

# Effects of Nandrolone Decanoate on Histological Healing of The Partial Tear Achilles Tendon in Wistar Rats

Mukharradhi Nanza<sup>a</sup>, Husnul Fuad Albar<sup>b</sup>, OK Ilham Abdullah Irsyam<sup>c</sup>

<sup>a</sup> drhamster@yahoo.com

<sup>a,b,c</sup>Department of Orthopaedics and Traumatology, Medical faculty, University of Sumatera Utara, Haji Adam Malik General Hospital, Medan, 20136, Indonesia

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**Abstract-** The increasing number of trauma cases resulting in tendon tears or degenerative tendinopathy leading to complicated ruptures require the replacement of damaged tissue, especially in elderly patients and athletes. Anabolic steroids stimulate muscle growth, which increases muscle protein synthesis, induces type I and II fiber hypertrophy, stimulates and activates satellite cell replication, and ultimately increases muscle strength. High-dose anabolic steroid treatment decreases prolyl 4-hydroxylase and hydroxyproline activity after three weeks, suggesting that anabolic steroids may harm collagen biosynthesis in tendons. This study aims to demonstrate the anabolic effect of steroids on the healing of partially torn Achilles tendon. This experimental study was conducted from February – June 2020. The inclusion criteria in this study were male Wistar rats; Non feverish (38.3 – 39.40C) rats; Weights 300 - 400 grams; About 2-3 months old. The dropout criteria in this study were total Achilles tendon rupture and all rats that died during the study. Rats in this study were divided into 3 groups, with the Achilles tendon surgically partial transected (<50%), were given Nandrolone Decanoate 3 times (day 0,7,14) and 5 times (day 0,7,14, 21,28). Group 1 was given 2 mg, group 2 was given 6 mg, and group 3 was given placebo (0.2 cc of 0.9% NaCl). Then the Achilles tendon tissue was taken for histological examination (Movin Score). Based on the analysis, the average Movin score for each parameter in each group showed no statistically significant difference either on the 17th day or on the 35th day of observation (p value > 0.05). However, the results of angiogenesis parameters of the three groups showed statistically significant result based on the duration of anabolic steroid administration (p<0.05). There is significant difference in angiogenesis observed by administering Nandrolone decanoate. Partial Achilles tendon rupture may heal spontaneously without administering Nandrolone decanoate.

**Index Terms-** Partial tear; Achilles tendon; Nandrolone decanoate; Histological

## I. INTRODUCTION

The increasing number of trauma cases resulting in tendon tears or degenerative tendinopathy leading to complicated ruptures require the replacement of damaged tissue, especially in elderly patients and athletes. Tendon ruptures are faced with two main problems: re-rupture and adhesion formation.

The use of anabolic steroids among competitive athletes is very common. Anabolic steroids stimulate muscle growth, which increases muscle protein synthesis, induces type I and II fiber hypertrophy, stimulates and activates satellite cell replication, and ultimately increases muscle strength. In a recent study, applying the anabolic steroid nandrolone decanoate prevented fat infiltration, pulling, and reducing functional muscle damage caused by myotendinous retraction in rabbit rotator cuff muscles. In the lamb model of a rotator cuff tendon tear, further muscle atrophy can be continued with anabolic steroids initiated immediately after tendon repair. In addition, muscle fat infiltration can largely be avoided if steroids are applied immediately after the tendon released [1,2].

High-dose anabolic steroid treatment decreased prolyl 4-hydroxylase and hydroxyproline activity after three weeks, suggesting that anabolic steroids may harm collagen biosynthesis in tendons. One of the considered harmful effects of anabolic steroids (SA) is the risk of tendon rupture. However, investigations have yielded inconsistent results, and it remains unclear how SA affects tendons. Small animal studies are the most common research publication, and conflicting results are often reported. All studies focused on the potential damaging effects of anabolic steroids on tendons. But surprisingly, SA has recently been used in patients following tendon injury [2,3]. This study aims to demonstrate the anabolic effect of steroids on the healing of the partially torn Achilles tendon.

