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Replacement of Synovial Fluid of the Induced Traumatic Arthritis by Autologous Normal Synovial Fluid Mixed with Autologous Conditioned Serum (ACS) in Dog

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Abstract

Man has primarily used dogs as a labor force with respect to the rationales for dog domestication. Even the first dogs seem to have offered general economic services, such as hunting, protecting or traveling. The aims of this study were to study the efficacy of ACS in canine traumatic arthritis treatment and to investigate the possibility of replacement of autologous normal SF instead of pathogenic SF. This study was conducted on twenty local straw dogs divided randomly into four groups , each group 5 dogs. Treatment group-1(G1) were treated with autologous conditioned serum(ACS), treatment group-2 (G2) were treated with autologous normal synovial fluid(SF), and positive control group-3(G3) were treated with normal saline and negative control group-4(G4) had no any treatment. These dogs weighing 15± 5 kg., their ages were 9±3 months. Each group kept in separated box and fed fresh meat with ad libitum clean water. Left stifle joint was chosen as the site of induced traumatic arthritis. Under aseptic conditions arthrocentesis was done and 1ml. of SF of all dogs was evacuated for SF analysis. The left stifle joints of all dogs were opened under routine surgery and the cranial cruciate ligament of the left stifle joint was crushed with forceps. The results showed that the replacement of autologous normal synovial fluid was superior than ACS.

Keywords: Synovial, fluid, traumatic, arthritis, autologous, conditioned, serum.

Introduction

In all joint cavities, synovial fluid is found where it supports the surfaces of the articular cartilage, in part by minimizing friction. In addition, Synovial fluid enables the transport between vascularized synovium and a vascular cartilage of nutrients and waste products including proteins and metabolites (Fernandez, *etal;* 2012; Hui,*etal;* 2012).

SF is secreted by the synovial membrane and is in close contact with both the articular cartilage and the synovial membrane. It is a fluid rich in Hyaluronic acid and lubricates and supplies articular cartilage with the vital nutrients required for the metabolism of chondrocytes under normal conditions (Cretu, *etal;* 2013) .

In joint injury and disease due to modifications directly to the SF, as well as the tissues lining the synovial joint, the structure and function of SF is altered (Gibson and Rooney; 2007) .

SF examination, computed mographic arthrography and magnetic resonance imaging should be considered (Sample *et al.*, 2017).SF is a plasma daily sate that comprises several variables and is modified from the joint tissue by components secreted. Hyaluronic acid (HA) and fibronectin are factors contributing to the viscoelastic nature of SF .(Blewis,*etal*,2007).

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Autologous Conditioned Serum (ACS) is an autologous blood substance enriched with an interleukin-1 receptor antagonist (IL-1Ra), which is a naturally occurring interleukin-1 inhibitor (IL-1).(Arend ,Evans ;2003 ; Gabay, *et al;* 2010) . In order to treat conditions in which IL-1 is thought to be an essential agent of pathological conditions, ACS is given locally. There have been many reviews written on this subject. (Wehling ,*et al*,2007; Alvarez-Camino *et al*,2013). The aims of this study was to replace autologous normal synovial fluid in induced traumatic arthritic joint and the efficacy of autologous conditioned serum.

Materials and methods

This project was carried out at random on twenty local straw dogs, divided into four .groups of five dogs in each group.

These puppies, 15± 5 kg. in weight, were 9±3 months old. Group-1(G1) treatment was treated with autologous conditioned serum(ACS), group-2 treatment was treated with autologous natural synovial fluid, group-3(G3) positive control was treated with regular saline and group-4(G4) negative control was not treated. Each population was kept in a separate box and fed fresh meat with clean water, ad libitum. Both stages of the work were followed by the Ethics Research Committee of the College of Veterinary Medicine/ Al-Qadisiyah University.

Induced arthritis:

The left stifle joint was chosen as the site of induced arthritis, and under strict aseptic conditions SF3ml arthrocentesis was performed. For laboratory study, the joint was evacuated and the left stifle joint cranial cruciate ligament was crushed with forceps as shown in Fig.-1 for induced traumatic arthritis.

