Original Article

Evaluation of the Performance of Melia Azedarach for Skin Wound Healing in Donkeys: Clinical and Histopathological Study

Al-Khafaji Nazar[1], Falah H. Khalaf[2], Ahmed H. AL-Zuhairi[1] and Ali Ibrahim Ali Al-Ezzy*[3]

[1]Department of Medicine, College of Veterinary Medicine, University of Diyala, Diyala, Iraq
[2]Department of Surgery, College of Veterinary Medicine, University of Diyala, Diyala, Iraq
[3]Department of pathology, College of Veterinary Medicine, University of Diyala, Diyala, Iraq

Address for Correspondence

Department of pathology, College of Veterinary Medicine, University of Diyala, Diyala, Iraq.
E-mail: alizziibrahim @gmail.com

ABSTRACT

Objectives: To determine clinical and histopathological efficacy of Melia azedarach in healing of surgical skin wound.
Methods: Incisions of 10 -12 cm length created bilaterally in the thigh, flank and neck in (6) donkeys, 4 – 5 years old, 150 kg body weight. Melia azedarach ointment (10%) applied on wounds daily for 14 days. Biopsies from wounds for histopathology taken on day 5, 10 and 15 respectively.
Results: Histopathological sections at 5th day for non-infected wounds treated with melia azedarach revealed PMNs infiltration and fibrin exudates. Acute inflammatory cell infiltration, necrosis of the epidermis as well as sloughing were detected in infected wounds treated with Melia azedarach. in10th day, in treating non infected wounds a fibrous connective tissue proliferation, formation of new blood vessels and collagen bundle formation were noticed.
Infected wounds treated with melia azedarach, inflammatory reaction extend from epidermal, dermal to the muscular layer noticed. In non-treated non-infected wounds, replacement with fibrous connective tissue noticed. At 15th day, in treating non infected wounds, regeneration of sweat and sebaceous glands, formation of smooth muscle fibers, mature connective tissue replacement, many newly formed blood vessels noticed. In treated infected wounds, inflammatory cell infiltration in subcutaneous tissue, fibrous connective tissue proliferation noticed. In non-treated non-infected wounds, slight inflammatory reaction, noticed.The wounds clinically healed on day 15, and it was better in both wounds in thigh regions, followed by that on flank regions.
Conclusion: Melia azedarach has an anti-inflammatory and antibacterial activity and used effectively in treatment of infected wounds, which completely heal in 15 days.

 ISSN 2321 – 2748

Keywords: Melia azedarach, Wound, Histopathology.

INTRODUCTION

Healing of wounds, whether acute or chronic pass through four stages or phases, Hemostasis, Inflammation, Proliferation or granulation and Remodeling or maturation[1].

The platelets play an important role in starting of hemostasis and healing process by closure any affected blood vessels via secretion of vasoconstructive substances under the influence of adenosine diphosphate leaking from damaged tissues. Aggregation and adherence of platelets to collagen takes place[2]. Inflammatory phase persists for 4 days after the initial injury. Cleaning process for the cellular debris and invading microbes takes place via macrophages as well as polymorphonuclear cells (PMNs) that escape from the blood vessel wall due to the vasoactive amines produce from mast[3]. Chemotactic and growth factors secreted from macrophages, such as transforming growth factor beta; epidermal growth factor; fibroblast growth factor and interleukin-1 play cardinal role in inflammatory phase[4].

Four days after the initial injury in acute wounds, the granulation stage starts and commonly persist for three weeks according to the size of wound[5]. In this stage, in wound base have a gritty red tissue, dermal and sub dermal tissues were replaced and contraction of the wound was takes place via collagen fiber framework produced by fibroblasts. The fibroblasts secrete the collagen framework on which further dermal regeneration occurs[6]. Angiogenesis was started by regeneration of the outer layers of blood capillaries and the endothelial cells via activity of Pericytes[6]. The final stage of epithelization occurs due to activity of keratinocytes, forming contracture as the keratinocytes differentiate to form stratum corneum[4]. In remodeling of the dermal tissues fibroblasts were the key cells for production of skin stretch strength represent the final stage in wound healing which may persist for two years after healing[7].

