



## Effect of Ultrasound Therapy on the Hydroxyproline Content in Experimental Tendon Injuries in Horses

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### Abstract

**Objective-**To determine the effect of ultrasound therapy on hydroxyproline (HP) content in superficial digital flexor tendon (SDFT) in horses.

**Design -** Experimental in vivo study.

**Animals -** Eight adult castrated horses

**Procedure-** The mediocaudal surface of right metatarsal bone in was exposed and after complete separation of SDFT, it was splitted (striking 15 times) in full thickness in longitudinal fashion of 10 cm in length in the mid-tendon area using BP blade no 24. These animals were divided into two equal groups of control and experiment. No treatment was given to control one, whereas; treatment group was subjected to therapeutic ultrasound (Sonopulse 434) regimens 10 minutes daily with intensity of 1 Watt/Esq. at 3 MHz frequency over 14 days. The hydroxyproline concentration was measured by modified spectrophotometry. The collected data was analyzed using student t test at  $p < 0.05$  significant level.

**Results-** HP content of severed tendons in both groups was significantly less than normal tendons ( $P < 0.01$ ). Also, the HP content of treatment group was significantly more than the control group.

**Conclusion and Clinical Relevance-** The results of this study indicated that direct application of ultrasound therapy on severely injured or extensive lesion of superficial digital flexor tendon in horses will enhance HP content in the treated tendon.

**Key Words-** Hydroxyproline, Ultrasound Therapy, Tendon, Horse.

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## Introduction

The biomechanical characteristics of tendon are determined by the composition and organization of the extra cellular matrix, which is composed of a hierarchical structure of subunits.<sup>1</sup> Tendon has been shown to undergo remodeling in response to strength or endurance training.<sup>2,3</sup> The strength of superficial digital flexor tendon (SDFT) is quite essential and even slight change in the tissue collagen content may lead to severe lameness and make a risk factor for rupture during exercise.<sup>3</sup> Hydroxyproline is formed from the posttranslational hydroxylation of proline and its concentrations is used as an index for assaying collagen content in connective tissues and in collagen metabolism studies, and reflects collagen turnover in bone, cartilage and skin, too.<sup>4</sup> Despite of application of different local or systemic treatment being advocated for treatment of severe tendonitis<sup>5,6</sup> there is more demand for application of non-invasive physical modalities for early and full recovery of tendon disorders in horses, so the purpose of this experiment was to investigate the effect of ultrasound therapy on the hydroxyproline content in tendon as well as to study final correlation between functional activity and clinical signs of severed tendon for early training planning.

## Materials and Methods

This experiment was conducted on eight castrated horses of having 310 to 395 kg BW with 4 to 9 years of age. The superficial digital flexor tendon (SDFT) of the right hind limb was exposed under deep general anesthesia and was splitted (striking 15 times) completely in full thickness in longitudinal fashion in about 10 cm in length in the mid-tendon area using BP blade no 24 in each tendon, then the underlining tissues and skin closed as routine. These animals were divided into two groups of control (untreated) and experiment (treated with ultrasound therapy) having 4 animals each. No treatment was given to control one, whereas treated group was subjected to therapeutic ultrasound (Sonopulse 343, Enraf Nonius Co. Netherland) regimens 10 minutes daily with intensity of 1 Watt/Esq. at 3 MHz frequency over 14 days. The hydroxyproline concentration was measured by a modified spectrophotometer method<sup>7,8</sup> by collecting. Samples from mid splitted area of injured and normal tendons of each animal from treated and untreated tendons on 60 days post surgery after being euthanized were collected and their hydroxyproline concentration was measured by a modified spectrophotometer method.<sup>7,8</sup> The samples were hydrolyzed in 6 molar HCl at 105°C for 14-16 hr and hydroxyproline was oxidized by chloramines T, then by using Ehrlich's reagent and incubating at 60°C, a chromophore was formed. To remove interfering chromophores, hydroxyproline product in alkaline media was extracted into toluene and then into acid phase. The absorbance of acid phase was read at 543 nm and hydroxyproline content was calculated from calibration curve based on standard solutions run as the same as samples. In order to determine the percentage of the dry matter (DM) in each tendon sample, 50-100 mg of each sample, concurrent with sampling for hydroxyproline analysis were placed on a plate and dried at 100°C in an oven for 3 hr. Finally hydroxyproline content of tendon samples were expressed in mg/g DM.<sup>4</sup> The collected data was analyzed using student t test at  $p < 0.05$  significant level.

