Tendon Healing after *Echinacea purpurea* Intake: Histological and Biomechanical Evaluation

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ABSTRACT

Introduction. Tendon injury is a major problem in sports and occupational medicine. Various methods have already been utilized to accelerate healing process and to allow recovery of normal mo9rphology and function of tendon. Among those is the use of herbal medicine, including Echinacea. The purpose of this study was to evaluate effect of echinacea on tendon healing through histological and biomechanical evaluation.

Material and Methods. This is an experimental study with post-test only control group design. Thirty male white rats were included in the study and have their Achilles tendon injured and repaired. They were randomly allocated into 6 groups. Three groups received 15 mg echinachea, while the others three served as control. One group from echinachea and control group were sacrificed at week-2, -4, and -6 for histological evaluation using Bonar score. Biomechanical test was also performed at week-6.

Results. In biomechanical test, force required to rupture echinachea and control group was 49.68±6.73 and 30.8±1.04 N (p=0.003). In histological evaluation at week-6, tenocyte and collagen score between each group varied significantly (p=0.003 and p=0.005 respectively). At week-4, tenocyte score between each group varied significantly (p=0.004). The collagen score between each group also varied significantly (p=0.031). There was no difference in Bonar score between each group at week-2.

Conclusions. Echinacea gives tendon healing a better biomechanical property as well as higher tenocyte and collagen score. The higher scores are obvious after week-4.

Keywords: Echinacea, tendon healing, Bonar score, biomechanical study

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Penyembuhan Tendon Pascapemberian *Echinacea purpurea*: Evaluasi Histologis dan Biomekanis

ABSTRAK

Pendahuluan. Kerusakan tendon merupakan masalah utama dalam Kedokteran olah raga dan okupasi. Berbagai metode telah dikembangkan untuk mempercepat penyembuhan dan mengembalikan morfologi dan fungsi normal tendon. Salah satu upaya yang dilakukan adalah dengan menggunakan obat herbal, antara lain degnan echinachea. Tujuan dari penelitian ini adalah untuk mengevaluasi penyembuhan tendon secara biomekanis dan histopatologis.
Bahan dan cara kerja. Penelitian ini merupakan penelitian eksperimental dengan desain *post-test only control group*. Sebanyak 30 ekor tikus putih dialokasikan acak ke 6 kelompok. Setelah dilakukan pencederaan dan penyambungan tendon Achilles, tiga kelompok mendapat 15 mg echinachea dna tiga kelompok mendapat akuades sebagai kontrol. Tikus dikorbankan pada minggu ke-2, -4, dan -6 untuk pemeriksaan histopatologis dengan skor Bonar dan uji biomekanis.
Hasil. Di uji biomekanis, gaya yang diperlukan untuk memutus tendon pada kelompok echinachea dan kelompok kontrol berturut-turut adalah 49,68±6,73 dan 30,8±1,04 N (p=0,003). Pada minggu ke-6, skor tenosit dan kolagen berbeda antara kelompok echinachea dan perlakuan (p=0,003 dan p=0,005). Pada minggu ke-4, skor tenosit dan kolagen juga berbeda antar kelompok (p=0,004 dan p=0,031). Tidak terdapat perbedaan skor Bonar pada minggu ke-2.
Simpulan. Echinachea memberikan property biomekanis serta skor tenosit dan kolagen yang lebih baik dibandingkan kelompok kontrol. Skor yang lebih baik tampak setelah minggu ke-4.

Kata kunci: Echinacea, penyembuhan tendon, fibroblas, skor Bonar, uji biomekanis

Introduction

Tendon injury occurs in 40-50% sports injury.[1] The incidence was about 12 of 100 000 injuries in 1994. It remains a major problem in sports and occupational medicine.[1] Complicating the high incidence, tendon has poor vascularization and minimal cells, making it difficult to heal.[2,3]

There are two processes in tendon healing; intrinsic and extrinsic. Although remains controversial, intrinsic process takes place through diffusion of synovial fluid around injury without necessity for blood flow.[4] In intrinsic process, inactive tenocytes are transformed into active tenocyte.[4]

Extrinsic tendon healing involves three phases. Exudation phase is characterized by inflammatory process, presence of polymorphonuclear cells, dilatation of blood vessels, and exudation. It occurs until day-3. In fibroproliferation phase, fibroblasts accumulate and produce collagen. Collagen bond is weak initially, but subsequently becomes mature and strong. The fibroproliferation phase starts on day-4 and continue until week-3. The last phase is collagen remodeling. Collagen will be oriented longitudinally, similar to that in normal tendon.[4] All these phases take as long as 2.5 months.

