Evaluation of Polysulphated Glycoaaminoglycan and Dexaphenylarthrite in Treatment of Induced Tendonitis in Donkeys

Awad, M. A.*; Hassan, M. M.*; Saleh, Sh. A. **; Abuzeid, S. M.***; El-Daharawy, M.H.* and Hanan, A. H*.


Abstract
The study was carried out in the superficial digital flexor tendon (SDFT) of the right forelimb of nine clinically apparently donkeys for evaluation the efficacy of Polysulfated Glycosaminoglycan (PSGAG) and Dexaphenylarthrite in treatment of artificial induced tendonitis. To achieve this goal, clinical, ultrasonography, biochemical and histopathological examinations were concluded. Tendonitis of the SDFT was induced by using bacterial collagenase enzyme from Clostridium histolyticum. Donkeys were classified into three groups. Group I (after induction of tendonitis they left as control group= 3 donkeys). Group II, 3 days after induction of tendonitis, they received five IM injections of 5ml PSGAG (100 mg/ml) every four days. Group III, 3 days after induction of tendonitis, they received Dexaphenylarthrite in a dose of 20 ml/day for the first two days followed by 10 ml/day for the other three days. From this study, it can be concluded that PSGAG had beneficial effects in treatment of artificial induced tendonitis in donkeys rather than Dexaphenylartrite based on clinical, ultrasonography, biochemical and histopathological findings.

Introduction
Superficial digital flexor (SDFT) tendonitis is the most common cause of lameness that decreased performance and resulted in significant economic losses in the equine industry (Carvalho et al., 2013). This problem is characterized by high incidence, as it represents about 8-43% among the musculoskeletal injuries in equines (Bazzano et al., 2013). Most of affected horses sustaining re-injury and get early retirement while a few number of the affected horses were returned successfully to racing after a long period of layoff and rehabilitation lasts from 6 up to 18 months (Zuffova et al., 2013). The diagnostic approach to flexor tendonitis of the equine depends mainly on physical examination with palpation, ultrasonography, thermography, radiography, nuclear scintigraphy, computed tomography
or magnetic resonance imaging (Karlin, 2010). However, different types of treatments are available, none of these therapies results in complete tissue regeneration and the great problem is recurrence of injury after a short period of time that may result in an unexpected end to the horse’s athletic career (Guercio et al., 2015).

The present study was performed to compare between the efficacy of Polysulfated Glycosaminoglycan (PSGAG) and Dexaphenylarthrite in the treatment of artificial induced tendonitis in donkeys. To achieve this goal, clinical findings, ultrasonography, biochemical analysis and histopathological examination were concluded.

Materials and methods

This experimental study was carried out at the Department of surgery, Anesthesiology, and Radiology, Faculty of Veterinary Medicine, Suez Canal University after approval by the Committee of Animal Welfare and Ethics, Faculty of Veterinary Medicine, Suez Canal University. Nine apparently healthy donkeys aging from 2 to 3 years old with body weight of 130 to 140 Kg were used in the present study. After animal sedation with Xylazine (Xylaject- ADWIA Co., Egypt) 0.5 mg/kg by the intravenous route. Palmar metacarpal region of the right forelimb in a donkey was aseptically prepared. The SDFT was palpated and fixed bacterial collagenase solution (C0130, Sigma-Aldrich) (2.5 mg/ml) using a hypodermic needle into the core of the tendon in the palmar midline midway between the carpal and the metacarpophalangeal joints. The needle was removed and a sterile bandage was applied from the proximal metacarpal region to the metacarpophalangeal joint according to Nixon et al. (2008).

After induction of tendonitis, animals were divided randomly into three groups; each one consisted of three donkeys. In group I, animals were left as a control group without treatment. In group II, animals were injected with 5ml PSGAG (Ichon-Kinetic – Lexington) (100 mg/ml) every four days for five times. In group III, animals were injected with Dexaphenylarthrite (Vetoquinol-France) in a dose of 20 ml/day for the first two days followed by 10 ml/day for the other three days. Animals were evaluated on the following points:-

Clinical examination: -
Each donkey was clinically evaluated depending on (lameness degree, local temperature, pain on palpation and swelling). These examinations were performed 24 hours post induction and continued till the end of study (four weeks). The degree of lameness was reported and rated according to Stashak (2002).

Ultrasonography examination:-
The SDFT at the palmer metacarpal aspect of the fore limb was scanned
using (Pie scanner 100 LC Philipsweg 1 6227AJ Maastricht Netherlands) Ultrasonography Machine with a 6 – 8 MHz Linear transducer of 4 cm Foot-print. Sagittal scanning were performed before injection of collagenase enzyme and at the 3rd and the 7th days post induction, then weekly till the end point of the study.