## Results

The records of clinical signs indicated that the degree of lameness showed by individual horse was almost identical due to the similarity of the lesions. Lameness was quite obvious during the first 3 days post surgery and then gradually weakened in the treated limb until the time of euthanasia on 60<sup>th</sup> day. Local tenderness and swelling varied between individuals but it was less severe in the treated limbs. The hydroxyproline content was quite significantly different between untreated (control) tendons with that of normal limb of the same animal (Table 1) whereas difference was quite less when treated tendon was compared with that of normal limb (Figure 1) of the same animal. In treated limbs, there was marked increase in hydroxyproline content of each tendon using ultrasound therapy when compared to values of untreated ones.

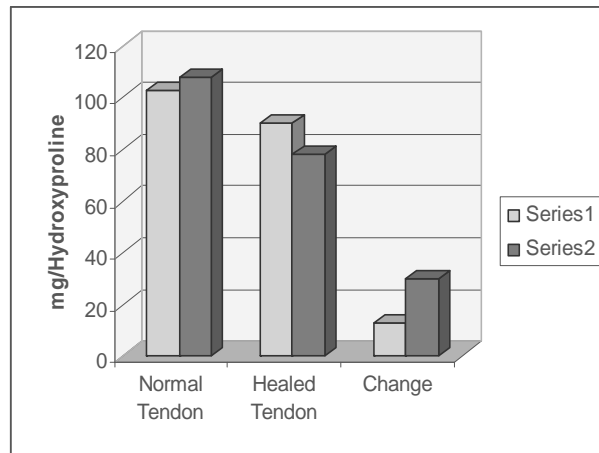
**Table1.** Statistical analysis on hydroxyproline content's changes between normal and treated tendons of two groups showed a significant differences ( $p<0/01$ ) on 60<sup>th</sup> day post surgery.

Group	No of Sample	Normal Tendon (mg/g DM)	Healed Tendon (mg/g DM)	Differences between normal and healed tendon (mg/g DM)
Control	1	108	71.2	36.8
	2	115.3	82.7	32.6
	3	90.6	69.1	21.5
	4	118.1	88.7	29.4
	Mean±SD	108.0 ±12.4	77.9 ± 9.3	30.1 ± 6.5
Treatment	1	98.6	78.7	19.9
	2	95.3	90.3	5.0
	3	96.7	80.6	16.1
	4	120.7	109.8	10.9
	Mean±SD	102.8 ±12.0	89.8 ±14.2	13.0 ± 6.5

## Discussion

The objective of this work was to determine the effect of ultrasound therapy on the severed tendon healing, especially by changes expected to occur in the hydroxyproline content of each tendon in treated and untreated tendon. The stimulated limb showed to be in better functional status on second week of treatment as compared to untreated limbs. The daily application of ultrasound therapy in the site of defined injury considered to be a part of rehabilitation plan for successful treatment of different disorders in the musculoskeletal system.<sup>9,10,11</sup> The results of the effect of ultrasound therapy at the site of injured tendon clinical reduced inflammatory process and lameness and also achieves pain relief via increasing blood flow.<sup>12,13,14</sup> The differences of hydroxyproline content between normal tendon and untreated one was quite significant ( $p<0.01$ ) whereas there was very narrow differences with those of treated limbs. It has been shown that the treated limb receiving ultrasound therapy resolved inflammation, increased tenocyte proliferation

