Effect of aloe vera gel on articular cartilage regeneration in dogs

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Article information

Abstract

The study aimed to assess the effectiveness of using aloe vera gel on articular cartilage regeneration in a canine model. About 6 mm osteochondral defects were induced on the trochlear groove of the distal extremity of the left femur bone of 18 adult dogs. These animals were divided into two equal groups, of 9 animals. The induced osteochondral defects were left without treatment for the first group (control group “C G”) While for the other group (treated group “T G”), the defect was occupied with 1 ml of aloe vera gel. Clinical, macroscopic, and histopathological evaluations were conducted on the 15th, 30th, and 45th day post-surgery. Clinical outcomes exhibited rapid and clear improvement in animals’ ability to use their limbs in standing and walking positions since the end of the first week following surgery in the treated group compared to the control group. Besides, the gross observation showed a relative disappearance of the osteochondral defect in the treated group rather than the control group at the end of the study. The histopathological data showed rapid and profound proliferation of the chondroblast and new cartilage formation with more angiogenesis and little development of granulation tissue at the end of the study in the treated group compared to the control group. In conclusion, aloe vera gel can enhance and accelerate the articular regenerative healing process to repair dog osteochondral defects.

Keywords: Aloe vera gel, Articular cartilage degeneration, Healing of articular cartilage

Introduction

The renewal of joint surface injuries and defects still represents a scientific challenge, where the healing of articular cartilaginous tissue has a restricted capacity (1). Most researchers agree that untreated cartilage defects lead to early osteoarthritis, as cartilage injuries are compound and difficult to repair (2). The cartilage tissue is designed to tolerate and allot loads across the various diarthrodial joints within the body. This specific structure displays a sole mechanical performance and little regenerative ability (3). The chondrocytes within the articular cartilage tissue proliferate and produce an extracellular matrix to keep the cartilage structure, while the cartilage extracellular matrix separates these cells from each other (4). They respond to external stimuli and tissue injury, and are responsible for degenerate status, such as osteoarthritis (5). The cartilage of the stifle joint is hyaline cartilage that shelters the articulation surfaces. The cartilage is a spongy, viscoelastic tissue that depends on a complex collaboration and arrangement of its elements to offer strong load-bearing, power-dissolving frictional and lubrication characteristics (2,6). The role of articular cartilage within synovial joints is crucial for its normal act. The capability of cartilage to minimize surface rubbing on articular surfaces and to bear high repeated loads permits it to be a shock absorbent and a lubricant (7,8). Nevertheless, articular cartilage is still a matter of injury by different causes, such as trauma, and joint diseases. When the hyaline articular is damaged, the ability to repair appears very limited (9,10) because of its little ability to repair itself, and thus, even slight damages may cause advanced injury and degeneration, resulting in significant pain and disability. Osteoarthritis (OA) and related degenerative diseases of joints result in an intense...
disease problem and distress many people word wide annually (11). In dogs, articular cartilage injury and osteoarthritis (OA) are regarded as common diseases. Once articular cartilage damage occurs, natural restoration seldom happens due to a percentage of chondrocytes besides its basic characteristic properties of lacking enough blood vessels, nerves, and lymphatics that make this extremely specialized connective tissue have a restricted ability for self-repairing after the occurrence of injury and the damage. Canines have distinguishing features compared to other laboratory species in that they develop articular cartilage lesions and pathology like humans. So far, dogs also require treatment for naturally developed articular cartilage damage; therefore, therapeutic OA methods are desired in veterinary and human medicine (12,13). Injury to articular cartilage is a chief source of disability. Animal research is fundamental to emerging active therapies for cartilage injuries (14), especially when a critical devastating fact implies that articular defects greater than 4–16 mm² hardly heal spontaneously, even with nonstop passive exercise. Oldness, gender, and anatomic location are vital in the defect healing process (15). Recently, several studies were done to repair articular cartilage damage (2,16,17).