**Biochemical examination:**
Synovial fluid was collected from digital flexor tendon sheath (DFTS) by using axial sesamoidean approache (ASA) to the DFTS as it was described by Hassel et al. (2000). Serial synovial fluid samples were obtained from the same site before induction of tendonitis (Zero time) time and 24 hours, 3rd, 7th, 15, 21, 30 days after induction of tendonitis. Lactate dehydrogenase (LDH) and C-reactive protein (CRP) levels were determined by using calorimetric method after Cabaud et al. (1958). Glucose concentration was determined by using calorimetric method after Torlotin (1966). Total protein was determined by using calorimetric method after Kuksis et al. (1968).

**Histopathological examination:**
Histopathological specimens were taken from the SDFT. Specimens were immediately fixed in 10% buffered formalin, routinely processed, sectioned and stained with Hematoxylin and Eosin (H&E) after Jones et al. (2008).

**Statistical analysis:**
Data of the present study were analyzed using Two-way Analysis of Variance (ANOVA) procedures according to (Snedecor and Cochran, 1989) for testing of significance between the studied groups. Means separation and pairwise comparisons were done by Duncan's Multiple Range test according to (Duncan, 1955). Statistical analyses were conducted with SPSS for Windows (SPSS version 20). Results are considered significant at probability level of 0.05 for each (P ≤ 0.05).

**Results**
Donkeys were apparently healthy depending on general physical examination before induction of tendonitis. Twenty four hours, post induction of tendonitis, these animals showed signs of inflammation which were represented by hotness, pain on palpation, swelling at the site of injection and lameness. Hotness and pain on palpation were disappeared on the 5th day post induction in all groups. Mild swelling at the injection sites was noticed 24 hours in all groups. This swelling, increased on the 3rd day post injection (Fig.1). At the end of the experiment, donkeys in group I showed severe swelling at the induction site. On the other hand, swelling disappeared from all animals in group II and group III. All animals in all groups showed lameness with mean degree (2.67±0.33) 24 hours post
induction. In group I, mean degree of lameness increased on the 3rd day post induction (3.67± 0.33) till reached (4±0.001) on the 7th day post induction and continued till the 2nd week post induction. After that it began to decrease on the 3rd week (3±0.001) till reached (2.67±0.33) on the 4th week post induction. In group II, mean degree of lameness showed a significant increase on the 3rd day post induction (4±0.001) then it began to decrease on the 7th day post induction (1.67±0.33) till it reached (1±0.001) on the 2nd and the 3rd week post induction and lameness disappeared on the 4th week. Group III, showed a significant increase in lameness degree on the 3rd day post induction (4±0.001) then it began to decrease on the 7th day post induction (2.67±0.33) till it reached (2±0.001) on the 2nd week while on the 3rd week it reached (1±0.001). On the 4th week post induction lameness mean degree was (0.67±0.33) as illustrated in (Fig.2).

Ultrasonography imaging of the normal SDFT, showed a uniform hyper-echogenic parallel pattern (Figs. 3A, 4A and 5A). The ultrasonography examination of group I showed progress increase in thickness of SDFT with irregular hyper-echogenic appearance of tendon fibers with synovial fluid distension 3 days post induction (Fig.3B), which continued up to the 7th day post induction (Fig. 3C). By the 2nd week post induction, the synovial fluid distention decreased gradually, with presence of hypo-echoic areas representing loss of normal fiber pattern (Fig.3D). On the 3rd through the 4th weeks, the SDFT showed increase in thickness with irregular hyper-echogenic pattern of tendon fibers (Fig.3E &F). Group II showed hypo-echoic to anechoic areas on the 3rd day post induction (Fig.4 B) while lesions responded to treatment earlier as the fluid content decreased and the early scar tissue formation begins as small patches of hyper-echoic areas with slight improvement in arrangement pattern of tendon fibers by the 7th day (Fig.4C). The density of fibers and its regularity alignment became clearer by the 2nd week (Fig.4D). On reaching the 3rd and the 4th weeks, the fibers of SDFT attained their normal pattern and echogenicity (Fig.4E&F).Group III showed decreased echogenicity (Fig.5B) with distinct increase in the synovial fluid (Fig.5C), but the normal fiber’s pattern is still normal. By the 7th Day post induction, typical areas of fiber damage appeared as hypo-echoic (Fig.5D). By the 2nd Week post induction, the fluid content decreased and the early scar tissue formation begins as small patches of hyper-echoic areas with slight improvement in the arrangement of tendon fibers pattern (Fig.5E) By the 3rd toward the 4th weeks, the SDFT showed improvement in echogenicity and pattern of its fibers alignment (Fig.5F).
Group I showed a significant increase in synovial fluid CRP, LDH, and total protein while it showed a significant decrease in synovial glucose. On the other hand group II showed a significant decrease in LDH, CRP, and total protein and it showed a significant increase in synovial glucose compared to group III as illustrated in (Table 1).

