Research Article

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# Effectiveness of Bovine Tunica Vaginalis Powder in the Prevention of Tendon Adherence Following Tendon Repair Process in Bucks

**Ali Hussein khudair * and Hassanain A. Emran**

Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Baghdad

* Corresponding email: ali.hussein1202a@covm.uobaghdad.edu.iq

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**Abstract**

The current study was designed to investigate the efficacy of lyophilized bovine tunica vaginalis powder on peritendinous adhesion of induced defect in superficial digital flexor tendon (SDFT) in goats. The study was carried out on 18 adult bucks, the surgical procedure was done under highly aseptic conditions. Prior to surgery, animals were sedated by intramuscular injection of 2 % xylazine in a dose 0.5 mg/kg body weight, then the site of the operation is anesthetized by linear infiltration of 2% lidocaine hydrochloride. Surgical incision is made on the skin on the palmer surface of the metacarpal bone of the left forelimb to expose the (SDFT), a (0.1 depth, 0.2 width, and l Long) cm defect is made on the exposed tendon, this gap is filled with drops of sterile normal saline in (9) animals, and mentioned as control group, while the gap of the rest (9) animals is filled with the lyophilized powder as treated group. Sub cutaneous tissue was closed using absorbable suture material by simple continuous suture technique, and simple interrupted suture mattress using non absorbable suture material for closing the skin. The macroscopic findings exhibited a significant difference in adhesion scores between the 2nd, and 3rd periods of assessment post operation in first and second groups, with no significant differences between groups. The conclusion of this study is the tunica vaginalis scaffold has no obvious effect on decreasing adhesion between tendon and surrounding tissues.

**Keywards: Peritendinous Adhesion, Tendon Repair, Biological Scaffold.**

## تاثير مسحوق الغلاله الغمديه للثيران على منع الالتصاق خلال عمليلة اصلاح الوتر في ذكور الماعز

**الخلاصة**

صممت الدراسة الحالية للتحقق من فعالية مسحوق الغلاله الغمديه المجففة بالتجميد للثيران على التغيرات النسيجية المرضية للضرر المحدث في وتر القابضة الإصبعية السطحي (SDFT) في الماعز. أجريت الدراسة على 18 حيوانا من ذكور الماعز ، وتم إجراء العملية الجراحية في ضل ظروف شديدة التعقيم. قبل اجراء العملية الجراحة ، تم تخدير الحيوانات عن طريق الحقن العضلي بنسبة 2٪ زايلازين بجرعة 0.5 ملجم / كجم من وزن الجسم ، ثم تم تخدير موقع العملية موضعيا باستخدام المخدر الموضعي ليدوكائين هيدروكلوريد بنسبة 2٪. تم عمل شق جراحي في الجلد على السطح الراحي لعظم المشط للطرف الأمامي الأيسر لكشف (SDFT) ، تم عمل ضرر (0.1 عمق ، 0.2 عرض ، 1 طول) سم على الوتر المكشوف ، هذه الفجوة ملئت بقطرات من المحلول الملحي الطبيعي المعقم في (9) حيوانات ، وتم ذكره كمجموعة سيطره ، في حين تم ملىء فجوة البقية (9) حيوانات بالمسحوق المجفف بالتجميد ، وتم ذكرها كمجموعة معالجة. تم إغلاق الأنسجة الجلدية الفرعية باستخدام خيط قابل للامتصاص بتقنية خياطة مستمرة بسيطة ، و خياطة المنجد البسيط المتقطع باستخدام خيط غير قابل للامتصاص لغلق الجلد. أظهرت النتائج العيانية اختلافًا كبيرًا في درجات الالتصاق بين الفترتين الثانية والثالثة من التقييم بعد العملية في المجموعتين الأولى والثانية ، مع عدم وجود فروق ذات دلالة إحصائية بين المجموعات. استنتجت هذه الدراسة أن سقالة الغلالة الغمديه للثيران ليس لها تأثير واضح على تقليل الالتصاق بين الأوتار والأنسجة المحيطة.

## Introduction

Tendons are the physical connection among the active then the static portion of the musculoskeletal structure, passing muscle reduction to the skeletal structure, and thereby contributing to movement (1). Superficial digital flexor tendon, deep digital flexor tendon and suspensory ligament places on the palmar surface metacarpal bone and phalanx (PI, PII and PIII)). Superficial digital flexor tendon is inserted on the proximal extremity of PII and deep digital flexor tendon inserted on the flexor surface of the (PIII) (2).

