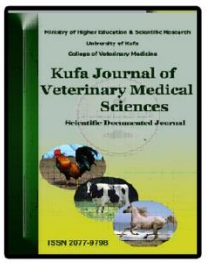
Kufa Journal for Veterinary Medical Sciences Vol.(8). No.(2) 2017

Kufa Journal for Veterinary Medical Sciences

vetmed@uoKufa.edu. iq



Comparative Study Between The Effects Of Henna (*Lawsonia Inermis*) Alcoholic Extract And The Autologous Platelet Rich Plasma (PRP) On The Cartilage Healing Of Rabbits.

Rafid Hadi Farman

University of Al- Qadisyah Veterinary Medicine College

rafid.farman@qu.edu.iq

Mobile No.: 07801269926

Abstract:

It's very interested to study new herbal therapy as henna and compare its effect on cartilage healing with another modern therapy as Platelets – Rich Plasma (PRP)**.**

Twenty seven domestic male rabbits was included in this study, divided into three groups of 9 animal randomly, groups of henna 20%, groups of (PRP), and control groups, and also each group subdivided in to 3 subgroups depending on the time of sampling 7,21,45 day, 5 mm in diameter of injury full-thickness skin wound was created on the ear of each rabbit. The wounds in first group were treated with Henna after operation for 7 days, while its in second group treated with (PRP) either for 7 days , and the control group the wounds was abdicated by distal water only.

The results of this study are very important to find a new cheap herbal therapy. The results publicized that the wound healing in Henna treated groups happened faster than the (PRP) groups, and the complete healing happened in 49 days after treated by henna extract.

Grossly and histological evaluations shows clearly, the superiority of henna extracts 20% effects on cartilage healing in compare with (PRP).

Key words: henna- extract- platelets- plasma- cartilage- healing.

دراسة مقارنة لتأثير مستخلص الحناء الكحولي والبلازما الغنية بالصفيحات الدموية على التئام الغضروف في الارانب

رافد هادي فرمان

جامعة القادسية / كلية الطب البيطري

الخلاصة:

ان من المهم دراسة تأثير عقار حديث مستخلص من الحناء على شفاء الغضروف مقارنة مع تأثير البلازما غني الصفيحات الدموية.

تضمنت الدراسة استخدام سبع وعشرون من ذكور الارانب المحلية , قسمت عشوائيا الى ثلاث مجاميع من تسع حيوانات , مجموعة الحناء بتركيز 20% , مجموعة البلازما الغنية بالصفيحات الدموية ومجموعة السيطرة ومن ثم تم تقسيم كل من المجموعات الى ثلاث مجاميع ثانوية اعتمادا على وقت اخذ العينات وهو (45,21,7) يوم. تم القيام باستحداث جرح بقطر (5) ملم شمل طبقات الجلد كله والغضروف في اذن الارانب .تم معاملة الجروح في المجموعة الاولى بالحناء بعد العملية ولمده سبعة ايام ,بينما المجموعة الثانية عولج الجرح بالبلازما غني الصفيحات الدموية ايضا لسبعة ايام ,ومجموعه السيطرة تم معاملتها بالماء المقطر فقط.

اظهر التقييم العياني والنسيجي بوضوح التأثير الكبير لمستخلص الحناء20% على شفاء الغضروف مقارنة مع البلازما الغنية بالصفيحات الدموية, كذلك اعلنت النتائج على ان شفاء الجروح في مجموعة المعالجة بالحناء حدث اسرع من مجموعة البلازما الغنية بالصفيحات الدموية , كما ان الشفاء التام حدث في 49 يوم بعد العلاج بمستخلص الحناء.

الكلمات الافتتاحية: حناء- مستخلص- صفيحات- بلازما- غضروف- التئام .

Introduction

The leaves of henna (*Lawsonia inermis*) commonly used in the form of an ointment or decoction in the treatment of wounds, skin inflammations, burns, and ulcers due to antifungal and antibacterial activities(1).Oral administration as well as topical application of alcoholic extract of henna leaves showed significant healing process, but topical application of henna was more effective than the oral route. Thus, topical application of alcoholic extract of henna can be successfully formulated for wound healing (2,3).

Henna extracts have antimicrobial effect on the bacteria that causes skin infections. Alcoholic and oily henna extracts have highly effects like antibiotics that commonly used in clinical practice(4,5).

Towfik, et.al (6)in his experimental found that henna has good bactericidal effect on wound due to that henna caused high wound tension and make wounds dry which prevent bacterial growth and formation of abscess and they found that the alcoholic henna extract concentration at 20% is the more effective and act as bactericidal for all the types of bacteria, while henna extract at 10% concentration inhibit the bacterial growth. These results support the use of henna in the management of wound infection(6). Use of henna to treat burns may decrease the complication that arise in the use of conventional wound dressings such as silver compounds (7,8).

Platelets are cells that flow in the blood stream which is help in hemostasis and wound healing and have two types of granules, the first one increase permeability of blood vessels and increase the access of inflammatory cells to the site of injury and the second one of granule that release growth factors which stimulate the immigration of cells to the area of trauma, thus facilitating tissue healing (9).

The platelet-rich plasma(PRP) it's a high number of platelets in a small volume of plasma. Its job Stimulate to form a fibrin clot, that adheres the site of wound to prevent bleeding, minimize oozing and hold or improve healing in tissues in a short period of time, either haemostatic and healing properties (10). The autologous PRP is a source of several growth factors, like vascular endothelial growth factor, transforming growth factor, platelet-derived growth factor, and fibroblast growth factor. These factors response on stimulation angiogenesis, matrix production and deposition as well as on tissue regeneration. Many studies have confirmed that PRP response on cell proliferation, collagen synthesis, and vascularization(11,12,13,14)

The treatment of wound by PRP was effective, minimally invasive, fast, easy, cheap and, able to accelerate and improve the quality of the healing(15,16). PRP has application in controlling of skin wounds, and in orthopedic illnesses, primarily for ligament and tendon repair, and cartilage repair (17,14), its enhance formation of chondrocytes and collagen deposition, when used with calcium-activated thrombin, provides a self-adherent platelet gel that combines augmented growth factors with chondrocytes that help on cartilage repair (14)

PRP has the ability to treatment of cartilage degeneration, and has been proved to have optimistic effects on the repair of cartilage lesions. Because its poor blood supplying and self-renewal capacity, the

structural and functional of cartilage are defected when it's injured or damaged (16).

The inflammation of tendon decrease when treated by PRP by regulate the COX-1, COX-2 that response on increases of blood supply to defected area. This anti-inflammatory function of PRP is at least partially mediated through Hormonal Growth Factor, a major growth factor in PRP, which produces anti-inflammation results similar to PRP(18). While Monteiro, found that PRP improve the quality of repair of wound and avoid the development of extensive granulation tissue, at least in small granulating wounds in horse . either this therapy may better for wounds that suffering from losing of massive tissue or chronic wounds(19).

Treatment of the articular wound by collagen-PRP causes increasing of repair tissue that filling the wound, this tissues which had a similar profiles of growth factor and protein expression to the extra articular ligament wounds. So collagen-PRP scaffold can enhance histological differentiation between healing extra-articular wounds and non-healing(20,21).

Material and methods

Preparation of alcoholic henna extract:

To Prepare henna extract, (500)gm. of henna leaves dissolved in (1500) ml. of ethanol for (24) hours at room temperature. Then the mixture filtered by fine filter paper and put in a water bath to dry at (40 c˚) to get a clear henna extracts which diluted with ethanol as 20%(6).

Preparation of autologous platelet-rich plasma:

Blood (5ml.) was collected from the heart of each rabbit into a collection tube containing 0.5 mL of the anticoagulant (10% sodium citrate), centrifuged at 1,000 rpm for 10 minutes, the upper two third of fluid was discard, the lower third was centrifuged again at 5,000 rpm for 10 minutes . The supernatant plasma was then removed, leaving 0.5 mL of centrifuged product(13).

Twenty seven domestic male rabbits was included in this study, housing and feeding equally then divided into three groups of 9 animals randomly, group of henna 20%, group of PRP, and control group, and also each group subdivided in to 3 subgroups depending on the time of sampling 7,21,45 day.

