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Türk Hijyen ve Deneysel Biyoloji Dergisi

Intraarticular injectional evaluation of dextrose prolotherapy; an experimental study in rat knee osteoarthritis model

Dekstroz proloterapisinin eklem içi enjeksiyonla değerlendirilmesi; sıçan diz osteoartrit modelinde deneysel bir çalışma

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ABSTRACT

Objective: Osteoarthritis (OA) of the knee with a prevalence of 365 million is the most common joint disorder in the world and is frequently encountered in the older population. However, the pathogenesis of osteoarthritis remains unclear, and yet it is not possible to effectively prevent the progression of OA. Therefore, it is of great importance to find more appropriate and effective treatment modalities for osteoarthritis. Although Hypertonic Dextrose Prolotherapy (HDP) appears to be a promising interventional treatment for knee OA, the dosage and immediate effects of this application require preliminary clinical as well as clinical studies.

Methods: In this study, to provide new information in the treatment of OA, animal models of OA were developed and used for assesment of different concentration of Dextrose prolotherapy. In accordance with this purpose and before starting the model development and treatment, the volume optimization of injectable solution in knee joint performed using trypan blue dye.

Results: According to the results, injection of more than 50 µl has the capacity of leakage out of the knee joint. Animal models of OA were developed by intra-articular

ÖZET

Amaç: Diz osteoartriti (OA) 365 milyon prevalansı ile dünyada en sık görülen eklem hastalığıdır ve sıklıkla yaşlı popülasyonda görülmektedir. Ancak osteoartritin patogenezi belirsizliğini korumasına rağmen OA'nın ilerlemesini etkili bir şekilde önlemek mümkün değildir. Bu nedenle osteoartritte daha uygun ve etkili tedavi yöntemlerinin bulunması büyük önem taşımaktadır. Hipertonik Dekstroz Proloterapisi (HDP), diz OA'sı için umut verici bir girişimsel tedavi gibi görünse de bu uygulamanın dozajı ve anlık etkileri, klinik çalışmaların yanı sıra ön klinik çalışmalar da gerektirmektedir.

Yöntem: Bu çalışmada, OA tedavisinde bilimsel destek sunmak amacıyla, OA hayvan modelleri geliştirilmiş ve bu modeller farklı konsantrasyonlarda dekstroz proloterapisi değerlendirilmesinde kullanılmıştır. Bu amaç doğrultusunda model geliştirmeye ve tedaviye başlamadan önce tripan mavisi boya kullanılarak diz eklemine enjekte edilebilir solüsyonun hacim optimizasyonu gerçekleştirildi.

Bulgular: Sonuçlara göre 50 µl'den fazla enjeksiyonun diz eklemi dışına sızma kapasitesi vardır. OA'nın hayvan modelleri, 50 µl'lik nihai hacime

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injection of 1 mg freshly prepared single dose Sodium Monoiodoacetate (MIA) in a final volume of 50 µl. Then, for the first time, the effect of different concentrations of Dextrose prolotherapy on the treatment of OA was investigated on these rat knee OA models.

Conclusion: After 28 days of follow-up, all applied concentration of Dextrose (5%, 10%, 15% and 25%) demonstrated the capacity to significantly (p<0.05) decrease hind paw weight distribution in a dose-independence manner. In conclusion, dextrose prolotherapy appears to be a safe treatment method for effective treatment, recovery and pain control in knee osteoarthritis. Future studies may also demonstrate the synergistic effect of dextrose prolotherapy with other therapeutic methods.

Key Words: Dextrose Prolotherapy (DP), Knee Osteoarthritis (OA), Sodium Monoiodoacetate (MIA), Hypertonic Dextrose Prolotherapy (HDP)

sahip ve 1 mg taze hazırlanmış tek doz Sodyum Monoiyodoasetat (MIA) içeren solusyonun eklem içi enjeksiyonu yoluyla geliştirildi. Daha sonra ilk kez farklı konsantrasyonlarda dekstroz proloterapisinin OA tedavisine etkisi bu sıçan deney hayvanı diz OA modelleri üzerinde araştırıldı.