Treatment of tendon injuries represents major challenge because of the restoration of normal function of the flexor tendon after injury requires not only the continuity of the tendon fibers, but also the process of gliding between the tendon and its surrounding structures (3). As many other tissues, tendons regenerate from scar tissue accumulation at the injury site. The development of scar tissue between the ends of the tendon provides physical continuity at the interruption point, while the accumulation, and distribution of scar tissue between the tendon and surrounding tissues are undesirable since these attachments restrict tendon gliding ability, and can be the cause of limiting tendon mobility, which is of considerable therapeutic significance (4).

Several studies have recently revealed the use of tissue engineering technologies and their beneficial effects in full-thick tendon and ligament injuries; thus, different forms of biomaterials have been used as developmental technologies (5,6). This study was aimed to verify the effect of lyophilized bovine tunica vaginalis powder on peritendinous adhesion of induced defect in superficial digital flexor tendon in goats.

## Materials and Methods

### Experimental animals.

Eighteenth apparently healthy adult local breed bucks, aged 1-2 years, weighted 20- 25 kg were used in this study. These animals were examined clinically, all bucks were free of lameness based on observation of their walk, inspection and palpation of their tendons. During the test interval all animals were kept under same circumstances and dewormed with Ivermectin (VETOMEC, Holland) administrated subcutaneously at a dose of 0.2 mg/Kg B.W.

### Experimental design.

A total of (18) bucks used in this study, all animals were received the same surgical procedure to create a defect in the superficial digital flexor tendon, these animals divided randomly into two equal groups, Nine animals each according to the experiment duration of Four months and labeled as group 1 (treated group), and 2 (control group), (n=9) for each group.

In the 1st group, the gap filled with tunica vaginalis scaffold powder. The 2nd group, the gap filled with normal saline as control positive group.

### Pre-operative concern.

1. Off food from the bucks for 24 hours and stopped of water for 12 hours.
2. The animals were secured in right lateral recumbence then calmed by intramuscular injection of xylazine (0.5 ml).
3. The left forelimb was surgically prepared (starting from carpal joint reaching the hoof), anaesthetized by subcutaneous linear infiltration of Lidocaine to the palmer surface of the limb (1 ml\ 1 cm length), and tourniquet above the carpal joint to reduce the hemorrhage at the site of operation.

### Surgical procedure.

After draping of the limb, an (5-7 cm length) incision was made in the skin on the palmer surface over the middle third of the metacarpal bone of the left limb, the superficial digital flexor tendon was exposed, a parallel longitudinal incisions (0.1 cm depth, 0.2 cm

width, and 1cm length) full thickness defect was created on the middle part of the tendon (7). The defect was filled with previously prepared biological scaffold as designed.

Closure of the surgical incision includes: suturing of the subcutaneous tissue by simple continuous suture technique using 3-0 absorbable suture material polyglactin 910 (vicryl), and interrupted horizontal mattress for skin incision using non absorbable suture material size 0 Silk.

In all groups that underwent surgery, the operation site was bandaged and the operated limb was immobilized with a plaster of Paris (with window) for two weeks. Penicillin-streptomycin was administered intramuscularly for five days after surgery at doses of 20,000 IU and 10 mg/kg, respectively.

**Preparation of scaffolds.**

The fresh (Testes) were harvested from slaughtered bull, and then transferred by preservative ice bag. Tunica vaginalis was separated from the testes. The final flattened sheets prepared as scaffolds, a decellularization was made as described by (8,9)

The decellularization and disinfection process were made by immersion the sheets in a mixture of 0.1% Peracetic Acid (PAA) and 4% Ethanol solution on a shaker for two hours. After that, the sheet rinsed in Phosphate Buffered Saline (PBS) (pH 7.4) to returned the pH to 7.4, containing 100 IU/ml Penicillin, 100 µg/ml Streptomycin and 100 µg/ml Amphotericin together at 25 °C with trembling, then in two changes deionized water, and finally one change of PBS, 15 min each.

The resulted decellularized sheets scaffolds finally sterilized by immersion in PBS containing antibiotics and antifungal drugs and preserved at 4 °C for five hours.

The powder of the scaffolds after that obtained by transferring the decellularized sheets to −20°C for 24 hours then transferred to the deep freezer at -80°C for 5 days, then lyophilized till it is completely dried, then chopping it into small sheets for immersion in liquid nitrogen and reduced to small pieces by a rotary knife mill. (10) (Figure 1).

