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IJPBS |Volume 4| Issue 3|JUL-SEPT|2014|11-23

Research Article
Biological Sciences

EFFECTIVENESS THE HYALURONIC ACID AFTER TENOTOMY AND TENORRHAPHY OF SDFT IN DONKEYS

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ABSTRACT

Eight healthy male donkeys were used, they were divided into two groups (control and Hyaluronic acid group), were anesthetized with (Ketamine 3 mg/kg B.W and Xylazine 0.5 mg/kg B.W) administered intravenously. Clinically observations were recorded after recovery from anesthesia. The biopsies were taken from each group at 30 and 60 days postoperative for histopathological examination. Histologically the tendon of control group was reveals vacuolated and slightly new blood vessels and present oedema, that is on 30 days, but on 60 days there was vacuolated tendon cells with infiltration of inflammatory cells .on 30 and 60 days in HA group, was showed new blood vessels and there was present collagen fibers and increase in fibroblast and tendinocytes. The sonography observation in control group on 30 and 60 days showing moderate fluid in the SDFT and absence of normal fibers and short croppy fibers. In HA group on 30 days the images indicates moderate fluid of the SDFT and showing fiber disruption and enlargement of DDFT. And showing lesion has a scar tissue in center of tendon that was on 60 days. The conclusion, that appear all results were recorded in HA group was the best from control group due to short time to healing of SDFT because the functions and advantages of HA.

KEY WORDS

Tendon, sonography, Hyaluronic acid, Donkeys.

INTRODUCTION

A hyaluronic acid is highly hydrophilic; it is a polymer that is well suited to applications requiring minimal cellular adhesion. Postoperative adhesions, which form between adjacent tissue layers following surgery, impede wound healing and often require additional surgical procedures to berepaired successfully. Barriers made from cross-linked HA have been effectively used to prevent such adhesions from forming. Furthermore, the adhesion of bacteria to biomaterials can induce infections and constitute a great risk to the patient; with this in mind, esterified HA has also been used to prevent bacterial adhesion to dental implants, intraocular lenses, and catheters (1).

Improvement in the weight bearing capacity together with reduction in the tissue swelling and adhesion following HA therapy could be due to the pain relief (2), anti- inflammatory (3) and inhibitory effects of HA on peritendinous adhesions. These beneficial effects could facilitate the structural organization and may result in improved mechanical properties of the injured tendons. These findings are in accordance with the previously reported investi- gations (4).

Hyaluronic acid is one of the primary components of the extracellular matrix that plays an active role in wound healing and contributes to cell migration and proliferation (5), Common sources of exogenous hyaluronic acid are the rooster comb and bacterial fermentation (6).

This study was conducted to evaluate effects of HA on tendon regeneration of SDFT in donkeys.

MATERIALS AND METHODS

A total of 8 donkeys of both sexes, age of 2–3years with body weight of 60–140 kg (mean ±SD, weight 97.77 ± 8.11 kg), were used for this study. Donkeys were purchased from different localities of Basra city. These animals were examined clinically and by ultrasonography to ensure that they presented no preexisting tendon damage and to exclude the presence of any locomotors disorder. Animals were kept at the Veterinary animal house, Basra University, Iraq, and were fed on a straw and bran, had free access to water. Two weeks before the start of the experiment, all animals were dewormed. During the entire experimental period, all animals were kept under similar management and feeding practices.

Donkeys were divided randomly into two groups (four of each), one of them was used as a control group, and tendons were tenectomized and lifted for healing without applied any material to serve as control. While the other as hyaluronic acid group was put in the site of severed tendon to the second treatment group Sedation was inducted via intravenous injection of xylazine Hcl, (at 0.5 mg/kg.) Then, the animals were generally anesthetized using the ketamine hydrochloride at the dose (3 mg/kg) administered intravenously into the jugular vein (7).

In control group a 10-12-cm mid-metacarpal linear skin incision was made over the plantar aspect of the right fore- limb, and the paratenon was longitudinally incised for exposure of SDFT. Blunt dissection was performed to separate the SDFT from the deep digital flexor tendon. When this has been achieved, curved artery forceps were inserted under the superficial tendon to expose it. A stay suture was done at the proximal side of the SDFT, under the carpal joint, using nylon thread No 2.0 to prevent slipping the tendon upward and this was considered as the first stitch of tenorrhaphy. The superficial digital flexor tendon then was transverse cutting with the scalpel. The cut ends of the tendon were approximated by Bunnell-technique suture using nylon suture material size (2.0), and Skin closure was accomplished using silk suture material size (2.0) in a routine manner (Fig -1). The site of operation was casting with window, removed after 30 days from operation in last two periods, while in first period within 14 days after operation for the removal of skin suture and assessment of clinical parameters.

In hyaluronic acid treated group the same technique mentioned in control group except the application 2 ml of sodium hyaluronate, on the sites of tendon anastomosis.

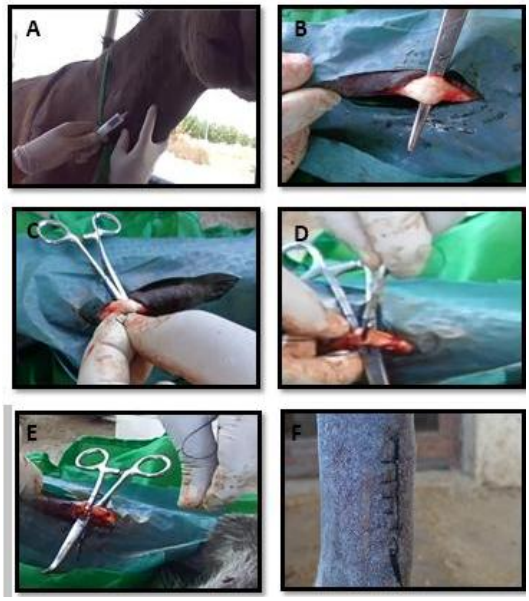

Fig.1: SDFT tenorrhaphy (A) I/v injection of anesthetized material(B) Mid metatarsal incision Including the skin, s/c and paratenon (c) for exposure of the SDFT and curved artery forceps were Inserted under the superficial tendon to expose it,(C) A stay suture was done at the proximal side of The SDFT and this was considered as the first stitch of tenorrhaphy, (D)

The transverse cutting of SDFT with the scalpel, (E)The cut ends of the tendon were approximated by Bunnell-te1chnique suture using nylon, (F) Skin closure was accomplished using silk suture material.

Ultrasonographic examinations of SDFT were carried out just before the surgical interference, and at 30 and 60 days postoperatively using 6.5 MHz mechanical linear scanner. For this purpose, donkeys were prepared by clipping and shaving of the hair at the area that needed to be examined. Scanning of the limb was done from just distal to the carpal joint to the level of the proximal sesamoid bones.

