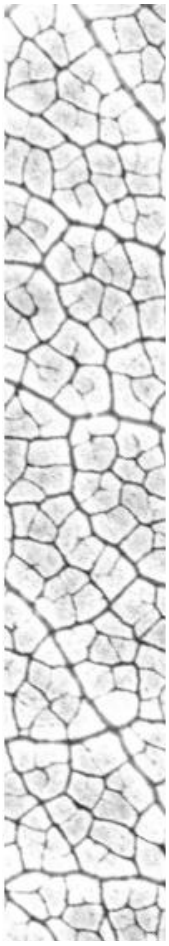

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Use of topical administration of interleukin-1 receptor antagonist on equine cutaneous wound healing

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Abstract

The present study was conducted on 16 local Arabian mares divided randomly into 4 groups, four for each. The aim was to evaluate the use of topical administration of IL-1Ra on equine cutaneous wound healing. Autologous interlukin-1 receptor antagonist (IL-Ra) were prepared. Under routine surgery complete square cutaneous wound 5x5mm^2 were done in the back region of G1 and G3, and the neck region of G2 and G4. G2and G4 had no any treatment. The wounds of G1 and G2 treated topically with the extracted serum 1ml. daily for one week. By caliper the ribs of the square wounds were measured weekly for one month and recorded. Biopsies were taken for histopathological evaluation at 1,2,3, and 4 weeks. The results proved the superiority potential effect of IL-1Ra on wound healing of G1 than G2.

Keywords: topical, administration, interleukin, receptor, antagonist, wound, healing

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INTRODUCTION

Many researches in the skin, interleukin-1 receptor antagonist (IL-1Ra) has been introduced in cutaneous wound healing and fibrosis which is indicated of its reduced skin fibrosis (Ishida et al. 2006., Thomay et al. 2009). These studies focus on the unbalanced production of IL-1 and IL-1Ra in re- epithelization.

The importance of immune system in wound healing although not completely understood, the wound healing is classified into 4 stages: hemostasis, inflammation, proliferation, and remodeling. These stages show the wound healing processes are controlled by a complex set of signals from the wound itself and its microenvironment (Guo, & DiPietro, 2010).

Cytokines, chemokines and growth factors are released by both resident cells and leukocytes which arrive newly to the site of injury (Strbo, Yin, Stojadinovic 2014; Khodaie et al., 2019). Reflex vasoconstriction and the components of the coagulation cascade control the hemostasis resulting in release of pro-inflammatory cytokines and the clot formation. Tissue injury trigger the inflammation stage and is important in readying the wound for closure (Palta, Saroa, & Palta, 2014; Fidelis et al., 2019). The proliferative stage is ended in wound closure and is followed by remodeling during which the tensile strength of the scar tissue is increased (Peter, N., 2013).

IL-1Ra has the therapeutic target for wound healing during the inflammatory stage (Koh, DiPietro, 2011; Saidi et al., 2018). IL-1Ra modulates a variety of inflammatory events and is a potent regulator of IL-1 (Perrault, 2018).

Few studies have shown the interference in IL-1 signaling can improve wound healing and reduce scar tissue formation (Thomay, et al. 2009). The little information, however about the local use of IL-1Ra in skin wound healing(Mirza, et al. 2013) therefore the aim of this study was to evaluate the use of topical administration of IL-1Ra on equine cutaneous wound healing.

MATERIALS AND METHODS

Under the ethics of the College of Veterinary Medicine committee 16 local Arabian mares were divided randomly into 4 groups, four for each treatment group-1 (G1), treatment group-2 (G2), control group-3 (G3), and control group-4 (G4). Their ages 10-12 y., weighted 400±30kg., and lived in separated boxes fed with standard feed grains, hay, straw, vitamins and minerals with ad labium clean water.

