The Effect of Pirfenidone on Peyronie Plaques and Erectile Function in a Peyronie's Rat Model

Gokhan Cevik[1], Atinc Tozsin[1], Ebru Tastekin[2], Murat Dursun[3], Tevfik Aktoz[1] and Ates Kadioglu[3]

[1]*Department of Urology, Trakya University, Edirne, Turkey*
[2]*Department of Pathology, Trakya University, Edirne, Turkey*
[3]*Department of Urology, School of Medicine, Istanbul University, Istanbul, Turkey*

ABSTRACT

Objective: To investigate the anti-fibrotic effects of pirfenidone on Peyronie's disease in an experimental rat model with intracavernosal injection of TGF-β and whether pirfenidone improves erectile function.

Study Design: Experimental study.

Place and Duration of the Study: Faculty of Medical Experimental Animals and Research Laboratory, Trakya University, from January to March 2021.

Methodology: In this study, 27 male Sprague Dawley rats were used, and three groups were randomly identified. The rats in Group 1 served as the control group. Group 2 was not treated, and Group 3 was treated with pirfenidone therapy. The rats in Group 3 were administered pirfenidone 30 mg/kg/day by oral gavage, every day for four weeks, three weeks after the start of the experiment. At the end of seven weeks, a haemodynamic study was performed with cavernosal nerve stimulation to evaluate the erectile function, the rats were sacrificed, and the penile tissues were evaluated immunohistochemically.

Results: MeICP/MIBP values were found to be higher in treated rats compared to rats in the untreated group but no statistically significant difference was found in MeICP/MIBP values between the control, Peyronie model, and treatment groups (p=0.25). According to the histopathological examination, the rate of fibrosis with H&E staining was mild (100%) in the control group, severe (100%) in the Peyronie group, and severe (87.5% severe and 12.5% moderate) in the Peyronie + treatment group.

Conclusion: In the study, pirfenidone used in the treatment of Peyronie's disease had a positive effect on erectile function, though not considered statistically significant. It has been shown that it has no histopathological effect on Peyronie's plaques.

Key Words: *Anti-fibrotic agent, Erectile function, Experimental study, Peyronie's disease, Pirfenidone.*

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INTRODUCTION

Peyronie's disease (PD) is a chronic condition of the penis that causes penile curvature, erectile dysfunction, and penile fibrosis resulting in pain. This condition includes two phases: the acute phase with painful erections, palpable plaques, and erectile dysfunction, and the chronic phase, in which the disease and penile deformity stabilise.[1]

Penile microtrauma leads to subtunical venous tears, triggering the release of various factors such as clotting factors, serotonin, platelet-derived growth factor (PDGF-A and PDGF-B), transforming growth factor-β1 (TGF-β1), and fibrinogen. TGF-β1, which affects fibroblast function, induces differentiation of fibroblasts into myofibroblasts. Continuous production of myofibroblasts causes collagen production and accumulation, causing tissue contraction, fibrosis, and plaque formation.[2]

Correspondence to: Dr. Gokhan Cevik, Department of Urology, Trakya University, Edirne, Turkey
E-mail: gokhancevik59@hotmail.com

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Conservative treatment is the first-line treatment for Peyronie's acute phase, and a variety of medications are available for this purpose. The primary goal of medical treatment is to prevent the progression of inflammation in the acute phase, improve deformity, and reduce pain. Randomised clinical trials have shown limited efficacy in preventing disease progression and reducing deformities.[3]

Pirfenidone (PFD), an agent with anti-fibrotic and anti-oxidant properties, is commonly used to treat idiopathic pulmonary fibrosis. Experimental studies have shown that pirfenidone can prevent fibrosis by reducing the formation of tumour necrosis factor-α (TNF-α), TGF-β, and PDGF. There are few studies that investigated the anti-fibrotic and anti-oxidant effects of pirfenidone on various organs such as the bladder, skin, kidney, and lungs.[4-7] The aim of this study was to investigate the anti-fibrotic effects of pirfenidone on Peyronie's disease in an experimental rat model with intra-cavernosal injection of TGF-β and whether pirfenidone improves erectile function or not.