Ultrasound transmission gel was liberally applied at the site to prevent air trapping between transducer and the skin surface. Longitudinal and transverse scans were obtained for the examined tendon at each specific time point to observe the different echogenic texture, fibers alignment as healing indicators and any other soft tissue changes.

RESULTS

Soon after the animal had recovered from sedation we noticed the following they stand and walk with an obvious lameness, which continued for 5-7 days, then they suppressed gradually on 8th-10th days and animals showed normal gait.

Histological section on 30 days reveals vacuolated with swelling of cells and slightly new blood vessels and there is empty space of edema (Fig. 2 and 3). On 60 days, there was vacuolated tendon cell with infiltration of leukocyte and presence of fibroblast as well as there is new blood vessels are formed when staining with van Gieson (Fig.4 and 5)

In HA group, on 30 days, show numerate new blood vessels and there is vacuolated of tenocyte and there is collagen fiber compare with control which they are color with red by Van Gieson stain that clear in figure (6 and 7).but on 60 days, histological section reveals increase leukocyte with fibroblast for healing normal tissue (Fig. 8 and 9)

Ultrasound image in control group, on 30 days, showing the presence of moderate fluid in SDFT and absence of normal fiber pattern, and short, croppy fiber (Fig. 10) and the hole where the tendon fibers are injured over time this area of fiber damage will fill in fluid ((Fig. 11). On 60 days, there is moderate fluid and the large anechoic (black)area within the SDFT (Fig. 12) and the black area is a core lesion are a common section where the Centre of the tendon has a visible hole. (Fig. 13)

The Hyaluronic acid group, on 30 days, the Ultrasound image indicates moderate fluid of the SDFT appear as dark aria (with enlargement of DDFT (Fig. 14) and showing fiber disruption and enlargement of the DDFT (Fig. 15) On 60 days, there is mild fluid the superficial Digital flexor tendon, separation

of fiber plus a hole was found in the SDFT, with enlargement of DDFT (Fig. 16) and indicates core lesion has a visible hole where the center of the tendon nearly from the edges of the SDFT (Fig. 17).

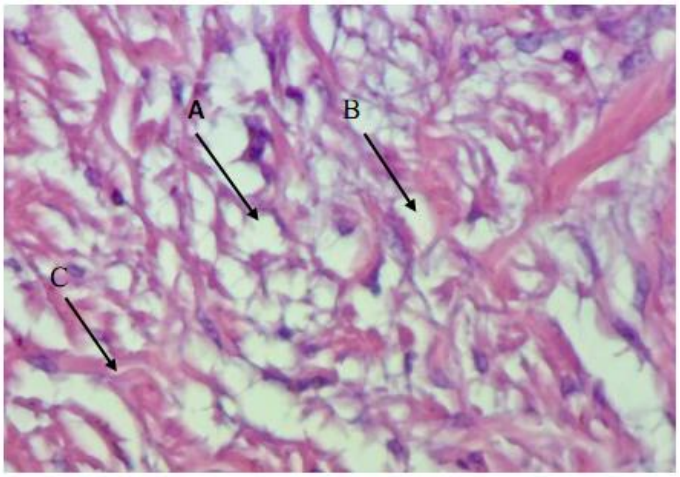

Fig. 2: Histopathlogical section in the SDFT of control group at 30 days postoperatively showing (A) tendon fiber, (B) edema, (C) collagen fiber (Van-Gieson stains 10 X).

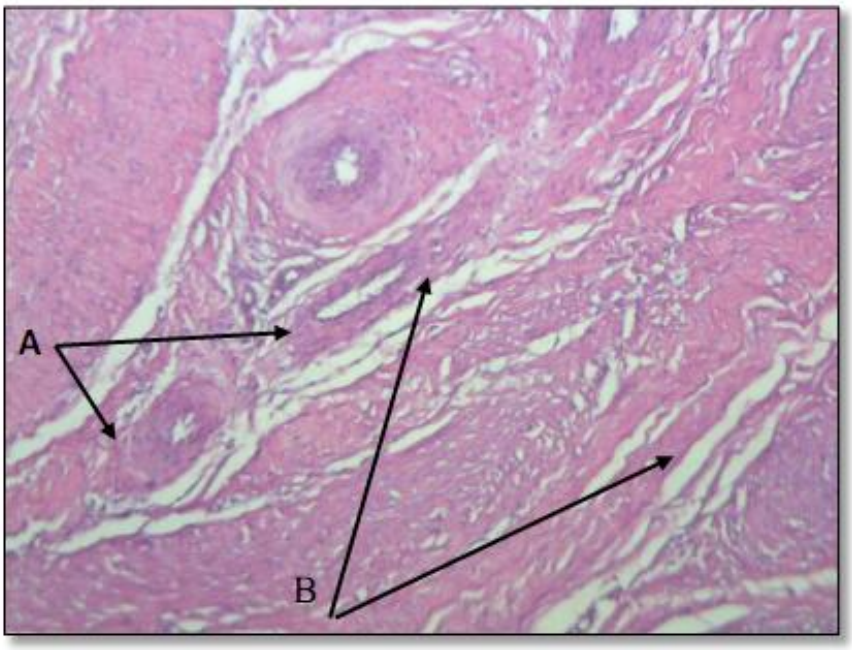

Fig. 3: Histopathlogical section in the SDFT of control group at 30 days postoeratively showing (A) new blood vessels formed, (B) edematous fluid (H&E)