## II. METHODS

This experimental study was conducted from February – June 2020 in the Integrated Laboratory of the Faculty of Pharmacy, University of North Sumatra. The population of this study was adult wistar rats weighing 300-400 grams. From the research population, samples were selected randomly based on the estimated sample size calculation results. The inclusion criteria in this study were male Wistar rats; Rats did not have fever (38.3 – 39.40C); Weights 300 - 400 grams; About 2-3 months old. The dropout criteria in this study were total Achilles tendon rupture and all rats that died during the study.

Rats in this study were divided into 3 groups who were given Nandrolone Decanoate 3 times (day 0,7,14) and 5 times (day 0,7,14,21,28) . Group 1 was given 2 mg, group 2 was given 6 mg, and group 3 was given placebo (0.2 cc of 0.9% NaCl). The three groups of rats were killed on the 17<sup>th</sup> and 35<sup>th</sup> day of observation. Then the Achilles tendon tissue was taken for histological examination (Movin Score).

### 1.1 Achilles tendon partial transection procedure in study rats

Skin was incised longitudinally lateral to the midline and 5 mm proximal to the Achilles tendon insertion in the calcaneus. The peritendon and tendons are exposed. Achilles tendon transection was performed partially and transversely (transverse hemisection) using scalpel no. 15 perpendicular to the collagen fibers, 2.5 mm from the point of insertion in the calcaneus and directed to the lateral side of the Achilles tendon as much as <50% of the total width of the Achilles tendon. The lateral transection aims to avoid the flexor halucis longus being medial to the Achilles tendon. After transection, the skin was closed with continuous sutures with Polypropylene 5.0 and covered with sterile dressing. After surgery, analgesics were administered (paracetamol 200 mg/kg) orally every 24 hours for three days. The rats were left without immobilization casting and then administered Nandrolone Decanoate according to the dose intramuscularly every week. The general condition of rats and surgical wounds was monitored and evaluated daily.



Fig.1. Achilles tendon partial transection in rats

(a) preparation of the operating field with asepsis and antiseptics; (b-c) longitudinal incision lateral to the midline and 5 mm proximal to the insertion of the Achilles tendon on the calcaneus; (d-f) Transect of the Achilles tendon partially and transversely <50% of the total width of the Achilles tendon; g&h = suturing and dressing the wound)

The follow up did not show any neurological improvement yet but the pain complaint was subsided and now she able to sit and ambulate using wheel chair, which made her daily activity much easier. Some sample also taken during the operation and the macroscopic histopathologic study showing 2cc sample in volume with brown color and tender consistency. As the microscopic study showing groups of epithelial cell's with enlarged nucleus, coarse chromatin and loose eosinophilic cytoplasm between the fibromyxoid connective tissue's stroma. All evidence's suggesting adenocarcinoma metastasis.

### III. RESULTS

A total of 3 groups research subjects amounted to 8 rats, namely the first group as a control (n=8), the second group was terminated on the 17th and 35th days after administration of nandrolone decanoate dose of 2 mg (n=8), and the third group was terminated on the 17th and 35th days after administration of 6 mg nandrolone decanoate (n=8)—histological assessment of tendons using the Movin score