and restored tendon integrity leading to restoration of biomechanical properties correlated to clinical signs of having full limbs weight bearing. The findings in this study provide the first indirect evidence that ultrasound therapy promote SDFT tendonitis repair due to increased fibroblastic/ tenoblastic activity.<sup>15,16</sup> Cumulative increased in hydroxyproline content has direct correlation in early maturation of fibroblasts and early parallel arrangement of collagen fibers and bundle formation.<sup>17,18</sup> The similar findings has been reported by sharifi et al<sup>18</sup> concerning the effect of transcutaneous electrical stimulation on hydroxyproline content in tendon of horses and Robert et al<sup>19</sup> regarding the positive effect of shock wave therapy (SW) on adult tendon in rat. He reported an increase in mitogenic and anabolic responses of tendon tissue that brought about the clinical success of using SW treatment in resolving tendonitis.<sup>19</sup> No doubt the etiology of tendonitis is multifactorial including avascular changes<sup>20</sup>, degenerative changes and metabolic disturbances, neural factors and neovascularization, but the acute swelling, inflammation and matrix destruction in tendon are similar to those seen in naturally occurring tendon injuries.<sup>21</sup> The ultrasound therapy increases temperature of soft tissue and increases with more activity of Alkaline Phosphates and ATPase<sup>14,22</sup> and blood flow in the area and intensifying cellular metabolism by significantly increased tenocyte growth. In this regard, tissue regeneration indicated that some growth factors during application of ultrasound therapy were induced mitogenic and morphogenic response of injured tendon<sup>14,22</sup>. Application of ultrasound therapy shown to have beneficial effects when applied to injured tendon.<sup>23</sup> There was a significant increase in collagen production with a model of an accelerated rate of collagen turn over and had a direct effect on biochemical properties of the tissue<sup>13</sup>. Early studies by Gigante et al<sup>22</sup> and Heidia Eriksen et al<sup>24</sup> showed a correlation exists among number of collagen fibrils, size, their organization, and mechanical strength. The amount of regenerating tendons considered to be good indication for relationship between mechanical strength and the absolute amount of collagen being determined as hydroxyproline content in this study, whereas reported findings relied on the collagen profiles of mature tendons.<sup>8,21,22</sup> In conclusion, this study indicates that direct application of ultrasound therapy on severely injured or extensive lesion of SDFT tendon in horses will be highly useful and enhance hydroxyproline content in the treated tendon. This study suggests using hydroxyproline content as a direct marker of the effect of ultrasound therapy on collagen content in injured tendon.



**Figure 1.** The mean hydroxyproline content (mg/g DM) in normal as well as control and treated healed tendons on day 60 post surgery (S1-treated group, S2-control group)

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## بررسی اثرات اولتراسونوترپی در میزان هیدروکسی پرولین بعد از ایجاد جراحات تجربی در تاندون خم کننده سطحی در اسب

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**هدف-** بررسی تاثیر امواج اولتراسوند بر روی میزان هیدروکسی پرولین در تاندون

**طرح مطالعه-** تجربی در حیوان تک سمی

**حیوانات-** ۸ راس اسب اخته بالغ

**روش کار-** تاندون خم کننده سطحی پای راست در تمام حیوانات تحت بیهوشی عمومی در دسترس قرار گرفت و با استفاده از تیغ بیستوری قسمت وسط تاندون تمام قطر به طول ۱۰ سانتیمتر تخریش گردید (۱۵ بار تیغ بصورت طولی کشیده شد) و سپس حیوانات به دو گروه ۴ تائی کنترل و گروه درمان با اولتراسوند تقسیم شدند. در گروه کنترل از هیچگونه درمانی استفاده نگردید در صورتیکه گروه دوم تحت تابش امواج اولتراسوند با شدت ۱ Watt/Esq و فرکانس ۳ MHz روزانه به مدت ۱۰ دقیقه برای ۱۴ روز متوالی قرار گرفتند. در روز ۶۰ نمونه بافتی از تاندون سالم پای مجاور و تاندون درمان شده و تاندون درمان نشده برای ارزیابی میزان هیدروکسی پرولین با استفاده از روش اسپکتروفتو متری تهیه گردید و داده های بدست آمده با روش Student -t test مورد تجزیه و تحلیل آماری قرار گرفت.

**نتایج-** میزان هیدروکسی پرولین در تاندون تخریش شده با تاندون های سالم در گروه کنترل پس از ۶۰ روز از جراحی تفاوت معنی داری را در مقایسه میزان هیدروکسی پرولین در تاندون های تخریش شده با تاندون های سالم گروه درمان نشان داد ( $P < 0.01$ ).

**نتیجه گیری و کاربرد بالینی-** بر اساس نتایج حاصل از این مطالعه می توان اظهار داشت که استفاده از اولتراسوند می تواند در تحریک افزایش هیدروکسی پرولین و کمیت کلاژن نقش مهم داشته باشد.  
**کلید واژگان-** هیدروکسی پرولین، اولتراسوند تراپی، تندون، اسب.