Original Article

Effects of Apixaban, Rivaroxaban, Dabigatran and Enoxaparin on Histopathology and Laboratory Parameters in Achilles Tendon Injury: An *in vivo* Study

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Abstract

Objectives: To compare the effects of apixaban, rivaroxaban, dabigatran and enoxaparin on histopathology and blood parameters in rats with Achilles tendon injury.

Materials and Methods: Thirty adult, male Wistar albino rats weighting 220–240 g were randomly divided into five (one control and four treatment) groups and placed in a controlled environment. The Achilles tendon was incised and re-sutured in each rat, after which each group was provided the following treatment for 28 days: a) 2 ml saline to the control group, b) apixaban in 1 ml of saline (10 mg/kg/day) +1 ml of saline, c) rivaroxaban in 1 ml of saline (2 mg/kg/day) +1 ml saline, d) dabigatran in 1 ml of saline (30 mg/kg/day) +1 ml of saline, e) enoxaparin (80 μg/kg/day) + 2 ml of saline.

Results: Hemogram, biochemical and coagulation parameters differed significantly between the control and treatment groups ($P < 0.05$). Compared with the control group, in the apixaban group, type I and type III collagen immunoreactivity were severe and moderate, respectively. In the rivaroxaban and dabigatran groups, both type I and type III collagen immunoreactivity were medium and severe, respectively. In the enoxaparin group, type I and type III collagen immunoreactivity were mild and severe, respectively.

Conclusion: The higher concentration of type I collagen in the apixaban and dabigatran indicates faster tendon healing in these groups, and the higher concentration of the type III collagen in the enoxaparin group indicates slower healing in this group.

Keywords: Achilles tendon, apixaban, collagen, dabigatran, enoxaparin, rivaroxaban

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INTRODUCTION

Achilles tendon is the thickest and most frequently ruptured tendon of the body. In the Unites States, the incidence of Achilles tendon rupture is approximately 5.9–9.9 per 100,000 population.[1] It is formed by the fusion of gastrocnemius and soleus muscle fibers, attaching to the

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calcaneus medially and laterally, taking part in locomotor movements such as walking, running, jumping and climbing.[1,2] Collagen is the main component that affects the strength and stability of the tendon, and about 85–95% of the dry weight of the Achilles tendon is composed of elastin fiber and extracellular matrix-rich type I collagen produced by tenocytes.[3]

Immobilization of the extremity by surgical operation or conservative treatment after the Achilles tendon rupture provides a basis for deep venous thrombosis. Low-molecular-weight heparins (LMWHs) such as enoxaparin and warfarin (vitamin K antagonist) are used as the standard in the prophylaxis and treatment of deep vein thrombosis resulting from surgical operation, stroke, non-valvular atrial fibrillation and pulmonary thromboembolism. However, these drugs are increasingly being replaced in clinical practice by new-generation anticoagulants such as apixaban (factor Xa inhibitor), dabigatran (thrombin inhibitor), rivaroxaban (factor Xa inhibitor) and edoxaban (factor Xa inhibitor), as they do not need monitoring and have fewer side effects compared to warfarin.[4-6]

Previously, studies have assessed the effects of LMWH and new-generation anticoagulants on Achilles tendon healing in rats; however, limited studies have assessed and compared multiple drug groups.[6] This study was conducted with the aim of comparing the effects of apixaban, rivaroxaban, dabigatran and enoxaparin on histopathology and blood parameters in rats with Achilles tendon injury.

MATERIALS AND METHODS

Study design

This *in vivo* study was carried out in the Experimental Animals Laboratory at the Faculty of Medicine, Sivas Cumhuriyet University, Turkey. The protocol was approved by the institutional Animal Experiments Local Ethics Committee and the study adhered to the EU Directive 2010/63/EU. This study has been reported in accordance with the ARRIVE (Animals in Research: Reporting In Vivo Experiments) statement.

Animal groups

A total of 30 adult, male Wistar albino rats with an average weight of 220–240 g (mean: 232 g) were used. The rats were obtained from the Experimental Animals Laboratory at the Faculty of Veterinary Medicine of the study institution. The rats were randomly divided (simple randomization) in the following five groups (six each): control, apixaban, rivaroxaban, dabigatran and enoxaparin treatment groups. All animals were placed in propylene cages in a controlled environment (temperature, 25 ± 3°C; humidity, 45–50%; 12/12 hours light-dark cycle), fed with standard rat chow and water *ad libitum*.

Surgical technique

Rats were administered intraperitoneal 91 mg/kg ketamine + 9.1 mg/kg xylazine combination for general anesthesia before surgical procedure. The right leg of each rat was cleaned with a razor blade, disinfected with 10% of povidone-iodine, closed with a surgical drape and approximately 1 *cm* longitudinal incision was applied to the skin. Transection of the paratenon and Achilles tendon fascicles was achieved with a transverse incision approximately 5 *mm* proximal to the calcaneal insertion area.[7] The incised Achilles tendon was re-sutured with atraumatic USP 4/0 silk (Neosilk Ultra, İzmir, Turkey) [Figure 1a and b]. After the primary repair of the tendon, the rats were released in the cages without immobilization.