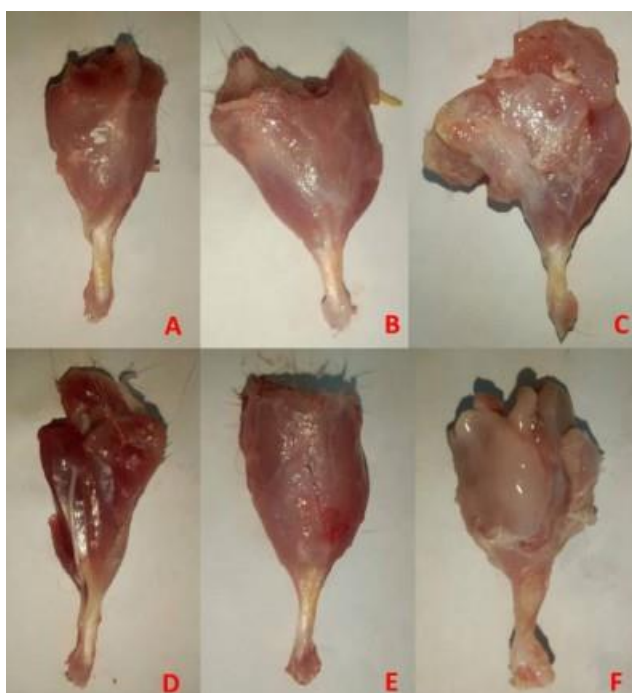


Fig.2. Macroscopic findings of isolated Achilles tendon and Gastrocnemius muscle. (a-b) samples taken on the 17<sup>th</sup> day; a: control, b: administered 2 mg of SA; (c) administered 6 mg of SA; (d-f): sample taken on 35<sup>th</sup> day; d: control, e: administered 2 mg of SA, f: administered 6 mg of SA)

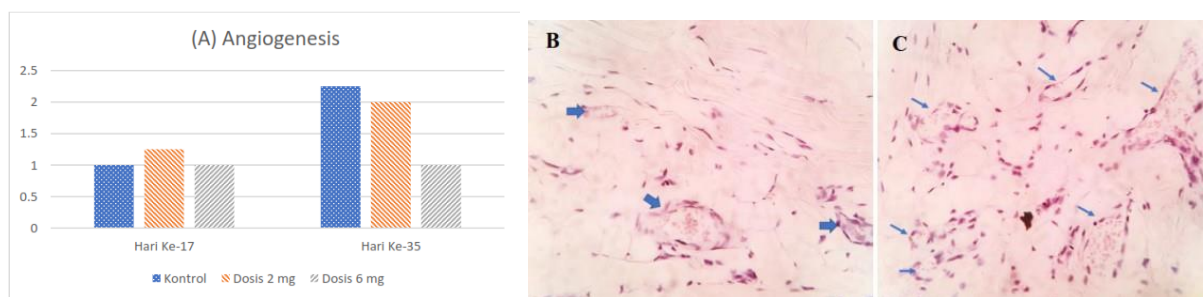
From the macroscopic findings, we could see that all the tendons that were taken from the samples were healed and minimal scarring occurred in all of the samples.

Based on the analysis, the average Movin score for each parameter in each group showed no statistically significant difference either on the 17th day of observation or on the 35th day of observation ( $p$  value > 0.05). The analysis results in this study also showed differences in angiogenesis parameters in the three groups based on the duration of anabolic steroid administration ( $p < 0.05$ ).

**Table 1. The average of Movin score for each parameter in each group**

Parameters	Group	Scoring (Mean±SE)		
		Day -17	Day-35	p-value
Fiber Arrangement	Control	1,25±0,47	1,25±0,25	0,308
	2 mg	1,00±0,41	1,50±0,28	
	6 mg	1,75±0,47	1,25±0,47	
	p-value	0,522	0,849	
Fiber Structure	Control	1,50±0,50	2,75±0,25	1,0
	2mg	2,50±0,25	2,75±0,25	
	6mg	2,50±0,50	1,75±0,75	
	p-value	0,153	0,178	
Angiogenesis	Control	1,00±0,40	2,25±0,25	0,015
	2mg	1,25±0,25	2,00±0,57	
	6mg	1,00±0,40	1,00±0,40	
	p-value	0,767	0,153	
Nuclear rounding	Control	0,75±0,47	1,50±0,28	0,243
	2mg	1,25±0,25	1,25±0,47	
	6mg	0,50±0,28	1,00±0,57	
	p-value	0,354	0,755	
Cell density	Control	1,00±0,40	1,50±0,28	1,0
	2mg	0,75±0,25	1,50±0,28	
	6mg	0,25±0,25	1,00±0,40	
	p-value	0,274	0,500	

Based on descriptive analysis, the mean score of Movin Fiber arrangement on the 17<sup>th</sup> day of observation seemed to increase in the group given nandrolone decanoate with a dose of 6 mg, while on the 35<sup>th</sup> day, the observation seemed to increase in the 2 mg nandrolone decanoate group. Meanwhile, the mean score of Movin Fiber structure on the 17<sup>th</sup> and 35<sup>th</sup> days of observation seemed to increase in the group given nandrolone decanoate with a dose of 2 mg.