Aloe barbadensis Miller, known as Aloe vera, is a medical juicy herbal plant belonging to the Liliaceae family. Aloe vera extract has been used for many years for its therapeutic and beneficial properties, as it can improve the growth of fibroblasts and raise the resistance of newly-formed tissues to outer stimulus (18,19). The topical use of aloe on skin wounds increases fibroblast activity, granulation tissue production, and collagen proliferation (20-22). The extract also holds angiogenic factors (22) that exert many pharmacological characteristics, such as anti-inflammatory (23,24). Anti-edema (25) and antimicrobial, antidiabetic and immune-boosting properties (26,27). Aloe vera has more than 75 active elements of therapeutic properties (28). The aloe vera gel's biochemical structure is complex and carries up to 75 potentially active ingredients, including sugars, and minerals like sodium, aluminum, phosphorous, boron, strontium, magnesium, silicon, iron, calcium, and barium. Vitamins such as vitamins B6, B2, B1, and C, enzymes like alkaline phosphatase, acid phosphatase, lipase, lactic dehydrogenase, and amylase have also been found within aloe vera extract. Other chemical constituents, include essential amino acids, lignin, salicylic acids, saponins, essential amino acids, numerous monosaccharides and polysaccharides, niacinamide, and choline. As well as, several inorganic ingredients and several organic compounds such as barbalo, a loin, and emodin, different polysaccharides (29). The aloe vera gel can treat minor burns, cutaneous irritations, and abrasions (29). Although, aloe vera can treat some external conditions, the internal application of aloe vera remains questionable (30). The significance of OA therapy and prophylaxes is accelerating because of the progressively aging society. However, some conservative alternatives, such as non-steroidal anti-inflammatory drugs (NSAIDs) administration and hyaluronan, are usable (31). Other recent options that only deal symptomatically to relieve pain without addressing the underlying causes of osteoarthritis include some drug joint injections such as steroids, physical therapy, and even arthroscopic lavage (32). The cartilage of joints is considered an essential structure to protect joint tissues. Because of the reservoir of blood vessels and nerves, lymphatics, and even restriction of the extracellular matrix (ECM) on the cartilage cells, the self-ability of articular cartilage to heal following damage is very restricted. when left without treatment, osteoarthritis can occur after articular cartilage damage. Injury can cause osteoarthritis and affect the physical status of the patients. Unfortunately, the recent surgical therapy procedures mostly applied in the clinic cannot regenerate articular cartilage (33). Therefore, several surgical interventions and alternative therapeutic options for repairing small cartilaginous defects include abrasion arthroplasty, multiple drilling, and mosaicplasty (34). So far, most of these therapeutic techniques pose many side effects, including multiple surgeries that need to be done for each patient, immune reaction, and disease transmission (34). As well as, most of these techniques lead to fibrous cartilagelike tissue formation rather than proper regenerated cartilaginous tissue. Thus, new alternatives that enhance true cartilage regeneration are still urgently necessary for better healing of cartilage defects (15). Cartilage injuries are caused by trauma, severe overload, and autoimmune diseases. Because of the avascular nature and little metabolic activities of chondrocytes, cartilage usually does not self-repair after an injury (35).

Therefore, the aim and the importance of this research are to show the ability of aloe vera gel for articular cartilage regeneration in dogs.

Materials and methods

Animals

Eighteen (N=18), adult male local breed dogs with an average weight of 18±1.8 kg were acclimatized for seven days before the start of the experiment. Then, the dogs were divided randomly into two equal experimental groups of nine animals each. The animals were subjected to knee surgery under general anesthesia. A 6-mm-diameter osteochondral defect was fashioned in the trochlear groove of all animals’ distal end of the femur of the left knees. The osteochondral defects of the first group (control group) were left without using any treatment, while in the second group (treated group), the defects were filled with 1 ml of aloe vera gel. These defects were examined clinically, grossly, and subjected to histopathological examination at days 15,30, and 45 postoperatively.
Ethical approve

Animal handling and surgical protocols of this research were approved by the Veterinary medicine/ Mosul University Ethics Committee, Iraq. UM.VET.2022.043.