The histopathological examinations of the tendon specimens which were obtained from apparently healthy donkey revealed normal SDFT with regular arrangement of parallel collagen fibrils (Fig.6). Group I, showed disruption of collagen fibers and consequently, necrosis and fibrosis. Hyperplasia of epitendon and endotenon were obvious. The entity of damage was degenerative rather than inflammatory. Moreover, mononuclear cell infiltration, dilation in blood vessels and newly formed capillaries were seen (Fig.7). Group II, showed normal appearance in the majority of bundles and focal hyalanisation that is related to the periphery with dilated blood vessels (Fig.8). Group III, showed thickening of endotenon and edema. Organization of the periphery with disruption of collagen bundles were evident and 50% of bundles looked normal (Fig.9).

**Table (1):** Mean ±SE of the parameters which were measured in synovial fluid (CRP, LDH, Glucose and total protein) at different times of the study.

<table>
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<tr>
<th></th>
<th>CRP (mg L)</th>
<th>LDH (U/L)</th>
<th>Glucose (mg)</th>
<th>Total protein (g/l)</th>
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<tr>
<td></td>
<td>Group I</td>
<td>Group II</td>
<td>Group III</td>
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<tr>
<td>Zero time</td>
<td>0.70 ± 0.01</td>
<td>0.62 ± 0.01</td>
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<td></td>
<td>50.5 ± 0.37</td>
<td>51.5 ± 0.44</td>
<td>50.5 ± 0.37</td>
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<td></td>
<td>29.33 ± 0.01</td>
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<td>3 days PI</td>
<td>8.0 ± 0.29</td>
<td>7.8 ± 0.77</td>
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<td>19.3 ± 1.53</td>
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<td></td>
<td>194 ± 2.08</td>
<td>194.67 ± 2.4</td>
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<td></td>
<td>19.0 ± 0.35</td>
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<td>7 days PI</td>
<td>10.23 ± 0.36</td>
<td>10.28 ± 0.36</td>
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<td>253.33 ± 1.76</td>
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<td>2nd week PI</td>
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<td>103.67 ± 2.03</td>
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<td>19.6 ± 0.58</td>
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<td>3rd week PI</td>
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<td>81.67 ± 0.88</td>
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<td>19.9 ± 0.57</td>
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<td>4th week PI</td>
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<td>72.33 ± 2.33</td>
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<td>20.5 ± 0.29</td>
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<td>Total</td>
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<td>3.1 ± 0.14</td>
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Fig. (1): Swelling of the palmar aspect of the mid-metacarpal region 3 days after induction of tendonitis in a donkey of group I.

Fig. (2): Degree of lameness in all groups at different time of the study.

Fig. (3): Longitudinal ultrasonography images of the SDFT of donkeys of group I, showing density and fiber pattern of the tendon at; zero time (A), 3 days post induction (B), 7 days post induction (C), 2 weeks post induction (D), 3 weeks post induction (E) and 4 weeks post induction (F).
**Fig. (5):** Longitudinal ultrasonography images of the SDFT of donkeys of group III, showing density and fiber pattern of the tendon at; zero time (A), 3 days post induction (B), 7 days post induction (C), 2 weeks post induction (D), 3 weeks post induction (E) and 4 weeks post induction (F).
Fig. (6): showing normal SDFT with regular arrangement of parallel collagen fibrils, H&E stain, x

Fig. (7): Showing fibrosis, hyperplasia of epitenon and endotenon, mononuclear cell infiltration and dilation in blood vessels in Group H&E stain, x 200.

Fig. (8): Showing normal appearance in the majority of bundles and focal hyalanisation is related to the periphery with dilated blood vessels in Group II H&E stain, x 200.

Fig.(9): showing thickening of endotenon, edema, organization of the periphery disruption of collagen bundles and 50% of bundles looked normal in Group III H&E stain, x 200

Discussion
Tendonitis of the SDFT is a serious equine injury with a high rate of recurrence which resulted in decreased athletic performance or removal from sports activities (Carvalho et al., 2014). The prognosis for complete recovery to the previous level of performance is often poor, horses that return to high performance activity following injury and reparative scar formation are more likely to become lame with moderate work and re-injury is common (Wilson et al., 1996).

