

A histological study for the effect of Platelet-Rich Plasma (PRP) therapy on muscular contusions in Iraqi Arabian horses.

دراسة نسيجية لتأثير علاج البلازما غني الصفائح الدموية على الكدمات العضلية للخيل العربية العراقية.

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Abstract

Platelet-rich plasma (PRP) is regarded as a modern therapeutic technique in horses, so there are few researches .

The study is conducted on eight Iraqi Arabian horses, which are divided equally and randomly into two groups, treatment and control group. Autologous PRP is prepared for treatment group. All experimental horses are exposed to hard trauma causing muscular contusions. Treatment group were treated two times a week for six weeks.

Due to the effect of many growth factors, the results show benefit effect of PRP, perfuse granulation tissue which is characterized by excellent proliferation of fibroblasts and highly proliferation of endothelial cells to form new blood vessels.

This study according to our knowledge is the first research in muscular contusions of Arabian horses, which is focusing on the histological effect of autologous PRP on the muscular contusions which is very common acute injuries in Iraqi Arabian horses.

Key words: PRP- therapy- muscular- contusion- Arabian- horses.

الخلاصة

يعد علاج البلازما غني الصفائح الدموية من العلاجات الحديثة للخيل لذا تميز بقلة الدراسات المنشورة عنه. شملت الدراسة ثمانية خيول عربية عراقية قسمت عشوائيا، وبصورة متساوية مجموعة العلاج ومجموعة السيطرة. تم تحضير بلازما غني الصفائح الدموية الذاتي لمجموعة العلاج. تعرضت جميع خيول التجربة تجريبيا الى كدمة شديدة أدت الى كدمة عضلية، عولجت مجموعة العلاج مرتين في الاسبوع ولمدة ستة اسابيع.

ونتيجة لتأثير عوامل النمو المتعددة اظهرت النتائج أفضلية لبلازما غني الصفائح الدموية، نمو مفرط للنسيج الحبيبي والذي تميز بنمو واضح للخلايا الليفية ونمو مفرط للخلايا البطانية لتكوين اوعية دموية جديدة. تعتبر هذه الدراسة هي الأولى حسب معلوماتنا في الكدمات العضلية للخيل العربية والتي سلطت الضوء على التأثير النسيجي للبلازما غني الصفائح الدموية على الكدمات العضلية والتي تعد احدى الإصابات الحادة الشائعة للخيل العربية العراقية.

Introduction

In spite of the high occurrence of muscle strains, there was a limited signal base for the majority of management techniques and treatment. In specific minimizing the risk for repeated muscle injuries, had progressed little in the previous thirty years. Furthermore, numerous risk factors for muscle injury had been known (1), evidence recommended that the highest risk factor for a recurrence remains a previous injury to that muscle, possibly a result of scar tissue formation at or near the injury spot (2).

The effects of autologous (PRP) was studied by (3) with recovery in the tibialis anterior muscle in rats. PRP was isolated by a centrifugation of whole blood, permitting extraction of the specific part of the plasma comprising a high concentration of platelets. These platelets contain growth factors that could activate myogenesis (4), (5), (6), and alleviate inflammation (7), (8).

Healing had been revealed to happen in response to local submission of growth factors (9), (10), these factors are stored in alpha granules of platelets which considered as an important component involved in haemostasis, the factors at the site of injury were released by activation (11).

There were at present frequent methods to concentrate growth factors. Platelets rich plasma (PRP) involved removing the protein of plasma that comprises larger concentration of platelets after centrifugation of autologous complete blood. PRP had been discovered to have between (4-8) times the standard platelet concentrations in comparison with whole blood (4).

Preparations of PRP had confident phases in general. The first step was the aspiration of the peripheral blood, followed by centrifugation to harvest three layers : the red layer {including erythrocytes}; white layer {leukocytes as well as inflammatory cytokines} and the yellow layer {including plasma; platelets and growth factors} (12).

