

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(9): 108-110
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www.thepharmajournal.com
Received: 28-07-2021
Accepted: 30-08-2021

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Comparative evaluation of “Histopathological changes on infected wound healing in dogs” with the use of propolis and povidone iodine

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Abstract

This study was design to compare wound healing properties of traditional healing agent povidone with propolis in respect to histopathological differences. The study was conducted on 12 dogs more than 8 month of age, divided into two groups having 6 dogs in each. The animals of group 1 were treated with propolis ointment and the animals of group 2, treated with povidone iodine dressing for 7 consecutive days. A tissue was harvested from the wound area by pinch biopsy method on 0, 7th and 14th day and histology was performed by using H&E and Van Geison staining technique. Better histopathological change was shown by group 1 as compare to group 2 with hyperplastic regeneration of stratum corneum, showed marked re-epithelialization, well organized granulation tissue, and reduction in inflammation. Therefore propolis showed better healing response in infected wound healing in dogs as compared to povidone iodine.

Keywords: histopathological changes, hyperplastic regeneration, infected wound, Re-epithelialization, wound healing

1. Introduction

Wound healing is depicted and described in a discrete timeline of physical attributes (phases) comprising the post trauma repair process. In undamaged skin, the epidermis (surface layer) and dermis (deeper layer) form a protective barrier against the external environment. When the barrier is broken, an orchestrated cascade of biochemical events is set into motion to repair the damage (Orgill and Blanco, 2009)^[12]. Wound repair includes migration and proliferation of epidermal cells and keratinocytes, fibroblast adherence, and extracellular matrix contraction (Olczyk *et al.*, 2014)^[11]. Various agents which recently used in wound healing included Glycerol, Collagen, Tri-peptide Copper Complex (TCC) hydrogel, Silver nano gel, Platelet Rich Plasma (PRP), Zinc compounds, Aloe vera (Oryan, 2008)^[13], Sildenafil, Tocopherol, Pomegranate, Lantana, Chitosan, Stemcell therapy, Honey, and Hydroethanolic extract of ribwort plantain leaves (Farahpour and Heydari, 2015)^[5]. Aristotle has coined the word propolis from the Greek words pro (before) and polis (city), meaning, before the city or defender of the city. Hippocrates, a Greek physician, considered the father of modern medicine, recognized the healing properties of propolis (Martinotti and Ranzato, 2015)^[7]. Propolis is a resinous material collected by Apismellifera bees from plants exudates and buds and mixed with wax and bee enzymes (Neto *et al.*, 2019)^[10]. It consisted of 30% beeswax, 50% resins and vegetable balsams, 10% essential oils, 5% pollen, and 5% other substances. Its color varies from green and red to dark brown. Propolis has a characteristic pleasant smell and shows adhesive properties because it strongly interacts with oils and proteins of the skin (Ahmed *et al.*, 2018)^[3]. It is generally believed that honeybees produce propolis to help protect the hives.

Pereira and Bartolo (2016) studied the ability of propolis to promote the keratinocytes proliferation, the stimulation of glycosaminoglycan deposition in the wound, and the modification of the chondroitin/dermatan sulfate structure. As the antioxidant, anti-inflammatory and antimicrobial activities of the natural product constituents are the most important properties for wound healing and different natural substances on propolis may have all these actions, the wound healing effect of propolis is explicable. Therefore the current study was designated with the objective of comparing wound healing property of propolis with povidone in reference to histopathological changes at healing site.

2. Material and Method

The present study was carried out during the period of September 2017 to March 2018 in Teaching Veterinary Clinical Complex (T.V.C.C.) of College of Veterinary Science & A.H. Mhow in the Department of Veterinary Surgery and Radiology. The study was conducted on 12 young dogs with infected wound, more than 8 month of age irrespective of sex, and breed. These dogs were divided in two groups having 6 dogs in each group. The Ethanolic extract of propolis was used to prepare 30% w/w an ointment using petroleum jelly/Vaseline and the ointment was kept in sterile glass container, sealed properly and preserved at 4°C till use for topical application. Flushing of wound was done with normal saline in both the groups. Group 1 and group 2 dogs treated with daily dressing of open wound with propolis ointment and povidone iodine for 7 consecutive days respectively, along with parenteral injection of Cefotaxim @ 20mg/ kg bwt I/M q24h for 5 days. If required the dressing continued till healing of wound.

3. Histopathological studies

A tissue was harvested from the wound area by pinch biopsy method on 0, 7th and 14th day. The tissue was preserved and fixed in 10% formal saline and was processed, sectioned and stained with haematoxyline and eosine (H & E) to study the histological changes as per standard procedure recommended by Lillie (1954). A special stain Van Gieson's was used for demonstration of collagen fibers as per the standard procedure recommended by Lillie (1954).