In order to accelerate tendon healing, various techniques have been developed. These include the use of mesenchymal stem cells,[5,6] physical modalities such as hyperbaric therapy,[7] as well as herbal medicine such as E*chinacea.*[8]

Echinacea is a plant traditionally known to Indian tribe since 1600 AD and has been used by Indians for various therapies.[9-11] In rural Missouri valleys and plains, it is used to treat syphilis, malaria, blood disorders and diphtheria. [12,13]

Echinachea stimulates phagocytosis.[14] It also acts as immune-modulator.[14] It increases number of circulating white blood cells, stimulates phagocytosis and cytokine production and triggers complement pathway.[14] *In vitro*, echinacea has bacteriostatic as well as anti-viral activity and stimulates production of cytokines (interferon, TNF, Il-1 and Il-6).[15]

Since echinachea affects pathway involved in tendon healing, we hypothesized that echinachea would accelerate tendon healing as measured in histological and biomechanical test.

Material and methods

This is a randomized experimental study with *Post Test Only Control Group Design*. The sample was 30 male white rats strain *Sprague Dawley*, aged 3-4 months old, weighted approximately with healthy lower limb.

Rats were randomly allocated into six groups; echinachea 2 week, echinachea 4 week, echinachea 6 week, control 2 week, control 4 week, and control 6 week. Tenotomy was performed on Achilles tendon, followed by tendon repair with Kessler core suture using atraumatic 5.0 nylon and peripheral continuous suture using 6.0 nylon. First three groups received 15 mg echinacea dissolved in aquades, the rest received aquades only. Rats were sacrified at week-2, week-4, and week-6 and their Achilles tendon were harvested along with distal femur, calcaneus and leg. Those bones served as a jig in biomechanical test.

Biomechanical test was done between the Achilles tendon and musculotendineous junction harvested on week-6. Ends of the bone were connected to aluminum clamp fixed to base of testing machine. Before the test begins, the specimens were given initial load of 1 N. Pull was performed at the speed of 1 mm/s until the tendon ruptured and force required to rupture the tendon weas recorded in Newton/kg.

Tendons were then preserved in 10% formalin for one day and decalcified using 10% HCL solution. Tendons were dehydrated using alcohol 70%, 80%, 95%, and 100%. They were cleared by soaking into xylol solution, and put into paraffin blocks at temperature of 45° C. The blocks were cut longitudinally by microtome and stained using hematoxylin-eosin. Bonar scores (table 1) for these slides were applied by an expert of musculoskeletal specialist form Department of Histology, University of Indonesia.

The scores between control and echinachea group were statistically tested using unpaired t-test. Statistical tests were performed using SPSS v.17.

Results

In biomechanical test, force required to rupture echinachea and control group was 49.68±6.73 and 30.8±1.04 N ($p=0.003$). In histological evaluation at week-6, tenocyte and collagen score between each group varied significantly ($p=0.003$ and $p=0.005$ respectively). At week-4, tenocyte score between each group varied significantly ($p=0.004$). The collagen score between each group also varied significantly ($p=0.031$). There was no difference in Bonar score between each group at week-2 (figure 1).

Discussions

Tendon repair process after rupture often causes morphological and biomechanical changes that are worse than normal tendon. Impaired healing process has decreased biomechanical tensile strength and absorption.[16] Tenocytes on tissue regeneration are described as having numerous endoplasmic reticulum and contractile proteins (actin and myosin). They are also more numerous and less uniformly distributed. Little is known about the collagen restitution process that occurs in the healing process.

Tensile strength is influenced by formation of col-

Table 1 . Bonar histological score

Variables	Grade 0	Grade 1	Grade 2	Grade 3
Tenocytes	Inconspicuous elongated spindle shaped nuclei with no obvious cytoplasm at light microscopy	Increased roundness: nucleus becomes more ovoid to round in shape without conspicuous cytoplasm	Increased roundness and size: the nucleus is round, slightly enlarged and a small amount of cytoplasm is visible	Nucleus is round, large with abundant cytoplasm and lacuna formation (chondroid change)
Ground substance	No stainable ground substance	Stainable mucin between fibers but bundles still discrete	Stainable mucin between fibers with loss of clear demarcation of bundles	Abundant mucin throughout with inconspicuous collagen staining
Collagen	Collagen arranged in tightly cohesive well-demarcated bundles with a smooth dense bright homogeneous polarization pattern with normal crimping	Diminished fiber polarization: separation of individual fibers with maintenance of demarcated bundles	Bundle changes: separation of fibers with loss of demarcation of bundles giving rise to expansion of the tissue overall and clear loss of normal polarization pattern	Marked separation of fibers with complete loss of architecture
Vascularity	Inconspicuous blood vessels coursing between bundles	Occasional cluster of capillaries, less than one per 10 high-power fields	1–2 clusters of capillaries per 10 high power fields	Greater than two clusters per 10 high-power fields