Fig.-1: Induced traumatic arthritis by damage the cranial cruciate ligament of stifle joint.

Autologous conditioned serum(ACS) preparation:
Whole blood, 10 ml. According to the procedure (RachitaDhurat, Sukesh, 2016), the blood was incubated 37oC, CO2 5 percent for 8 hours, then the blood centrifuged for 10 minutes at 5000 rpm, the supernatant stored when used at-20oC, was collected from cephalic vein of G1 in tubes containing glass beads and calcium chloride 10 percent at 1:10 ratio.

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Fig.-2: Collection of autologous interleukin-1 receptor antagonist.

Synovial fluid collection :

Local anesthesia with Lidocaine 2% 5ml.under routine strict aseptic conditions. The proper stifle joint in both dogs and 2 ml of synovial fluid had arthrocentesis. 2ml synovial fluid acquired in the same matter. It was obtained from the correct stifle joint for laboratory testing.

Experimental design:

The caused arthritic left stifle joint of G1 2 ml. was evacuated after 48 hrs. of SF and replaced with 2ml of ACS. Whereas 2 ml of SF was withdrawn from the proper G2 joint and replaced with 2 ml of normal SF, removed from the right stifle joint. 2ml of normal saline solution was supplemented with the SF of the proper stifle joint of G3, and the left stifle joint of G4 was left without any treatment.

Fig.-3: Replacement of normal synovial fluid in the left stifle joint.

Lameness evaluation :

All dogs were tested for lameness on a scale of 0 -5 degrees according to severity, mild, moderate, and severe.

Clinical examination of the stifle joint :

For 1, 2, and 3 weeks, the clinical symptoms of traumatic arthritic joints were reported daily. At 1, 2, and 3 weeks, the physical markers of SF redness, swelling, and angle of the stifle joint were reported. Biochemical synovial fluid markers were

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measured at 1, 2, and 3 weeks with a commercial kit of total protein, glucose, alkaline phosphatase, and uric acid (Fa. R & D Systems, Minneapolis, USA).

Measurent of the stifle joint angle :

As the cranial tibial plateau landmark, the proximal part of the cranial extent of the medial tibial plateau was identified, and the caudal extent of the medial tibial plateau was identified as the caudal landmark. To assess the tibial plateau slope, a line was drawn connecting the anterior and posterior extensions of the tibial plateau (line a). From the center of the intercondylar eminences to the center of the talus, a second line was drawn (line b). Line b is the tibia's long axis on the plane of the sagittal. A third line (line c) at the intersection of lines a and b was drawn perpendicular to the long tibial axis. The tibial plateau was determined between lines a and c as the angle (fig.-4).

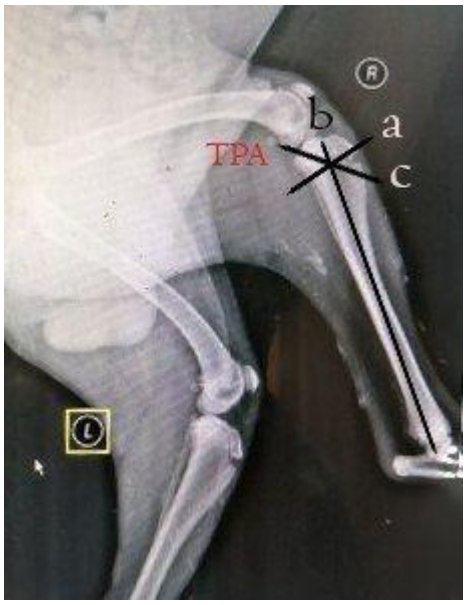

Fig- 4 : Radiograph of the stifle joint showed the drawn lines for measurement of joint angle.

Radiographic examination:
All joints were tested at 0, 1, 2, and 3 weeks with X-rays.

Statistical analysis:

The findings were evaluated statistically with the SPSS program one way software of ANOVAs. Version 32, at $P \leq 0.05$, the variances were considered significant.

