrotic effects of pirfenidone on colorectal anastomotic stenosis, abdominal wall wound healing, and adhesion formation in rats, using bursting pressure analysis, evaluation of histological parameters, and immunohistochemical and molecular

analyses of specific gene expression. This study also aimed to compare the efficacy of different pirfenidone administration routes (intraperitoneal, oral, and rectal). There is no specific information regarding the effect of oral, rectal, or intraperitoneal administration of pirfenidone on anastomotic healing.

Pirfenidone has been studied using both oral and intraperitoneal routes of administration. Walker *et al.*[22] reported on oral pirfenidone for multiple sclerosis, showing significant improvements in neurological rating scales and reduced relapses compared with placebo. Hasdemir *et al.*[23] compared intraperitoneal and oral pirfenidone for preventing postoperative adhesions and found that intraperitoneal administration was more effective in reducing inflammation markers. In our study, pirfenidone was found to be two times higher after oral administration, while bursting pressure was found to be three times higher after intraperitoneal and rectal administration. The healing-enhancing effect of pirfenidone on colonic anastomosis is associated with its anti-inflammatory and antifibrotic properties. It regulates TGF-β-mediated fibroblast activity and collagen synthesis, thus providing balance in wound healing.[24] Differences between administration routes are related to bioavailability and tissue distribution. From a pharmacokinetic standpoint, pirfenidone exhibits an oral bioavailability of approximately 60%, allowing efficient entry into the systemic circulation and thereby ensuring a more uniform and sustained distribution across target tissues.[25] The compound has an elimination half-life of approximately 2-3 h, and its plasma concentrations can be effectively maintained through regular dosing schedules.[25] These pharmacokinetic characteristics are likely to underlie the more pronounced systemic and local therapeutic efficacy observed with oral administration of pirfenidone. The more even and continuous delivery of pirfenidone to the systemic circulation with oral administration may explain the achievement of the widest anastomotic diameter by homogeneously stimulating fibroblast activity and neoangiogenesis in the mucosal and submucosal layers.[26] This finding suggests that the oral bioavailability of pirfenidone may be more clinically advantageous.[7]

Inflammatory cell infiltration, fibroblastic activity, collagen deposition, and neoangiogenesis are crucial processes in the healing of colonic anastomoses. Studies have shown that these factors play a significant role in the strength and integrity of anastomosis. Infiltration of inflammatory cells is an early and essential step in the healing process. However, excessive inflammation may be detrimental. In a study using amniotic membranes to cover colonic anastomoses, researchers found significantly lower inflammatory cell infiltration scores in the treated groups than in the control groups.[27] Fibroblast activity and collagen deposition are critical for wound healing and anastomotic strength. The same study reported significantly higher fibroblast activity and collagen deposition in groups with amniotic membrane coverage.[27] Neoangiogenesis is vital for providing oxygen and nutrients to healing tissues. The study found significantly higher neoangiogenesis in the groups with amniotic membrane coverage.[27]

In our study, in terms of fibroblast activity, collagen deposition, and neoangiogenesis, a significant increase was observed between the 1st and 14th d. A significant increase was also observed in all treatment groups in terms of these parameters compared to day 1. However, no significant difference was found between control and treatment groups on day 14.

FGF-2, VEGF-A, and TGF-β play crucial roles in the healing process of colonic anastomoses. These growth factors are involved in various stages of wound healing, including inflammation, proliferation, and remodeling.[28] They contribute to cell proliferation, angiogenesis, and extracellular matrix production, which are all essential for successful anastomotic healing. FGF-2 and VEGF-A are particularly important for promoting angiogenesis and cell proliferation in healing anastomoses. FGF-2 has been shown to stimulates endothelial cell proliferation and increases the expression of other growth factors, including VEGF-A.[29,30] In turn, VEGF-A promotes angiogenesis and indirectly stimulates smooth muscle cell proliferation and migration by stimulating FGF-2 expression.[30] TGF-β, on the other hand, has a more complex role. While it can inhibit endothelial cell proliferation in some contexts,[31] it also promotes fibroblast differentiation and collagen production, which are crucial for wound strength.[28] In our study, TGF-β mRNA levels were increased by all three pirfenidone administration routes. The highest increase occurred in the RAP form of pirfenidone. The decrease in VEGF-A and FGF-2 mRNA levels indicated decreased vascularization. The decrease in gene expression in the anostoma line at 14 d after surgery indicated that the vascularization mechanism was complete.