Each rabbit was anesthetized by combination of ketamine hydrochloride (Duopharma, Malaysia) at dose of 50mg/kg B.W. I/M and xylazine hydrochloride2%(Alfasan, Holland) at a dose of 10mg/kg B.W.(22). Under routine surgery, 5 mm. in a diameter, circle hole was made in the ears cartilage of all animals (Fig.3), treatment of each group was done daily and measurements of the holes diameters was recorded daily.

The biopsies were taken for histopathologcal evaluation of the cartilages healing at 7,21,45 days

.

Fig.(1) : Shows the circle shape hole in the ear cartilage of rabbit..

Results

Generally animals of all groups were recovered without complications, and with no any mortality. Group of henna 20% were healed faster than other groups the opening of ear was closed after 49±2 days (Fig,2),then follow by the PRP group(Fig,3), ,while the control group was last group of healing.

After 1st week , the diameter of all groups decrease until reach 4.5(mm) while at the 3rd week mean diameter was 2.44 (mm)in the henna groups ,and 3.94(mm)in PRP groups while the mean diameter was 4.5(mm) in control groups. After the 6th week the mean diameter was 0.25 (mm)in the henna groups ,and 3.25(mm)in PRP groups while the mean diameter was 3.58(mm) in control groups. Table(1).

Table-1 shows the diameters (mm)of holes of each group.

Time / Treatment	1st Week $\pi \pm_{SE}$	2nd Week $\pi \pm_{SE}$	3rd Week $\pi \pm_{SE}$	4th Week $\pi \pm_{SE}$	5th Week $\pi \pm_{SE}$	6th Week $\pi \pm_{SE}$
Control	4.58 ±0.103 A	4.5 ±0.057 A	4.5 ±0.059 A	4.25 ±0.07 A	4± 0.1157 A	3.58 ±0.103 A
PRP	4.5 ±0.056 A	4.25±0.714 B	3.94±0.965 B	3.58 ±0.102 B	3.47 ±0.061 B	3.25 ±0.099 B
Henna	4.5 ±0.055 A	3.58±0.104 C	2.44 ±0.82 C	1.5 ±0.058 C	1.0 ±0.057 C	0.25 ±0.019 C

Different litter mean Significance variance (P≤0.05).

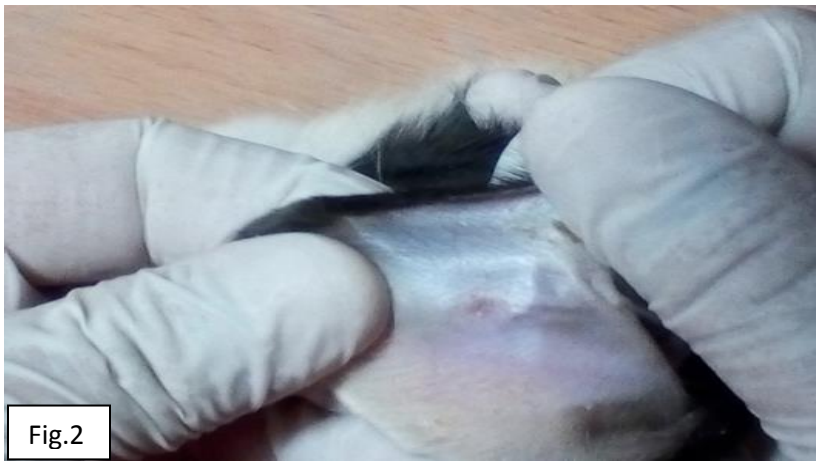


Fig.(2): Henna groups 49 days after wounded show that the hole of wound closed completely.

Fig.(3): PRP groups 49 days after wounded show that the hole of wound was presence.

The histopathologcal changes were seen in henna group at 7 days post-wounded includes complete sloughing of epidermis with hyperplasia of epidermal layer, hyper atrophy of chondrocytes, profuse collagen and infiltration of inflammatory cells in dermis Fig.(4). At 21 days, the histopathologcal lesions appearance in the incised site were partial healing of cartilage with extended edge and hypertrophied chondrocytes also there is there was granulation tissue formation consisting from new blood vessels and fibroblast, deposit collagen fiber in the incision, also showed fibrin deposition in the incision site Fig.(5). At 45 days post-surgery, the site showed the marked of complete healing of cartilage like proliferation of chondrocytes , hypertrophied and fused with another piece with presence of blood vessels and normal fibrosis, also there is presence of keratin layer and normal epidermis. Fig.(6-7).

In PRP group at 7 days post-wounded. The incised site showed complete cutting of

cartilage of ear , infiltration of inflammatory cells, presence of collagen fiber either, there is hyper atrophy of chondrocytes toward the another edge of cutting Fig.(8,9) . at 21 days post-surgery, we found marked healing of cartilage which characterized by hyper atrophied chondrocytes and another which are rupture .resulting in extended edge toward another piece also there is profuse fibrosis in dermis, marked hyper atrophied chondrocytes and ruptured chondrocytes that discharge calcium from it Fig.(10,11). At 45 day the incised site showed partial healing of cartilage which showed hyperplasia and hypertrophy of chondrocytes in all edges of the hole with profuse collagen in the center of incision . atrophied with ruptured chondrocytes and deposition of calcium in the ruptured chondrocytes Fig.(12, 13).

In control groups at 7 days, we found hemostasis with profuse fibrosis and collagen fiber. increase infiltration of inflammatory cells Fig.(14). In control group at 21 day. the incised site showed profuse granulation tissue characterized by formation of blood vessels and profuse fibrosis with complete absence of cartilage Fig.(15,16).While control group at 45 day, the incised site showed partial healing of cartilage which show as extended edge resulting of proliferation of chondrocytes and profuse granulation tissue which characterized by formation new blood vessels and fibrosis Fig.(17).

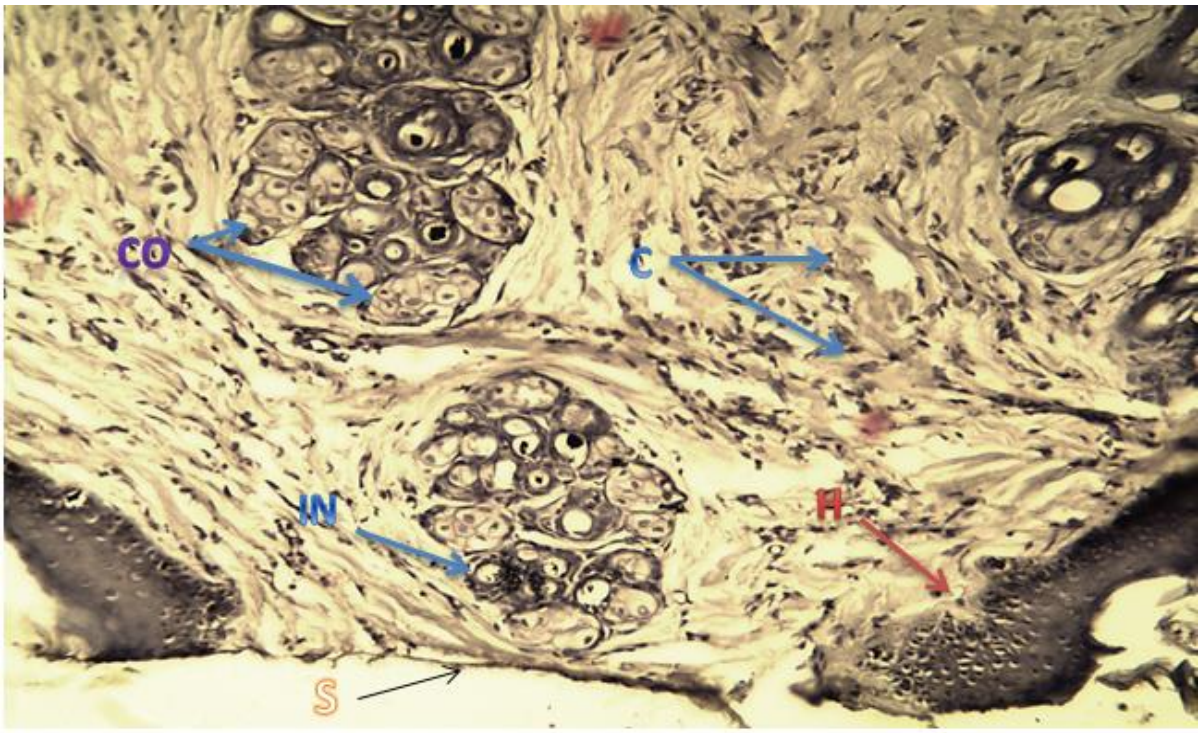


Fig.4 : Henna group at 7 days, showed complete sloughing of epidermis (S)with downward hyperplasia (H)of epidermal layer .Also there was chondrocytes show with marked hyper atrophy(Co), profuse. collagen(C) and infiltration of inflammatory cells in dermis(IN). 4X H&E