Sonuç: 28 günlük takipten sonra, uygulanan tüm Dekstroz konsantrasyonları (%5, %10, %15 ve %25), dozdan bağımsız bir şekilde arka pençe ağırlık dağılımını önemli ölçüde (p<0.05) azaltma kapasitesini gösterdi. Sonuç olarak dekstroz proloterapi, diz osteoartritinde etkili tedavi, iyileşme ve ağrı kontrolü için güvenli bir tedavi yöntemi olarak görünmektedir. Gelecekte yapılacak çalışmalar dekstroz proloterapinin diğer terapötik yöntemlerle sinerjik etkisini de gösterebilir.

Anahtar Kelimeler: Dekstroz Proloterapisi (DP), Diz Osteoartriti (OA), Sodyum Monoiyodoasetat (MIA), Hipertonik Dekstroz Proloterapisi (HDP)

INTRODUCTION

Knee Osteoarthritis (OA) is the most common chronic, progressive, and disabling joint disease, often resulting in a poor quality of life. According to clinical and radiographic assessments, the prevalence of OA is 50% over the age of 60 and over 80% at the age of 75 (1,2). The main clinical symptom of OA is pain, which is one of the leading causes of disability in OA (3,4).Current treatments of OA mainly include analgesic agents and viscosupplementation. However, these treatment and supplementations only reduce OA symptoms such as joint pain. Furthermore, some studies suggest that these drugs are not sufficiently beneficial and may cause adverse drug reactions (5,6).

Osteoarthritis has often been described as a non-inflammatory, and simply a degenerative joint disease that predominantly caused by mechanical factors and genetic predisposition. However, there is increasing evidence that shows inflammation is high in OA and the pathogenesis of this disease is much more complex than just a degenerative process that may contribute to the progression of this disease (7,8).

Prolotherapy is one of the promising options for the treatment of painful musculoskeletal conditions such as knee OA, particularly when other standard treatments have proved to be ineffective (9). Intra-articular or extra-articular applications of hypertonic dextrose (a natural form of glucose normally found in the body) as prolotherapy agent is an effective injectional therapy with few adverse effects, that can be used in treating many chronic musculoskeletal problems, including osteoarthritis (OA) (10–12). Initially, Dextrose prolotherapy was thought to

contribute to the treatment of OA by inducing inflammatory pathways. Studies in this direction reveal that the inflammatory environment created after dextrose prolotherapy is very short-term and transient. The study conducted by Jensen and colleagues, shows that although the inflammatory effect of Dextrose prolotherapy is seen initially (at 6 and 24 hours), this effect is not seen after 72 hours. More importantly, when the inflammatory effects were compared, Dextrose prolotherapy and saline (control group) produced the same level of inflammatory effect. This study suggests that the inflammatory effect is more likely to be due to damage to the injection site (13). Dextrose itself works through repairing injured musculoskeletal tissue by stimulating the body's natural healing mechanisms. Dextrose prolotherapy stimulates different growth factors such as platelet derived GF, ILGF and transforming growth factor ß, which in turn results with the expression of type I and III collagens. According to recent studies, adequate supply of glucose to chondrocytes during inflamatory conditions and matrix degradation can interrupt the detrimental inflammatory cycle and induce synthesis of hyaluronan, thereby promoting cartilage repair (14,15). Despite its long history and widespread use as a form of a single or complementary therapy, there are still disparities over Dextrose optimal effective concentrations. In the light of the abovementioned information, in this study it was aimed to analyse different concentrations of Dextrose to determine the optimal effective concentrations of this cheap, easy-to-use and harmless prolotherapy factor for the treatment or relieving pain of knee OA (Figure 1).