Initial goal in wound treatment is proscription of infection, rapid reestablishment, contraction and epithelization[8]. During a process of skin wound healing a Series of cellular and molecular episodes takes place including cell proliferation and differentiation as well as cells migration and augment of biosynthetic activity[1].

In first stage wounded area loaded with blood clot as well as fibrin to counteract the secondary infections. Necrotic tissue and cellular debris were get rid by granulocytes. In the second stage, formation of granulation tissue started. One of the most crucial phases in dermal wound healing is the cumulative increased in tissue strength by granulation tissue formation[9]. Dermis structure comprising from collages as well as elastic fibers reflecting good strength and mechanical properties of the skin[1].

Cutaneous wound healing affected by locally produced reactive oxygen species (ROS). Numerous antioxidants have recorded to get over tissue injury. Antioxidants were proposed as successful strategy for treatment of wound due to ability to free radical scavenging such as flavonoids which comprise the main part of Melia azedarach[9-11]. A flavonoid posse's anti-inflammatory activities[12].

Melia azedarach is a perennial tree of Meliaceae family. Aqueous extract of Melia azedarach have antioxidant properties[13]. Melia azedarach contain a number of organic molecules; terpenoids, flavonoids, steroids, acids, anthraquinones triterpenoids, meliantriol, melianone, melianol[13].

The objective of this study was to evaluate the Performance of Melia azedarach fruit ointment in surgical wound healing compared to normal wound healing without any medication in Donkeys Clinically and Histopathologically.

MATERIALS AND METHODS

The study conducted in Teaching Farm-College of Veterinary Medicine, University of Diyala-Iraq from January 2014 to September 2014. Approval of ethic committee in College of Veterinary Medicine, University of Diyala achieved before starting of current study.

Preparation of ointment

Melia azedarach fruit Vaseline ointment (10%) was prepared to this study[14].

Animals

This study performed on six donkeys, 4-5 years old, weighing 150 kg. The animals anaesthetized by local infiltration of 2% lidocaine hydrochloride at a dose rate of 1 ml/1 cm in the site of incision, intravenous sedation via acepromazine (2%) at dose 0.1 mg/kg.[15].

Wounds preparation

Surgical incisions of about 10-12 cm under aseptic conditions made bilaterally on skin of thigh, flank, and neck of 6-shedonkey. The incision was closed by using interrupted horizontal suture pattern using 2 non absorbable silk suture materials[16].

Treatment

Melia azedarach fruit Vaseline ointment applied on the incision daily, until complete healing in right side wounds of thigh, flank, and neck, in the same time, left side wounds of thigh, flank, and neck left without treatment as a control. Infected wounds treated with the ointment for 5 days. A biopsy taken from treated and untreated wounds for histopathological examination on days 5, 10 and 15 of the experiment. The wounds observed during daily wound care for the presence of exudates and wound healing until 15 day[16].

Histopathological evaluation

Samples of 1-2 cm length were took from the incision of both treated and control sites under the anesthesia and sedation, post of which the wound were closed by interrupted method. Tissue samples fixed in 10% formalin, dehydrated in alcohols, and embedded in paraffin wax. Serial sections of 5 µm were prepared, and stained with hematoxylin & eosin for routine histopatho-logical examination[17].

RESULTS

Histopathological findings at day 5 of experiment

A. Histopathological samples from treated non infected surgical wounds at 5th day post-operative, showed severe hemorrhage with proteinaceous material and acute inflammatory cells infiltration mainly PMNs, in other sections fibrins exudates also seen with PMNs infiltration Figure (1-A).

B. Histopathological samples from treated infected wounds at 5th day showed severe acute inflammatory reaction which extend to the subcutaneous tissue (Muscular layers) (Figure-1-B1). In other sections appearance of sloughing and necrosis of epidermal layer, with infiltration of inflammatory cells (Figure 1-B2).

C. Histopathological samples from non-treated non-infected surgical wounds at 5th day revealed: inflammatory reaction with infiltration mainly of PMNs (Figure 1-C).