Besides producing greater concentrations of growth factors; PRP compounds also presented some benefits with other methods of getting growth factors. Platelets play an important part in clotting and hemostasis (12).

The platelets' alpha granules correspondingly secreted other cytokines ; proteins (endostatins; platelet factor 4) that were included in the process of healing (13).

Platelets had also found out to have analgesic effects; freeing protease activated receptor 4 peptides (14).

Muscle healing occurred in consequent steps, which overlapped inflammation; proliferation as well as remodeling. The steps were controlled by the incidence of growth factors and cell communications. There was a huge concentration of cytokines presented in healing muscle; evidence for the importance of growth factors in muscle healing. Growth factors enhanced muscle regeneration; and also, improved muscle force (15).

The goal of this study was to demonstrate the beneficial use of (PRP) in the treatment of muscular contusion of the local Arabian horses.

Materials and methods

Eight local Arabian horses are used 2 stallions and 6 mares, their ages are 6-6.5 years their weights ranged 350-400kg , which divide randomly and equally into two groups, treatment group and control group.

Whole blood one hundred milliliters are aspirated from the peripheral blood circulation (jugular vein) of each horse of treatment group {autologous blood}, twice centrifugation at (4000) rpm for ten minutes. The yellow layer {including plasma; platelets as well as growth factors} is aspirated gently, adding 10% calcium chloride then keeping the solution in incubator at 37°C for 24 hours to activate platelets, to release high concentration of growth factors and preserve in (- 80 ° c).

All the experimental horses had sedation intravenously by acepromazine maleate (2-4 mg/lb.B.W.). Their muscles (superficial gluteal muscle) are exposed to hard trauma by a metal object causing muscular contusion.

The treatment group had injected locally 4ml.of plasma rich platelets (PRP) preparations, two times a week for six weeks, directly after contusion (Fig- 1).times a week for (6) (2)) weeks.reparations, (2) times a week for (6) weeks. Their muscles

Biopsies were taken from both groups (7, 14, and 21) days for histo-pathological examination. The histo- pathological slides were stained with hematoxiline and eosin stains and examed under light microscope (Olympus Incorporation - Japan) .



Fig.-1: injection of PRP in the superficial gluteal muscle

Results

On day seven of the treatment group with (PRP), the histological assessment shows significant changes, particularly regarding wound healing . There are marked irregular muscle fibers and scattered infiltration of inflammatory cells, with presence of few hemorrhage between skeletal muscle fibers (Fig. 2).

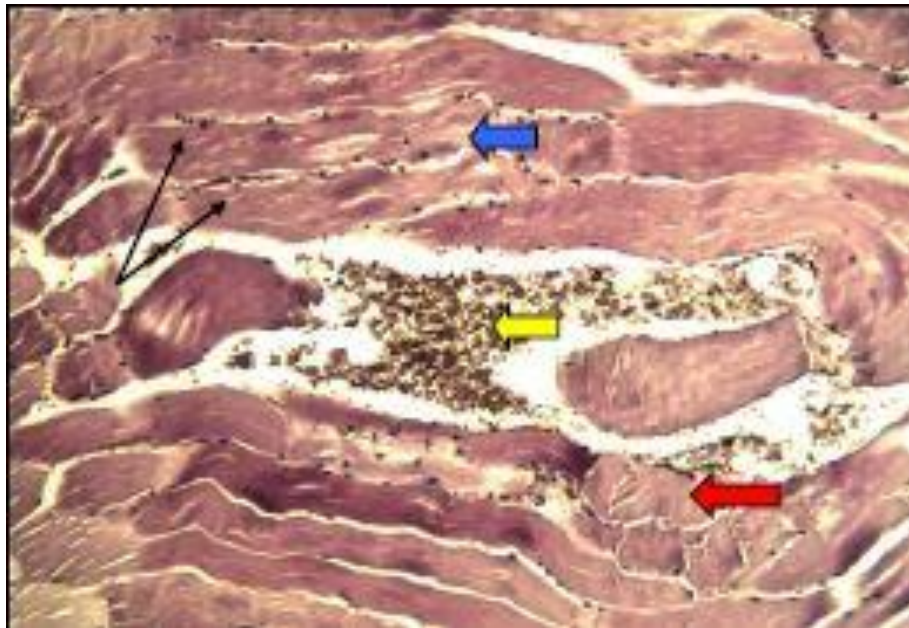
After fourteen days of treatment with (PRP), shows higher infiltration of inflammatory cells and abundant irregular arrangement of skeletal muscle fiber, also presence of granulation tissue which characterized the wound with formation of new blood vessels (Fig. 3).

