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Comparison Study of Autologous Saliva Extract as a Biological Therapy with Low Level Laser Therapy (LLL) Effects on Canine Cutaneous Wound Healing

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Abstract

Humans have attempted a variety of wound-healing treatments, including chemical, biochemical, and irradiation therapies, among others. The aim of this study was to compare the effects of saliva extract and low-level laser (LLLT) on cutaneous wound healing. Four groups of twenty straw dogs were formed. Saliva extract was prepared after G1 was obtained, G2 was treated with LLLT, G3 with saline, and G4 was not treated at all. Many of the groups had surgical cutaneous incisions on their backs that were treated. The wound contraction measurements of G1 were less, the tensile strength of G1 was higher, and histological examination showed that by the third week, the normal contour of the skin surface had been restored, with the development of new hair follicles, reduced cellularity of the wound site, and the growth of new blood vessels. The results showed the superiority was for saliva extract than LLLT.

Keywords: autologous, saliva, laser, canine, cutaneous, wound, healing.

Introduction

Saliva is a fluid produced primarily by salivary glands in the mouth (Schenkels*et al*. 1995,Parra*et al*.2005, Krasteva*et al*.2011). It comprises a complex mixture of electrolytes and proteins with various biological functions in digestion, host defense, lubrication, and oral and general health maintenance (Parra *et al*.2005, Sanchez*et al*. 2013).Many researchers in human medicine have used sialochemistry to diagnose systemic illnesses and track general patient health, as well as as a disease risk predictor, demonstrating that oral and systemic health are closely linked (De Almeida *et al*.2008). In veterinary medicine, there are compelling reasons to use saliva as a diagnostic fluid. The method's greatest benefit is its non-invasiveness: saliva from various animal species is easily collected (Lavy ,*et al*.2012,Tvarijonaviciute*et al*. 2014).

Saliva samples have been used to determine cortisol in dogs, avoiding the stress caused by venipuncture for blood collection (Wenger-Riggenbach*et al*. 2010), as well as IgGs assessment (German*et al*. 1998), rabies virus antigen identification (Kasempimolporn*et al*. 2000), drug screening (Dunnett*et al*. 2002), and CRP quantification (Parra*et al*.2005).Despite the fact that periodontal disease is the most common oral disease in dogs, no research has looked into the connection between salivary biochemistry and the severity of periodontal disease in this species (Marshall *et al*. 2014). Furthermore, little is known about the salivary composition of healthy dogs that do not have any dental problems.

The exact mechanism of action of LLLT is unknown, but it has been shown to alleviate pain associated with inflammation by reducing levels of prostaglandin E2 (PGE2), interleukin-1 beta (IL-1 beta), tumor necrosis factor-alpha (TNF-alpha),

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cellular influx of neutrophils and granulocytes, oxidative stress, edema, and bleeding in a dose-dependent manner (Gordon , Surrey ,1960).

The dose of LLLT varies between 0.3 and 19 J/ cm2. At low doses, low-level laser therapy stimulates cells, and at high doses, it suppresses them. Another mechanism proposed is stimulating the mitochondrion to increase adenosine triphosphate (ATP) production in order to increase reactive oxygen species (ROS), which regulates redox signaling and affects cell proliferation intercellular homeostasis (Lubart*et al*.,2005). Low-level laser therapy also affects microcirculation, which helps to relieve edema by altering capillary hydrostatic pressure (Yamada *et al*., 2004).

The aim of the study is to compare the effects of saliva extract and LLLT on canine cutaneous wound healing.

Materials and methods:

This research was performed from July 2020 to July 2021, according to evidence from the committee of the College of Veterinary Medicine /Al-Qadisiyah University. The research was performed on twenty straw dogs of both sexes, randomly divided int o four groups of five dogs and living in separatecages for each species. For one week, deworming and veterinary tests were carried out daily to check their proper health. T heir ages were11.5±0.5 months, fed with ad libitum clean water on the normal amount of half cooked beef.