METHODOLOGY

This experimental study was conducted at Faculty of Medical Sciences Experimental Animals and Research Laboratory,

Trakya University, from January to March 2021. Twenty-seven Sprague Dawley male rats (3 months, 280-320 g weighted) were included in this randomised, blind trial. Rats aged less than 3 months, older than 4 months, less than 280 grams, more than 320 grams, and female rats were excluded from the study. Three groups were identified (n = 9). The rats were kept in rooms with a 12/12 hour light-dark period, ventilated 15 times per hour, a relative humidity of approximately 50±3%, and an average temperature of 22±1°C. The Clinical Research Institute Committee at Trakya University approved the protocol of this study (Approval number: TUHDYEK-2020.05).

Before injection, 50 mg/kg ketamine hydrochloride and 7.5 mg/kg xylazine were administered intraperitoneally for anaesthesia.[8]

In Group 1 (control/sham), 0.2 mL saline (NaCl 0.9%) was injected into the tunica albuginea and followed up for seven weeks. In Group 2 (Peyronie's model + no treatment), 0.1 mL TGF-β (TGF-β, Thermo-Fisher, cat. No. PHG9214) + 0.1mL disodium phosphate dihydrate 3% (Aethoxysklerol®) was injected and followed up for seven weeks. In Group 3 (Peyronie's model + Pirfenidone), 0.1 mL of TGF-β + disodium phosphate dihydrate 3% was injected, and after three weeks, pirfenidone (Esbriet®, 267 mg capsule, Roche) was administered to the rats by oral gavage at a dose of 30 mg/kg/day once a day for four weeks (Figure 1).[8] During the follow-up, one rat was lost due to aspiration because of oral drug administration, and the remaining eight rats completed the study. No disease was observed in any rats during the experiment.

At the end of the seven weeks, anaesthesia was given and continued with an intraperitoneal injection of pentobarbital sodium. Before the abdominal incision, the carotid artery of the rats was dissected and a 26-gauge cannula was inserted (Figure 1) to assess arterial blood pressure (BIOPAC MP 35 System, Santa Barbara, CA, USA).

Heparin solution (250 ml) was used to fill a 26 gauge cannula to monitor the intracavernous pressure (ICP) and was placed into the left corpus cavernosum. ICP, BICP (basal intracavernosal pressure), MICP (maximum intracavernosal pressure), PICP (peak intracavernosal pressure (calculated by MICP–BICP)), MeICP (mean intracavernosal pressure), MIBP (mean intracavernosal pressure), MICP/MIBP parameters were measured.

At seventh week, an abdominal midline incision was made to evaluate the erectile function of rats after the injection of TGF-β + disodium phosphate dehydrate 3%. Pelvic ganglions and bilateral cavernous nerves were revealed after bladder and prostate dissection. The penile shaft was exposed by peeling off the skin of the penis. Stimulation was applied with a 15 ms pulse width, 20 Hz frequency, and 7.5 V voltage parameters (BIOPAC Systems, Inc., BSLSTM) for 1 minute through the cavernous nerves. In the protocol, a current of 10 mA was applied for a consistent erectile response. Electrical stimulation was repeated thrice on both sides of each rat. The maximum ICP amplitude was calculated from the baseline values during nerve electrostimulation and included in the statistical analysis. All rats were sacrificed, and the penile tissues were dissected from the most proximal part and collected for immunohistochemical analysis. The tissue samples were obtained by a pathologist after fixation with 10% neutral formaldehyde. Each sample was used for tissue tracking with a single cassette. Tissues were dehydrated, cleared, and paraffinised. The tissues were prepared on 5-micron slides from 23 paraffin blocks and stained with Hematoxylin & Eosin (H&E), Masson Trichrome, Picro-Sirius red. Erectile dysfunction assessment was evaluated according to the ratio of mean intracavernosal pressure (MeICP) to mean arterial pressure (MIBP).[9] All the examined sections were photographed.

SPSS (v19.0) package programme was used for the statistical analysis. Variables were defined individually on the variable page. Categorical variables were expressed as counts and percentages, and continuous variables were expressed as mean and standard deviation (SD). The single-sample Kolmogorov–Smirnov test was used to evaluate the conformity of the data to the normal distribution. Because the data did not show a normal distribution, the results were given as medians (minimum-maximum). The Kruskal-Wallis test was used to compare the BICP, MICP, PICP, meICP, MIBP, MeICP/MIBP, and MICP/MIBP values between the groups and Collagen/SM, Collagen III/Collagen I ratio. When a significant difference was detected, Dunn's test with Bonferroni correction was used to determine the differences between the groups. The Chi-square test was used for the comparison of fibrosis scores by H&E staining. The p-value less than 0.05 was considered significant.