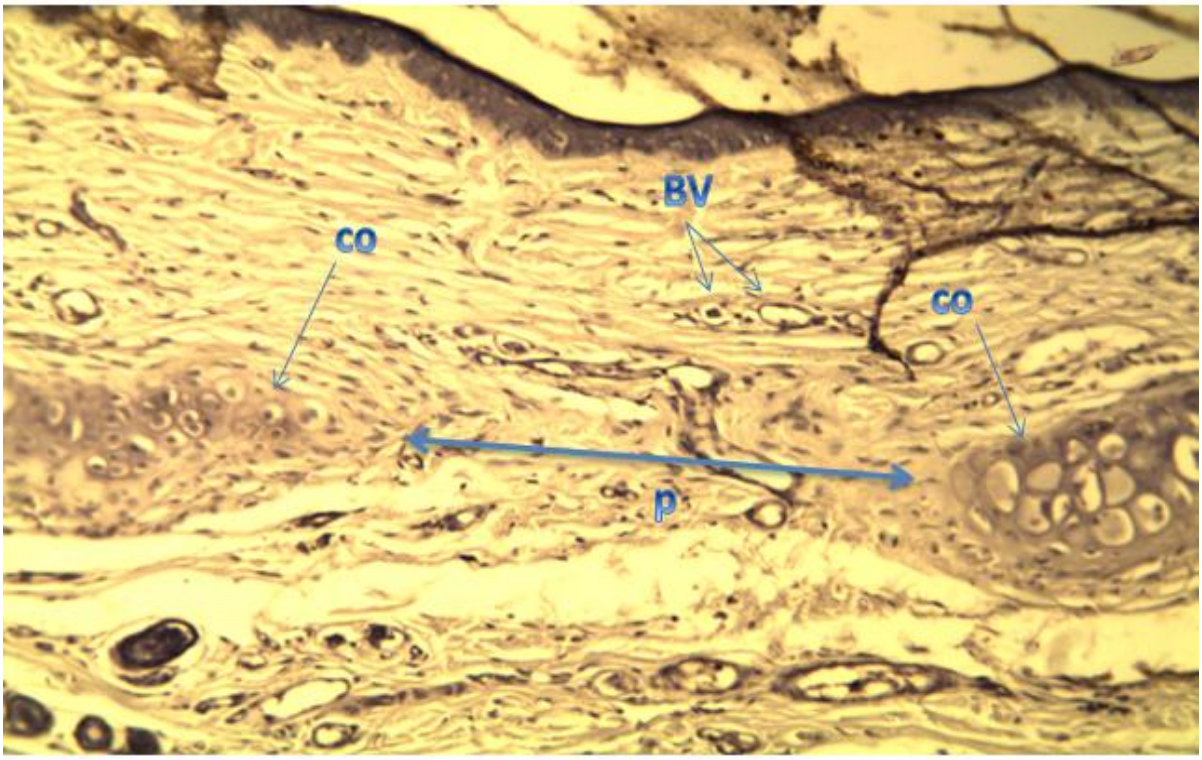


Fig.5 : Henna group at 21 days, showed partial healing of cartilage with extended edge and hypertrophied chondrocytes (CO)also there was presence of granulation tissue which characterized by formation of new blood vessels(BV) and fibrosis(F). 10X H&E.

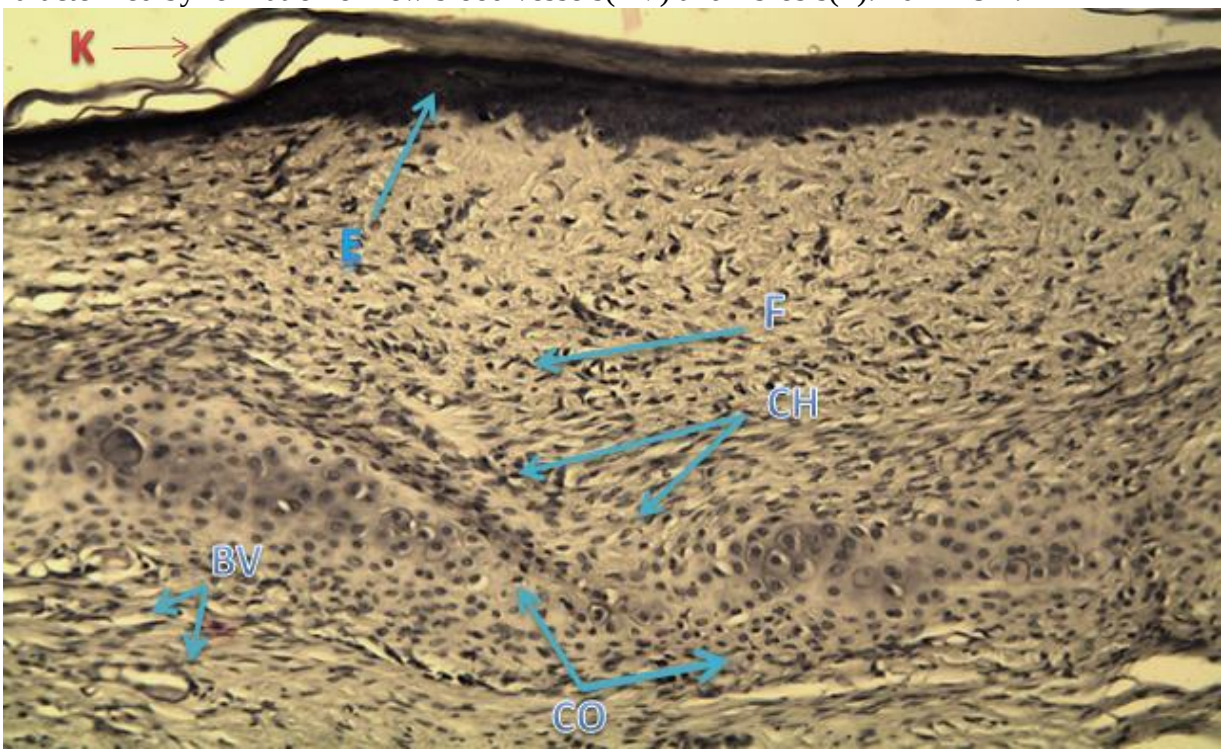


Fig.6 : Henna group at 45 days, showed there was complete healing of cartilage(CH) which showed as proliferation of chondrocytes (CO)with presence of normal blood vessels(BV) and normal fibrosis(F) , also there was presence of keratin layer (K)and normal epidermis(E). 10X H&E.