Twenty one days of treatment with (PRP), the histo-pathological exam shows profuse granulation tissue which characterize by proliferation of fibroblast to form fibrosis and highly proliferation of endothelial cells to form new blood vessels (Fig. 4).

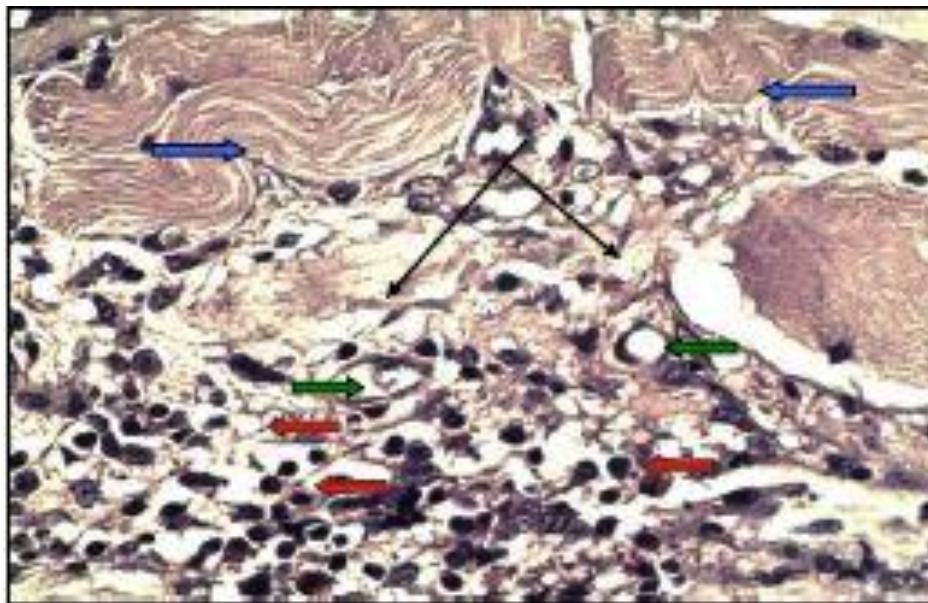
While the histological assessment of the control group on day seven shows severe Hemorrhage between skeletal muscle and slightly infiltration of inflammatory cells also there is irregular muscle fiber (Fig.5).

After fourteen days post wound in control group, there are marked irregular arrangement with severe degeneration of striated muscle fiber. Also presence of hemorrhage between muscle fiber (Fig.6).