Pirfenidone treatment was associated with decreased expression of TGF-β and matrix metalloproteinase-9 (MMP-9) in a mouse model of intestinal fibrosis.[32] However, this study showed that TGF-β mRNA and protein levels decreased on the sixth day of pirfenidone administration in a heterotopic transplantation model. Pirfenidone was orally administered. Hasdemir *et al.*[23] compared intraperitoneal and oral pirfenidone for preventing postoperative adhesions and found that intraperitoneal administration was more effective in reducing inflammation markers. In this study, intraperitoneal and oral administration of pirfenidone reduced tissue levels of inflammatory markers (TGF-β and Interleukin-17) in the parietal and visceral peritoneum compared to those in the control group.

Intra-abdominal adhesions occurred postoperatively. Pathological adhesions prevent proper functioning of the gastrointestinal system.[33] The effects of pirfenidone on MMP-9, tissue inhibitor of metalloproteinase-1, TNF-α, and TGF-β1 protein levels were studied in an experimental rat model. While MMP-9 levels were found to be high, decreases were found in other parameters, emphasizing its adhesion-reducing effect.[34] In another study, interleukin 17 and TGF-β levels in pirfenidone were investigated, and decreases were found in both parameters. The decrease in intra-abdominal adhesions was attributed to these two parameters.[23] In our study, when the control groups compared with the other groups, intra-abdominal adhesion decreased in all groups. In another study, they examined the effects of pirfenidone on an esophageal stricture model; collagen, TGF-β1, plasminogen activator inhibitor-1, MMP1 inhibitor tissue inhibitor, and MMP2 gene expression values were examined.[35]

We concluded that it prevented stricture formation by improving fibrosis in the chronic period by reducing profibrogenic gene expression. In a study conducted by Yang *et al.* in rabbits, the therapeutic effect of pirfenidone on benign bile duct strictures was investigated. In this study, rapamycin and pirfenidone groups were compared with the control group using physiological serum. Bile duct strictures were significantly prevented in both the study groups. Moreover, proliferation precursors, such as Proliferating Cell Nuclear Antigen, collagen, and TGF-β, were significantly reduced.[36] In our study, pirfenidone was administered intraperitoneally, orally, and rectally to prevent colonic anastomotic strictures. In all three study groups, the anastomosis was significantly wider than that of the control group. The group with the widest anastomosis was administered oral pirfenidone. This is thought to be because of its suppressive effect on fibroblast activity and collagen synthesis, similar to its effect on adhesion. Our results based on measurements are similar to those of studies conducted with pirfenidone. TGF-β levels were found to be high in our study, in contrast to the literature. However, when the study designs were examined, this was thought to be due to the much longer experimental end-of-life terms.

In this study, only female rats were included. This approach provided consistency with previous experimental models of colonic anastomosis and helped reduce variability that may arise from sex-related behavioral and hormonal fluctuations. However, it is acknowledged that wound healing may exhibit biological differences between males and females, particularly in processes such as inflammatory regulation, collagen remodeling, and angiogenesis. Therefore, the findings of the present study should be interpreted with this consideration in mind. Future research that includes both sexes will be valuable to determine whether the effects observed with pirfenidone are consistent across biological sex and to further understand any potential sex-specific aspects of colonic wound repair.

Based on our findings, pirfenidone demonstrated a significant antifibrotic effect in preventing colorectal anastomotic stenosis, reducing intra-abdominal adhesion, and promoting optimal wound healing. While previous studies have primarily focused on its effects in other fibrotic conditions, our research expands its potential applications in gastrointestinal surgery. The differences in TGF-β expression levels compared to the existing literature suggest that the timing and duration of pirfenidone administration play a crucial role in its mechanistic effects. Further studies are needed to determine the long-term impact of pirfenidone on anastomotic healing and to optimize its dosing regimen for maximal therapeutic benefits.

## Supplementary Materials

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jss.2025.11.050.

## Disclosure

The authors declare no competing financial interest.