RESULTS

Among haemodynamic parameters, an increase in ICP was observed following electrical stimulation of the cavernosal nerve. The haemodynamic values were consistent with each other. The MICP/BICP ratios were similar between the Peyronie and treatment groups. The systemic pressure values measured at this time were close to each other in all three groups. A significant difference was found between the control group and the Peyronie + treatment group (p=0.003), but there was no statistically significant difference between the Peyronie group and the treatment group (p=0.946). Basal intracavernosal pressures were similar in the Peyronie and treatment groups (p=0.604) but lower in the control group (Table I). There was a statistically significant difference in all parameters between the Peyronie group and treatment groups when compared with the control group. MeICP/MIBP values were found to be higher in treated rats compared to rats in the untreated group but no statistically significant difference was found in MeICP/MIBP values between the control, Peyronie model, and treatment groups (p=0.25). Although the MICP and PICP values in the treatment group were higher than those in the Peyronie model group, no statistically significant difference was found in the MICP values (Table I).

Table I: Intracavernosal pressure levels; Median (minimum-maximum).

	Control	Peyronie	Treatment	p-value*	p-values for multiple comparisons		
					Control-treatment	Control-Peyronie	Peyronie-treatment
BICP	6 (1-12.5)	17 (7-60)	17 (3-21)	0.003	0.004	0.027	>0.99
MICP	15.3 (6-25)	20 (9-66)	29 (16-32)	0.012	0.038	0.024	>0.99
PICP	7.5 (2.9-7.5)	5 (2-10)	12 (3-17)	0.016	0.24	0.73	0.013
MeICP	8 (1-15)	17.5 (8-58)	21.25 (8.5-24)	0.012	0.005	0.009	>0.99
MeICP/MIBP	0.14 (0.12-0.5)	0.3 (0.12-0.9)	0.29 (0.13-0.1)	0.25	0.001	0.001	>0.99

**The Kruskal Wallis test was used for comparison. When a significant difference was detected, Dunn's test with Bonferroni correction was used to determine the group which showed the difference. ICP: Intracavernosal Pressure, BICP: Basal intracavernosal pressure, MICP: Maximum intracavernosal Pressure, PICP: Peak intracavernosal pressure (calculated by MICP–BICP), MeICP: Mean intracavernosal pressure, MIBP: Mean intra-arterial pressure.*

Table II: Fibrosis scores by H&E staining; Median (±SD).

	Control	Peyronie	Treatment	p-value*
H&E (fibrosis)				
Mild (+)	9	0	0	<0.001
Moderate (++)	0	0	1	<0.001
Severe (+++)	0	9	7	<0.001
Collagen/SM, median	1.2 (±0.1)	3.5 (±0.2)	3.5 (±0.2)	<0.0001**
Collagen III/Collagen I, median	1.0 (±0.1)	3.0 (±0.1)	2.9 (±0.2)	<0.0001**

**Chi-square test was used for comparison. **Kruskal-Wallis test was used for comparison.*

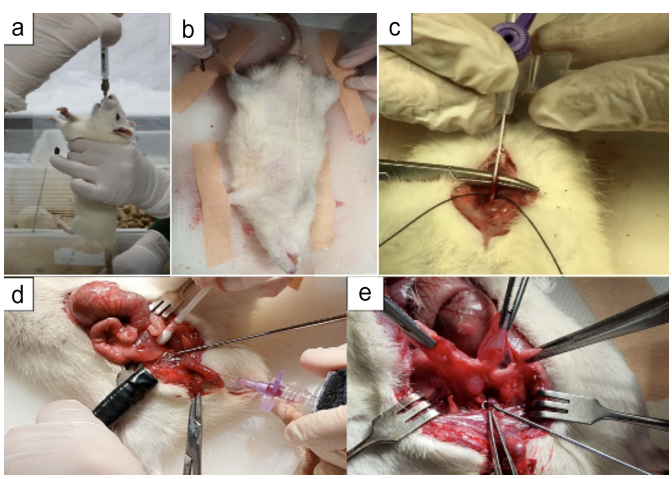

Figure 1: (a) Oral gavage administration of pirfenidone; (b) Sedated and positioned preprocedural rats; (c) Catheterisation of the carotid artery; (d) Electrical stimulation was provided by suspending the cavernosal nerve; (e) Dissection of the cavernosal nerve after laparotomy.