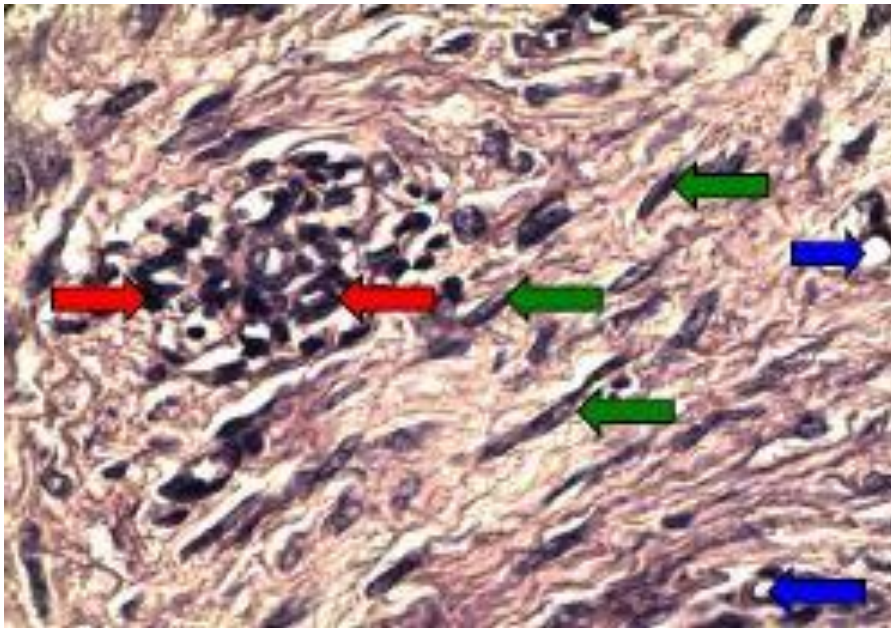
On day twenty-one post wound in control group, there are clear higher infiltration of inflammatory cells, also shows weak granulation tissue and characterize by the muscle fiber which visible distraction and are separated with degeneration of myocytes (Fig.7).



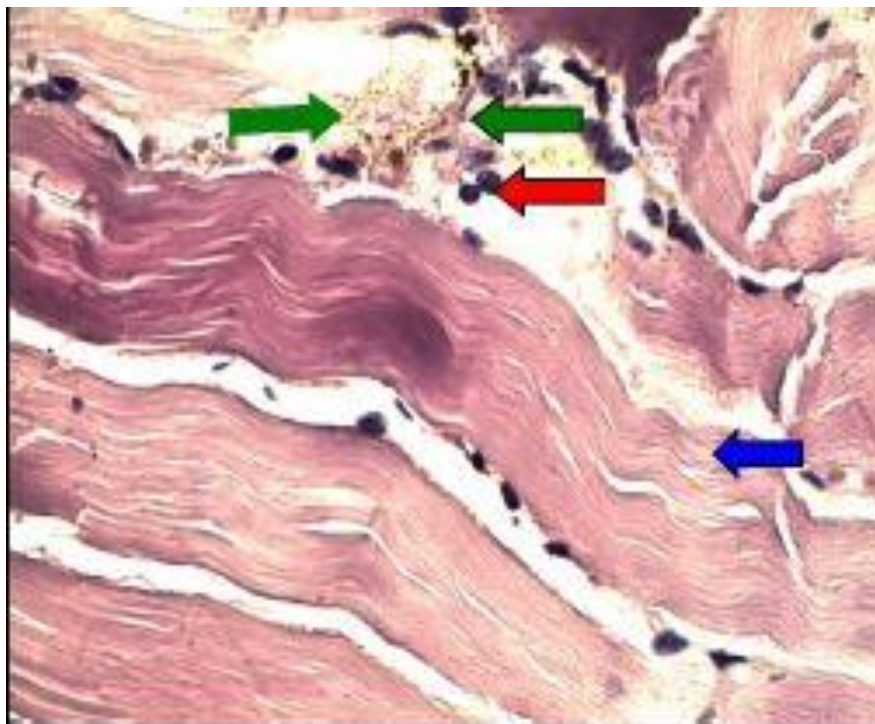
Fig, -2: treated group (PRP) histopathological section of the superficial gluteal muscle at seven days shows there is marked irregular muscle fiber (blue arrow) and *scattered* infiltration of inflammatory cells (thin arrows). Necrosis and degeneration muscle fiber (red arrow). also there is hemorrhage between the skeletal muscle fibers (yellow arrow). 10X H&E.