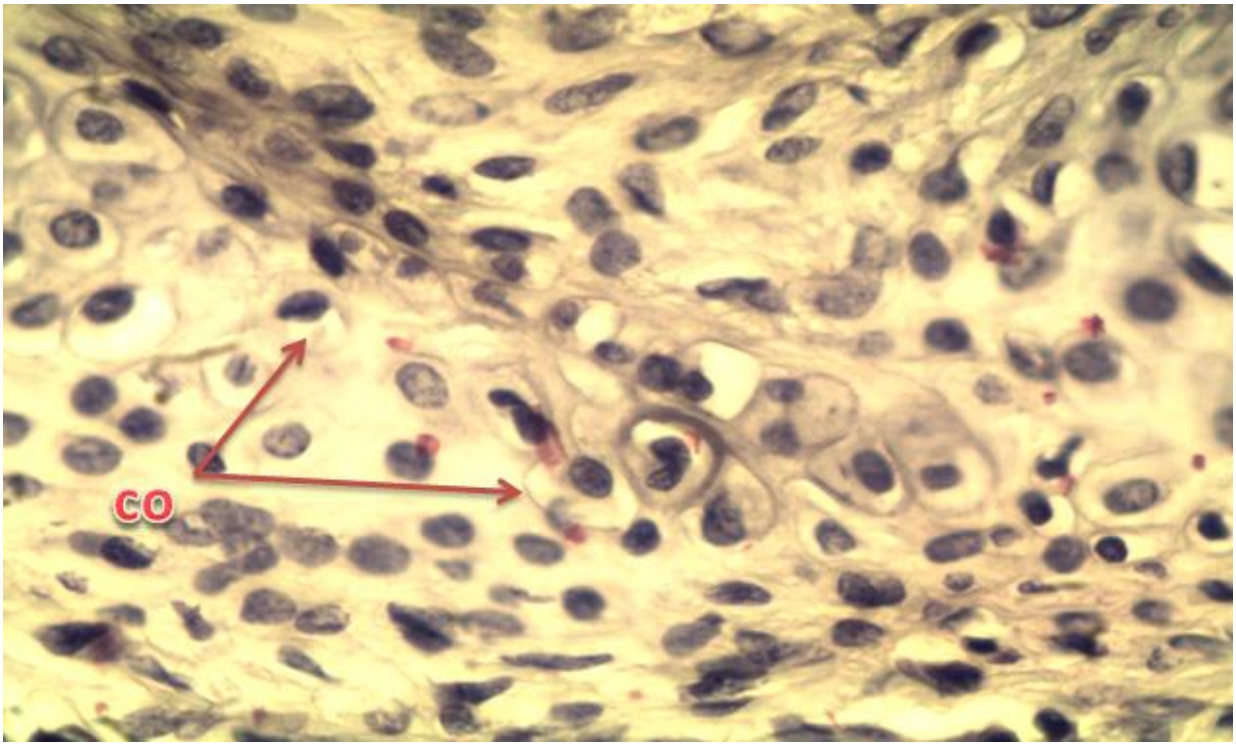


Fig.7: Henna group at 45 days, showed higher magnification showed there was marked proliferation of chondrocytes(CO) which showed hypertrophied and fused with another piece. 40X H&E.

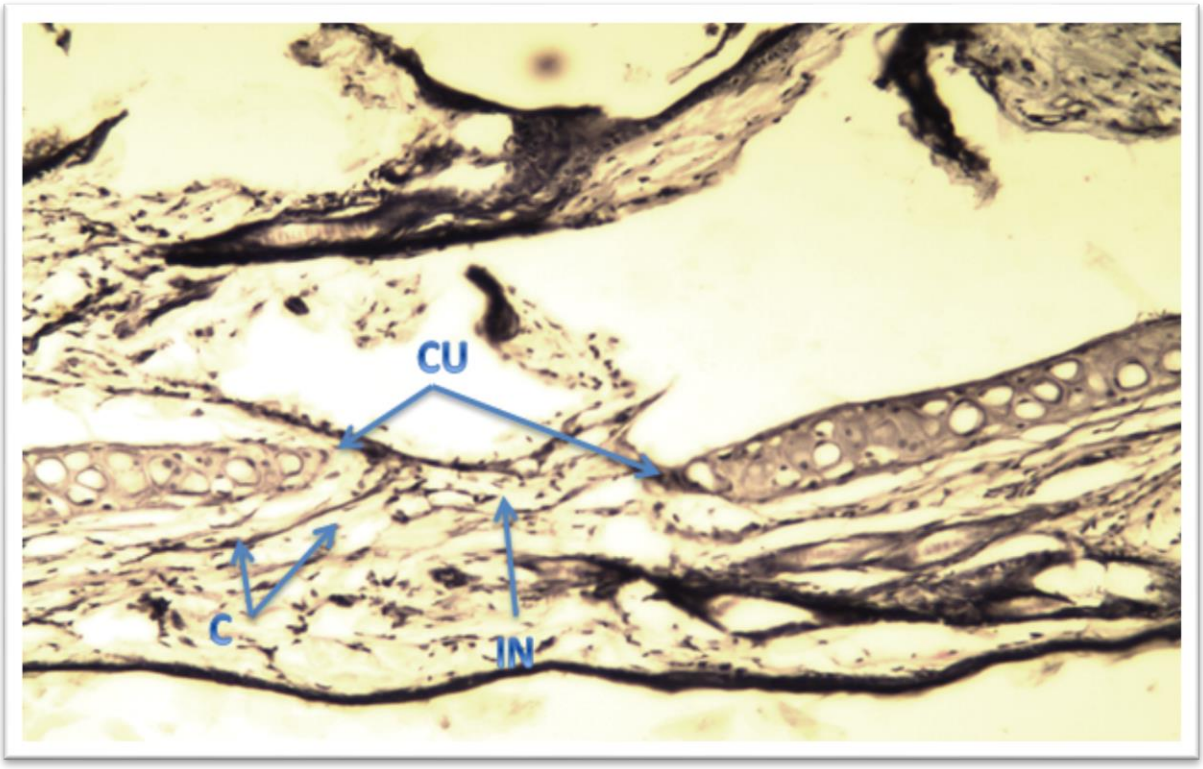


Fig.8 : PRP group at 7 days showed there was complete cutting of cartilage of ear (CU)with infiltration of inflammatory cells(IN) , presence of collagen fiber(C) . 4X H&E.

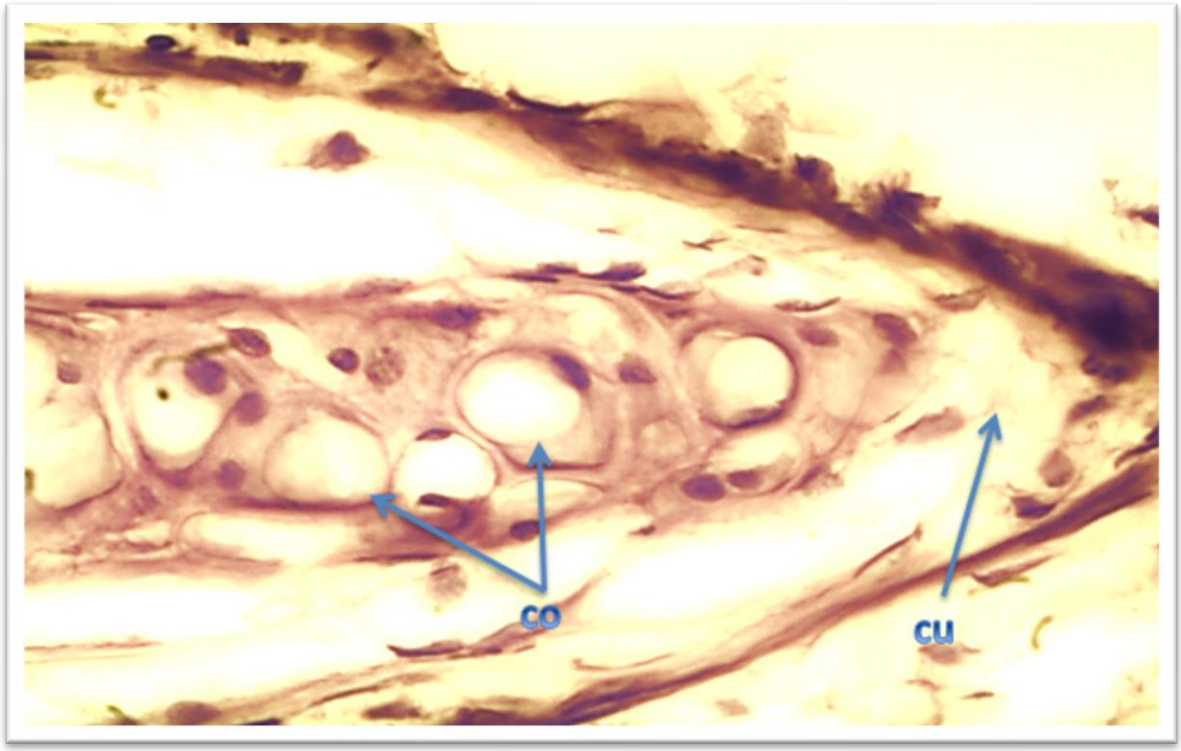


Fig.9 : PRP group at 7 days, showed higher magnification showed complete cutting of cartilage of ear(CU) there was hyper atrophy of chondrocytes(co) toward the another edge of cutting. 40X H&E.