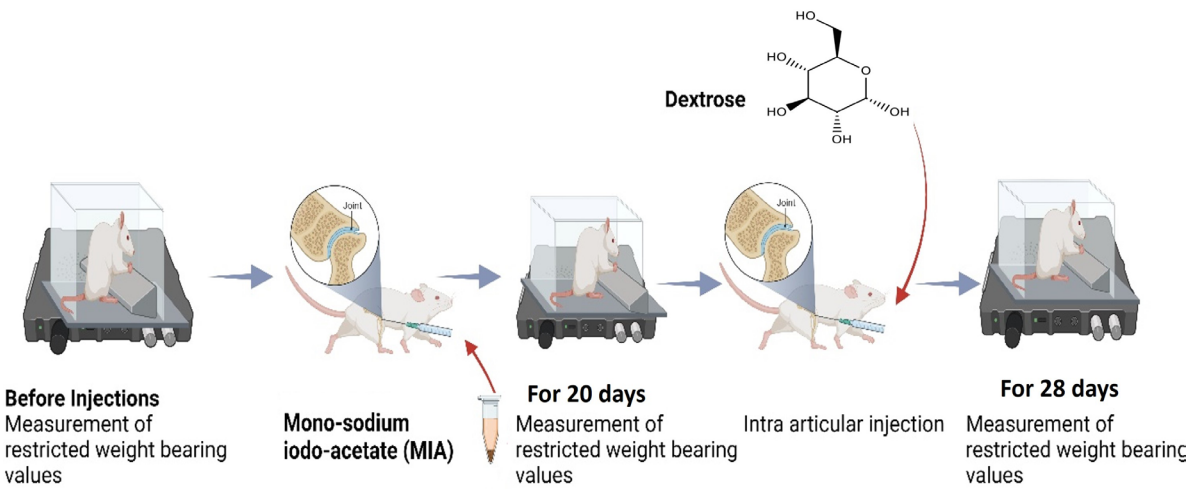

Figure 1. Flowchart of the in vivo experiment. Created by biorender.com

MATERIAL and METHOD

Creating the OA Model

In this study, 40 male 10-12 weeks Wistar rats (200 ± 30 g) were supplied by Koç University- Animal Facility and kept under animal breeding conditions. The experimental protocol was approved by the Local Ethics Committee for Animal Experimentation of Koc University (2018-31). Animals were fed with standard rat chow and provided with pure water. They were kept in individually ventilated cages under 22 °C room temperature with air filter.

Single intra-articular injection of mono-sodium iodoacetate (MIA), an inhibitor of glycolysis, into the rat knee femorotibial joint causes cartilage damage and degeneration, similar to the signs of human

OA (1,2,16). To create an animal model of OA, the Restricted Weight Bearing values in the left and right legs of rats were measured with the incapacitance device (Figure 1a) and their suitability for the study was checked by the % hind left leg weight (17). Both legs of the animals to be included in the study should have equal or very close weight bearing power and the hind left leg weight value should be 50%. During the experiment, rats were allowed to get used to the apparatus and were obtained five times and the average were considered as a data. For this purpose, the rats were placed in the chambers of the apparatus (made of angular plexiglass). Thus, the animals placed their hind left and right legs on two separate pressure plates as shown in the figure (Figure 1a). A video on the formation of the OA model, available on the Jove website, was used to visually be trained and perform the in vivo experiment (18,19). Then, evaluation of the volume and optimization of the point of intraarticular injection was assessed applying Intraarticular injection of water soluble blue stain (Trypan Blue) solution.

The limited weight bearing value was calculated by incorporating the following formula into the microsoft exel-2010 program (Formula 1).

$$\text{Hind left leg weight \%} = \left[\frac{\text{Hind left leg weight}}{(\text{Hind left leg weight} + \text{Hind right leg weight})}\right] X\ 100$$

Formula 1. Formula for measuring the limited weight bearing value of the left leg (the leg to be administered physiological serum or MIA)

For induction of MIA-induced osteoarthritis, rats were anesthetized with isoflurane and positioned on their backs.After shaving and disinfection of knee area, the knee was positioned at a 90° angle to reveal the white patellar tendon below the patella. Pressing the patellar tendon with the fingertip 1 mg of freshly prepared MIA (experimental) and blank (control) solution in 50 µL sterile saline was injected vertically (5 mm) into the joint cavity in the junction of the gap and the lateral patellar tendon of male wistar rats using 26 G needle. It was important to not felt a resistance when the needle was in the articular space (19). The pain related behavior were tested at 2, 5, 8, 12, 16, and 20 days after injection.