Preparation of saliva extract:

Until the saliva diluents were collected, a citric acid solution was forced into each dog's mouth cavity as a stimulant to extract a large amount of saliva. The saliva was collected in sterile test tubes with tiny cotton parts and sent to the lab right away. The saliva was centrifuged at 5000 rpm for 5 minutes, then micro-filtered using a 0.45 m microfiltration membrane and stored in 4Co before use.

Study design:

In the back region of the dogs, full thickness circular skin incisions 3 Cm in diameter were made under routine surgery with general anesthesia by combination of xylazine 1 mg/kg and Ketamine 10 mg/kg, IM . Group-1(G1) was treated daily for one week with autologous saliva extract, collected from sterile soft sponge pieces, centrifuged at 3000 rpm for 5 minutes, filtered with a micro filter of 0.22 μm and processed at 4 Cº until used. Group-2(G2) was treated with low-level laser therapy at a dose of 850 nm, exposure time was 10 s, energy density was 8 J/Cm2 with a pulsing rate of 146 Hz every day for one week with Laser Diode (Omega laser systems Ltd., UK).

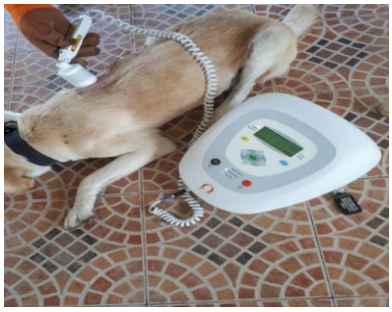

Fig.-1: Treatment of G2 with LLL therapy.

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Group-3(G3) was topically treated for one week with normal saline of 3 ml. daily. And it was seen as positive control. Group-4(G4) is known to be a negative control and had not been treated. The outline of each wound was recorded every 24 hours for the next 21 days. The wound contraction was calculated by following a formula:

WC= WC on day n- WC on day 0.n = the day when WC measured.Tensiometer-measured wound tensile strength healing within 21 days and every 3 days reported. The tensile strength was calculated by following a formula: TS= TS on day n – TS on day 0 n = the day when TS measured.

Fig.-2: Measurement of tensile strength of wound healing by Tensiometer.

Histological evaluation: Biopsies 1mmx1mm were taken at 1, 2, 3 weeks from the edges of wounds, fixed with formalin 10%, cut into smears and stained with hematoxylin and eosin stains (H&E) and examined under light microscope X40, and X100.

Statistical analysis:

The findings were statistically evaluated in one way by SPSS software version 32, and the differences were considered significant at P 0.05.

Results :

Wound contraction: In the 21-day post-operative cycle, the mean ±SE values of the full thickness wound contraction rate were higher for G1 (1.1±0) than for the other groups, while they were (1.6±0) for G2, (2.35±0) for G3, and (2.65±0) for G4, as shown in Table-1.

Table-1: The mean ±SE of the rates of the full thickness wound contraction for various dog groups.

Groups	Post-operative period(days)							
	0	3	6	9	12	15	18	21
G1	3±0 A	2.95±0.05 A	2.8±0.05 A	2.6±0.06 A	2.55±0.05 A	2.3±0.05 A	2.2±0.05 A	1.1±0.12 A
G2	3±0	3±0 A	2.95±0.	2.8±0.0	2.65±0.	2.6±0.0	2.4±0.0	1.6±0.1

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	A		05 AB	5 B	06 AB	6 B	6 B	6 B
G3	3±0 A	3±0 A	2.95±0.05 AB	2.8±0.05 B	2.65±0.06 AB	2.65±0.06 B	2.55±0.05 BC	2.35±0.06 C
G4	3±0 A	3±0 A	3±0 A	2.85±0.06 B	2.8±0.05 B	2.7±0.05 B	2.65±0.06 C	2.65±0.06 D

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

Tensile strength measurement: In the 21-day post-operative cycle, the mean ±SE values of the full thickness wound contraction rate were higher for G1(133±4.81) than for the other groups, while they were (105±2.04) for G2, (98.4±2.11) for G3, and (86.8±0.37) for G4, as shown in Table-1.

Table-2: The mean values± SE of the tensile strength rates 0ver 21 days of the full thickness wounds of all various dog groups.