Treatment protocol

The treatment provided for each group for 28 days after the surgical procedure were as follows:

a. 2 ml saline to the control group (orogastric gavage)
b. Apixaban in 1 ml of saline (10 mg/kg/day) +1 ml of saline (orogastric gavage)[8]
c. Rivaroxaban in 1 ml of saline (2 mg/kg/day) +1 ml saline (orogastric gavage)[8]
d. Dabigatran in 1 ml of saline (30 mg/kg/day) +1 ml of saline (orogastric gavage)[9]
e. Enoxaparin (80 μg/kg/day) +2 ml of saline.[10]

The wound site and the general physical activities of the rats were checked daily. After the experimental applications, approximately 7 ml of blood was taken from the rats by puncture from the hearts under anesthesia (10 mg/kg xylazine and 90 mg/kg ketamine hydrochloride) and euthanasia was performed by the cervical dislocation method. Achilles tendons of all rats were removed from the adhesion site in the femoral

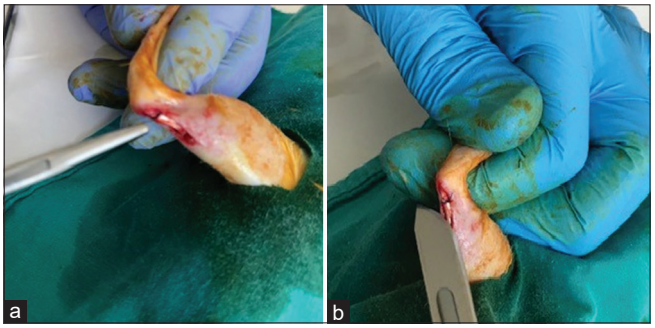

Figure 1: (a and b) Repair of the incised Achilles tendon

condyle and calcaneus for histopathological evaluation, and the tendons were placed in a 10% formaldehyde solution.

Blood analysis

A blood sample of 1 ml was taken into a purple top tube containing anticoagulant and ethylenediaminetetraacetic acid for complete blood count analysis (BC-2800 Vet, Mindray, China). In addition, 2 ml blood serum was taken to the yellow top separator tube and centrifuged at 2500 rpm for 10–15 minutes (BS-200 Chemistry Analyzer, Mindray, China). Finally, 3 ml of blood was taken into a blue top tube containing anticoagulant and citrate for coagulation analysis (MTI Diagnostic Analyzers, Germany) and centrifuged at 1500 rpm for 15 minutes, and then the plasma was separated and transferred to a plastic tube as citrated plasma.

Immunohistochemical and immunofluorescence method

The rats were necropsied and the tendon tissues were fixed in a 10% neutral formalin solution. Tissues were taken into paraffin blocks after routine alcohol-xylol follow-up. The 5-μm sections taken on the slides were passed through the xylol and alcohol series, and endogenous peroxidase inactivation was achieved by keeping it in 3% H_2O_2 for 10 minutes after washing with phosphate-buffered saline (PBS). It was treated with an antigen retrieval solution at 500 W for 2 × 5 minutes to expose the antigen in the tissues. Tissues washed with PBS were then incubated for 20 min at room temperature with primary antibodies of type I collagen (Abcam, catalog number: ab90395 1/200 dilution rate) and type III collagen (Abcam, catalog number: ab6310, 1/200 dilution rate; Cambridge, UK). Secondary, Large Volume Detection System: anti-Polyvalent, HRP (ThermoFisher, catalog number: TP-125-HL; Massachusetts, USA) was applied, as recommended by the manufacturer. 3.3'-Diaminobenzidine was used as a chromogen. It was closed with entellan and examined under a light microscope after contrasting with Mayer's hematoxylin (Sigma-Aldrich, Missouri, USA). Immunoreactivity was applied in the review as follows: none (−), mild (+), moderate (++) and severe (+++). Goat Anti-Mouse IgG H and L (FITC) (Abcam, Catalog number: Ab6785, dilution 1/50, Cambridge, UK) secondary antibodies were applied to the sections, and then washed with PBS at the end of the incubation with primary antibody for 45 minutes in the immunofluorescence method. The sections washed at the end of the period were covered with 4',6-diamidino-2-phenylindole fluorescence medium. Fluorescence was evaluated under the microscope as none (−), mild (+), moderate (++), and severe (+++).

Statistical analysis

All statistical calculations were performed with IBM SPSS Statistics software (version 22, IBM Corporation, Armonk, NY, USA). All continuous variables were expressed as mean ± standard deviation. Kruskal–Wallis test and Mann–Whitney *U* test were used to compare parameters that do not show the normal distribution in quantitative data. The results were evaluated in the 95% confidence interval and the significance level was $P < 0.05$.

RESULTS

Blood analysis

The complete blood count parameters of the five groups is compared in Table 1. White blood cell (WBC), lymphocyte count, lymphocyte percentage, granulocyte percentage, red blood cell (RBC), hemoglobin, hematocrit, red cell distribution width (RDW), platelet, plateletcrit (PCT) measurements showed significant differences between the groups ($P < 0.05$).

In the between-group comparison of electrolytes, lipid panel, and liver–kidney function tests, cholesterol, creatinine, blood urea nitrogen (BUN), phosphorus, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, direct bilirubin, alkaline phosphatase (ALP), total protein, triglyceride, calcium, creatine kinase, low-density lipoprotein (LDL), high-density lipoprotein (HDL), indirect bilirubin, very-low-density-lipoprotein (VLDL) and glucose significantly differed between the groups ($P < 0.05$) [Table 2].

In terms of the coagulation parameters, prothrombin time (PT) ($P = 0.000$), international normalized ratio (INR) ($P = 0.004$) and activated partial thromboplastin time (aPTT) ($P = 0.003$) were found to be significantly different between the groups [Table 3].