No alterations were observed in the tunica albuginea or corpus cavernosum in the control group, except for signs of injection-induced trauma. Haemorrhagic areas were observed in the cavernous body, and the tunica albuginea was normal. The microscopic findings were consistent with the normal tunica albuginea and corpus cavernosum histology in the control group (Figure 2).

When the penile tissues of the rats in Group 2 were examined, a severe thickness increase and fibrosis were observed in the corpus cavernosum and tunica albuginea. Mild haemorrhagic areas and oedema were observed in the corpus cavernosum.

In Group 3, the thickness of the tunica albuginea and fibrosis were observed in the penile tissues of rats. Although the fibrotic areas in this group were smaller than those in Group 2, the degree of fibrosis was similar (Figure 2).

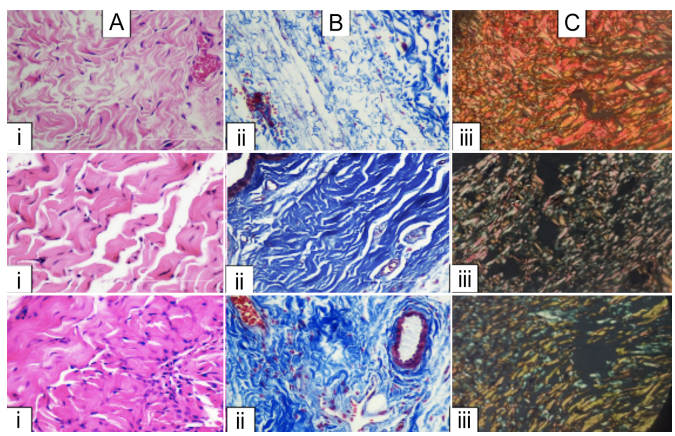

Figure 2: A (i) Changes in the control group by H&E staining; A (ii) Changes in the Peyronie group by H&E staining; A (iii) Changes in Peyronie + PFD group by H&E staining; B (i) Evaluation of collagen/smooth muscle ratios in the control group by Masson's trichrome staining (x400); B (ii) Evaluation of collagen/smooth muscle ratios in the Peyronie group by Masson trichrome staining (x400); B (iii) Evaluation of collagen/smooth muscle ratios in the Peyronie +PFD by Masson trichrome staining (x400); C (i) Evaluation of the Type III/Type I collagen ratio in the control group under polarised light (x400) with Picro-Sirius red colour; C (ii) Evaluation of the Type III/Type I collagen ratio in the Peyronie group under polarised light (x400) with Picro-Sirius red colour; C (iii) Evaluation of the Type III/Type I collagen ratio in the Peyronie + PFD group under polarised light (x400) with Picro-sirius red colour.

According to the histopathological examination, the rate of fibrosis with H&E staining was mild (100%) in the control group, severe (100%) in the Peyronie group, and severe (87.5% severe and 12.5% moderate) in the Peyronie + treatment group (Table II). The fibrosis intensity was higher in the Peyronie and treatment groups than in the control group. No significant differences were found between Peyronie and the treatment groups. After Masson's trichrome staining of collagen/smooth muscles, red staining was observed in smooth muscle fibers, and blue staining was observed in collagen fibers. The collagen/smooth muscle ratio was 1.2 ± 0.1 in the control group, 3.5 ± 0.2 in the Peyronie group, and 3.5 ±

0.2 in the treatment group (Table II). When the Peyronie group and all treatment groups were compared, no significant differences were found between the groups.

DISCUSSION

TGF-β1 was used for the Peyronie's disease model. In this study, histopathological results similar to those of Peyronie's were obtained by immunohistochemical examination of rats injected with TGF-β1. An increase in Collagen Type-1 and Type-3 levels was detected in the cavernosal tissue. Sodium tetradecyl sulfate 3% is a sclerosing agent, and it has been shown to accelerate the process for two weeks when used together with TGF-β1 in the formation of Peyronie's plaque.[10] Chung *et al.* used sodium tetradecyl sulfate in 12 Sprague-Dawley rats to create a Peyronie animal model. They showed that tissue plaque formation occurred as early as two weeks after combining TGF-β1 with a sclerosing agent such as sodium tetradecyl sulfate 3%.[11] This study also demonstrated tissue plaque formation in an earlier period by using a sclerosing agent, disodium phosphate dihydrate 3%.