Groups	Post- operative measurement(kg/Cm^2)							
	0	3	6	9	12	15	18	21
G1	93±5.25 A	100±5.66 A	105.6±6.18 A	111±5.43 A	116±5.3 A	121.4±5.11 A	125.4±4.95 A	133±4.81 A
G2	82.6±2.03 B	84.2±1.98 B	88.8±2.05 B	91.8±2.17 B	95.6±2.2 B	98.8±2.05 B	101.4±2.11 B	105±2.04 B
G3	83.2±2.57 B	84±2.42 B	85.4±2.35 B	87.6±2.18 BC	89.8±2.51 BC	92.2±2.43 BC	95±2.19 B	98.4±2.11 B
G4	80.2±0.91 B	80.6±0.74 B	81.2±0.72 B	82.2±0.73 C	82.8±0.58 C	83.8±0.58 C	84.6±0.60 C	86.8±0.37 C

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

Gross evaluation:

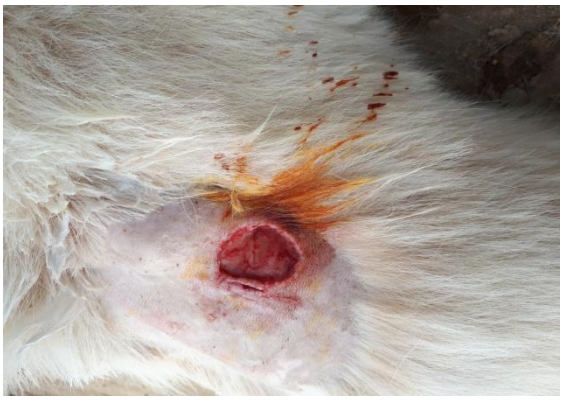

Fig.-3: G1 circular cutaneous wound at 0 day. Fig.-4: G1 wound over 21 days.

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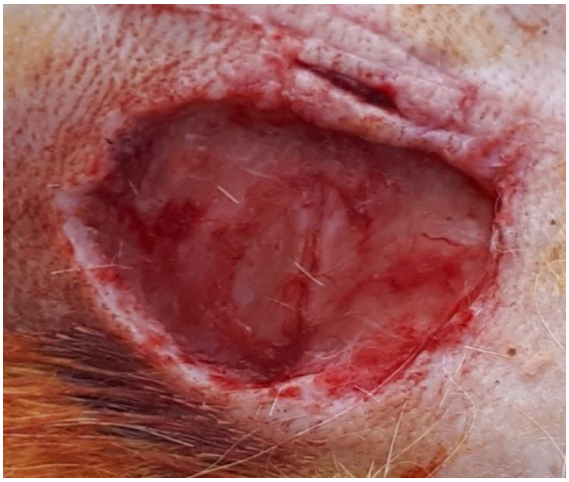


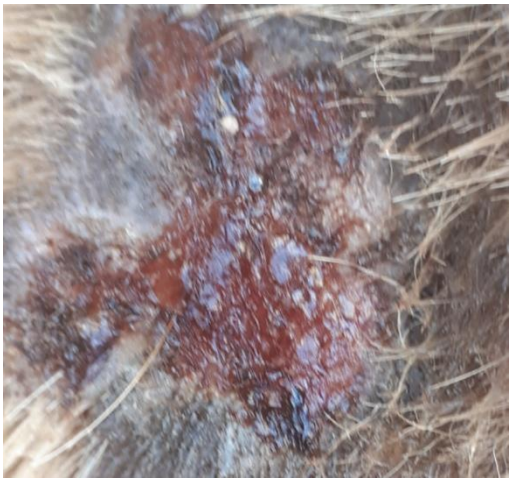


Fig.-6: G2 wound over 21 days.Fig.-5: G2 circular cutaneous wound at 0 day.