Binary comparisons of the complete blood count between the control and drug groups

The number and percentage of granulocytes and RBC, hemoglobin, hematocrit, RDW, platelet, and PCT were significantly higher in the control group than the apixaban group ($z = -2.729$, $P < 0.01$; $z = -2.882$; $P < 0.01$; $z = -2.082$; $P < 0.05$; $z = -2.892$, $P < 0.01$; $z = -2.887$, $P < 0.01$; $z = -2.887$, $P < 0.01$; $z = -20334$, $P < 0.05$; $z = -2.324$; $P < 0.05$, respectively). The lymphocyte percentage was significantly lower in the control group than the apixaban group ($z = -2.882$; $P < 0.01$).

Hemoglobin and hematocrit were significantly higher in the control group than the dabigatran group ($z = -2.812$,

Table 1: Comparison of complete blood count parameters between the control and treatment groups

Parameter	Mean rank	χ^2	SD	*P*
WBC ($10^3/\mu L$)				
Control	18.75	11.6	4	0.02*
Apixaban	13.08			
Rivaroxaban	22.33			
Dabigatran	17.00			
Enoxaparin	6.33			
Lymphocyte ($10^3/\mu L$)				
Control	17.42	12.8	4	0.01*
Apixaban	16.50			
Rivaroxaban	21.83			
Dabigatran	17.08			
Enoxaparin	4.67			
Monocyte ($10^3/\mu L$)				
Control	16.17	5.6	4	0.2
Apixaban	9.25			
Rivaroxaban	20.33			
Dabigatran	15.33			
Enoxaparin	16.42			
Granulocyte ($10^3/\mu L$)				
Control	15.25	13.2	4	0.01*
Apixaban	6.42			
Rivaroxaban	22.67			
Dabigatran	20.58			
Enoxaparin	12.58			
Lymphocyte (%)				
Control	16.33	15.2	4	0.004**
Apixaban	26.75			
Rivaroxaban	13.83			
Dabigatran	7.83			
Enoxaparin	12.75			
Monocyte (%)				
Control	11.92	2.4	4	0.6
Apixaban	18.50			
Rivaroxaban	14.58			
Dabigatran	18.08			
Enoxaparin	14.42			
Granulocyte (%)				
Control	13.33	17.5	4	0.002**
Apixaban	3.58			
Rivaroxaban	18.67			
Dabigatran	23.08			
Enoxaparin	18.83			
RBC ($10^6/\mu L$)				
Control	22.58	10.0	4	0.04*
Apixaban	11.75			
Rivaroxaban	20.42			
Dabigatran	13.17			
Enoxaparin	9.58			
Hemoglobine (g/dL)				
Control	23.67	17.2	4	0.002**
Apixaban	7.25			
Rivaroxaban	20.58			
Dabigatran	8.08			
Enoxaparin	17.92			
Hematocrit (%)				
Control	24.42	14.4	4	0.006**
Apixaban	9.17			
Rivaroxaban	19.83			
Dabigatran	8.67			
Enoxaparin	15.42			
MCV (fL)				
Control	13.67	3.8	4	0.4
Apixaban	17.17			
Rivaroxaban	16.67			
Dabigatran	10.50			
Enoxaparin	19.50			
RDW (%)				
Control	18.25	20.2	4	0.000**
Apixaban	4.42			
Rivaroxaban	17.33			
Dabigatran	11.50			
Enoxaparin	26.00			
Platelet ($10^3/\mu L$)				
Control	17.00	17.4	4	0.002**
Apixaban	6.50			
Rivaroxaban	17.83			
Dabigatran	22.33			
Enoxaparin	7.83			
Mean platelet volume (fL)				
Control	22.33	7.0	4	0.1
Apixaban	13.83			
Rivaroxaban	16.42			
Dabigatran	15.67			
Enoxaparin	9.25			
PDW (%)				
Control	9.92	9.2	4	0.05
Apixaban	17.42			
Rivaroxaban	10.42			
Dabigatran	22.92			
Enoxaparin	16.83			
PCT (%)				
Control	18.00	15.4	4	0.004**
Apixaban	5.50			
Rivaroxaban	16.33			
Dabigatran	16.83			
Enoxaparin	4.67			

*$P<0.05$, **$P<0.01$. WBC – White blood cell; RBC – Red blood cell; MCV – Mean corpuscular volume; RDW – Red cell distribution width; PDW – Platelet distribution width; PCT – Plateletcrit; SD – Standard deviation

$P < 0.01$; $z = -2.887$; $P < 0.01$, respectively). WBC ($z = -2.562$; $P < 0.05$) and lymphocyte ($z = -2.727$; $P < 0.01$) values were significantly higher in the control group than the enoxaparin group. RDW was higher in the enoxaparin group than the control group ($z = -2.169$; $P < 0.05$).

Binary comparisons of biochemical parameters between the control group and drug groups

BUN ($z = -2.714$; $P < 0.01$) and VLDL ($z = -2.898$; $P < 0.01$) were significantly higher in the apixaban group. AST, total bilirubin, triglyceride, calcium, creatine kinase, LDL, HDL, indirect bilirubin, and glucose were significantly lower in the control group than the apixaban group ($z = -2.562$, $P < 0.05$; $z = -3.083$; $P < 0.01$; $z = -2.882$; $P < 0.01$; $z = -2.326$; $P < 0.05$; $z = -562$; $P < 0.05$; $z = -2.882$, $P < 0.01$; $z = -2.892$, $P < 0.01$; $z = -3.083$; $P < 0.01$; $z = -2.882$; $P < 0.01$, respectively).