## CRediT authorship contribution statement

**Ali Duran:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ferhat Çay:** Methodology, Investigation. **Nelin Hacıoğlu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis. **Esra Tokay:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis. **Feray Köçkar:** Writing – review & editing, Writing – original draft, Validation, Formal analysis, Conceptualization. **Eren Altun:** Methodology, Investigation, Formal analysis. **Azad Gazi Şahin:** Formal analysis. **Hüseyin Pülat:** Formal analysis. **Alev Çetin Duran:** Formal analysis.

## Acknowledgments

The study was financially supported by Balıkesir University Research Project (2020/065).

## REFERENCES

1. Biraima M, Adamina M, Jost R, Breitenstein S, Soll C. Long-term results of endoscopic balloon dilation for treatment of colorectal anastomotic stenosis. *Surg Endosc*. 2016;30:4432–4437.
2. Neutzling CB, Lustosa SA, Proenca IM, da Silva EM, Matos D. Stapled versus handsewn methods for colorectal anastomosis surgery. *Cochrane Database Syst Rev*. 2012CD003144.
3. Kraenzler A, Maggiori L, Pittet O, Alyami MS, Prost À la Denise J, Panis Y. Anastomotic stenosis after coloanal, colorectal and ileoanal anastomosis: what is the best management? *Color Dis*. 2017;19:O90–O96.
4. Garcea G, Ch B, Sutton CD, et al. Management of benign rectal strictures A review of present therapeutic procedures. *Dis Colon Rectum*. 2023;46:1451–1460.
5. Genser L, Manceau G, Karoui M, et al. Postoperative and long-term outcomes after redo surgery for failed colorectal or coloanal anastomosis: retrospective analysis of 50 patients and review of the literature. *Dis Colon Rectum*. 2013;56:747–755.
6. Kim ES, Keating GM. Pirfenidone: a review of its use in idiopathic pulmonary fibrosis. *Drugs*. 2015;75:219–230.
7. Azuma A. Pirfenidone treatment of idiopathic pulmonary fibrosis. *Ther Adv Respir Dis*. 2012;6:107–114.
8. Chan DD, Li J, Luo W, Predescu DN, Cole BJ, Plaas A. Pirfenidone reduces subchondral bone loss and fibrosis after murine knee cartilage injury. *J Orthop Res*. 2018;36:365–376.
9. Bisen AC, Agrawal S, Rayiti R, et al. Pirfenidone: a promising drug in ocular therapeutics. *Chem Biodivers*. 2024;21:e202301389.
10. Duran A, Çay F, Hacıoğlu N, et al. Investigation of the effects of post-operative intraperitoneal, oral, and rectal phenytoin administration on colorectal anastomosis in rats. *Ulus Travma Ve Acil Cerrahi Derg*. 2022;28:1214–1222.