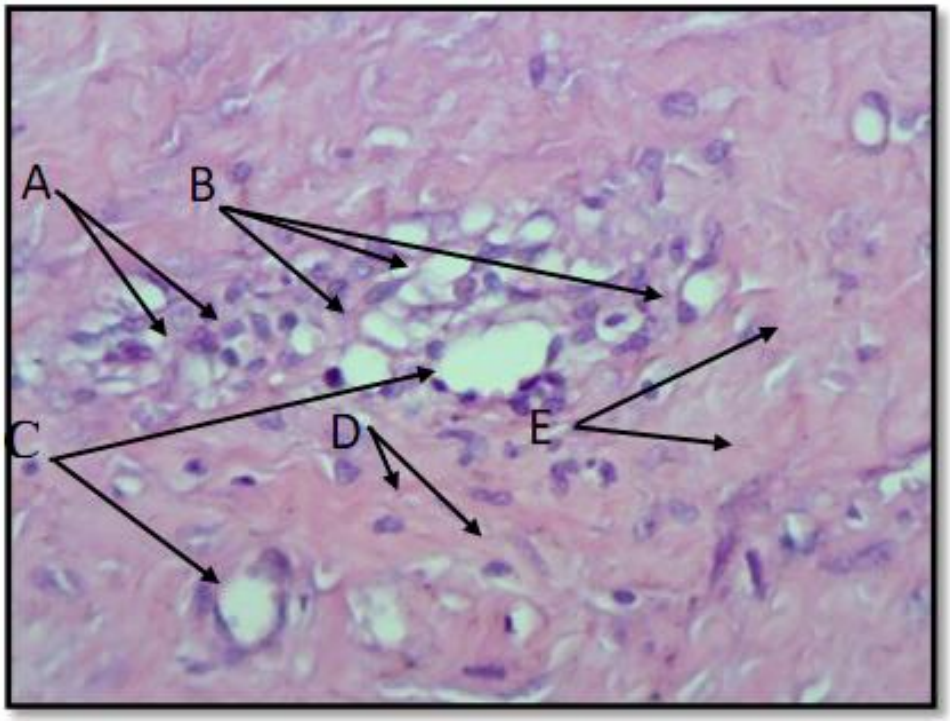

Fig. 4: Histopathlogical section in the SDFT of control group at 60 days post-surgery (A) leuckocyte, (B) vacuolated tendenocyte, (C) new blood vessels formed, (D) fibroblast, (E) collagen fiber (Van-Gieson stains 40 X

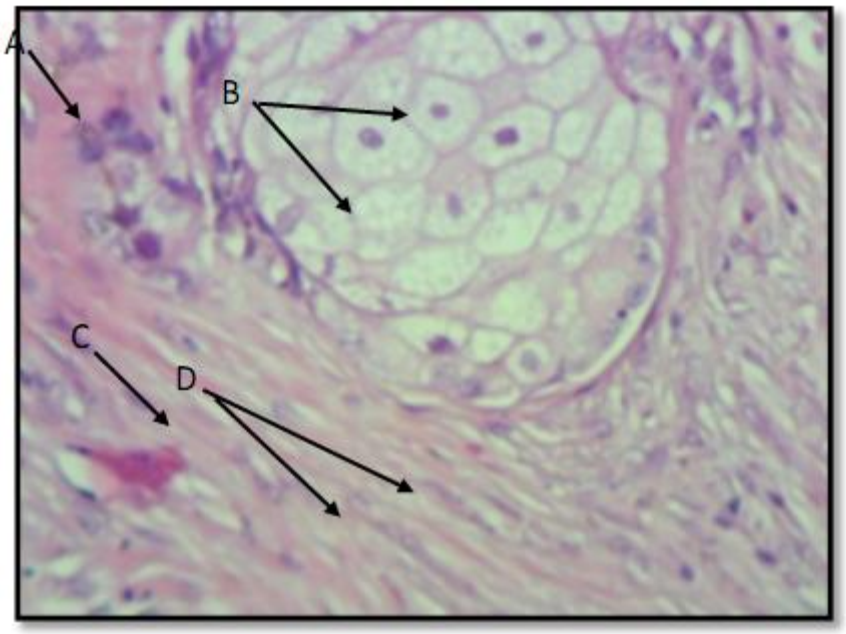

Fig. 5: Histopathlogical section in the SDFT of control group at 60 days post0peratively showing (A) leukocyte, (B) swelling of cells, (C) collagen fiber, (D) fibroblaste (H &E stains 40 X).

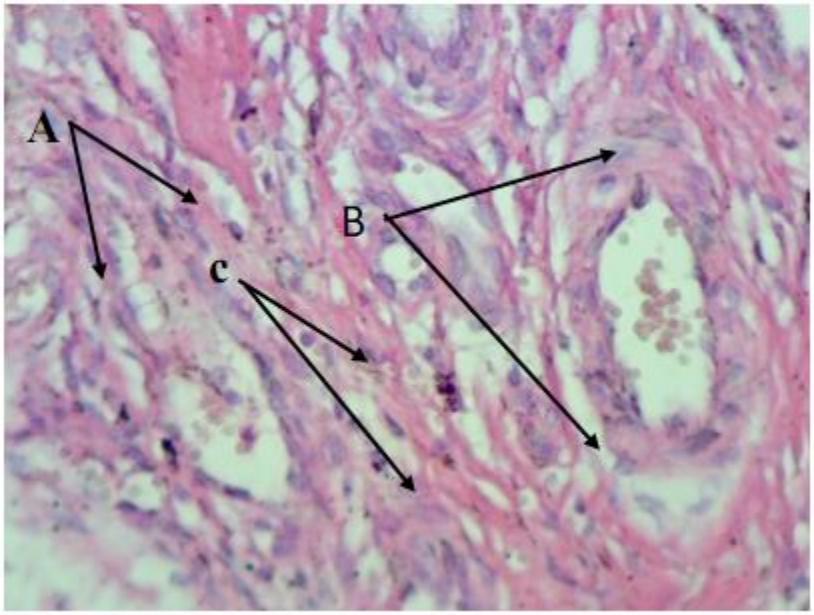

Fig. 6: Histopathological section in the SDFT in the hyaluronic acid treated group at 30 days postoperatively showing (A) vacuolated of tenocyte ,(B) vascularization, (C) collagen fiber (H&E stain 10X).