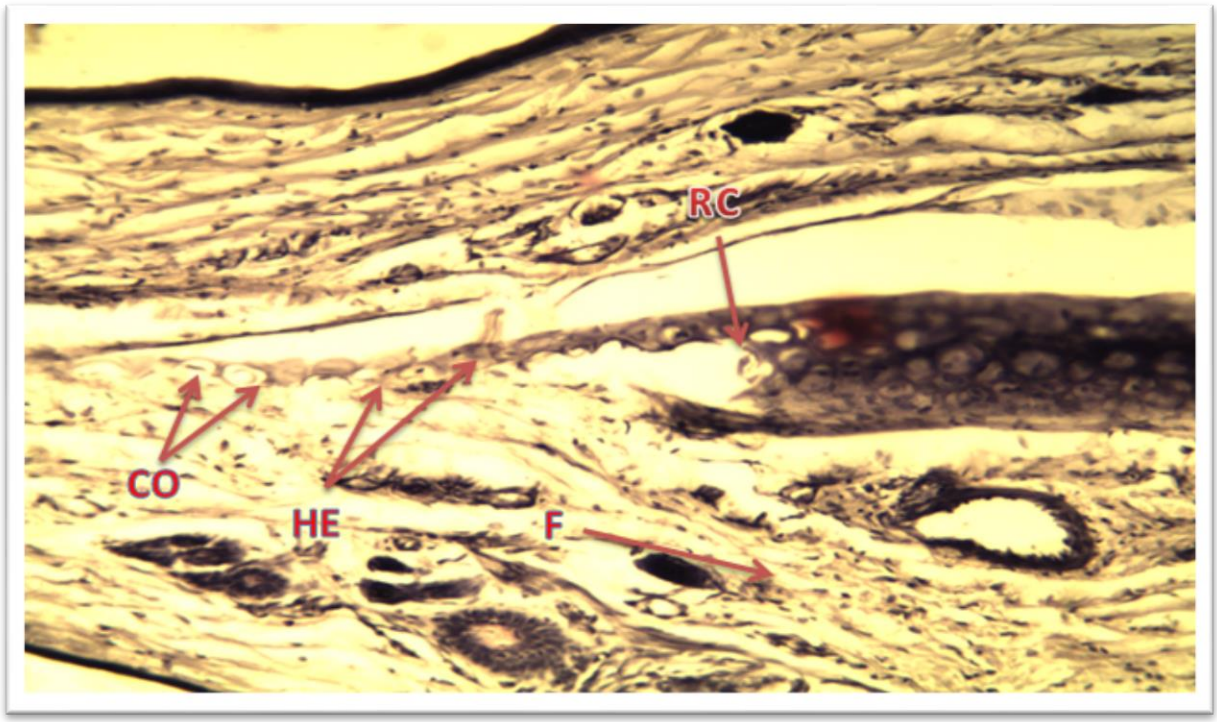


Fig.10 : PRP group at 21 days, showed there was marked healing of cartilage(HC) which characterized by hyper atrophied chondrocytes (CO)and another which were rupture (RC) resulting in extended edge toward another piece also there was profuse fibrosis in dermis(F).10X H&E.

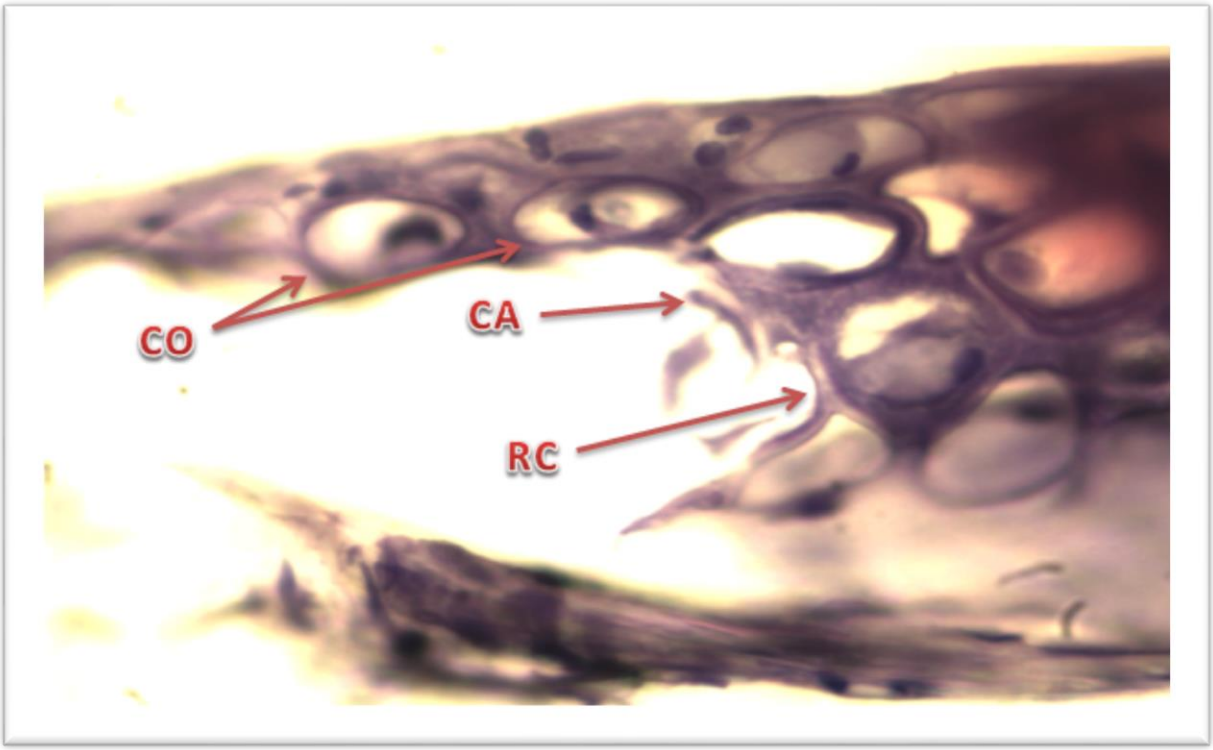


Fig.11 : PRP group at 21 days, there were marked hyper atrophied chondrocytes (CO), ruptured chondrocytes (RC) and deposition of calcium within ruptured chondrocytes CA).40X H&E.

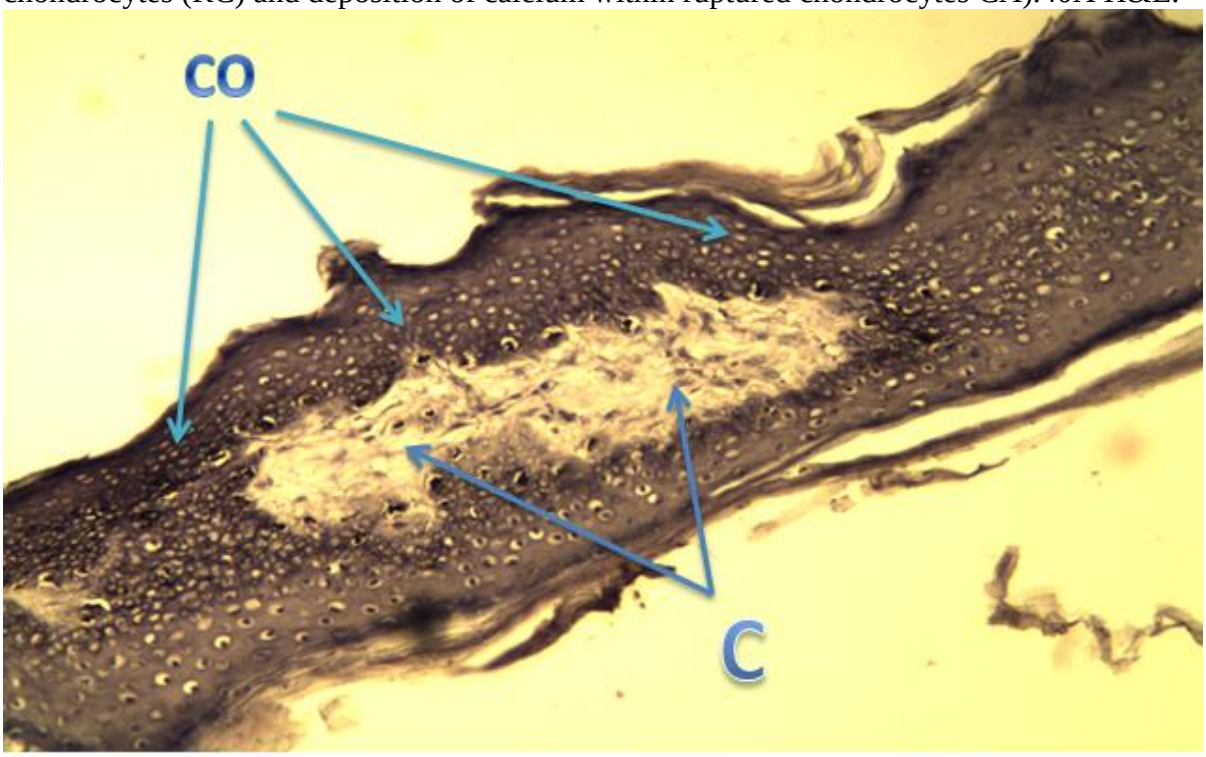


Fig.12: PRP group at 45 days, there was partial healing of cartilage which shoed hyperplasia and hypertrophy of chondrocytes (CO) in all the hole edges with profuse collagen(C) in the center of incision . 10X H&E.