**Assessments of study parameters.**

**Electron microscope.** To determine the pore structure, and size of particles.

**light microscope examination.** To ensure complete destruction of cells (decellularization). (Figure 2).

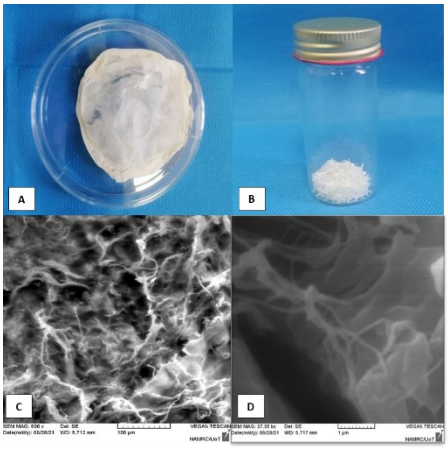


**Figure 1: Bovine tunica vaginalis sample. (A) After lyophilization. (B). After milling. (C) SEM image MAG. Power 500x. (D) SEM image MAG. Power 37.35 kx.**

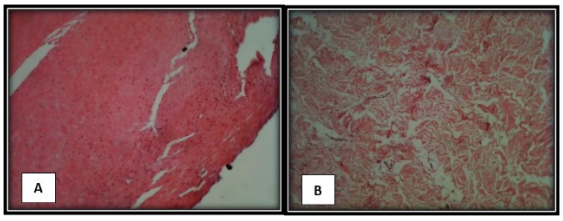


**Figure 2: Bovine tunica vaginalis sample. (A) Before decellularization. (B). After decellularization.**

**Macroscopic evaluation.**

A second surgical procedure for adhesion assessment, which were scored according to the method described by (11), was used to assess fibrous tissue formation between tendon and surrounding tissues.

The evaluation of adhesions was performed at (30, 60 and 120 days) post-treatment through a second surgical operation. For these purposes,

buck was sedated and the site of original operation was clipped, aseptically prepared then anesthetized locally with Lidocaine Hydrochloride. The skin was incised and the operated area were inspected to determine the peritendinous adhesions scores which may happened between the SDFT and surrounding structures which was classified and described by (12) such as (Table 1).

**Table 1: Shows the adhesion scores (9).**

| Scores level | Status |
|---|---|
| 0 | Negative |
| 1 | Thin |
| 2 | Thick |
| 3 | Thick wide spread |

## Results and Discussion

In orthopedics, suitable grafting materials have been investigated as alternatives to tendon repair, as well as new biological grafts have now been proposed for clinical use. Use of decellularized tendon-derived arrays as a biological scaffold for the treatment of tendon ruptures is a promising approach (13).

Adhesions are a common side effect of tendon surgery, resulting in pain, rigidity, and decreased function.

The gross appearance of the SFDT at the time of harvesting biopsies in control group revealed thick to thick and wide spreading of adhesions between the SDFT and the surrounding tissues at 30, 60, while the animals showed thin adhesion after120 days of the study as in figures (3, 4 and 5),

The results of the current study are agreed with (14) Who discovered that adhesions form as a result of inflammatory and thrombotic processes that damage the monolayer of cells placed on the basement membrane in tissues, making them susceptible to fibrin deposition, which leads to further fibroblast attachment and angiogenesis. These issues, combined with decreased fibrinolytic activity, result in the formation of adhesions and the deposition of the organized extracellular matrix (ECM).

Since tendon healing is typically divided into three phases: inflammatory, proliferative, and fibrotic, in which tendon cells and macrophages direct the deposition of the primary matrix, particularly type III collagen; And then there's the remodeling phase as described by (15), External healing, or the infiltration of surrounding fibroblasts during the inflammatory phase, leads to the formation of adhesions. Endotenon and adhesive tendon cell repair, on the other hand, promotes proper healing, prevents adhesion formation, and preserves tendon slip properties (16). Also, these findings agreed with (17) who find that the severity of peritoneal adhesion was found to be higher in the control group than in the other treated groups, which could be due to the severity of the tendon sheath trauma and the gap formation between the severed limbs, which increased adhesion formation.

External healing is the result of the invasion and proliferation of fibroblasts and inflammatory cells from the surrounding tissues and tendon sheath in order to produce a new collagen matrix. Tendon cells and fibroblasts at the site of injury will lead to self-healing, whereas internal healing is the result of the invasion and proliferation of fibroblasts and inflammatory cells from the surrounding tissues and tendon sheath. The extent to which these exogenous cells are involved is determined by the vascular perfusion at the injury site. In general, these external cells will outnumber resident cells, causing tissue to adhere to the repair site, with cell adhesion as a side effect. Intrinsic healing factors are required for adequate vascularization and nutrition with surrounding fluid, the absence of cell adhesion, and the proliferation of resident

cells (18), in the other hand animals of treatment group showed thin adhesion after 30 days, while after 60 and 120 days the adhesion was thin in only one animal (Figure 6) with no adhesion in the rest of animals (Figure 7).