Results

Lameness evaluation:

According to the severity of lameness, lameness tests were reported after 3 weeks on a scale of 0 to 5 to compare the groups. Two puppies displayed mild lameness of 3 degrees in G1, while G3 showed one moderate lame puppy of 3 degrees, three extreme lame puppies of 4 degrees and one very severe lame puppy of 5 degrees in

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G1, although G4 showed one moderate lame puppy of 4 degrees with four very severe lame puppies of 5 degrees.

Table-1: The lameness evaluation after 3 weeks post the treatments.

No.	Groups	Degree					
		0	1	2	3	4	5
1	G1			+			
2					+		
3			+				
4				+			
5					+		
6	G2		+				
7				+			
8			+				
9		+					
10		+					
11	G3						+
12						+	
13						+	
14						+	
15					+		
16	G4						+
17							+
18						+	
19							+
20							+

Lameness evaluations were according to the severity: mild, moderate, severe, and very severe..

Clinical signs:

In the 1st, 2nd and 3rd weeks, Table -2 mentioned the clinical signs of all groups. At the 3rd week the swollen disappeared in G2, while at the 3rd week it was mild in G1 and at week 3 in G4 it was moderate. The same results were obtained for redness and lameness, except for chronic severity in G3 and G4. The angle degree of the stifle joint was decreased in G1 and G2 at 35 °,53 ° at week 3, respectively, while in G3 and G4 it was 31 ° and 30 ° respectively.

Table-2: The clinical signs of the stifle joints of the dogs after 1, 2, and 3 weeks.

Clinical signs	G1			G2			G3			G4		
	1st week	2nd week	3rd week	1st week	2nd week	3rd week	1st week	2nd week	3rd week	1st week	2nd week	3rd week
Swollen	++++	++	+	++++	++	-	++++	+++	++	++++	+++	++
Redness	++++	++	+	++++	++	-	++++	+++	++	++++	+++	++
Angle degree of the joint	42	39	35	59	55	53	25	27	31	22	28	30
lameness	++++	++	+	+++	+	-	++++	+++	+++	++++	+++	+++

* Degrees scale of severity: +, ++, +++, and ++++

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Physical markers of the SF:

For all groups shown in table-3, the physical SF markers were straw in G1 at week 3, pale straw in G2, dark straw in G3, and G4 at week 3. The viscosity of the 3rd week SF was viscous for G1, G2, and very poor for both G3 and G4. The pH values of the SF were 7.2 in G1, 7.5 in G2, 6.6 in G3, and 6.4 in G4.

Table-3: The physical markers of the synovial fluid of the stifle joints.

Physical markers	G1			G2			G3			G4		
	1st week	2nd week	3rd week	1st week	2nd week	3rd week	1st week	2nd week	3rd week	1st week	2nd week	3rd week
Color	Dark straw	Straw	Straw	Straw	Pale Straw	Pale Straw	Dark Straw	Dark Straw	Dark Straw	Dark Straw	Dark Straw	Dark Straw
viscosity	Low viscid	viscid	viscid	Low viscid	viscid	viscid	Very low	Very low	Very low	Very low	Very low	Very low
pH	6.6	6.9	7.2	6.9	7.3	7.5	6.2	6.3	6.6	6.1	6.1	6.4

* Degrees scale of color were: pale straw, straw, and dark straw.

* Degrees scale of viscosity were: low viscid, viscid, very low viscid.

Biochemical markers of the SF:

The biochemical SF markers of all groups were recorded for the 1st, 2nd and 3rd weeks, as shown in Table-4. In the third week, TP variances were non-statistically significant at P≤ 0.05, 3.2±0.12 in G1, 5.6±0.09 in G2, 7.4±0.13 in G3 and 7.2±0.03 in G4, respectively, while in the third week, glucose variances were significant at P≤ 0.05, 61.3±0.42 in G1, 60.6±0.23 in G2,134.8±0.75 in G3, 150.4±0.76 in G4. Although variances in ALP values were statistically significant at P≤ 0.05 in the third week, 172.9±0.34 for G1, 161.7±0.24 for G2, 173.7±0.18 for G3, 190.5±0.09 for G4 were statistically significant at P≤ 0.05,and differences in WBC count values were relevant at P≤ 0.05, 13341.6 ± 64.1 for G1, 9859.4 ± 36.6 for G2, 14359 ± 21.8 for G3,and 15336±51.2 in G4 in the third week.