Histopathological findings at day 10 of experiment

A. Samples from treated non-infected wounds at 10th day, showed proliferation of fibrous connective tissues (F.C.T.) consist mainly from newly formed and dilated blood vessels filled with inflammatory cells, collagen bundles formation also noted parallel to each of them. In other sections still few RBC had seen (Figure -2-A1& 2-A2).
B. Samples from treated infected wound at 10 day showed, continuous inflammatory reaction extend from epidermal and dermal to the muscular layer (Figure 2-B1&2-B2).
C. Histopathological samples from non-treated non infected surgical wounds at 10th day revealed that the injuring sites replaced with fibrous C.T. consist mainly from collagen fibers and fibrocystic parallel to each other and to the surface of skin , beneath it still there is inflammatory reaction (figure 2-C1-&2- C2).

Histopathological findings at day 15 of experiment

A. Samples from treated non infected surgical wounds at 15th day showed; regeneration of sweat glands and sebaceous glands and formation of smooth muscle fibers (Figure 3-A), the injurious parts of skin replaced with mature C.T. (scar tissue) **(**Figure 3A-1), in other sections many newly formed blood vessels dilated and filled with blood, with few inflammatory cells (Figure 3A-2).
B. Samples from treated infected wounds at 15th day showed; still there are inflammatory reaction extend from epidermal to dermal to muscle (figure 3-B), other sections showed infiltration of many inflammatory cells in subcutaneous tissue (lymphocytes, macrophages and PMNs (figure3-B1), in other section there is proliferation of fibrous C.T. consist from collagen bundles of small blood vessels, with inflammatory cells (figure 3-B2).
C. Samples from non-treated non-infected wounds at 15th day showed slight inflammatory reaction (Figure 3-C1&3-C2).

The wounds clinically healed on day 15, and it was better in both wounds in thigh regions, followed by that on flank regions.

DISCUSSION

The study revealed that healing processing were according the standard steps, at the 15-day post operation the wound in both treated non-infected and non-treated non-infected wound were clinically and histologically were normal.

Wound healing pass through four phases, initial stage starting at the moment of injury which is a coagulation process which hold back blood loss; then a stage of inflammatory reaction and debridement of wound area; followed by cellular proliferation, tissue remodeling and collagen deposition that constitute a repairing stage[18]. For complete wound healing persistent interactions takes place at cellular level as well as at cells and extracellular matrix that allow the healing steps to finish[19].

Various cells play a vital role in healing process and return the original architecture for skin such as leucocytes, monocytes, macrophages, fibroblasts, endothelial cells and epidermal cells .The presence of muscarinic receptors has been confirmed in fibroblast[20], endothelial cells[21] and keratinocytes[22], and thus in cells involved in the process wound healing of skin.

In current study the collagen bundles formation start 10 day post-operative in both

treated non infected and non-treated non infected wound, while in infected treated it appear in 15 day post-operative which is within normal healings time.

Motivation wound strength considered as one of the most important factors in the healing process and can be detected by measuring of total amount as well as quality of collagen whether new or deposited collagen[23] well as degradation of performed collagen[24]. Collagen is essentially in-charge-of the mechanical attributes of the skin[25]. The total collagen deposition in wound relies on differences between turnover and degradation of collagen[26]. Scar collagen replaced the normal one at initial time of wound and the original collagen does not regained by connective tissue which cause weakness of tensile strength in healed skin compared to the normal skin[27,28].

Re-epithelization, collagen deposition and revascularization are among the parameters that used for evaluation of healing wounds[28-30]. The immature vessels are originated from mature vessels of deeper tissues. Adequate blood supply to the wounded tissue may happed via anastomoses between mature and immature vessels to furnish the wound[1]. In the present study, increase in revascularization was observed in the melia fruit treated wounds on 10th day. Leaves paste prepared from Melia azedarach was used to treatment of wounds[28], others reported different therapeutic uses[30] against carbuncles and abscess as well as scabies.

CONCLUSION

Melia azedarach has an anti-inflammatory and antibacterial activity and can be used effectively for treatment of infected wounds, that heal clinically and histopathologically in 15 day.