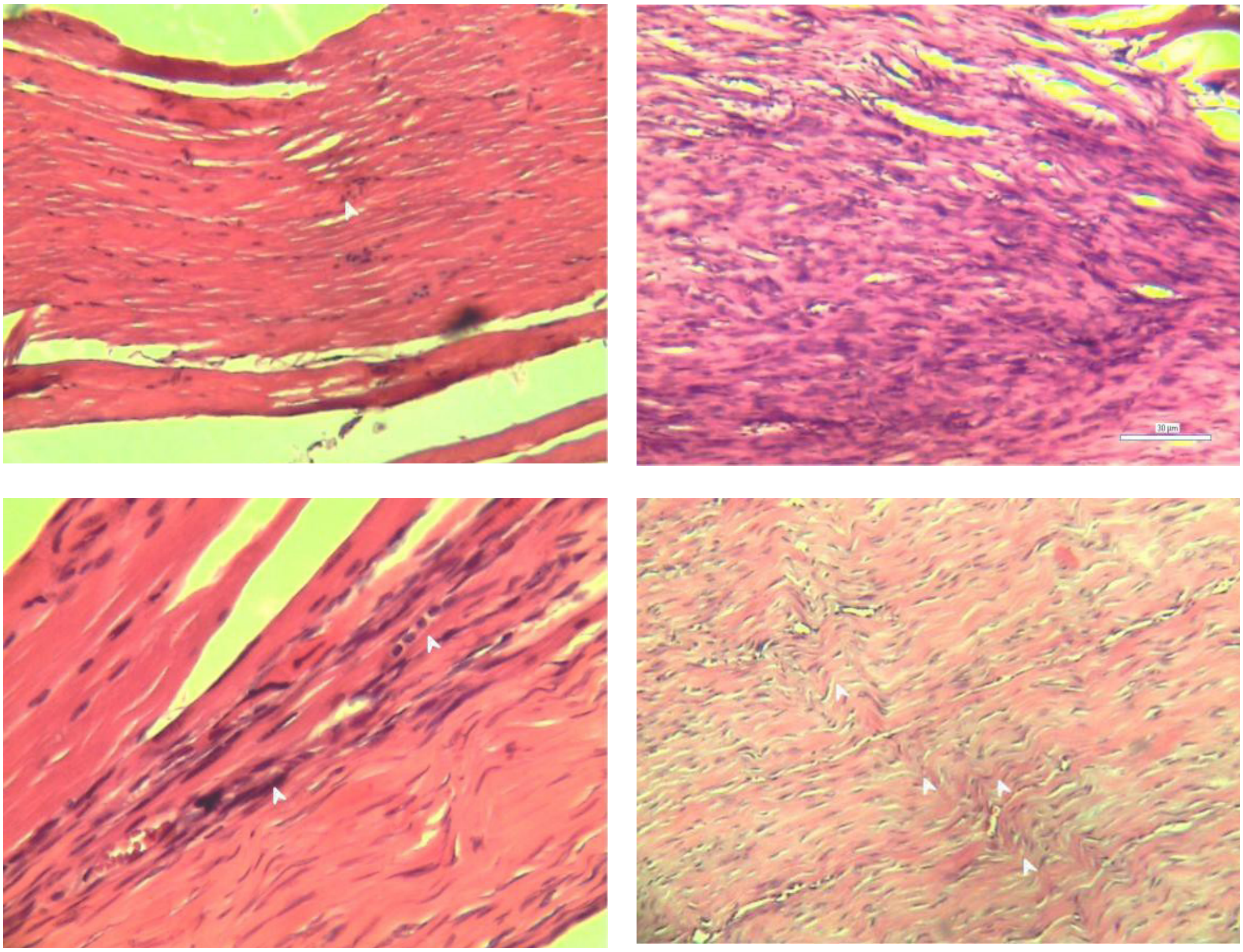

Figure 1. Histological finding of the specimens; control group at week-2 (left top), echinachea group at week-2 (right top), control group at week-4 (left bottom), echinachea group at week-4 (right bottom)

lagen fibers after injury. Dense sheath fibrils form collagen fibers and bring them together to form a unit of collagen fiber bundles frequently called collagen fascicle. Fiber bundle can move relative to each other. In general, elasticity of tendon tissue is very small, about 3-8%, partly because of the texture of the molecular composition.[17] Proteoglycans play role in inhibiting transmission and tensile stress on tendon thus adding strength to network.[18]