Autologous interlukin-1 receptor antagonist (IL-Ra) preparation:

Blood samples 50 ml. were collected from each mare by syringe contained glass beats and calcium chloride

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Table 1. Mean values ± SE of WBC count and IL-Ra concentrations in plasma for all experimental groups

Groups	WBC count Before 24 hrs. of surgery ($x10^3$cells/µL)	WBC count After 1wk. of surgery ($x10^3$cells/µL)	IL-1 Ra before 24hrs.of surgery (pg/mL)	IL-1 Ra After 1wk.of surgery (pg/mL)
G1	6.15±0.427 A	6.725±0.4421 A	121.375±1.59863 A	134.275±3. 69422 B
G2	7.925±0.718 A	8.55±0.8752 A	120.1± 3.19818 A	136.025±2.44996 B
G3	6.9±0.4544 A	7.45±0.4267 A	122.25 ±2.7575 A	139.0±3.1051 B
G4	7.7±0.7547 A	8.275±0.66211 A	122.95± 2.33184 A	140.55± 2.67099 B

*Different letters mean significant variances at ≤ 0.05

Table 2. Mean values± SE of the measurements of the wound ribs (Cm) weekly for all experimental groups

Week/Group	1st wk.	2ndwk.	3rdwk.	4thwk.
G1	4.5625 ± 0.0587 **B**	4.0625 ± 0.0512 **C**	3.6250 ± 0.0677 **B**	3 ± 0.2806**B**
G2	4.6875 ± 0.0588 **B**	4.4375 ± 0.1530 **B**	4.5 ± 0.0967 **A**	4.0625 ± 0.0591 **A**
G3	4.9375 ± 0.0612 **A**	4.6875 ± 0.0609 **B**	4.6250 ± 0.0709 **A**	4.1875 ± 0.1186 **A**
G4	5 ± 0.00 **A**	4.8750 ± 0.704**A**	4.6875 ± 0.0598 **A**	4.5± 0.00 **A**

*Different letters mean significant variances at ≤ 0.05

solution 10% added at ratio 1:10ml of blood. The syringes were incubated for 6 hours at 37ºC, the clot blood were removed, serum immediately transferred into polyethylene tubes, centrifuged 1500xg for 10 min. by cold centrifuge, rapidly filtered with micro filter 22µm.and serum were preserved at - 8ºC. The concentrations of IL-1 Ra in the extract serum were measured by a double sandwich ELISA with use of a commercial kits (Ray biotech, USA).

Experimental design

Under routine surgery with lidocaine 2% 10ml, complete square cutaneous wound 5x5mm^2 were done in the back region of G1 and G3, and the neck region of G2 and G4 with no any antibiotics or anti-inflammatory drugs were used. The wounds of G1 and G2 treated topically with the extracted serum 1ml. daily for one week. By caliper the ribs of the square wounds were measured weekly for one month and recorded. Biopsies were taken 1mm^3 for histo-pathological evaluation at 1,2,3, and 4 w., stained the smears with eosin and hematoxillin stains and examined under light microscope.

Statistical analysis

The results were analyzed with SPSS program version 32 and different variances were regarded significant at ≤ 0.05.

RESULTS

Mean values ± SE of WBC count after 24 hrs. and IL-Ra concentrations in plasma after one week have an considerable increase for all experimental groups.

The results of measurement of the ribs of wounds of all groups showed significant variances at ≤ 0.05 between the groups and the superiority was clear for G1, G2 respectively as showed in **Table 2**.

Histo-pathological evaluation

See **Figs. 1**-**16**.