Anesthetic protocol

The dogs were off feed for 12 hours prior to the surgical operation. The surgical events were accomplished under general anesthesia using pre-medication with atropine sulfate at a dose of 0.044mg/kg, followed by the premixed combination of 2% Xylazine at a dose of 5 mg/kg, and 10% Ketamine Hydrochloride at a dose of 10 mg/kg respectively intramuscularly (36).

Surgical procedure and creation of cartilage defects

Food and water were withheld from the dogs for 12 hours before the anesthesia. All animals were prepared surgically by clipping, shaving, and disinfecting the area around the surgical incision on the left knee. Then, the animal is positioned laterally. The lateral side of the stifle joint with luxation of the femoral patellar ligament was used to expose the surface of the joints. The joint was flexed during the surgical operation to establish the cartilage defect. An osteochondral defect about 6 mm in diameter was established in the trochlear groove of the distal femoral bone using an electric drill after incised skin, subcutaneous tissue, and joint capsule (Figure 1). The defect was washed with a normal saline solution. Then, these created defects were left empty in the control group, and filled with one ml of aloe vera gel in the treated group. After finishing inducing osteochondral defect, the patella with its ligaments was returned to its normal position, and the joint capsule was sutured by vicryl size 3/0 using a simple continuous pattern. Then, the incised surgical wound was sutured routinely. The surgical wound was dressed daily with a clean wound bandage.

Figure 1: A photograph displaying an osteochondral defect about 6 mm in the trochlear groove of the distal femoral bone.

Postoperative care

All animals received i.m. injection of penicillin-streptomycin in a dose of 1ml /10 kg and metalgen in a dose of 1ml/ day as a prophylactic and analgesic, respectively, for five days postoperatively, in addition to the dressing of wounds. The dogs were permitted to gait without being limited after surgical operation recovery. No external fixation, such as casts, supported the limb. In addition, the dogs were left to use their affected limb during exercise during the experimental study. The biopsy was taken after euthanizing the animals by giving a high dose of general anesthetic agents.

Assessment of healing

The progress of the healing process was evaluated during the whole period of this study on the 15th, 30th, and 45th postoperative days. The clinical evaluation was based on assessing the functionality of the left hind limb during standing and walking, besides observation of the animal’s ability for weight bearing. In contrast, the postmortem gross observation focused on the degree of disappearance of the created osteochondral defects. The histopathological evaluation was based on chondroblast proliferation, new cartilage formation, granulation tissue formation, and angiogenesis (new blood vessel formation).

Results

Clinical results

The clinical observation for the animals of the control group following surgery exhibited loss of left hind limb function with incapability to put, nor use, the limb during standing and walking within the first two weeks. However, the animals showed mild ability to put and use their affected limb during the third week of walking. Besides, the use of the left hind limb was improved progressively at the end of the fourth week after the surgical operation. At the same time, the functional use of the affected limb of this group was returned to the normal style relatively during the fifth and sixth weeks postoperatively. On the other hand, the aloe vera treated group animals revealed the ability to use their affected hind limb during standing and walking at the end of the first week following surgery. The functional use of that limb increased at the end of the second week post-operation and was ultimitly returned to normal function at the begining of the third week post-operation.

Gross results

The postmortem gross examination displayed no degenerative or pathological signs of joints postoperatively in all animals during the biopsy from the defect site. The defect in the control group after the 15th and 30th postoperative day was still present (Figures 2 and 3), while after the 45th postoperative day, the defect partially disappeared compared to the defect of the treated group of
the same postoperative timepoint (Figure 4). On the other hand, the defect at 15 and 30 days postoperative of the aloe vera-treated group, still appeared relatively just like the control group (Figures 5 and 6), but on day 45 postoperatively, the defect disappeared relatively (Figure 7).