Animals with a left leg weight bearing value in the range of 42 ± 3% were considered as OA developed and suitable for the study, and rats below (advanced OA) and above (no OA or initial OA) this value were removed from the study with the least painful method within the framework of ethical rules.

OA Model Treatment

From 40 animals with MIA injected as OA model in left leg and saline injected as control in right legs, those with a limited restricted weight bearing values for left leg closest to 42% were selected and randomly assigned to four different treatment groups consisting of six animals each, for therapeutic administration in left legs. Dextrose injections were performed intraarticularly into the knee joint (lateral) as a single dose under anesthesia (3% isoflorane).

Post-treatment Pain Assessment (posterior left leg limited weighted pressure value)

After the treatment, the limited weight bearing value was measured five times a day at different intervals in all animals and the average was calculated. The results obtained were then compared with the pre-treatment results and the recovery rates of the different groups calculated. The recovery curves were compared by evaluating the differences within and then between the groups.

This study was approved by the Koç University Local Ethics Committee for Animal Experimentation (Date: 07.11.2018 and Number: 2018-31).

RESULTS

Creating the OA Model

Rats are one of the mostly used animal models in the preclinical studies for both investigating the pathophysiology and developing treatments for joint disorders namely, osteoarthritis, rheumatoid arthritis, etc (20). The injection volume of solutions differs

in wide ranges, from 20 to 200 µL in intraarticular injection experiments that was carried out with rat knees. Large differences in applied volume between the samples can certainly effect the outcomes of the studies. In addition excess amount of injection will end up with leakage from the articular (21). Therefore, not only the site of injection, but also the volume of injectible solution are important in knee joint injections. According to our study injection over 50 µl will pave the way for the leakage of blue stain out side of the joint into the leg (figure 2b). Therefore freshly prepared MIA (OA model leg), saline (control leg) and dextrose (treatmemt) solution were injected into the joint cavity in the junction of the gap and the lateral patellar tandons of animals in 50 µl final solutions using 26 G needle.

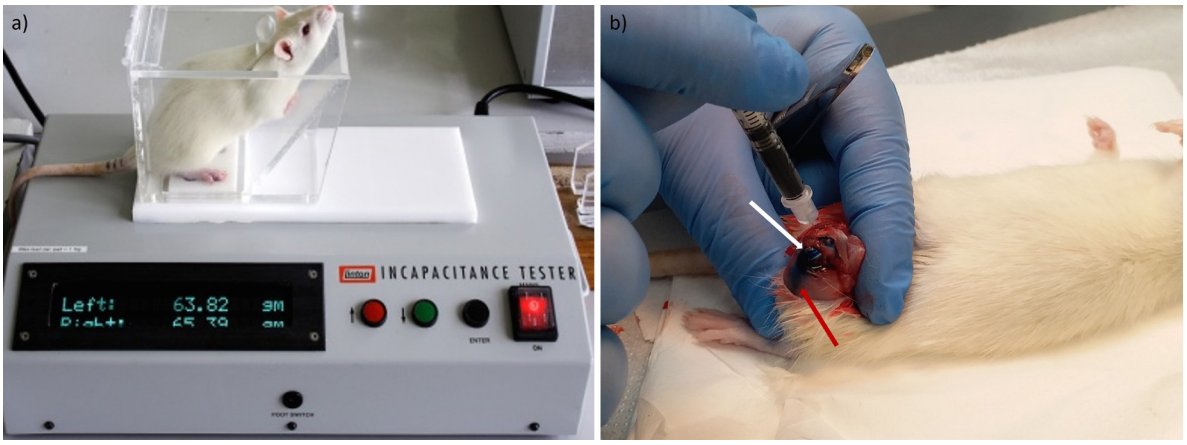

Figure 2. a) Position of the rat's legs pressed against the plate of the incapacitance device. b) injection of water-slouble blue stain material into rat knee joint. red arrow shows the stain leaking in the leg through the ankle and white arrow indicated the stainin in the injection site of the joint

According to Formula 1, a healthy Wistar rat should have equal or very close weight-bearing power of both legs around 50% (17). However, this value was expected to decrease by about 8% from the normal value for the leg that developed model OA due to MIA injection and thus to be around 42%. Animals with a left leg weight bearing value in the range of 42 ± 3% were considered as animals with osteoarthritis and suitable for the study. The left and right leg values measured for each rat with incapacitance meter and calculated according to Formula 1. Each formula result was summed and averaged. Standard deviation data prepared from Excel were added below these values. For 24 Wistar male rats, graphs were obtained from each average formula value (Figure 3).