DISCUSSION

In the present study involving 16 mares have different sites of incisions to compare the effect of topical administration of IL-1Ra on wound healing of G1 fixed wounds and G2 movable wounds according to their

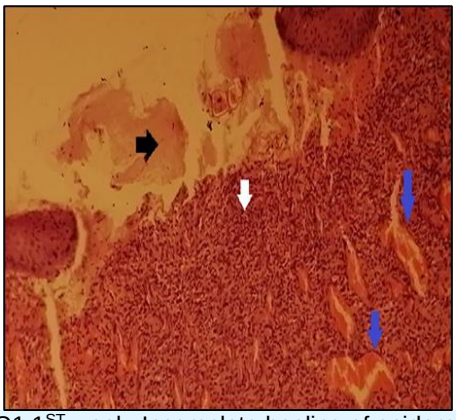

Fig. 1. G1,1ST week, Incomplete healing of epidermal layer (black arrow), the new dermis of wound showing disorganized fibroblast proliferation with blood vessels (blue arrows), angioblasts proliferation and moderate infiltration of the inflammatory cells (white arrow) and free RBCs infiltration. H& E, X100

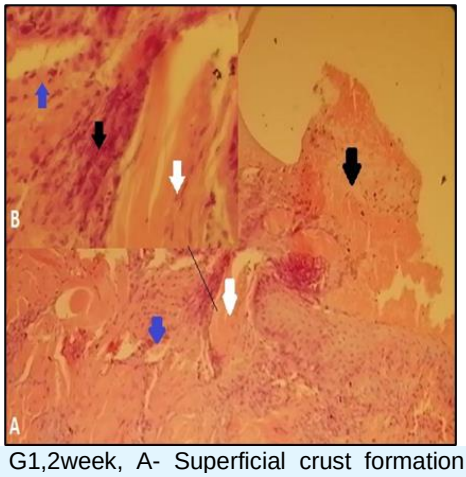

Fig. 2. G1,2week, A- Superficial crust formation (black arrows) and early stage of sub epithelial wound healing (white arrow), as well as newly formed blood vessels (blue arrow). H& E, X100. B- early stage of re-epithelization (black arrow) and formation of granulation tissue (white arrow) as well as congested newly formed blood vessel (blue arrow). H& E, X100

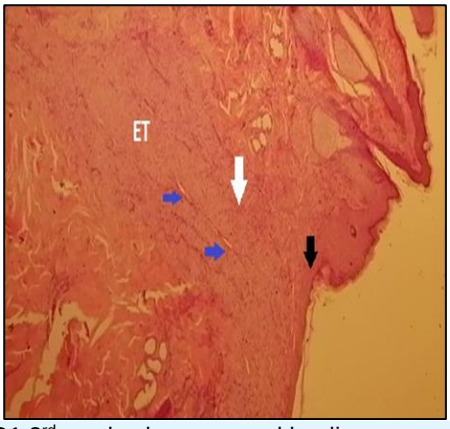

Fig. 3. G1,3rd week, shows a good healing process, where there are complete healing of epidermal layer (black arrows) covered by a thin layer of scar tissue (yellow arrow), as well as a good re-epithelization process in the dermal layer (White arrow) with forming epithelial tongue (ET) and blood vessels (blue arrows). H& E, X100

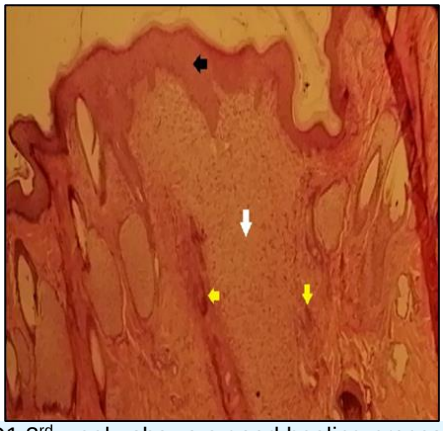

Fig. 4. G1,3rd week, shows a good healing process, where there are complete healing of epidermal layer (black arrows) covered by a thin layer of scar tissue (yellow arrow), as well as a good re-epithelization process in the dermal layer (White arrow) with forming epithelial tongue (ET) and blood vessels (blue arrows). H& E, X100