Pirfenidone is a powerful anti-fibrotic agent.[12] It shows anti-fibrotic properties by blocking TGF-β expression and activity. Thus, it inhibits Type I and IV collagen expression induced by TGF-β and reduces ROS production in mesangial cells. Positive effects of pirfenidone have been reported in studies conducted in patients with idiopathic pulmonary fibrosis.[5] Pirfenidone treatment has been shown to significantly reduce rat kidney mesangial matrix expansion and the expression of renal matrix genes in rats with diabetic nephropathy. Pirfenidone treatment may be a new method for the treatment of fibrotic diseases, such as Peyronie's.[13] Various studies have investigated the anti-fibrotic effects of pirfenidone on fibroblast proliferation and activation.[6,14-17] In Europe and Japan, pirfenidone is administered to patients with idiopathic pulmonary fibrosis (IPF).[5,12] Studies on Crohn's disease have shown that it inhibits the secretory activity and proliferation of fibroblasts, and it is assumed that it can also be used as an anti-fibrotic agent in patients with intestinal fibrosis.[7,18] Pirfenidone can be orally administered for therapeutic purposes. It has been shown to slow down and prevent fibrosis, and these changes in penile tissue are consistent with findings in different animal models.[6,9,14-16] Medical treatment is preferred during the acute phase of Peyronie. Currently, the use of many medical agents has been described. The first study on colchicine, one of the most preferred agents, was published by El-Sakka *et al.* Colchicine partially prevented fibrosis in the tunica albuginea and reduced TGF-β expression.[19] In a study by Kadioglu *et al.*, it was reported that with oral colchicine treatment administered in the acute period, the deformity improved in 30% of patients, the deformation remained stable in 48% of patients, and the pain disappeared in 95% of patients.[20] Medical treatment options are preferred in the acute phase of Peyronie's disease. While determining the oral treatment to be chosen, preference should be made according to patient compliance.[21] There are few evidence-based studies to support their efficacy, but none have demonstrated definitive clinical benefit. The efficacy of various pharmacological agents in the treatment of Peyronie's disease has also been evaluated.[22] PRP is another treatment option currently used in the treatment of Peyronie's disease. However, more preclinical and clinical studies are required for the standard protocol.[23] Culha *et al.* investigated the effectiveness of platelet-rich plasma (PRP) treatment after applying TGF-β1 to the penis of rats in a Peyronie animal model. The degree of fibrosis (Haematoxylin and Eosin staining), collagen/smooth muscle ratio (Masson Trichrome staining), and Type III/Type I collagen ratio (Picro-Sirius red staining) of the tissues were evaluated histologically. In this study, PRP had no therapeutic effect in rats in which Peyronie's disease was induced.[8]

The effect of sildenafil on the penis was evaluated using a rat model of cavernous nerve damage. The mean arterial pressure (MAP) and intracavernosal pressure (ICP) were measured to evaluate erectile function (EF) in response to cavernosal nerve stimulation. In addition, the maximum ICP/MAP ratio was calculated as the highest recorded ICP during stimulation divided by the corresponding MAP and expressed as a percentage.[13] Similar to this study, electrical stimulation of the cavernosal nerve and cavernosal pressure values in rats in all experimental groups were recorded and compared the pirfenidone group with the other groups. In another study by Cinar *et al.*, fibrosis evaluation and haemodynamic studies were performed after pirfenidone treatment in rats in an ischemic priapism (IP) model.[9] MeICP/MIBP values were found to be higher in treated rats compared to rats in the untreated group. This was interpreted as an improvement. However, it was not statistically significant. The pirfenidone dose, the doses used in the idiopathic pulmonary fibrosis and severe pulmonary hypertension models, were taken as examples. On the basis of these studies, the pirfenidone dose was calculated. Cinar *et al.* showed that pirfenidone administered *via* oral gavage once a day caused a decrease in cavernosal fibrosis. The average amount of collagen regressed at the end of the 6-week treatment period. In addition, statistically significant improvements were observed in ICP measurements in the pirfenidone-treated group compared to the other groups. Regarding cavernosal histology and ICP variants, the findings showed that pirfenidone effectively inhibited fibrosis and improved erectile function in the current animal model.[9] This study showed that erectile function was better in the group treated with PFD than in the group treated with only Peyronie's disease, but there was no statistically significant difference.