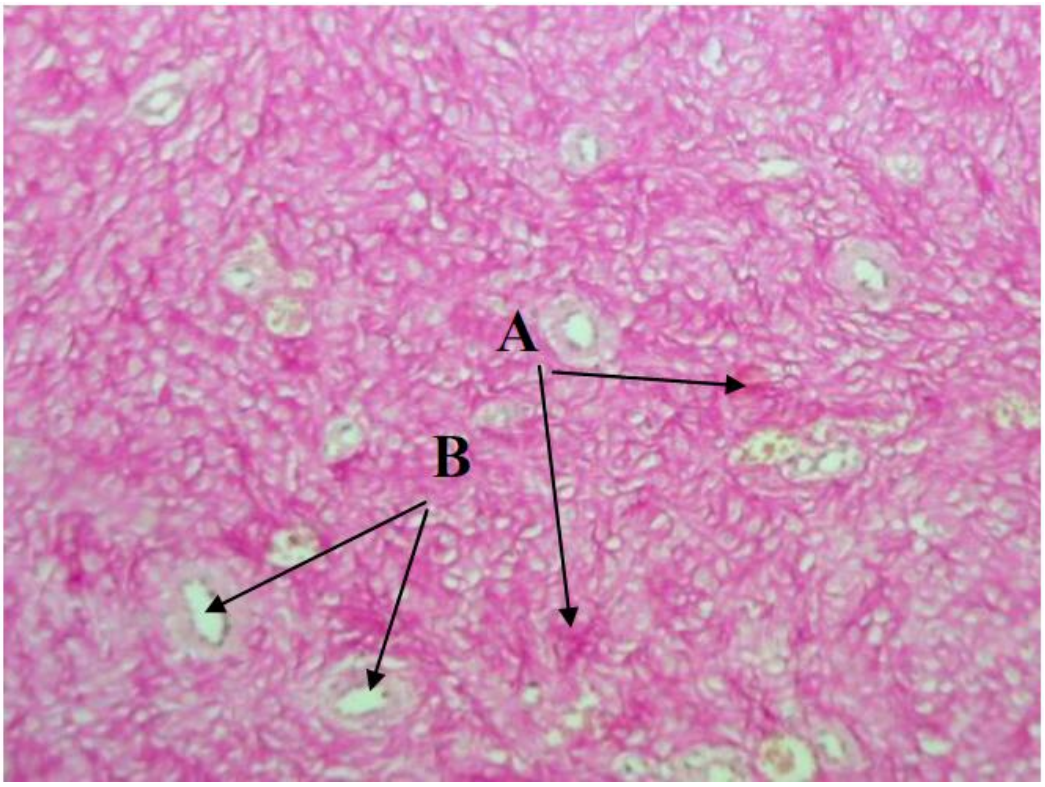

Fig. 7: Histopathological section in the SDFT of the hyaluronic acid treated group at 30 days postoperatively showing (A) moderate increase of collagen which take red color, (B) blood vessels (C) edema (Van-Gieson stains 10 X).

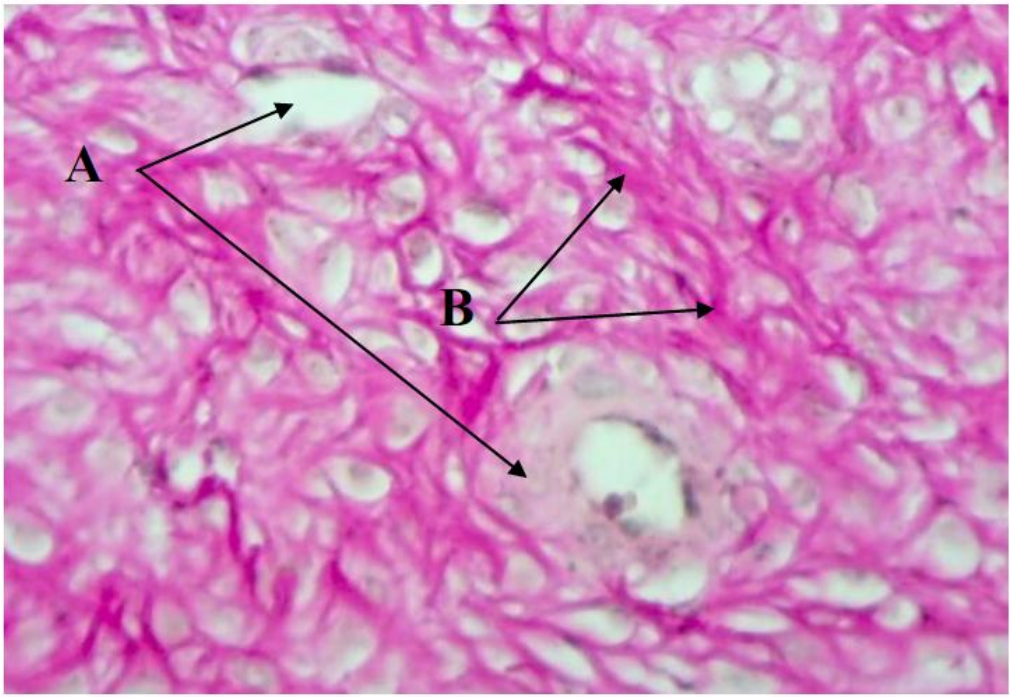

Fig. 8: Histopathological section in the SDFT of the hyaluronic acid treated group at 60 days postoperatively showing, (A) new blood vessels, (B) infiltrational leukocyte(C) vacuolated cells (H&E stains 10X).

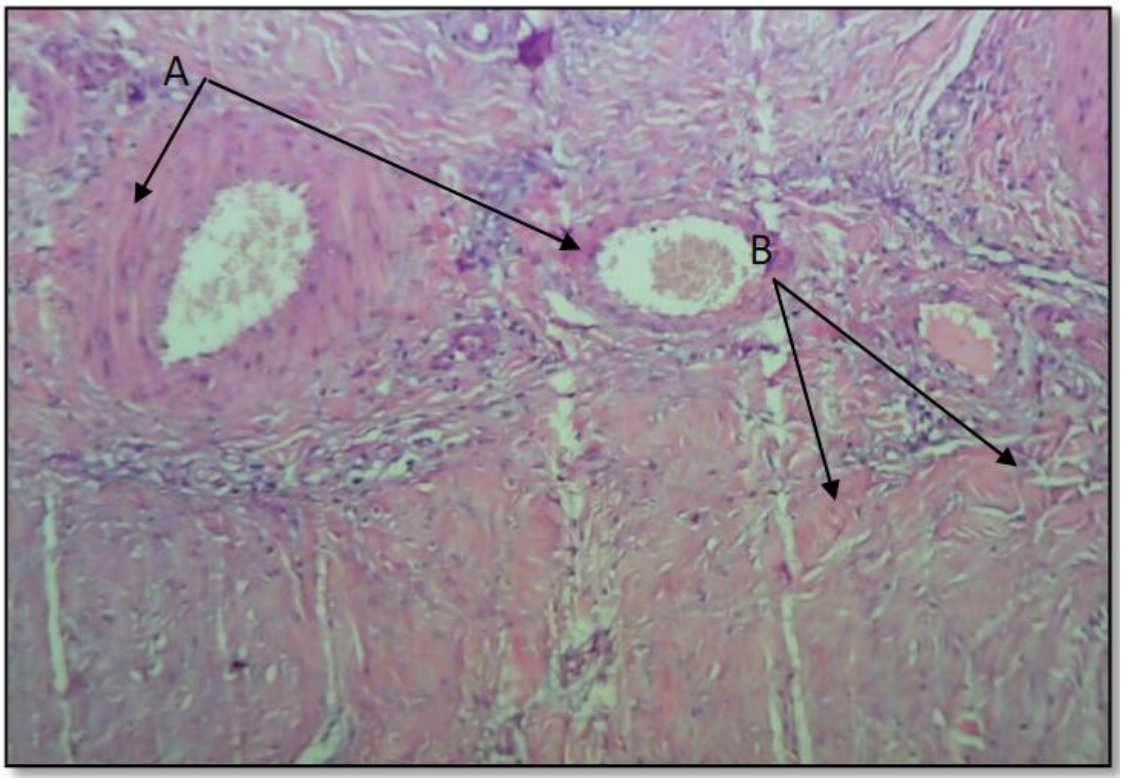

Fig. 9: Histopathological section in the SDFT of the hyaluronic acid treated group at 60 days postoperatively showing (A)infiltrational fibroblast , (B) edema, (C) leukocytes (D) vacuolated of cells ((Van-Gieson stains 40X)