11. Malekhosseini SA, Alizadeh B, Hosseinzadeh A, et al. Comparison of the preventive effect of colchicine versus diphenhydramine, prednisolone, and a combination therapy on intraperitoneal adhesion bands: an experimental study in rats. *BMC Surg*. 2023;23:79.
12. Tokay E, Kockar F. Identification of intracellular pathways through which TGF-β1 upregulates URG-4/URGCP gene expression in hepatoma cells. *Life Sci*. 2016;144:121–128.
13. Hacıoğlu N, Güngör T, Tokay E, Önder FC, Ay M, Köçkar F. Synthesis and biological evaluation of 2,4,6-trinitroaniline derivatives as potent antitumor agents. *Monatsh Chem*. 2020;151:1629–1641.
14. Tokay E. Epidermal growth factor mediates Up-Regulation of URGCP oncogene in human hepatoma cancer cells. *Mol Biol*. 2021;55:618–623.
15. Yildirim H, Köçkar F. TGF-β upregulates tumor-associated carbonic anhydrase IX gene expression in Hep3B cells. *Cell Biol Int*. 2009;33:1002–1007.
16. Çay F, Duran A, Tokay E, et al. Is phenytoin a safe agent for staple line recovery after gastric sleeve surgery in rats? *Ulus Travma Ve Acil Cerrahi Derg*. 2023;29:1321–1328.
17. Türkmen E, Pata YS. Prevention of tracheal stenosis with pirfenidone after tracheotomy: an experimental study. *Laryngoscope*. 2019;129:e178–e186.
18. Mathew AJ, Wann VC, Abraham DT, et al. The effect of butyrate on the healing of colonic anastomoses in rats. *J Investig Surg*. 2010;23:101–104.
19. Albahrawy M, Abass M, Mosbah E, Karrouf G, Awadin W, Zaghloul A. Reinforcement of colon anastomosis healing with leukocyte platelet-rich fibrin in rabbit model. *Life Sci*. 2023;333:122146.
20. Mortensen A, Cherrier L, Walia R. Effect of pirfenidone on wound healing in lung transplant patients. *Multidiscip Respir Med*. 2018;13:1–5.
21. Leuschner G, Behr J. Acute exacerbation in interstitial lung disease. *Front Med*. 2017;4:1–13.
22. Walker JE, Giri SN,MS, Margolin SB. A double-blind, randomized, controlled study of oral pirfenidone for treatment of secondary progressive multiple sclerosis. *Mult Scler J*. 2005;11:149–158.
23. Hasdemir PS, Ozkut M, Guvenal T, et al. Effect of pirfenidone on vascular proliferation, inflammation and fibrosis in an abdominal adhesion rat model. *J Investig Surg*. 2017;30:26–32.
24. Conte E, Gili E, Fagone E, Fruciano M, Iemmolo M, Vancheri C. Effect of pirfenidone on proliferation, TGF-β-induced myofibroblast differentiation and fibrogenic activity of primary human lung fibroblasts. *Eur J Pharm Sci*. 2014;58:13–19.
25. Cho ME, Kopp JB. Pirfenidone: an anti-fibrotic therapy for progressive kidney disease. *Expert Opin Invest Drugs*. 2010;19:275–283.
26. Betancourt-Vicencio S, Prieto-Aldape MR, Alvarez-Villaseñor AS, et al. Effects of oral pirfenidone on Colon Anastomosis healing and adhesion Formation in rats. *Eur Surg Res*. 2022;63:241–248.
27. Uludag M, Citgez B, Ozkaya O, et al. Effects of amniotic membrane on the healing of primary colonic anastomoses in the cecal ligation and puncture model of secondary peritonitis in rats. *Int J Colorectal Dis*. 2009;24:559–567.
28. Morgan RB, Shogan BD. The science of anastomotic healing. *Semin Colon Rectal Surg*. 2022;33:100879.
29. Li Y, El Mozen LA, Cai H, et al. Transforming growth factor beta 3 involved in the pathogenesis of synovial chondromatosis of temporomandibular joint. *Sci Rep*. 2015;5:8843.
30. Li D, Zhang C, Song F, Lubenec I, Tian Y, Song QH. VEGF regulates FGF-2 and TGF-β1 expression in injury endothelial cells and mediates smooth muscle cells proliferation and migration. *Microvasc Res*. 2009;77:134–142.
31. Fràter-Schröder M, Müller G, Birchmeier W, Böhlen P. Transforming growth factor-beta inhibits endothelial cell proliferation. *Biochem Biophys Res Commun*. 1986;137:295–302.
32. Meier R, Lutz C, Cosín-Roger J, et al. Decreased fibrogenesis after treatment with pirfenidone in a newly developed mouse model of intestinal fibrosis. *Inflamm Bowel Dis*. 2016;22:569–582.
33. Arung W, Meurisse M, Detry O. Pathophysiology and prevention of postoperative peritoneal adhesions. *World J Gastroenterol*. 2011;17:4545–4553.
34. Bayhan Z, Zeren S, Kocak FE, et al. Antiadhesive and anti-inflammatory effects of pirfenidone in postoperative intra-abdominal adhesion in an experimental rat model. *J Surg Res*. 2016;201:348–355.
35. Orozco-Perez J, Aguirre-Jauregui O, Salazar-Montes AM, Sobrevilla-Navarro AA, Lucano-Landeros MS, Armendáriz-Borunda J. Pirfenidone prevents rat esophageal stricture formation. *J Surg Res*. 2015;194:558–564.
36. Yang Q, Wang J, Liu F, et al. A novel rabbit model for benign biliary stricture Formation and the effects of medication infusions on stricture Formation. *Dig Dis Sci*. 2018;63:2653–2661.