Tendon is dominated by fibroblasts.[19] It is also a structure of dense connective tissue consisted of collagen fibrils, fibers, fiber bundles, and fasciculus, as well as other extra-cellular matrix proteins. The nature of individual components of tendon is equipped to withstand tensile strength. Division of fibers and fiber bundles into fibrils ensures that minor damage does not always spread to the entire tendon. It also provides high structural tendon strength. Tendon consists of 55-70% water, and most of it is associated with proteoglycans in the extracellular matrix. Of tendon dry weight, 60-85% is collagen, mainly type I (60%) which consists of two $\alpha 1$ and one $\alpha 2$ chain, and collagen type III (10%).[19]

Fibroblast activation resulting from the use of echinacea has been studied previously, especially in wound healing.[11,12,20,21] The study showed that there were meaningful biomechanical differences between the control and experimental groups. This could due to activation of fibroblasts and collagen synthesis affecting the structural strength of the tendon.

Process of tendon healing starts from inflammation, namely infiltration of inflammatory cells, capillary dilation and exudation. Proliferation of granulation tissue from outer line of injury will penetrate two ends of tendon and collagen deposit begins to occur.[4,22]

Fibroblast density will continue to increase until third week where there is a significant build-up of the mass of surrounding granulation. When tendons that are exposed to trauma have been sutured, suture material will withstand tendon fibroblasts to produce collagen, which enough to form a “tendon callus”. Tensile strength of tendon that has been repaired does not depend on concentration and orientation of collagen fibrils. Longitudinal collagen fibrils will be present in approximately 4 weeks, and within 2,5 months remodeling will begin.[6,23,24,25]

Objective picture of tendons described in abnormalities in epidemiological and imaging can sometimes be very difficult to determine. Some studies tried to explain histopathological findings that are subjective and semi-quantitative. There are two methods that are commonly used, Movin score and Bonar score. Both scores are based on semi-quantitative criteria to assess changes related to process of tendinopathy on four points ranging from 0-3. This study uses Bonar score as histological assessment.[27]

European Medicines Agency stated that Echinacea herbal products had effect of inhibiting prostaglandin E2. In vivo, it also mentioned that activation of other mediators of inflammatory response occurs, such as nitric oxide, TNF-alpha and IL-1 beta, which are known mediators that is useful in process of angiogenesis. Angiogenesis is a process of growth and development of blood vessels that occurs either during embryogenesis, normal tissue growth, healing process or female reproductive cycle. This process is stimulated by several factors, among others: bFGF, TGF-β, TNF-α, angiogenin, IL-1, and angiopoetin.[5,26,28]

Other factor that plays a role in process of angiogenesis is vascular endothelial growth factor (VEGF). VEGF expression is stimulated by presence of hypoxia, oncogene and several cytokines. VEGF can act to induce endothelial cell proliferation, cell migration, and it also inhibit apoptosis. Some factors that increase production of VEGF include: FGF-4, PDGF, TNF-α, TGF-β, kgf, IGF-1, IL-1B and IL-6. While factor that block production are: IL-10 and IL-13.[29] Other compounds that increase VEGF was *Nitric Oxide* (NO). NO is synthesized by three types of enzyme nitric oxide synthase (NOS) namely, neuronal NOS, endothelial NOS, and *inducible* NOS.[29]

Echinacea sp is a polysaccharide that has active ingredients that can increase production of IL-1 macrophages. While purely conceived alkamide will spur an increase of TNFα and nitric oxide. presence of IL-1, TNFα and nitric oxide would increase VEGF. VEGF plays a role in collagen synthesis and fibroblast activation.[19]

From histological assessment, we found that there were increased density of fibroblasts in echinachea group, with tenocyte and collagen increased at week-4 and -6. Fibroblast density increased at week-3 and more longitudinal collagen fibrils with a tight cohesion after week-4. Synthesis of collagen through collagen fibrils is enough to form a “tendon callus”. Tensile strength of tendon that has been healed does not depend on concentration and orientation of collagen fibrils, this association with biomechanical testing of results of this study also showed significant differences between echinachea and control group.

From assessment of biomechanical and histologic testing, results obtained can be said to be synergistic. We believe this information is still useful clinically to accelerate healing in cases of patients with tendon injury. However, further study specifically related to assessment of matrix metalloproteinase, VEGF and other intrinsic linkage elements still need to be done.

Conclusions

Echinacea gives tendon healing a better biomechanical property as well as higher tenocyte and collagen score. The higher scores are obvious after week-4.

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