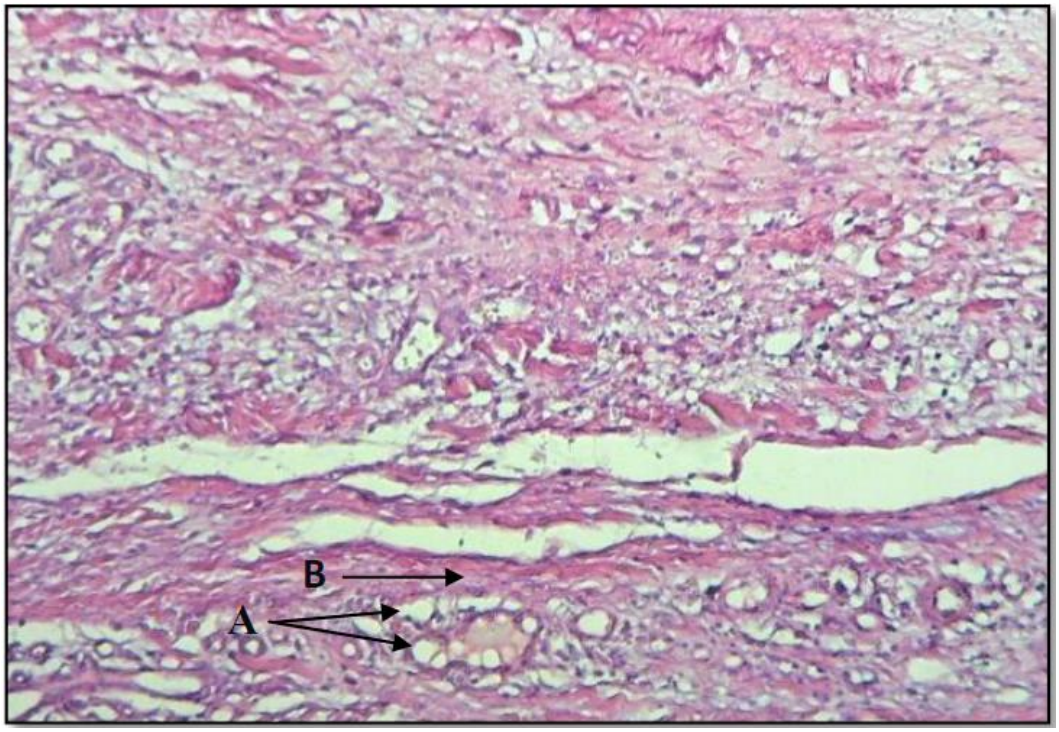

Fig. 10: Ultrasound image(longitudinal) in the control group at 30 post operatively showing the presence of moderate fluid in SDFT and absence of normal fiber pattern, and short, croppy fiber.

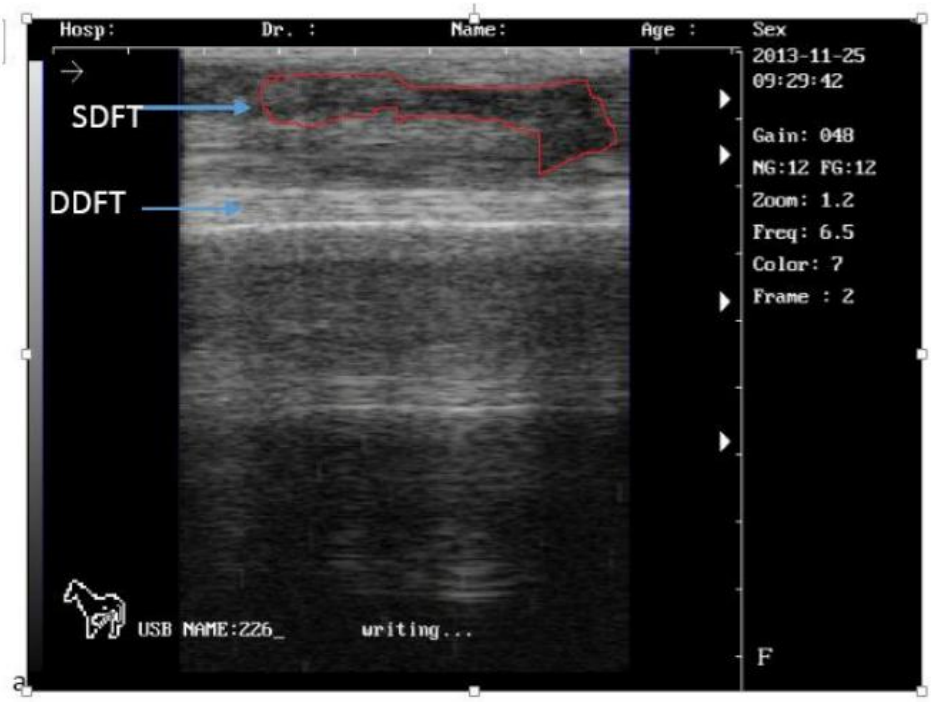

Fig-11:: Imaged (Cross section) of a superficial digital flexor tendon of control group at 30 days post operatively indicates the hole where the tendon fibers are injured (Red) ,over time this area of fiber damage will fill in fluid (A).