In general, pattern formation was observed. As can be seen from the sharp decrease in the values, a response occurred immediately after the first injection in the majority of the animals. Although there was a recovery period in animals afterwards, the model maintained its success. Response results were generally in the range of 42 ± 3 (Figure 3).

OA Treatment

The t-test and two-way analysis of ANOVA (Graphpad Prism 9 Software) were used for statistical evaluation after Dextrose treatment. As seen in Figure 2, statistically significant ($p<0.05$) improvement was observed in 24 animals after Dextrose prolotherapy treatment compared to MIA injection (Figure 4a). On the other hand, when compared according to the Dextrose ratios injected at different rates (5%, 10%, 15% and 25%); although improvement (increase in weight bearing capacity) was observed after injection of each Dextrose ratio, no statistical significant difference was observed between the groups (Figure 4b).

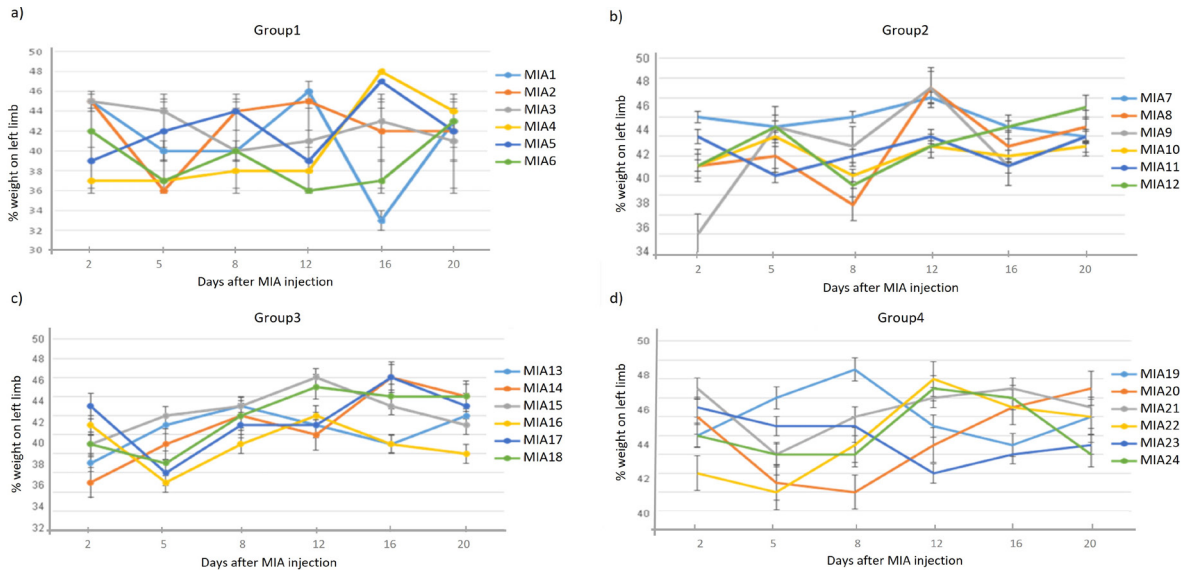

Figure 3. Effect of MIA injection into the rat left knee in different groups (1-4). Left leg weight bearing percentages of four different groups (a-d) at different days after MIA injection

Figure 4. a) Statistical analysis showing the effect of Dextrose injection on the Left leg weight bearing capacity of animals (24 rats) with OA after Dextrose injection $p<0.05$ b) Statistical analysis showing the effect of Dextrose injection at different ratios (5%, 10%, 15%, 25%) on the left leg weight bearing capacity of animals (6 rats in each ratio) with OA after Dextrose injection

Incapacitance measurements of Dextrose-treated animals (0.5 ml/1 dose) were performed starting from day 2 for four weeks. According to the left leg weight bearing measurments, the improvement mostly starts from day 14 after treatment (Figure 5a-d). When leg weights at the end of MIA injection were compared with those of at the end of Dextrose treatment, some, but not complete, recovery was observed.