4. Result and Discussion

4.1 Histopathological observations

The section taken before treatment on day 0, revealed heavy infiltration of inflammatory cells, tearing of dermis, and epidermis, haemorrhage and pigmentation also observed with necrosis of surface epithelium in both the group. (Plate- 1). After treatment on 7 day, marked decrease of inflammatory cells, neovascularization observed in both the group and granulation is immature in group 2 (povidone iodine treated group), while group 1 (propolis treated group) shows well organized granulation tissue. There was presence of loose connective tissue and change in epithelia with hyperplastic regeneration of stratum corneum in group 1. In group 2 on day 7 there was decrease in the inflammatory cells at oedematous granulation tissue however, that shows presence of immature epithelia growth along with presence of some blood clots beneath the epithelia and some necrotic tissue is observed. Therefore, wound treated with propolis ointment in group 1 showed better healing as compared to group 2, wounds treated with Povidone-iodine (Plate- 2, 3). These findings were also supported by Castaldo and Capsso (2002)^[4], Nayak (2006)^[9], Pereira and Bartolo (2016), who reported that the ability of propolis to promote the keratinocytes proliferation, the stimulation of glycosaminoglycan deposition in the wound, and the modification of the chondroitin/dermatan sulfate structure. Propolis is also has ability of increasing vascularization, preventing necrosis of cell, and synthesizes new cells along with thick vascular granulation tissues, more fibroblast and collagen deposition and epithelial migration. In comparison on day 14, in group 1 Van geison staining revealed large amount of collagen deposition on wound surface, well organized mature granulation tissue, highly vascularized area of wound, keratin layer well developed reaches almost up to the dermis with

almost complete re-epithelialization (Abel-wahed *et al.*, 2013)^[1], deposition of heavy amount of collagen in place of maximum damage, whereas in group 2, re-epithelialization was complete with minimal amount of collagen deposition, indicates low tensile strength of wound as compared to group 1 (Plate- 4, 5, 6). These finding were supported by findings of Abreu *et al.* (2012)^[2] and Olczyk *et al.* (2014)^[11], who reported that the propolis showed positive collagen metabolism in the wound during the healing process by increasing the collagen content of tissues, therefore propolis generate a favorable biochemical environment supporting faster wound healing process.

4.2 Figures

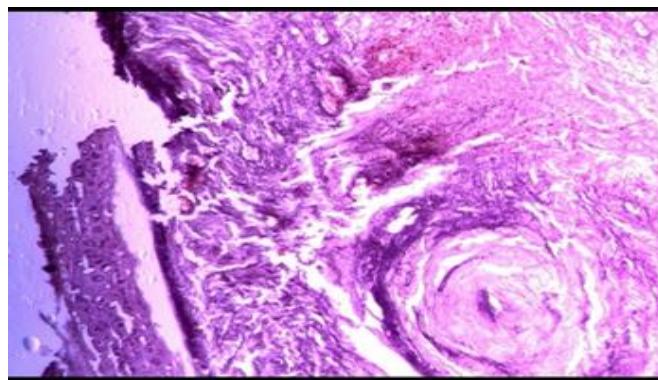


Plate 1: Tissue section showing infiltration of inflammatory cells, tearing of epithelia, presence of haemorrhages, and narcotic tissue on day 0, in both the groups (H & E 4X).

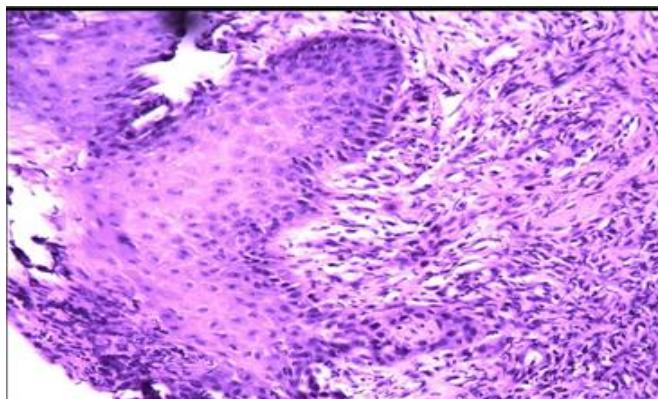


Plate 2: Photomicrograph showing hyperplastic re-epithelialization, and well organized granulation tissue at 7th day in group 1 (H & E 4X).