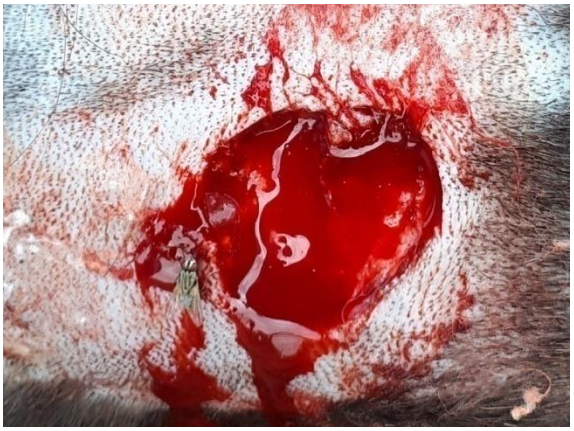
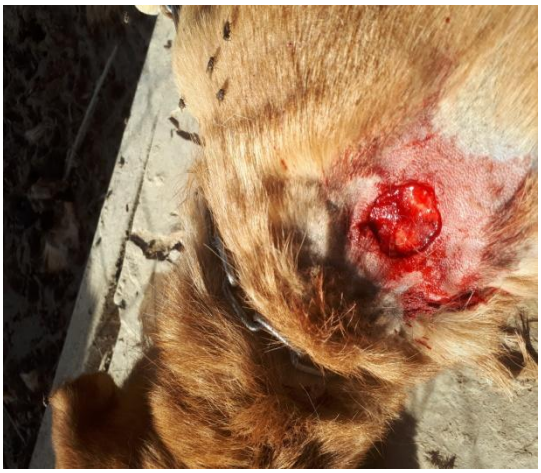


Fig.-7: G3 circular cutaneous wound at o day.Fig.-8: G3 over 21 days



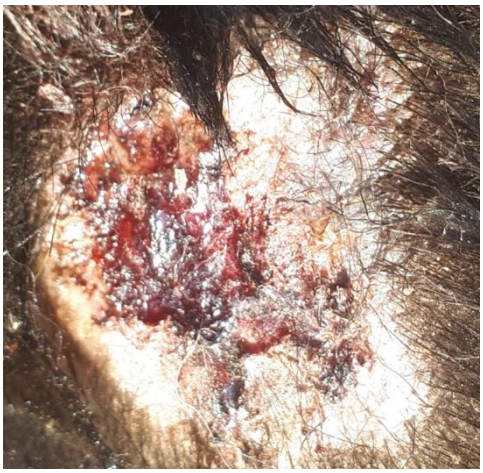


Fig.-9: G4 circular cutaneous wound at 0 day.

Fig.-10: G4 over 21 days.

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Histopathological examinations:

Saliva treated group:

The thick scar tissue was informed in the 1st week after injury as well as epithelial tongue formation in the incision linethat leads to full wound closure. In addition, new blood vessels are a clear indicator of the regeneration process, as is the invasion of inflammatory cells and fibroblasts (Fig.-11).The scar tissue became thinner and full re-epithelization of epidermal tissue by the 2nd week of the saliva extract-treated wounds. In addition, a regenerated dermal layer characterized by newly developed blood vessels and fibroblast aggregation and mild edema were observed (Fig.-15). The normal contour of the skin surface was restored by the 3rd week, including the development of new hair follicles and reduced cellularity of the wound site (Fig.- 19).

Laser treated group:

There was dramatically increased cellularity of the tissue around the wound site by the 1st week after injury, creating "heaped up" margins. The "crater" had an amorphous clot on the edge of the cut. From the edges of the wound, epithelial keratinocytes begin to migrate. Edema and infiltration of a few inflammatory and fibroblast cell numbers at the edge of the incision line were also recorded at this point (Fig.-12).By the second week, most of the wounds treated with laser tended to be re-epithelialized microscopically, while a scab was still adherent to the surface (Fig.-16). Thick scar tissue is still present by the 3rd week, incomplete epidermal layer regeneration, tremendous inflammatory infiltration (Fig.-20).