Figure 2: Osteochondral defect at 15th day postoperatively in CG1.

Figure 3: Osteochondral defect at 30th day postoperatively in CG1.

Figure 4: Osteochondral defect at 45th day postoperatively in CG1.

Figure 5: Osteochondral defect at 15th day postoperatively in TG2.

Figure 6: Osteochondral defect at 30th day postoperatively in TG2.

Histopathological results
In the control group, the histopathological changes at the cartilage defect site on the 15th postoperative day showed poor chondroblast proliferation, scanty granulation tissue, and few new blood vessels (Figure 8). At 30 days postoperatively, the sections did not differ from the 15th day postoperatively and also revealed proliferation of the chondroblasts, a scanty amount of granulation tissue, and few new blood vessels formation (Figure 9). In the same group, on the 45th postoperative day, there is a proliferation of the chondroblasts and new cartilage formation with a profound amount of granulation tissue more than cartilage formation and moderate new blood vessel formation (Figure 10).
In the treated group, the histopathological section at the cartilage defect site at 15 days postoperatively was characterized by good proliferation of the chondroblasts and new cartilage formation, well-developed granulation tissue, and vast new blood vessel formation (Figure 11). On the 30th postoperative day, the changes exposed abundant proliferation of the chondroblasts and new cartilage formation with reduced granulation tissue formation and vast new blood vessel formation (Figure 12). On the 45th postoperative day, there is more proliferation of the chondroblasts and new cartilage formation, which connect to the well-developed granulation tissue and vast new blood vessel formation (Figure 13).

Figure 6: Osteochondral defect at 30th day postoperatively in TG2.

Figure 7: Osteochondral defect at 45th day postoperatively in TG2.

Figure 8: Micrograph at 15th day post-operation in CG showing poor proliferation of the chondroblasts (black arrow), scanty amount of granulation tissue (yellow arrow), and few fresh blood vessels (red arrow). H&E, 40X.

Figure 9: Micrograph at 30th day post-operation in CG1 showing proliferation of the chondroblasts (black arrow), scanty amount of granulation tissue (yellow arrow), and few blood vessels (red arrow). H&E, 40X.

Figure 10: Micrograph at 45th day post-operation in CG1 showing proliferation of the chondroblasts and new cartilage formation (black arrow), a profound amount of granulation tissue (yellow arrow) and moderate new blood vessels (red arrow). H&E, 40X.
Figure 11: Micrograph at 15\textsuperscript{th} day post-operation in TG2 showing well proliferation of the chondroblasts and new cartilage formation (black arrow), well-developed granulation tissue (yellow arrow) and high formation of angiogenesis (red arrow). H&E, 40X.

Figure 12: Micrograph at 30\textsuperscript{th} day post-operation TG2 showing well thick proliferation of the chondroblasts and new cartilage formation (black arrow), reduction of granulation tissue (yellow arrow), and high angiogenesis (red arrow). H&E, 40X.

Figure 13: Micrograph at 45\textsuperscript{th} day post-operation TG2 showing the more and well thicker proliferation of the chondroblasts with new cartilage formation (black arrow) connects to the well-developed granulation tissue (yellow arrow) and high angiogenesis also (red arrow). H&E, 40X.

Discussion

In the current research, dogs were used as an experimental model for studying articular cartilage healing because of their close relation to humans, as they naturally can develop articular cartilage degenerative disease just like us. Besides, the thickness of articular cartilage 0.95-1.3 mm (37) is almost resembles that of humans 1.0-2.6 mm (38,39). This suitable thickness allows the creation of articular cartilage defects without causing damaging changes in the subchondral bone (37). Additionally, dogs can receive various postoperative magement like humans, including splinting, bandaging, walking, or even training (40), which can prevent OA or regulate its progression after the experimental interventions (13).