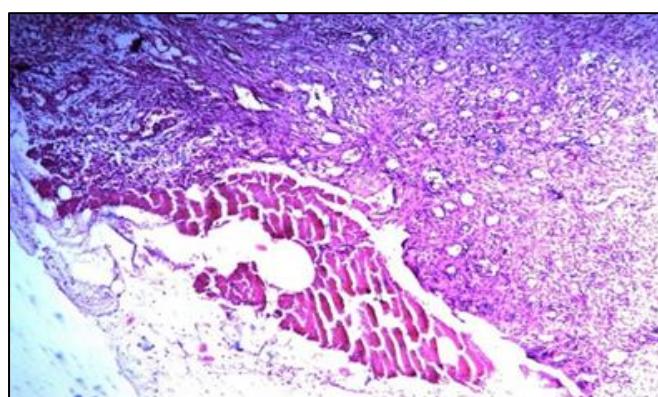


Plate 3: Photomicrograph showing immature re-epithelialization, presence of blood clot and immature granulation tissue on day 7 in group 2 (H & E 4X).

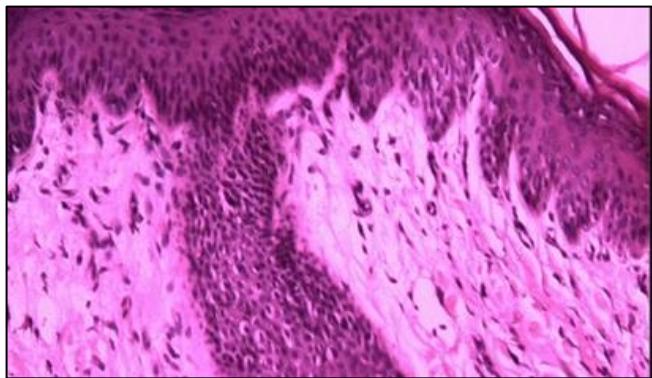


Plate 4: Photomicrograph showing mature epithelium, hyperplasia of stratum corneum, and well organized granulation tissue at day 14, in group 1 (H & E 4X).

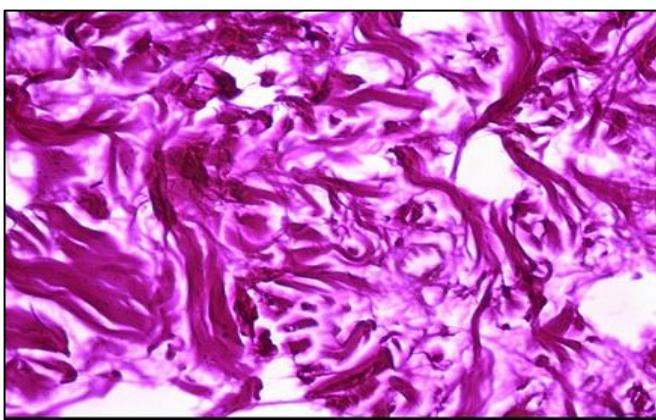


Plate 5: Photomicrograph showing mature fibrous tissue and well organized collagen fiber deposition appears characteristically red in colour at day 14 in group 1 (VG 4X).

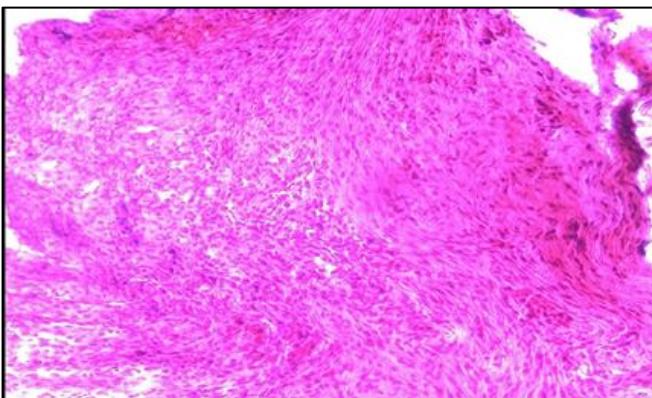


Plate 6: Photomicrograph showing mature fibrous tissue and minimal collagen deposition at day 14 in group 2 (VG 10X).

5. Conclusions

Although povidone iodine is extensively used in wound healing in dogs as traditional means of healing but in infected and non-healing wounds it revealed inappropriate outcomes. In such cases this study showed that propolis with their better healing capability of hyperplastic regeneration of stratum, large amount of collagen deposition, better keratinization with extend almost up to dermis with minimal time as revealed in Histopathological observations, a better option may be chosen for treatment of infected or deep non healing wounds in daily clinical practices in dogs.

6. Acknowledgments

Kuldeep Gautam express profound sense of gratitude to College of Veterinary Science & A.H. Mhow (NDVSU Jabalpur), for funding and supporting my research work.

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