Saline group:

The incision line filled with a very thick granulation tissue at the 1st week of skin wound induction (Fig.-13) and the clots covering the wounds in this community contained much larger quantities of red blood cells (RBCs). Also noted was strong infiltration of inflammatory cells in the under layer (Fig.-13) as well as extravasated RBCs. Neutrophils were the most common inflammatory cells, and macrophages were less numerous (Fig.-13).The granulation tissue is still very thick after 2 weeks with the beginning of deterioration (Fig.-21) and the epithelial tongue starts to develop (Fig.-21) with the presence of hemorrhage and extravascular RBCs in the dermal layer, but few inflammatory cells in the wound region have been detected (Fig.-21). A granulation tissue is still present after 3 weeks of skin wound, incomplete epidermal layer regeneration, enormous inflammatory cell infiltration has been found (Fig.-21).

Control group:The wound displays edema at week 1 and accumulation of a few inflammatory cell and fibroblast amounts. Epithelial keratinocytes are starting to migrate from the edges of the wound, however (Fig.-14). By week 2, the control group had demonstrated thick granulation tissue formation (Fig.-18) as well as inflammatory cell infiltration (Fig.-18). A dense scar tissue is still present after 3 weeks of skin wound induction, incomplete regeneration of the epidermal layer, major infiltration of inflammatory cells (Fig,-22).

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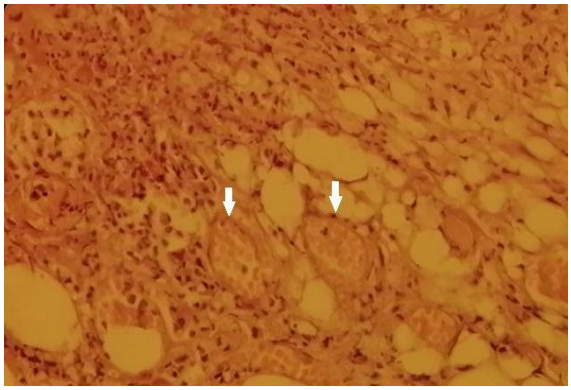

Fig.-11: G1-1st week: In the incision line, the skin wound shows epithelial tongue formation marked by newly developed blood vessels (white arrows) and inflammatory cell and fibroblast infiltration, H&E, 400X.

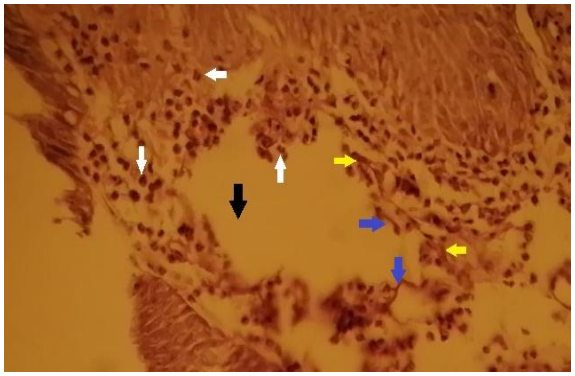

Fig.-12:G2-1st week:The skin wound displays edema and penetration of inflammatory cells (white arrows) as well as the inflammatory cells of Langerhans (blue arrows) and the aggregation of fibroblasts (yellow arrows) at the edge of the incision line (black arrows), H&E, 400X.

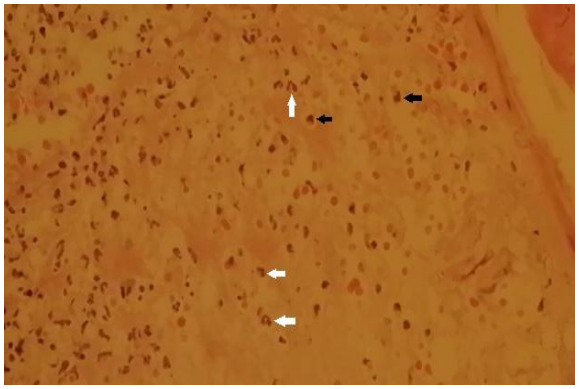

Fig.-13:G3- 1st week: Heavy infiltration and extravasation of neutrophils (white arrows) and macrophages (black arrows) and RBCs, H &E, 400X.