Cholesterol, total bilirubin, direct bilirubin, triglyceride, and LDL were significantly lower in the control group than

Table 2: Comparison of biochemical and coagulation parameters between the groups

Parameter	Mean rank	χ^2	SD	*P*
Cholesterol (mg/dL)				
Control	15.00	20.6	4	0.000**
Apixaban	22.25			
Rivaroxaban	24.00			
Dabigatran	12.42			
Enoxaparin	3.83			
Creatinin (mg/dL)				
Control	19.58	18.1	4	0.001**
Apixaban	24.58			
Rivaroxaban	15.08			
Dabigatran	4.08			
Enoxaparin	14.17			
BUN (mg/dL)				
Control	27.25	19.9	4	0.001**
Apixaban	19.17			
Rivaroxaban	7.08			
Dabigatran	10.83			
Enoxaparin	13.17			
Phosphorus (mmol/L)				
Control	25.00	23.4	4	0.000**
Apixaban	23.33			
Rivaroxaban	11.67			
Dabigatran	4.00			
Enoxaparin	13.50			
ALT (U/L)				
Control	21.92	15.8	4	0.003**
Apixaban	19.33			
Rivaroxaban	19.75			
Dabigatran	11.75			
Enoxaparin	4.75			
AST (IU/L)				
Control	20.17	22.6	4	0.000**
Apixaban	27.17			
Rivaroxaban	14.75			
Dabigatran	10.17			
Enoxaparin	5.25			
Total bilirubin (mg/dL)				
Control	7.50	24.4	4	0.000**
Apixaban	27.00			
Rivaroxaban	15.50			
Dabigatran	20.00			
Enoxaparin	7.50			
Direct bilirubin (mg/dL)				
Control	10.50	16.1	4	0.003**
Apixaban	15.00			
Rivaroxaban	25.25			
Dabigatran	16.25			
Enoxaparin	10.50			
GGT (U/L)				
Control	15.50	0.000	4	1.000
Apixaban	15.50			
Rivaroxaban	15.50			
Dabigatran	15.50			
Enoxaparin	15.50			
Alkaline phosphatase (IU/L)				
Control	21.17	14.4	4	0.006**
Apixaban	15.17			
Rivaroxaban	23.50			
Dabigatran	8.17			
Enoxaparin	9.50			
Total protein (g/L)				
Control	25.08	22.8	4	0.000**
Apixaban	22.75			
Rivaroxaban	9.42			
Dabigatran	5.08			
Enoxaparin	15.17			
Triglyceride (mg/dL)				
Control	12.83	15.2	4	0.004**
Apixaban	21.50			
Rivaroxaban	24.33			
Dabigatran	10.33			
Enoxaparin	8.50			
Calcium (mg/dL)				
Control	14.58	21.9	4	0.000**
Apixaban	25.58			
Rivaroxaban	3.67			
Dabigatran	21.08			
Enoxaparin	12.58			
Creatin kinase (U/L)				
Control	17.83	20.9	4	0.000**
Apixaban	26.83			
Rivaroxaban	16.33			
Dabigatran	12.17			
Enoxaparin	4.33			
LDL (mg/dL)				
Control	11.25	19.6	4	0.001**
Apixaban	22.33			
Rivaroxaban	24.67			
Dabigatran	13.83			
Enoxaparin	5.42			
HDL (mg/dL)				
Control	14.33	19.3	4	0.001**
Apixaban	25.50			
Rivaroxaban	21.00			
Dabigatran	11.00			
Enoxaparin	5.67			
Indirect bilirubin (mg/dL)				
Control	13.00	18.8	4	0.001**
Apixaban	27.17			
Rivaroxaban	7.67			
Dabigatran	16.67			
Enoxaparin	13.00			
VLDL (mg/dL)				
Control	23.42	20.0	4	0.001**
Apixaban	6.17			
Rivaroxaban	20.58			
Dabigatran	19.67			
Enoxaparin	7.67			
Glucose (mg/dL)				
Control	20.58	21.1	4	0.001**
Apixaban	27.50			
Rivaroxaban	10.08			
Dabigatran	8.08			
Enoxaparin	11.25			
PT (%)				
Control	15.33	20.3	4	0.000**
Apixaban	6.42			
Rivaroxaban	24.75			
Dabigatran	22.33			
Enoxaparin	8.67			

Contd...

Contd...

Table 2: Contd...

Parameter	Mean rank	χ^2	SD	*P*
INR				
Control	16.75	16.2	4	0.003**
Apixaban	6.83			
Rivaroxaban	20.50			
Dabigatran	23.83			
Enoxaparin	9.58			
aPTT (s)				
Control	14.42	15.3	4	0.004**
Apixaban	15.67			
Rivaroxaban	26.67			
Dabigatran	7.25			
Enoxaparin	13.50			

**$P<0.01$. SD – Standard deviation; BUN – Blood urea nitrogen; ALT – Alanine aminotransferase; AST – Aspartate aminotransferase; GGT – Gamma glutamyl transferase; LDL – Low-density lipoprotein; HDL – High-density lipoprotein; VLDL – Very-low-density lipoprotein; PT – Prothrombin time; INR – İnternational normalized ratio; aPTT – Activated partial thromboplastin time

Table 3: Comparison of histopathology results between control and treatment groups