In the left legs measurements several days after Dextrose injection, the rats put less weight on their left legs in a stable state may be due to the damage to the injection site (13). Although, the recovery starts several days after Dextrose injection, the rate of recovery increases after two weeks. The effects of dextrose percentages on treatment from day 2 are given in Figures 6 (a-d).

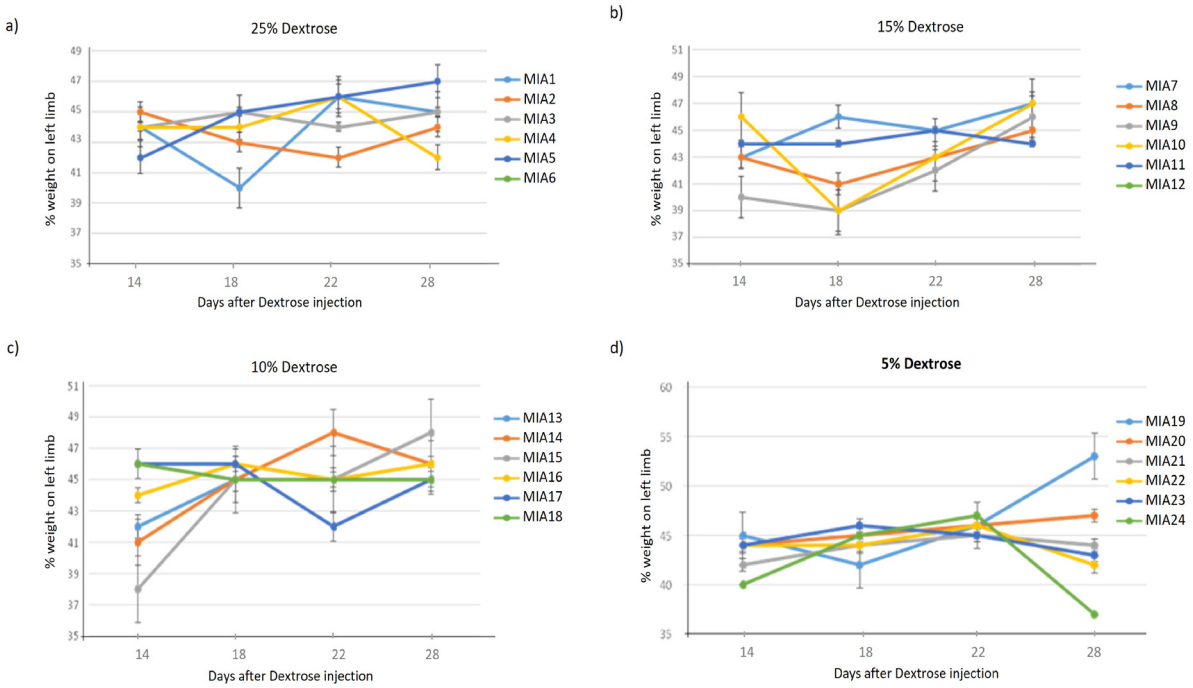

Figure 5. Left leg weight bearing percentages after a)25%, b)15%, c)10% and d)5% Dextrose injection