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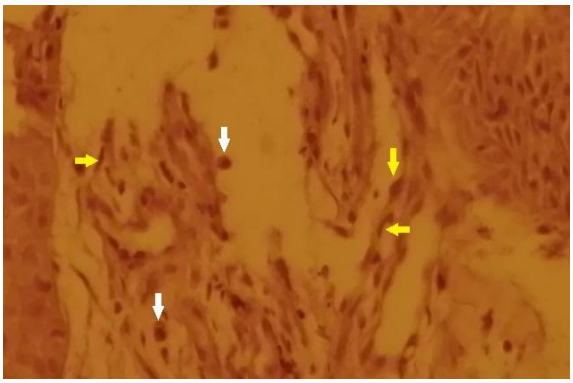

Fig.-14:G4-1st week:Skin wounds show edema and a few inflammatory cell numbers (white arrows) and fibroblast aggregation (yellow arrows), H&E, 400X.

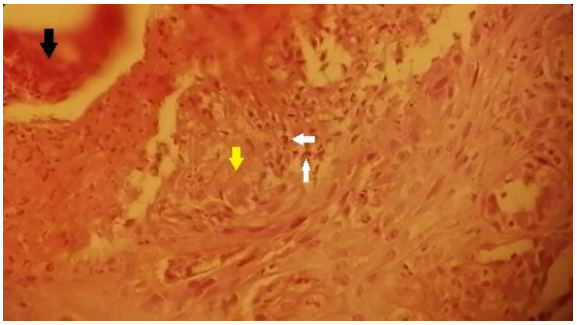

Fig.-15:G1-2nd week: A well-regenerated epidermal and dermal layer, a regenerated dermal layer marked by newly developed blood vessels (blue arrows) and fibroblast (yellow arrow) accumulation and mild edema are seen in the skin wound, H&E, 400X.

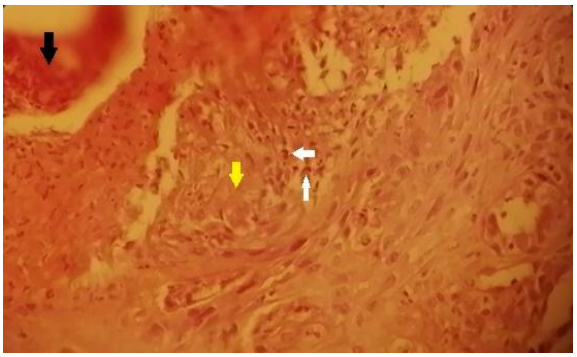

Fig.-16:G2-2nd week: Skin wounds seemed to be completely re-epithelialized microscopically, although a thick scab (black arrow) was still adherent to the surface, sub-epidermal edema was also noted, as well as infiltration of a few inflammatory cell numbers (white arrows) and fibroblast accumulation (yellow arrows),H&E, 400X.

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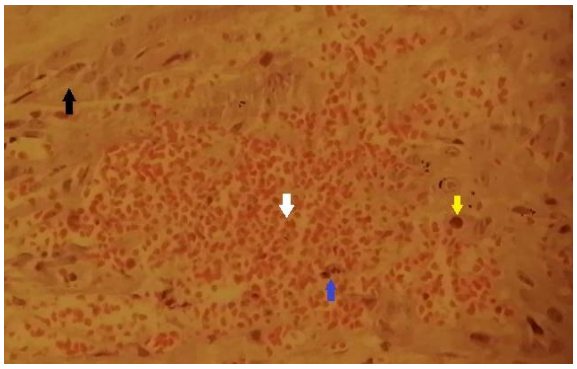

Fig.-17:G3- 2nd week:In the wound region, the skin wound showed extreme hemorrhage in the dermal layer (white arrow) as well as infiltration of macrophages (yellow arrow) and few neutrophils (blue arrow), and accumulation of fibroblast (black arrow) are found, H&E, 400X.

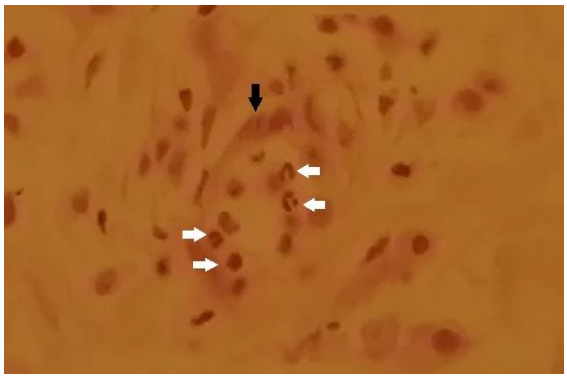

Fig.-18:G4- 2nd week:Skin wound suggests inflammatory cell infiltration at the injury site under the granulation tissue (white arrows), H&E, 400X.