IHC-IF	Type I collagen	Type III collagen
Control	0.16±0.40[a]	0.33±0.51[a]
Apixaban	2.83±0.40[b]	1.83±0.40[c]
P	<0.05	<0.05
Control	0.33±0.51[a]	0.16±0.40[a]
Rivaroxaban	2.16±0.40[b]	2.33±0.51[b]
P	<0.05	<0.05
Control	0.33±0.51[a]	0.16±0.40[a]
Dabigatran	2.83±0.40[b]	2.66±0.51[b]
P	<0.05	<0.05
Control	0.16±0.40[a]	0.33±0.51[a]
Enoxaparin	1.16±0.40[b]	2.83±0.40[c]
P	<0.05	<0.05

[a,b,c]Differences between the groups ($P<0.05$).
IHC-IF – Immunohistochemical-immunofluorescence

the rivaroxaban group (z = −2.005; $P < 0.05$; z = −2.298, $P < 0.05$; z = −3.102; $P < 0.01$; z = −2.085; $P < 0.05$; z = −2.562; $P < 0.05$, respectively). BUN, phosphorus, AST, total protein, calcium, and glucose values were significantly higher in the control group than the rivaroxaban group (z = −2.913; $P < 0.01$; z = −2.722, $P < 0.01$; z = −2.005; $P < 0.05$; z = −2.242; $P < 0.05$; z = −2.722; $P < 0.01$; z = −2.892; $P < 0.01$, respectively).

Creatine, BUN, phosphorus, AST, ALP, total protein, and glucose values were significantly higher in the control group than the dabigatran group (z = −2.913; $P < 0.01$; z = −2.929; $P < 0.01$; z = −2.882; $P < 0.01$; z = −2.882; $P < 0.05$; z = −2.887; $P < 0.01$; z = −2.903; $P < 0.01$; z = −2.722; $P < 0.01$, respectively). Total bilirubin (z = −3.140; $P < 0.01$) was significantly higher in the dabigatran group.

Cholesterol, creatinine, BUN, phosphorus, ALT, AST, ALP, total protein, creatine kinase, VLDL, and glucose levels were significantly higher in the control group than the enoxaparin group (z = −2.741; $P < 0.01$; z = −2.049; $P < 0.05$; z = −2.913; $P < 0.01$; z = −2.882; $P < 0.01$; z = −2.887; $P < 0.01$; z = −2.882; $P < 0.01$; z = −2.887; $P < 0.01$; z = −2.918; $P < 0.01$; z = −2.882; $P < 0.01$; z = −2.670; $P < 0.01$; z = −2.166; $P < 0.05$, respectively).

Binary comparisons of coagulation parameters between the control group and drug groups

PT (z = −2.722, $P < 0.01$) and INR (z = −2.704; $P < 0.01$) were significantly higher in the control group than the apixaban group. PT (z = −2.562, $P < 0.05$) and aPTT (z = −2.882; $P < 0.01$) were significantly higher in the rivaroxaban group. The INR value was significantly higher in the dabigatran group than the control group (z = −2.282; $P < 0.05$).

Histological analysis

Control group and apixaban

There was a significant difference between the two groups in terms of type I collagen and type III collagen immunoreactivity in the immunohistochemical staining findings ($P < 0.05$) [Table 3]. No significant difference was found in the tendon tissues of the control group rats in terms of type I collagen and type III collagen immunoreactivity. While the type I collagen immunoreactivity was severe in the tendon tissues of apixaban group animals, the type III collagen immunoreactivity was moderate [Figure 2a-d]. Similar results were seen in immunofluorescence and immunohistochemical staining findings [Figure 3a-d].

Control group and rivaroxaban

A significant difference was detected between the two groups in terms of type I collagen and type III collagen immunoreactivity in immunohistochemical staining findings ($P < 0.05$) [Table 3]. No significant difference was found in the tendon tissues of the control group rats in terms of type I collagen and type III collagen immunoreactivity. Type I and III collagen immunoreactivity were detected at a medium level in the tendon tissues of animals in the rivaroxaban group [Figure 4a-d]. Immunofluorescence staining findings were similar to those of the immunohistochemical staining findings [Figure 5a-d].

Control group and dabigatran

A significant difference was detected between the groups in terms of type I and type III collagen immunoreactivity in immunohistochemical staining findings ($P < 0.05$) [Table 3]. No significant difference was found in the tendon tissues of the control group rats in terms of type I and type III collagen immunoreactivity. Type I and III collagen immunoreactivity were severely detected in the tendon tissues of the dabigatran group animals [Figure 6a-d]. Immunofluorescence staining

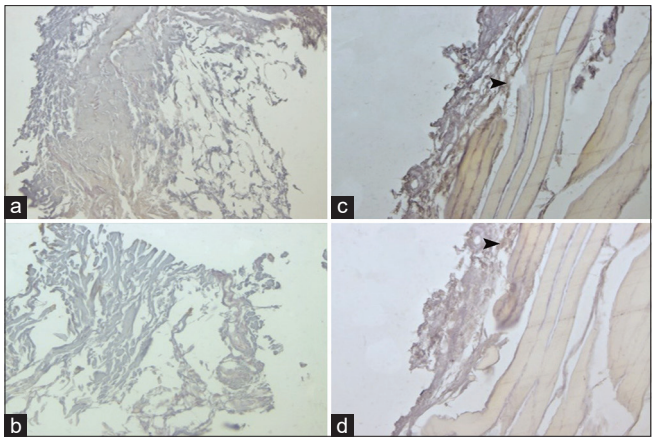

Figure 2: (a and b) Type I and type III collagen immunonegativity in the control group. (c) Severe Type I collagen immunopositivity in the apixaban group (arrowhead). (d) Moderate Type III collagen immunopositivity in the apixaban group (arrowhead) (immunohistochemical-tendon)