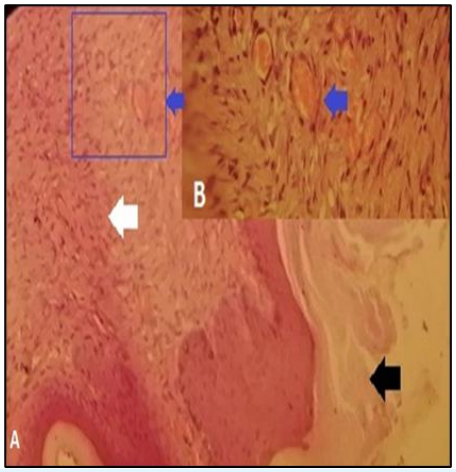

Fig. 5. G2, 1st week, The wound area (black arrow) showing approximation of the wound edges with weak epithelization of wound surface. A- H& E, X100. B- H& E, X100

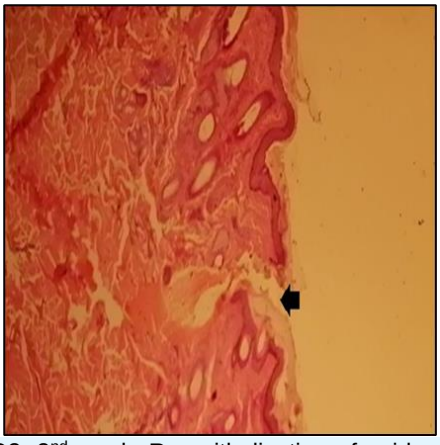

Fig. 6. G2, 2nd week, Re-epithelization of epidermal layer and the scab still present (black arrow), the neodermis of wound (white arrow) showing rich vascularization in regenerated wound area (blue box and arrow). A- H& E, X100. B- H& E, X100

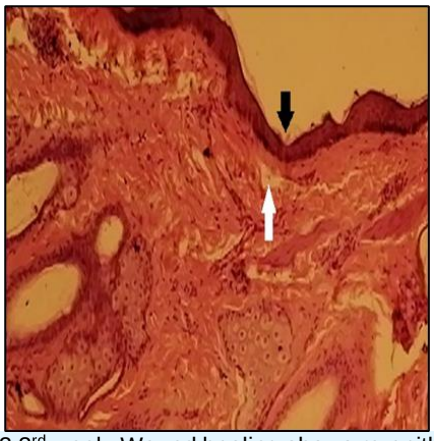

Fig. 7. G2,3rd week, Wound healing shows re-epithelization of epidermal layer (black arrow) with mild dermal edema (white arrow). H& E, X100

control groups G3 and G4 respectively. In previous study (Perrault, et al. 2018, Abdulhameed et al., 2018) who use local administration of IL-1Ra to improve the diable wound healing.

The results of **Table 1** show an considerable increase of the WBC count after one week of injuries which are accompanied with the findings of (de Souza1, et al. 2017) even this increase is not significant. The macroscopic closure of incisions take maximum time 43 days in general for all mares. The macroscopic signs observe during the daily examinations accompanied with (Ferreira, et al. 2007) who find a dark crust from the clot formation in the incisions, followed by granulation tissue formation, contractions, and re-epithelization with reduce scar tissue specially in G1 due to their wounds are fixed which may give good chance for fibroblasts migration and fill the wound pad where as G2 may less due they are movable.

In this study **Table 1** show the maximum values of IL-1Ra concentrations after one week of injuries, significant increase after one week of injuries, indicate to

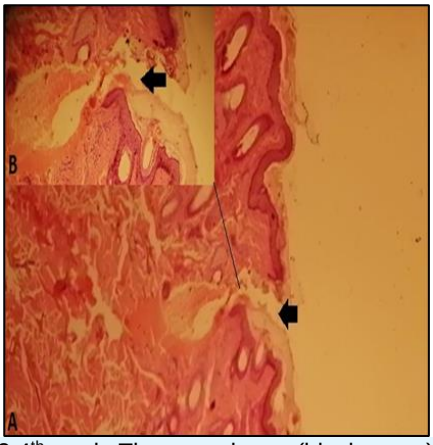

Fig. 8. G2,4th week, The wound area (black arrow) showing approximation of the wound edges with weak epithelization of wound surface. H& E, X100