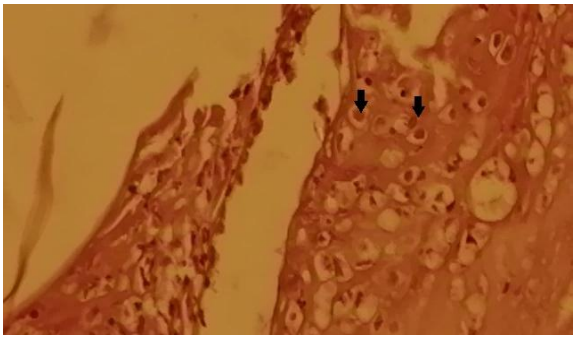

Fig.-19:G1- 3rd week:A well-dermal layer is seen in the skin wound; a regenerated dermal layer characterized by newly formed blood vessels (white arrow), fibroblast (black arrow), and few inflammatory cells (yellow arrow), H&E, 400X.

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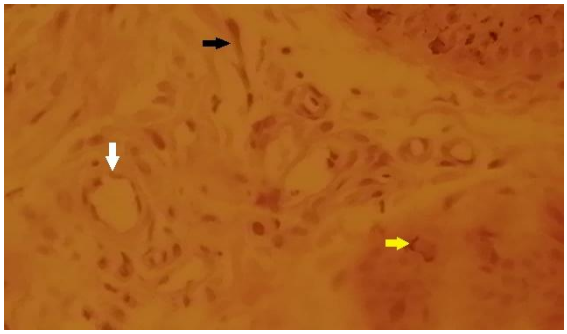

Fig.-20:G2-3rd week: Skin wound regeneration of the epidermis layer of the stratum spinosum (black arrows),H&E, 400X.

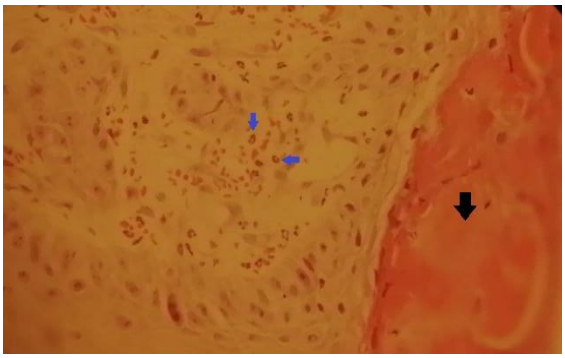

Fig.-21:G2-3rd week:The scar tissue (black arrow) and heavy infiltration of inflammatory cells directly under the granulation tissue in the superficial layer of the epidermis are seen in the skin wound, H&E,400X.

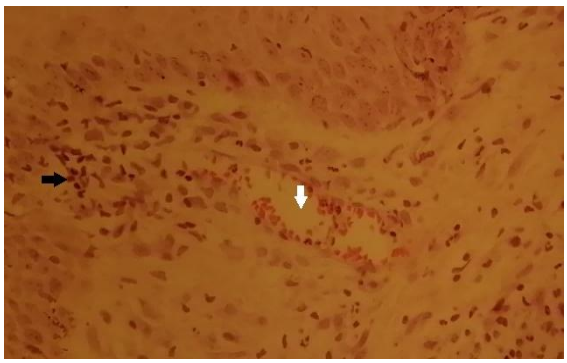

Fig.-22:G4-3rd week: Skin wounds indicate inflammatory cell infiltration. (black arrow) and blood vessel engorgement (white arrow) inside the regenerated dermal epithelial layer, H&E, 400X.

Discussion

Although the therapeutic effects of saliva and LLLT on wound healing are well documented, this study was conducted to compare their therapeutic effects in addition to a positive control of local application of saline.