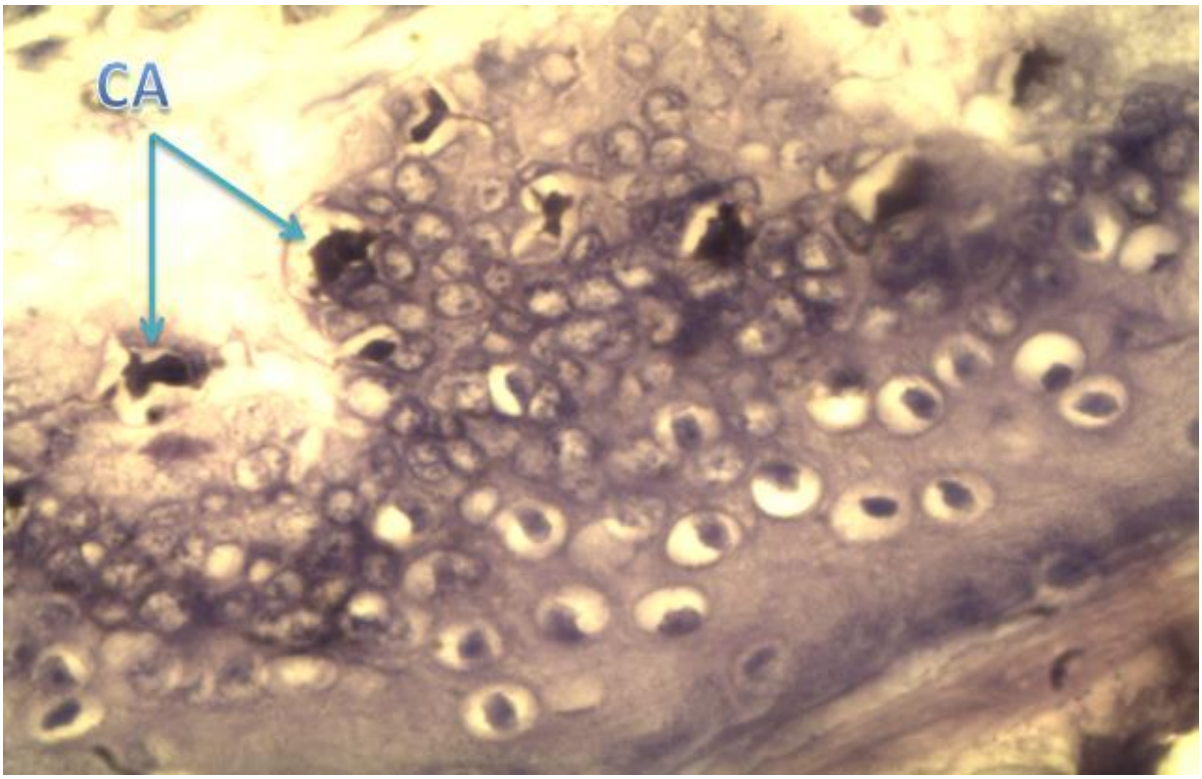


Fig.13 : PRP group at 45 days, there was marked healing and atrophied with ruptured chondrocytes and deposition of calcium(CA) in the ruptured chondrocytes . 40X H&E.

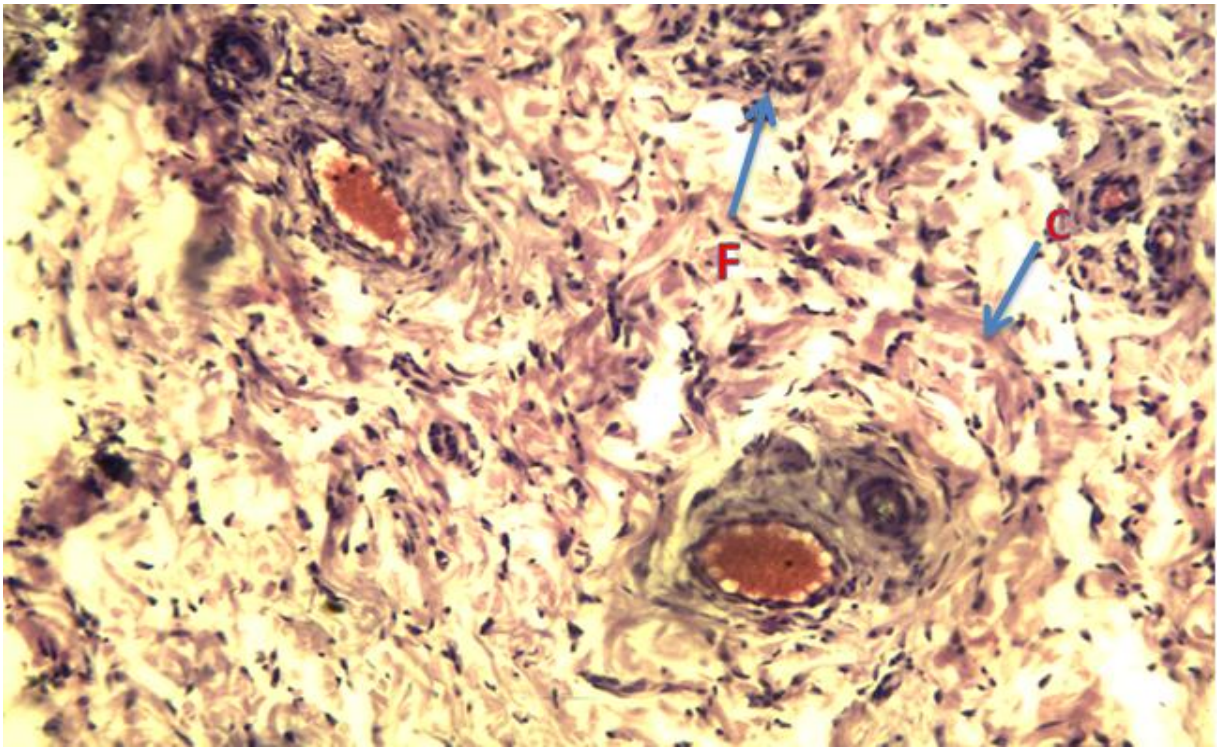


Fig. 14: Control group at 7 days, there was hemostasis with profuse fibrosis and collagen fiber and increase infiltration of inflammatory cells.