Table-4: Biochemical markers of the synovial fluid of the experimental dogs.

Biochemical markers	G1(n=5)			G2(n=5)			G3(n=5)			G4(n=5)		
	1st week	2nd week	3rd week	1st week	2nd week	3rd week	1st week	2nd week	3rd week	1st week	2nd week	3rd week
TP (g/dl)	4.4±0.1 Aa	4.3±0.14 Aa	3.2±0.12 Aa	6.4±0.09 Aa	5.3±0.12 Aa	5.6±0.09 Aa	6.6±0.18 Aa	6.9±0.09 Aa	7.4±0.13 Aa	8.5±0.14 Aa	7.4±0.06 Aa	7.2±0.03 Aa
GLU (mg/dl)	69.1±0.53 Ba	65.7±0.18 Aa	61.3±0.42 Aa	73.8±0.89 Ba	63.6±0.24 Aa	60.6±0.23 Aa	154.5±0.66 Bb	142.8±0.28 Bb	134.8±0.75 Bb	158.4±0.52 Bb	154.1±0.79 Bb	150.4±0.76 Bb
ALK (U/L)	183.2±0.63 Ca	178.9±0.81 Ba	172.9±0.43 Ba	174±0.41 Ca	165.3±0.28 Ba	161.7±0.24 Ba	195.5±0.12 Ca	182.3±0.21 Ba	173.7±0.18 Ba	198.6±0.08 Ba	194.1±0.05 Ba	190.5±0.09 Ba
WBC count(cells/µl)	15232±37.7 Da	14382.6±78.8 Cb	13341.6±64.1 Cb	12492.8±65.6 Dc	11275.2±48.1 Cd	9859.4±36.6 Ce	16587±43.7 Dc	15375.8±18.8 Cg	14359±21.8 Cb	17243.4±30.2 Ca	16078±31.4 Ch	15336.6±51.2 Cg

* Different letters denote the variances were significant at P≤0.05.

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Radiographic views of the groups:

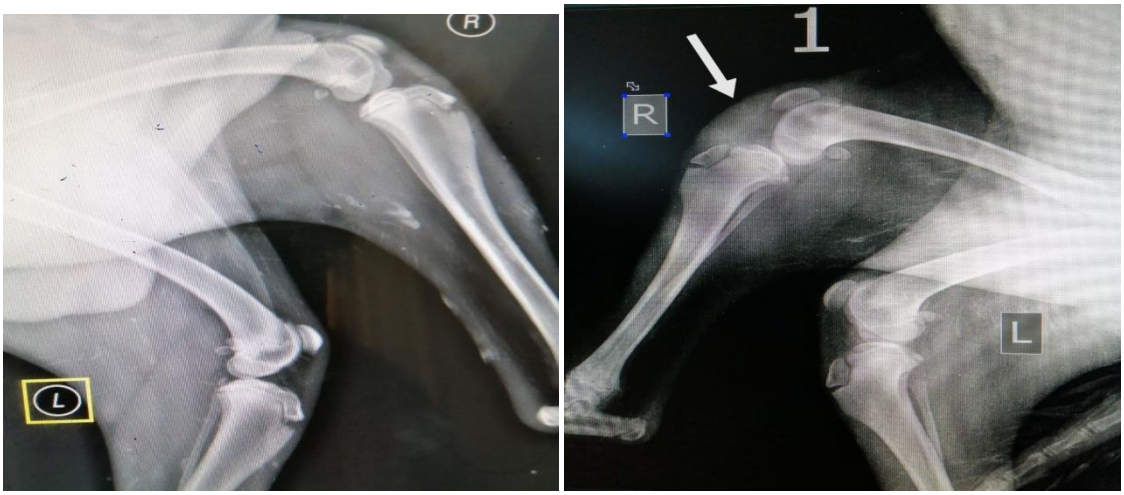

Fig.-5:The radiographic views of G1 at 1st week (left) and 3rd week (right) showed the effect of ACS on the resolution of traumatic arthritis.