The close clinical and macroscopic examination of the treated group showed rapid positive progress in using their affected hind limb in both standing and walking since the first week after surgery, with noticeable functionality at the end of the second week. These massive outcomes enabled these animals to use their injured hind limb for walking and standing since the end of the first week following surgery in comparison to animals of the control group, which suffered from the inability to use their affected limb within the first two weeks with delayed limb functionality until the third week could be explained as a result of adding aloe vera gel into the induced articular cartilage defects that behaved as an anti-inflammatory agent which inhibited any inflammatory reaction following articular cartilage defects besides its antimicrobial activities that suppressed development of any infection following procedure for injury induction. These results were in the same line as Vázquez et al. (41), who studied the effects of different physical preparations of aloe vera extracts on carrageenan-induced edema in the rat paw and concluded that aloe vera had an anti-inflammatory activity. Also, Olaleye and Bello-Michael (42) tested the antimicrobial activities of the aloe vera leaf and gel against several species of bacteria and fungi and observed the powerful antimicrobial activity against these microorganisms. While Langmead et al. (43) found that oral administration of aloe vera gel had an anti-inflammatory action and therapeutic effect against inflammatory bowel disease. Other researchers Abid et al. (44) mentioned its beneficial effects on tendon healing in donkeys that enhanced tendon healing by preventing wound infection and protecting the tissues from contamination.

On the other hand, the gross results of the treated group exhibited relative disappearance of the induced osteochondral defect on day 45 post-operation as a result of the influence of aloe vera gel that enhanced the proliferation of chondroblasts and formation of new cartilage tissue that filled that defect and made it less apparent in treated group comparable to the control group which showed more apparent cartilaginous defects because of use nothing to fill that induced space.
Furthermore, the histopathological examination of treated group samples revealed that there were many indicative cardinal signs of an ideal healing process, including gradual progress in the proliferation of the chondroblasts, presence of new cartilage formation, angiogenesis, and granulation tissue existence since the 15th day following surgery. These identified histopathological features, especially chondroblasts are considered necessary for cartilage regeneration as chondroblasts and chondrocytes are the main cellular structures of normal tissue cartilage, along with the ECM (3).

ECM, which makes up the most cartilage matrix, is produced by chondroblasts and chondrocytes (3,45). Inside the ECM, chondroblasts are metabolically dynamic cells that produce and secret extracellular matrix (ECM) elements, including glycoproteins, collagen, proteoglycans, and hyaluronan (5). This secreted ECM improves compressional and stretchy forces across the diarthrodial joint. Besides, these ECM elements, particularly collagen, and proteoglycan, may support the repair of cartilage (35) as collagens are the most constituent supermolecules of the ECM, make up more than 60% of the dry mass of the cartilage, and about 10-20 % of the wet weight of the articular cartilage (46) that provide stretch, shear and tensile strength to the tissue and stabilizes the matrix. Furthermore, another positive impact of aloe vera is its ability to enhance collagen synthesis due to having an element known as mannose-6-phosphate in aloe vera leaf gel (28). The products present in the mannose can increase the activity of macrophages and, therefore, stimulate the synthesis of collagen (47).

Besides, the documented anti-inflammatory properties of aloe vera played a crucial role in providing the appropriate and ideal suitable environment for chondroblasts to accomplish their vital regenerative role and function because its metabolic activities are powerfully changed by several factors that are found within their chemical and mechanical environment especially the pro-inflammatory elements which have anabolic and catabolic effects that may lead to degradation of matrix macromolecules (5). Furthermore, the histopathological feature, especially the proliferation of chondroblasts, extracellular matrix synthesis, and new cartilage formation, are highly agreed with those of Khaleefah and Emran (48) who studied the histopathological changes associated with articular cartilage repair in rabbits using repeated doses of plateletrich plasma and noticed that articular cartilage regeneration was happening through increasing chondrocytes proliferation, and Dahlin et al. (49) who focused on the vital role of chondrocytes for in vivo repairing of cartilage defects.