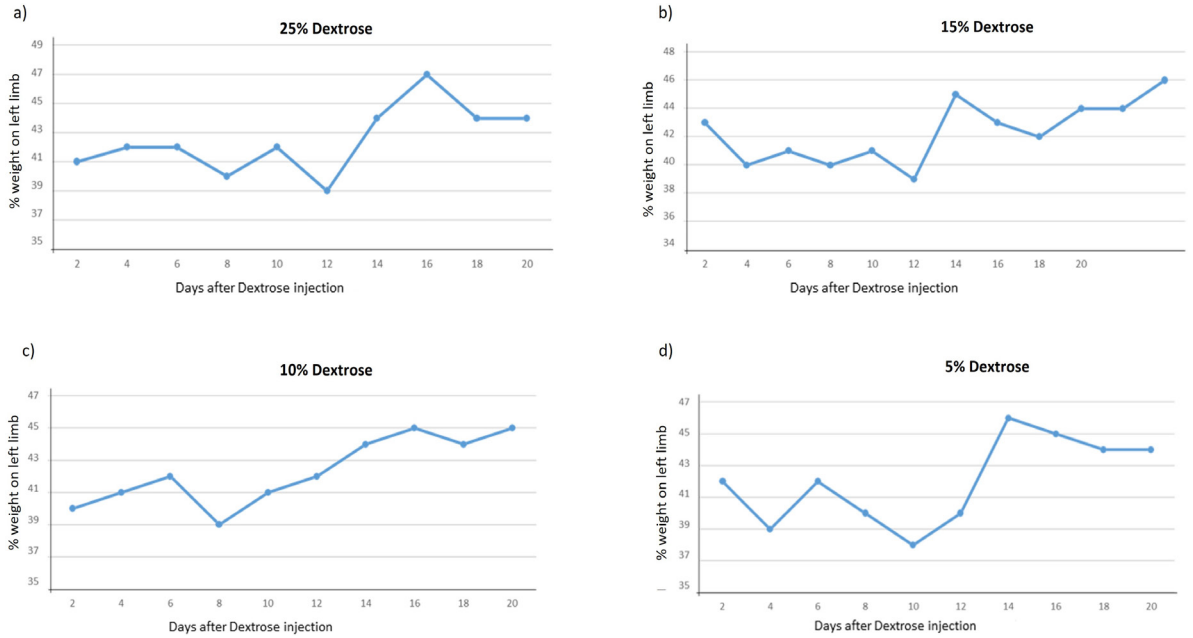

Figure 6. Graph of arithmetic mean values of incapacitance measurements before and after a)25%, b)15%, c)10% and d)5% Dextrose injection

DISCUSSION

Knee osteoarthrit remains as one the most common degenerative disease and pain associated with this disease still continues to remain as a major unmet medical need. Therefore, effective therapeutic options to treat OA pain are still very much warranted.

In vitro studies on the dose of Dextrose to be administered have shown that lower doses are effective in the treatment of OA, while higher doses trigger the inflammatory environment (22).

In *in vivo* studies on dextrose prolotherapy injections demonstrated early inflamatory response overall, similar to that of saline injections or needlestick procedures which was resolved by 72 h ostinjection (13). Prolotherapy injections create an inflammatory response, but this response is variable and overall, not uniformly different from that caused by saline injections or needlestick procedures (13). Although short term therapeutic effects of dextrose prolotherapy were more abundant, there are some studies that show improvements with dextrose prolotherapy treatment in the long term up to two years (23).

Our current in vivo study on rat demonstrated that injection of more than 50 µl has the capacity of leakage out of the knee joint and pave the way for fake results. Our study showed no significance diffirence in the therapeutic effects of different concentration of dextrose on developed rat animal OA model. The current study adds two important findings. Considering the post-treatment results in our in vivo study, in general, it can be suggested that Dextrose treatment was statistically and significantly effective four weeks after injection at all doses of Dextrose. However, no significant difference in pain reduction efficiency between Dextrose doses was observed. Therefore, dextrose at any concentration between 5% to 25% can be used as treatment strategy in OA patients alone or in combination with other therapeutic techniques. However, generalisability of this statement requires further studies in combination with other therapetic techniques and clinical studies

In conclusion; Dextrose prolotherapy is one of the most inexpensive and safe therapeutic methods for effective treatment, recovery and pain control of knee osteoarthritis. In experiments for which rat is used in developing and treatment of animal Knee OA model, application of 50 µl injecable solutions seams to be the optimal effective volume. It also comes out that no significant difference presence in the effeciency of applied concentration of Dextrose alone. However, there is a need for further studies related to synergistic effect of prolotherapy together with other therapeutic methods.

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ETHICS COMMITTEE APPROVAL

* This study was approved by the Koç University Local Ethics Committee for Animal Experimentation (Date: 07.11.2018 and Number: 2018-31).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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