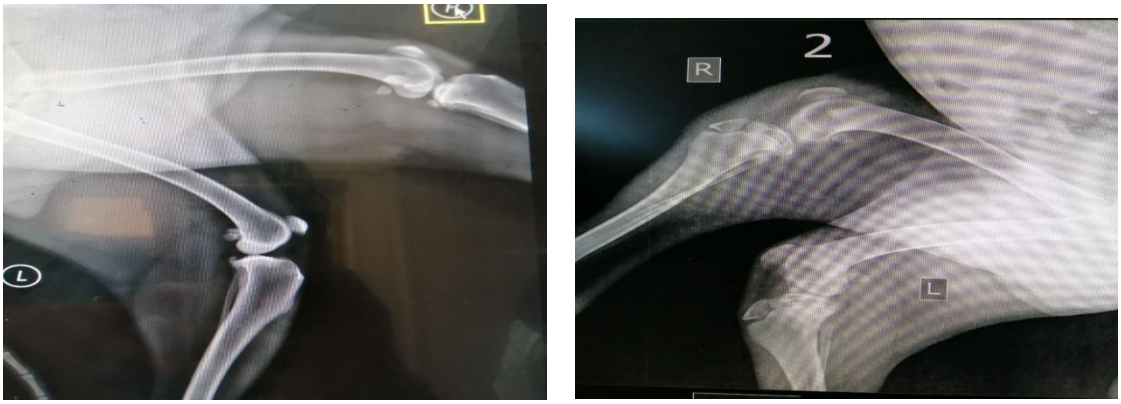

Fig.-6:The radiographic views of G2 at 1st week (left) and 3rd week (right) showed the effect of normal synovial fluid on the resolution of traumatic arthritis.

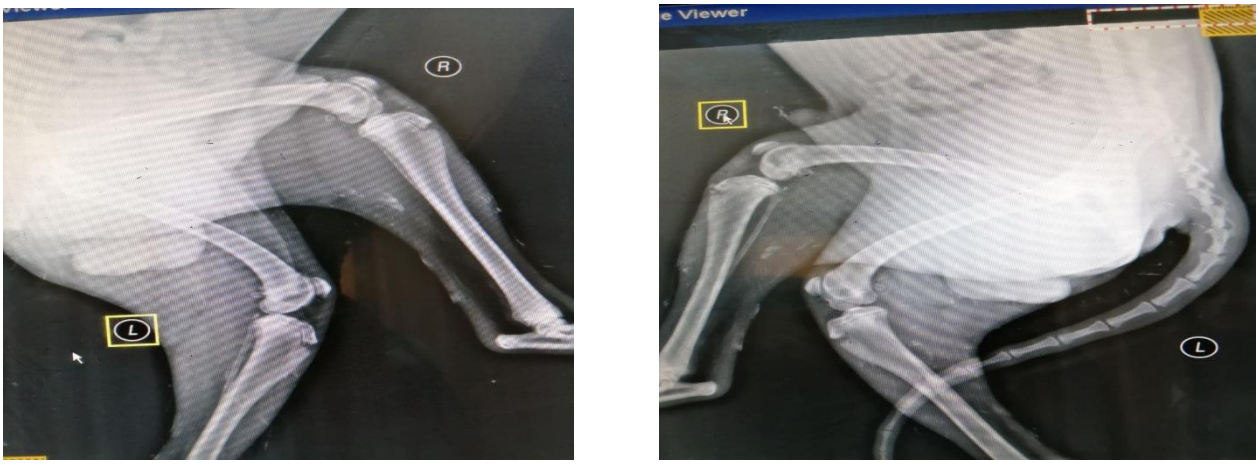

Fig.-7:The radiographic views of G3 at 1st week (left view) and 3rd week (right view) showed that there was no effect of normal saline on the resolution of the traumatic arthritis.