REFERENCES

1. Cowin A, Waters J. The science of wound healing. Wound Management for the Advanced practitioner 2014:1.
2. Kumar ks, reddy be. Wound image analysis classifier for efficient tracking of wound healing status. Signal 2014.
3. Stephen-haynes j, gibson e, greenwood m. Chitosan: a natural solution for wound healing. *J comm nurs* 2014; 28(1):48-53.
4. Zheng z, lee ks, zhang x, nguyen c, hsu c, Wang JZ, et al. Fibromodulin-Deficiency Alters Temporospatial Expression Patterns of Transforming Growth Factor-²ζ Ligands and Receptors during Adult Mouse Skin Wound Healing. *Plos one* 2014; 9(3):e90817.
5. Kuck M, Strese H, Alawi SA, Meinke MC, Fluhr JW, Burbach GJ, et al. Evaluation of optical coherence tomography as a noninvasive diagnostic tool in cutaneous wound healing. *Skin Research and Technology* 2014; 20(1):1-7.
6. Beal Gw, Cocke D. process and composition of a gel for wound disinfection and promotion of healing. *US Patent* 20,140,314,873; 2014.
7. Yousefi S, Qin J, Dziennis S, Wang RK. Assessment of microcirculation dynamics during cutaneous wound healing phases in vivo using optical microangiography. *Journal of biomedical optics* 2014; 19(7):076015.
8. Tredget EE, Levi B, Donelan MB. Biology and Principles of Scar Management and Burn Reconstruction. *Surgical Clinics of North America* 2014; 94(4):793-815.
9. Kerstein MD. The scientific basis of healing. *Advances in Skin & Wound Care* 1997; 10(3):30-6.
10. Chow O, Barbul A. Immunonutrition: Role in Wound Healing and Tissue Regeneration. *Advances in wound care* 2014; 3(1):46-53.
11. Moor AN, Tummel E, Prather JL, Jung M, Lopez JJ, Connors S, et al. Consequences of age on ischemic wound healing in rats: altered antioxidant activity and delayed wound closure. *Age* 2014; 36(2):733-48.
12. Xiao J, Zhang Q, Gao Y-Q, Tang J-J, Zhang A-L, Gao J-M. Secondary Metabolites from the Endophytic Botryosphaeria dothidea of Melia azedarach and Their Antifungal, Antibacterial, Antioxidant, and Cytotoxic

Activities. *Journal of agricultural and food chemistry* 2014; 62(16):3584-90.
13. Rupa BA, Azam MNK, Mannan MA, Ahmed MN, Hasan MN. Phytochemistry and Pharmacological Appraisals of Persian Lilac (Melia azedarach Linn.): A Quick Comprehensive Review. *American Journal of Ethnomedicine* 2014; 1(3):152-63.
14. Dos Santos AC, Rodrigues OG, de Ara¼jo lcv, Santos sbd, Guerra Rd, Feitosa ML, et al. Use of neem extract in the control of acariasis by Myobia musculi Schranck (Acari: Miobidae) and Myocoptes musculinus Koch (Acari: Listrophoridae) in mice (Mus musculus var. Albina L.). *Neotropical entomology* 2006; 35(2):269-72.
15. Clarke KW, Trim CM. Veterinary anaesthesia: Elsevier Health Sciences; 2013.
16. Mair TL, Sandy; Schumacher, James; Smith, Roger KW; Frazer, Grant,. Equine medicine, surgery and reproduction: Elsevier Health Sciences; 2013.
17. Du Z. Technique of practical histology. Beiging: *People's Medical Publishing House* 1998:132-4.
18. Onah JA, Eze CA, Aba PE, Shoyinka SV. Histopathology assessment of incisional wound-healing behaviour of peritoneum-sutured and not-sutured techniques following laparotomy and omentopexy in West African dwarf (WAD) goats. *Comparative Clinical Pathology* 2014:1-6.
19. Baum CL, Arpey CJ. Normal cutaneous wound healing: clinical correlation with cellular and molecular events. *Dermatologic surgery* 2005; 31(6):674-86.
20. Buchli R, Ndoye A, Rodriguez JG, Zia S, Webber RJ, Grando SA. Human skin fibroblasts express m 2, m 4, and m 5 subtypes of muscarinic acetylcholine receptors. *Journal of cellular biochemistry* 1999; 74(2):264-77.
21. Kirkpatrick CJ, Bittinger F, Nozadze K, Wessler I. Expression and function of the non-neuronal cholinergic system in endothelial cells. *Life sciences* 2003; 72(18):2111-6.
22. Kurzen H, Berger H, J¼ger C, Hartschuh W, N¼her H, Gratchev A, et al. Phenotypical and molecular profiling of the extra neuronal cholinergic system of the skin. *Journal of Investigative Dermatology* 2004; 123(5):937-49.
23. Wojtowicz AM, Oliveira S, Carlson MW, Zawadzka A, Rousseau CF, Baksh D. The importance of both fibroblasts and keratinocytes in a bilayered living cellular construct used in wound healing. *Wound Repair and Regeneration* 2014; 22(2):246-55.
24. Goswami S, Kandhare A, Zanwar AA, Hegde MV, Bodhankar SL, Shinde S, et al. Oral glutamine administration attenuated cutaneous wound healing in Wistar rats. *International wound journal* 2014.
25. Duli¦„ ska-Molak I, Pasikowska M, Pogoda K, Lewandowska Mg, Eris I, Lekka Mg. Age-Related Changes in the Mechanical Properties of Human Fibroblasts and Its Prospective Reversal After Anti-Wrinkle Tripeptide Treatment. *International journal of peptide research and therapeutics* 2014; 20(1):77-85.
26. Barrientos S, Brem H, Stojadinovic O, tomiccanic M. Clinical application of growth factors and cytokines in wound healing. *Wound Repair and Regeneration* 2014; 22(5):569-78.
27. Greenhalgh DG. Skin, Soft Tissue, and Wound Healing in the Elderly. Geriatric Trauma and Critical Care: *Springer*; 2014. P. 37-44.
28. Abbasi AM, Khan MA, Ahmad M, Qureshi R, Arshad M, Jahan S, et al. Ethnobotanical study of wound healing herbs among the tribal communities in Northern Himalaya Ranges District Abbottabad, Pakistan. *Pak J Bot* 2010; 6:3747-53.
29. Tanaka A, Nagate T, Matsuda H. Acceleration of wound healing by gelatin film dressings with epidermal growth factor. *The Journal of veterinary medical science/the Japanese Society of Veterinary Science* 2005; 67(9):909-13.
30. Ikram S, Bhatti KH, Parvaiz M. Ethnobotanical studies of aquatic plants of district Sialkot, Punjab (Pakistan). *Journal of Medicinal Plants* 2014; 2(1).