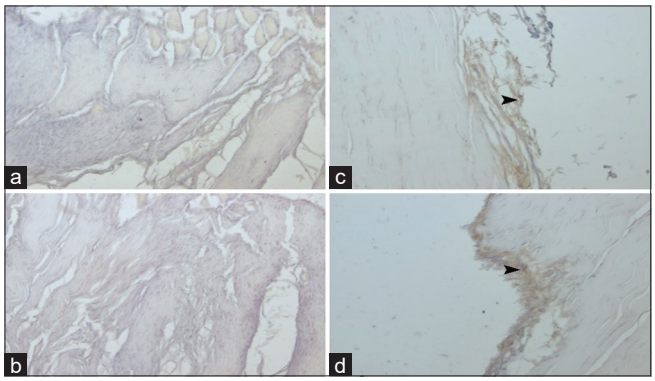

Figure 4: (a and b) Type I and Type III collagen immunonegativity in the control group. (c and d) Moderate level Type I and III collagen immunopositivity in the rivaroxaban group (arrowhead) (immunohistochemical-tendon)

findings showed similar results to immunohistochemical staining findings [Figure 7a-d].

Control group and enoxaparin

A significant difference was detected between the groups in terms of type I and type III collagen immunoreactivity in immunohistochemical staining findings ($P < 0.05$) [Table 3]. No significant difference was found in the tendon tissues of the control group rats in terms of type I and type III collagen immunoreactivity. Type I collagen immunoreactivity was determined mildly, while type III collagen immunoreactivity was detected at a severe level in the tendon tissues of animals in the enoxaparin group [Figure 8a-d]. Immunofluorescence staining findings showed similar results to immunohistochemical staining findings [Figure 9a-d].

DISCUSSION

New-generation anticoagulants such as apixaban, rivaroxaban and dabigatran are alternatives to reduce

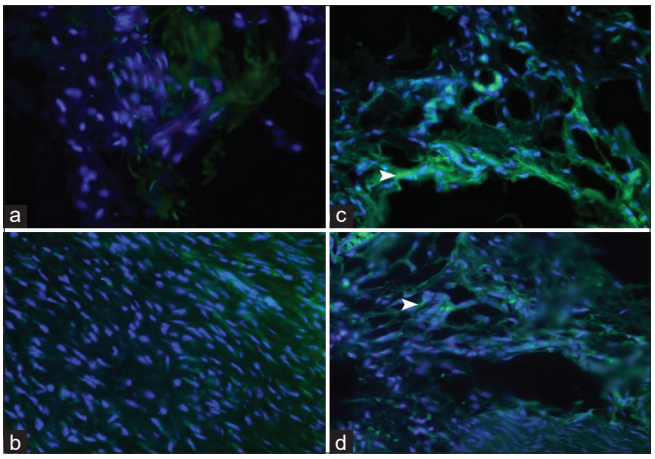

Figure 3: (a and b) Type I collagen and Type III collagen immunonegativity in the control group. (c) Severe Type I collagen immunopositivity in the apixaban group (arrowhead). (d) Moderate Type III collagen immunopositivity in the apixaban group (arrowhead) (immunohistochemical-tendon)

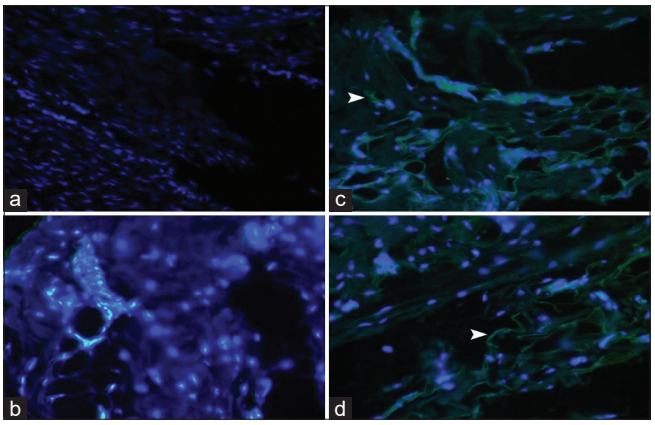

Figure 5: (a and b) Type I and Type III collagen immunonegativity in the control group. (c and d) Moderate level I and Type III collagen immunopositivity in the rivaroxaban group (arrowhead) (immunofluorescence-tendon)

complications due to vitamin K antagonists (warfarin) and LMWHs used in thromboembolism prophylaxis and treatment.[11] However, few studies have assessed the effects of these drugs on histopathological findings and blood parameters during the healing process of Achilles tendon, which was the basis of the current study.