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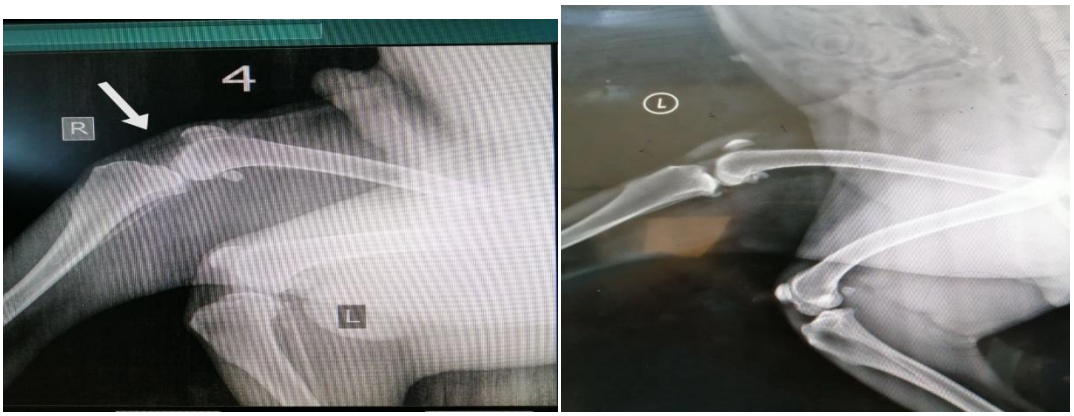

Fig.-8:The radiographic views of G4 at 1st week (left) and 3rd week (right)

Discussion

This study focuses on the effect of replacing inflamed synovial fluid with ACS that contains prepared IL-1Ra in G1 and replacing autologous normal SF from another limb in treating acute arthritis and preventing its progression to osteoarthritis and its chronic pathological drama of the joint cartilages. This is the first attempt to use autologous logos, according to our understanding. For the study of canine arthritis, the stifle joint was chosen as a suitable joint model, which, as stated in several previous studies (Stefania Pinna et al. 2019), can easily induce arthritis by crushing the cranial cruciate ligament and deciding lameness (Emmanuel Kuyinu et al.,2016).

WBC release IL-1Ra after incubation and activation with a 10% calcium chloride solution in an IL-1Ra preparation (Bruce Hamilton et al. 2016). The glass beads are used to absorb IL-1Ra adherent on their surfaces, which is then centrifuged. In the healing of arthritis, IL-1Ra, GFs and cytokines that are present in ACS can play an important role. By inducing cartilage anabolism, intra-articular application of ACS is thought to have a protective effect on the development of the Osteoarthritis agonist (Carlota Salgado et al.2021).

Lameness checking reveals that replacing autologous normal SF with autologous normal SF as in G2 is superior to using ACS as in G1. We think that this may be due to its hyaluronic acid quality, which is a natural anti-inflammatory treatment.

The first, second, and third weeks after the surgical operation were used to document the gross signs that occurred in both groups' stifle joints. It was assumed that all joints were swelling, redness, and lameness as shown in Table-2, as mentioned by (Leonardo Punzi et al.2016) at the 1st week, although these symptoms differed according to the various therapies used. At week 3, moderate swelling, redness, and lameness of G1 were observed, with none of these symptoms occurring in G2 at the end of the study, but very severe in G3 and G4 at week 3. The angle level of the stifle joints was reduced following the progress of joint inflammation (Jason Bleedorn, et al. 2011), as shown in Table-1, due to the damage to joint structures such as the joint capsule, collateral ligaments, cruciate ligaments. Such results have been accompanied by (Jerram, Walker,2003).

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As shown in Tables- 3 and 4, the physical and biochemical biomarkers of SF constituents (Evelien de Bakker et al. 2017) are included in the SF review in the present study.