**Fig.3. The graph showed comparison in Angiogenesis level on 2 observational day. B and C is the microscopic finding of angiogenesis level, score 2 (B), score 3 (C)**

The mean score of Movin Angiogenesis on the 17<sup>th</sup> day of observation seemed to increase in the group given nandrolone decanoate at a dose of 2 mg, while on the 35<sup>th</sup> day the observation showed the same results in the 6 mg nandrolone decanoate group.

The mean score of Movin Nuclear rounding on the 17<sup>th</sup> day of observation seemed to increase in the group given nandrolone decanoate with a dose of 2 mg, while on the 35<sup>th</sup> day of observation, it seemed to increase in the 6 mg nandrolone decanoate group.

The mean score of Movin cell density on the 17<sup>th</sup> and 35<sup>th</sup> days of observation showed an increase in all groups. Histological scoring chart and histology of cell density tissue.

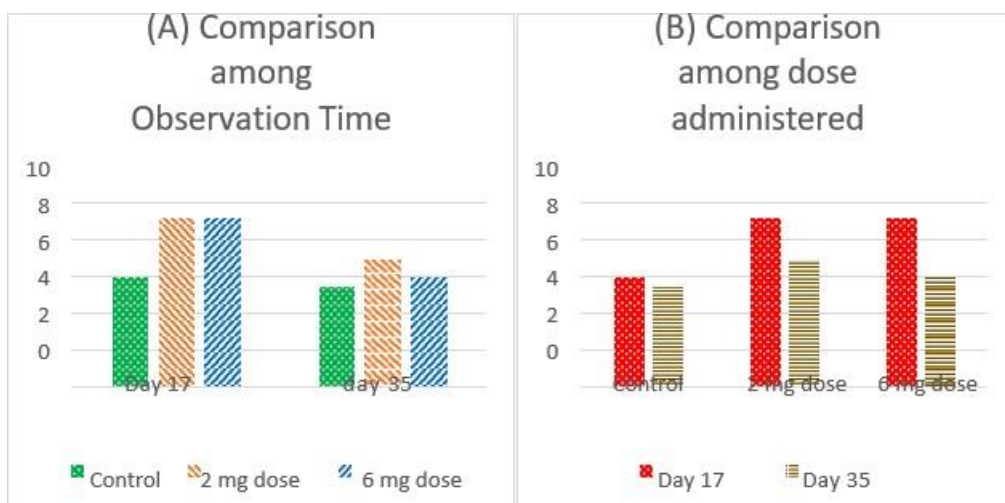
### 1.2 Effect of Anabolic Steroids on Achilles Tendon Histology Based on Movin Score

Based on statistical analysis, the effect of anabolic steroids on the histology of the Achilles tendon based on the total Movin score, there was no statistically significant difference between the Movin scores based on the observation day group and the nandrolone decanoate dose group, with  $p > 0.05$ . So it was concluded that there was no significant difference in the administration of different doses Nandrolone decanoate and the duration of observation on the tendon tissues histological changes based on the Movin score.

**Table 2. Effect of anabolic steroids on Achilles tendon histology based on Movin score**

*Movin Scoring Tendon Repair*

Days	Control	Dose 2 mg	Dose 6 mg	p-value**
17 days	6,00±4,24	9,25±0,95	9,25±2,21	0,224
35 days	5,50±3,10	7,00±0,81	6,00±2,16	0,641
Δ	0,50±2,38	2,25±1,70	3,25±2,50	-
p-value*	0,703	0,078	0,080	



**Fig. 4. Graph (A) showed the data comparison between the observation day, while (B) is the comparison of the dose administered**

The graphic above showed that on the 17<sup>th</sup> day of observation, there is no difference in the Movin score of group administered with 2 mg and 6 mg, on the 35<sup>th</sup> day of observation, there is increased of Movin score. However Movin score on the 35<sup>th</sup> day of observation decreased compared to 17<sup>th</sup> observation day. Analytic test to compare histological features based on Movin score between low and high doses showed  $p = 0.092$  on the 17<sup>th</sup>-day evaluation and  $p = 0.207$  on the 35<sup>th</sup>-day evaluation. It shows no significant difference between the administration of low and high doses of histology based on the Movin score.