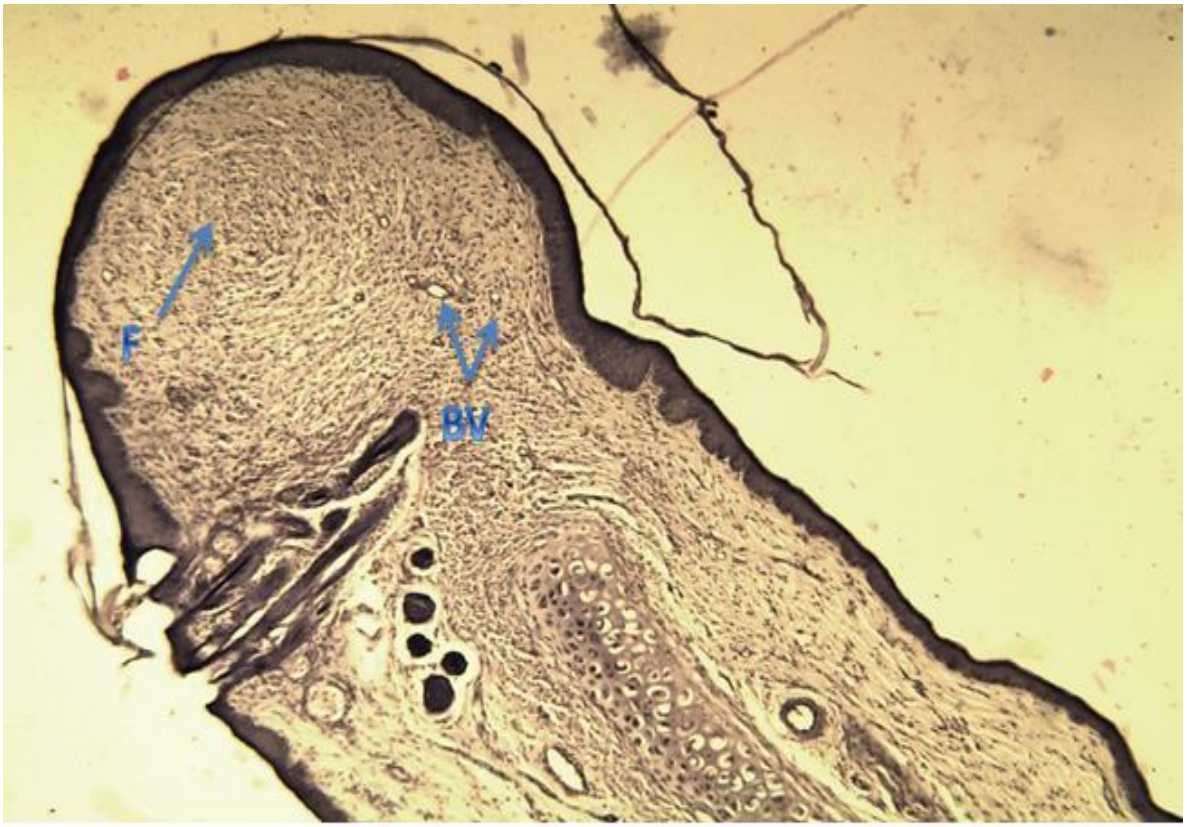


Fig. 15: Control group in 21 days, showed there was profuse granulation tissue characterized by formation of blood vessels(BV) and profuse fibrosis (F)with complete absence of hair follicles and cartilage. 4X H&E.

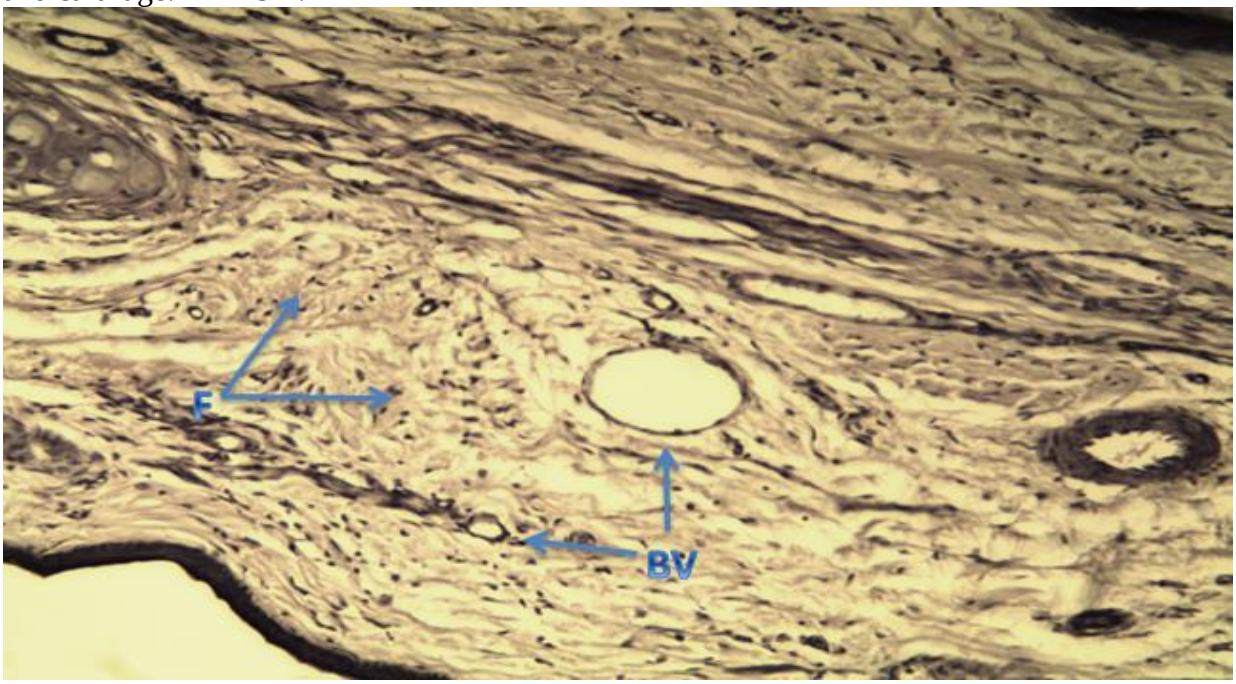


Fig.16: Control group in 21 days showed there was profuse granulation tissue characterized by formation of blood vessels(BV) and profuse fibrosis (F). 10X H&E

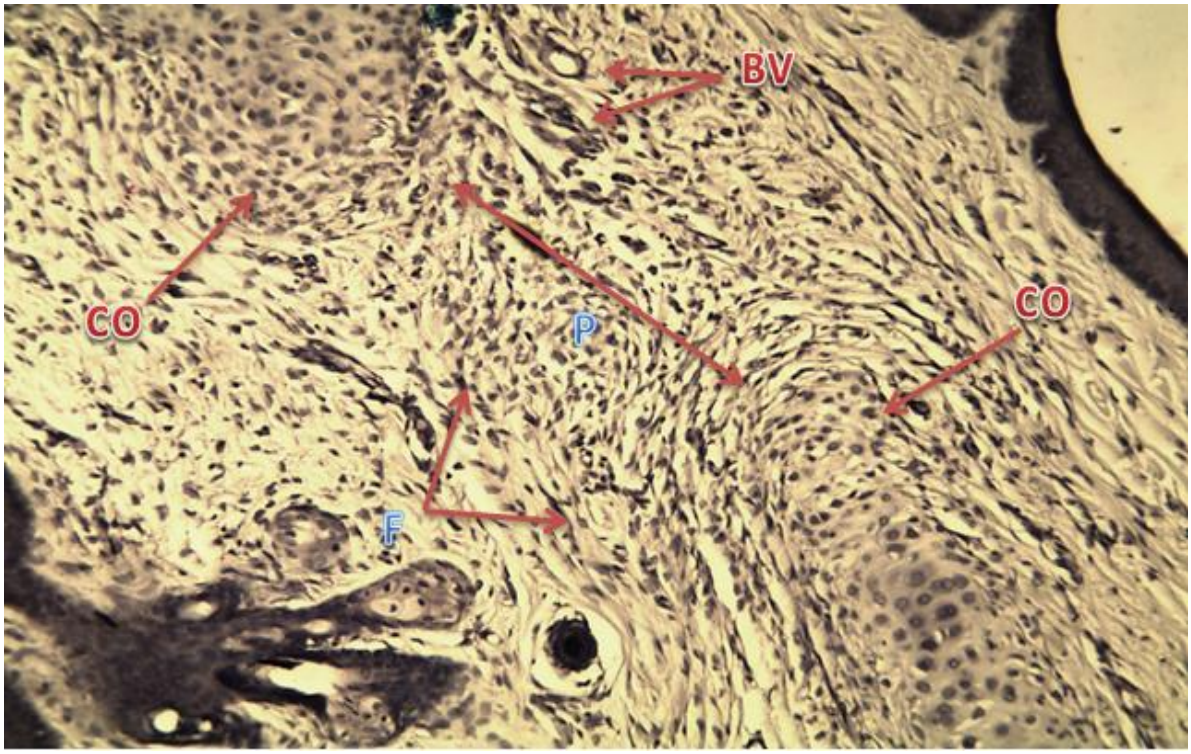


Fig. 17 : Control group at 45 days, showed partial healing of cartilage(P) which showed as extended edge resulting of proliferation of chondrocytes (CO) and profuse granulation tissue which characterized by formation of new blood vessels (BV)and fibrosis(F). 10X H&E.

Discussion

From the clinical finding of this experiment and Statistically found that, the diameter of wound shown significant differences at the level of $p< 0.05$ between all groups, the diameter of henna group less than the PRP and control groups, due to that we believe that, henna extract has strong wound tension (contain tannic acid as astringent factor) and dryness and it accelerate the proliferation of chondrocytes. as mentioned by(6), so these features will prevent the bacterial growth and prevent pus formation in addition henna extracts at 20% concentration have bactericidal effect(4,5) Table(1).