 ISSN 2321 – 2748

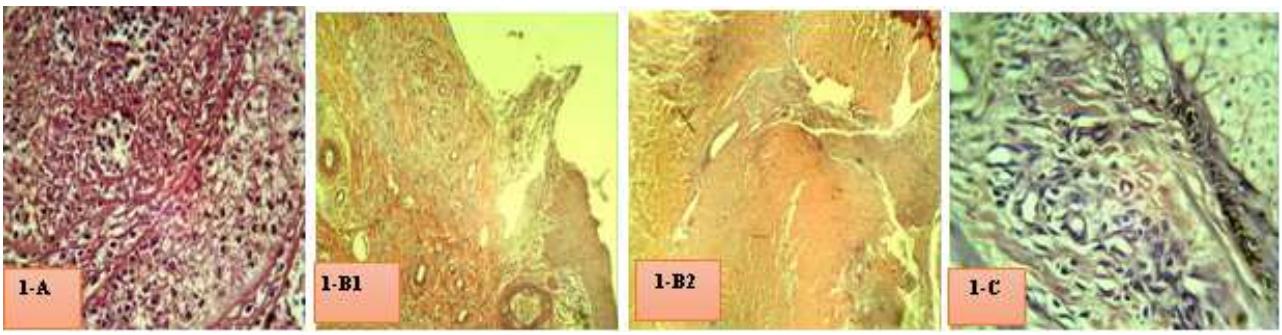

Figure 1. Histopathological findings of surgical wounds at 5th day post operation, 1-A: Histopathological samples from treated non infected surgical wounds at 5th day, 1- B: Histopathological samples from treated infected wounds at 5th day, 1- C: Histopathological samples from non-treated non-infected surgical wounds at 5th day