To the best of the authors' knowledge, this is the first study to investigate the effect of pirfenidone on Peyronie's disease. This study investigated whether pirfenidone is effec-

tive against penile plaques in an animal model. Histopathological findings in the Peyronie and control groups were consistent with previous studies on this subject.[13] In this study, no significant improvement was observed in penile sections, tunica albuginea, and corpus cavernosum of rats treated with pirfenidone due to the immunohistochemical examination. This is because orally administered pirfenidone therapy may not have reached the therapeutic dose in the area of inflammation in the penile tunica albuginea. In this study, pirfenidone was administered 3 weeks after the TGF-B1 injection. The treatment period may have begun later. The effect of pirfenidone may be better in the earlier stages of Peyronie's disease if administered earlier.

CONCLUSION

Although this study showed that pirfenidone used in the treatment of Peyronie's disease had a positive effect on erectile function, this was not found statistically significant. It has been shown that it has no histopathological effect on Peyronie's plaques and that more comprehensive information can be provided about the effectiveness of this drug by applying it in different animal models and at different doses.

ETHICAL APPROVAL:
The Clinical Research Institute Committee at Trakya University approved the protocol of this study and the ethical committee approval number is TUHDYEK-2020.05.

COMPETING INTEREST:
The authors declared no competing interest.

AUTHORS' CONTRIBUTION:
GC: Drafting the manuscript, acquisition, analysis, and interpretation of data.
AT: Supervision, review, writing, and editing.
ET: Result analysis.
MD: Critical revision of content.
MO: Data curation, supervision, review, writing, and editing.
TA: Formal Analysis, investigation, and resources.
AK: Drafting, conception, designing the study content, review, writing, and editing.
All authors approved the final version of the manuscript to be published.