#### IV. DISCUSSION

This experimental study aims to determine the effect of administering anabolic steroids using nandrolone decanoate to increase the activity of increasing regeneration and remodeling activities in the histology of the Achilles tendon of Wistar rats with partial tears, examined on the 17th and 35th days.

Nandrolone decanoate is an anabolic steroid, which is a derivative of testosterone that has a number of therapeutic functions. In addition to being used in treating hormonal disorders, anabolic steroids have anabolic effects that counter the muscle wasting associated with a number of diseases, anabolic steroids have also been studied in patients suffering from traumatic injuries [3,4].

In this study, it was found that all observed tendons were healed. Previous research conducted by Bruns et al., which used a sample of sheep treated with partial tendon rupture, showed that there was spontaneous healing in the sample; even on observation after 12 months, the researchers found that based on histology examination showed a structure that was almost similar to normal tendons. The initial healing mechanism was the thickening of the scar tissue with the improvement in the orientation of the tendon fibers until they were similar to normal tendon structures until the end of the observation at 12-month intervals. Limb immobilization was not even performed [5]. It indicates that there is an ability of the tendon to undergo spontaneous healing from partial rupture without any specific treatment.

In this study, it was found that the mean score of the Movin Fiber arrangement increased on the 17th day of observation in the 6 mg dose group and on the 35<sup>th</sup>-day in the 2 mg dose group. The mean score of Movin Fiber structure increase on the 17th and 35<sup>th</sup>-day observations in the 2 mg dose group. All groups showed increase of mean Movin score in angiogenesis on the 17th day of observation. The mean score of Movin Nuclear rounding increase in the 2 mg dose group. The mean score of Movin cell density on the 17th and 35th days of observation decreased in the 6 mg dose group. Thus it showed there is differences in angiogenesis parameters of the three groups based on the duration of anabolic steroid administration. There was no significant difference between the administration of low and high doses of histology based on the Movin score.

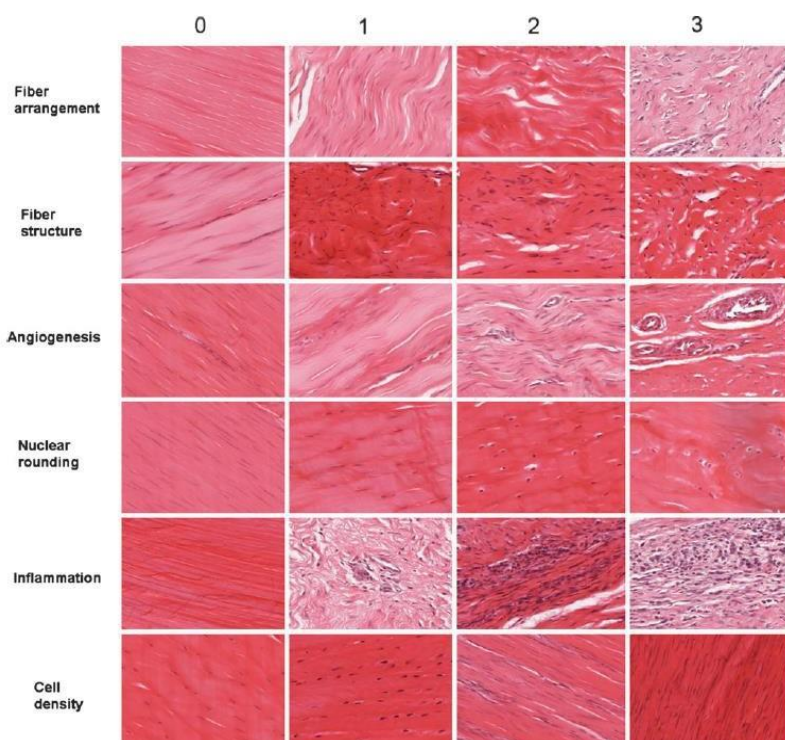


Fig 4 Movin Score scoring system (he 40x) [6]