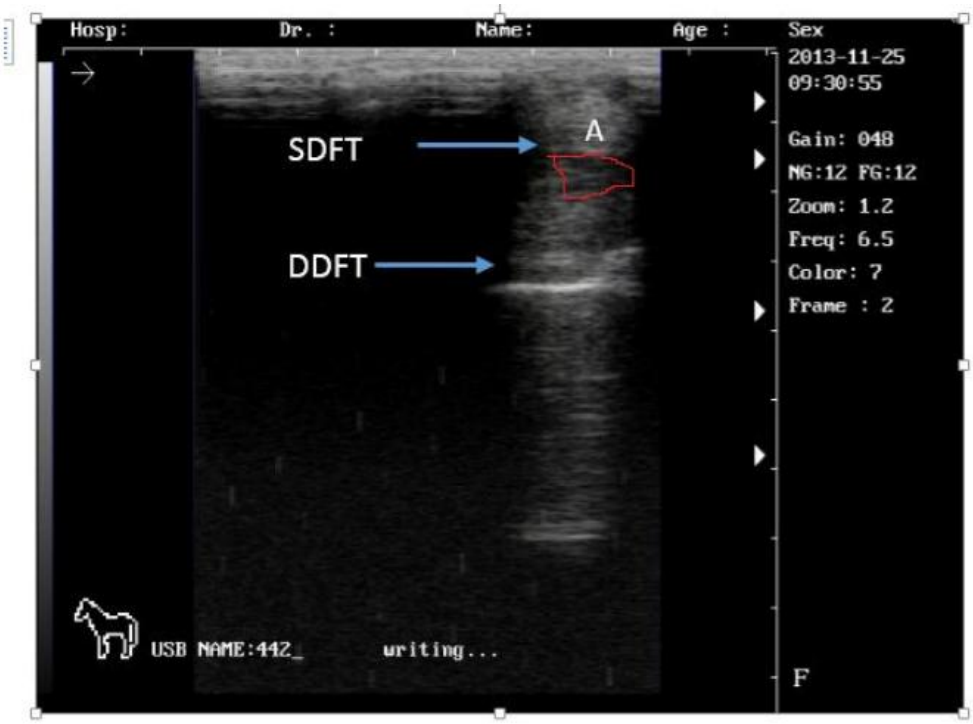

Fig. 12: Longitudinal image of a superficial digital flexor tendon of control group at 60 days post operatively indicates moderate fluid and the large anechoic (black) area within the SDFT(Red).core lesion(A).

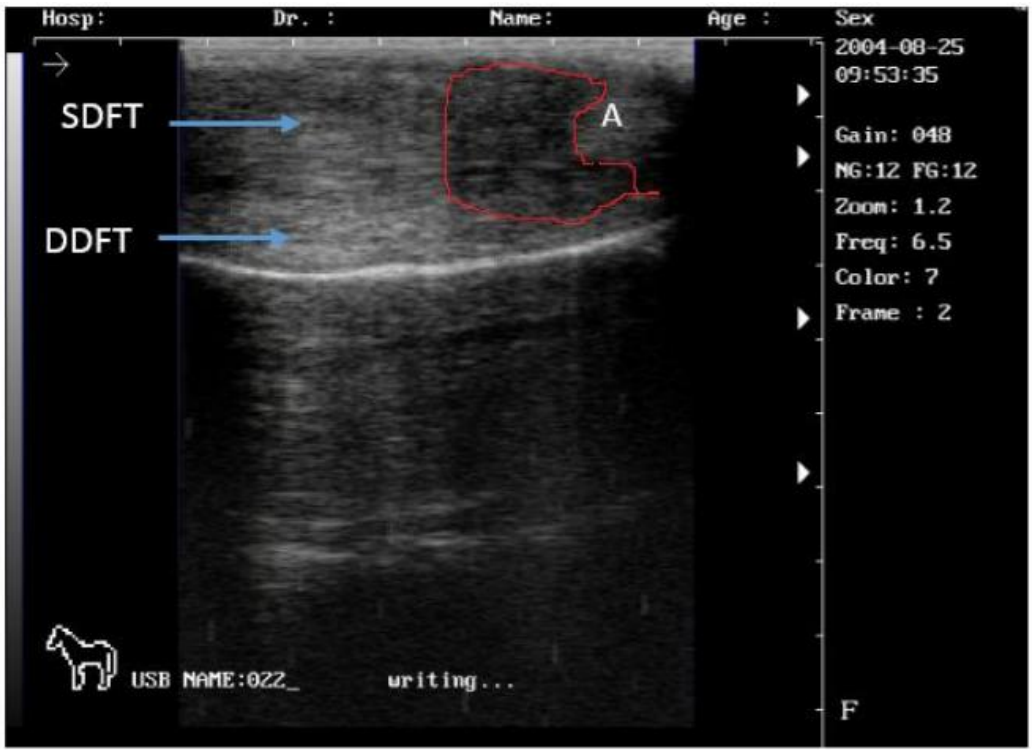

Fig. 13: Images(Cross section) of a superficial digital flexor tendon of control group at 60 days post operatively indicates ,the black area is a core lesion are a common section where the Centre of the tendon has a visible hole(Red).

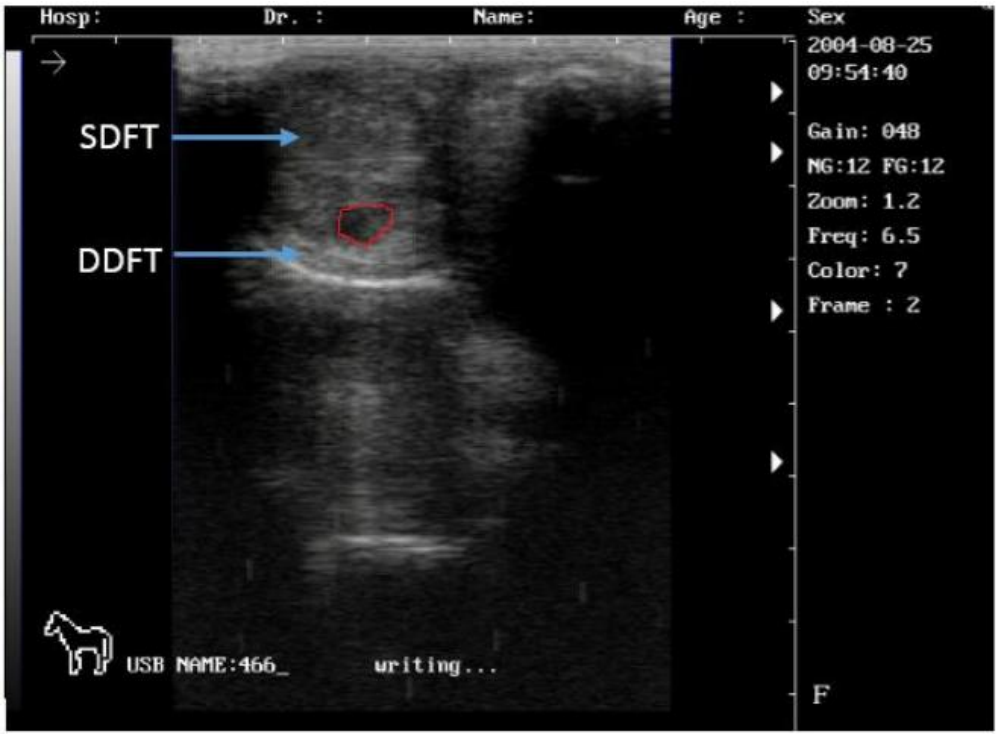

Fig. 14: Ultrasound longitudinal image tendon of hyaluronic acid treated group at 30 days post operatively indicates moderate fluid of the superficial Digital flexor tendon appear as dark aria (Red),with enlargement of DDFT (yellow arrow).