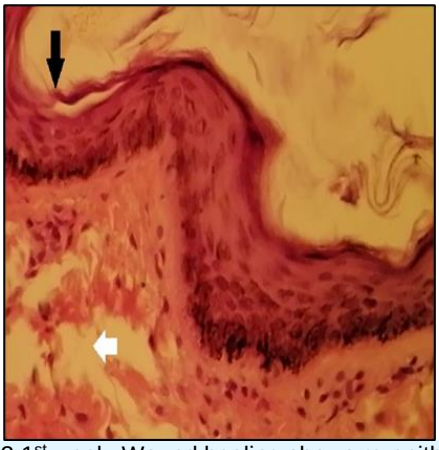

Fig. 9. G3,1st week, Wound healing shows re-epithelization of epidermal layer and forming of scab (black arrow) with mild dermal edema (white arrow). H& E, X100

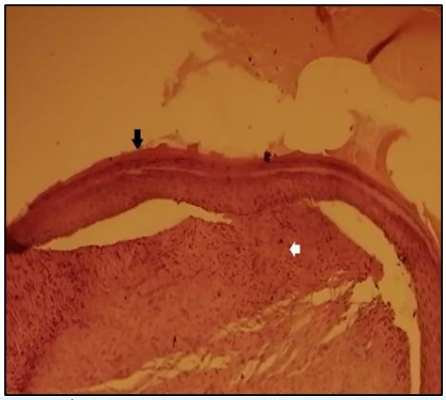

Fig. 10. G3,2nd week, Wound healing shows scab with re-epithelization and epithelial tongue forming (white arrow) of neodermis in the wound area (black arrows). H& E, X100

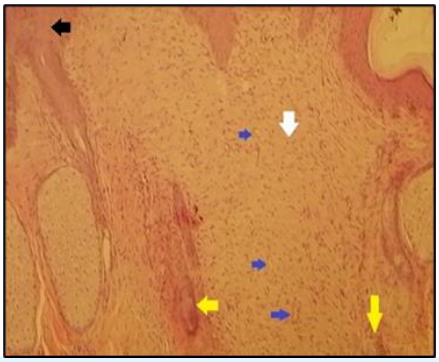

Fig. 11. G3,3rd week, Complete epidermal healing (black arrow), good re-epithelization process in the dermal layer and the epithelial tongue (white arrow) is contain a newly formed blood vessels and hair follicles (yellow arrow). H& E, X100

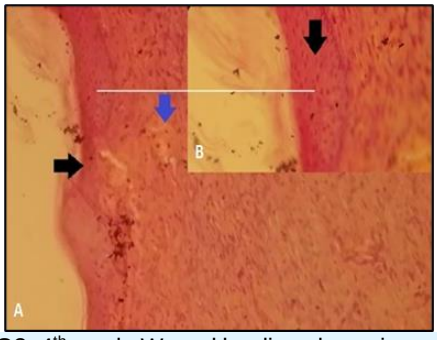

Fig. 12. G3, 4th week, Wound healing shows incomplete re-epithelization of epidermal layer, where thin epidermal layer that showing vacuolization of the prickle cells with absence of keratin (black arrow) and neodermis in the wound area is rich in blood vessels(blue arrows). A- H& E, X100. B- H& E, X100