Histopathologcal evaluation at 7days post-surgery in both groups, observe the similar event with a little differentiation in stages of healing specially in henna and PRP groups like, show absence of the keratinization with mild fibrosis and scant collagen in PRP groups, while in the 20% henna treatment group there are narrow scar tissue, fibrosis and thick keratinization. These events means that two groups in middle of proliferation stage of heeling while in control groups the healing just in the inflammatory stage of wound healing these agreed with (2,8) whom said that both material henna or PRP accelerated the wound healing.

At 21 days post treated, histopathologcal examination showed formation of granulation tissue in Henna treated group was faster than PRP group and the regular abundant collagen fiber noticed with well orientation and formation of new blood vessels. high proliferation of epidermal layer and profuse fibrosis the amount of collagen in the wound more than the PRP group with the infiltration of inflammatory cells as well as the group sampled after 7 days was the formation of angiogenesis more than the

PRP. These facts indicate that there are fibroblast proliferative activity with epithelization during short period. So the proliferative fibroblasts will induce large amount of collagen which gives strength integrity to the tissue matrix and play role in homeostasis and epithelization at latter phase of healing, these results agreed with (6), while in control groups the delay of healing was more clear compare with other groups represents by profuse granulation tissue characterized by delay formation of blood vessels and profuse fibrosis with complete absence of hair follicles and cartilage either there is no proliferation in chondrocytes, these results agreed with (8),

At 45 days post treated Histopathologcal examination showed clarity in healing of cartilaginous layer and complete connection to the cartilage layer was obtained in the henna groups compare with PRP groups which was still in the end stage of proliferation and division of the chondrocytes either the edges of the cartilage tissue did not get attached together either the wound edges. In control groups the delay in healing was clear and obvious, where we note that it is early stages of proliferation phase with the presence of blood vessels and decline in the proliferation of chondrocytes failure in decrease the space between the two ends of the wound this delay maybe because the nature of cartilage tissue is weak in blood supply resulting in difficulty of self-renewal and healing, the structural and functional of cartilage are defected when it's injured or damaged (16).

From the clinical finding and histological evaluations of this experiment found that, the henna and PRP groups have power of healing more than the control groups ,because PRP that concentrate with platelets, these platelets according to (11,12,13,14) secrete a bioactive factors which called (growth factors). I think, these growth factors act as an important role in chondrocytes proliferation by acceleration of the chondrocytes proliferation, but it has less power than the henna extract 20% due to the pharmaceutical actions of henna.so henna extract has superiority effect on the healing of the holes than PRP which have been done in the elastic cartilages of the rabbit ears. This superiority is very clear specially at the 4th week, and at the end of the study (6th week) the holes of the henna extract group are clearly complete healing.

Conclusion

The histological evaluations of henna extract effects on the healing of the elastic cartilage in compare with PRP effects shows superiority of henna extract.

References

1- Alam G, Singh M, and Anita S(2011). Wound Healing Potential Of Some Medicinal Plants. *International Journal of Pharmaceutical Sciences Review and Research. 9(1):136-145.*

2- Shiravi A H, Alebooyeh M , Hojati V, Akbari H (2011).The Effect Of Extract Of Henna Leaves (LawsoniaInermis) On Skin Wound Healing In Wistar Rats. *Journal Of Animal Biology. 3(4) 45-51.*

3- Sakarkar DM , Sakarkar UM, Shrikhande VN, Vyas JV, Mandavgade S, Jaiswal SB, and Purohit RN (2004) Wound healing properties of Henna leaves, *Natural Product Radiance, 3(6)406-412.*

4- Slovis N M (2014). Lawsonia intracellularis Proliferative Enteropathy in Foals. *Proceedings of the American Association of Equine Practitioners.www.ivis.org.*

5- Al-Rubiay K K, Nawres NJ, Al-Mhaawe BH, and Alrubaiy L K (2008). Antimicrobial Efficacy of Henna Extracts. *Oman Medical Journal , 23(4): 253-256.*

6- Towfik AI, Hamza A S, and Munahi A K(2015). The effect of Henna (Lawsoniainermis) on the wound healing of Local Arabian Horses. *Journal of Kerbala University , 13 (1): 78-91*

7- Muhammad H S, and Muhammad S (2005). The use of Lawsoniainermislinn. (henna) in the management of burn wound infections .*African Journal of Biotechnology , 4 (9):934-937.*

8- Mohammad A J(2016). Histological and surgical study of the henna on wound healing in animals Field (Sheep). *Kufa Journal For Veterinary Medical Sciences , 7 (1):259-268.*

9- Canapp SO(2015). Prp/Msc For Orthopedic Conditions. *Proceedings of the Southern European Veterinary Conference &CongresoNacional AVEPA, 2015 - Barcelona, Spain. www.ivis.org.*

10- Gimeno FL, Silvia G, Ferro J, Juan O, and Juan E G(2006). Preparation of platelet-rich plasma as a tissue adhesive for experimental transplantation in rabbits.*Thrombosis Journal, 4:18.*

11- Lee H, Shon O, Park S, KimH, Kim S, AhnM, and Do S (2016). Platelet-Rich Plasma Increases the Levels of Catabolic Molecules and Cellular Dedifferentiation in the Meniscus of a Rabbit Model. *Int. J. Mol. Sci., 17, 120.*

12- Gui K, Ren W, Yu Y, Li X, DongJ, and Yin W(2015). Inhibitory Effects of Platelet-Rich Plasma on Intervertebral Disc Degeneration: A Preclinical Study in a Rabbit Model. *Med. Sci. Monit., 21, P.p.1368-1375.*

13- Choi HN, Han YS, Kim SR, Kim HK, Kim Hand ParkJH(2014). The Effect of Platelet-Rich Plasma on Survival of the Composite Graft and the Proper Time of Injection in a Rabbit Ear Composite Graft Model.*Archive of Plastic surgery,41(6): 647-653.*

14- NixonAJ(2010). Critical review of the clinical use of PRP and BMAC.*15th Annual European ESVOT Congress - Bologna, Italy 233-235.*

15- Spadari A, RicciardiG, RinnovatiR, andRomagnoliN (2010). Single injection of autologous platelet rich plasma (PRP) in suspensory ligament lesions in horses: a clinical trial. *15th Annual European ESVOT Congress - Bologna, Italy . 611-612.*

16- Liu J, Song W, Yuan T, XuZ, Jia W, and Zhang C (2014).A Comparison between Platelet-Rich Plasma (PRP) and Hyaluronate Acid on the Healing of Cartilage Defects. *PLoS ONE 9(5).*

17- Cook JL(2013). Use Of Platelet rich Plasma (Prp) In Small Animal Orthopedic Practice *Proceedings of the Southern European Veterinary Conference &Congreso Nacional AVEPA, Barcelona, Spain.*

18- Zhang J, Middleton KK Fu F H, ImH, andWangJ (2013). HGF Mediates the Anti-inflammatory Effects of PRP on Injured Tendons. *PLoS ONE 8(6).*

19- MonteiroS, LepageO, and TheoretC (2008). Effects of platelet rich plasma (PRP) on the repair of wounds on the distal limb in horses. *14th Proceedings of the European Society of Veterinary Orthopaedics and TraumatologyESVOT Congress, Munich,306-307.*

20- Yin W, Xu H, Sheng J, An Z, Guo S, Xie X, and Zhang C (2016). Advantages of Pure Platelet-Rich Plasma Compared with Leukocyte- and Platelet-Rich Plasma in Treating Rabbit Knee Osteoarthritis. *Med SciMonit, 22: 1280-1290.*

21- Martha MM, Palmer M, Abreu E, Spindler K P, Zurakowski D, and Fleming B C (2008). Platelet-Rich Plasma Alone Is Not Sufficient to Enhance Suture Repair of the ACL in Skeletally Immature Animals: An In Vivo Study. *Journal Of Orthopaedic Research 27:639-645.*

22- Shin K H, Lee H, Kang S, KoY, Lee S, Park J, and Bae J (2015). Effect of Leukocyte-Rich and Platelet-Rich Plasma on Healing of a Horizontal Medial Meniscus Tear in a Rabbit Model. *BioMed Research International. Volume 2015.*