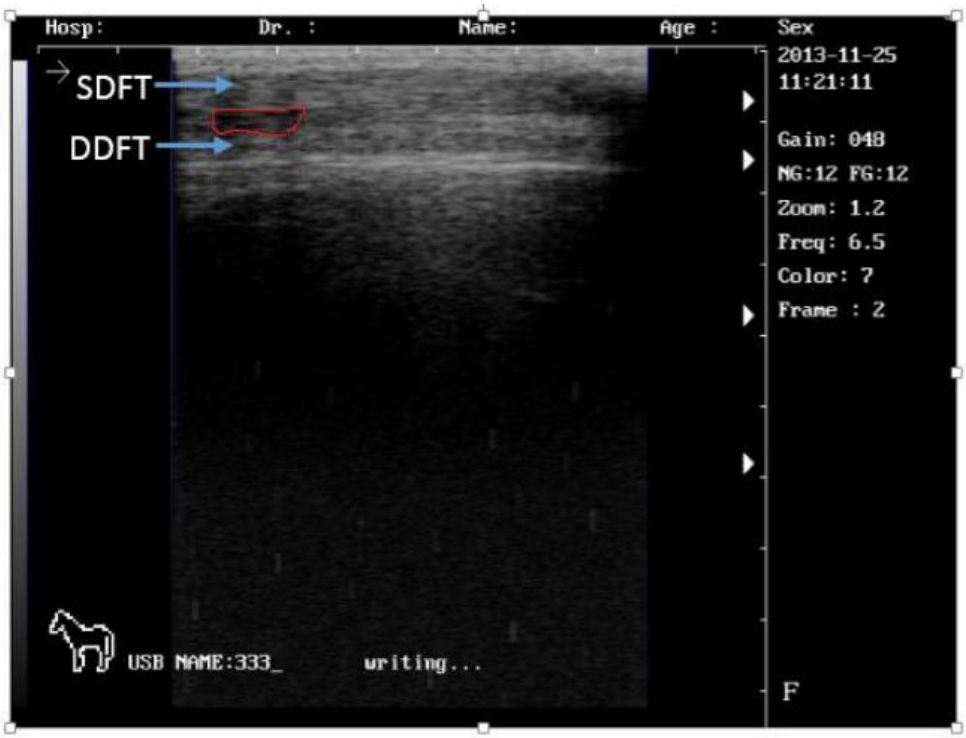

Fig. 15: Ultrasound image cross section of SDFT of hyaluronic acid treated group at 30 days post operatively showing fiber disruption and enlargement of the DDFT(indicated with yellow arrow), moderate fluid in SDFT(Red).

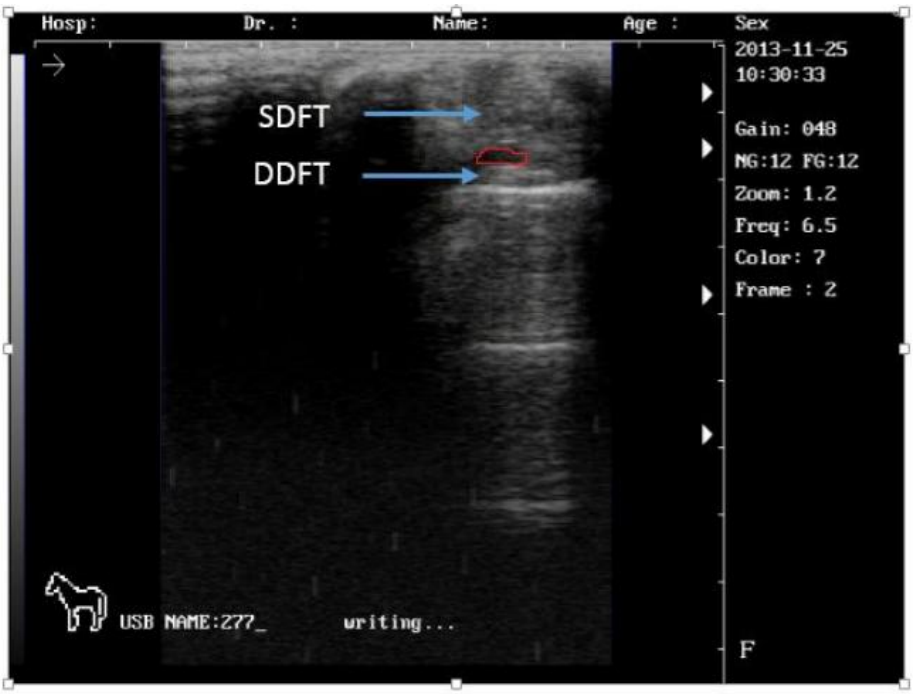

Fig. 16: Ultrasound longitudinal image tendon of hyaluronic acid treated group at 60 days post operatively indicates mild fluid the superficial Digital flexor tendon (green), separation of fiber plus a hole was found in the SDFT(Red),with enlargement of DDFT (yellow arrow).