We found that in the apixaban group, the type I collagen (mature) and type III collagen (immature) immunoreactivity was severe and moderate, respectively, compared with controls. In the rivaroxaban group, type I collagen and type III collagen immunoreactivity was moderate, and in the dabigatran group, the immunoreactivity was severe. In the enoxaparin group, type I immunoreactivity was mild, and type III collagen was severe. The type I collagen density was higher in the apixaban and dabigatran group when compared to the

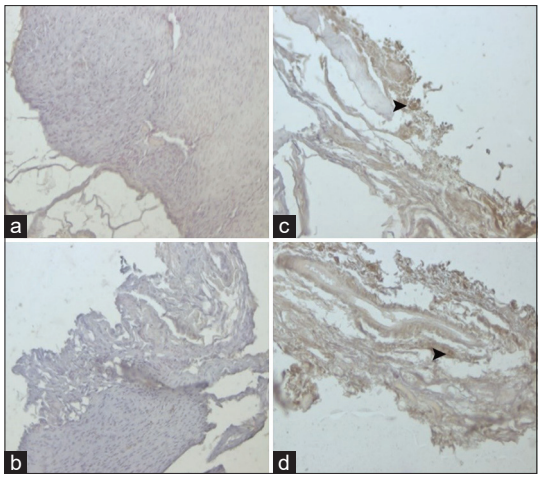

Figure 6: (a and b) Type I and Type III collagen immunonegativity in the control group. (c and d) Severe Type I and Type III collagen immunopositivity in the dabigatran group (arrowhead) (immunohistochemical-tendon)

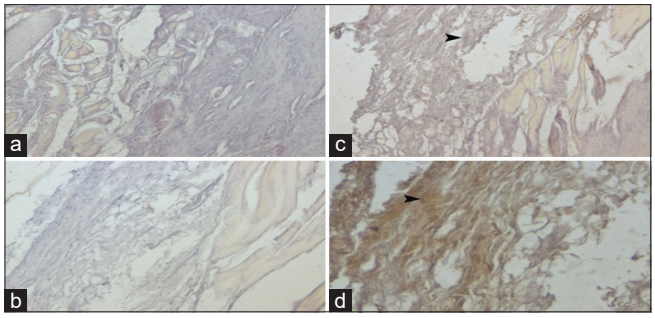

Figure 8: (a and b) Type I and Type III collagen immunonegativity in the control group. (c) Mild Type I collagen immunopositivity in the enoxaparin group (arrowhead). (d) Severe Type III immunopositivity in the enoxaparin group (arrowhead) (immunohistochemical-tendon)

control group, whereas the type III collagen density was higher in the enoxaparin group.

As our study found that type I collagen immunoreactivity was more severe in the apixaban and dabigatran groups and the type III collagen immunoreactivity more severe in the enoxaparin group, it indicates that that the healing process is faster and more effective in the apixaban and dabigatran group and slower in the enoxaparin group. In the study of Eren *et al*.,[6] the amount of type I collagen was higher in the rivaroxaban and LMWH group, and on the contrary, the type III collagen was higher in the control group.

The healing process of the tendon after surgical repair consists of a short inflammatory phase lasting about a week, followed by a proliferative phase lasting several weeks and, finally, remodeling lasting for months.[12] Vascular permeability increases in the inflammatory phase and inflammatory cells accumulate in the wound area, providing the production of cytokines and growth

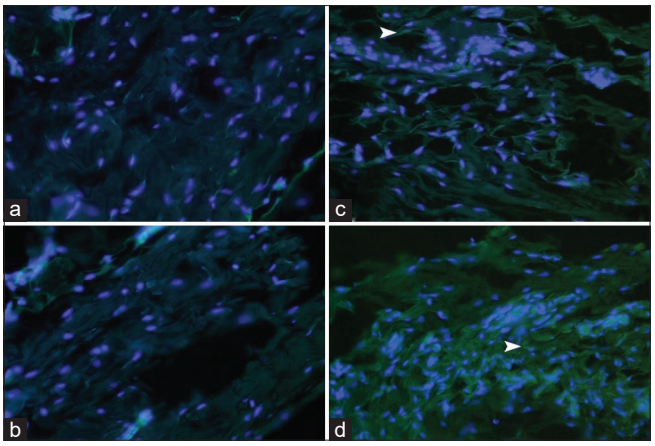

Figure 7: (a and b) Type I and Type III collagen immunonegativity in the control group. (c and d) Severe Type I and Type III collagen immunopositivity in the dabigatran group (arrowhead) (immunofluorescence-tendon)

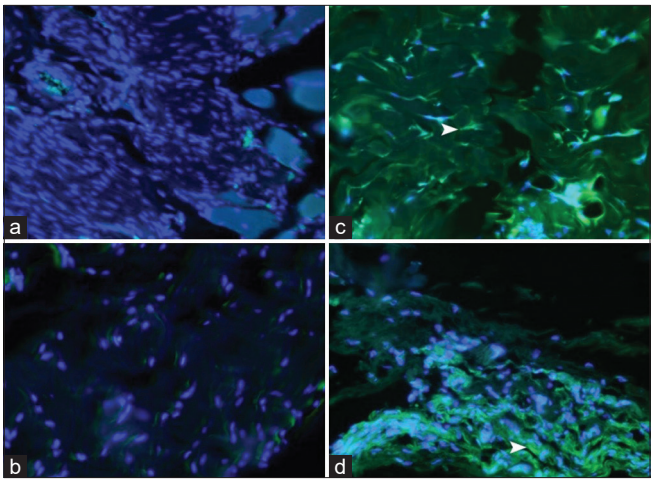

Figure 9: (a and b) Type I collagen and Type III collagen immunonegativity in the control group. (c) Mild Type I immunopositivity in the enoxaparin group (arrowhead). (d) Severe Type III immunopositivity in the enoxaparin group (arrowhead) (immunofluorescence-tendon)

factors, leading to clustering and proliferation of macrophages and tendon fibroblasts.[12] Fibroblasts, which are in the proliferation and remodeling phase of healing, provide cross-fibrillar collagen production and storage.[12] According to Levine,[13] oral anticoagulants such as apixaban, rivaroxaban and dabigatran delay the wound healing by inhibiting factor production in the coagulation phase, which is the beginning of wound healing initiated by platelet-derived growth factor and transforming growth factor.[13] In clinical practice, apixaban and dabigatran are used twice a day, and rivaroxaban is used once a day to achieve effective doses in thromboembolism prophylaxis and these are oral anticoagulants with similar half-life.[14] Gómez-Outes *et al*.[15] reported more major bleeding in the apixaban and dabigatran group of patients using standard dose oral anticoagulants. Unlike the standard dose