Adhesions in samples of treated groups could be explained by the role of the SFDT surrounding tissue in healing process (extrinsic healing), the results of our study are agreed with other researchers whom says that, although significant morbidity was observed at the donor site, causing tendinitis, pain, and muscle deterioration, flexor tendon grafting procedures will also result in adhesion formation, with all of the previously described negative outcomes such as limited supply (19). Even though the process of tendon graft rebuild is unknown, it is thought that autograft adhesion formation is caused by intrinsic fibrosis (tendon necrosis) and extrinsic fibrosis (influx of synovial cells and inflammation), resulting in high scarring (20, 21). Further research on bovine flexor tendon repair using allografts revealed very little adhesion forming (22). It is frequently observed in autograft tissue, implying that acellular allografts can heal without a self-healing mechanism.

The samples of treated group which showed no signs of adhesion, may be due to resolve of fibrosis occur during the proliferative phase, which then resolved subsequently during remodeling. These results are agreed with (23), as hypothesized, that treated groups showed adhesion at 14-to-28-day post-grafting, the quantity of fibrotic tissue close the tendon is markedly reduced by 42 days.

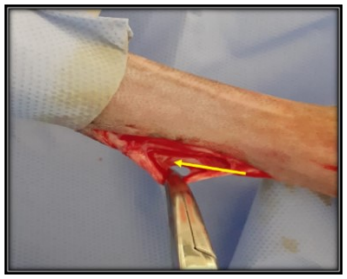

**Figure 3:** Shows macroscopic appearance of SDFT after (30) days of treatment in control group (yellow arrow).

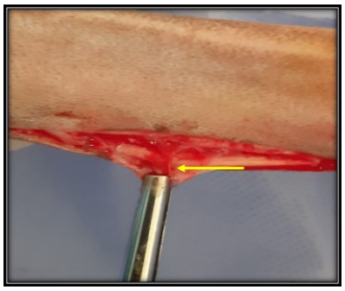

**Figure 4:** Shows macroscopic appearance of SDFT after (60) days of treatment in control group (yellow arrow).

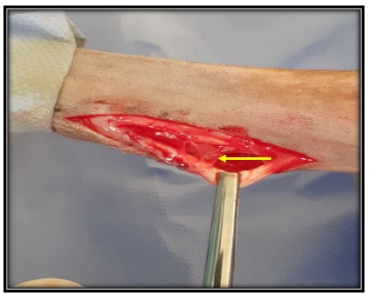

**Figure 5:** Shows macroscopic appearance of SDFT after (120) days of treatment in control group (yellow arrow).

**Figure 6:** Shows macroscopic appearance of SDFT after (120) days of treatment in treatment group (yellow arrow).

**Figure 7:** Shows macroscopic appearance of SDFT after (120) days of treatment in treatment group (yellow arrow).

The (table 2), and (Figure 8) shows adhesion scoring system used in this study, which are revealed that there are no significant differences between groups, while there were significant differences among periods at the same group (P≥0.05).

**Table 2: Comparison between control and treatment groups in Adhesionwith difference period**

| Group | Mean ± SE of Adhesion | | |
|---|---|---|---|
| | 30 Day | 60 Day | 120 Day |
| **Control** | 2.33 ±0.33 | 1.67 ±0.33 | 1.33 ±0.33 |
| **Treatment** | 1.67 ±0.33 | 0.67 ±0.33 | 0.33 ±0.17 |
| **T-test** | 1.308 NS | 1.308 NS | 1.309 NS |
| **NS: Non-Significant.** | | | |

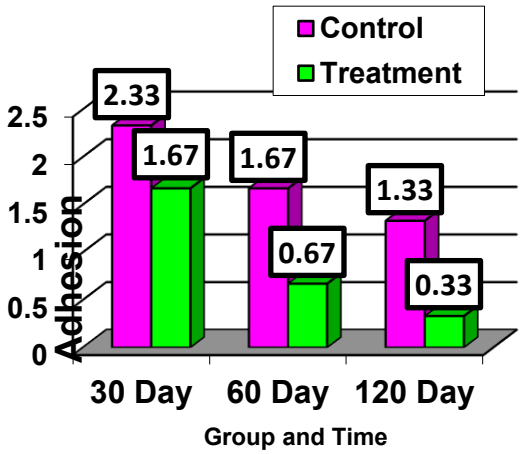


**Figure 8: Comparison between difference Groups and Time in Adhesion**

## Conclusion

From all the results obtained from this research concluded that the tunica vaginalis scaffold has no obvious effect on decreasing adhesion between tendon and surrounding tissues.

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