REFERENCES

1. Brock G, Hsu GL, Nunes L, von Heyden B, Lue TF. Anatomy of the tunica albuginea in normal penis and Peyronie's disease. *J Urol* 1997; **157(1)**:276-81.
2. El-Sakka AI, Hassan MU, Nunes L, Bhatnagar RS, Yen TS, Lue TF. Histological and ultrastructural alterations in an animal model of Peyronie's. *Br J Urol* 1998; **81(3)**:445-52. doi: 10.1046/j.1464-410x.1998.00529.x.
3. Gonzalez-Cadavid NF. Mechanisms of penile fibrosis. *J Sex Med* 2009; **6(Suppl 3)**:353-62. doi: 10.1111/j.1743-6109.2008.01195.x.
4. Gur S, Limin M, Hellstrom WJ. Current status and new developments in Peyronie's: Medical, minimally invasive, and surgical treatment options. *Expert Opin Pharmacother* 2011; **12(6)**:931-44. doi: 10.1517/14656566.2011.544252.
5. Takeda Y, Tsujino K, Kijima T, Kumanogoh A. Efficacy and safety of pirfenidone for idiopathic pulmonary fibrosis. *Patient Preference Adherence* 2014; **8**:361-70. doi: 10.2147/PPA.S37233.
6. Shi Q, Liu X, Bai Y, Cui C, Li J, Li Y, *et al*. *In vitro* effects of pirfenidone on cardiac fibroblasts: proliferation, myofibroblast differentiation, migration, and cytokine secretion. *PLoS One* 2011; **6(11)**:e28134. doi: 10.1371/journal.pone.0028134.
7. Meier R, Lutz C, Cosín-Roger J, Fagagnini S, Bollmann G, Hünerwadel A, *et al*. Decreased fibrogenesis after pirfenidone treatment in a newly developed mouse model of intestinal fibrosis. *Inflamm Bowel Dis* 2016; **22(3)**:569-82. doi: 10.1097/MIB.0000000000000716.
8. Culha MG, Erkan E, Cay T, Yucetas U. Effect of platelet-rich plasma on PD in rat model. *Urol Int* 2019; **102(2)**:218-23. doi: 10.1159/000492755.
9. Cinar O, Bolat MS, Erdem S, Kaymaz E, Demirkiran ED, Girgin R, *et al.* Effect of the antifibrotic agent pirfenidone on penile erectile function in an experimental rat model of ischemic priapism. *Int J Impot Res* 2020; **32(2)**:232-8. doi: 10.1038/s41443-019-0152-9.
10. Chung E, De Young L, Brock GB. Rat as an animal model for Peyronie's disease research: A review of current methods and peer-reviewed literature. *Int J Impot Res* 2011; **23(6)**:235-41. doi: 10.1038/ijir.2011.36.
11. Chung E, Garcia F, Young LD, Solomon M, Brock GB. A comparative study of the efficacy of intralesional verapamil *versus* normal saline injection in a novel animal model of PD: Assessment of immunohistopathological changes and erectile function outcome. *J Urol* 2013; **189(1)**:380-4. doi: 10.1016/j.juro.2012.08.191.
12. Meyer KC, Decker CA. Role of pirfenidone in the management of pulmonary fibrosis. *Ther Clin Risk Manag* 2017; 13:427-37. doi: 10.2147/TCRM.S81141.
13. Tavukçu HH, Akbal C, Tinay I, Simşek F, Türkeri L. Outcome of preemptive penile rehabilitation before bilateral cavernosal nerve injury in rats. *World J Urol* 2010; **28(6)**:735-40. doi: 10.1007/s00345-009-0495-9.
14. Duan LJ, Qi J, Huang T, Gu X, Xu D, Kong XJ, *et al*. Pirfenidone attenuated bladder fibrosis and mitigated deterioration of bladder function in a rat model of partial bladder outlet obstruction. *Mol Med Rep* 2015; **12(3)**:3639-47. doi: 10.3892/mmr.2015.3814.
15. Azuma A, Nukiwa T, Tsuboi E, Suga M, Abe S, Nakata K, *et al*. Double-blind placebo-controlled trial of pirfenidone in patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2005; **171(9)**:1040-7. doi: 10.1164/rccm.200404-571OC.
16. Hewitson TD, Kelynack KJ, Tait MG, Martic M, Jones CL, Margolin SB, *et al*. Pirfenidone reduced *in vitro* rat renal fibroblast activation and mitogenesis. *J Nephrol* 2001; **14 (6):**453-60.

17. Lin X, Yu M, Wu K, Yuan H, Zhong H. Effects of pirfenidone on the proliferation, migration, and collagen contraction of human Tenon fibroblasts *in vitro*. *Invest Ophthalmol Vis Sci* 2009; **50(8)**:3763-70. doi: 10.1167/iovs.08-2815.

18. Kadir SI, Wenzel Kragstrup T, Dige A, Kok Jensen S, Dahlerup JF, Kelsen J. Pirfenidone inhibits fibroblast proliferation in patients with active Crohn's disease. *Scand J Gastroenterol* 2016; **51(11)**:1321-5. doi: 10.1080/00365521.2016.1185146.

19. El-Sakka AI, Bakircioglu ME, Bhatnagar RS, Yen TS, Dahiya R, Lue TF. Effects of colchicine on a Peyronie-like condition in an animal model. *J Urol* 1999; **161(6)**:1980-3.

20. Kadioglu A, Tefekli A, Köksal T, Usta M, Erol H. Treatment of Peyronie's with oral colchicine: Long-term results and predictive parameters of successful outcome. *Int J Impot Res* 2000; **12(3)**:169-75. doi: 10.1038/sj.ijir.3900519.

21. Chung PH, Han TM, Rudnik B, Das AK. Peyronie's disease: what do we know and how do we treat it*? Can J Urol* 2020; **27(3)**:11-9.

22. Carson C. Peyronie's disease: New paradigm for the treatment of a unique cause of erectile dysfunction. *Postgrad Med* 2020; **132(4)**:4-8. doi: 10.1080/00325481.2020.1805865.

23. Alkandari MH, Touma N, Carrier S. Platelet-rich plasma injections for erectile dysfunction and peyronie's disease: A systematic review of evidence. *Sex Med Rev* 2022; **10(2)**: 341-52. doi: 10.1016/j.sxmr.2020.12.004.