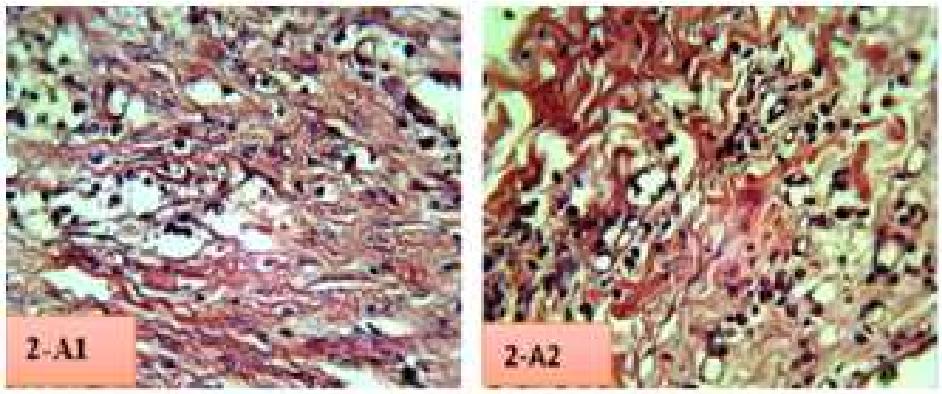

Figure 2-A. Histopathological findings of Treated non-infected wounds at 10th day post operation

 ISSN 2321 – 2748

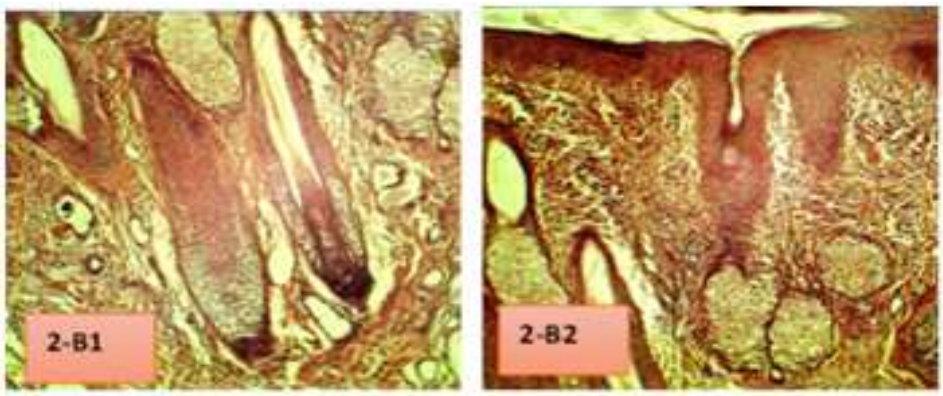

Figure 2-B. Histopathological findings of Treated infected wounds at 10th post operation

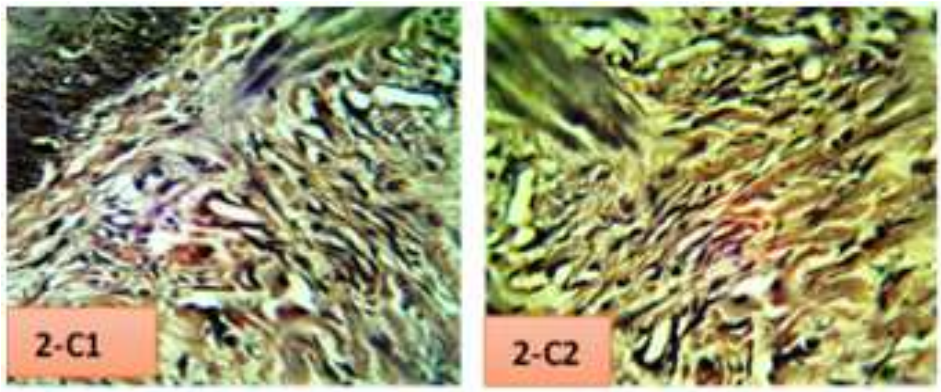

Figure 2-C. Histopathological samples from non-treated non infected surgical wounds at 10th day post operation