used in thromboembolism prophylaxis, using low-dose anticoagulants may speed up wound healing and not inhibit the coagulation phase. Oken *et al*.[16] also reported that factor Xa inhibitor enoxaparin, a LMWH species, decreased vascularity formation, inflammation, fibrosis and delayed epithelization in tissue healing model, similar to our study. In the LMWH (nadroparin) group used by Eren *et al*.,[6] type I collagen amount is higher and tendon healing is faster. Durmaz *et al*.[17] found that enoxaparin was better in the 7th and 10th days of wound healing than unfractionated heparin and the control group but did not compare with an oral anticoagulant agent. Although the findings of enoxaparin in the wound healing process differ in the literature, the difficulty of subcutaneous administration compared to oral drug intake restricts the use of this drug.

Hemoglobin, hematocrit, RBC, platelet, RDW, granulocyte count and percentage, and PCT were lower in the apixaban group than the control group in the current study. Hemoglobin and hematocrit were lower in the dabigatran group. WBC and lymphocyte count was lower in the enoxaparin group compared to the control group, while RDW was higher. PT and aPTT were higher in the rivaroxaban group and INR in the dabigatran group compared to the control group. AST, total bilirubin, triglyceride, calcium, creatine kinase, LDL, HDL, indirect bilirubin, and glucose were higher in the apixaban group than the control group. Cholesterol, total bilirubin, direct bilirubin, triglyceride, and LDL and dabigatran group were significantly higher in the rivaroxaban group than the control group. Apixaban lowers hemoglobin, hematocrit, and RBC but does not affect PT and aPTT. Rivaroxaban does not decrease hemoglobin and hematocrit but increases PT and aPTT. Dabigatran decreases hemoglobin and hematocrit by increasing INR; all these suggest that apixaban causes more minor bleeding and causes anemia in rats where we do not detect major bleeding.

Pujadas-Mestres *et al*.[18] have demonstrated that apixaban inhibits platelet aggregation and reduces the number of platelets in a dose-dependent manner. The hemoglobin, hematocrit, RBC, platelet, RDW, granulocyte count and percentage, and low PCT in the apixaban group suggest that the drug may perform bone marrow suppression, but there is insufficient evidence in the literature regarding the same. Turkoglu[19] reported that aPTT is more sensitive to the anticoagulant effect of dabigatran, rivaroxaban extends PT and aPTT, but PT is more sensitive to the anticoagulant effect of the drug, and apixaban extends PT and aPTT but aPTT is not sensitive. Since apixaban does not affect thrombin, it is thought that it does not affect PT among the synthetic functions of the liver, but it can increase AST by causing hepatocellular damage and increases the value of bilirubin, which shows cholestasis by increasing hemoglobin breakdown with dabigatran and rivaroxaban.[20] Considering the effects of apixaban and rivaroxaban on the lipid profile, it is thought to form a dyslipidemia. Among the causes of non-alcoholic fatty liver disease are insulin resistance, dyslipidemia, and the use of drugs such as glucocorticoids, amiodarone, aspirin, ibuprofen, protease inhibitors, valproic acid and carbamazepine.[21,22] Both drugs can reveal this clinical manifestation by steatosis in the liver, and the fact that apixaban increases blood glucose substantiates this theory. Non-alcoholic fatty liver disease was associated with insulin resistance and hyperglycemia.[23]

This study had some limitations. As the study was designed experimentally, it was not possible to compare clinical and functional results. Achilles tendon injuries and ruptures usually occur after compulsive physical activity in the human body. Iatrogenic rupture model was developed in rat tendon in this study. At the end of the experiment, the biomechanical effects including the structure, function, and motion elements related to the tendon were not evaluated and the collagen structure emerging during the healing process was evaluated. Histopathological findings were compared individually with the control group and drug groups were not compared within themselves. While creating the treatment protocol in drug groups, the studies in the literature were taken as examples. Bone marrow and liver biopsy is required in future studies to support the blood results obtained. Finally, the findings of these drugs were examined only at the end of the experiment, and not as early or late terms.

CONCLUSION

The higher concentration of type I collagen in the apixaban and dabigatran groups indicates that tendon healing is faster, and the higher concentration of the type III collagen in the enoxaparin group shows that the healing in this group is gradual. Notably, apixaban and dabigatran lowers hemogram and hematocrit values, which may cause minor bleeding, in addition to apixaban affecting multiple hemogram parameters may cause bone marrow suppression, but apixaban, unlike rivaroxaban and dabigatran, is not effective on coagulation parameters. The effects of apixaban and rivaroxaban on the lipid profile strengthens the theory that they can lead to dyslipidemia, and also, the fact that apixaban raises blood glucose further suggests that it can cause fatty liver disease.

Ethical considerations

The study protocol was approved by the Animal Experiments Local Ethics Committee of Sivas Cumhuriyet University, Sivas, Turkey (Ref no.: 65202830-050.04.04-260), on 21 February, 2019, and the study adhered to the EU Directive 2010/63/EU.

Peer review

This article was peer-reviewed by two independent and anonymous reviewers.

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Conflicts of interest

There are no conflicts of interest.

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