The physical markers included the color, viscosity and pH values as shown in Table-3. Yellow or straw is typically the standard color of canine SF (James Anderson, et al. 2018), but in this study the colors ranged from dark straw to pale straw. It was straw in G1, straw pale in G2 after three weeks, straw dark in G3, and straw dark in G4.

These results may be attributable to the therapeutic effect of ACS for G1 on healing pathways, to the therapeutic effect of autologous SF in G2, and to mild saline therapy in G3, while G4 treatment was not available. The standard SF is viscid in order to lubricate the joints and lower the stress between the joint cartilage (Denis Furmann,et al. 2020). The results of this analysis showed that the viscosity return was normal for G2 at weeks 2 and 3, whereas it was low for G1 at week 3, while it was very low for G3 and G4.These findings showed that for G2, the level of hyaluronic acid returned to normal and the GFs of ACS were not linked to the level of hyaluronic acid. The pH values were also followed by a description of the viscosity due to the degree of hyaluronic acid.

Biochemical markers are very useful in the diagnosis of arthritis (Rubén Daniel Arellano Pérez Vertt. et al. 2015). The biochemical markers which were measured in this study were TPs, glucose levels, ALP concentrations and WBC counts. The most accurate tests for arthritis diagnosis have been described by (Jih-Chen Yeh et al. 2018). The TP was higher than average at the first week of G1 and G2, as seen in table-4, while at very high levels of G3 and G4 this spontaneously gave the hands the correct division of the dogs, but the values gradually decreased at the 2nd and 3rd weeks to regular values. At the 1st week, the TP value was 4.4±0.1 g/dl for G1 and 6.4±0.09 g/dl for G2, respectively, which were higher than normal because of arthritis but decreased when the adjustment was directed to the resolution at the 3rd week. We believed that the rise in TP was due to the increased permeability of the blood vessel wall, which nourishes the joint to improve protein passage into the synovial fluid (Michael Shang Kung et al.,2015). The glucose concentrations in arthritis (Erik Toonen et al., 2014) were reduced, as shown in table-4. It was less than normal in the first week for G1 69.100.53mg/dl and G2 73.80.89mg/dl, respectively, due to its conception by synovial excretion cells that contain the SF. (IratxeMacas et al., 2020) finds ALP to be a byproduct of cell damage (IratxeMacas et al., 2020). In this study, the concentrations of alkaline phosphatase (ALP) for G1 183.2±0.63 U/L and G2 174±0.41 U/L were increased at week 1 due to the growth of the damaged cells during arthritis. At week 1, the WBC counts for G1 (1523237.7 cell/ml3) and G2 (12492.865.6 cell/ml3) were both very high. We believed that because of its blood aggregation, the WBC increased inflammation for pathogen phagocytosis and the release of cytokines, growth factors, and interleukin receptors, which it battles.

The CCL rupture is the most common canine stifle joint problem. For cranial drawer or tibial compression tests, palpation may be difficult to diagnose in some dogs (de Rooster ,and van Bree , 1998). Intra-articular swelling, cranial tibia

displacement with tarsal flexion applied in the medial lateral view (Cazieux positive sign) and recurrent cases, as in the current research, are radiographic signs (Kealy and McAllister , 2000), During radiography, for G1 and G2 respectively, the stifle

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joints were flexed at an angle of 35,53 ° after 3 weeks. According to the improvement and assistance in the diagnosis of arthritis, inflammatory degrees were clearly shown by the radiographic studies, Fig.-7,8,9, and10, respectively. The results demonstrated the power of the usual effect of SF superiority resolution.

Conclusions

1- Autologous ACS is a modern traumatic arthritis treatment .
2- The superior therapeutic effect of autologous normal SF on traumatic arthritis .
3- The preparation of biological therapies is easy and fast .
4- There are a lot of benefits to biological therapies, such as quick, easy preparation and no side effects.

Recommendations

1- Precisely, the current study requires further research to concentrate on biological therapeutic effects .
2- Use of substitution of diseased SF with autologous normal SF applied clinically .
3- For other animals, use these therapeutic protocols .
4- These useful biological therapies have been updated .

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