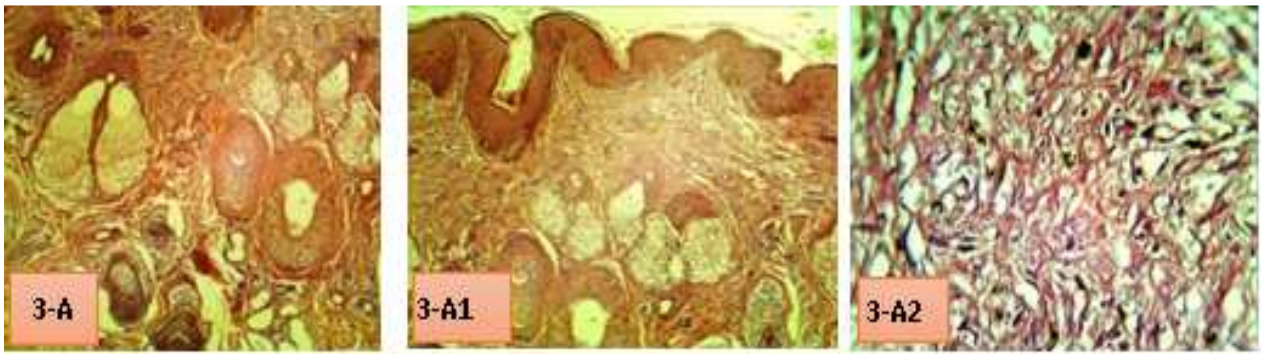

Figure 3-A. Histopathological findings of treated non infected surgical wounds at 15th post operation

 ISSN 2321 – 2748

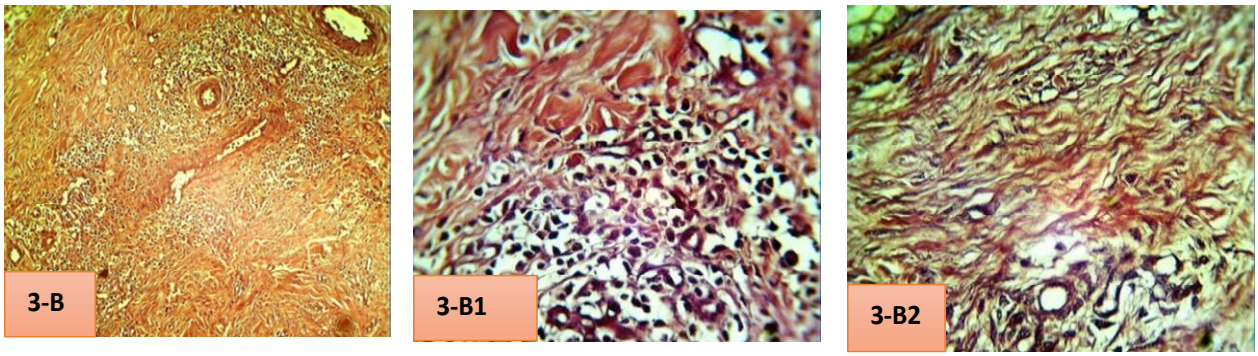

Figure 3-B, B1&, B2. Histopathological findings of treated infected surgical wounds at 15th post operation

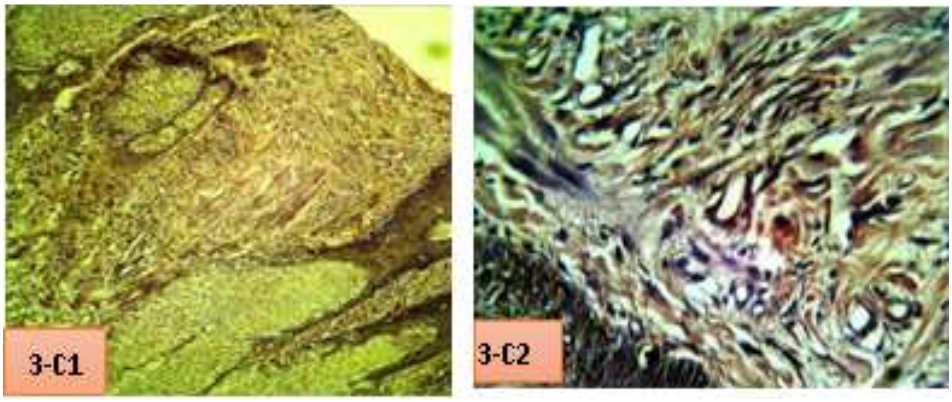

Figure 3-C. Histopathological findings of non-treated non-infected wounds surgical wounds at 15th post operation