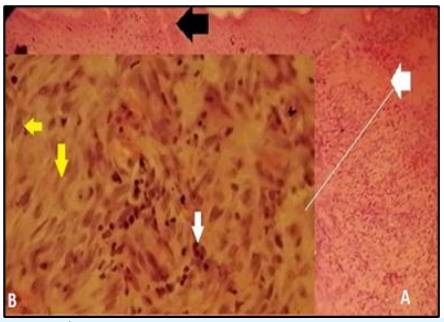

Fig. 13. G4, 1st week, Complete healing of epidermal layer (black arrow) and the neodermis of wound showing disorganized fibroblast proliferation (yellow arrows) with blood vessels, angioblasts proliferation and infiltration of the inflammatory cells (white arrow). A- H& E, X100. B- H& E, X400

the releasing by leukocytes in blood circulation wounds. In addition presence of macrophages in the incision sites may the transition of inflammatory stage to the proliferative stage during the complex healing process and these cells may phagocytize the wound area and devitalize tissue as well as its job of phagocyte the bacteria and may release growth factors that stimulate fibroblasts migration inside incisions (Schultz, et al. 2011).

Histopathological evaluations show too many facts which explain clearly the effect of IL-1Ra on the healing of all experimental incisions from one week of topical use to four weeks. **Fig. 1** of G1 at one week show that there are Incomplete healing of epidermal layer, the new dermis of wound showing disorganized fibroblast

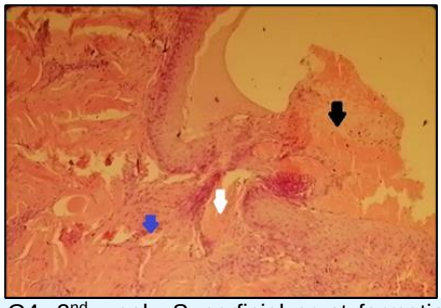

Fig. 14. G4, 2nd week, Superficial crust formation (black arrows) and early stage of sub epithelial wound healing (white arrow), as well as newly formed blood vessels (blue arrow). H& E, X100

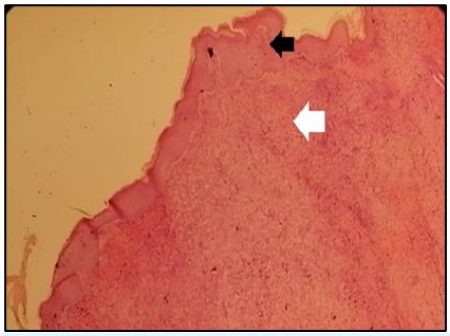

Fig. 15. G4,3rd week, Complete healing of epidermal layer (black arrow) the neodermis of wound showing disorganized fibroblast proliferation with blood vessels (blue arrows), angioblasts proliferation (blue arrows) and moderate infiltration of the inflammatory cells(white arrow). H& E, X100

proliferation with blood vessels, angioblasts proliferation and moderate infiltration of the inflammatory cells and free RBCs infiltration whereas after four weeks **Fig. 4** show that complete epidermal healing, good re-epithelization process in the dermal layer and the epithelial tongue is contain a newly formed hair follicles.

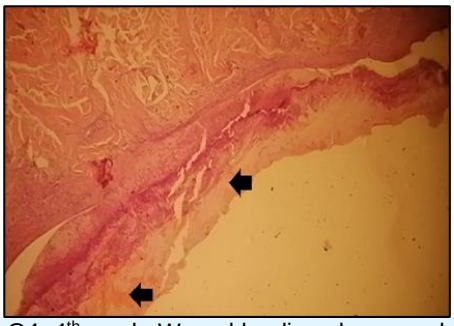

Fig. 16. G4, 4th week, Wound healing shows scab forming and hemorrhage in the wound area (black arrows). H& E, X100

These results indicate the superiority effect of IL-1Ra on fixed wounds healing. While **Fig. 5** of G2 at one week the wound area show approximation of the wound edges with weak epithelization of wound surface. Whereas after four weeks **Fig. 6** re-epithelization of epidermal layer and the scab still present, the neodermis of wound showing rich vascularization in regenerated wound area. The Histopathological results support the results in **Table 1** and **2** and accompanied with histological findings of (Thomay, et al. 2009) who find In skin wound models, IL-1 receptor knockout reduced inflammatory cell infiltration and fibrosis, and suggesting that IL-1 inhibition can provide therapeutic value in attenuating scar formation.

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