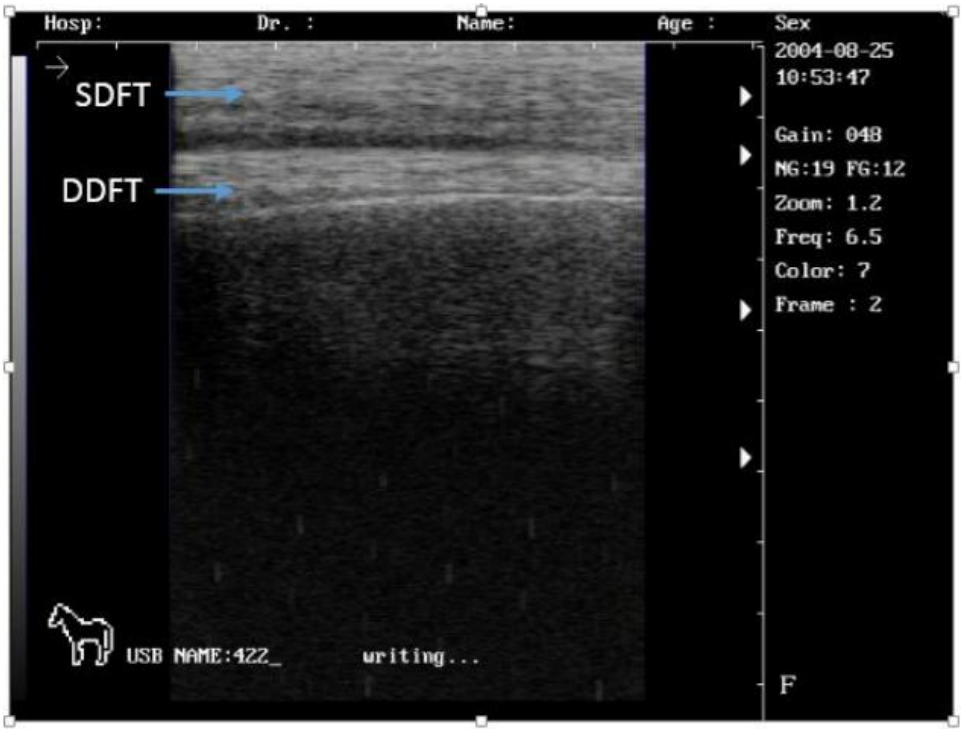

Fig. 17; Ultrasound image cross section of SDFT of hyaluronic acid treated group at 60 days post operatively indicates core lesion has a visible hole where the center of the tendon nearly from the edges of the SDFT(Red).

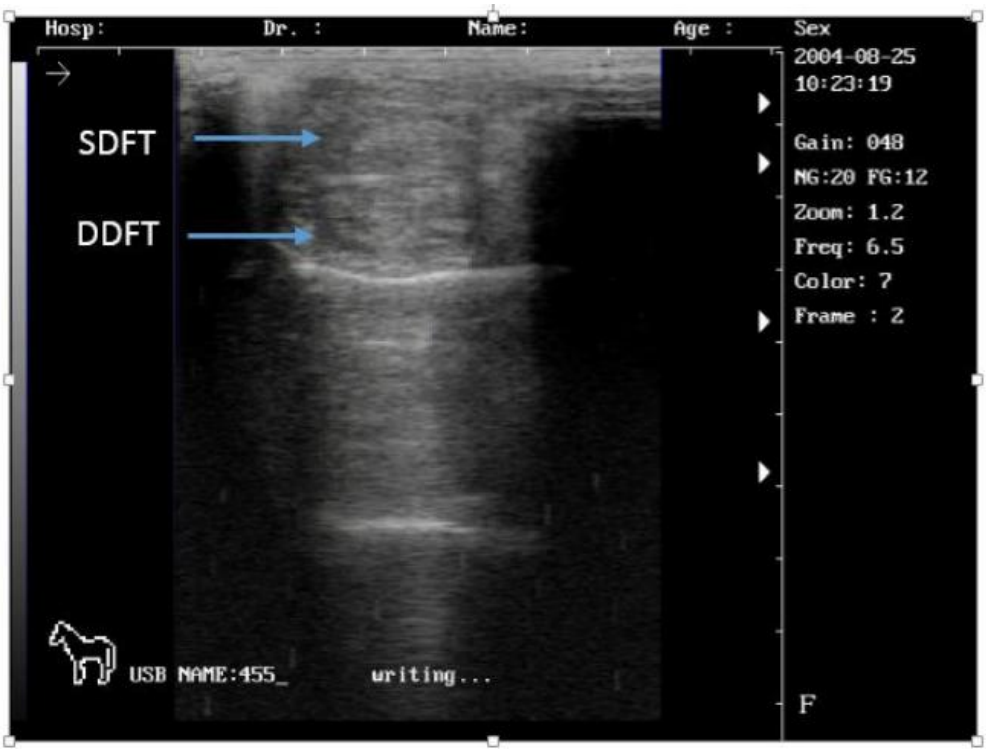

Fig. 18: Cross section of tendons of bone marrow treated group at 60 days post operatively showing normal tendons and ligaments demonstrate a homogenecity echogenic (evenly white) appearance on ultrasound when viewed on cross-section) fiber pattern is evaluated by placing the transducer parallel to the tendon ligament fiber.

DISCUSSION

The present study explored the effect of HA gel with an increased tissue residence time) on postoperative adhesion formation and repair healing in an tentomoy and tenorrahapy of SDFT in donkeys. Most importantly, our results show that healing of flexor tendons after primary tendon repair can be accelerated by an HA gel, without increasing adhesions. This was demonstrated by a significantly faster restoration of tendon breaking strength compared to tendons which were control group, that is agree with (8).

In previous reports, stimulatory effects of exogenous agents, like hyaluronic acid, on aspects of tendon healing have been proposed. However, we believe that this is the first study, to our knowledge, to demonstrate a significant effect on the healing of end-to-end tendon repairs.

According to previous reports, HA may stimulate wound healing in several ways. It has been shown that, during the early inflammatory phase of wound healing, a high concentration of HA in the wound matrix leads to increased infiltration and proliferation of cells in this area. This is consistent with our histological findings of a more disorganized appearance and a significantly increased cellular density in the area of repair in HA gel-treated compared with control group at 30 and60 days postoperatively. The present study about the results for histopathology I control about vacuolated and swelling cells and edema on 30 and 60 days as infiltration of leukocytes and fibrocytes and appear new vascular blood vessels, but in HA group on 30 and 60 days as present new blood vessels appear early and tenocytes as well as appear collagen fibers this is due to density of tendinocytes in the SDFT was the largest and that is proliferation assays of collagen production and collagenase synthesis, that agreement with (9). About the role of HA stimulatory effect on cytokines such as IL-1B and stimulate fibroblast and fibroblast-derived cells to synthesis > The different between the control and HA groups about incomplete collagen and edema in control but vacuolated is present and new blood vessel in HA is early establishment, that lead to progressively improved with time scince remodeling of tendon need at least 3-6 months to have a complete healing that is in control group , that agree with (10), but when added the HA that is accelerating healing through enhance the proliferation the tendinocytes and proliferation the collagen fibers in early stage. In sonography evaluation the results of healing the SDFT by detect fine textural abnormalities of these structure and also recorded dynamic evaluation of structures the artifacts that may be mimic tendon , injury and tendon disorder tears or tendonitis as well as tenosynovitis, that these results appear in our study and agree with (11 and 12).

The conclusions of the our study that is the HA group is the best results due to the characterization of this agent through the glycosaminoglycans are made in the cells Golgi networks ,naturally synthesized by hyaluronan synthesis enzymes, also HA capable of interacting with a number of receptor resulting in the activation of signaling cascades that influence cell migration ,proliferation and gene expression, and due the highly hydrophilic, polymer that is well suited to applications requiring minimal cellular adhesion to prevent adhesions that is agree with (14).

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