Fig,- 3: treated group (PRP) histopathological section of the superficial gluteal muscle at fourteen days. There is higher infiltration of inflammatory cells mainly macrophage irregular arrangement of skeletal muscle fibers (blue arrows). Also, there is marked granulation tissue which characterized by proliferation of fibroblast and formation of new blood vessels (green arrows). 40X H&E.



Fig,- 4: treated group (PRP) histopathological section of the superficial gluteal muscle at twenty-one days there is presence of granulation tissue which characterized by proliferation of fibroblast to form fibrosis (green arrows) and proliferation of endothelial cells to form new blood vessels (blue arrows) also there is infiltration of inflammatory cells (red arrows).(10XH&E)



Fig,- 5: control group histopathological section of the superficial gluteal muscle at seven days There are severe heamorrhage between skletal muscle (green arrows) and shows slitley inflammatory cells (red arrow) also there is visible irregular muscle fiber(blue arrow) (10 XH& E)

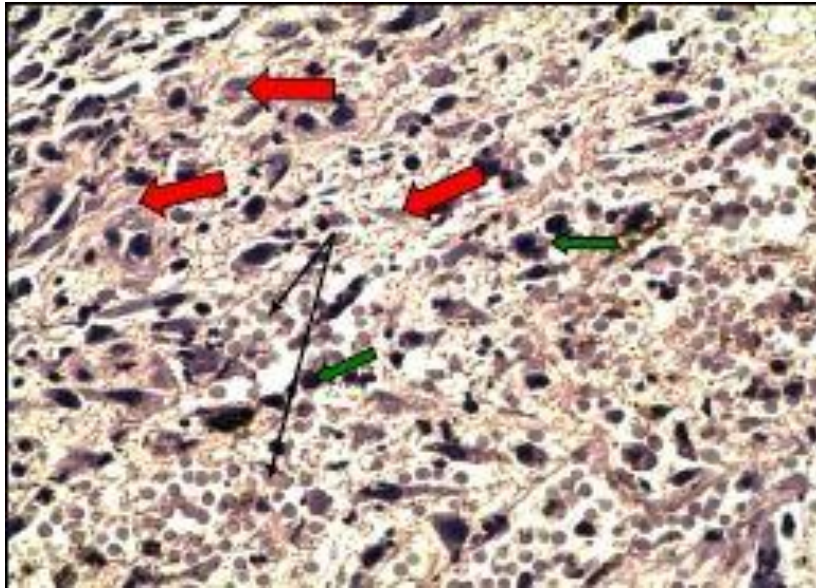


Fig-6: control group histopathological section of the superficial gluteal muscle at fourteen days there is marked irregular arrangement with severe degeneration of striated muscle fibers (red arrows) also there is hemorrhage between muscle fiber (thin arrows) also infiltration of inflammatory cells (green arrows).