	<i>Tendon repair assessment score</i>			
	0	1	2	3
Fiber structure	Continue, long fiber	Slightly fragmented	Moderately fragmented	Severely fragmented
Fiber arrangement	Compacted and parallel	Slightly loose and wavy	Moderately loose, wavy and cross to each other	No identifiable pattern
Rounding of the nuclei	Long spindle shape cells	Slightly rounding	Moderately rounding	Severely rounding
Inflammation (area infiltrated by Inflammation cells)	< 10%	10%–20%	20%–30%	> 30%
Increased vascularity (area infiltrated by neo-vascular)	< 10%	10%–20%	20%–30%	> 30%
Cell density	Normal pattern	Slightly increase	Moderately increase	Severely increase

Fig 5 Movin Score

Similar results were found in a histological study by Tsitsilonis et al., in which nandrolone decanoate was administered for 12 weeks at a dose of 5 mg/kg twice a week, then the tendons were examined using an electron microscope. The results showed histological changes in the form of dysplasia. Especially in the group of rats given anabolic steroids and undergoing some physical activity, other findings found were increased vascularity and cellularity, micro destruction of collagen fibers, and increased synovial layer. In a study by Marques et al., nandrolone decanoate was administered for 8 weeks at 25 mg weekly. It was found that the use of nandrolone decanoate in a group of rats that did not perform physical activity resulted in the disorganization of collagen fibers similar to that found in a group of trained rats. but not given nandrolone decanoate. The combination of physical activity and the use of nandrolone decanoate resulted in more significant changes in tissue regarding collagen disorganization and MMP concentrations, the largest disorganization observed in all studies [7,8].

Previous study evaluating the effect of anabolic steroids on angiogenesis conducted by Tagarakis et al. showed different results. This study found that the group given anabolic steroids did not significantly increase in angiogenesis. It was found that the control group had a significantly higher density of blood capillaries [9].

In this study, statistical analysis showed no significant relationship between the administration of nandrolone decanoate in different doses and the duration of observation on the histological changes of tendon tissue based on the Movin score. Similar results were found in the study of Inhofe et al., where after administration of anabolic steroids for 6 weeks, then reassessment at week 12, analysis of the Achilles tendon using light microscope showed no changes in the histology of the fibrils. Analysis using electron microscope had no changes in the diameter or shape of the fibrils. Meanwhile, in the histological study of Marqueti et al., wistar rats that were given nandrolone decanoate and testosterone decanoate for 5 weeks at a 5 mg/kg dose once a week, respectively a thick fibrotic layer covering the tendons was observed in the group receiving anabolic steroids. In addition, the administration of anabolic steroids decreased the concentration and active form of MMP-2, thus showing inhibition of tendon (collagen) remodeling. Wood et al published a report showing that administration of anabolic steroids, in this case, nandrolone decanoate administered for 6 weeks at a dose of 5 mg, increased crimp angles and decreased collagen fibril length, especially when anabolic steroid use was combined with physical activity [10-12]. Several other factors that may be the cause of absence of an association between the administration of anabolic steroids with Achilles tendon remodeling is

the lack of monitoring duration. Physiologically, tendon remodeling is divided into 3 phases, where the remodeling phase occurs between 1-2 months after the injury [13].

## V. CONCLUSIONS

There is significant difference in angiogenesis observed by administering Nandrolone decanoate. Partial Achilles tendon rupture may heal spontaneously without administering Nandrolone decanoate.

## ACKNOWLEDGEMENTS

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## AUTHORS

**First Author** – Mukharradhi Nanza, Department of Orthopaedics and Traumatology, Medical faculty, University of Sumatera Utara, Haji Adam Malik General Hospital, Medan, 20136, Indonesia, drhamster@yahoo.com

**Second Author** – Husnul Fuad Albar, Department of Orthopaedics and Traumatology, Medical faculty, University of Sumatera Utara, Haji Adam Malik General Hospital, Medan, 20136, Indonesia

**Third Author** – OK Ilham Abdullah Irsyam, Department of Orthopaedics and Traumatology, Medical faculty, University of Sumatera Utara, Haji Adam Malik General Hospital, Medan, 20136, Indonesia