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The Creeks used snake saliva to treat open cutaneous wounds topically before 2000 years, according to tradition. Saliva was also well known for treating oral wounds with less scar tissue.

We used citric acid to extract a significant amount of canine saliva. For those researchers who need to collect large volumes of saliva or at the very least ensure minimum volumes of saliva (and not discard samples) for experimental experiments with dogs, this approach was critical to consider. Our findings support the use of citric acid as a significant stimulant of saliva flow in dogs, as previously stated in several studies (Kobelt*et al*., 2003, Dreschel and Granger, 2009, Lensen*et al*., 2015, Cobb *et al*.2016).

The findings showed that saliva extract out performed LLLT. As shown in table-1, the wound contraction measurements of G1 were less 1.10.12 than those of the other classes G2 1.60.16, G3 2.350.06, and G4 2.650.06. This parameter is critical for assessing wound healing. Following that, epithelial cells crawl over the wound bed to cover it, and myofibroblasts grasp the wound edges and contract using a process close to that of smooth muscle cells (Esimonz*et al*.2005, Nayak*et al*.2006, Bhat*et al*.2007).

Wound healing, we believe, is a complex and dynamic process that restores cellular structures and tissue layers in damaged tissue to their natural state as nearly as possible. Wound contraction is a phenomenon that happens during the healing process, beginning with the fibroblastic stage, where the wound shrinks. The wound contracts in the maturational process, the final phase of wound healing, resulting in a reduced amount of visible scar tissue and accompanying pain (Karan Gupta *et al*.2011).

These wound contraction findings were similar to those shown in the gross evaluation of wound healing by the incremental narrowing of the circular wound diameter, as seen in figures 4, 6, 8, and 10.

We used a Tensiometer made by the researchers to calculate tensile strength at 0 day and every 3 days until 21 days to determine another important parameter of wound healing. Table 2 also revealed that saliva extract 1334.81 outperformed the other classes G2 1052.04, G3 98.42.11, and G4 86.80.37. The wound's mechanical properties match the findings of our research. As a result, we believe that the initial increase in the TS of a wound is linked to the inflammatory phase's vascular reaction (via the formation of a fibrin network).

The dynamic, self-remodeling macromolecular complex – extracellular matrix ECM – synthesized by fibroblasts had a critical impact on the increase in wound TS (Kadler, 1995, Cotran*et al*., 1999,Menetrey*et al*., 2000). The greatest amount of new structured collagen was found in our parallel histological analysis after wounding, which correlates with the increase in TS.

It is well known that LLLT has limited success for wound healing, the use of lasers may vary in the form of activation means, the power and dosage, and also on the manner and time of irradiation and number of applications(BashardoustTajaliet al.2010).

The histological examination of biopsies taken at 1, 2, and 3 weeks after surgery revealed that saliva extract outperformed the other categories. By the third week, the

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natural contour of the skin surface had been restored, with the production of new hair follicles, decreased cellularity of the wound site, and the growth of new blood vessels. In general, we conclude that saliva extract's superiority is due to the presence of growth factors EGF, FGF, TGF, EVGF, and histatin. These natural ingredients promoted wound healing by interfering with the three stages of wound healing: inflammation, proliferation, and remodeling (Mohammad rezaPakyari,*etal*.2013). Epidermal cells migrate, proliferate, and differentiate as a result of these elements. In the early stages of wound healing, migration and proliferation are common. Because of the role of growth factors in wound healing and their deficiency in chronic wounds, topical administration of growth factors is a promising strategy for promoting wound healing (Gainza, G.*et al*.2015).

Conclusions:

1 . Saliva extract has a greater effect on wound healing than LLLT.

2 . It is possible to make saliva extract therapy for wound care in a realistic manner.

3 . The application of saliva extract therapy topically is simple.

Recommendations:

1 . Because this study only used Iraqi straw dogs, it would be interesting to expand the study to include more breeds in order to gain a better understanding of behavior and salivary composition.
2 . Use of saliva extract as a modern biological therapy in veterinary field for future.
3. Foundation of new collection methods of saliva.

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