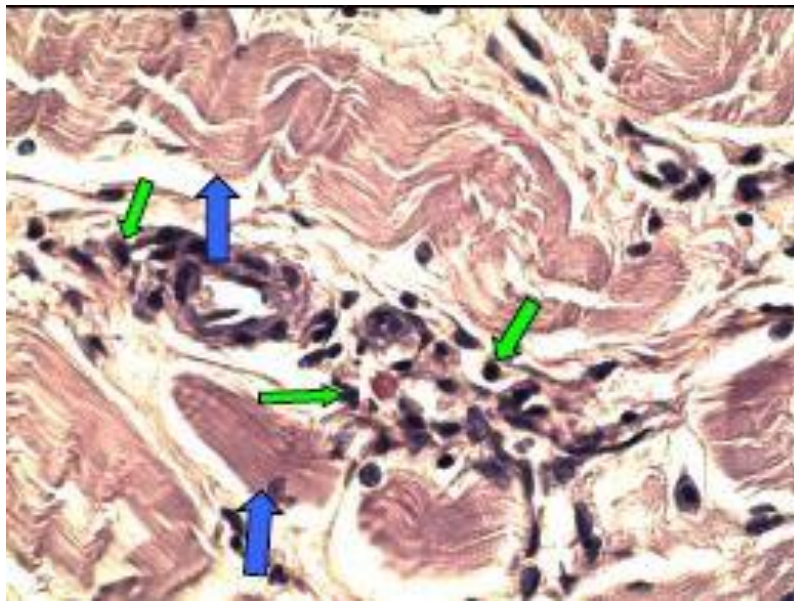


Fig-7: control group histopathological section of the superficial gluteal muscle at twenty-one days shows high infiltration of inflammatory cells (green arrows) also irregular muscle fiber weak granulation tissue and this characterize by the muscle fibers which shows distracted and separated with degeneration of myocytes .

Discussion:

There are limited studies of the PRP effect on the muscle injuries because PRP remedy has full-fledged in acceptance in the past few years. PRP is a safe therapy assumed its autologous nature and long term application with out any serious complications. It is formed from the animals' peripheral blood and is centrifuged to attain a high concentration of platelets with in a little volume of plasma. It is then re-inject at a location of muscular contusion {acute trauma}. There are many preparation procedures of PRP and activation of platelets to release mean growth factor like {TGF β 1, TGF β 2, TGF β 3, Vascular Endothelial GF} that responsible of acceleration the proliferation of cells {myogenesis}. According to (16) double- centrifugation method is able to achieve higher platelet concentrations than the single- centrifugation.

The histological evaluation of PRP effect is very clear as shows by the results of the present study specially after twenty one days, there is perfuse granulation tissue which is characterized by proliferation of fibroblasts to form fibrosis and highly proliferation of endothelial cells to form new blood vessels (Fig.- 4) while the histological assessment of the control group at the same period shows, clear higher infiltration of inflammatory cells, weak granulation tissue, visible distractions of myocytes. These results are accompanied with (17).

The real mechanisms of action of PRP are widespread because of the production of a myriad of bioactive agents.

The benefit use of PRP for the healing acceleration of muscular contusion may be due to the injection of concentrated platelets in the injured muscle, once is stimulated; results in an exponential increase of abundant growth factors. Though; the purpose of many growth factors; chemokines; cytokines; and ion of muscular sin the injured muscle, once activate at ccelerat 3 contusion may be due to the injection of concentrated plateleinflammatory mediators has not been illuminated; and have not the interactions between agents and their effect on adjacent cells; specially the transforming growth factor (TGF β 1) (17), (18).

Many questions are related to PRP remain unanswered, such as the maximum concentration of platelets in PRP, ideal procedures of preparation, the correct PRP formulations, the types of cells which are contained in PRP, its frequency of applications, so further researches are needed to explain the quantification of specific growth factors release by PRP which effect on angiogenesis and myogenesis as well as the functional recovery before PRP can be used in a wide clinical application (19).

This technique is still need focusing on many questions, the ideal procedures preparation, frequency of application, the correct PRP formulation, types of cells which are contained in PRP and the quantification of the specific growth factors release by PRP which effect on angiogenesis and myogenesis.

This study according to our knowledge is the first research in muscular contusion of Arabian horses, which is focusing on the histological effect of autologous PRP on the muscular contusion which considers the most common acute injury in the Iraqi Arabian horses.