As well as, Funayama et al. (50), who depend on embedded collagen II gel and cultured chondrocytes to repair full-thickness articular cartilage defects of a rabbit model due to their well-known role in cartilage regeneration. Another histopathological inspection of aloe vera cartilage samples revealed the presence of new blood vessels since the end of the first week following surgery that became more developed with the progress of the healing process, which is a very crucial step in cartilage regeneration as angiogenesis and growth of newly capillaries is a basic phenomenon of any tissue regeneration as new blood vessels would not only bring oxygen and nutrients to the highly metabolically active regenerating tissue but also serve as a route for stem cells to reach the injury site and be differentiate into cartilage precursor cells (51-54).The data regarding the presence of new blood vessels since the early time of the experiment for the aloe vera group followed those of Fujisato and, Ikada (55), who noticed the existence of much angiogenesis during the new formation of cartilage in vivo using chondrocytes seeded on poly (L-lactide).

Other studies revealed, that the local application of aloe vera gel enabled the re-establishment of the vascularity of the burned tissue in guinea pigs. The presence of well-developed granulation tissue was also evident in the articular cartilage defects of the treated group during the whole period of the regenerative healing process, which agreed with that of Al-Sabaawy and Al-Hyani (56), who noticed the occurrence of granulation tissue while he was studying the effect of aloe vera gel on the healing of skin wounds in donkeys. This vital histopathological feature is critical for the formation of true cartilaginous tissues rather than fibrocartilaginous, which is associated with other therapeutic options (57), as the true hyaline cartilage of articulation had superior biomechanical properties comparable to those associated with fibrocartilage and hyaline-like cartilage repairs (58).

All these observed beneficial histopathological manifestations suggest that aloe vera could be considered a brilliant chondrogenic promoter that improves the process of cartilage regeneration. The accelerating ability for articular cartilage regeneration and the anti-inflammatory properties of aloe vera make it a magical agent (59). Besides, articular cartilage lesions usually lead to fibrillation and consequent surrounding articular surface degradation (60). The histological features of the aloe vera treated group greatly supported both the clinical and gross outcomes, including improved limb functionality and less apparent of cartilaginous defect.

Conclusions

This study's clinical, gross, and microscopic results showed that the direct application of fresh aloe vera gel on the articular cartilage defect improved the healing process through the proliferation of chondroblast and new cartilage formation.

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Conflict of interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

References


Tأثير جل الصبار على تجدد الغضروف المفصلي في الكلاب

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إن الهدف من هذه الدراسة هو تقييم فعالية استخدام هلام الصبار لتحفيز تجدد الغضروف المفصلي في الكلاب. تم إجراء معالجة واحدة على 40 كلبًا بالغًا، وتم تقسيمهم إلى مجموعتين متساويتين. تم إجراء عملية إزالة الأربطة الغضروفية للفخذ، ثم تم إجراء عملية إزالة الأربطة الغضروفية للفخذ في المجموعة الأولى، بينما تم إجراء عملية إزالة الأربطة الغضروفية للفخذ في المجموعة الثانية. تم إجراء عملية إزالة الأربطة الغضروفية للفخذ في المجموعة الأولى، بينما تم إجراء عملية إزالة الأربطة الغضروفية للفخذ في المجموعة الثانية.

Über die Wirkung von Aloe vera Gel auf die Wundeheilung


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تأثير جل الصبار على تجدد الغضروف المفصلي في الكلاب

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إن الهدف من هذه الدراسة هو تقييم فعالية استخدام هلام الصبار لتحفيز تجدد الغضروف المفصلي في الكلاب. تم إجراء معالجة واحدة على 40 كلبًا بالغًا، وتم تقسيمهم إلى مجموعتين متساويتين. تم إجراء عملية إزالة الأربطة الغضروفية للفخذ، ثم تم إجراء عملية إزالة الأربطة الغضروفية للفخذ في المجموعة الأولى، بينما تم إجراء عملية إزالة الأربط...