Dyson (2004) and Waselau et al. (2008) mentioned that SDFT of the forelimb is much more susceptible for tendonitis, due to its small cross-sectional area and high tensile loads. As well as they bear 60% of the total body weight during rest and more during galloping, so the SDFT of the right forelimb was used for this study.

Mid palmar metacarpal region of the SDFT of the forelimb was used for injection of collagenase enzyme and induction of tendonitis because of most clinical cases of tendonitis usually occur in this region and the same was referred by Al_Jeboury (2002). Patterson-Kane and Firth (2009) added that mid-metacarpal region of the SDFT of the forelimb is the most susceptible to injury because it is far from the bloody dispensation sources and has a poor blood supply, relying on the tiny vessels of the paratendon, as well as narrowing of the tendon in this region.

In accordance with Dirks and Warden (2011) collagenase enzyme was used for induction of tendonitis in the present study as collagenase method exhibited several advantages over the other methods such as development of hypercellularity, loss of organization of extracellular matrix and increased vascularization. Dehghan et al. (2007) reported that that collagenase model is a well simulator to clinical tendon injury as collagenase enzyme takes some time to induce lesions as endogenous collagenase and proteases are responsible for degradation of normal tendon matrix, lesion enlargement and initial mechanical injury to collagen
fibers. Schnabel et al. (2009) and Wallis et al. (2010) added that surgical lesions do not mimic the natural pathogenesis of tendon injury, as they are usually followed by partial central tendon rupture and increased degradative enzymes. In this study, all animals in all groups showed hotness and pain on palpation at the induction site 24 hours post induction and these signs disappeared in all groups on the 5th day post induction. These results may be attributed to leakage of the enzyme to peritendinous tissues. Similar results were obtained by Oloumi et al. (2011) and Watts et al. (2012). Mild swelling at the injection site 24 hours post induction and it became more severe on the 3rd day post induction in all groups. The local swelling may be attributed to formation of hematoma and edema as a part of inflammatory process. These results were close to the results which were described by Dahlgren et al. (2002) and Dehghan et al. (2007).

Presence of lameness was recorded in group I because these animals did not receive any treatment so they developed more severe lesion within tendon core. Keg et al. (1992); Alves et al. (2001) and Barreira et al. (2008) mentioned that collagenase enzyme resulted in dissolution and mechanical injury of collagen fibers with inflammatory cells and degenerated tissues together with persistent angiogenesis, produce large amounts of metalloproteases (MMP) and proteases that destroy the surrounding collagen and extracellular matrix of the connective tissue. Group II showed absence of lameness and swelling at the induction site at the 4th week post induction. O’Sullivan (2007) reported that PSGAG inhibited the activity of (MMP), so it is resulted in suppression of inflammatory processes and prevention of further degradation of healthy collagen fibrils proteoglycans and stimulate tenocyte to produce collagen. These results were in accordance with. Group III showed absence of swelling at the injection site, but lameness was still present until the 4th week of the study with mean degree (0.67). These results may be attributed to treatment with Dexaphenylarthrite which consisted of both phenylbutazone and dexamethasone. Tobin et al. (1986) mentioned that both agents resulted in inhibition of the cyclooxygenase enzyme system, which is responsible for the synthesis of prostanoids such as PGE$_2$ so it is resulted in reduction of prostanoid-dependent swelling, edema, erythema, and hypersensitivity to pain in inflamed tissues.

Ultrasonography examination of the 3rd day post induction in all groups showed decreased echogenicity and distinct synovial fluid distention. The anechoic and hypoechoic lesions observed at this moment may be corresponding to areas of hemorrhage, edema and fibril
disruption. These results agreed with the results of Rantanen et al. (1998) and Avella et al. (2009). The ultrasonography examination of the SDFT of group I showed increasing in thickness with irregular hyper echogenic pattern of tendon fibers. These results may be attributed to the repaired tissue changed from cellular to fibrous. The same results were reported by Thorpe et al. (2010). Group II showed that core lesions respond to treatment earlier as hyperechogenic patches appeared on the 7th day post induction in addition to increased echogenicity and a decreased lesion. By the 4th week post induction, the SDFT attained its normal pattern and echogenicity. These results may be attributed to earlier treatment with PSGAG; 3 days post induction as there is a direct relationship between echoic intensity and the amount of collagen fiber. These results were in accordance with Oryan et al. (2008) and Dahlgren et al. (2009). While group III, showed early scar tissue formation on the 2nd week post induction. At the end of the study, slight improvement in echogenicity and fiber alignment was noticed. Hossain et al. (2008) attributed these results to treatment with dexaphenylarthrite as Dexamethasone may result in delayed healing by inhibiting fibroplasia, collagen and glycosaminoglycan synthesis, both of which are important for tendon repair.