References

1. Orchard, J.W. (2001). Intrinsic and extrinsic risk factors for muscle strains in Australian football. *Am.J.Sports Med.* 29:300-303.
2. Jarvinen, T. ; Kaarianen, M. and Jarvinen, M. (2000). Muscle strain injuries. *CurrOpinRheumatol.* 12:155-161.
3. Jason, W. ; Hammond, M. ; Richard, Y. ; Leigh, A. C. ; Joaquin, M. M. and Richard, M. L. (2009). Use of autologous platelet-rich plasma to treat muscle strain injuries. *Am. J. Sports Med.* 37(6): 1135-1142.
4. Creaney, L. ; Hamilton, B. (2008). Growth factor delivery methods in the management of sports injuries: the state of play. *Br.J. Sports. Med.* 42:314-320(Pubmed).
5. Schanbel, L. V. ; Mohammed, H. O. and Miller, B.J. (2007). Platelet rich plasma (PRP) enhances anabolic gene expression patterns in flexor digitorumsuperficialis tendon. *J. Orthop. Res.* 25:230-240. (PubMed).
6. Wright-Carpenter, T. ; Opolon, P. and Appell, H.J. (2004). Use of autologous conditioned serum: animal experiments using a muscle contusion model. *Int. J. Sports Med.* 25:582-587. (PubMed).
7. Al-Sharkawy, H. ; Kantarici, A. and Deady, J. (2007). Platelet-rich plasma growth factors and pro-and anti-inflammatory properties. *J. Periodontol.* 78:911-921. (PubMed).
8. Meijer, H. ; Reinecke, J. and Becker, C. (2003). The production of anti-inflammatory cytokines in whole blood by physico-chemical induction. *Inflamm. Res.* 52:404-407. (PubMed).
9. Menetre, J. ; KasemkijWattana, C. ; Day, C. S. ; Bosch, P. ; Vogt, M. and Fu, F.H. (2000). Growth factors improve muscle healing in vivo. *J. Bone Joint Surg. Br. :* 82(1): 131-137.
10. Molloy, T. ; Wang, Y. and Murrell, G.A.C. (2003). The roles of growth factors in tendon and ligament healing. *Sports Med:* 33(5): 381-394.
11. Epply, B.L. ; Woodell, J. E. and Higgins, J. (2004). Platelet quantification and growth factor analysis from platelet rich plasma: implications for wound healing. *PlastReconstrSurg:* 11(6): 1502-8.
12. Lopez-Vidriero, E. ; Goulding, K.A. ; Simon, D. A. ; Sanche, Z.M. and Johnson, D.H. (2010). The use of platelet-rich plasma in arthroscopy and sports medicine optimizing the healing environment. *Arthroscopy:*26(2):269-278.
13. Anitua,E. ; Andia, I. ; Ardanza, B. ; Murden, P. and Nurden, A. T. (2004). Autologous platelets as a source of protein for healing and tissue regeneration. *Thromb Haemost:*91(1):4-15.
14. Asfaha, S. ; Cenac, N. ; Houle, S. Alter, C. Papez, M.D. and Nguyen, C. (2007). Protease-activated receptor-4: Anovel mechanism of inflammatory pain modulation. *Br.J. Pharmacol:* 150(2):176-85.
15. Kasemkijwattana, C. ; Menetre, J. Bosch, P. ; Somogyi, G. and Moreland, M.S. and Fu, F. H. (2000). Use of growth factors to improve muscle healing after strain injury. *ClinOrthopRelat Res.:* (370): 272-85.
16. Tamimi, F.M.; Montalvo, S.;Tresguerres, I.;Blanco Jerez, L.(2007).A comparative study of 2 methods for obtaining platelet-rich plasma. *J. Oral Maxillofac Surg.;*65(6):1084-93.
17. Middleton, K.K.;Barro, V.; Muller, B.; Terada, S.; Fu, F.H.(2012). Evaluation of the effects of platelet-rich plasma therapy involved in the healing of soft tissue injuries. *Iowa Orthop. J.;*32:150-163.
18. Hammond, J.W.; Hinton, R.Y.; Curl, L.A.et al.(2009). Use of autologous platelet-rich plasma to treat muscle strain injuries. *Am. J. Sports Med. ;* 37:1135-1142.
19. Gigante, A.; Cianforlini, M.; Manzotti, S.; Ulisse, S.(2013). The effect of growth factors on skeletal muscle lesion. *Joints;*1(4):180-186.