Synovial fluid analysis of group I showed a significant increase in LDH, CRP, total protein and it showed a significant decrease in synovial glucose compared to results which were recorded at zero time before induction of tendonitis and compared to treated groups. These results may be attributed to continued inflammatory processes as animals did not receive any treatment. Similar results were obtained by Reijno (1976). Synovial fluid analysis of group II showed a significant decrease in LDH, CRP, Total protein and sugar returned back to its normal level compared to group III. These results may be attributed to potent inflammatory action of PSGAG and its role in suppression of inflammatory processes. These results were in agreement with Smith (1992).

Histopathological results in group I showed disarrangement of collagen fibers, fibrosis and hyperplesia of epitendon and endotenon and the entire damage was degenerative rather than inflammatory. These results may be attributed to fibroblasts had differentiated into new tenocytes which resulted in production of collagen type III rather than type I which is weaker than type I, so the orientation of the newly formed fibrils will be random instead of longitudinally aligned as was in the original tendon tissue. Thickened areas of endotenon were attributed to due to increased number of blood vessels. These results were in accordance with
Dowling et al. (2000) and Oloumi et al. (2011). On the other hand, the histopathological specimen of the SDFT of group II showed normal architecture of the majority of collagen bundles. Glade (1990) and Redding et al. (1992) mentioned that treatment with PSGAG earlier 3 days post induction as it resulted in improvement in collagen fibril organization in addition to inhibition of thrombin and fibrin formation together with accelerated clot removal so resulted in improvement of tissue organisation and strength in the early stages of healing.

Histopathological findings of group III showed that 50 % bundles looked normal with organization of the periphery, disruption of collagen bundles, thickening and edema of the endotenon. These results may attributed to treatment with Dexaphenylarthrite 3 days post induction, as this drug reduced inflammatory reaction, but not have effect on collagen fibers architecture. On the other hand, these results may be attributed to serious adverse effects of Dexamethasone treatment, as it may be resulted in impaired tendon healing and complete suppression of collagen type I. Marqueti et al. (2006) and Hossain et al. (2008) illustrated the action of dexamethasone.

From the present study we can be concluded that PSGAG showed beneficial results in tendon healing than Dexaphenylarthrite based on clinical, ultrasonography, biochemical and histopathological examinations.

References


Oloumi, M. M.; Vosough, D.; Derakhshanfar, A. and


Wallis, T. W.; Baxter, G. M.; Werpy, N. M.; Mason, G. L.;


دراسات تشخيصية وعلاجية علي التهاب الأوتار في الفصيلة الخيلية

محمود عبد التواب عوض - محسن محمد حسن - شريف أحمد صالح - صابر محمد أبو زيد - محمد حسين الدهراوي - حنان عمرو هنداوي

اقتمل الجراحة والتخدير وإزالة الأشعة من الكيمياء الحيوي والتشريح والأخون - كلية الطب البيطري - جامعة قناة السويس

تهدف هذه الدراسة إلى تقديم فعالية مادة البولي سلافانتليكوز أمينوجليكان ومادة الدكسافيل ارثرث في علاج التهاب الوتر المثلثي الإصبعي المستحدث في الحميربات على التغيرات الكيميائية والموجات الفوق صوتية والبيوكيماوية والتغيرات المرضية النسيجية وقد تم استعداد التهاب

استخدام أنزيم كولاجيناز البكتيري (2.5 ملغ / مل).

ويقاس لمدة 9 حمير صحيحة أكثيفيما كما قسمت الحيوانات عشوائيا إلى ثلاث مجموعات تتألف كل مجموعة من ثلاثة حمير. بعد أحداث التهاب الوتر حيث تلتث خمس حقات عضل كل حقة تمتثل 5 مل من مادة البولي سلافانتليكوز أمينوجليكان وكل مل يحتوي على (100 ملغ / مل) كل أربع أية كما تم علاج المجموعة (3) بعد ثلاثة أيام بعد أحداث التهاب الوتر حيث تلتث خمس حقات عضل (20 ملغ) من مادة الدكسافيل ارثرث لمدة يومين و تلتها ثلاث جرعات كل منها مكون من 10 مل كل الأيام.

وقد أظهرت الدراسة أن المجموعة الثانية المعالجة بمادة البولي سلافانتليكوز أمينوجليكان أظهرت تحسن ملحوظ في النتائج البارزة عند الفحص بالموجات فوق صوتية وعلى مستوى التغيرات النسيجية المرضية مقارنة بالمجموعة الثالثة المعالجة بمادة الدكسافيل ارثرث.